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REVIEW

Fine-tuning plant valuable secondary metabolite biosynthesis via small RNA manipulation: strategies and potential

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Abstract

Plants produce secondary metabolites that serve various functions, including defense against biotic and abiotic stimuli. Many of these secondary metabolites possess valuable applications in diverse fields, including medicine, cosmetic, agriculture, and food and beverage industries, exhibiting their importance in both plant biology and various human needs. Small RNAs (sRNA), such as microRNA (miRNA) and small interfering RNA (siRNA), have been shown to play significant roles in regulating the metabolic pathways post-transcriptionally by targeting specific key genes and transcription factors, thus offering a promising tool for enhancing plant secondary metabolite biosynthesis. In this review, we summarize current approaches for manipulating sRNAs to regulate secondary metabolite biosynthesis in plants. We provide an overview of the latest research strategies for sRNA manipulation across diverse plant species, including the identification of potential sRNAs involved in secondary metabolite biosynthesis in non-model plants. We also highlight the potential future research directions, focusing on the manipulation of sRNAs to produce high-value compounds with applications in pharmaceuticals, nutraceuticals, agriculture, cosmetics, and other industries. By exploring these advanced techniques, we aim to unlock new potentials for biotechnological applications, contributing to the production of high-value plant-derived products.

Keywords Downregulation · MiRNA · Overexpression · Secondary metabolite · SiRNA · Small RNA

Introduction

Plants produce secondary metabolites that are crucial for defense, growth, and reproduction, and facilitate interactions with other organisms. These metabolites, known as plant specialized metabolites (PSMs), play vital roles in growth, development, signal transduction, and protection against biotic and abiotic challenges (Erb and Kliebenstein 2020; Xu et al. 2021). These secondary metabolites are considered end products derived from primary metabolites, while primary metabolites are the main precursors of secondary metabolites. The distinction between primary and secondary metabolites in plants is often unclear due to overlapping intermediates in their metabolic pathways, making differentiation challenging (Thirumurugan et al. 2018; Twaij and Hasan 2022). Some intermediates act as precursors for both types of metabolites, indicating shared metabolic pathways. Secondary metabolites in plants serve as a buffer for excess carbon and nitrogen, which can be reconverted to primary metabolites when needed. This balance is regulated by developmental processes, tissue differentiation, and stress responses (Thirumurugan et al. 2018).

Plants produce numerous secondary metabolites, with around 50,000 identified (Erb and Kliebenstein 2020). Precursors for PSMs originate from pathways like the Krebs cycle and the shikimate pathway. For instance, the shikimate pathway synthesizes phenolic compounds, while the mevalonic pathway produces terpenes (Jan et al. 2021). Sulfur-containing metabolites are derived from precursors like methionine and cysteine (Sobieszczuk-Nowicka et al. 2022). These metabolites can be further categorized based on chemical structures, functions, and biosynthesis pathways

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(Thirumurugan et al. 2018). Extensive research has been conducted on secondary metabolic pathways, such as phenylpropanoid, lignin, flavonoid, and glucosinolate pathways (Na et al. 2019; Liu et al. 2021). Well-studied PSM classes include terpenoids, steroids, fatty acid-derived substances, and alkaloids (Hussein and El-Anssary 2018; Thirumurugan et al. 2018).

For over a century, the biosynthesis of PSMs has been extensively studied in model plant species, including *Arabidopsis thaliana, Nicotiana tabacum,* and *Solanum lycopersicum* (Qin et al. 2022; Twaij and Hasan 2022). Certain economically valuable plant secondary metabolites are frequently generated in high concentrations in specific tissues or cell types of a specific species or among closely related species. Metabolites of economic significance, notably nicotine, and morphine, for example, are selectively synthesized in specialized cells within specific tissues (Jin et al. 2020; Pei et al. 2021). The biosynthesis of PSMs is intricately regulated at both the transcriptional and post-transcriptional levels, demonstrating precise temporal and spatial control.

sRNA fragments, typically ranging in size from 20 to 24 nucleotides, have been shown to play a crucial role in the regulation of various biological processes, including plant secondary metabolites (PSMs) biosynthesis. These sRNA fragments are produced by dicer-like (DCL) enzymes from double-stranded RNA substrates, and can also originate from single-stranded RNA or double-stranded RNA precursors with stem-loop structures (Morgado and Johannes 2018). Plants have been found to produce thousands of sRNAs, which regulate gene expression by causing post-transcriptional silencing of specific target genes through the inhibition of translation or cleavage of mRNA (An et al. 2022).

Recently, there has been a growing interest in using sRNAs, including miRNA and small interfering RNA (siRNA), to regulate PSM biosynthesis at the post-transcriptional level. This approach is seen as a promising way to better understand the mechanisms involved in regulating PSM biosynthesis (Hudzik et al. 2020; Ražná et al. 2022). The breakthrough study came from Hamilton and Baulcombe (1999) discovered sRNAs derived from plant transgenes experiencing RNA silencing (Hamilton and Baulcombe 1999). Several studies have shown the involvement of sRNA in the regulation of various metabolites, such as flavonoids, alkaloids, terpenoids, and other secondary metabolites (Marcela et al. 2019). Understanding the mechanisms involved in the sRNA-mediated regulation of PSM biosynthesis is essential for improving our knowledge of these important metabolites and potentially enhancing their production for various applications. Additionally, it should be noted that crops engineered with appropriate sRNAs have been deemed safe for consumption by the US Food and Drug Administration (FDA 1992), presenting an opportunity to explore the potential applications of sRNAs in manipulating valuable PSMs. Thus, this review aims to provide a comprehensive overview of the recent advancements in sRNA manipulation for enhancing secondary metabolite biosynthesis in plants, focusing on novel strategies and their potential applications in non-model plants. This review also integrates the latest research findings with practical applications and future directions, providing a unique perspective on this rapidly evolving field.

Roles of sRNAs in regulating PSM biosynthesis

PSMs are synthesized through a complex network of genes regulated at multiple levels, including transcriptional, post-transcriptional, translational, and post-translational stages (Jan et al. 2021). sRNAs act as both negative and positive regulators of gene expression (Hör et al. 2020). Transcription factors (TFs) like WRKY, MYB, bHLH, NAC, AP2/ERF, and bZIP play crucial roles in regulating PSM biosynthesis in response to stress (Jan et al. 2021). Significant correlations exist between sRNAs and TFs, forming regulatory routes involved in the transcriptional control of PSM biosynthesis (Hu et al. 2020). miRNAs, such as miR858, miR159, and miR828, regulate MYB expression, affecting secondary metabolite production in *Taxus chinensis* (Hu et al. 2020).

PSMs, such as alkaloids, terpenoids, phenolics, and glucosinolates, are vital for plant defense against biotic and abiotic stresses, acting as toxins, repellents, antioxidants, or signaling molecules (Waheed et al. 2021). The changes in sRNA expression in response to environmental changes can impact the synthesis and accumulation of PSMs, which play a crucial role in plant defense against biotic and abiotic stresses (Waheed et al. 2021). For example, miRNAs in *Brassica napus* have been shown to regulate secondary metabolism in response to cadmium-contaminated soil (Fu et al. 2019). miRNAs also help plants cope with abiotic stresses like temperature changes, salinity, UV exposure, and drought by regulating genes involved in photosynthesis and metabolism (Jan et al. 2021).

sRNAs are pivotal regulators in PSM biosynthesis, affecting gene expression at multiple levels. Various miRNAs and siRNAs have been identified in the biosynthesis of key metabolites like flavonoids, alkaloids, and terpenoids. However, the regulatory mechanisms for many of these sRNAs remain underexplored, especially in non-model plants. Future research should focus on comprehensive sRNA profiling across diverse species to uncover novel sRNAs. High-throughput sequencing and advanced bioinformatics can help identify sRNA-target interactions. Understanding these mechanisms and developing advanced manipulation techniques can unlock new potential for using sRNAs in biotechnology. This approach provides opportunities to improve

crop traits, support sustainable agriculture, and enhance the production of high-value plant products.

Strategies for manipulating small RNAs to regulate plant specialized metabolite (PSM) biosynthesis

Current approaches for manipulating PSM biosynthesis through the use of functional sRNAs have been widely adopted to investigate the specific roles of targeted genes in PSM biosynthesis, thus enabling the manipulation of useful metabolites in plants. Recent advances in genetic engineering have facilitated the development of more sophisticated methodologies, given the growing understanding of the impact of sRNA-mediated regulation on plant metabolism (Ražná et al. 2022). Manipulation of secondary metabolites through sRNAs, using both overexpression and downregulation strategies, can offer a promising approach to enhance or suppress the production of specific metabolites in plants. Several strategies can be implemented using overexpression and downregulation of sRNA (Fig. 1). The strategies applied in genetic manipulation include overexpressing the key biosynthetic genes, suppressing the competitive pathways, inhibiting the further conversion of the targeted compounds and subcellular localization of key gene expression (Majdi et al. 2016; Abbas et al. 2017; Majeed and Rehman 2022).

Alternative approach that potentially be applied in non-model plants is by manipulating the regulatory networks that regulate multiple genes in PSM biosynthesis through transcription factors and non-coding RNAs, including miRNAs. Upregulation of miRNAs can be achieved through the overexpression of endogenous miRNA genes or artificial miRNA (amiRNA) (Sharma et al. 2019). The application of amiRNA-mediated knockdown technology has enabled the downregulation of target genes of interest while avoiding off-target silencing issues commonly encountered with siRNAs (C. Wang et al. 2017a, b). Overexpression of endogenous miRNA genes also can be achieved via *Agrobacterium*-mediated transformation, transfection of synthetic miRNA mimics and virus-induced gene silencing (VIGS) (Alvarez et al. 2006; Adhikary et al. 2019; M. Wang et al. 2019a, b). Downregulation of miRNA can be achieved through RNAi targeting promoters of MIR genes (Vaistij et al. 2010) or other genetic engineering techniques such as short tandem target mimics (STTMs) which can cause significant reduction and degradation of targeted miRNA (Peng et al. 2018; Othman et al. 2023). Another approach is CRISPR/Cas9-mediated genome editing which can be used to specifically target and modify miRNA genes (Hong et al. 2021; Sharma et al. 2023). In addition to miRNA-mediated regulation, transcription factors (TFs) also play a crucial role in the regulation of terpenoid biosynthesis in plants (Samad et al. 2017). The combination of miRNA and TF-based approaches can provide a powerful tool for fine-tuning the biosynthesis of PSM. The regulation of gene expression can also be manipulated using synthetic siRNA with several inexpensive delivery methods into cells including *Agrobacterium*-mediated transformation, particle bombardment, and viral vectors. (Demirer et al. 2020; Ozyigit and Yucebilgili Kurtoglu 2020). Research has shown that DNA nanostructures can serve as effective cargo carriers for direct siRNA delivery and subsequent gene silencing in mature plants (H. Zhang et al. 2020). Short hairpin RNA also can be used as RNAi technology to negatively regulate gene expression by delivery of plasmids, viral and bacterial vectors into cells (Xu et al. 2019). This innovative approach holds promise for advancing plant biotechnology and improving crop traits.

Manipulating sRNAs to regulate PSM biosynthesis has shown great promise, with techniques, such as *Agrobacterium*-mediated transformation, synthetic miRNA mimics, and CRISPR/Cas9 genome editing, being effectively employed. Despite these advancements, challenges remain in achieving precise and efficient sRNA manipulation, particularly in non-model plants. Future research should aim to optimize these techniques, potentially through the development of more specific and less off-target sRNA mimics or CRISPR variants. Additionally, investigating the effects of sRNA manipulation under different environmental conditions could provide valuable insights into the stability and scalability of these approaches.

Manipulation of small RNA in alkaloid biosynthesis

Alkaloids have gained significant importance as secondary metabolites owing to their therapeutic potential. They are extensively utilized in the development of drugs due to their anti-proliferative, anti-bacterial, anti-viral, and anti-oxidant properties (Kaur and Ahmed 2021). They can be classified into true alkaloids, protoalkaloids, and pseudoalkaloids based on their chemical structure (Dey et al. 2020). Table 1 summarizes the sRNAs involved in alkaloid biosynthesis and their manipulation in various plants.

Pyridine and piperidine alkaloids

Alkaloids can be classified into different groups based on their parent bases, such as pyridine and piperidine alkaloids. Alkaloids are found in various plant species, including model plants like *Nicotiana tabacum* (tobacco) and non-model plants, such as *Piper nigrum* (black pepper), *Papaver somniferum* (Opium poppy), and *Erythroxylum coca* (Coca Plant) (Hu et al. 2019; Lewis et al. 2020).

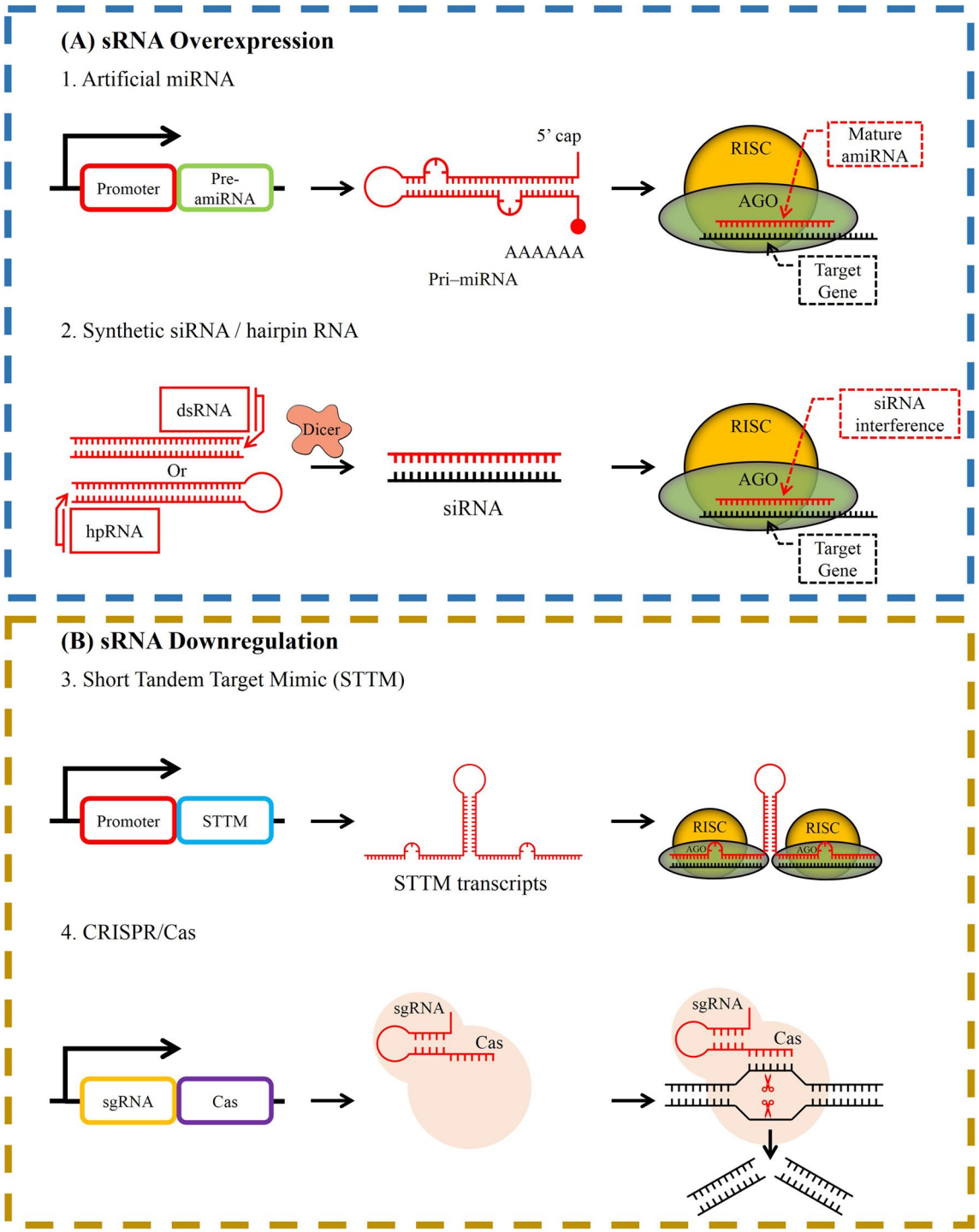

Fig. 1 **A** To overexpress sRNAs, including amiRNAs, a promoter is often used to enhance the expression of the pre-amiRNA, which is designed to have a hairpin structure similar to endogenous miRNA. This allows for targeted regulation of gene expression in specific tissues or cell types. Once transcribed, the pre-amiRNA is processed by the RNA silencing machinery to generate the mature amiRNA, which can then regulate target gene expression. Synthetic siRNA and short hairpin RNA can also be utilized for sRNA overexpression, which involves the enzyme Dicer processing the double-stranded RNA or hairpin RNA, followed by RISC guiding the siRNA to target and degrade specific mRNA molecules. **B** The STTM technique can be used to downregulate sRNAs by designing a sRNA molecule to mimic the miRNA-binding site on the target mRNA. This sRNA is then cloned downstream of a promoter in a plasmid and introduced into the plant genome, where it downregulates the target mRNA without affecting endogenous miRNA expression. CRISPR/Cas9 is an alternative approach for downregulating sRNA, utilizing a complex of single guide RNA (sgRNA) and Cas9 protein. The sgRNA is responsible for directing the Cas9 protein to the specific target site in the genome where it is intended to cut

Table 1 Type of sRNAs, target and manipulation of alkaloid in various types of plant

Secondary metabolites	Type of plant	Type of sRNAs	Target	Manipulation	References
Nicotine	*Nicotiana tabacum* L	MiR164, Nov-miR902, Nov-miR1646, miR167d, miR827, miRX13, miRX17, miRX20 miRX19,	Putrescine methyltransferase (PMT), BBL, WRKY family transcription factor, PMT2, QPT1, CYP82E4, and NAC_148	No	(Qi et al. 2012; Fu et al. 2013; Li et al. 2015; Jin et al. 2020)
		miRx27	QPT	Overexpression and silencing of nta-miRX27	(Li et al. 2015)
Piperine	*Piper nigrum*	miR166, miR396, and miR397	4CL, Peroxidase (PER) and Cinnamoyl-CoA reductase (CCR)	No	(Ding et al. 2021)
BIA	*Papaver somniferum* L	pso- miR t0047847, pso-miR2161, pso-miR13, pso-miR t0013376, and pso-miR t0000199	codeinone reductase (COR), S-adenosyl-L-methionine:30-hydroxy-N-methylcoclaurine 40-O-methyltransferase 2 (4-OMT), 7-O-methyltransferase (7-OMT), salutaridinol-7-O-acetyltransferase (SAT), and tyrosine/dihydroxyphenylalanine decarboxylase (TYDC)	No	(Boke et al. 2015)
Camalexin	*Arabidopsis thaliana*	miR1015	SUPERROOT1 (SUR1)	Overexpression of miR1015	(Kong et al. 2015)
		miR827	NITROGEN LIMITATION ADAPTION (NLA)	Overexpression of miR827	(Val-Torregrosa et al. 2022)
Camptotechin	*Nothapodytes nimmoniana*	pre-miRNA 159a	Geraniol-10-hydroxylase (NnCYP76B6)	Silencing of NnCYP76B6 using amiRNA	(Rather et al. 2018)
Artemisinin	*Artemisia annua*	miR160	Auxin Response Factor 1 (ARF1)	Silencing and overexpression of miR160	(Guo et al. 2022)

Nicotine, a pyridine alkaloid, is a naturally occurring compound found in species of the *Nicotiana* genus, most notably in *Nicotiana tabacum* L. Nicotine usually translocated to the aerial parts of tobacco plants under stresses due to plant wounding or loss of the apical inflorescence. This accumulation of nicotine serves as a natural defense mechanism against potential herbivores and protects the plant (Lewis et al. 2020). Despite being a well-known toxicant and primary addictive substance in most combustible cigarettes, nicotine has also been used in clinical settings as an analgesic and anti-inflammatory medication (Ribisl et al. 2019; Heinrich et al. 2021). Nicotine is also present in the leaves of other plant species, such as *Duboisia leichhardtii* (*D. leichhardtii*). This plant, which belongs to the nightshade family and is native to Australia, is known to have high levels of nicotine in its leaves (Singh et al. 2018).

Nicotine biosynthesis of the tobacco plant primarily occurs in the roots (Zenkner et al. 2019). "Topping" and "wounding" are common techniques used by tobacco growers to increase nicotine biosynthesis in the upper leaves of the plant. The biosynthesis of nicotine in *N. tabacum* is regulated through the activity of genes encoding key enzymes, such as putrescine methyltransferase (PMT) and quinolinate phosphoribosyltransferase (QPT), while jasmonic acid (JA) acts as a positive regulator of JA-induced alkaloid production (Shoji 2020). Previous studies have shown that miRNAs play a significant role in the biosynthesis of nicotine in tobacco, particularly during wounding or topping treatments (Tang et al. 2012; Jin et al. 2020). For instance, miR164 increases in the roots under these conditions, which influences nicotine biosynthesis through the regulation of NAC transcription factors (Qi et al. 2012; Tang et al. 2012).

In tobacco, nta-miRX27 regulates nicotine biosynthesis by targeting *QPT2*. Overexpression of nta-miRX27 decreases *QPT2* expression, while its silencing increases QPT2 levels, impacting nicotine accumulation. The regulation of nicotine biosynthesis involves multiple layers: one layer is the degradation of *QPT2* transcript by miRNA nta-miRX27 through a gene silencing mechanism, while the second layer involves the control of eTMs on nta-miRX27 and its impact on the *QPT2* and the biosynthesis of nicotine (Xie and Fan 2016). Another application of using sRNA to manipulate QPT can be seen in a study involving transgenic hairy root clones of *Duboisia leichhardtii* (Singh et al. 2018). The study implemented intron hairpin RNA (ihpRNA) to suppress the QPT gene, leading to a decrease in the production of nicotine and an increase in the synthesis of scopolamine (Singh et al. 2018).

Piperine, an alkaloid in *Piper nigrum*, is known for its sharp taste and therapeutic properties. It is concentrated in the berries, particularly at 8 months of development, and has potential as a drug target for neurodegenerative diseases (Hu et al. 2019; Azam et al. 2022). This 'king of spices' has been found to have 256 miRNAs that may be involved in piperine biosynthesis (Hu et al. 2019). Phenylalanine was identified as a precursor for the biosynthesis of piperine and other secondary metabolites (Ding et al. 2021). Three miRNAs and their target on the biosynthesis of piperine in black pepper fruits have been found to be enhanced in the berries of pepper. The findings reveal an upregulation of miR396, whereas miR166 and miR397 are found to be downregulated in fruits (Ding et al. 2021). The full scale of genome reference in black pepper distinguished the genes that upregulated for the biosynthesis of piperine including aminotransferase (PPA-AT), arogenate dehydratase (ADT), cinnamate 4-hydroxylase (C4H/CYP73), HCT, p-coumarate 3-hydroxylase (C3H), caffeic acid-3-O-methyltransferase (CAOMT), p-coumarate-CoA-ligase (4CL), glycosyltransferases (GTF) (Hu et al. 2019). To date, there is no manipulation of sRNAs in the biosynthesis of piperine. Thus, it is highly recommended to conduct further research and experimentation on the manipulation of piperine using sRNAs.

Benzylisoquinoline alkaloids

Benzylisoquinoline alkaloids (BIA) are a group of plant metabolites with medicinal properties that have been the subject of research for centuries. The structure of benzylisoquinoline is formed by replacing the heterocyclic isoquinoline with a benzyl group at the C1 position through the action of specific enzymes (Hagel and Facchini 2013). *Papaver somniferum* L. (*P. somniferum*), frequently known as opium poppy or breadseed poppy, is a flowering plant (Aragón-Poce et al. 2002). Historically, the opium poppy has been recognized as a medicinal plant that has been used for a very long time and cultivated as a traditional Chinese herbal for the past millennium. This has brought immense advantages and significant importance to human civilization (Heinrich et al. 2021). This ancient herbal remedy produces various secondary metabolites, such as morphine, sanguinarine, and codeine. Morphine alkaloids are also known well for anesthetics, cardiac protection, and anti-inflammatory prescription for modern medication (Heinrich et al. 2021).

In *Papaver somniferum*, studies have identified multiple miRNAs involved in BIA biosynthesis. miRNAs such as pso-miR t0000199 target codeinone reductase (COR), a key enzyme in morphine and codeine biosynthesis levels (Allen et al. 2004; Unver et al. 2010; Boke et al. 2015; Carr et al. 2021; Pei et al. 2021). RNAi-mediated silencing of COR increases morphine and codeine levels (Allen et al. 2004). In another study, Kempe et al. (2009) demonstrated that RNAi inhibition of salutaridinol 7-O-acetyltransferase (SalAT), an enzyme crucial for morphine biosynthesis in transgenic Papaver somniferum, led to increased levels of salutaridine and salutaridinol. Despite the reduction in SalAT transcripts, the quantity of salutaridine reductase (SaIR), which

converts salutaridine to salutaridinol, remained unchanged (Kempe et al. 2009). This step is followed by the acetylation by SalAT in the biosynthesis of morphine. Additionally, Pourmazaheri et al. (2019) identified nine miRNA precursors (mir477, mir319, mir396, mir159, mir828, mir171, mir167, mir4376, and mir169) across various families, suggesting potential regulatory roles in benzylisoquinoline alkaloid (BIA) biosynthesis. However, there is currently no evidence of targeted miRNA manipulation in the biosynthesis of morphine and other BIAs (Pourmazaheri et al. 2019).

Indole alkaloid

Camalexin (3-thiazol-2'-yl-indole) is a secondary metabolite that falls under indole alkaloid that poses the indole ring structure obtained from tryptophan (Zook and Hammerschmidt 1998). The evolution of plants toward protecting themselves against specific fungi, bacteria, or herbivores has been discovered with the involvement of camalexin (Nguyen et al. 2022). Camalexin is a crucial phytoalexin in *Arabidopsis*, playing a significant role in preventing bacterial and fungal pathogens. Glawischnig (2007) demonstrates that the infection by both biotrophic and necrotrophic plant pathogens induces camalexin production in *Arabidopsis* leaves. It was found that Flagellin peptide Flg22 treatments on *Arabidopsis* increased the level of miR393, which targets auxin receptors thus revamping the metabolic signaling into the production of glucosinolates and decreasing the synthesis of camalexin. An overexpression study of miR393 shows an increase in the concentrations of mRNAs that code for genes in the glucosinolate pathway. This study has examined the role of miRNA in prioritizing plant resources and defense against specific pathogens (Robert-Seilaniantz et al. 2011). Glucosinolates can be detrimental to bacteria (Sikorska-Zimny and Beneduce 2021), while camalexin does not contribute to necrotroph defense (Garcia et al. 2021). Biosynthesis of camalexin, Indole-3-acetic acid (IAA), and glucosinolates involve the participation of Indole-3-acetaldoxime (IAOx) as an intermediate in the biosynthesis pathway of indole alkaloids. The miRNA miR10515 has been identified as a specific regulator of the SUPERROOT1 (SUR1) gene (Kong et al. 2015). The SUR1 gene is responsible for encoding an enzyme involved in the biosynthesis of indole glucosinolate. Interestingly, when miR10515 is overexpressed, it enhances the production of IAA through the IAOx pathway. This was achieved by inhibiting the synthesis of glucosinolate and camalexin. The findings of this study support the notion that miR10515 directly targets SUR1 to regulate the biosynthesis of IAA and related compounds (Kong et al. 2015). *Arabidopsis* exhibits elevated levels of inorganic phosphate upon the overexpression of miR827 targeting the NITROGEN LIMITATION ADAPTION (NLA) gene. This overexpression also correlates with an increased biosynthesis of camalexin when the plant is challenged with *Plectosphaerella cucumerina* (Val-Torregrosa et al. 2022).

Nothapodytes nimmoniana is rich in anti-cancer properties, specifically camptothecin (CPT), classified as monoterpene indole alkaloid (MIA). Transcriptome study of *N. nimmoniana* discovered the role of geraniol-10-hydroxylase (NnCYP76B6) in the biosynthesis of CPT which converts geraniol into secologanin in MIA pathway. AmiRNA (pre-miRNA 159a) was used to silence the NnCYP76B6, resulted in the reduction of CPT content. This study indicates the possible regulatory role of NnCYP76B6 in biosynthesis of CPT (Rather et al. 2018).

Sesquiterpene alkaloid

Artemisinin is an example of sesquiterpene alkaloid which found abundant in the aerial part of *Artemisia annua*. Isolation of artemisinin were done by Chinese scientist in 1972 and used as antimalarial agent causing by *Plasmodium* (Badshah et al. 2018). Recent studies by Guo et al. (2022) reveal that the suppression of miR160 using Short Tandem Target Mimic (STTM) leads to an increase in the rate of artemisinin biosynthesis, concurrent with the growth of glandular trichomes. The activation of Auxin Response Factor 1 (ARF1) triggers the expression of AaDBR2 when miR160 is repressed, thereby amplifying the artemisinin pathway. This finding is corroborated by contrasting results observed in the overexpression of miR160. These findings suggest the involvement of miR160 in regulating both the biosynthesis of artemisinin and the development of glandular trichomes.

While the manipulation of sRNAs in plants like tobacco, *Arabidopsis*, and few medicinal plants has shown promising results in manipulating alkaloid biosynthesis, further research is needed to fully understand their regulatory mechanisms. Known sRNAs and miRNAs in plants, such as tobacco, *Papaver somniferum* (opium poppy), and *Piper nigrum* (black pepper), remain largely unmanipulated, offering substantial potential for future studies. Future studies should explore the use of CRISPR/Cas9 and synthetic miRNA mimics to target specific miRNAs involved in alkaloid biosynthesis, with a focus on increasing the production of medicinal alkaloids. Additionally, exploring the role of sRNAs in lesser-studied alkaloid-producing plants could uncover new regulatory pathways and broaden the application of these biotechnological tools.

Manipulation of small RNA in terpenoid biosynthesis

Terpenoids are one of the major groups of secondary metabolites in plants, which can be further classified according to the number of five carbon units in their skeleton, such

as hemi- (C_5), mono- (C_{10}), sesqui- (C_{15}), di- (C_{20}), sester- (C_{25}), tri- (C_{30}) and tetra- (C_{40}: carotenoids) terpenoids (Yazaki et al. 2017). The chemically diverse terpenoid compounds occur in all living organisms while flowering plants pose a high diversity of terpenoids compared with other organisms. Herbivory defense mechanisms in some plants can be mediated by terpenoids as signal molecules (He et al. 2020) and poisons to prevent the consumption by insects (Mumm et al. 2008), or by releasing the volatile terpenoids to attract natural enemies of the insect (Turlings et al. 1990). Terpenoid-based secondary metabolites are beneficial for human consumption and have been widely used for food, cosmetic and medical purposes (Pichersky and Raguso 2018). Such terpenoids include diosgenin, which has been used as a mimic of progesterone (Trauner and Novak 2020) while digitoxigenin is useful for heart disease treatment (Agrawal et al. 2012). Terpenoids also play important roles in biomaterials and biofuels (Tong 2013).

However, many terpenoid-producing plants are non-model plants. Manipulating rate-limiting step in non-model plants is limited due to the lack of genomic information, low transformation efficacy, and the duration to generate transgenic plants is time-consuming to manipulate multiple genes to get the optimal level of terpenoid (Majdi et al. 2016; Abbas et al. 2017). Nevertheless, manipulation of terpenoids through sRNAs has been reported in multiple plant species (Fig. 2).

Previous research has shown that manipulating miRNA to target transcription factors can effectively affect terpenoid biosynthesis in plants. Through overexpression and down-regulation using the mimicry of miR156, Yu et al. (2015) found that the biosynthesis of (E)- β-caryophyllene in *Arabidopsis* inflorescence is regulated by miR156 by targeting transcription factor *SQUAMOSA PROMOTER BINDING PROTEIN LIKE* (SPL), which activates the sesquiterpene synthase gene TPS21 expression. Through further study, they found that the overexpression of miR156 in the perennial fragrant herb *Pogostemon cablin* has led to the reduced expression of the sesquiterpene synthase gene PatPTS, and PatPTS major product, (−)-patchoulol (Yu et al. 2015). Withanolides can be classified as triterpenoids which naturally have a 30-carbon compound (Dhar et al. 2015). This

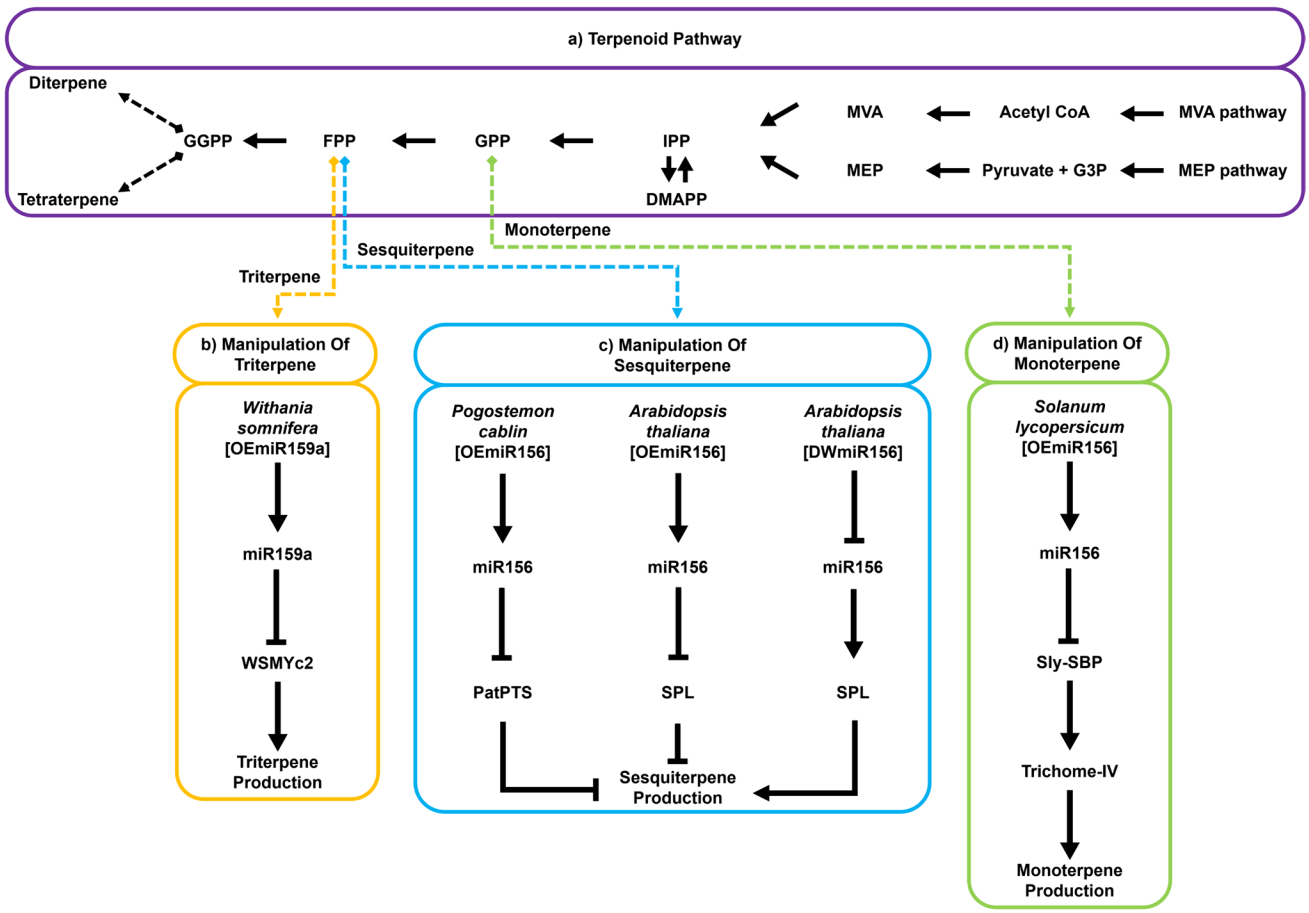

Fig. 2 The manipulation of terpenoids biosynthesis pathway through sRNA-mediated targeting the key genes has been demonstrated in numerous plant species

bioactive was shown to be synthesized in different parts of *Withania somnifera* (Sangwan et al. 2008). It has been reported that several miRNAs may regulate withanolide biosynthesis in *Withania somnifera*. In drought conditions, there was a discovery of decreased levels of endogenous miR5140 and miR159 situated in the roots. These microRNAs were observed to target genes responsible for cycloartenol synthase (CAS1) and sterol delta-7 reductase 1 (DWF1), respectively. Whereas miRNA families identified in the leaf namely miR530 and miR477 were highly upregulated during drought, targeting secoisolariciresinol dehydrogenase (ABA2) and zeatin o-glycosyl transferase (UGTs) (Srivastava et al. 2018). An amiRNA constructed from the backbone of *Arabidopsis* pre-miRNA159a has been expressed to silence the gene encoding MYC2 transcription factor in *Withania somnifera* WsMYC2. The miRNA-silencing of WsMYC2 affected the expression of several terpenoid biosynthesis genes, such as WsCAS, WsCYP85A, WsCYP90B, and WsCYP710A, while resulting in a significant reduction of withanolides and stigmasterol levels (Sharma et al. 2019). The roles of miRNA in targeting genes encoding other transcription factors, such as AP2/ERF, bZip, bHLH, NAC, WRKY, and MYB families, which may regulate terpenoid biosynthesis have been discovered in several plant species (Samad et al. 2019; Khan et al. 2020; Song et al. 2022). The identification of potential miRNA-targeted transcription factors and target genes that regulate terpenoid biosynthesis in plants opens opportunities for the manipulation of terpenoid biosynthesis in plants.

Trichomes are known to produce secondary metabolites, such as flavonoids, and terpenes, which play important roles in defense against herbivores as well as attracting pollinators. Type-VI trichomes produce mainly sesquiterpenes, monoterpenes, methylketones (Fridman et al. 2005; Besser et al. 2009) and flavonoids (Kang et al. 2014). Vendemiatti et al., (2017) found that the overexpression of miR156 has led to the abundance of type-VI trichome in *Solanum lycopersicum* (tomato) (Vendemiatti et al. 2017). This discovery highlights the potential for using miRNA manipulation to regulate the development of trichomes and the production of their associated allelochemicals.

The discoveries of sRNAs regulating terpenoid biosynthesis are not recent, even in non-model plants. Mevalonate (MVA) and non-mevalonate, or methylerythritol 4-phosphate (MEP) pathways are usually involved in the biosynthesis of terpenoids (Kong et al. 2022). Induction of terpenoid synthesis has been done previously by inoculation of *Persicaria minor* with *Fusarium oxysporum* (Samad et al. 2019). Novel miRNAs, such as pmi-Nov_13, and miRNAs: pmi-miR530, pmi-miR6173, and pmi-miR6300, were predicted to target transcripts that involved in the MVA pathway, while several genes that are involved in the MEP pathway were predicted to be targeted by miR396a and miR398f/g in *Persicaria minor* (Samad et al. 2019). Terpenoids derived from *Cinnamomum camphora* have been studied, and target identification analyses revealed that miR4995, miR5021, and miR6300 might be associated with the production of terpenoids in the plant. It was found that the expression of miR4995 was significantly upregulated (Chen et al. 2020). Terpenoids also contribute to the pigments, flavours and the scents of tea beverages (Zhao et al. 2018). *Camellia sinensis* leaves, that widely used in tea beverages, contain four classes of TFs that may regulate the expression of genes encoding terpenoid synthase under the regulation of miRNAs. These TFs include MYB, MYC, SPL, and AP2/ERF which have been targeted by various miRNAs (Zhao et al. 2018). A previous study by Fan et al., 2015 on the differential miRNA expression in young leaves and glandular trichomes of *Xanthium strumarium* L. provides some key findings on the roles of miRNAs in regulating terpenoid biosynthesis in plants through targeting genes that are involved in the upstream of the MEP and MVA pathway (Fan et al. 2015). More recently, a comparative analysis of miRNA expression in two varieties of tea plants *Toona sinensis* sprouts, Black Youchun (BYC) and Green Youchun (GYC), have identified eight miRNAs, including a novel miRNA regulating terpenoid biosynthesis, that were highly expressed in BYC compared to the GYC variety (Zhao et al. 2022). The biosynthesis of centellosides in *Centella asiatica* was increased as upregulation of most genes participating in triterpenoid pathway after elicitor treatment using salicylic acid. Expression of miRNAs (miR156, miR159, and miR1171) of multiple shoots in vitro grown cultures was downregulated which may suggest the probability of affecting triterpenoid pathway (Ranjith et al. 2022). *Gleditsia sinensis* Lam. contains various types of terpenoids including gleditsioside, thiamine, and brassinosteroids (Yang et al. 2022). The first report of miRNA identification in (*G. sinensis*) demonstrated that the targets of miR2093-5p, miR4414b, miR5037a, miR829-3p.1, and miR838-3p may be involved in regulating the biosynthesis of triterpenoid saponin and monoterpenoid (Yang et al. 2022). Small RNA profiling studies in *Chlorophytum borivilianum* (C. *borivilianum)* have identified mcliRNAs that involved in terpenoid biosynthetic pathway which focus on saponin. KEGG pathway mapping of the targeted genes predicted that miR9662, miR894, miR172, and miR166 may be participating in controlling saponin production in C. *borivilianum* by targeting terpenoid backbone biosynthesis, particularly the MEP and MVA pathway (Kajal and Singh 2017).

The discovery of sRNAs, target gene and manipulation of terpenoid in various types of plant are listed in Table 2. Despite the progress made in identifying miRNAs involved in terpenoid biosynthesis, precise manipulation approaches, particularly in non-model plants, remain limited. Future research should prioritize the identification and functional characterization of miRNAs and siRNAs involved in the

Table 2 Type of sRNAs, target and manipulation of terpenoid in various types of plant

Type of secondary metabolite	Type of plant	Type of sRNAs	Target	Manipulation	References
Terpenoid	*Solanum lycopersicum*	miR156	SlySBP	Overexpression of miR156	(Vendemiatti et al. 2017)
	Xanthium strumarium L	miR7539, miR5021, miR1134, miR6435, miR7540, miR5183, miR6449, miR5255, miR5491, and miR6435	1-deoxy-d-xylulose-5-phosphate synthase (DXS), isopentenyl diphosphate (IPP)/dimethylallyl diphosphate (DMAPP) synthase (IDS), isopenteyl diphosphate isomerase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR), R- linalool synthase, gibberellin 3-oxidase, ent-kaurene synthase, squalene epoxidase, beta-amyrin synthase, and germacreneA oxidase	No	(Fan et al. 2015)
	Cinnamomum camphora	miR5021, miR6300	Chalcone synthase (CHS), abscisic acid 8′-hydroxylase 4 and novel_10 target cytochrome P450 724B1-like	No	(Chen et al. 2020)
	Camellia sinensis	ath-miR858b, ath-miR858b_R-3, vvi-miR3630-3p_L-3_1ss22CA, stu-miR156a, mtr-miR156e, rco-miR156f and mdm-miR535a, PC-3p-81_33418 and ptc-MIR156f-p3_2ss3CT22CT	MYB, MYC, SPL, and AP2/ERF	No	(Zhao et al. 2018)
triterpenoid	*Centella asiatica*	miR156, miR159, and miR1171	SPL, MYB	No	(Ranjith et al. 2022)
	Persicaria minor	pmi-Nov_13, pmi-miR530, pmi-miR6173, pmi-miR6300, Pmi-miR396a, and Pmi-miR398f/g	mevalonate kinase (MVK), diphosphomevalonate decarboxyl- ase (MVD), sesquiterpene synthase and farnesyl diphosphate synthase (FDS), HMGR, DXS, and 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR)	No	(Samad et al. 2019)
	Withania somnifera	miR5140, miR159, miR530, and miR477	Cycloartenol synthase (CAS1), DWF1, secoisolariciresinol dehydrogenase (ABA2), zeatin o-glycosyl transferase (UGTs), MYC	Silencing of EsMYC2 using amiRNA from pre-miRNA159a	(Srivastava et al. 2018; Sharma et al. 2019)

Table 2 (continued)

Type of secondary metabolite	Type of plant	Type of sRNAs	Target	Manipulation	References
	Toona sinensis	Tsi-miR-1260, Novel_28, tsi-miR390b-5p, tsi-miR-107b, tsi-miR156g, tsi-miR8155, tsi-miR482d-5p and tsi-miR395b-3p	DXS-1, 4-Hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR), Farnesyl diphosphate synthase (FPPS), Geranyl diphosphate synthase (GPPS), (Geranylgeranyl pyrophosphate synthase) GGPPS -1, ((E)-β-farnesene synthase) FAS, Terpene synthases (TPS) and (-)-(Germacrene D synthase) GDS		(Zhao et al. 2022)
	Chlorophytum borivilianum	miR172 and miR166	HDR and Squalene synthase (SQS)	No	(Kajal and Singh 2017)
Sesquiterpenoid	*Pogostemon cablin*	miR156	patchoulol synthase (PatPTS)	Overexpression of miR156	(Yu et al. 2015)
	Arabidopsis	miR156	SPL	overexpression and downregulation of miR156	

rate-limiting steps of terpenoid biosynthesis pathways. For instance, targeting miRNAs that regulate key transcription factors like MYB, MYC, and SPL, which modulate terpenoid synthase gene expression, could significantly enhance terpenoid production. Furthermore, developing high-throughput screening methods to evaluate the effects of sRNA manipulation on terpenoid profiles under different environmental conditions can provide valuable insights into optimizing these approaches.

Manipulation of small RNA in flavonoid biosynthesis

Flavonoids are a type of secondary metabolite that possess variable polyphenolic structures and are commonly found in fruits, vegetables, and various other parts of plants. Flavonoids have evolved to serve various functions in plants, including acting as pigments, providing defense against pathogens, and functioning as signaling molecules (Chen et al. 2023). Flavonoids can be classified into different subgroups based on the position of the B ring attachment to the C ring, and the degree of unsaturation and oxidation in the C ring. These subgroups include isoflavones, neoflavonoids, flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones (Ekalu and Habila 2020). In general, flavonoids are synthesized in the phenylpropanoid pathway from phenylalanine, which is derived through the shikimate pathway (Liu et al. 2021). The key enzymes that involved in flavonoid biosynthesis include chalcone synthase (CHS), chalcone isomerase (CHI), flavone synthase (FNS), flavonoid 3′-hydroxylase (F3′H), flavanone 3-hydroxylase (F3H), flavonoid 3′,5′-hydroxylase (F3′5′H), flavonol synthase (FLS), dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase (ANS) (Deng et al. 2018). The flavonoid biosynthesis pathway is regulated by genes, whose expression is influenced by various factors, such as plant development, light, hormones, and stress signals (Ye et al. 2021). Transcription factors like MYB, bHLH, TTG1, GL3, and PAP1 are crucial in regulating the expression of these genes (Liu et al. 2015). Several plant species have shown successful manipulation of flavonoid biosynthesis through sRNA-mediated approaches (Fig. 3). Genetic engineering has been widely used to manipulate the production of flavonoids. For instance, transgenic tobacco plants that expressed grape MYB114 exhibited increased flavonoid biosynthesis, suggesting that MYB114 is a positive regulator that specifically controls the branch point of the phenylpropanoid pathway, which is involved in the biosynthesis of flavonols (Tirumalai et al. 2019). However, the manipulation of flavonoid biosynthesis can also have unintended consequences. Silencing the chalcone synthase (CHS) gene in tomato fruits results in reduced flavonoid content, leading to

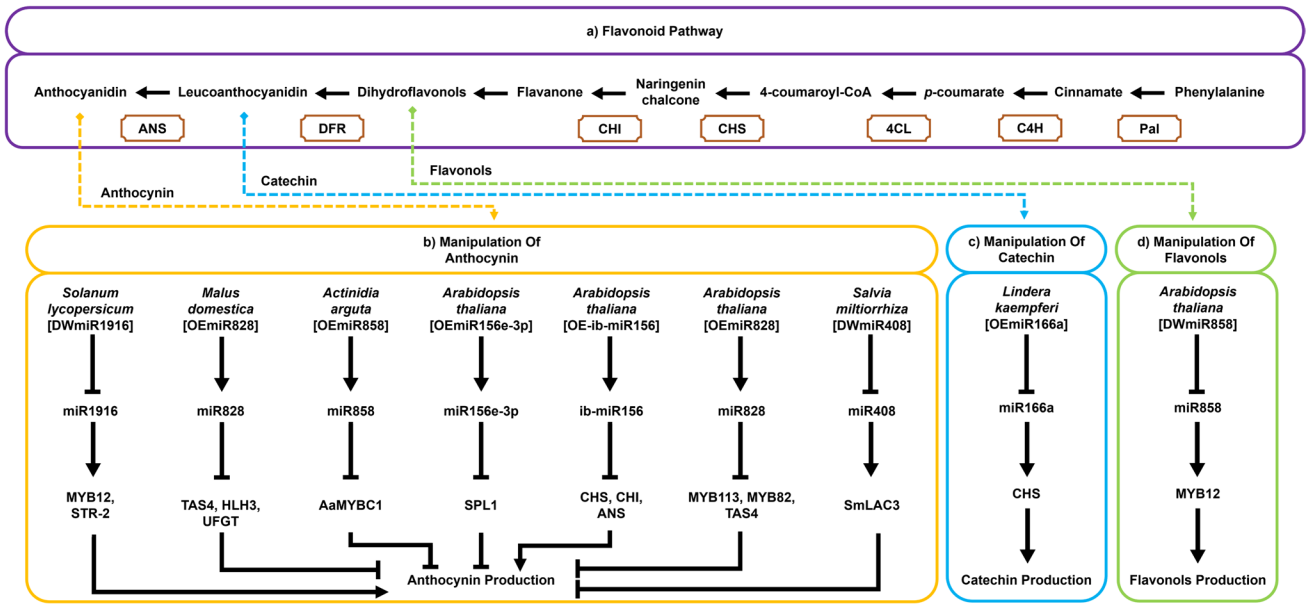

Fig. 3 Manipulation of flavonoid biosynthesis through sRNA-mediated approaches in several plant species

parthenocarpic fruit development and impaired pollen tube growth due to auxin deficiency. This ultimately restricts fruit expansion (Schijlen et al. 2007). These findings highlight the dual nature of genetic interventions in flavonoid biosynthesis. While it is possible to enhance flavonoid production by targeting specific regulatory genes, such manipulations must be carefully managed to avoid adverse effects on plant development and reproductive processes.

Previous studies have shown that variations in flavonoid production in the intergeneric hybrid of *Brassica rapa* and *Raphanus sativus*, known as *Brassicacoraphanus*, are caused by the regulation of miRNA expression levels. Specifically, miR157 targets genes encoding SPL, while miR858a targets genes encoding MYB, suggesting their role in regulating flavonoid biosynthesis in this hybrid. Furthermore, it has been found that miR858a is downregulated in hybrid plants (zhang et al. 2022)**.** It is proven that miR858 targets the transcripts of *AtMYB11*, *AtMYB12*, and *AtMYB111*, which encode the transcription factors of genes involved in flavonoid biosynthesis, such as CHS, CHI, F3H, and Flavonol Synthase1 (FLS1) (Liu et al. 2015). Furthermore, overexpressing miR858a in *Arabidopsis*, which targets R2R3-MYB, leads to the downregulation of several MYB transcription factors, including MYB11, MYB12, and MYB111, which regulate flavonoid biosynthesis enzymes, such as CHS, CHI, and FLS1. This downregulation shows a negative correlation with the expression level of miR858a (Sharma et al. 2016). Through high-throughput sequencing of flowers and leaves of *Osmanthus fragrans*, Shi et al. (2021) revealed that the high level of flavonoids in the flowers was correlated with the downregulation of miR858a and the up-regulation of MYB1, CHI, CHS, and FLS genes. Other studies have demonstrated that sRNA can be targeted to modulate the biosynthesis of different flavonoid classes in plants, presenting an alternative approach to control flavonoid production in plant products (Shi et al. 2021).

Chalcone

Chalcones are a class of flavonoids that have been investigated for their potential health benefits in various fields of medicine due to their reported anti-inflammatory, anticancer, anti-bacterial, and antioxidant properties (Salehi et al. 2021). RNAi was used to suppress CHI, which is an enzyme that converts chalcone into flavanones in *N. tabacum*. This manipulation resulted in lower pigmentation and altered the flavonoid concentration in tobacco flower petals. Transgenic lines with suppressed CHI also showed high chalcone concentration in pollen, which led to the yellow coloration of the flowers (Nishihara et al. 2005). The overexpression of Ptr-MIR397a in *Populus trichocarpa* has led to an increase in the abundance of chalcone synthase present in xylem (Lu et al. 2013). The regulation of CHS by sRNAs, which catalyzes the first reaction in forming chalcone, is not well studied. However, evidence from dahlia transgenic CHS gene families (*DvCHS1*, *DvCHS2*, *DvCHS3*, and *DvCHS4*) suggests that genes encoding CHS may be regulated by sRNAs post-transcriptionally, affecting red petal coloration and chalcone content in the plant. Additionally, it has been observed that specific unidentified sRNAs downregulate DvCHS2 in flavonoid-poor leaves of *Dahlia*, indicating that these particular sRNAs specifically regulate the DvCHS2

gene family, and possibly other CHS gene families as well (Ohno et al. 2018). While the current studies provide valuable insights into the manipulation of sRNAs in regulating chalcone and flavonoid biosynthesis in plants, further investigations will be necessary as we have very limited knowledge of the different plants that could potentially benefit from this approach. Expanding our research to include a wider variety of plant species will help us better understand the broader applications and implications of sRNA manipulation in plant biochemistry.

Flavanones

Conversion of chalcones to flavanones occurs via intramolecular cyclization catalyzed by CHI, which interacts with the 2′ OH group of chalcones to produce a 2′ oxyanion and subsequent cyclization (Liu et al. 2021). Understanding CHI regulation is crucial for modulating flavanone biosynthesis. Therefore, understanding the regulation of CHI is essential to modulate flavanone biosynthesis. Although miRNAs targeting CHI genes involved in flavonoid biosynthesis have been identified, no significant manipulation has been done to modulate CHI or flavanones through specific miRNAs. Further research is needed to determine the feasibility and effectiveness of using miRNAs to regulate the production of these compounds. An instance of miRNA targeting CHI genes was observed in *Murraya koenigii*, where mko-miR858 was identified as targetting chalcone synthase and chalcone isomerase (Gutiérrez-García et al. 2021). 239 miRNAs were identified to target 11 CHI genes in cotton plants (Zu et al. 2019). Specifically, *GbCHI05* was targeted by one miRNA, whereas *GbCHI01* was targeted by 72 miRNAs. These observations suggest that miRNAs are significant regulators of CHI genes and their expression, which can potentially affect the production of secondary metabolites in this plants. Further research is necessary to fully understand the feasibility and effectiveness of these miRNA interventions, which could pave the way for innovative approaches in agricultural biotechnology.

Isoflavones

Isoflavones are commonly present in leguminous plants, and they consist of a phenyl ring (B ring) that is bonded to another ring (C ring) at the C3 position (Nankar et al. 2016). Isoflavones have been extensively studied in the *Glycine max* plant (soybean). For instance, in the hairy roots of soybean, the turnover of TF was manipulated through GmMYB176-RNAi, resulting reduced level of phenylalanine compared to the control plants, which led to a decrease in the production of isoflavonoids (Anguraj Vadivel et al. 2019). Additionally, GmbZIP5-RNAi also caused the reduction of isoflavonoids concentration in soybean hairy roots (Anguraj Vadivel et al. 2021). On the other hand, when cotyledon tissues of soybean were transformed with *Agrobacterium rhizogenes* carrying an RNAi construct for Isoflavone synthase (IFS), it led to reduced production of isoflavones in the roots. The RNAi construct binds to the IFS gene and suppresses its activity, resulting in lower levels of the targeted gene. Interestingly, the silencing of IFS in the transgenic hairy root of soybean is expected to cause nearly complete suppression of mRNA accumulation in IFS1, IFS2 and also accumulation of isoflavone (Subramanian et al. 2005). Another study on soybean demonstrated that the silencing of IFS (isoflavone synthase) during infection with *Phytophthora sojae* leads to the inhibition of 5-Deoxyisoflavone production, which in turn suppresses cell death in the roots (Graham et al. 2007). In a more recent study aimed at increasing the production of isoflavones, bivalent RNAi was used to simultaneously silence both F3H and GmFNSII genes. The downregulation of these genes significantly increased the biosynthesis of isoflavones in transgenic soybean plants (Jiang et al. 2014). A previous study by Gupta et al. (2019) has suggested that miRNA plays an important role in regulating the production of isoflavones in soybeans, with varying expression levels observed between different genotypes. This miRNA was found to regulate various TFs involved in isoflavone biosynthesis, including MYB65-Gma-miR159, MYB96-Gma-miRNA1534, MYB176-Gma-miRNA5030, SPL9-Gma-miRNA156, TCP3 families, TCP4-Gma-miRNA319, WD40-Gma-miRNA162, flavonoid3-O-glucosyltransferase-Gma-miRNA396, and CHI3-Gma-miRNA543. Further studies are necessary to fully understand how miRNA can precisely regulate the production of isoflavones in plants.

Flavonol

Flavonols are a type of flavonoid that have a backbone composed of 3-hydroxyflavone, with phenolic -OH groups in varying positions. In *Pyrus bretschneideri* Rehd (pear) fruit, the regulation of flavonols biosynthesis involves various TFs, including PbMYB12b. Zhai et al. (2019) found that the overexpression of PbMYB12b increased the accumulation of most flavonol glycosides, except for quercetin 3-arabinoside, which is induced by the upregulation of PbFLS. The silencing of PbMYB12b by RNAi was found to affect the concentration of flavonol glycosides more in the leaves than in the fruits (Zhai et al. 2019). One subclass of flavonols, namely quercetin, acts as a phytochemical involved in the plant defense system against pathogens, herbivores, and insects and abiotic stresses (Singh et al. 2021). In rice, overexpression of miR156 was found to target the quercetin-related transcription factor SPL9, resulting in a lower growth rate of seedlings under cold temperatures (Cui et al. 2015). In a recent study, it was found that miR156 acts as a negative regulator of SPL expression, indirectly affecting male fertility

in rice during temperature changes. It was also discovered that infertile males in rice have lower levels of quercetin (Sun et al. 2022). Furthermore, kaempferol, another subclass of flavonol, is known to exhibit a plant defense mechanism against fungal infections. In *Arabidopsis*, it was discovered that plant lines with downregulated miR858 (MIM858) were more tolerant to necrotrophic or hemibiotrophic fungal parasites compared to those with overexpressed miR858 and wild-type plants (Camargo-Ramı´rez et al. 2018). The increased tolerance is induced by the upregulation of flavonoid content, particularly kaempferol, which is found to be most abundant in fungal-infected plants. Moreover, transgenic plant lines were found to be tolerant to fungal infections even without a lignification defense system, as kaempferol possesses antifungal activity without lignin (Camargo-Ramı´rez et al. 2018). In another study, researchers used CRISPR-Cas9 to inhibit the activity of miPEP858a, which resulted in the upregulation of AtMYB12 and its target genes. This increase in gene expression ultimately led to enhanced flavonol biosynthesis in *Arabidopsis* (Sharma et al. 2020).

Catechin

Catechins, a subclass of flavonoids derived from flavonols, are abundant in different plant organs including fresh tea leaves, fruits, and vegetables (Bernatoniene and Kopustinskiene 2018). Silencing MYB134 using RNAi resulted in reduced catechin biosynthesis in *Populus* spp. (poplar) leaves (Gourlay et al. 2020). In *Larix kaempferi* (*L. kaempferi*), miR166a was found to be a positive regulator in flavonoid biosynthesis (Li et al. 2016; Fan et al. 2020). Overexpression of LaMIR166a *L. kaempferi* resulted in an increase in flavonoid biosynthesis, with upregulated genes and metabolites related to these pathways, including CHS, FLS, DFR, and cytochrome P450 (CYP75A) (Fan et al. 2020). Additionally, this upregulation also resulted in an enhancement of metabolites involved in the flavonoid biosynthesis pathway, such as dihydroquercetin, catechin, and procyanidins (Fan et al. 2020).

The tea plant, *Camellia sinensis*, is an example of a plant that is rich in catechin content (Sun et al. 2017). Sequencing analyses in the leaves of *C. sinensis* at different developmental stages revealed negative correlation of expression pattern of six miRNAs (*cs*-miRNA167a, *cs*-miR2593e, *cs*- miR4380a, *cs*-miR3444b, *cs*-miR5251 and *cs*-miR7777-5p.1) and their target genes that involved in catechin biosynthesis, suggesting that these miRNAs may negatively regulate the catechin biosynthesis in C. *sinensis* (Sun et al. 2017). A more recent study by Li et al. (2021) found that the biosynthetic of gallated catechins in *C. sinensis* was negatively correlated with the expression of *cs*-miR156, while positively correlated with the expression of *cs*-miR172 and *cs*-miR166 (Li et al. 2021). Additionally, the expression of *cs*-miR169, *cs*-miR171, and *cs*-miR319 was found to be positively correlated with the production of nongallated catechin, along with the expression of genes encoding leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), which were predicted to be targeted by *cs*-miR169a, *cs*-miR169l, and *cs*-miR319h (Li et al. 2021).Additionally, cs-miR319b was found to be targeting two TF genes, *CsTCP3* and *CsTCP4*, that are related to catechin biosynthesis. The findings reveal an inverse relationship between cs-miR319b and *CsTCP4* transcripts, where *CsTCP4* expression increases when cs-miR319b is suppressed. Consequently, the upregulation of *CsTCP4* also increases the accumulation of catechin in leaves (Yu et al. 2021). Further research is essential to explore the potential of sRNA-mediated manipulation of catechin levels in plants, particularly in *C. sinensis*, since catechin content is vital for enhancing the taste and quality of tea. Previous studies have identified several miRNAs that directly target genes involved in catechin production in *C. sinensis*, and these miRNA targets have been verified using degradome analysis (Zhao et al. 2020)..

Anthocyanin

The involvement of several miRNAs, including miR156 and a novel miRNA in regulating genes encoding DFR has been predicted to occur during the sRNA-mediated regulation of anthocyanin biosynthesis in *Podophyllum hexandrum* following treatment with methyl jasmonate. DFR is a key enzyme in the biosynthesis of anthocyanins and also influences the production of other flavonoids, such as flavonols and proanthocyanidins (Biswas et al. 2022). The biosynthesis of anthocyanin in *Ipomoea batatas* L. tuberous sweet potato was found to be influenced by the differential expression of miRNAs in the roots. Specifically, ib-miR156a-5p and ib-miR858b were expressed at higher levels in purple-fleshed sweet potato compared to white-fleshed sweet potato, indicating their potential role in anthocyanin biosynthesis (He et al. 2019). Interestingly, in blackberry, miR858 was found to be involved in regulating most target genes involved in the biosynthesis of flavonoids and anthocyanins (Wu et al. 2022). Furthermore, sRNA sequencing of *Actinidia arguta* (kiwi) fruits has revealed the involvement of miR858 and its target gene *AaMYBC1* in regulating the production of anthocyanin. In the green flesh color phase of the fruits, there is a higher expression of miR858 which leads to a lower level of anthocyanin biosynthesis as compared to the red flesh color phase (Li et al. 2019). Transient expression assay confirmed the *AaMYBC1* were also targeted by miR858 in *Nicotiana benthamiana* (Li et al. 2020). Overexpression of miR858 resulted in suppression of coloration in kiwi fruit, whereas reduction in anthocyanin biosynthesis was also observed

when *AaMYBC1* was silenced through a virus-induced approach (Li et al. 2020).

In grapes, miR828 and miR858 were found to target the transcript encoding novel MYB called VvMYB114, which represses anthocyanin biosynthesis. Colored grape varieties were found to have low expression of, with high anthocyanin levels, while both miRNAs are highly expressed (Tirumalai et al. 2019). It was found previously that the overexpression of miR828 in *Arabidopsis* also resulted in a decrease in anthocyanin accumulation due to the targeting of key genes involved in anthocyanin synthesis, such as MYB113, MYB82, and TAS4, by miR828, which led to a reduction in the transcript levels that stimulate anthocyanin synthesis (Yang et al. 2013). In addition, miR828 was found to be involved in a negative feedback regulatory process associated with the biosynthesis of anthocyanin in *Malus domestica* (apple) peel. This was demonstrated in *Arabidopsis* transformants and apple peel overexpressing mdm-miR828, which showed decreased expression levels of target genes related to anthocyanin biosynthesis, such as MdTAS4, MdbHLH3, MdDFR, MdANS, and MdUFGT, resulting in lower accumulation of anthocyanin compared to the wild type (B. Zhang et al. 2020).

To gain a comprehensive understanding of the role of miR156e-3p in regulating anthocyanin biosynthesis, manipulation of sRNA was conducted (Zhao et al. 2017). Transgenic *Arabidopsis* plants that overexpressed miR156e-3p exhibited a distinct purple color in their lateral branches, whereas the wild-type plants had green branches. Further analysis revealed differences in the chromatographic peaks of flavonoids, particularly anthocyanins, and anthoxanthins, in the transgenic plants that overexpressed miR156e-3p (Zhao et al. 2017). Moreover, transgenic *Arabidopsis* seedlings that overexpressed ib-pri-MIR156 exhibited an upregulation of genes encoding anthocyanin-related enzymes (CHS, CHI, DFR, and ANS) and repression of SPL, resulting in a purplish phenotype (He et al. 2019).

Manipulation via miRNA silencing using STTM and amiRNA, as well as overexpression of sly-miR1916 targeting STR-2, modulated α-tomatine and anthocyanin biosynthesis in tomato, impacting immune response against *Phytophthora infestans* or *Botrytis cinerea* (Chen et al. 2019). Overexpression of miR1916 led to necrotic lesions in detached leaves, while silenced miR1916 plants showed higher anthocyanin levels due to its negative regulatory function (Chen et al. 2019). Zou et al. (2021) found that the flavonoid content in *Salvia miltiorrhiza* can be increased by suppressing miR408, which targets *SmLAC3*, leading to upregulation of the enzyme and enhanced synthesis of phenolic acids, such as rosmarinic acid and salvianolic acid B. Conversely, inhibiting miR408 results in lower levels of anthocyanin content (Zou et al. 2021). Genetic engineering, specifically the manipulation of sRNAs, has been a promising approach to enhance the production of anthocyanins in various plants. Additionally, studies could investigate the effects of manipulating sRNAs on anthocyanin production in a wider range of plant species, including both model organisms and economically important crops. Research could also focus on the environmental and physiological conditions that optimize the effectiveness of sRNA-based genetic modifications, ensuring stable and enhanced anthocyanin production under various growing conditions. Table 3 provides a summary of the target gene and manipulation involved in the biosynthesis of flavonoids, as well as the discovery of sRNAs in different types of plants that produce flavonoids.

Overall, our understanding of the complex regulatory networks involved in flavonoid biosynthesis pathways is still in its early stages. Future research should prioritize identifying sRNA targets within the flavonoid biosynthesis pathway, particularly those that regulate key enzymes, such as CHS and FLS. Additionally, exploring the interactions between sRNAs and transcription factors, including MYB, bHLH, and WD40, can provide deeper insights into the coordinated regulation of flavonoid biosynthesis. Investigating the influence of environmental factors, such as light and temperature, on sRNA-mediated regulation of flavonoid production can further aid in developing strategies to enhance flavonoid accumulation in plants.

Biosynthesis of fatty acid derivatives and other compounds

Fatty acid-derived

Fatty acid-derived secondary metabolites are a relatively small group of organic compounds found in plants. They are different from other plant-derived compounds that provide energy and make up cellular membranes. Fatty acid-derived compounds are produced through the fatty acid synthase (FAS) pathway, in which acetyl-CoA units are linked together to create a lengthy chain of carbon atoms with a carboxyl group at one end. These compounds can be saturated or unsaturated. In flowering plants, it is common to find long-chain fatty acids that contain up to three cis double bonds, with C16 and C18 being the most frequently produced (Böttger et al. 2018). Fatty acids-derived can be beneficial toward the preparation of herbal remedies from dried roots of *Echinacea purpurea* as immunostimulators for bacterial and virus infection (Madariaga-Mazón et al. 2019). Fatty acid-derived compounds play a crucial role in the production of oil composition in plants, regardless of whether the resulting oil is saturated or unsaturated. This contributes to the overall health and well-being of the plant (Böttger et al. 2018).

Table 3 Type of sRNAs, target and manipulation of flavonoid in various types of plant

Type of secondary metabolite	Plant species	Type of sRNAs	Target	Manipulation	References
Flavonoid	*Brassica coraphanus*	miR157 and miR858a	SPL and MYB	No	(zhang et al. 2022)
	Osmanthus fragrans	miR858	MYB1, CHI, CHS and FLS	No	
Chalcone	*Nicotiana tabacum*	NtCHI1-derived small RNA	CHI	Inhibition of CHI using siRNA	(Nishihara et al. 2005)
	Populus trichocarpa	Ptr-MIR397a	CHS	Overexpressing of Ptr-MIR397a	(Lu et al. 2013)
Isoflavone	*Glycine max*	GmMYB176-RNAi silenced (GmMYB176-Si)	MYB176	Inhibition of MYB using siRNA	(Anguraj Vadivel et al. 2019)
		GmbZIP5-RNAi (GmbZIP5-Si)	ZIP5	Inhibition of ZIP5 using siRNA	(Anguraj Vadivel et al. 2021)
		IFS-RNAi	IFS	Inhibition of IFS using siRNA	(Subramanian et al. 2005)
		Bivalent RNAi	F3H and GmFNSII	Inhibition of F3H and GmFNSII using bivalent RNAi	(Jiang et al. 2014)
		Gma-miR159, Gma-miRNA1534, Gma-miRNA5030, Gma-miRNA156, Gma-miRNA319, Gma-miRNA162, miRNA396, Gma-miRNA543	MYB65, MYB96, MYB176, SPL9, TCP3, TCP4, WD40, flavonoid3-O-glucosyltransferase, and CHI3	No	(Gupta et al. 2019)
Flavonol	*Pyrus bretschneideri* Rehd		MYB12b	Silencing of PbMYB12b using RNAi	(Zhai et al. 2019)
	Arabidopsis	miR156	SPL9	Overexpression of miR156	(Cui et al. 2015)
		miR858	AtMYB11, AtMYB12, AtMYB13, AtMYB20 and AtMYB111	Inactivation and overexpression of miR858	(Camargo-Ramı´rez et al. 2018; Sharma et al. 2020)
Catechin	*Populus* spp.	MYB134-RNAi	MYB134	Silencing of MYB134 using siRNA	(Gourlay et al. 2020)
	Larix kaempferi	miR166a	CHS, FLS, DFR, and cytochrome P450 (CYP75A)	overexpression of LaMIR166a	(Li et al. 2016; Fan et al. 2020)
	Camellia sinensis	*cs*miRNA167a, *cs*-miR2593e, *cs*- miR4380a, *cs*-miR3444b, *cs*-miR5251, *cs*-miR7777-5p.1, *cs*-miR156, *cs*-miR172, *cs*-miR166, *cs*-miR169, *cs*-miR171, and *cs*-miR319	leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), *CsTCP3* and *CsTCP4*	No	(Sun et al. 2017; Li et al. 2021; Yu et al. 2021)

Table 3 (continued)

Type of secondary metabolite	Plant species	Type of sRNAs	Target	Manipulation	References
Anthocyanin	*Podophyllum hexandrum*	miR156	DFR	No	(Biswas et al. 2022)
	Ipomoea batatas L	ib-miR156a-5p and ib-miR858b	ibSPL and ibMYB	No	(He et al. 2019)
	Vitis spp.	miR828 and miR858	VvMYB114	No	(Tirumalai et al. 2019)
	Arabidopsis	miR156e-3p	SPL1	Overexpression of miR156e-3p	(Zhao et al. 2017)
		ib-pri-MIR156	CHS, CHI, DFR and ANS	Overexpression of ib-pri-MIR156	(He et al. 2019)
		miR828	MYB113, MYB82, and TAS4	Overexpression of miR828	(Yang et al. 2013)
	Actinidia arguta	miR858	*AaMYBC1*	Overexpression of miR858	(Li et al. 2020)
	Solanum lycopersicum	miR1916	strictosidine synthase (STR-2), UDP-glycosyltransferases (UGTs), late blight resistance protein homolog R1B-16, disease resistance protein RPP13-like, and MYB transcription factor (MYB12)	Overexpression and silencing of miR1916	(Chen et al. 2019)
	Salvia *miltiorrhiza*	miR408	SmLAC3	Silencing of miR408	(Zou et al. 2021)
	Malus domestica	Mdm-miR828	MdTAS4, MdbHLH3, MdDFR, MdANS, and MdUFGT	Overexpression of Mdm-miR828	(B. Zhang et al. 2020)

In several plant species, a range of sRNA molecules have been found to target genes responsible for the biosynthesis of fatty acids. In *Lonicera japonica* (commonly known as honeysuckle), the expression of certain miRNAs namely U436803, U977315, U805963, U3938865, and U4351355 are predicted to be involved in the biosynthesis of secondary metabolites, such as flavonoids, and fatty acids. These miRNAs are involved in processes, such as long-chain acyl-CoA synthetase (LACS), acyl carrier protein (ACP), and fatty acid hydroxylase (FAH), contributing to the regulation and synthesis of these essential compounds (Liu et al. 2017). In another research, the identification of several miRNAs tree peony plant (*Paenia* section *Moutan*) including novel-m0027, miR414, miR7826, miR2673b, miR156b, miR399f, miR477g, miR159, and miR159b which are directly involved in the regulation of fatty acid in two developing seed of different cultivar (Yin et al. 2018). Unfortunately, there are currently no studies that focus on the manipulation of the miRNAs involved in these plants. This lack of studies on the manipulation of miRNAs and sRNAs in these plants presents both a challenge and an opportunity. On the one hand, it highlights a critical area where more targeted research is needed to unlock the potential of these regulatory molecules for crop improvement, disease resistance, and environmental adaptation.

Recent research has revealed that the use of amiRNA to target the genes responsible for producing saturated fatty acids in *Camelina sativa* oilseed can lead to an increase in unsaturated fatty acids, particularly oleic acid. The downregulation of the fatty acyl-ACP thioesterases B (FATB) genes through the replacement of Csa-miRNA159a resulted in a decrease in saturated fatty acids and a significant increase in polyunsaturated fatty acids and oleic acid biosynthesis compared to non-transgenic seeds. These findings demonstrate the potential of amiRNA technology for improving the fatty acid composition of *C. sativa* oil seed (Ozseyhan et al. 2018). Overexpressing of miRNA167a in *C. sativa* caused the change in the seed size following lower production of α-linolenic acid (ALA) and slightly higher linoleic acid content compared to the wild-type seed (Na et al. 2019). In this study, the decreased level of fatty acid desaturation caused by the downregulation of *C. sativa* fatty acid desaturase3 (CsFAD3) in the transgenic seed (Na et al. 2019). Furthermore, in *B. napus* miRNA–target pair suggests that several miRNAs including miR9563a-p3, miR9563b-p5, miR838-p3, miR156e-p3, miR159c, and miR1134 may target genes that probably involved in regulating the concentration of long chain fatty acids, transportation of lipid and β-oxidation and various type of process metabolism (Z. Wang et al. 2017a, b). The manipulation of amiR159b by silencing of targeted genes encoding FATB, Δ12-desaturase (FAD2), and fatty acid elongase (FAE1) increases oleic acid in transgenic *Arabidopsis* plants when expressed with *Brassica napus* (*B. napus*) truncated napin (FP1 promoter) but decreases palmitic acid and eicosenoic acid relative to wild-type plants due to downregulation activity of FATB and FAE1 (Belide et al. 2012). Overall, these findings highlight the effectiveness of amiRNA technology as a tool for improving the nutritional quality of oilseed crops by modifying their fatty acid composition, offering promising implications for agricultural biotechnology and food industry applications.

The downregulation of genes encoding *Chlamydomonas reinhardtii* phosphoenolpyruvate carboxylase (CrPEPC) results in a significant increase in the production of C16-C22 fatty acids when amiRNAs are used to replace endogenous miRNA-cre-MIR1162 (C. Wang et al. 2017a, b). These results are likely to be related to the downregulation of phosphoenolpyruvate carboxylase (PEPC) which is one of the key enzymes involved in the flow of carbon to fatty acid synthesis that helps in enhancing the oil content of cells (Hurtado-Gaitán et al. 2021). Using the STTM approach, another manipulation of this secondary metabolite fatty acid-derivative was found. By utilizing STTM to downregulate miR1432 and expressing a miR1432-resistant form of Acyl-CoA thioesterase (OsACOT), there is a significant alteration in rice grain size due to the influence of OsACOT gene activity, which plays a crucial role in the production of medium-chain fatty acids. The most notable result of the study is that it promotes grain filling by stimulating the biogenesis of IAA and ABA (Zhao et al. 2019). These findings underscore the potential of using genetic manipulation to improve oil content and quality in microalgae and crops, providing valuable insights for agricultural biotechnology and crop improvement strategies.

Lignin

Another one of the most significant secondary metabolites is lignin, which is produced in plant cells by the phenylpropanoid pathway that shares the same pathways as other secondary metabolites, such as flavonoids, hydroxycinnamic acid esters, hydroxycinnamic acid amides, and tannins (Yadav and Chattopadhyay 2023). The biosynthesis of phenylpropanoid metabolites begins with the amino acid phenylalanine, which is produced via the shikimate pathway (Dong and Lin 2021). In contrast to fatty acid derivatives, lignin is a complex polymer that is present in plant cell walls and offers structural support as well as defense against pathogens and herbivores (Al-Khayri et al. 2023). The biosynthesis of lignin monomers, transport, and polymerization comprises a highly intricate network that is essential to the biosynthesis of lignin (Liu et al. 2018). Lignin monomers are synthesized in the cytoplasm and then transported to the apoplast after undergoing a series of reactions including deamination, hydroxylation, methylation, and reduction (Liu et al. 2018). In the secondary cell wall, peroxidase (POD) and laccase

(LAC) polymerize lignin with sinapyl alcohol, coniferyl alcohol, and p-coumaryl alcohol, the three main types of monolignols (Zeng et al. 2020).

Previously studies have been done on this lignin synthesis, especially on the gene regulation to identify and modify lignin content and composition in transgenic plants using sRNAs (Fig. 4). One of the studies that has been done is through key enzymes in lignin biosynthesis including CHS and hydroxycinnamoyl: CoA transferase (HCT) which participated in the phenylpropanoid pathway (Besseau et al. 2007). The study explores the trend in transgenic lines producing amiRNA (*MIM858*) related to increased MYB expression, which in turn changes the direction in the biosynthetic of lignin. The concentration of lignin is lower in the *MIM858* transgenic line due to the upregulation of CHS causing abnormal lignification of the stem and vessel compared with overexpression of miR858a in *Arabidopsis* (Sharma et al. 2016). These findings highlight the effectiveness of sRNA-based genetic manipulation in controlling lignin biosynthesis, offering valuable insights for developing transgenic plants with tailored lignin content and composition for various industrial applications (Peracchi et al. 2024).

Additionally, *IbMYB1* is a transcription factor belonging to the MYB subfamily that plays a crucial role in this process by controlling the expression of genes involved in lignin biosynthesis. By regulating the expression of these genes, *IbMYB1* helps to determine the amount and type of lignin that is produced, which in turn affects the mechanical strength and growth behaviour of the plant (Cesarino 2019). A sRNA namely miR828 found to play a role in the manufacture of lignin upon wounding in *Ipomoea batatas* which are intended to strengthen the cell wall with suppression of *IbMYB*. Overexpression of miR828 on transgenic plants without wounding shows the decrease of *IbMYB* expression thus significantly enhancing the content of lignin (Lin et al. 2012). Other studies showed that overexpression of pre-sRNA8105 in sweet potatoes with no wounding resulted in lower expression of the IbMYB1 gene thus increasing the lignin biosynthesis (Lin et al. 2013). These findings underscore the effectiveness of manipulating transcription factors and sRNAs to control lignin biosynthesis.

Overexpression of transient Novel-miR1 and Novel-miR7 inhibited the expression of target genes *4CL* and *PAL* in *Cucumis sativus* L. (cucumber) thus leading to the downregulation of lignin synthesis which is also lower the resistance to *Corynespora cassiicola* (*C. cassiicola*). Silencing of miR164d, miR396b, Novel-miR1, and Novel-miR7 using STTM caused the increased rate of the biosynthesis of lignin and resistance in *C. cassiicola* (X. Wang et al. 2019a, b). The discovery of sRNAs targeting genes and manipulation of other secondary

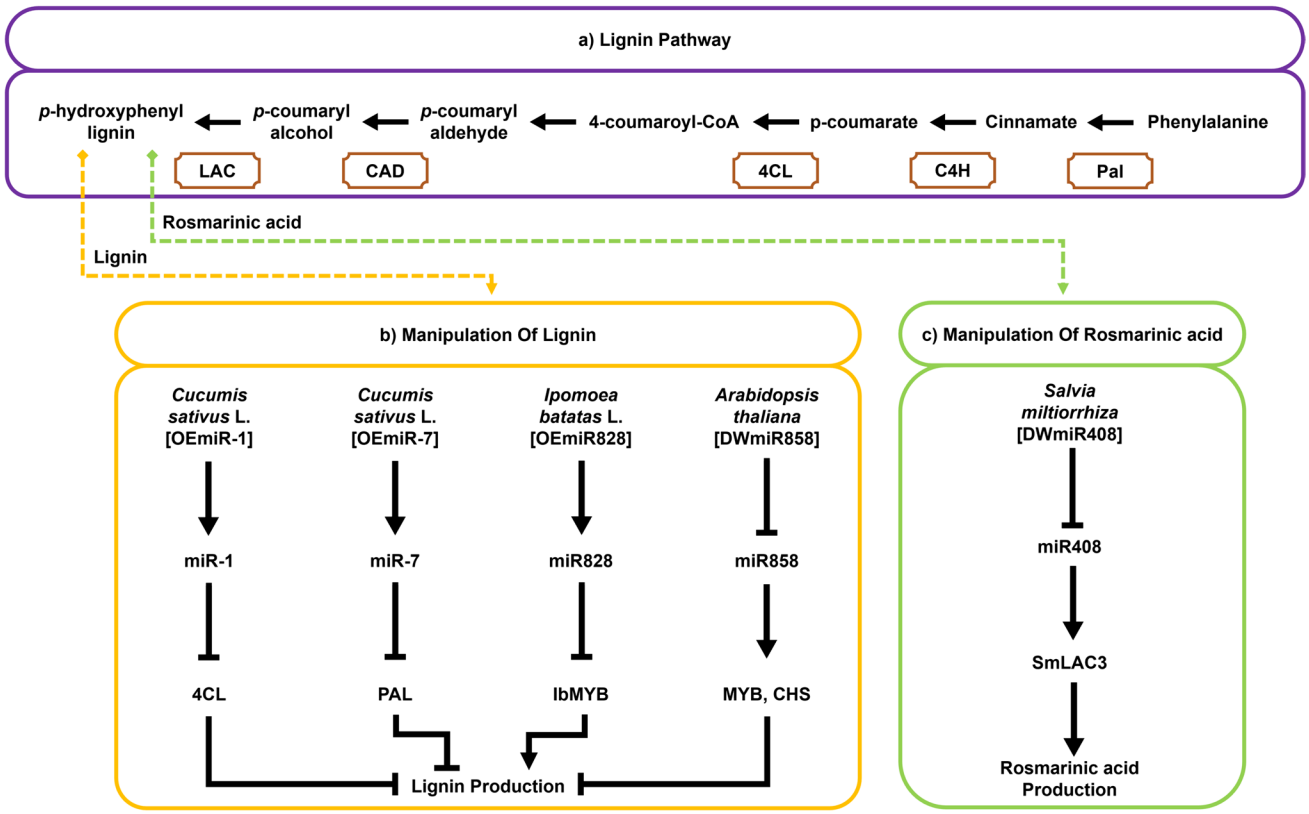

Fig. 4 Manipulation of lignin biosynthesis through sRNA-mediated approaches in several plant species

Table 4 Type of sRNAs, target and manipulation of fatty acid and other compounds in various types of plant

Type of secondary metabolite	Type of plant	Type of sRNAs	Target gene	Manipulation	References
Fatty acid-derived	*Camelina sativa*	Csa-miR159a	FATB	Knocked down of FATB gene using amiRNA	(Ozseyhan et al. 2018)
		miRNA167a	FATB	Overexpression of miRNA167a	(Na et al. 2019)
	Arabidopsis	miR159b	FATB, FAD2, and FAE1	Silencing of FATB, FAD2, and FAE1 using amiR159B	(Belide et al. 2012)
	Brassica napus	bna-5p-163957_18, bna-5p-396192_7, miR9563a-p3, miR9563b-p5, miR156e-p3, miR159c, bna-5p-396192_7, miR838 and miR1134	LACS9, MFPA, ADSL1 (stearoyl-CoA desaturase/ delta-9 desaturase), ACO32 (acyl-CoA oxidase), C0401, GDL73, PlCD6, OLEO3, PDP, and WSD1	No	(Z. Wang et al. 2017a, b)
	Lonicera japonica	U436803, U977315, U805963, and U805963	long-chain acyl-CoA synthetase (LACS), acyl carrier protein (ACP), and fatty acid hydroxylase (FAH)	No	(Liu et al. 2017)
	Paenia section *Moutan*	novel-m0027, miR414, miR7826, miR2673b, miR156b, miR399f, miR477g, miR159, and miR159b	choline-phosphate cytidylyltrans-ferase (CCT), APETALA2/ ethylene-responsive element binding proteins (AP2-EREBP) and pyru-vate dehydrogenase E1 component alpha subunit (PDHC), 1-acyl-sn-glycerol-3-phosphate, APETALA2/ ethylene-responsive element binding proteins (AP2-EREBP)acyltrans-ferase (LPAAT), very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase (PHS)	No	(Yin et al. 2018)

Table 4 (continued)

Type of secondary metabolite	Type of plant	Type of sRNAs	Target gene	Manipulation	References
	Chlamydomonas reinhardtii	miRNA-cre-MIR1162	Phosphoenolpyruvate carboxylase (PEPC)	Replacing endogenous miR1162 with amiRNAs	(C. Wang et al. 2017a, b)
	Oryza sativa L	miR1432	cyl-CoA thioesterase (OsACOT)	Overexpression and suppression of miR1432	(Zhao et al. 2019)
Lignin	*Arabidopsis thaliana*	miR858	MYB and CHS	Overexpression mir858 using amiRNA	(Sharma et al. 2016)
	Ipomoea batatas L	miR828	IbMYB1 and IbTLD	Overexpression of pre-miR828	(Lin et al. 2012)
		sRNA8105	IbMYB1	Overexpression of pre-sRNA8105	(Lin et al. 2013)
	Cucumis sativus L	Novel-miR1, Novel-miR7, miR164d and miR396b	4CL, PAL, NAC, and Anthranilate phosphoribosyl-transferase (APE)	Overexpression of Novel-miR1, Novel-miR7 and silencing of Novel-miR1, Novel-miR7, miR164d and miR396b	(X. Wang et al. 2019a, b)

metabolites by various types of plants are briefly listed in Table 4.

Future prospect and potential

In the realm of future research, there is exciting potential to explore how sRNAs influence the production of valuable PSMs in a variety of plant species. To move forward, it is essential to better understand how sRNAs precisely control PSM biosynthesis, especially in non-model plants where our current knowledge falls short. By harnessing the potential of sRNAs, researchers can employ both overexpression and downregulation strategies to fine-tune the production of specific secondary metabolites, paving the way for the creation of plants tailored to possess desirable traits.

Moreover, using sRNAs to manipulate secondary metabolites, we can also gain insights into the complex molecular processes involved in secondary metabolism. This could lead to practical biotechnological tools for improving crops and potentially finding new uses for these compounds. For instance, in the pharmaceutical field, the ability to precisely control the production of specific PSM in medicinal plants could streamline the extraction of therapeutic compounds. In agriculture, this technology could be harnessed to enhance crop protection, flavor, or nutritional content, catering to evolving consumer demands for more sustainable and nutritious food sources (Hussein and El-Anssary 2018). All in all, this research holds the promise of a more sustainable and environmentally friendly future.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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