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Micro-RNA Mastery: Boosting Secondary Metabolite Production in Plants

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ABSTRACT

Plants have been indispensable for their nutritional and health benefits, serving as primary sources of natural products used in pharmaceuticals, agrochemicals, flavors, fragrances, and more. The beneficial properties of plants are largely due to secondary metabolites, compounds essential for modern medicine and various industries. This article examines the role of microRNAs (miRNAs), small non-coding RNAs that regulate gene expression, in controlling the biosynthesis of these valuable secondary metabolites. The miRNAs influence plant developmental activities, stress responses, and various biological processes, including secondary metabolite production. By understanding and manipulating miRNA pathways, the production of these compounds can be enhanced. Effective techniques such as artificial miRNAs (amiRNAs) are widely used for targeted gene silencing and modification of small RNA biosynthesis pathways. The article discusses the potential of miRNA technology to revolutionize the production of plant-derived compounds, paving the way for innovations in medicine, agriculture, and industry through more efficient and sustainable practices.

INTRODUCTION

Throughout human history, plants have consistently provided not only nutritional benefits but also significant health advantages. Today, as we explore the intersection of traditional wisdom and scientific research, the importance of plants in promoting health and wellness remains as relevant as ever. Plants are the major sources of natural products used in pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives, and pesticides. However, humanity's curiosity about the origins of these plant properties has persisted. Various methodologies have been developed in our quest to uncover the factors responsible for the healing properties of plants. Central to the beneficial properties of plants are their 'secondary metabolites'—compounds that do not play a direct role in the growth, development, or reproduction of the plants. These metabolites are crucial in modern medicine, serving as antibiotics and substitutes for agrochemicals. They also hold significant economic value as flavours, fragrances, dyes, pigments, and food preservatives. Additionally, many contemporary drugs are synthetic modifications of naturally occurring substances. These secondary metabolites are of different classes: phenolic compounds, derived from the phenylpropanoid pathway in plants, exhibit a wide range of structures and impart color to flowers, fruits, and vegetables. Alkaloids and flavonoids are significant groups of secondary metabolites known for their diverse biological functions. Various phenolic metabolites possess properties such as antioxidant, anticancer, and anti-inflammatory effects. Alkaloids, another major class of metabolites, are notable for their pharmacological and recreational impacts. Additionally, there is a third category of nitrogen-based organic compounds, characterized by a heterocyclic ring derived from aromatic amino acids. Notably, the aromatic amino acids phenylalanine and tryptophan serve as common metabolic precursors for both phenolic compounds and alkaloids.

Considering the importance, there is substantial scientific and industrial interest in plant secondary metabolites. The growing commercial significance of these chemical compounds has led to heightened interest in secondary metabolism, especially in exploring methods to modify the production of bioactive plant metabolites. These secondary metabolites are synthesized by plants to meet specialized needs in specific ecological conditions, as their biosynthesis is highly energy-consuming. The biosynthesis and accumulation of secondary metabolites in plants result from the tight regulation of their biosynthetic machinery. However, achieving stable production of secondary metabolites in many plant cell cultures using classical techniques is challenging. Novel approaches should be developed to remove the inhibitory blocks that prevent pathway activation and to shift the regulatory balance towards activating entire biosynthetic pathways. Notably, the advanced strategies have demonstrated that secondary metabolic pathways are highly regulated by factors such as miRNAs – the ultimate regulators in plants. With this understanding, we can delve deeper into the role of miRNAs in managing these critical biological processes.

MICRO RNAs – THE MASTER REGULATORS OF GENE EXPRESSION

Micro RNAs are small RNAs composed of 20–23 nucleotides in length, that are not translated into proteins. miRNAs can target individual genes or coordinate the regulation of various gene families. They can inhibit the expression of specific genes by binding to the messenger RNA.

Additionally, a single miRNA gene can regulate multiple targets within tissues, while a single target can be regulated by multiple miRNAs. Thousands of these miRNAs have been identified in plants. Research has shown that miRNAs influence various plant developmental activities, including leaf blooming, seed emergence, root morphology, and hormonal responses. They play a critical role in a wide range of biological processes such as developmental timing, cell and tissue differentiation, proliferation, apoptosis, and metabolism. miRNAs enhance growth, development, and biological responses at the plant's biological level. At the gene expression level, they effectively inhibit the translation of their target mRNAs. Moreover, miRNAs can regulate the expression of genes that encode transcription factors and stress response proteins, impacting various biological processes in the plant. miRNAs are well-known for their role in regulating various plant processes under biotic and abiotic stresses.

MICRO RNAs IN SECONDARY METABOLITE PRODUCTION

Recent studies have indicated that numerous miRNAs play a role in regulating secondary metabolite production in various plants. These miRNAs target either the activators or repressors involved in metabolic pathways. When miRNAs target repressors or other inhibitory factors, they downregulate the expression of these inhibitor genes, thereby accelerating the production of secondary metabolites. Conversely, if miRNAs target activator genes or key genes, the production of the corresponding secondary metabolites is inhibited. In such cases, through artificial inhibition of specific miRNAs, the production of secondary metabolites can be increased. Thus, a deeper understanding of miRNAs and their target genes is essential for exploiting them in metabolic engineering in order to increase the production of secondary metabolites.

The following table shows various miRNAs identified in different plants, highlighting their role in regulating a variety of secondary metabolites.

miRNAs	**Plant**	**Regulation of Expression**
miR156	Arabidopsis	Regulates the accumulation of anthocyanin
miR828, miR858	Grape	Promote the biosynthesis of Anthocyanin
miR172i, miR5298b, miR369b, miR828a	Himalayan Mayapple, Common yew, Oriental persimmon	Involved in the regulation of various genes in phenylpropanoid biosynthesis
miR156	Arabidopsis	Involved in the biosynthesis of terpenoids and positively regulates the expression of terpene synthase.
miR4995	*Picrorhiza kurroa*	Involved in the synthesis of the compound picroside
miR2161, miR13	Opium poppy	Regulation of benzylisoquinoline alkaloid biosynthesis pathway
miR5140, miR159, miR477, miR1534, miR5030	Ashwagandha	Regulation of the biosynthesis of withanolides

miR159, miR1534, miR5030	Soybean	Involved in isoflavonoid biosynthesis
miRX17, miRX27, miRX20, miRX19	Tobacco	Enhancing the biosynthesis of Nicotine in topping treated tobacco plants
miR166, miR396, miR397	Black pepper	Involved in the regulation of corresponding genes in piperine biosynthesis
miR169	Black pepper	Anthocyanin Biosynthesis

HOW DO WE IDENTIFY MIRNAs AND THEIR TARGETS?

Typically, the identification of miRNAs in non-model plants begins with sequencing the small RNAs followed by identification of their targets. The next step is to experimentally validate these interactions and design bioengineering strategies to enhance secondary metabolism using miRNAs. There are at least two approaches to enhancing secondary metabolism through miRNAs: directly targeting the mRNAs of genes encoding biosynthetic enzymes or targeting the genes encoding transcription factors or their regulators. The latter method tends to be more effective. Enhanced expression of a single miRNA can lead to either an increase or decrease in secondary metabolite production. If activation is the goal, the miRNA will repress a gene that encodes a negative regulator of secondary metabolism.

UTILIZING MIRNAS FOR ENHANCED PLANT METABOLITE PRODUCTION

With the comprehensive understanding of the key miRNAs and their corresponding target genes, various bioengineering strategies can be employed to enhance the production of secondary metabolites. One effective approach is the use of artificial miRNAs (amiRNAs) for gene silencing in plants, particularly when multiple related target genes need to be downregulated. Compared to conventional gene knockout approaches, the amiRNA technique offers greater specificity and safety. In this context, achieving perfect complementarity between amiRNA and its target gene can significantly enhance the efficiency of amiRNA-based gene silencing in plants. Another strategy involves manipulating key genes involved in small RNA biosynthesis and processing. Silencing a single gene responsible for miRNA biosynthesis could reduce the levels of several miRNAs. If these miRNAs negatively regulate the biosynthesis of certain secondary metabolites, this could activate an entire branch of a biosynthetic pathway. Additionally, the suppression or activation of specific miRNAs can increase secondary metabolite production. Ultimately, manipulating miRNAs can redirect the flow of metabolites within a plant cell's biosynthetic pathway, boosting the production of economically important compounds.

CONCLUSION

The complex interplay between miRNAs and secondary metabolites reveals a promising frontier in plant science and bioengineering. As our understanding deepens, the potential to harness these tiny regulators for enhancing plant-derived compounds becomes ever more tangible. This research not only bridges traditional botanical wisdom with cutting-edge science but also paves the way for innovations in medicine, agriculture, and industry. The future holds exciting possibilities, where the precise manipulation of miRNAs could lead to more efficient and sustainable production of valuable secondary metabolites, ultimately benefiting human health and the global economy.

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