Li Y *et al.* (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 2, Article number 12604
DOI:10.15835/nbha50212604
Review Article

Regulation mechanism of long non-coding RNA in plant secondary metabolite biosynthesis

Yuting LI, Huan HAN, Jiabao YE*, Feng XU*, Weiwei ZHANG, Yongling LIAO

*Yangtze University, College of Horticulture and Gardening, Jingzhou 434025, Hubei, China; liyutingxi@163.com; hanhuan20190922@163.com; yejiabao@yangtzeu.edu.cn (*corresponding author); xufeng@yangtzeu.edu.cn (*corresponding author); wwzhangchn@163.com; liaoyongling@yeah.net*

Abstract

Long non-coding RNAs (lncRNAs) are widely available transcription products of more than 200 nucleotides with unrecognizable coding potential. A large number of lncRNAs have been identified in different plants. lncRNAs are involved in various basic biological processes at the transcriptional, post-transcriptional and epigenetic levels as key regulatory molecules, including in the regulation of flowering time and reproductive organ morphogenesis, and they play important roles in the biosynthesis of plant secondary metabolites. In this paper, we review the research strategies of lncRNAs and lncRNAs related to the biosynthesis of plant secondary metabolites, focusing on the research strategies for studying lncRNAs and the effects of lncRNAs on the biosynthesis of terpenoids, alkaloids and flavonoids, aiming to provide new ideas for the study of the regulation of plant secondary metabolite biosynthesis.

Keywords: alkaloid; flavonoids; lncRNA; secondary metabolites; terpenoids

Introduction

More than 90% of genomic DNA in eukaryotes can be transcribed into RNA, of which only 1-2% is coding RNA that can be translated into protein, and most of the rest is non-coding RNA (Ariel *et al.*, 2015). According to expression patterns and functional differences, ncRNAs can be classified into housekeeping non-coding RNAs and regulatory non-coding RNAs (Figure 1). Housekeeping non-coding RNAs contribute to ribosomal and cellular activities and include ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs), which are usually constitutively expressed. Regulatory non-coding RNAs include small RNAs, such as microRNAs (miRNAs: 20-24 nt), which are the most abundant class of small non-coding RNAs, small interference RNAs (siRNA: 20-24 nt), piwi-interacting RNAs (piRNAs: 24-32 nt), long noncoding RNA (lncRNAs: >200 nt) (Ponting *et al.*, 2009; Nejat and Mantri, 2018). According to the length, ncRNAs are mainly classified as small ncRNAs (18-30 nt), medium-sized ncRNAs (31-200 nt) and long ncRNAs (lncRNAs: >200 nt) (Wang *et al.*, 2017; Song *et al.*, 2021). lncRNAs account for about 80% of all ncRNAs and play an important role or are a key component in various biological processes (Fok *et al.*, 2017). Specifically, lncRNAs are usually eukaryotic RNAs greater than 200 nucleotides,

Received: 21 Dec 2021. Received in revised form: 14 Apr 2022. Accepted: 19 Apr 2022. Published online: 23 May 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

open reading frames (ORFs) of less than 100 amino acids, and no apparent protein-coding ability (Nejat and Mantri, 2018). The first eukaryotic lncRNA was discovered in mice in 1984 (Pachnis *et al.*, 1984); then, the first identified plant lncRNA was isolated in Medicago plants (Crespi *et al.*, 1994). Initially, lncRNA was regarded as "transcriptional noise," but in numerous studies, lncRNAs have been revealed to play an important role in many life activities and developmental processes (Ponjavic *et al.*, 2007), such as the regulation of flowering time (Kim and Sung, 2017; Kim *et al.*, 2017), modulation of reproductive organ development (Gao *et al.*, 2016; Fang *et al.*, 2019), and response to biotic and abiotic stress in plants (Qin *et al.*, 2017; Seo *et al.*, 2017). In addition, lncRNAs play an important role in regulating the generation of secondary metabolites such as flavonoids, terpenoids, and alkaloids, in plants. Currently, studies on the regulation of secondary metabolite biosynthesis have mainly focused on the key structural genes and transcription factors in the synthetic pathway, while little research has been reported on the effects of lncRNAs on plant secondary metabolites. According to the position of lncRNAs in the genome relative to neighboring protein-coding genes, lncRNAs can be classified into five types (Figure 2) : (1) sense lncRNA; (2) antisense lncRNA, when overlapping one or more exons of another transcript on the same or opposite strand; (3) bidirectional lncRNA, when its expression and that of a neighboring coding transcript on the opposite strand are initiated in close genomic proximity; (4) intron lncRNA, when it originates from introns of protein-coding genes; and (5) intergenic lncRNA (lincRNA), when it lies within the genomic interval between two genes (Ponting *et al.*, 2009). In addition, lncRNAs can be divided into three groups based on their length distribution (Figure 1): small-lncRNA (200~950 nt), medium-lncRNA (950~4,800 nt), large-lncRNA (4,800 nt~) (Ma *et al.*, 2013).

Secondary metabolites refer to a large class of small organic compounds that are not essential for the growth and development of plants, and they vary in content in plants and have their own unique metabolic pathways. The distribution of secondary metabolites in plants is usually specific to species, organs, tissues, and growth (Hartmann, 2007). At first, secondary metabolites were considered to be the byproducts of various plant primary metabolic processes, but later studies revealed that these compounds are as important as primary metabolites, and they play an indispensable role in plant growth, development, symbiosis, and reproduction, especially under adverse conditions, and their main functions include attracting insects as pollinators and seed dispersal agents, preventing herbivores from eating them, resisting insects and pathogens, acting as plant antitoxins, producing growth hormones and signaling compounds, and stimulating the formation of nodules (Kutchan, 2001; Wink, 2015; Takshak and Agrawal, 2019). Not only are secondary metabolites important to plants, they are also widely used commercially. Many of them are used by humans, including in cosmetics, perfumes, dyes, fragrances, and health products (Zhou *et al.*, 2011). In addition, the secondary metabolites have some physiological activities and pharmacological effects. The alkaloids have antibacterial, anti-inflammatory, anti-cancer, vasodilating and other effects. Flavonoids have anti-cancer, anti-bacterial, antioxidant, anti-inflammatory, and other physiological activities or pharmacological effects, which are important for the prevention and treatment of human tumors, aging, and cardiovascular diseases (Wu *et al.*, 2012). Currently, people rely mainly on plant secondary metabolites as medicine. Plant secondary metabolites can be classified into seven major groups: phenylpropanoids, quinones, flavonoids, tannins, terpenoids, steroids and their glycosides, and alkaloids. Based on the biogenic pathway of secondary products, They have also been classified into three major groups: phenolic compounds, terpenoids, and nitrogenous compounds (Takshak and Agrawal, 2019).

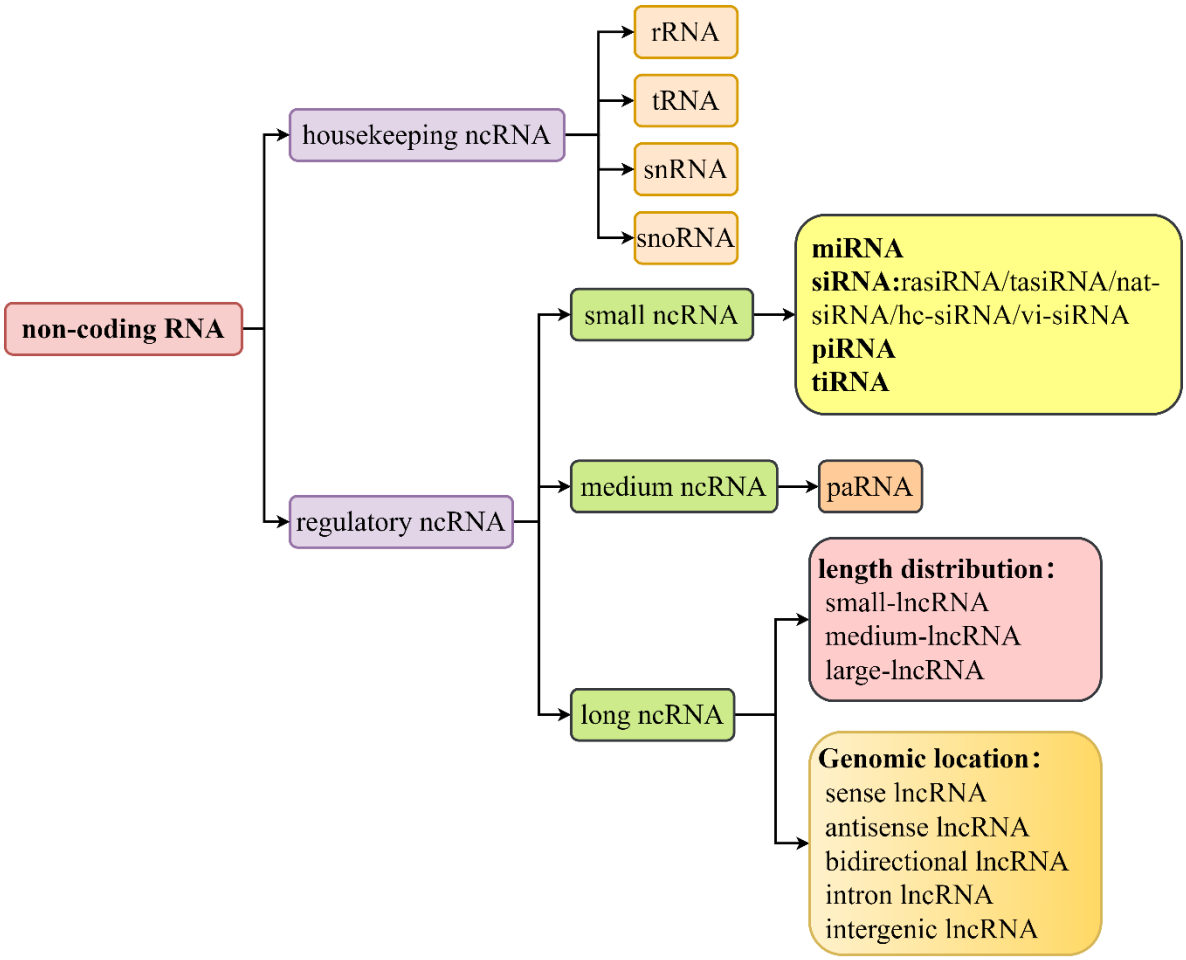

Figure 1. Classification of non-coding RNAs

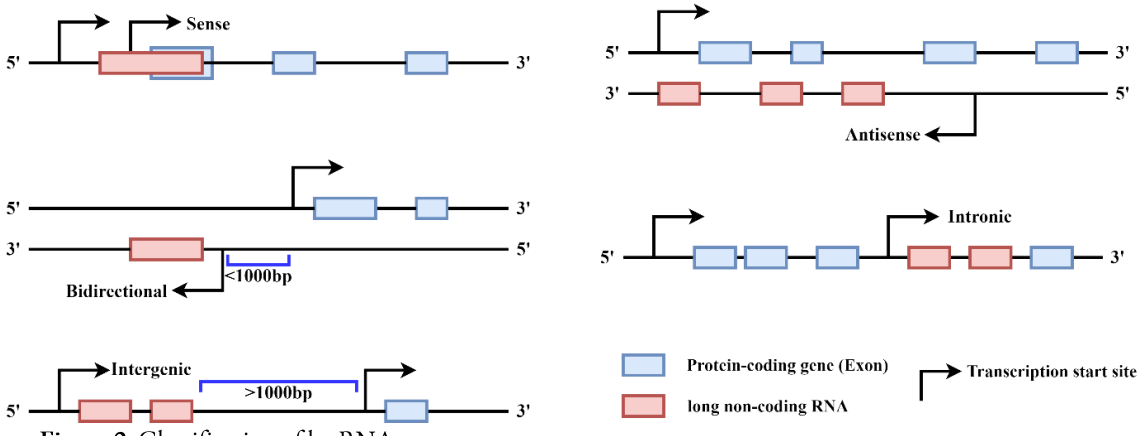

Figure 2. Classification of lncRNAs

In recent years, with the advancement of high-throughput sequencing technology, the increasing number of lncRNA databases, the rapid development of computer bioinformatics, and advanced bioinformatics tools, a large number of lncRNAs have been identified and characterized in plants, and research on lncRNAs has gradually advanced. lncRNAs are considered important regulators in many essential biological processes, and there is increasing evidence that lncRNAs are involved in a wide range of biological processes, including development and stress responses, and have already become a popular research topic in the field of biology.

Research methods for studying lncRNAs

Compared with miRNAs, lncRNAs were studied later, especially for specific functional verification. In recent years, with the rapid development of high-throughput sequencing technology and bioinformatics, research on lncRNAs has gradually deepened and has become a popular topic. At present, the research methods of lncRNA are mainly in the following aspects (Figure 3).

Screening and identification of lncRNA

With the rapid development of strand-specific RNA-seq libraries (ssRNA-seq) and prediction software, a large number of potential lncRNAs have been screened and identified in *Arabidopsis thaliana* (Zhao *et al.*, 2018), tomato (Liao *et al.*, 2020), cotton (Zou *et al.*, 2016), and *Ginkgo biloba* (Ye *et al.*, 2019). In addtion, Iso-seq, a single-molecule real-time sequencing based on PacBio, which has the feature of ensuring the integrity of RNA without interrupting splicing, has been widely used in the screening and identification of lncRNAs. Currently, numerous lncRNAs have been identified in *A. thaliana* (Cui *et al.*, 2020), rice (G Zhang *et al.*, 2019), and safflower (Chen *et al.*, 2018). Compared to other sequencing technologies, Iso-seq technology has significant advantages: longer read length, higher accuracy, lower offset, and the ability to locate methylation bases (Roberts *et al.*, 2013).

Localization technology of lncRNA

mRNA is generally exported to the cytoplasm for translation; in contrast, after being processed, lncRNAs can reside in the nucleus, be exported to the cytoplasm, and be located in both the nucleus and the cytoplasm (Carlevaro-Fita and Johnson, 2019). The function of lncRNA depends on its subcellular localization. Therefore, studying the subcellular localization of lncRNAs and their dynamic changes is the key to clarifying the function and mechanism of newly discovered lncRNAs, and providing new ideas for further study of their roles in cells (Chen, 2016).

There are many methods for lncRNA subcellular localization, such as RNA fluorescence in situ hybridization (RNA FISH), nuclear-cytoplasmic fractionation, Apex-RIP, and fluorescent in situ RNA-sequencing (FISSEQ), and multiplexed error-robust fluorescence in situ hybridization (MERFISH) (Carlevaro-Fita and Johnson, 2019). The methods commonly used in plants are RNA-FISH and nuclear-cytoplasmic fractionation. RNA FISH is one of the oldest and most extensive methods for RNA localization, and it involves incubating intact cells with labeled complementary oligonucleotide probes to make them visible under a microscope. It has the advantages of safety, speed, high sensitivity, strong specificity, and can display a variety of colors at the same time (Cui *et al.*, 2016). Nuclear-cytoplasmic fractionation has the advantages of low cost and simple operation. Through RNA-FISH and nuclear-cytoplasmic fractionation, Zhang X *et al.* (2019) found that lncRNA973 was mainly located in the nucleus of cotton, which provided a basis for subsequent studies on the function and mechanism of lncRNA973. In cotton, lncRNA354 was found in the nucleus and cytoplasm by nuclear-cytoplasmic fractionation, and it acted as a negative regulator of plant response to salt stress (Zhang *et al.*, 2021).

Subcellular localization is also of practical significance for the functional study of lncRNAs. Compared with antisense oligonucleotides (ASOs), RNA interference (RNAi) is more effective on cytoplasmic RNA populations. In contrast, ASOs and CRISPR-Cas9 are effective on nuclear RNA populations (Lennox and Behlke, 2016; Stojic *et al.*, 2018). Therefore, an understanding of lncRNA subcellular localization can guide it in more effectively selecting appropriate methods for lncRNA functional studies.

Study on the function and mechanism of lncRNA

Currently, most studies on lncRNAs focus on high-throughput sequencing, while there are relatively few studies on the cloning and functional verification of lncRNAs. Similar to miRNAs, the basic strategies for

exploring the functions of lncRNAs include studies of the acquisition and loss of function, and then analyzing and comparing phenotypes. The study of functional acquisition is mainly through the introduction of an overexpression vector. There are many methods for studying lncRNA loss of function, including RNAi, VIGS, and CRISPR/Cas9, ASOs.

RNAi uses small, double-stranded RNA molecules to combine with a variety of protein factors to form an RNA-induced silencing complex to inhibit the translation or degradation of target mRNA (Lennox and Behlke, 2016). VIGS is a technique that uses recombinant virus specificity to reduce endogenous gene activity. The mechanism of RNA degradation is very similar to that of the RNAi pathway, both of which are posttranscriptional gene silencing methods (Baulcombe, 1999; Becker and Lange, 2010). CRISPR/Cas9 is a powerful gene-editing technology that can edit any DNA sequence of any organism (Hussain *et al.*, 2018). The CRISPR/Cas9 system is considered a revolutionary gene editing toolbox due to its advantages of simple design, rapid implementation, low cost, and strong scalability (Zhang H *et al.*, 2021). For example, in tomato, overexpression and silencing of lncRNA33732 showed that lncRNA33732 was a positive regulatory factor that could enhance the resistance of tomato to late blight (Cui *et al.*, 2019). In cotton, silencing lncRNA354 by VIGS enhanced resistance to salt stress (Zhang X *et al.*, 2021).

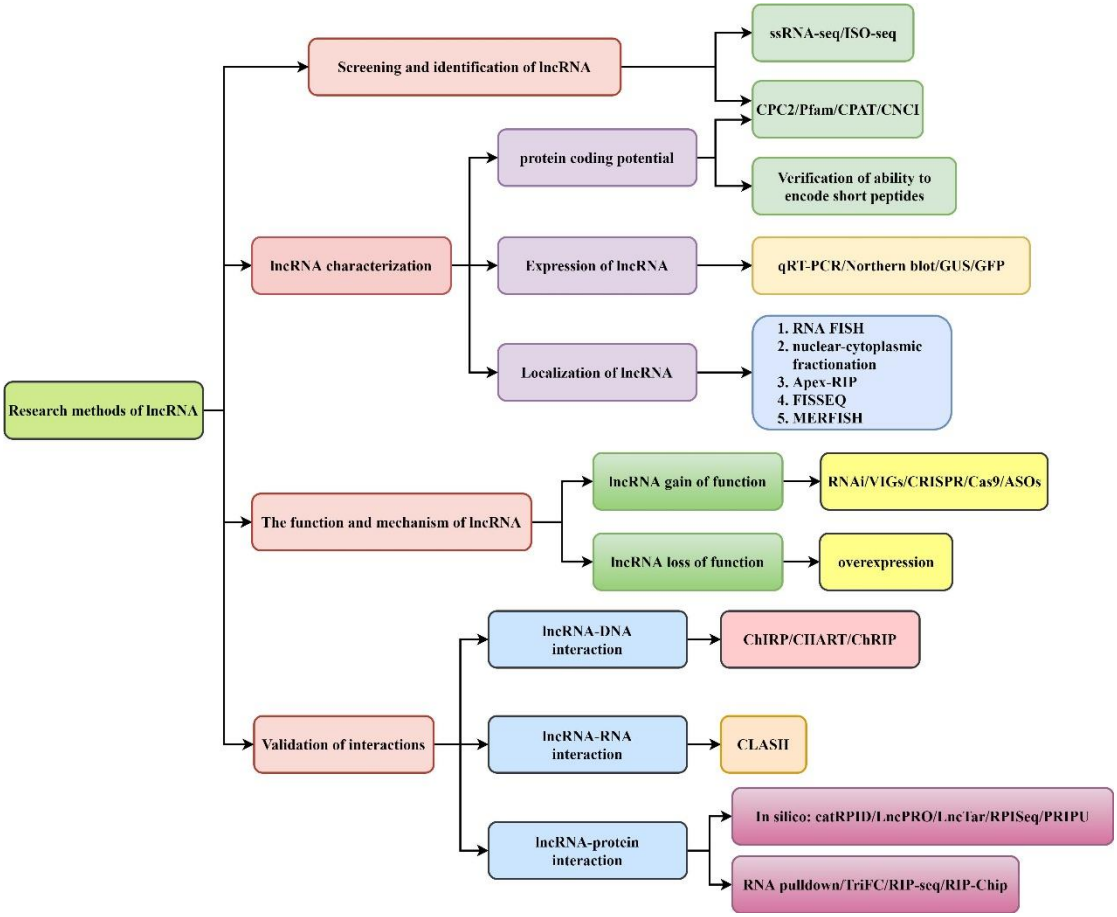

Figure 3. Research methods for studying lncRNAs

lncRNA-DNA/RNA/protein interaction

LncRNA functions mainly through interactions with other molecules, including DNA, RNA, and proteins, among which the interaction between lncRNA and proteins is a topic of interest of current research. Many lncRNAs interact with proteins to form regulatory complexes. RNA protein interactions are crucial in

controlling core cell processes, such as transcription and translation. Therefore, identifying the proteins that interact with lncRNAs is the first step in deciphering lncRNA function (Seo and Chua, 2019a).

At present, there are many prediction tools for lncRNA and protein interaction, such as catRAPID (Armaos *et al.*, 2021), LncPro (Lu *et al.*, 2013) and LncTar (Li J *et al.*, 2015). In addition, there are many methods to verify the interaction between lncRNAs and proteins, such as trimolecular fluorescence complementation (TriFC), RNA pulldown, and RNA binding protein immunoprecipitation (RIP). Among them, RNA pulldown combined with mass spectrometry (MS) analysis is an important means of studying the interaction between lncRNAs and proteins. RNA pulldown is a process in which RNA is synthesized by an in vitro transcription method, and biotin is labeled as a probe to enrich proteins in cell lysates by binding with streptavidin magnetic beads (Seo and Chua, 2019a). The TriFC system has been used for in vivo visualization of RNA-protein interaction by transient expression in tobacco leaves (Seo and Chua, 2019b).

The regulation mechanism of lncRNA interaction with miRNAs

lncRNAs can act as key genetic components of gene expression and play an important role in many life activities and developmental processes. They may function as cis-acting factors acting on neighboring genes on the same strand or as trans-acting factors operating far from the site of biosynthesis, blocking expression. Moreover, recent evidence suggests the roles of lncRNAs and miRNAs in regulating the expression of mRNAs in many species. miRNAs can regulate gene expression at the transcriptional and post-transcriptional level by binding to the target sequences, resulting in cleavage, decoy, or translation repression of targeted mRNA (Dalmay, 2013). At present, several studies have uncovered the interactions among lncRNAs and miRNAs. In plants, lncRNAs can interact with miRNAs in three different ways (Figure 4).

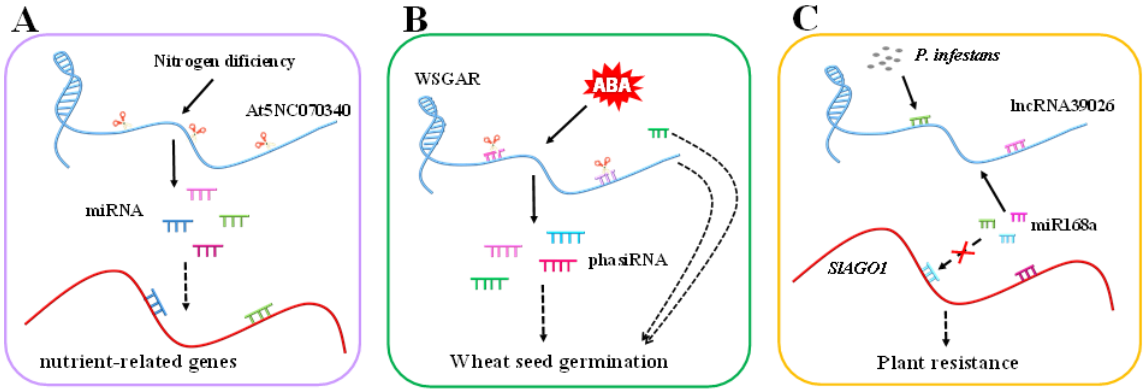

Figure 4. The regulation mechanism of LncRNA interaction with miRNAs. (A) Under nitrogen dificiency, lncRNA (At5NC070340) acts as the precursor of miRNA that negative regulates nutrient-related genes in response to nitrogen deficiency (Fukuda *et al.,* 2019). (B) Under abscisic acid (ABA), miR9678 targets a lncRNA (WSGAR) and triggers the generation of phasiRNAs that play a role in the delay of seed germination (Hou *et al.,* 2020). (C) lncRNA39026 positively regulates the tomato defense response by acting as an eTM for miR168a to regulate *SlAGO1*, and influences tomato resistance to *P. infestans* (Guo *et al.,* 2018)

First, lncRNA can function as an miRNA precursors for the generation of miRNA (Figure 4A) (Wang *et al.*, 2017). At present, lncRNAs have been found to play a role as miRNA precursors in many plants. At5NC070340 and AT1G67105 from lncRNA were predicted might be precursors for miRNAs and respond to low nitrogen in *A. thaliana* (Fukuda *et al.*, 2019). The same phenomenon has been observed in other plant species; in Strawberry, 130 lncRNAs were identified as putative precursors of 80 miRNAs in Strawberry (Lin *et al.*, 2018). Another study in wheat reported four lncRNAs as precursors of miRNAs, three (TalnRNA5,

TapmlnRNA19, and TapmlnRNA8) of which were responsive to powdery mildew infection. Both TalnRNA5 and TapmlnRNA19 were precursors of miR2004, and TapmlnRNA8 was the precursor of miR2066 (Xin *et al.,* 2011).

Second, miRNAs can cleave lncRNAs (Figure 4B). In wheat, for example, *miR9678* targets lncRNA (WSGAR) and triggers the generation of phased siRNA (phasiRNA), which negatively affects the expression of genes involved in seed germination (Guo *et al.*, 2018). Similarly, in mulberry, lncRNA (MuLnc1) was targeted and cleaved by mul-miRNA3954 and generated siRNAs (Gai *et al.*, 2018). In addition, Pto-miR167a may cleave lncRNA (ARFRL) to reduce its expression in *Populus* (Quan *et al.*, 2018). Mature Sl-miR482a may cleave Sl-lncRNA15492 to remove the inhibitory activity of Sl-lncRNA15492 on pre-Sl-miR482a, thus increasing the accumulation of mature Sl-miR482a to decrease tomato resistance (Jiang *et al.*, 2020).

Third, lncRNAs can function as endogenous target mimics (eTMs) of miRNA to compete for miRNA binding with mRNA targets (Figure 4C) (Wu *et al.*, 2013). For example, in sea buckthorn, target prediction analysis revealed that 22 lncRNAs may act as eTMs of 25 DE-miRNAs (Zhang *et al.*, 2018). In wheat, three lncRNAs that acted as eTMs were identified through in silico analysis of RNA-seq data, thus regulating the expression of target genes (Jain *et al.*, 2020). In addition, lncRNA23468 and lncRNA39026 could function as miR482b and miR168a eTMs, respectively, to regulate tomato resistance to *Phytophthora infestans* (Jiang *et al.*, 2019; Hou *et al.*, 2020).

LncRNA is involved in flavonoid biosynthesis

At present, lncRNAs have been identified to be plants involved in the induction of flavonoid biosynthesis in a variety of plants. For example, Qiao *et al.* (2019) predicted 9052 lncRNAs through PacBio Iso-seq and RNA-seq analysis, suggesting that they may play a role in the regulation of secondary metabolism in tea plants. Several lncRNAs were predicted from *Ginkgo biloba* by second-generation sequencing (SGS), single-molecule real-time sequencing (SMRT), and high-throughput sequencing (Li *et al.*, 2014; Ye *et al.*, 2019; Liu *et al.*, 2020). Further analysis revealed that lncRNAs not only target structural genes (*F3H*, *ANR*, *CHI*, *PAL*) related to flavonoid biosynthesis but also regulate transcription factors (MYB, bHLH, WD40) related to flavonoid biosynthesis (Ye *et al.*, 2019; Liu *et al.*, 2020). By using RNA-seq to analyze differential lncRNAs in land cotton and GO annotation and pathway enrichment analysis of potential cis-target genes of these differential lncRNAs, it was found that lncRNA (ltcon_00004262) cis-regulates the target gene Ghir_A01G005150.1 to participate in flavonoid biosynthesis, thus playing an important role in another development (Li *et al.*, 2019). Analysis of the KEGG pathway revealed the presence of differentially expressed lncRNAs that are abundantly enriched in the carotenoid biosynthetic pathway (Zhou *et al.*, 2020). RNA-seq and physiological and biochemical analyses of *Dendrobium officinale* indicate that lncRNA is involved in flavonoid, alkaloid, and carotenoid metabolism, thus affecting the effect of far-red light on the shade avoidance response of *D. officinale* (Li *et al.*, 2021). The fruit of sea buckthorn contains many flavonoids. In ripe fruits of sea buckthorn, 3428 lncRNAs were identified and 61 differentially expressed lncRNAs were obtained from by the RNA-seq technique, and further analysis of the differentially expressed lncRNAs indicated that seven of them are involved in flavonoid biosynthesis by regulating *TT4* and *CYP98A3* (Zhang *et al.*, 2017). The flavonoid, alkaloid, and carotenoid contents were significantly higher in *D. officinale* after far-red light treatment than in the control group (Li *et al.*, 2021). Among these, the target gene of lncRNA (MSTRG.63384.1), *ZSD1*, is a key gene in the carotenoid biosynthesis pathway, and the expression of MSTRG.63384.1 and *ZSD1* showed a positive correlation and a significant upregulation of carotenoid content under far-red light treatment (Li *et al.*, 2021). These findings laid the foundation for further exploring the role and regulatory mechanism of lncRNA in plant flavonoid biosynthesis.

It has been proven that lncRNA is also regulated by transcription factors and is involved in the regulation of specific transcription factors. Anthocyanins are derived from the branching synthetic pathway of flavonoids, which are water-soluble pigments and belong to the class of flavonoids (Andersen and Jordheim, 2010). The biosynthesis of anthocyanins is regulated by structural genes related to enzymatic reactions and by transcription factors. In apple, when *MdWRKY1* is induced by light, it can increase the transcription of MdLNC499 by regulating the W-box cis-elements in the MdLNC499 promoter, which induces the downstream ethylene response factor protein *MdERF109*, and the *MdERF109* transcription factor is involved in light-induced anthocyanin biosynthesis and can further induce anthocyanin-related gene expression and anthocyanin accumulation in early apple coloration (Ma *et al.*, 2021). In addition, MdLNC610 can be induced by high-light, and to regulate anthocyanin accumulation by mediating regulation of *MdACO1* expression (Yu *et al.*, 2022).

In plants, lncRNAs can also interact with miRNAs. Similarly, lncRNAs and miRNAs can interact with each other to induce anthocyanin accumulation. However, compared with miRNAs, there are fewer studies on the mechanism of lncRNA involvement in anthocyanin biosynthesis during fruit development.

At present, it has been demonstrated that several lncRNAs can act as ceRNAs that bind to miRNAs through specific complementary sequences, blocking the interaction of miRNAs with their target mRNAs and thus affecting the expression of repressed target genes (Wu *et al.*, 2013). For example, under light, SPLs interact with MYB TFs to promote anthocyanin biosynthesis in the apple pericarp by inducing the expression of anthocyanin biosynthetic genes, whereas *miR156a* overexpression negatively regulates the expression of SPLs. The lncRNAs MLNC3.2 and MLNC4.6, as eTMs of miR156a, were induced by white and blue light. MLNC3.2 and MLNC4.6 interact with miR156a and block the cleavage of its target genes, *SPL2-like* and *SPL33*, by miR156a, thus enhancing the anthocyanin content (Yang *et al.*, 2019). The silencing of LNC1 and LNA2 and expression analysis revealed that LNC1 and LNA2 acted as eTMs for miR156a and miR828a, which reduced the expression of *SPL9* and induced *MYB114*, respectively, which regulated the accumulation of anthocyanins in sea buckthorn fruit (Zhang *et al.*, 2018). Studies have demonstrated that lnaRNAs can not only act aseTMs for miRNA and compete for the binding of miRNA to mRNA targets, but can also act as precursors for miRNA production (Wang *et al.*, 2017). In strawberry, for example, 130 lncRNAs were identified as putative precursors of 80 miRNAs. Among these, several lncRNAs were identified as putative precursors of miRNAs, which are known as regulators of flavonoid pathways including miRNA858a, miRNA156, and miRNA396 (Lin *et al.*, 2018).

LncRNA is involved in terpenoid biosynthesis

In plants, terpenoids are mainly biosynthesized by the mevalonic acid pathway (MVA) and 2-C-methyl-D-erythritol 4-phosphate (MEP) (Narnoliya *et al.*, 2019). By analyzing the RNA-seq data of *Ganoderma lucidum*, it was found that three lncRNAs were adjacent to *GL24088* and encoded hydroxymethylglutaryl-CoA reductase (HMGR). HMGR is involved in isopentenyl diphosphate production in the MVA pathway, which is an important precursor of triterpenes (Li *et al.*, 2014). Five lncRNAs related to the biosynthesis of several important secondary metabolites have been identified in *Digitalis purpurea*. For example, mlncR8 may regulate terpenoid backbone biosynthesis, and mlncR31 is involved in the biosynthesis of the isoprenoid side chain of ubiquinone and plastoquinone (Wu *et al.*, 2012).

The lncRNA sequences with transcriptional potential for targeting the terpene biosynthesis pathway were identified from the transcriptome database of rose-scented geranium by the LNCTAR tool. A total of 321 unique lncRNA sequences were identified as being involved in the transcriptional regulation of terpene pathway enzymes, and 78 lncRNA sequences that may be related to gene regulation of terpenoid biosynthesis pathway were identified (Narnoliya *et al.*, 2019). The ssRNA-seq technique was used to identify the target

genes with different lncRNAs in *Cinnamomum camphora* that were enriched in the terpene biosynthesis pathway, and 17 of them were associated with monoterpene and sesquiterpene biosynthesis (Ni *et al.*, 2021). Previous studies have found that lncRNAs can act as miRNA targets (Wang *et al.*, 2017). Through ssRNA-seq analysis of tomato, a total of 20 lncRNAs were predicted by PSMIMIC software as the assumed targets of 12 known miRNAs (Liao *et al.,* 2020). Among these miRNA targets, LncRNA000551 is the sly-MIR1917 target, which has been found to be essential for terpene accumulation in tomato fruit (Karlova *et al.*, 2013).

LncRNA is involved in alkaloid synthesis

Many plants produce alkaloids, which are major secondary metabolites. However, there are few reports on the involvement of lncRNAs in alkaloid biosynthesis. Nicotine accounts for about 90% of the total alkaloid content in *Nicotiana tabacum* (Saitoh *et al.*, 1985). Several key genes encoding enzymes of the nicotine biosynthesis pathway have been cloned and characterized. In addition, recent reports have shown that miRNAs and lncRNAs are involved in the regulation of alkaloid biosynthesis in plants (Xie and Fan, 2016). As the eTMs of miRNAs, some lncRNAs compete with miRNAs to block the regulation of miRNAs on target genes and then participate in alkaloid biosynthesis. *QPT* converts quinolinic acid to nicotinic acid mononucleotide and serves as the entry point into the pyridine nucleotide cycle that leads to the production of nicotinic acid and consequently nicotine and is a key gene of the nicotine biosynthesis pathway (Dewey and Xie, 2013). miRNA (NTA-mirX27) is involved in nicotine biosynthesis by negatively targeting the regulation of the *QPT2* gene, while lncRNA (NTA-ETMX27) competes with NTA-mirX27 as an endogenous target mimicry (*QPT2*) and attenuates the targeting of *QPT2* by the nta-miRX27 effect, which in turn affects nicotine accumulation (F Li *et al.*, 2015; Chen *et al.*, 2019).

Table 1. LncRNAs regulating the synthesis of secondary metabolites in plants

Plant species	lncRNA	Target gene	Secondary metabolite	Reference
Gossypium hirsutum L.	ltcon_00004262	*Ghir_A01G005150.1*	Flavonoid biosynthesis	(Li *et al.,* 2019)
sea buckthorn	XLOC_035993, XLOC_037048, XLOC_270088, XLOC_312485, XLOC_312902, XLOC_340909	*TT4*	Flavonoid biosynthesis	(Zhang *et al.,* 2017)
	XLOC_194466	*CYP98A8*		
	LNC1	miR156a	Anthocyanin biosynthesis	(Zhang *et al.,* 2018)
	LNC2	miR828a		
Dendrobium officinale	MSTRG.63384.1	*ZSD1*	Carotenoid biosynthesis	(Li *et al.,* 2021)
	MSTRG.22972.1	*CCD4*		
kiwifruit	TCONS_00005212	*Achn270471*	Carotenoid, flavonoid metabolism	(Chen *et al.,* 2021)
Apple	MdLNC499	*MdERF109*	Anthocyanin biosynthesis	(Ma *et al.,* 2021)
	MdLNC610	*MdACO1*		(Yu *et al.,* 2022)
	MLNC3.2	*SPL2-like*		(Yang *et al.,* 2019)
	MLNC4.6	*SPL33*		

Populus tomentosa	TCONS_00003480	ptc-miR6464	Lignin biosynthesis	(Ci *et al.*, 2019)
Ganoderma lucidum	TU4312	*GL24088*	Terpenoid biosynthesis	(Li *et al.*, 2014)
	TU4313	*GL24089*		
	TU4314	*GL24090*		
Digitalis purpurea	mlncR8	*JO463639*	Terpenoid backbone biosynthesis	(Wu *et al.*, 2012)
	mlncR31	*JO463507*	Biosynthesis of isoprenoid side chain of ubiquinone andplastoquinone	
	FXAT9O005FZSI2	*FXAT9O005FZ5UJ*	Flavonoid biosynthesis	
	FXAT9O005GD1W7	*FXAT9O005GCP3D*	Carotenoid biosynthesis	
	FXAT9O005FYD4C	*FXAT9O005FK4AG*	Alkaloid biosynthesis	
Cinnamomum camphora	HMGS_like		Sesquiterpenoid biosynthesis	(Ni *et al.*, 2021)
	FPPS_like			
	TPS21_like			
	DXS_like		Monoterpenoid biosynthesis	
	TPS04_like			
	TPS-CIN_like			
Tomato (*Solanum lycopersicum*)	lncRNA1459		Carotenoid biosynthesis	(Li *et al.*, 2018)
	ACoS-AS1	*SlPsy1*		(Xiao *et al.*, 2020)
	LncRNA000551	sly-MIR1917	Terpene biosynthesis	(Karlova *et al.*, 2013)
Tobacco (*Nicotiana tabacum*)	NTA-ETMX27	*QPT2*	Nicotine biosynthesis	(F Li *et al.*, 2015)

Conclusions

With the rapid development of high-throughput sequencing technology and bioinformatics, significant progress has been made in prediction and functional studies of plant lncRNAs. Mounting evidence has shown that lncRNAs are emerging regulatory factors of various biological processes and play a potentially important role in the various basic biological processes of plants and animals. Most studies on lncRNAs focus on humans and animals, and they are relatively few studies on plants. In recent years, a large number of potential lncRNAs have been screened and identified in a variety of plants, and many lncRNAs have been confirmed to participate in and play an important regulatory role in the biosynthesis of plant secondary metabolites (Table 1). However, most of the current research is focused on high-throughput sequencing and other aspects, and there are few studies on its specific functional verification. Therefore, it is necessary to further systematically analyze and verify the regulatory role and related pathways of lncRNA in the biosynthesis of plant secondary metabolites; the analysis of the lncRNA-miRNA-mRNA regulatory relationship is particularly important. In addition, lncRNAs interact with proteins to form regulatory complexes and play a crucial role in controlling core cellular processes such as transcription and translation. Although many methods have been used to screen lncRNA-interacting proteins in plants, the specific mechanisms of lncRNA-protein interactions have not been studied.

Secondary metabolites have important medicinal and commercial value, but their yield in plants is low, and they are environmentally susceptible. LncRNAs, as important regulatory factors in plants, play an

important role in the biosynthesis of plant secondary metabolites. Therefore, an in-depth study on the regulatory mechanism of lncRNA in the biosynthesis of plant secondary metabolites can lay a good foundation for using biotechnological means to improve the production of plant secondary metabolites.

Authors' Contributions

Conceptualization: YTL, JBY; Funding acquisition: JBY, FX; Project administration: JBY; Supervision: WWZ, YLL; Validation: JBY; Writing - original draft: YTL; Writing - review and editing: YTL, HH, JBY. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by the National Natural Science Foundation of China, grant number 32101563.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Andersen ØM, Jordheim M (2010). Chemistry of flavonoid-based colors in plants. In: Comprehensive Natural Products II. Elsevier, Oxford, pp 547-614.

Ariel F, Romero-Barrios N, Jégu T, Benhamed M, Crespi M (2015). Battles and hijacks: noncoding transcription in plants. Trends in Plant Science 20:362-371. *https://doi.org/10.1016/j.tplants.2015.03.003*

Armaos A, Colantoni A, Proietti G, Rupert J, Tartaglia GG (2021). catRAPID omics v2.0: going deeper and wider in the prediction of protein-RNA interactions. Nucleic Acids Research 49:W72-W79. *https://doi.org/10.1093/nar/gkab393*

Baulcombe DC (1999). Fast forward genetics based on virus-induced gene silencing. Current Opinion in Plant Biology 2:109-113. *https://doi.org/10.1016/S1369-5266(99)8002-3*

Becker A, Lange M (2010). VIGS – genomics goes functional. Trends in Plant Science 15:1-4. *https://doi.org/10.1016/j.tplants.2009.09.002*

Carlevaro-Fita J, Johnson R (2019). Global positioning system: understanding long noncoding RNAs through subcellular localization. Molecular Cell 73:869-883. *https://doi.org/10.1016/j.molcel.2019.02.008*

Chen J, Tang X, Ren C, Wei B, Wu Y, Wu Q, Pei J (2018). Full-length transcriptome sequences and the identification of putative genes for flavonoid biosynthesis in safflower. BMC Genomics 19:548. *https://doi.org/10.1186/s12864-018-4946-9*

Chen L (2016). Linking long noncoding RNA localization and function. Trends in Biochemical Sciences 41,761-772. *https://doi.org/10.1016/j.tibs.2016.07.003*

Chen X, Sun S, Liu F, Shen E, Liu L (2019). A transcriptomic profile of topping responsive non-coding RNAs in tobacco roots (*Nicotiana tabacum*). BMC Genomics 20:856. *https://doi.org/10.1186/s12864-019-6236-6*

Chen Y, Cheng C, Feng X, Lai R, Gao M, Chen W, Wu RJ (2021). Integrated analysis of lncRNA and mRNA transcriptomes reveals the potential regulatory role of lncRNA in kiwifruit ripening and softening. Scientific Reports 11:1671. *https://doi.org/10.1038/s41598-021-81155-1*

Ci D, Tian M, Song Y, Du Q, Quan M (2019). Indole-3-acetic acid has long-term effects on long non-coding RNA gene methylation and growth in *Populus tomentosa*. Molecular Genetics and Genomics 294:1511-1525. *https://doi.org/10.1007/s00438-019-01593-5*

Crespi MD, Jurkevitch E, Poiret M, d'Aubenton-Carafa Y, Petrovics G, Kondorosi E, Kondorosi A (1994). *enod40*, a gene expressed during nodule organogenesis, codes for a non-translatable RNA involved in plant growth. The EMBO Journal 13:5099-5112.

Cui C, Shu W, Li P (2016). Fluorescence in situ hybridization: cell-based genetic diagnostic and research applications. Frontiers in Cell and Developmental Biology 4:89. *https://doi.org/10.3389/fcell.2016.00089*

Cui J, Shen N, Lu Z, Xu G, Wang Y, Jin B (2020). Analysis and comprehensive comparison of PacBio and nanopore-based RNA sequencing of the Arabidopsis transcriptome. Plant Methods 16:85. *https://doi.org/10.1186/s13007-020-00629-x*

Cui J, Jiang N, Meng J, Yang G, Liu W, Zhou X, ... Luan Y (2019). LncRNA33732-respiratory burst oxidase module associated with WRKY1 in tomato- *Phytophthora infestans* interactions. The Plant Journal: For Cell and Molecular Biology 97(5):933-946. *https://doi.org/10.1111/tpj.14173*

Dalmay T (2013). Mechanism of miRNA-mediated repression of mRNA translation. Essays in Biochemistry 54:29-38. *https://doi.org/10.1042/bse0540029*

Dewey RE, Xie J (2013). Molecular genetics of alkaloid biosynthesis in *Nicotiana tabacum*. Phytochemistry 94:10-27. *https://doi.org/10.1016/j.phytochem.2013.06.002*

Fang J, Zhang F, Wang H, Wang W, Zhao F, Li Z, ... Chu C (2019). *Ef-cd* locus shortens rice maturity duration without yield penalty. Proceedings of the National Academy of Sciences of the United States of America 116(37):18717-18722. *https://doi.org/10.1073/pnas.1815030116*

Fok ET, Scholefield J, Fanucchi S, Mhlanga MM (2017). The emerging molecular biology toolbox for the study of long noncoding RNA biology. Epigenomics 9(10):1317-1327. *https://doi.org/10.2217/epi-2017-0062*

Fukuda M, Nishida S, Kakei Y, Shimada Y, Fujiwara T (2019). Genome-wide analysis of long intergenic noncoding RNAs responding to low-nutrient conditions in *Arabidopsis thaliana*: possible involvement of trans-acting siRNA3 in response to low nitrogen. Plant and Cell Physiology 60(9):1961-1973. *https://doi.org/10.1093/pcp/pcz048*

Gai Y, Yuan S, Zhao Y, Zhao H, Zhang H, Ji X (2018). A novel lncRNA, MuLnc1, associated with environmental stress in Mulberry (*Morus multicaulis*). Frontiers in Plant Science 9:669. *https://doi.org/10.3389/fpls.2018.00669*

Gao R, Liu P, Irwanto N, Loh DR, Wong S (2016). Upregulation of LINC-AP2 is negatively correlated with AP2 gene expression with *Turnip crinkle virus* infection in *Arabidopsis thaliana*. Plant Cell Reports 35(11):2257-2267. *https://doi.org/10.1007/s00299-016-2032-9*

Guo G, Liu X, Sun F, Cao J, Huo N, Wuda B, ... Yao Y (2018). Wheat miR9678 affects seed germination by generating phased siRNAs and modulating abscisic acid/gibberellin signaling. The Plant Cell 30(4):796-814. *https://doi.org/10.1105/tpc.17.00842*

Hartmann T (2007). From waste products to ecochemicals: fifty years research of plant secondary metabolism. Phytochemistry 68(22-24):2831-2846. *https://doi.org/10.1016/j.phytochem.2007.09.017*

Hou X, Cui J, Liu W, Jiang N, Zhou X, Qi H, ... Luan Y (2020). LncRNA39026 enhances tomato resistance to *Phytophthora infestans* by decoying miR168a and inducing *PR* gene expression. Phytopathology 110(4):873-880. *https://doi.org/10.1094/PHYTO-12-19-0445-R*

Hussain B, Lucas SJ, Budak H (2018). CRISPR/Cas9 in plants: at play in the genome and at work for crop improvement. Briefings in Functional Genomics 17(5):319-328. *https://doi.org/10.1093/bfgp/ely016*

Jain N, Sinha N, Krishna H, Singh PK, Gautam T, Prasad P, ... Gupta PK (2020). A study of miRNAs and lncRNAs during Lr28-mediated resistance against leaf rust in wheat (*Triticum aestivum* L.) Physiological and Molecular Plant Pathology 112:101552. *https://doi.org/10.1016/j.pmpp.2020.101552*

Jiang N, Cui J, Shi Y, Yang G, Zhou X, Hou X, ... Luan Y (2019). Tomato lncRNA23468 functions as a competing endogenous RNA to modulate *NBS-LRR* genes by decoying miR482b in the tomato-*Phytophthora infestans* interaction. Horticulture Research 6:28. *https://doi.org/10.1038/s41438-018-0096-0*

Jiang N, Cui J, Hou X, Yang G, Xiao Y, Han L, ... Luan Y (2020). Sl-lncRNA15492 interacts with Sl-miR482a and affects *Solanum lycopersicum* immunity against *Phytophthora infestans*. The Plant Journal: For Cell and Molecular Biology 103(4):1561-1574. *https://doi.org/10.1111/tpj.14847*

Karlova R, van Haarst JC, Maliepaard C, van de Geest H, Bovy AG, Lammers M, ... de Maagd RA (2013). Identification of microRNA targets in tomato fruit development using high-throughput sequencing and degradome analysis. Journal of Experimental Botany 64(7):1863-1878. *https://doi.org/10.1093/jxb/ert049*

Kim D-H, Sung S (2017). Vernalization-triggered intragenic chromatin loop formation by long noncoding RNAs. Developmental Cell 40(3):302-312. *https://doi.org/10.1016/j.devcel.2016.12.021*

Kim D-H, Xi Y, Sung S (2017). Modular function of long noncoding RNA, COLDAIR, in the vernalization response. PLoS Genetics 13(7):e1006939. *https://doi.org/10.1371/journal.pgen.1006939*

Kutchan TM (2001). Ecological arsenal and developmental dispatcher. The paradigm of secondary metabolism. Plant Physiology 125(1):58-60. *https://doi.org/10.1104/pp.125.1.58*

Lennox KA, Behlke MA (2016). Cellular localization of long non-coding RNAs affects silencing by RNAi more than by antisense oligonucleotides. Nucleic Acids Research 44(2):863-877. *https://doi.org/10.1093/nar/gkv1206*

Li F, Wang W, Zhao N, Xiao B, Cao P, Wu X, ... Fan L (2015). Regulation of nicotine biosynthesis by an endogenous target mimicry of microRNA in tobacco. Plant Physiology 169(2):1062-1071. *https://doi.org/10.1104/pp.15.00649*

Li H, Ye W, Wang Y, Chen X, Fang Y, Sun G (2021). RNA sequencing-based exploration of the effects of far-red light on lncRNAs involved in the shade-avoidance response of *D. officinale*. PeerJ 9:e10769. *https://doi.org/10.7717/peerj.10769*

Li J, Ma W, Zeng P, Wang J, Geng B, Yang J, Cui Q (2015). LncTar: a tool for predicting the RNA targets of long noncoding RNAs. Briefings in Bioinformatics 16(5):806-812. *https://doi.org/10.1093/bib/bbu048*

Li J, Wu B, Xu J, Liu C (2014). Genome-wide identification and characterization of long intergenic non-coding RNAs in *Ganoderma lucidum*. PLOS ONE 9(6):e99442. *https://doi.org/10.1371/journal.pone.0099442*

Li R, Fu D, Zhu B, Luo Y, Zhu H (2018). CRISPR/Cas9-mediated mutagenesis of lncRNA1459 alters tomato fruit ripening. The Plant Journal: For Cell and Molecular Biology 94(3):513-524. *https://doi.org/10.1111/tpj.13872*

Li Y, Qin T, Dong N, Wei C, Zhang Y, Sun R, ... Wang Q (2019). Integrative analysis of the lncRNA and mRNA transcriptome revealed genes and pathways potentially involved in the anther abortion of Cotton (*Gossypium hirsutum* L.). Genes 10(12):E947. *https://doi.org/10.3390/genes10120947*

Liao X, Wang J, Zhu S, Xie Q, Wang L, Yu H, ... Yang C (2020). Transcriptomic and functional analyses uncover the regulatory role of lncRNA000170 in tomato multicellular trichome formation. The Plant Journal: For Cell and Molecular Biology 104(1):18-29. *https://doi.org/10.1111/tpj.14902*

Lin Y, Jiang L, Chen Q, Li Y, Zhnag Y, Sun B, ... Tang Haoru (2018). Comparative transcriptome profiling analysis of red- and white-fleshed Strawberry (*Fragaria×ananassa*) provides new insight into the regulation of the anthocyanin pathway. Plant & Cell Physiology 59(9):1844-1859. *https://doi.org/10.1093/pcp/pcy098*

Liu S, Wang Lu, Cao M, Pang S, Li W, Kato-Noguchi H, ... Wang L (2020). Identification and characterization of long non-coding RNAs regulating flavonoid biosynthesis in *Ginkgo biloba* leaves. Industrial Crops and Products 158:112980. *https://doi.org/10.1016/j.indcrop.2020.112980*

Lu Q, Ren S, Lu M, Zhang Y, Zhu D, Zhnag X, Li T (2013). Computational prediction of associations between long non-coding RNAs and proteins. BMC Genomics 14:651. *https://doi.org/10.1186/1471-2164-14-651*

Ma H, Yang T, Li Y, Zhang J, Wu T, Song T, ... Tian J (2021). The long noncoding RNA MdLNC499 bridges *MdWRKY1* and *MdERF109* function to regulate early-stage light-induced anthocyanin accumulation in apple fruit. The Plant Cell 188. *https://doi.org/10.1093/plcell/koab188*

Ma L, Bajic VB, Zhang Z (2013). On the classification of long non-coding RNAs. RNA Biology 10(6):924-933. *https://doi.org/10.4161/rna.24604*

Narnoliya LK, Kaushal G, Singh SP (2019). Long noncoding RNAs and miRNAs regulating terpene and tartaric acid biosynthesis in rose-scented geranium. FEBS letters 593(16):2235-2249. *https://doi.org/10.1002/1873-3468.13493*

Nejat N, Mantri N (2018). Emerging roles of long non-coding RNAs in plant response to biotic and abiotic stresses. Critical Reviews in Biotechnology 38(1):93-105. *https://doi.org/10.1080/07388551.2017.1312270*

Ni Z, Han X, Chen C, Zhong Y, Xu M, Xu L, Yu F (2021). Integrating GC-MS and ssRNA-Seq analysis to identify long non-coding RNAs related to terpenoid biosynthesis in *Cinnamomum camphora*. Industrial Crops and Products 171:113875. *https://doi.org/10.1016/j.indcrop.2021.113875*

Pachnis V, Belayew A, Tilghman SM (1984). Locus unlinked to alpha-fetoprotein under the control of the murine *raf* and *Rif* genes. Proceedings of the National Academy of Sciences of the United States of America 81(17):5523-5527. *https://doi.org/10.1073/pnas.81.17.5523*

Ponjavic J, Ponting CP, Lunter G (2007). Functionality or transcriptional noise? Evidence for selection within long noncoding RNAs. Genome Research 17(5):556-565. *https://doi.org/10.1101/gr.6036807*

Ponting CP, Oliver PL, Reik W (2009). Evolution and functions of long noncoding RNAs. Cell 136(4):629-641. *https://doi.org/10.1016/j.cell.2009.02.006*

Qiao D, Yang C, Chen J, Guo Y, Li Y, Niu S, ... Chen Z (2019). Comprehensive identification of the full-length transcripts and alternative splicing related to the secondary metabolism pathways in the tea plant (*Camellia sinensis*). Scientific Reports 9(1):2709. *https://doi.org/10.1038/s41598-019-39286-z*

Qin T, Zhao H, Cui P, Albesher N, Xiong L (2017). A nucleus-localized long non-coding RNA enhances drought and salt stress tolerance. Plant Physiology 175(3):1321-1336. *https://doi.org/10.1104/pp.17.00574*

Quan M, Xiao L, Lu W, Liu X, Song F, Si J, ... Zhang D (2018). Association genetics in *Populus* reveal the allelic interactions of *Pto-MIR167a* and its targets in wood formation. Frontiers in Plant Science 9:744. *https://doi.org/10.3389/fpls.2018.00744*

Roberts RJ, Carneiro MO, Schatz MC (2013). The advantages of SMRT sequencing. Genome Biology 14(6):405. *https://doi.org/10.1186/gb-2013-14-6-405*

Saitoh F, Noma M, Kawashima N (1985). The alkaloid contents of sixty Nicotiana species, Phytochemistry, 24(3):477-480. *https://doi.org/10.1016/S0031-9422(00)80751-7*

Seo JS, Sun HX, Park BS, Huang CH, Yeh S, Jung C, Chua NH (2017). ELF18-INDUCED LONG-NONCODING RNA associates with mediator to enhance expression of innate immune response genes in Arabidopsis. The Plant Cell 29(5):1024-1038. *https://doi.org/10.1105/tpc.16.00886*

Seo JS, Chua N-H (2019a). Identification of long noncoding RNA-protein interactions through in vitro RNA pull-down assay with plant nuclear extracts. Methods in Molecular Biology (Clifton, N.J.) 1933:279-288. *https://doi.org/10.1007/978-1-4939-9045-0_17*

Seo JS, Chua N-H (2019b). Trimolecular fluorescence complementation (TriFC) assay for visualization of RNA-protein interaction in plants. Methods in Molecular Biology (Clifton, N.J.) 1933:297-303. *https://doi.org/10.1007/978-1-4939-9045-0_19*

Song L, Fang Y, Chen L, Wang J, Chen X (2021). Role of non-coding RNAs in plant immunity. Plant Communications 2(3):100180. *https://doi.org/10.1016/j.xplc.2021.100180*

Stojic L, Lun ATL, Mangei J, Mascalchi P, Quarantotti V, Barr A, ... Odom DT (2018). Specificity of RNAi, LNA and CRISPRi as loss-of-function methods in transcriptional analysis. Nucleic Acids Research 46(12):5950-5966. *https://doi.org/10.1093/nar/gky437*

Takshak S, Agrawal SB (2019). Defense potential of secondary metabolites in medicinal plants under UV-B stress. Journal of Photochemistry and Photobiology, B: Biology 193:51-88. *https://doi.org/10.1016/j.jphotobiol.2019.02.002*

Wang J, Meng X, Dobrovolskaya OB, Orlov YL, Chen M (2017). Non-coding RNAs and their roles in stress response in plants, Genomics. Proteomics & Bioinformatics 15(5):301-312. *https://doi.org/10.1016/j.gpb.2017.01.007*

Wink M (2015). Modes of action of herbal medicines and plant secondary metabolites. Medicines (Basel, Switzerland) 2(3):251-286. *https://doi.org/10.3390/medicines2030251*

Wu B, Li Y, Yan H, Ma Y, Luo H, Yuan L, ... Lu S (2012). Comprehensive transcriptome analysis reveals novel genes involved in cardiac glycoside biosynthesis and mlncRNAs associated with secondary metabolism and stress response in Digitalis purpurea. BMC Genomics 13:15. *https://doi.org/10.1186/1471-2164-13-15*

Wu H, Wang Z, Wang M, Wang X (2013). Widespread long noncoding RNAs as endogenous target mimics for microRNAs in plants. Plant Physiology 161(4):1875-1884. *https://doi.org/10.1104/pp.113.215962*

Xiao Y, Kang B, Li M, Xiao L, Xiao H, Shen H, Yang W (2020). Transcription of lncRNA ACoS-AS1 is essential to trans-splicing between *SlPsy1* and *ACoS-AS1* that causes yellow fruit in tomato. RNA Biology 17(4):596-607. *https://doi.org/10.1080/15476286.2020.1721095*

Xie J, Fan L (2016). Nicotine biosynthesis is regulated by two more layers: Small and long non-protein-coding RNAs. Plant Signaling & Behavior 11(6):e1184811. *https://doi.org/10.1080/15592324.2016.1184811*

Xin M, Wang Y, Yao Y, Song N, Hu Z, Qin D, ... Sun Q (2011). Identification and characterization of wheat long non-protein coding RNAs responsive to powdery mildew infection and heat stress by using microarray analysis and SBS sequencing. BMC Plant Biology 11:61. *https://doi.org/10.1186/1471-2229-11-61*

Yang T, Ma H, Zhang J, Wu T, Song T, Tian J, Yao Y (2019). Systematic identification of long noncoding RNAs expressed during light-induced anthocyanin accumulation in apple fruit. The Plant Journal: For Cell and Molecular Biology 100(3):572-590. *https://doi.org/10.1111/tpj.14470*

Ye J, Cheng S, Zhou X, Chen Z, Kin SU, Tan J, ... Zhu Y (2019). A global survey of full-length transcriptome of *Ginkgo biloba* reveals transcript variants involved in flavonoid biosynthesis. Industrial Crops and Products 139:111547. *https://doi.org/10.1016/j.indcrop.2019.111547*

Yu J, Qiu K, Sun W, Yang T, Wu T, Song T, ... Tian J (2022). A long noncoding RNA functions in high-light-induced anthocyanin accumulation in apple by activating ethylene synthesis. Plant Physiology kiac049. *https://doi.org/10.1093/plphys/kiac049*

Zhang G, Duan A, Zhang J, He C (2017). Genome-wide analysis of long non-coding RNAs at the mature stage of sea buckthorn (*Hippophae rhamnoides* Linn) fruit. Gene 596:130-136. *https://doi.org/10.1016/j.gene.2016.10.017*

Zhang G, Chen D, Zhang T, Duan A, Zhang J, He C (2018). Transcriptomic and functional analyses unveil the role of long non-coding RNAs in anthocyanin biosynthesis during sea buckthorn fruit ripening. DNA Research 25(5):465-476. *https://doi.org/10.1093/dnares/dsy017*

Zhang G, Sun M, Wang J, Lei M, Li C, Zhao D, ... Zhang B (2019). PacBio full-length cDNA sequencing integrated with RNA-seq reads drastically improves the discovery of splicing transcripts in rice. The Plant Journal 97(2):296-305. *https://doi.org/10.1111/tpj.14120*

Zhang H, Qin C, An C, Zheng X, Wen S, Chen W, ... Wu Y (2021). Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. Molecular Cancer 20(1):126. *https://doi.org/10.1186/s12943-021-01431-6*

Zhang X, Dong J, Deng F, Wang W, Cheng Y, Song L, ... Shen F (2019). The long non-coding RNA lncRNA973 is involved in cotton response to salt stress. BMC Plant Biology 19(1):459. *https://doi.org/10.1186/s12870-019-2088-0*

Zhang X, Shen J, Xu Q, Dong J, Song L, Wang W, Shen F (2021). Long noncoding RNA lncRNA354 functions as a competing endogenous RNA of miR160b to regulate *ARF* genes in response to salt stress in upland cotton. Plant, Cell & Environment 44(10):3302-3321. *https://doi.org/10.1111/pce.14133*

Zhao X, Li J, Lian B, Gu H, Li Y, Qi Y (2018). Global identification of Arabidopsis lncRNAs reveals the regulation of *MAF4* by a natural antisense RNA. Nature Communications 9(1):5056. *https://doi.org/10.1038/s41467-018-07500-7*

Zhou M, Zhu X, Shao J, Tang Y, Wu Y (2011). Production and metabolic engineering of bioactive substances in plant hairy root culture. Applied Microbiology and Biotechnology 90(4):1229-1239. *https://doi.org/10.1007/s00253-011-3228-0*

Zhou W, Shi H, Wang Z, Zhao Y, Gou X, Li C, ... Liu Y (2020). Identification of lncRNAs involved in wheat tillering development in two pairs of near-isogenic lines. Functional & Integrative Genomics 20(5):669-679. *https://doi.org/10.1007/s10142-020-00742-z*

Zou C, Wang Q, Lu C, Yang W, Zhang Y, Cheng H, ... Song G (2016). Transcriptome analysis reveals long noncoding RNAs involved in fiber development in cotton (*Gossypium arboreum*). Science China. Life Sciences 59(2):164-171. *https://doi.org/10.1007/s11427-016-5000-2*