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Review

Recent Research Advances of Small Regulatory RNA in Fruit Crops

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Abstract: MicroRNAs (miRNAs) are endogenous noncoding small RNAs containing 21–24 nucleotides (nt) that regulate gene expression precisely and efficiently at the posttranscriptional level through the negative regulation of target messenger RNA (mRNA) expression, such as translational inhibition or degradation. Likewise, as a controlling element, miRNA itself is regulated by a variety of factors when performing its basic purposes, such as SNP detection, miRNA purging, methylation, and the circadian clock in model crops. In current years, miRNA-mediated controls have been intensely investigated in horticultural plants, leading to the discovery of numerous novel mechanisms that exhibit significantly greater mechanistic complexity and distinctive regulatory properties than those explored in model species. In fruit crops, miRNAs play a crucial role corresponding to various biological, metabolic functions and environmental challenges, including growth, expansion, response to biotic and abiotic stress, signaling of growth hormones, and the regulation of secondary product metabolism. In this study, we appraisal the current improvement of small regulatory RNA research in fruit crops, emphasizing miRNA mechanisms and their correlation with key trait rule. Considering that miRNAs engaged in the regulation of all aspects of fruit tree life activities, we focus here on their biosynthesis, target genes, function and regulatory network, as well as the mechanistic connection among them, to provide a theoretic base and breakthrough for upcoming exploration on miRNAs in fruit plants.

Keywords: fruit trees; small RNA; development; stress response

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1. Introduction

Plant small regulatory RNAs play critical roles in plant development and expansion, including fruit development, quality formation, signal transduction, abiotic stress, biotic stress, and secondary metabolism. Information regarding miRNA and phasiRNAs and regulatory networks involved in fruit development has been summarized recently [1–3]. MicroRNAs can enhance plant productivity by modulating crucial plant metabolic processes at the posttranscriptional level [4–6]. MiRNA pathways involved in fruit development, size, shape, flavor, and texture have been highlighted. However, the functional characterization of miRNAs in many fruit species remains largely unknown, mainly due to the genetic complexity and the long juvenility of many fruit species [2,7–9]. Although researches of model plants provide much of our knowledge about miRNAs, such as *Arabidopsis*, ongoing research is gradually extending to nonmodel systems, including an essential group of economic plants—horticultural plants. These investigations have significantly increased our knowledge of the synthesis, metabolism, and use of miRNAs in crops. Now, we aim to summarize the progress of research on these miRNAs to show an overview of the regulatory networks including miRNAs that are essential for the development of important economic features in fruit plants.

Pre-messenger RNA (mRNA) processing (capping, splicing, and polyadenylation), mRNA stability, and mRNA translation are all instances of posttranscriptional modifications that affect gene expression in plants. Such alterations are examples of posttranscriptional gene expression modulation. MicroRNAs play important roles in plant gene control, including de novo DNA methylation, regulation by transcription factors (TFs), and modulation of mRNA accumulation via small RNAs. Small RNAs are non-coding RNAs of 20–24 nucleotides (nt) that were first identified for their ability to silence target genes through base complementation. Small RNA processing has been discovered in *Caenorhabditis elegans* to regulate postembryonic progress. The lin-4 RNA regulates the genes *LIN-14* and *LIN-28*. Since the discovery of miRNA in *Caenorhabditis elegans* as a molecule affecting larval development time, it has been hypothesized that organisms can be used to regulate endogenous genes. RNA silencing has been postulated as a defense mechanism against viruses and transposons. Since then, research on small RNAs has quickly expanded to plant and mammalian systems. Small RNAs are divided into dual types, microRNA (miRNA) and short interfering RNA (siRNA), with two separate functional pathways: the RNA-induced silencing complex (RISC) path and the RNA-directed DNA methylation (RdDM) pathway [10–12]. MiRNAs are evolutionarily conserved across all significant plant lineages, according to numerous studies. While early miRNAs are frequently expressed at relatively low levels or are only triggered under certain circumstances, old miRNAs are frequently highly expressed and evolutionarily conserved [13].

Numerous short RNAs have been found in various plants, and it has been demonstrated that they control the genes responsible for fruit ripening. Small RNAs significantly impact plant improvement and abiotic/biotic opposition. They regulate organ integrity, trichome development, phase shift, and blooming period, and stem cell fate. MiR390 cleaves *TAS3* to produce tasiRNAs that control auxin response factors, which are essential for plant development [14]. Auxin response factors are also involved in fruit formation, lateral root development, and leaf polarization. Small RNAs can be used to induce species-specific phenotypes in plants. For example, in persimmon, *Diospyros kaki* lotus, a Y-chromosome-encoded short RNA serves as a sex indicator [15].

MicroRNAs have been found to regulate the development of many plants, including fruits (peach, grape, apple, citrus, blueberry, litchi, persimmon, pitaya, passion fruit) [16,17], vegetables (sponge gourd) [17]. It has been demonstrated that some small RNAs are conserved in higher plants and can be found in both model and horticultural plants [18–21].

Conserved small RNAs have similar effects on their target genes in various plants, for example, miR172 controls *AP2/ERF* TFs and *ARF*-tasiRNA affects *ARFs* in plants [22]. miR156-*SPL* regulates TFs, which are involved in phase shift and trichome development. Overexpression of sly-miR156a causes a variety of alterations in vegetative and reproductive features, as well as a limited phenocopy of the *sft* mutant [23]. To facilitate the production of lignin in plants, miR397 cleaves the expression of *LAC*. MiR390 directs the production of *ARF* tasiRNAs and *MYB* tasiRNAs that target *MYB* TFs involved in flower and fruit development by *TAS3* and *TAS4*, respectively [24]. *MYB TFs* control the phenylpropanoid pathway as well as other horticultural characteristics like color, flavor, and texture [25,26]. Studies have been conducted to clarify how small RNAs affect horticultural features such as those of apple (*Malus domestica*), citrus (*Citrus medica)*, grape (*Vitis vinifera*), peach (*Prunus persica*), strawberry (*Fragaria x ananassa*) and persimmon (*Diospyros kaki)* [15,27–31]. The processes linked to fruit size, fruit color, and flowering period are the main topics of sRNA studies for fruit trees.

2. Biological Anabolic Pathways of miRNA Genes

2.1. Biosynthesis of miRNA

In plant cells, the process of miRNA biosynthesis includes three main steps: transcription, processing and maturation, and complex functional assembly [32]. First, the *MIR* gene is transcribed under the action of RNA polymerase II (Pol II) to produce a primary transcript (primaRNA) with an uneven hairpin structure, cap structure, and poly-A tail [33].

Subsequently, the pri-miRNA is cleaved by Dicer-like 1 to form a precursor miRNA (pre-miRNA) with a typical neck ring structure and a length of 70–500 nt [34]. Unlike the transport of animal miRNA precursors from the nucleus to the cytoplasm, the gene transcription and processing of plant miRNA are completed in the nucleus, and pre-miRNA is very unstable and continues to be processed into a 22 nt miRNA/miRNA* double-stranded complex under the catalysis of Dicer-like 1 enzyme [35]. To resist destruction by micro exonucleases, the methyl transferase Hua Enhancer 1 methylates the 3′ end of either strand of the miRNA/miRNA* double-stranded complex [36]. The methylated miRNA/miRNA* double-stranded complex is transported into the cytoplasm with the assistance of the nuclear export protein Exportin-5 homologous protein HASTY, which is dissociated by a helicase. One strand forms a mature miRNA that binds to the RNA-induced silencing complex (RISC) to form the miRNA-induced silencing complex (miRISC), and the other strand is degraded [37].

The miR173 target genes could encode a series of siRNAs simultaneously, and these siRNAs can continue to act on the downstream target genes in the same way as miRNAs [38]. These siRNAs are also called trans-acting siRNA (tasiRNA), when analyzing the expression of tasiRNAs in miRNA and siRNA biosynthesis pathway of different genes mutants in *Arabidopsis*, these tasiRNAs were not expressed in *dcl1*, *hen1*, *hyl1*, *rdr6*, and *sds3* mutants, it indicates that *RDR6* and *SDS3* are essential genes for siRNA biosynthesis, and *DCL1*, *HEN1*, and *HYL1* are essential genes for miRNA biosynthesis, proving that the biosynthesis of these tasiRNAs is miRNA-dependent. Further investigation reveals that these tasiRNAs are produced by the degradation site of miRNA on the target gene, and continuously cleave to the 3′ end to produce 21 nt siRNA with phase arrangement (the dimer of siRNA produced on the positive chain and negative chain has a two-base extension at 3′) [38].

In addition to miR173, miR390 and miR828 targeting *TAS3* and *TAS4* triggers the production of tasiRNA, respectively [38,39]. In apple, 19 *MYBs* targeted by miR828 could produce secondary siRNAs, which target more than 70 genes that regulate apple growth and development [29]. In grapes, miR828 targets two *MYBs* genes (*VvMYBA6*, *VvMYBA7*), which can produce secondary siRNA to regulate flavonoid synthesis and grape fruit development [40]. Besides, tasiRNAs produced by *TAS1-4* act on *PPR* (pentatricopeptide repeat) gene, *ARF* (auxin response factor) gene, and MYB transcription factor, respectively.

2.2. Mechanism of Small RNA Action

Plant miRNAs regulate the expression of their target genes in two main ways: one is the degradation of their highly complementary target genes, and the other is the translation inhibition of their local complementary target genes [41,42]. Quantitative expression analysis of target genes can be performed by qRT–PCR to verify whether there is a negative regulatory relationship between a miRNA and its target genes [43]. An effective way to study the function of miRNAs is to inhibit the expression or overexpression of target miRNAs by transgenic methods and then analyze the changes in expression of the target genes.

A large number of researches suggested that miRNA not only specifically targets the 3′UTR region of the target gene, but also targets the 5′UTR region, coding sequence and promoter region, inhibiting the translation of the target gene [44]. When the miRNA recognizes and binds the 5′UTR and coding regions, it has silencing effects on gene expression, but it induces transcription when miRNA interaction with promoter region. The guide strand and AGO constitute the basal miRNA induced silencing complex (miRISC), the target specificity of which is decide on the interaction of miRISC and complementary sequences on target mRNA (MREs). Whether there is ago2-dependent target mRNA slicing or miRIC-mediated translation inhibition and target mRNA decay depends on the degree of MRE complementarity. However, this interaction breaks the link between AGO and the 3′ end of the miRNA that promotes its degradation.

In wild diploid strawberry, one of the miRNAs in the new cluster is 22 nucleotides and triggers six *FBX* genes to produce staged small interfering RNA, which amplifies silencing

of other *FBX* genes [45]. In *Medicago truncatula*, miR390 targets *TAS3* (trans-acting short interfering RNA3) transcripts to produce trans-acting small interfering RNA, which targets mRNA encoding *ARF2* (auxin response factor), *ARF3* and *ARF4*, promotes nodulation and rhizobia infection, changes the spatial distribution of nodules, and increases the percentage of nodules with multiple meristems [46].

3. Small RNA Function: The Role in Regulatory Network

3.1. Visual Properties of Fruits and the Effect of miRNAs

Small RNA sequencing has been used on various fruits and horticultural crops that grow fruit, even though most miRNA exploration focuses on model crops. In general, conserved miRNAs target genes that are likewise conserved, i.e., many plant species contain members of a family of conserved miRNA target genes. Highly conserved miRNAs in fruits often control fruit morphogenesis, including size and form. Instead, the high-quality properties of the species, such as the development of distinctive color, flavor, aroma, and fruit durability, are usually represented by pathways regulated by species-specific miRNAs.

3.1.1. Influence of miRNAs on Fruit Size

MiR172 shows a crucial part in phase change, fruit growing, and ripening, it depends on the type of fruit, and affects the size of the fruit in various species. Therefore, alteration the expression of miR172 could be an upright approach for producing fruits of the desired size. The miR172-*AP2* module regulates fruit growth in apples, *Arabidopsis*, and tomatoes, and depending on the tissue type from which the fruit is formed, this module influences the size of the fruit [47,48]. The expression of *AG* (AGAMOUS) and *FUL* (FRUITFUL), which are required for the growth of the ovary and pod, is impacted by microRNA miR172. When miR172 is overexpressed in apples, fruit size is greatly reduced, but the mechanism remains unclear. Further studies have demonstrated that miR172 affects fruit mass liable on the fruit variety and that the effect is different in *Arabidopsis* pods and fleshy tomato fruits [48]. Overexpression of apple miR156 in *Arabidopsis* outcomes in a squatter pod than WT plants, whereas slymiR156a overexpression in tomato lowers fruit mass and profit twofold [23]. It has been reported that miR396a/b and miR1917 regulate fruit size in tomatoes and that miR171 and *AGO1* interact to regulate fruit size [49,50]. A novel strawberry miRNA, Fa_novel6, targets *HERCULES1*, which may help determine fruit size at ripening [9] (Figure 1).

3.1.2. Impact of miRNAs on Fruit Shape

MiRNAs can exert an impact on fruit morphology; for instance, overexpressing miR172 causes parthenocarpic fruit to have ectopic ovaries [47]. Additionally, ectopic fruit production on receptacles is caused by overexpressing REVOLUTA (*REV*), a miR166-resistant variant, and the majority of secondary fruits lack a placenta and ovules. [51]. Additionally, miR160's target, *ARF10A/10B/17*, is substantially more expressed in fruits when miR160 is downregulated, giving rise to fruits with a prolonged, pear-shaped morphology. Additionally, transgenic flowers have ovaries that are larger and thinner than those found in wild-type flowers. These characteristics are noticeable at the early stages of bud development and last until flowering [52] (Figure 1).

3.1.3. Impact of miRNAs on Fruit Color

Fruit color is determined by the variety and amount of various pigments, containing chlorophyll, carotenoids, anthocyanins, and flavonoids. Previous researches have shown that miRNAs regulate *MYB* family members to control anthocyanin biosynthesis, which is highly conserved among various fruit species. In *Arabidopsis*, heterogeneous expression of vvi-miR828 results in lighter color leaves that are consistent with the phenotype of the control grape berry, demonstrating that miR828 is a repressor that affects anthocyanin biosynthesis. MiR828 and miR858 both target *MYB114*, which regulates anthocyanin accumulation in grapes [53]. MiR156, which targets *SPL9*, interferes with the MYB-bHLH-

WD40 complex to negatively control the production of anthocyanins [54,55]. Similar, miR5072 targets chalcone isomerase (*CHI*) and anthocyanidin reductase (*ANR*), two crucial enzymes in the metabolism of anthocyanins in apples. MiRNAs have been invented to regulate the biosynthesis of several essential plant pigments, including anthocyanins, lycopene, carotenoids, and betalain. These miRNAs might also play a part in the production of the pigment suberin, which is present in russet-skinned pears [56,57] (Figure 1).

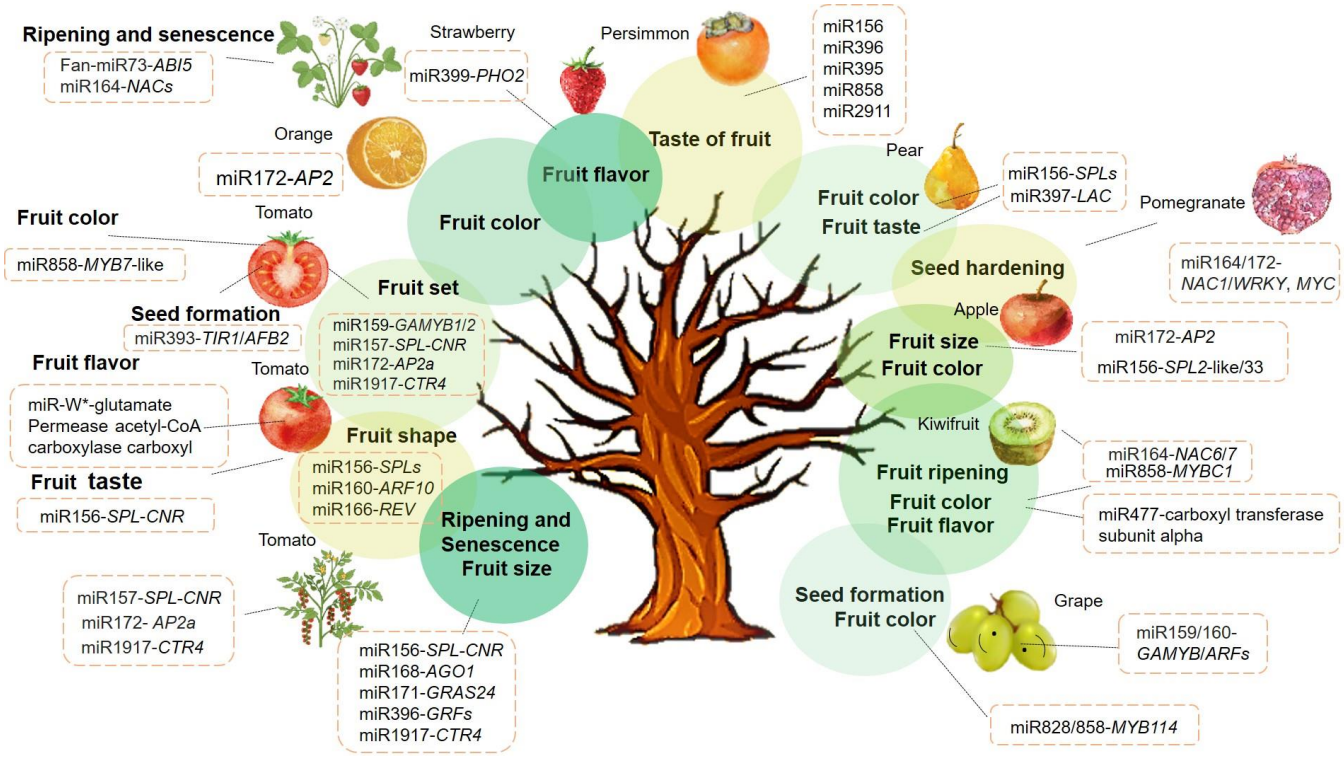

Figure 1. The main microRNA pathways—fruit development, size, color, shape, taste, flavor, ripening, and senescence—are divided into seven categories and are represented by a cartoon fruit tree. MicroRNA-integrated pathways are denoted in bold, while species- or lineage-specific pathways are denoted in circle.

3.1.4. Impact of miRNAs on Fruit Aroma and Flavor

Fruit flavor compounds are derived primarily include fatty acids, amino acids, and secondary metabolism. There are few researches on miRNA's role in fruit's aromatic anabolism. MiR172, miR159, miR160, miR395, miR399, miR535, and miR7120 negatively control the genes related to major fatty acid oxidation enzymes HPL, ADH, and LOXs [57]. One miRNA, miR1534, was found to be intricate in targeting a terpene synthase in tomatoes, and the expression of sesquiterpene synthase and patchoulol synthase was also moderated by miR156 [58]. A study revealed that miR399 regulates phosphorus metabolism in strawberry fruits and that miR319 negatively regulates tomato fruit flavor by targeting *LA* (LANCEOLATE), a TF from the *TCP* family [59,60]. Additional miRNAs have been well-known that regulate sugar and acid metabolism in fruit crops including grape, wolfberry, and peach [61–63]. It has been reported that miRNAs regulate fruit flavor, which strongly influences edible quality. MiR156, miR157, miR396g, miR395p, miR858b, and miR2911a might are essential in enhancing flavor of the fruits, likely by target genes of the tannin biosynthetic pathway [19] (Figure 1).

3.2. Participation in Growth Hormone Signaling

Plant hormones regulate plant development, differentiation, and growth, whereas miRNAs work in conjunction with hormones by suppressing target genes in hormone metabolism pathways. Almost every biological action that occurs during a plant's life cycle

involves phytohormones, which are significant signaling molecules. To date, the primary identified plant hormones are auxin, ethylene, gibberellic acid, cytokinin, jasmonic acid, and salicylic acid.

3.2.1. miRNA-Gibberellin Signaling

For developmental activities such as seed germination, stem lengthening, and flower initiation, GAs (gibberellin) are needed. Exogenous gibberellin has been shown to affect a number of conserved and nonconserved grapevine miRNAs, and research has shown that these miRNAs may participate in mediating gibberellin-induced control of proliferation, formation, and berry development in response to various conditions [64,65]. Another study found six novel miRNAs that were expressed in berries after exogenous gibberellin and/or ethylene treatment, indicating that miRNAs are involved in the growth and maturation of grapevine fruit [66]. For developmental activities such as seed sprouting, stalk lengthening, and blossom beginning, GAs remain essential. They are created by the action of GA-OXIDASES (*GA20OX* and *GA3OX*), and the receptor that senses them encourages the breakdown of DELLA proteins, important GA response repressors in grapevine [67]. In addition to changing the number of transcripts that encode proteins, handling plants with GA also causes synthesis of more than 100 miRNAs in apple [68].

3.2.2. miRNA-Auxin Signaling

MiRNAs might play a role in plant hormone and stress signaling. By modulating *ARFs* (auxin-responsive factors), miRNAs and phasiRNAs participate in the pathway of auxin, an important plant hormone. These *ARFs* are regulated by miRNAs, resulting in the regulation of auxin ripostes in plant growth via miRNAs. *ARF* transcription factors may have potential target genes for four apple miRNAs (miR169a, miR160e, miR167bg, and miR168a, b). MiR160 regulates several biotic approaches in plants, with flower identity specification, leaf development, fruiting, etc. [69,70]. Short tandem target mimic (STTM160)-mediated downregulation of miR160 results in elongated, pear-shaped tomatoes, and overexpression of miR160-targeted *ARFs* outcomes in reduced lamina and increased leaf complexity in young foliage [71]. MiR167 regulates *ARF6/8*, which is vital for flower and fruit development. MiR167 is also tangled in plant protection and the reaction to fungal infections. The miR390-*TAS3*-*ARF2/3/4* pathway regulates leaf and flower development, particularly leaf morphogenesis. To start miRNA-mediated regulation of auxin responses in plant development, miR-NAs control a large number of ARFs. It has been demonstrated that miR167 adversely regulates *ARF6/8* [72], and miR160 post-transcriptionally controls *ARF10/16/17* [73–75]. In addition, as already indicated, miR390 causes TAS3 genes to produce tasiRNA (tasiARF) with the specific goal of targeting *ARF2/3* [24,38,76]. Auxin inhibits ripening via *ARFs* and *ERFs* and has antagonistic effects with ethylene on fruit ripening. However, miRNAs may be involved in this regulation process, for example, miR160, miR167, and miR390 specifically target certain melon *ARFs*. MiR393 targets *TIR1* and *AFB2*, while miR160 and miR167 target a number of *ARF* genes [77]. Auxin receptors are produced by the genes *AFB2* and *TIR1*. The miR393-*AFB2* regulatory module is thought to be present in melon fruit and to influence the maturing process [78]. According to previous studies, miR160, miR167, and miR390 regulate *ARF* genes to modulate auxin signal transduction in papaya. It showed that auxin signaling F-box 2 (*AFB2*) of the peach (*Prunus persica*) intimate were correspondingly targeted by miR160, miR164, miR172, and miR393 in auxin signaling pathways and ultimately in fruit growth [79–81] (Figure 2).

3.2.3. miRNA-Cytokinin Signaling

The essential function of cytokinins in cell division led to their discovery. Cytokinin production and, to a lesser extent, cytokinin signaling appear to be impacted by sRNAs. For instance, in potato (*Solanum tuberosum*), st-miR156 raises cytokinin levels by subtly increasing the expression of *LOG1*, which causes more significant cytokinin-induced branching. With ath-miR165 development, sRNAs likewise mediated in regulating the auxin/cytokinin

stability in many tissues and is an extra participant in the auxin/cytokinin equilibrium that controls root development [82]. A fascinating question for future research is whether other sRNAs have an impact on this stability in other tissues or organs. Understanding the answer to this question could help explain why sRNA mutants take different sensitivities to both hormone [83].

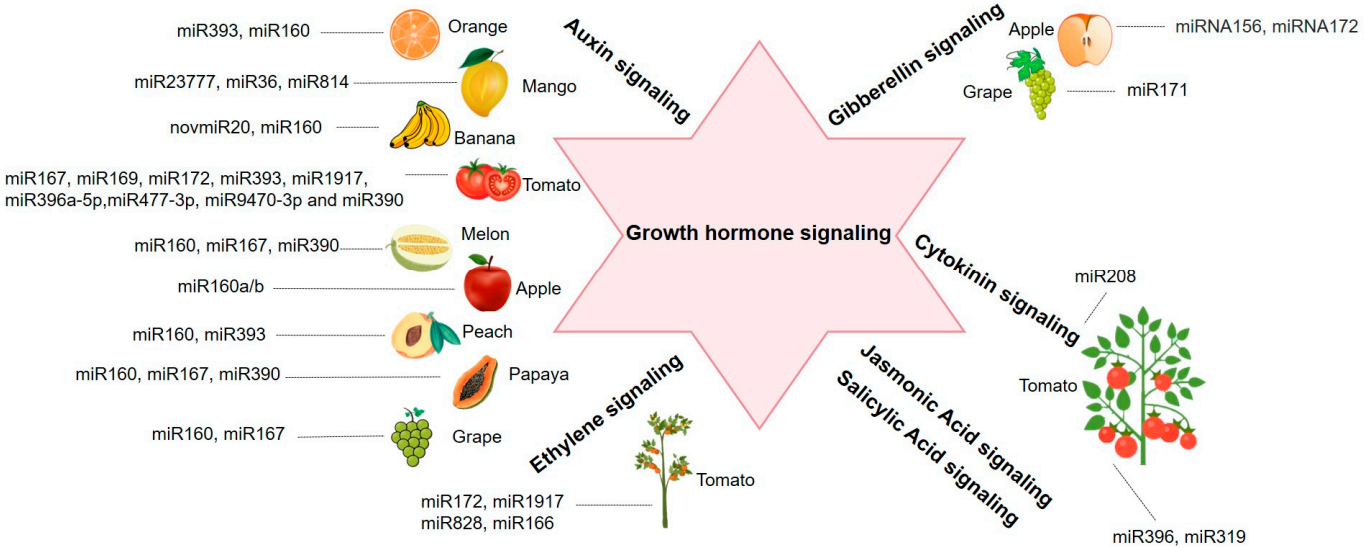

Figure 2. Role of conserved miRNAs in the well-designed regulation of growth hormone signaling in particular fruit tree.

3.2.4. miRNA-Ethylene Signaling

Ethylene-sensitive (EIN) proteins signaling pathway are activated or stabilized by ethylene, which is produced as a result of osmotic stress. Several cell wall hydrolysis processes, ethylene, the manufacture of fatty acids and esters, and several secondary plant products show significant roles in banana fruit maturity [84]. Numerous ethylene reaction factors (*ERFs*), which support the expression of stress-sensitive genes, are further induced by two downstream TFs, *EIN3* and *EIN3-LIKE1* (*EIL1*). Although the functions of ethylene in the reaction to abiotic stress are well understood, offering information on the variations in gene expression that ethylene controls, it is not known how or why ethylene also modifies the levels of sRNAs in various species. MiR396a-5p and miR477-3p (target ETHYLENE INSENSITIVE 3-like), miR172a (target *AP2* ethylene-responsive transcription factor-*TOE3*), and miR9470-3p (*ERF5* and *ERF021*) in particular revealed distinctive alterations in ethylene pathways [71]. The nonconserved tomato slymiR1917 controls *SlCTR4* splice variant (*SlCTR4sv*) degradation to control tomato ethylene responses [49,85]. The *DkERF8/16/18* genes in persimmon fruit might contribute to fruit maturing by increasing ethylene production and cell wall alteration [86].

3.2.5. miRNA-Salicylic and Jasmonic Acid Signaling

Under increased SA (salicylic acid) concentrations and *NPR* expression, tomato plants overexpressing sl-miR396 are additional vulnerable to *Phytophthora infestans* plus *B. cinerea* contagion [87]. Two sRNAs expression level influenced by JA, miR319 and ath-miR156, in turn supply the JA pathway with feedback control. *TCP4* is the first reported target of miR319 in tomato and *Arabidopsis* [88].

3.3. miRNA and Abiotic Stress in Fruit Trees

To date, many miRNAs have been predicted, and some have been confirmed experimentally to be involved in various abiotic stress responses, such as high temperature, drought, freezing damage and salt stress [89].

In peach under drought stress, high-throughput sequencing of sRNAs revealed that 104 miRNAs were upregulated and 158 miRNAs were downregulated in leaves, and 221 miRNAs were upregulated and 147 miRNAs were downregulated in roots [30]. A total of 152 conserved miRNAs from 36 miRNA families, 8 known but nonconserved miRNAs, and 64 candidate novel miRNAs from 54 miRNA families were screened from three sRNA libraries in Chinese white poplar (*Populus tomentosa*) under drought stress, waterlogging stress, and control conditions. Among them, 17 candidate miRNA families and 9 new miRNAs showed significant expression changes under drought stress, and 7 conserved miRNA families and 5 new miRNAs showed significant expression changes under waterlogging stress [90]. For example, the expression levels of miR156 and miR162 in switchgrass (*Panicum virgatum*) changed significantly under high drought stress [91]. In tomato, miR169c was upregulated under drought stress, and drought resistance was improved in tomato miR169c transgenic plants [92]. In poplar, the miR172d targets *PuGTL1*/*PuSDD1* that is a potential target for engineering improved drought tolerance [93]. In apple, the miR171i represses *SCL26.1* to enhance drought stress tolerance by regulating antioxidant gene expression and ascorbic acid metabolism [94], and the regulatory network of mdm-miR160-*MdARF17-MdHYL1* is thought to be critical for apple survival under drought stress [95].

Salt stress could induce high expression of miR398 in poplar, which is negatively correlated with the expression of the target gene *CDS1* [96]. The miR156/*SPL* upregulated *MdWRKY100* to regulate the salt tolerance of apple [97]. In tomato, miR156e-5p, sly-miRn23b and sly-miRn50a were verified to responded to salt stress in *S. pimpinellifolium* [98].

MiR397 and miR169 were upregulated in *Arabidopsis*, poplar, and *Brachypodium* after low-temperature stress, and miR172 was only upregulated in *Arabidopsis* and *Brachypodium* after low-temperature stress [99]. Sly-miR156e-3p targeted *SlMYB15* to regulate the ABA and ROS signals, which enhanced the survivals to cold stress [100]. In *Solanum lycopersicum*, sly-miR166 and sly-miR319 act as regulating *S. lycopersicum* responses to cold stress via repressing the expression of *HD-Zip III* and *GAMyb-like* [101].

Moreover, miR396a targeted growth-regulating factor 15 (*GRF15*), that play an important role in the heat-stress response, and knockout the target sites of miR396a on *GRF15* mRNA could enhance the heat tolerance and photosynthetic efficiency compared to wild-type plants [102]. In addition, some miRNAs participate in multiple stress responses at the same time, and miR172 and miR403 in sunflower may be involved in drought, high-temperature, salt, and chromium stress by regulating multiple pathways and cellular processes [103] (Figure 3).

3.4. miRNA and Biotic Stress in Fruit Trees

Infection by plant viruses and pathogens dramatically adversely impacts the growth and development of plants and the acquisition of economic traits. It often causes local organ necrosis and even early plant death, negatively impacting agricultural production. MiRNAs are involved not only in the plant stress response but also in the plant biotic stress defense response. Infection of plants with bacteria and viruses can induce the expression of some miRNAs, such as the flagellin of bacterial spot pathogen, which can induce the expression of miR393 in tomato. Plant nucleotide binding site (NBS)-leucine-rich repeat sequence (LRR) proteins have specific gene-gene relationships between host and pathogen, usually associated with immune response. NBS-LRR encodes an intracellular innate immune protein containing NBS and LRR domains. Some NBS-LRR proteins include an N-terminal domain, similar to Toll and interleukin-1 receptor (TIR–NBS–LRR) that mediate animal innate immunity. The Non-TNL structure NBS–LRR protein includes two types, the CC–NBS–LRR that contains a coiled-coil domain at the N-terminus, and XNBS–LRR that has an unknown structure (X) at the N-terminus (XNL). If the NBS–LRR gene is overexpressed in plants, it can induce plant resistance to pathogens. Recently, two papers reported the relationship between miRNAs and NBS–LRR genes, among which four miRNAs targeted NBS–LRR protein genes that were predicted to regulate the conserved P-loop domains of TIR-NB-LRR, CC-NB-LRR and NB-LRR in herbacter plants, respectively.

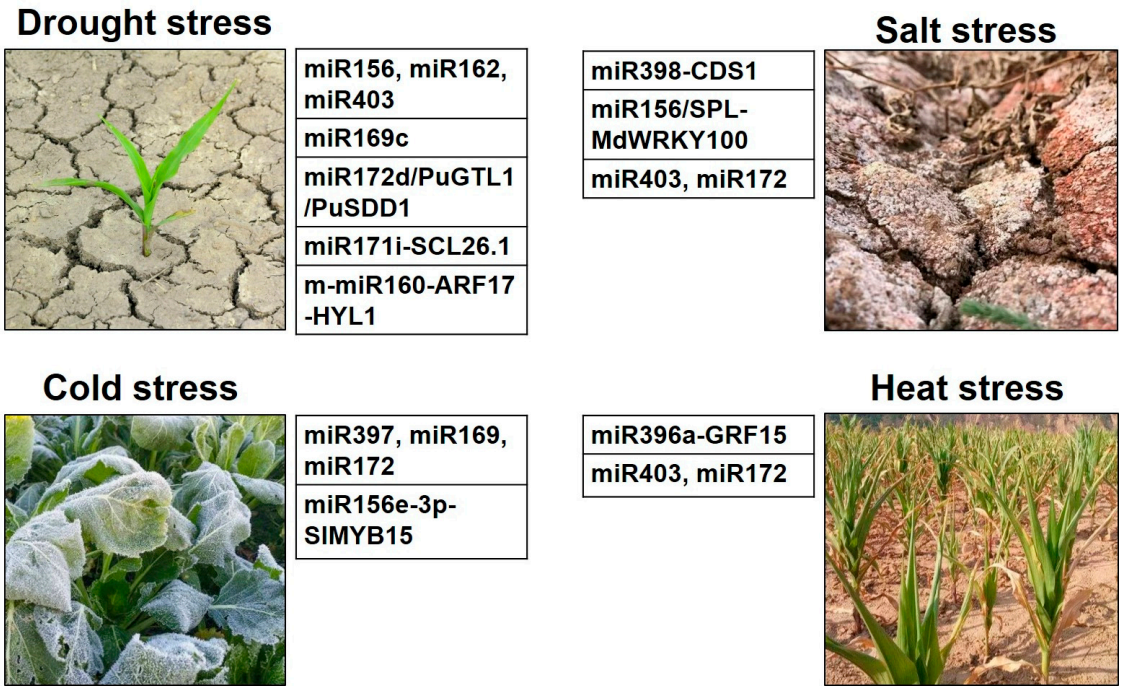

Figure 3. Summary of the miRNAs and their target families related to drought stress, salt stress, cold stress, and heat stress.

In 2009, a new miRNA was found in the poplar genome, and the target gene was an NBS-LRR resistance gene, which may regulate the expression of this kind of gene [104]. Moreover, deep sequencing of sRNA was performed in trifoliate orange flowers and leaves, and target gene prediction results included three resistance genes encoding NB-LRR proteins [28]. In tomato, two different miRNAs were identified targeting at least three NB domain protein genes, and they maintained plant immunity by affecting the stability or translation of mRNA [105]. In apple, md-miRRn11 targeting Md-NBS affects apple resistance to *Alternaria blotch* [106], and md-miR156ab and md-miR395 induced by *Alternaria alternata* f. sp. *mali* (ALT) inhibited the expressions of *MdWRKYN1* and *MdWRKY26*, thereby reducing the expression of part of PR (pathogenesis-related) gene, leading to susceptibility to ALT1 [107]. MiRcand137 inhibited the immune response of apples by affecting the antifungal defense mediated by gene *ERF14*, and the expression of miRcand137 in resistant and susceptible apples was negatively correlated with the sensitivity of *B. dothidea* [108]. In addition, studies have shown that miRNAs are associated with virus-mediated diseases and virus-induced gene silencing. Under the guidance of miRNAs, plants cut viral RNA, and viruses interfere with the gene expression of host cells by cutting a variety of regulatory target gene mRNAs in a manner similar to RNA interference. These results indicate that miRNAs contribute to the defense response of plants to biotic stress [109].

3.5. miRNA and Secondary Metabolism in Fruit Trees

Plant secondary metabolites are involved in resisting plant stress and can improve plant defense capabilities. Some secondary plant metabolites also have important health benefits for medicine and disease prevention. In addition to being a crucial regulator in plants, miRNA is also involved in the control of secondary metabolic pathways in two primary ways: first, miRNA acts directly on the genes involved in the secondary metabolic pathway, cleaving the target gene to negatively regulate the accumulation of secondary metabolites; second, miRNA controls transcriptional regulatory factors to control secondary metabolism. The effect of miRNA on potato secondary metabolism was discovered based on transcriptome and small RNA sequencing. The miRNA target genes

involved metabolic pathways such as flavonoids, carotenoids, and phenylpropane. Tea plant miR156 was found to be involved in the regulation of nitrogen formation during catechin synthesis [110]. Lemon miR396b regulates *PtrACO* gene expression and maintains the balance of ethylene and polyamine synthesis [111]. Differences in the abundance of some miRNAs exist between wild-type citrus and red-fleshed mutants, possibly related to carotenoid synthesis [112].

High-throughput sequencing and degradome sequencing revealed a new miRNA-*NEW41* target, *LcCHI*, in litchi, which may be implicated in the regulation of anthocyanin formation [113]. In apple, miR828 targets the *MYB* transcription factor to regulate anthocyanin synthesis, and the *MdMYB16*/*MdMYB1*-miR7125-*MdCCR* module co-regulates the homeostasis of anthocyanin and lignin biosynthesis under light induction [114]. In blueberry, miR156a–*SPL12* enhanced anthocyanin biosynthesis and chlorophyll degradation during fruit ripening and fruit coloration [115]. MiR828 and miR858 suppress *VvMYB114* transcription level in grapes to increase *ANR* and flavonoid formation [116]. Moreover, mdm-miR858 could regulate PA accumulation by negatively regulation of *MdMYB9/11/12*. Under light stress, *MdBBX22* inhibited the accumulation of PA by inducing the expression of mdm-miR858, and changed metabolism by enhancing anthocyanin synthesis and promotes rapid skin coloring [117]. In persimmon, DkmiR397 inhibits the expression of *DkLAC2* in PA biosynthesis [118], and miR858b inhibits the expression of *DkMYB19* and *DkMYB20*, negatively regulating PA biosynthesis [119] (Figure 4).

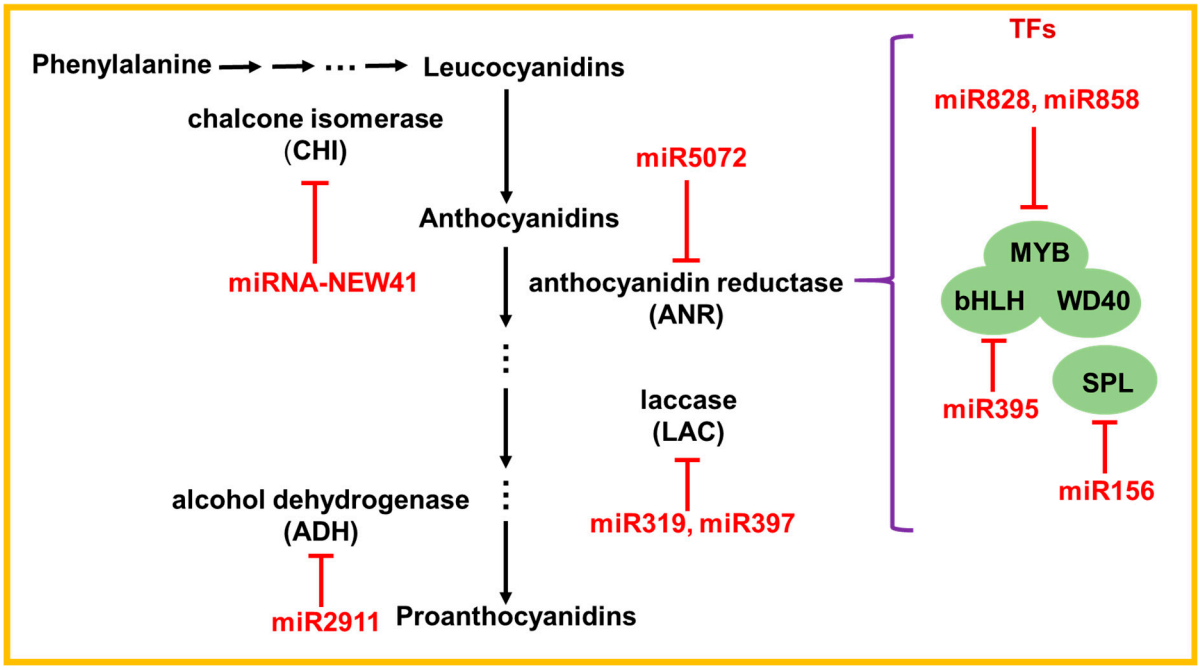

Figure 4. Schematic representation of the core flavonoid anthocyanin pathway leading to major branches of proanthocyanidin biosynthesis and their possible interaction with miRNAs.

4. Conclusions and Future Perspectives

Plant miRNAs play key roles in plant biology. They are also evolving as next-generation targets for fruit tree genetic engineering [120,121]. Significant improvement has been prepared in miRNA investigation in pomology tree, but this progress is limited to a few species and has not been made in many other fruits. Consequently, it is required to discover novel miRNAs and unravel their regulatory pathways. There are a variety of approaches to overcoming these barriers. One strategy is to accurately manage the look of particular miRNAs for aiming aspects that need to be improved by using tissue- or growth-specific promoters. Another strategy is to introduce a miRNA appreciation element into the genetic code of further key pathways, so generating artificial interaction. To meet

rising consumer demand, it is a huge task for the current fruit trade to create innovative fruit cultivars of first-rate value. In addition to sharing conserved miRNA purposes with model plants, fruits have also developed distinctive species-specific miRNA pathways that could contribute to developing particular fruit attributes. In practical, the major problem is the absence of quick, effective, and stable transformation methods, which makes functional analysis difficult in the majority of fruit-bearing species. Furthermore, it is anticipated that additional funding and effort will lead to new technologies in the upcoming, such as the foliar spraying miRNAs using nanotechnology. The biological activities and regulatory mechanisms of miRNA in fruit plants will be revealed further, laying a strong platform for explaining plant growth and development mechanisms and breeding high-yielding, high-quality, and stress-resistant plant products. In conclusion, over the past decade, we have had some insights into miRNAs in pomology crops; further research is needed, including detailed bioinformatics data mining and in-depth functional analysis to disclose their more complete picture.

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