OXFORD

Food Quality and Safety, 2020, 4, 1–8
doi:10.1093/fqsafe/fyz039
Review
Advance Access publication 27 March 2020

Review

Research advance in regulation of fruit quality characteristics by microRNAs

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Received 17 October 2019; Revised 26 November 2019; Editorial decision 25 November 2019.

Abstract

MicroRNAs (miRNAs) are short (19–24 nucleotides in length) noncoding RNAs that have a profound effect on gene expression. By completely or almost perfectly base-pairing with their individual target mRNAs they cause mRNA cleavage or repression of translation. As important regulators, miRNAs plays an important role in the regulation of fruit quality. Extensive studies have been reported in fruits, however current studies are mostly focused on the identification of miRNAs and the prediction and validation of target genes. This review summarizes research progress on the role of miRNAs in regulating fruit ripening and senescence and quality characteristics, such as coloration, flavor metabolism, and texture for providing information for future research.

Key words: miRNA; fruit; quality; regulation.

Introduction

MicroRNAs (miRNAs) are a class of short noncoding RNAs 19–24 nucleotides in length, involved in posttranscriptional gene regulation in many organisms (D'Ario *et al.*, 2017). The targets of plant miRNA are mainly transcription factors, enzymes, and signal proteins participating in physiological and biochemical processes, such as plant growth and development (Varkonyi-Gasicet *et al.*, 2010), plant hormone regulation and signal transduction (Xia *et al.*, 2012a), biotic stress and stress responses (Sun *et al.*, 2015), and their own negative feedback regulation (Millar *et al.*, 2005). Usually individual miRNAs can target transcription factors or structural genes including members of gene families (Song *et al.*, 2019). miRNAs also plays an important role in the regulation of fruit quality. A substantial number of miRNAs have been studied in the regulation of fruit quality to date. However, most of these reports have focused on the recognition and identification of miRNAs and the cloning and identification of target genes. There are few studies on the biological functions of miRNAs and their target genes relating to fruit quality. Here, we summarize the recent progress made in the area of miRNAs involved in regulating the formation and development of fruit quality characteristics and provides a reference for further studies on the role of miRNAs in regulating fruit quality.

Role of miRNAs in Fruit Ripening and Senescence

During postharvest ripening and senescence, fruit quality declines. This can be affected by environmental factors, such as temperature, the gas environment, the content and activity of plant hormones, and endogenous transcription factors. Abscisic acid (ABA) and ethylene (ETH) are important hormones and in conjunction with a decline in auxin, they have important effects during fruit ripening and senescence. The tomato ripening mutant *rin* (ripening inhibitor) affects the climacteric rise of ethylene and impairs the development of color, flavor, and texture change. Gao *et al.* (2014) screened differentially expressed miRNAs between wild type tomato and *rin* mutants and characterized MIR172a whose transcript was inhibited by *RIN*. Further chromatin immunoprecipitation and electrophoretic

mobility shift assays revealed that RIN directly inhibits MIR172a transcription and affects miR172 accumulation by binding to its promoter region, which demonstrated that *RIN* can regulate ripening-related genes through miRNA pathways at the posttranscriptional level, not just by directly targeting functional genes directly. In addition, *Le-CTR4*, the target gene of miR1917, was found to regulate tomato ripening and senescence by modulating the ethylene signal transduction pathway (Moxon *et al.*, 2008). Overexpression of Sly-miR1917 accelerated tomato fruit ripening, accompanied by a significantly up-regulation of ethylene signal transduction genes (Wang *et al.*, 2018). Meanwhile, the co-expression of Sly-miR1917 and *SlCTR4sv3* in tobacco showed that miR1917 causes cleavage of *SlCTR4sv3 in vivo*, indicating that Sly-miR1917 negatively regulates target gene *SlCTR4sv* to participate in plant ethylene signaling. Recently, Wang *et al.* (2019a) revealed the regulatory mechanism for postharvest ripening of kiwifruit that involved ethylene-miR164-NAC. At the same time, they suggested that the mir164-NAC pathway regulating fruit ripening and senescence may be widespread in different fruits.

ABA, in addition to ethylene, is also involved in fruit ripening and senescence. A novel miRNA, Fan-miR73, was identified in strawberry that targets an important transcription factor *ABI5* in the ABA signaling pathway (Li *et al.*, 2016a). Expression of Fan-miR73 was down-regulated in a dosage-dependent manner in response to exogenous ABA treatment, resulting in an accumulation of *ABI5* transcripts that was associated with the promotion of fruit ripening and senescence. In kiwifruit, miR172c-3p and miR162a-3p were identified and shown to respond to the ethylene-perception inhibitor 1-MCP and ABA, respectively. Both were found to regulate fruit ripening and senescence through a common target gene, *CTR1*. 1-MCP treatment down-regulated miR172c-3p, while expression of its target gene *CTR1* was up-regulated and the ethylene signaling pathway was shut down, thus delaying fruit maturation (Xu *et al.*, 2017). Meanwhile, ABA treatment of fruit up-regulated miR162a-3p expression, while expression of its target gene *CTR1* was down-regulated, thus activating the ethylene signaling pathway, eventually leading to accelerated ripening of the fruit (Xu *et al.*, 2017).

To preserve fruit quality after harvest, postharvest treatments are widely used. Low temperature storage is one of the main methods employed and effects of miRNAs are implicated in this process. According to Xu et al. (2013, 2015), PC-5p-176409_20 inhibited ABA signal transduction through the PYR1/PYL1-PP2C-SnRK2 network during low temperature storage of strawberry fruit, and miR167 reduced jasmonic acid (JA) by targeting auxin response factor 8 (*ARF8*), thereby participating in delaying fruit senescence. In addition, miR164, miR172, PC-5p-67794_53, and PC-5p-1004_3092 were involved in low temperature fruit quality preservation by up-regulating or down-regulating the expression of the transcription factors *NAC* and *APETALA2.7*, and the enzymes *alpha/beta hydrolase superfamily protein* and *glycosyl hydrolase 9B1*. Preharvest UV-C treatment delayed strawberry senescence by slowing the consumption rate of sugar and organic acids during strawberry storage and reducing the level of lipid peroxidation (Xu *et al.*, 2018a). These authors found the effect of UV-C treatment on the anti-oxidation system might operate by down-regulating the expression of miR398 and miR159, which target genes *FaCSD* and *FaPOX1/2*, respectively, promoting ROS removal, thus delaying the aging of strawberry fruits after harvest.

Controlled atmosphere is an important storage methods used in fruit postharvest. For example, 20% carbon dioxide (CO_2) can significantly postpone the ripening and senescence of sweet cherry fruit, and miRNAs sequencing identified differentially expressed miRNAs which might be involved in sweet cherry senescence (Wang *et al.*, 2019b).

Browning occurs during fruit storage and processing, which is a common postharvest problem that negatively affects the flavor, aroma, and nutritional value of the fruit. Xu *et al.* (2018b) sequenced anti-browning and susceptible-browning near-isogenic lines of *Luffa cylindrica* (sponge gourd), and identified eight known and eight novel miRNAs related to fruit browning. They also proposed a miRNA-mediated browning regulatory network model. Zhu *et al.* (2019) found miR528 targets genes encoding polyphenol oxidase (*PPO*). Expression of PPO genes was up-regulated by >100 fold in cold condition, leading to reactive oxygen species (ROS) surge and subsequent peel browning of banana fruit.

Role of miRNA in Fruit Coloration

Fruit coloration is related to the type and content of the pigments. The pigments in fruit include chlorophyll, carotenoids, anthocyanins, and flavonoids. Previous studies on miRNAs regulation of fruit color have been focused on anthocyanins, which are synthesized by the shikimic acid pathway. The amount and composition of anthocyanins is determined by enzyme-encoding structural genes and their regulatory genes (Zhang *et al.*, 2007), including enzymes involved in anthocyanin synthesis, such as phenylalanine ammonia lyase (*PAL*), chalcone synthase (*CHS*), dihydroflavonol reductase (*DFR*), anthocyanin synthase (*ANS*), and flavonoid 3-glucosyltransferase (*3GT*). The regulatory genes are highly conserved among different species and include the transcription factors *MYB*, *bHLH*, and *WD40* proteins, which synergistically regulate the above structural genes.

Tomato miR828 is a negative regulator of anthocyanin accumulation. The anthocyanin content of tomato fruit overexpressing miR828 was significantly lower than that in wild type tomatoes. miR828 significantly reduced the expression of key enzyme genes related to anthocyanin synthesis compared with those in wild type tomatoes by targeting *Sly-myb-like* (Liu, 2015). Tomato miR858 also negatively regulate the synthesis of anthocyanins by mediating cleavage of SLMYB7-like and SlMYB48-like transcripts in tomato (Jia *et al.*, 2015). Anthyocyanin-related miRNAs have also been identified in kiwifruit, where miR858 participates in the biosynthesis of anthocyanin (Li *et al.*, 2019). In apple, miR858 and miR828 may also be involved in the synthesis of anthocyanins (Xia *et al.*, 2012b; Qu *et al.*, 2016). In grape, miR828 and miR858 co-regulate a novel *MYB*, *VvMYB114*, which is a repressor of anthocyanins to promote flavonol accumulation (Tirumalai *et al.*, 2019).

The miRNAs mentioned above regulate anthocyanin by targeting MYB transcription factors, while miR156 targets SPL, who could interact with MYB, affecting the interaction of MYB and bHLH. The regulation of anthocyanin by a miR156-*SPL* module has been reported in pear and litchi. Qian *et al.* (2017) found that abundance of miR156a/ba/g/s/sa increased during anthocyanin accumulation and that transcripts of targets PpSPL2/5/7/9/10/13/16/17/18's were down-regulated. Further, yeast two-hybrid assays showed that PpSPL10 and PpSPL13 had protein interaction with PpMYB10, suggesting that the miR156-*SPL* module participates in the accumulation of anthocyanins by regulating the MBW (MYB-bHLH-WD40) transcription complex of pears. Litchi miR156a regulates anthocyanin in a similar way, *via* its target LcSPL1, which interacts with LcMYB1, the key regulatory gene of anthocyanin synthesis (Liu *et al.*, 2017).

There are also some reports of the regulation of fruit carotenoids by miRNAs. Xu *et al.* (2010) identified 51 known and 9 novel differentially expressed miRNAs by comparing a sweet orange red-flesh mutant and its wild type by Illumina sequencing. Among these miRNAs, the target genes of miR167 and miR1857 are EY752486, encoding geranylgeranyl pyrophosphate synthase (*GGPS*), and TC5 encoding lycopene β-cyclase (*LYCb*), a rate-limiting enzyme in the conversion of lycopene to downstream cyclic carotenes. It was suggested that the differential expression of miR167 and miR1857 may cause abnormal lycopene accumulation in the red-flesh mutan. Koul *et al.* (2016) studied the relationship between carotenoid content, biosynthetic genes, and miRNA expression in tomato and identified seven miRNAs that target important regulatory genes of the carotenoid pathway, including miR1911, miR482, miR172, miR396, miR395, osa-miR169i-5p.2, and ppt-miR1027a.

miRNAs Involved in Fruit Sugars and Organic Acids

Sugars and acids are major determinants of fruit quality. miR156 is also involved in regulating sugar metabolism and Zeng *et al.* (2015) found miR156 targets fructokinase (*FK*) and 1-deoxy-d-xylose-5-phosphate synthase (*DXS*) in *Lycium barbarum* L. (gogi berry). In the model plant *Arabidopsis thaliana*, miR164 targets β-fructofuranosidase (*β-FFase*), regulating sugar accumulation and researchers found that sugar is required for the phosphate starvation responses (Karthikeyan *et al.*, 2007). It was reported that there was a positive correlation between soluble solids and phosphorus content in ripened strawberry fruits (Cao *et al.*, 2015). Bari *et al.* (2006) showed that miR399d was involved in the phosphate-signaling pathway. According to Wang *et al.* (2017), strawberries overexpressing miR399a contain a higher level of soluble sugar, soluble solids, and vitamin C content and also the phosphorus content and photosynthetic rate of strawberry plants were improved, suggesting that the expression of miR399a has positive correlation with the content of sugars. However, there have also been some reports suggesting that the expression level of miR399a has a possible negative correlation with the content of sugars in strawberry (Li et al., 2013, 2016b). Tomato miR319 is a negative regulator of sugar and organic acid *via* regulating target LA (LANCEOLATE, a TCP transcription factor). Overexpression of miR319 in tomato resulted in lower fruit titratable acid and soluble sugar content compared to wild-type M82, while tomatoes overexpressing its target LA increased the content of titratable acid and soluble sugar in tomato fruit (Chang *et al.*, 2014).

Additional miRNAs involved in fruit sugar and acid metabolism have been identified. In pear, for example, miR1132, miR1318, miR2635, miR394a, miR396b, miR5077, miR5500, miR825*, and miR952b were identified by small RNA sequencing of different stages of pear fruit (Wu *et al.*, 2014). In peach, miR3627-5p and Pp_22312 respond to low-dose UVB radiation and then affect the sugar content of the fruit *via* regulation of downstream target genes involved in production of carbohydrates (sorbitol, fructose, glucose, etc.) and chlorophyll pathway genes (Li *et al.*, 2017). In Amur grapes, two novel miRNAs, miR081 and miR006, were identified as being involved in sugar anabolism and targeted genes for the enzymes UDP-glucose: glycoprotein glucosyltransferase, vacuolar invertase 1 (GIN1) (hexose), and rhamnose biosynthetic enzyme 1 (rhamnose), respectively (Wang *et al.*, 2012). In date palm, pda-nov-miR110 and pda-nov-miR215 was predicted to target sucrose-phosphate synthase (*SPS*) and pyrophosphate: fructose-6-phosphate 1-phosphate transferase (*PFP*), respectively, affecting starch/sucrose metabolism (Xin *et al.*, 2015).

Role of miRNAs in Fruit Aroma

The aroma of fruits is derived from volatile substances, including esters, alcohols, aldehydes, terpenes, and volatile phenols. The fatty acid pathway is the main source of aroma formation in many fruits. Lipoxygenase (LOX) in the fatty acid pathway catalyzes hydroperoxide production, using linoleic acid and linolenic acid as substrates, and then the corresponding alcohols are converted by hydroperoxide lyase (HPL) and alcohol dehydrogenase (ADH). Finally, esters are formed under the action of alcohol acyltransferase (AAT). Studies have shown that miRNAs affect aroma reduction during low temperature storage of "Nanguo" pears (Shi *et al.*, 2018). mdm-miR172a-h, mdm-miR159a/b/c, mdm-miR160a-e, mdm-miR395a-i, mdm/ppe-miR399a, mdm/ppe-miR535a/b, mdm-miR7120a/b, and mdm-miR156a-o negatively regulated the LOX pathway genes including *LOX2S*, *LOX1_5*, *HPL*, and *ADH1*. In addition, Liu *et al.* (2019) found that miR477 is a key miRNA involved in fatty acid synthesis genes during the development of *Camellia oleifera* fruit by small RNA sequencing.

Terpenoids also play an important role in the formation of aroma in some fruits. In the model plant *A. thaliana*, the miR159-targeted *SPL9* can directly bind to the sesquiterpene synthase gene *TPS21* promoter and activate its expression (Yu *et al.*, 2014). In tomato fruit, the target of sly-miR1534 was predicted to be a terpene synthase (Din *et al.*, 2014).

Role of miRNAs in Fruit Texture

Texture change is an irreversible feature of fruit maturity. Postharvest texture mainly includes fruit softening and lignification (Zhang *et al.*, 2012). Softening-related miRNA studies have been focused on the model fruit tomato. Both SlymiR157 and SlymiR156 have been shown to regulate the ripening and softening of tomatoes. SlymiR157 interfered with fruit ripening and softening by cleaving transcripts and inhibiting translation of of *LeSPL-CNR* and, although SlymiR156 did not affect the onset of maturation, it promoted fruit softening after the red ripe stage by induced *LeSPL-CNR* (Chen *et al.*, 2015). In addition, miRZ7, miR482, and miR396 are also involved in the softening process of tomato fruit by targeting β-galactosidase, pectate lyase, and β-glucanase encoding genes, respectively (Zuo *et al.*, 2012).

Postharvest lignification reduces the edible quality of the fruit, which happens in pear, loquat, and mangosteen fruits. Laccase (LAC) is a key biosynthetic enzyme involved in lignin biosynthesis. Transient overexpression of PbrmiR397a in pear fruit silenced three LAC genes, which reduced the lignin content and stone cell number in pear fruit. In addition, stable overexpression of PbrmiR397a in transgenic tobacco plants reduced the expression of the *LAC* gene and decreased the lignin content, indicating that miR397a regulates the lignification of pear fruit cells by inhibiting the *LAC* gene transcripts (Xue *et al.*, 2018). Juice sac granulation occurs during postharvest storage of citrus and is found in almost all citrus fruits. Studies have shown that lignin and phosphorus are closely related to the granulation of citrus juice, and csi-miR397/Cs6g06890.1 and N-miR828/Cs1g17590.1 may be involved in the juice sac granulation process by regulating lignification (Zhang *et al.*, 2016).

Table 1 List of miRNAs that regulate fruit quality.

Function	miRNA	Target gene	Fruit species	References
Ripening and senescence	miR172a	—	Tomato	Gao *et al.* (2014)
Ripening and senescence	miR1917	*Le-CTR4*	Tomato	Moxon *et al.* (2008); Wang *et al.* (2018)
Ripening and senescence	Fan-miR73	*ABI5*	Tomato	Li *et al.* (2016a)
Ripening and senescence	miR172c-3p; miR162a-3p	*CTR1*	Kiwi fruit	Xu (2017)
Ripening and senescence	miR164	*NAC*	Strawberry; kiwi fruit	Xu *et al.* (2015); Wang et al. (2019a)
Ripening and senescence	miR172	*AP27*	Strawberry	Xu *et al.* (2015)
Ripening and senescence	PC-5p-67794_53	*Alpha/beta-hydrolases superfamily protein*	Strawberry	Xu *et al.* (2015)
Ripening and senescence	PC-5p-1004_3092	*Glycosyl hydrolase 9B1*	Strawberry	Xu *et al.* (2015)
Ripening and senescence	miR167	*ARF8*	Strawberry	Xu *et al.* (2015)
Ripening and senescence	PC-5p-176409_20	*PP2C*	strawberry	Xu *et al.* (2015)
Browning	miR172a-3p	*TOE3* *SR34A* *RAP2-7*	*Luffa cylindrica*	Xu et al. (2018b)
Browning	miR858	*Myb4* *MYB308* *ODO1*	*Luffa cylindrica*	Xu et al. (2018b)
Browning	miR477a	*ACL5*	*Luffa cylindrica*	Xu et al. (2018b)
Browning	miR169b	—	*Luffa cylindrica*	Xu et al. (2018b)
Browning	miR169t	*NFYA*	*Luffa cylindrica*	Xu et al. (2018b)
Browning	miR171d	—	*Luffa cylindrica*	Xu et al. (2018b)
Browning	miR399h	*UBC24*	*Luffa cylindrica*	Xu et al. (2018b)
Browning	miR396b-3p	*PHYH* *RGLG*	*Luffa cylindrica*	Xu et al. (2018b)
Browning	Lc-miRn10-3p	—	*Luffa cylindrica*	Xu et al. (2018b)
Browning	Lc-miRn19-3p	*TCB3*	*Luffa cylindrica*	Xu et al. (2018b)
Browning	Lc-miRn24-5p	—	*Luffa cylindrica*	Xu et al. (2018b)
Browning	Lc-miRn41-5p	—	*Luffa cylindrica*	Xu et al. (2018b)
Browning	Lc-miRn52-5p	—	*Luffa cylindrica*	Xu et al. (2018b)
Browning	Lc-miRn54-3p	—	*Luffa cylindrica*	Xu et al. (2018b)
Browning	Lc-miRn60-3p	—	*Luffa cylindrica*	Xu et al. (2018b)
Browning	Lc-miRn66-3p	—	*Luffa cylindrica*	Xu et al. (2018b)
Browning	miR528	*PPO*	Banana	Zhu *et al.* (2019)
Anthocyanin biosynthesis	miR828	*Sly-myb-like*	Tomato	Liu (2015)
Anthocyanin biosynthesis	miR858	*S1MYB7-like*	Tomato	Jia *et al.* (2015)
Anthocyanin accumulation	miR858	*AaMYBC1*	Kiwi fruit	Li *et al.* (2019)
Anthocyanin biosynthesis	miR828; miR858	*MYB*	Apple	Xia *et al.* (2012b)
Anthocyanin biosynthesis	miR828; miR858	*VvMYB114*	Grape	Qu *et al.* (2016) Tirumalai *et al.* (2019)
Anthocyanin biosynthesis	miR156	*PpSPL*	Pear	Qian *et al.* (2017)
Anthocyanin biosynthesis	miR156a	*LcSPL1, LcSPL2*	Litchi	Liu *et al.* (2017)
Carotenoid biosynthesis	miR167	*EY752486*	Sweet orange	Xu *et al.* (2010)
Carotenoid biosynthesis	miR1857	*TC5*	Sweet orange	Xu *et al.* (2010)
Carotenoid biosynthesis	mir1911	*fpps*	Tomato	Koul *et al.* (2016)
Carotenoid biosynthesis	mir482	*zds*	Tomato	Koul *et al.* (2016)
Carotenoid biosynthesis	mir172	*crtISO*	Tomato	Koul *et al.* (2016)
Carotenoid biosynthesis	mir396	*lyc-b*	Tomato	Koul *et al.* (2016)
Carotenoid biosynthesis	mir395	*Zep*	Tomato	Koul *et al.* (2016)
Carotenoid biosynthesis	osa-miR169i-5p2	*psy1*	Tomato	Koul *et al.* (2016)
Carotenoid biosynthesis	ppt-miR1027a	*psy1*	Tomato	Koul *et al.* (2016)
Glycolysis and sucrose metabolism	miR156	*FK*	*Lycium barbarum* L.	Zeng *et al.* (2015)
Carotenoid biosynthesis	miR156	*DXS*	*Lycium barbarum* L.	Zeng *et al.* (2015)
Glycolysis and sucrose metabolism	miR164	*β-FFase*	*Lycium barbarum* L.	Zeng *et al.* (2015)
Sugar accumulation	miR399a	*E2 24-like*	Strawberry	Li *et al.* (2013); Li et al. (2016b)
Sugar accumulation	miR399a	*PHO2*	Strawberry	Wang *et al.* (2017)
Accumulation of sugars and organic acids	miR319	*LA*	Tomato	Chang *et al.* (2014)

Table 1 *Continued*

Function	miRNA	Target gene	Fruit species	References
Organic acids metabolism	miR1132	*Malate_PEPCK_PEPC*, *K-ATP*	Pear	Wu *et al.* (2014)
Organic acids metabolism	miR1318	*Citrate_ACL_ACLY*	Pear	Wu *et al.* (2014)
Organic acids metabolism	miR2635	*Malate_PEPC_PEPC*	Pear	Wu *et al.* (2014)
Organic acids metabolism	miR394a	*Malate_MalT*	Pear	Wu *et al.* (2014)
Organic acids metabolism	miR396b	*Malate_MalT*, *DNA_G6PDH_G6PDH*	Pear	Wu *et al.* (2014)
Sugar metabolism	miR5077	*Sugar_HT*	Pear	Wu *et al.* (2014)
Sugar metabolism	miR5500	*Sugar_HK_HK*	Pear	Wu *et al.* (2014)
Sugar metabolism	miR825*	*Sugar_ALD-ALD1*	Pear	Wu *et al.* (2014)
Sugar metabolism	miR952b	*Sugar_F16BP_F16BP1*	Pear	Wu *et al.* (2014)
Carbohydrate metabolism	miR3627-5p	—	Peach	Li *et al.* (2017)
Carbohydrate metabolism	Pp_22312	—	Peach	Li *et al.* (2017)
Sugar anabolism	miR081	UDP-glucose:glycoprotein glucosyltransferase	Amur grape	Wang *et al.* (2012)
Sugar anabolism	miR006	Rhamnose biosynthesis enzyme 1, *GIN1*	Amur grape	Wang *et al.* (2012)
Starch/sucrose metabolism	pda-nov-mir110	*SPS*	Date palm	Xin *et al.* (2015)
Starch/sucrose metabolism	pda-nov-miR215	*PFP*	Date palm	Xin *et al.* (2015)
Aroma	mdm-miR172a-h; mdm-miR160a-e; mdm-miR395a-i; mdm/ppe-miR535a/b	*LOX2S*	Pear	Shi *et al.* (2018)
Aroma	mdm/ppe-miR399; mdm-miR159a/b/c	*LOX1_5*	Pear	Shi *et al.* (2018)
Aroma	mdm-miR156a-o	*ADH1*	Pear	Shi *et al.* (2018)
Aroma	mdm-miR7120a/b	*HPL*	Pear	Shi *et al.* (2018)
Fatty acid synthesis	miR477	acetyl-CoA carboxylase carboxyl transferase subunit alpha	*Camellia oleifera*	Liu *et al.* (2019)
Terpene synthesis	sly-miR1534	*Terpene synthase*	Tomato	Din *et al.* (2014)
Bind the sesquiterpene synthase gene	miR159	*SPL9*	Arabidopsis	Yu *et al.* (2014)
Fruit ripening and softening	SlymiR157	*LeSPL-CNR*	Tomato	Chen *et al.* (2015)
Fruit ripening and softening	SlymiR156	*LeSPL-CNR*	Tomato	Chen *et al.* (2015)
Fruit ripening and softening	miRZ7	Pectate lyase	Tomato	Zuo *et al.* (2012)
Fruit ripening and softening	miR396	β-glucanase	Tomato	Zuo *et al.* (2012)
Lignification	PbrmiR397a	*LAC*	Pear	Xue *et al.* (2018)
Lignification	csi-miR397	*Cs6g068901*	Citrus	Zhang *et al.* (2016)
Lignification	N-miR828	*Cs1g175901*	Citrus	Zhang *et al.* (2016)

Summary

In summary, miRNAs have important regulatory effects on fruit quality (Table 1), but the current research focuses on the model fruit tomato and a few perennial fruits. At present, research has focused on the identification of miRNAs and the prediction and validation of target genes. The relationship between miRNA and their target genes has been established mainly by analyzing their temporal and spatial expression patterns. However, this approach is limited because miRNAs can inhibit target gene translation in addition to degrading target gene transcripts. Other studies have shown that miRNAs can also positively regulate target genes (Li *et al.*, 2006).

The most straightforward way to verify the function of miRNAs and target genes is by transgenic experiments. The function of the miRNA can be verified by comparing the phenotypes of the four sets of transgenic lines (Sun *et al.*, 2018). That is, 1. overexpression of miRNA; 2. silencing of miRNA by overexpression of an miRNA target mimic; 3. overexpression of key target genes; 4. RNAi of key target genes or CRISPR/cas9 knockout. However, transgenic systems for perennial woody fruit trees are at present not very successful. In previous studies, the transgenic system of model crops was used to overexpress fruits miRNAs. However, this approach is not particularly desirable because conserved miRNAs may perform different functions in different species and in different tissues. Nonconserved horticultural plant miRNAs may not even have target genes in model plants.

All in all, there is still large opportunity for exploration of the regulation of fruit quality by miRNAs worthy of in-depth systematic research. Further studies on miRNA in fruit will help to enrich our knowledge of the regulatory mechanisms of fruit quality regulation.

Funding

This work was supported by the National Key Research and Development Program (2016YFD0400100) and Fundamental Research Funds for the Central Universities (2019XZZX005-1-06 and 2019FZA6010).

Author Contributions

L.M. and Y.S. wrote the article. K.C. designed the scope of the review and edited the article. D.G. improved the article.

Conflict of interest statement. None declared.

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