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果树miRNAs研究进展

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摘 要:模式植物的研究表明,miRNA作为一种转录后调控因子,在植物的生长发育、逆境胁迫应答等生物学过程中发挥着重要的调节作用。截至目前,虽然已经从多种果树中鉴定了大量的miRNAs,但大多数miRNAs的靶基因和功能特性还不清楚。笔者总结了目前果树中miRNAs的研究进展,特别是miRNAs在葡萄(*Vitis vinifera*)、桃(*Prunus persica*)、梨(*Pyrus* spp.)、苹果(*Malus domestica*)和柑橘(*Citrus* spp.)等具有重要经济价值的果树等方面的作用,比如调控果树生长发育与果实品质、激素信号转导和环境胁迫应答。探讨了果树miRNAs的前景和研究方向。

关键词:果树;microRNA;调控;功能

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Research progress in miRNAs in fruit trees

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Abstract: The functional studies, mostly from model species, have revealed that miRNAs are major post-transcriptional regulators of gene expression in plants and are implicated in fundamental biological processes, such as plant development and abiotic/biotic stress responses. miRNAs have been detected in different parts of fruit trees, including leaves, inflorescence, roots and fruits, and there are significant differences in expression levels among different parts or in different developmental processes. With the rapid development of sequencing technology, a substantial number of miRNAs have been identified in a series of fruit crops sofar, while the target mRNAs and functions remain largely uncharacterized. The present review summarizes the progress in miRNA research in fruit crops, especially the role of miRNAs on the economically important species, such as grapes, peaches, pears, apples, oranges, and so on. Some studies show grapes have the ability of anti- abiotic/biotic stress attributed to the significant expression of miRNAs and the miRNAs also respond to exogenous hormones. The above-mentioned abiotic/biotic stress responses to miRNAs are also observed in apples, oranges, pears and peaches. In apples, miRNAs regulates the metabolic pathways of IAA, ABA, GA, and thus regulates the process of flower bud differentiation and juvenile period. Moreover, the activation of miRNAs influences the fruit size of apples as well. Boron and magnesium are implicated in citrus tree development and fruit quality, and some studies have found that miRNAs are widely involved in the response of citrus to boron and magnesium by regulating auxin synthesis, leaf morphogenesis, antioxidant system and boron transport. In citrus, miRNAs are also active in apomixis, somatic embryogenesis and cytoplasmic male sterile hybrids. Color is a part of fruit quality. The miRNAs are involved in carotenoid metabolism and then influence the citrus fruit color. Some research have showed orange juice sacs are related to the expression of

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miRNAs, while the quantity of juice sacs affects the taste of citrus. This function of fruit quality regulation is also found in pears. The target gene of miRNAs in pears encodes some enzymes, which is related to the formation of lignin and stone cells. The role of miRNAs in bud dormancy and dormancy release of pears was also detected. Except for the above-mentioned fruit trees, twith some additional fruit trees, the miRNAs and its targe genes have been researched, such as papayas, bananas, strawberries, litchi, *etc*. We also discuss the future miRNA research prospects in fruit trees: (Ⅰ) To use RLM-RACE, 3' PPMRACE, degradome sequencing and genetic transformation technology to enhance the ability of predicting and validating miRNAs target genes, (Ⅱ) to enhance the research on miRANs and its target genes in fruit trees by the creation of efficient transformation germplasm resources of fruit trees, transient transformation, virus vector-mediated transformation or natural variation materials of fruit tree varieties for some fruit trees with low efficiency of genetic transformation,, (Ⅲ) to construct molecular regulation of important biological processes mediated by target miRNAs-target genes by means of various genomics and other bioinformatics methods, to combine the results of functional genomics,and to systematically analyse the regulation mechanism of plant development or stress response, in which that target miRNAs and its Target genes are involved, (Ⅳ) to discover many conservative, non-conservative and fruit-specific miRNAs and (Ⅴ) to declare how miRNAs regulates some specific traits of fruit trees, such as flower bud formation, bud dormancy, juvenile transformation, fruit coloring and cell engineering breeding, etc.

Key words: Fruit trees; microRNA; Regulation; Functions

真核生物microRNA（miRNA）最早是Lee等[1]在研究秀丽新小杆线虫的突变体遗传性状时发现的，他们发现miRNA能够调控线虫细胞发育。植物中的miRNAs最早由Reinhart等[2]在拟南芥（*Arabidopsis thaliana*）中发现，截至目前已经发现了几百种植物miRNAs家族。大量研究也发现植物miRNAs具有非常多的生物学功能，可以参与调控生长发育、激素信号转导和环境胁迫应答等方面。近年来，随着对miRNAs功能的重要性和测序技术的飞速发展，人们也在多种果树中发现了大量miRNAs。因此，笔者主要综述了miRNAs在几种重要果树中的现状，并提出了前景和展望。

1 植物miRNAs的作用机制和鉴定方法

植物中的miRNA是一类长度为20~25核苷酸（nucleotide，nt）的单链非编码小分子RNA[3]。miRNA首先由RNA聚合酶Ⅱ转录形成初级转录物primary miRNA（pri-miRNA）[4-5]。pri-miRNA随后在DCL1（dicer like protein 1）蛋白的作用下，被剪切成可自身折叠成茎环结构的precursor miRNA（pre-miRNA）[6]。pre-miRNA然后被DCL1进一步剪切形成双链miRNA/miRNA*复合体。该复合体可在HEN1（HUAENHANCER1）蛋白的作用下在其3'端的2'-OH位置被甲基化以维持其稳定[7]。甲基化修饰后的miRNA/miRNA*双链复合体会在HASTY蛋白（HST）的辅助作用下被运输到细胞质中[8]。进入细胞质后，miRNA则与Argonaute（AGO）蛋白结合形成RNA介导的沉默复合体（RISC），RISC切割靶mRNA分子或是抑制靶mRNA翻译，从而使靶基因沉默[9]。miRNA*链可能会进入降解途径，但是在某些环境条件下，miRNA*也可能不被降解而发挥一定功能[10]。

最初在拟南芥和水稻（*Oryza sativa*）等模式植物中，主要是通过一些生物信息学工具预测和试验验证相结合的方法进行miRNAs的鉴定和验证。这些生物信息学验证工具一般都是基于miRNAs序列的特征和同源比对等进行预测，然后再通过克隆、杂交和定量PCR等试验方法进行验证[11]。

近些年来，使用新一代测序技术分析小RNA序列发掘miRNAs已日渐成熟，葡萄（*Vitis vinifera*）[12-14]、柑橘（*Citrus* spp.）[15-17]、苹果（*Malus domestica*）[18]、桃（*Prunus persica*）[19-20]和番木瓜（*Carica papaya*）[21-22]等果树均通过测序技术获得了很多miRNAs序列。一批植物miRNA数据库也相继建成，如miRBase version 20（mirbase.org）、plant miRNA data-

base（bioinformatics.cau.edu.cn/PMRD）等。

虽然测序技术的发展为植物miRNAs的发掘提供了便利，但通过测序技术获得的海量数据还需要一系列的生物信息学软件或服务器去进行miRNAs的分析、定性和靶基因预测。这样的分析软件或服务器有PatScan[23]、miRNAassist[24]、miRU[25]、psRNA-Target[26]、miARma- Seq[27]、naive Bayes classifier（NBC，https://github.com/smdouglass/mirBayes）[28]、miRFinder[29]、MirCheck[30]和MicroHARVESTER[31]等。利用高通量测序、降解组测序技术、生物信息学分析、PLM-RACE、PPM-RACE、qRT-PCR等方法进行靶基因鉴定研究的方法也逐渐成熟。

2 miRNAs在果树中的研究现状

2.1 葡萄

葡萄是果树中最先完成基因组测序的树种。早在2007年，通过‘黑比诺’葡萄构建的基因组草图为在葡萄中挖掘miRNAs序列奠定了良好的基础[32]。Wang等[14]通过高通量测序在山葡萄中鉴定出一批miRNAs，其中有72个是山葡萄特有的。Kullan等[33]在分析了70个来源于葡萄果实、花序、卷须、芽、子房和雄蕊等组织的小RNA文库后，鉴定出110个已知的和175个新发现的miRNAs，并研究了它们的表达模式。

大量研究表明，葡萄中的miRNAs广泛分布于不同部位，包括叶、花序、卷须、根和果实[12-13]，但不同部位间或不同发育进程中表达量差异显著，如miR397a、miR398a和miR408在根中的表达比叶和花序中的高100倍，而miR171、miR172、miR395、miR396、miR319和miR535则在果实发育的不同进程呈现不同的表达模式[12]。

miRNA也参与葡萄对逆境胁迫的响应。最近的研究发现，miR156、miR162、miR166和miR167A的表达与葡萄卷叶病相关[34]。Pagliarani等[35]的研究发现，不同抗性的2个葡萄基因型中的miRNAs数量和种类在干旱胁迫下呈现不同的表达模式。Paim等[36]也认为葡萄种类和生长条件对miRNAs的种类、表达量和表达模式有影响。

葡萄是一种对外源生长调节剂处理非常敏感的树种。外源乙烯处理后，从葡萄果实中鉴定出124条已知的和78条新发现的miRNAs，其中有162条miRNAs能够响应乙烯处理[37]。Wang等[38]发现在葡萄花期使用GA_3处理可以抑制Vv-miR061的表达，从而促进Vv- miR061的2个靶基因*VvREV*和*VvHOX32*的表达，最终影响到果实无籽化等进程。

2.2 苹果

苹果是一种重要的果树，关于苹果miRNAs的研究也早已开始。Xia等[18]通过深度小RNA测序发现了23个保守的、10个较保守的和42个苹果特异的miRNAs。研究还发现这些miRNAs的靶基因涉及多个代谢途径。Ye等[39]通过苹果基因组测序结果和miRNAs的保守性，从苹果中发现了154条miRNAs，并发现这些miRNAs属于26个家族，一些miRNAs是进化过程中基因组复制产生的。

miRNAs在苹果中的表达模式也很丰富。如保守型的miR156、miR159、miR160、miR167和miR172苹果的不同组织和发育阶段具有不同的表达模式[40-41]，非保守型的miR477、miR482、miR828、miR845和miR535也有同样的特点[18]。还发现miRNAs有组织富集表达的特点，如miR156在根中、miR165、miR166和miR167在叶片中大量表达[18]。

多个研究聚焦于miRNAs在苹果花芽形成和童期转化过程中的作用。平邑甜茶童期转化的研究中发现，在童期叶片中，mdm-miR156较高的表达和mdm-miR172较低的表达可能调控童期阶段转化，而且还发现mdm-miR160和miR393可以通过作用于生长素信号转导相关基因来调控这种转化进程。miR156还被发现可以作用于*MxSPL26*基因而与苹果不定根形成相关[42]。Guo等[43]分别对苹果叶芽和花芽进行了小RNA测序，结果发现了33个已经注释的和6个新发现的miRNAs在2种组织中的表达差异显著，这些差异基因的表达趋势大多与花芽形成的进程相反。苹果枝条拉枝处理后，分别有68和27个已知的miRNAs的表达下调和上调。其中一些miRNAs及其靶基因与生长素、脱落酸、赤霉素和开花等代谢通路相关[44]。对‘长富2号’苹果及其短枝型芽变品种‘烟富6号’枝条顶端组织小RNA的测序分析共发现了700个miRNAs，其中有135个miRNAs在2个品种中的表达存在差异，大部分在‘烟富6号’苹果中表现出下调。研究还发现miR164、miR166、miRNA171及其靶基因可以调控茎尖分生组织的生长，miR159、miR167、miR396及其靶基因可以调控细胞分化和节间长度[45]。

在生产中，嫁接可以有效地改善果树的性状，

An等[46]鉴定了自根‘富士’、自根M9和嫁接‘富士’/M9之间差异表达的miRNAs，在这3种材料中检测到206个已知的miRNAs和976个新的miRNAs，并确定了那些在嫁接反应中上调或下调的miRNAs。其中与开花相关的miR156和miR172在嫁接‘富士’/M9内的数量介于‘富士’和M9之间，这些表达模式表明这些miRNAs与嫁接有关。基因本体论（GO）分析表明，差异表达的miRNAs控制基因参与细胞生物合成和代谢等多种生物学过程。

miRNA也参与苹果抗病响应。Yu等[47]通过高通量测序分析了轮纹病侵染沙果（*Malus hupenhensis*）和‘富士’苹果之后miRNA的种类和表达模式（以正常条件为对照），结果共鉴定出了59个miRNA基因家族的108条miRNAs，并发现其中5条miRNAs可能响应轮纹病胁迫。Ma等[48]发现苹果抗病基因Md-NBS基因在抗性品种中表达较高，而一种miRNA（Md-miRLn11）则在感病品种中表达较高。研究还发现Md-miRLn11可以在11~12 nt位置裂解Md-NBS基因，研究认为Md-miRLn11可以通过作用于Md-NBS基因来响应真菌侵入。在另一项研究中，通过对比苹果火疫病抗病品种和易感品种中的差异表达miRNA发现了4个与抗火疫病相关的miRNAs[49]。在平邑甜茶的研究中还发现，轮纹病病菌和水杨酸能够激活miR168的表达，而miR168可以通过作用于一个反向作用因子*MhAGO1*来提高抗病性[50]。

研究还发现苹果miRNA172的一个转座子插入等位基因与苹果的大果型性状有关，而且在苹果中过表达miRNA172明显减小果实大小，表明miRNA172是控制苹果果实大小的关键因素[51]。

2.3　柑橘类

柑橘类果树的miRNAs鉴定工作，最初是利用公共数据库中的EST序列获得的[52-54]，之后随着测序技术的发展和柑橘基因组数据的发布，又发现了大量miRNAs序列。研究发现这些miRNAs在叶片、茎和根中均有表达[55-57]。另外，miR-RACE、RLM-RACE（RNA ligase-mediated rapid amplification of cDNA ends）和qRT-PCR等技术现在也广泛应用于柑橘类果树miRNAs序列的精准识别及其靶基因鉴定[58]。

硼和镁元素对柑橘类果树生长和果实品质影响很大，一些研究发现miRNAs广泛参与柑橘对硼和镁元素的响应。Lu等[59]从硼亏缺的根系中分离了52个上调的和82个下调的miRNAs，并发现miRNAs可能通过钝化和清除活性氧信号、增加侧根数目、增加细胞转运能力和提高渗透调节能力等几个方面参与柑橘对硼亏缺的响应。Lu等[60]分析对比了硼在亏缺和充足状态下血柑实生苗中miRNAs的差异，结果发现大量的差异表达miRNAs。这些miRNAs的靶基因涉及生长素合成、叶片形态建成、抗氧化系统、硼元素运输等多个方面。而当柑橘类果树处于硼超量供给时，植物会通过miR397a作用于2个参与植物次生细胞壁生物合成的漆酶基因（*LAC17*和*LAC4*）提高植物对硼胁迫的耐受性[61-62]。

柑橘miRNAs也广泛参与其对逆境胁迫的响应。在有些研究中发现一些柑橘miRNAs的靶基因可能是转录因子和抗病相关基因[15-16]，如NBS-LRR抗性基因家族[17]。还有研究发现csi-miR167、csi-miR396和csi-mir399可以通过离子运输和防御响应途径提高柑橘对黄龙病的影响[63-64]。此外，Xie等[65]也通过综合分析干旱和盐胁迫下香橙根系的mRNA和miRNA的数据，发现了一批响应干旱和盐胁迫的miRNAs。

在柑橘无融合生殖、体细胞胚发生和雄性不育胞质杂种等方面，也有关于miRNAs的研究。Wu等[66]分析比较了甜橙（Valencia）非胚性组织和胚性组织中的miRNAs，发现一些差异表达的miRNAs在胚性组织或者体细胞胚发生过程中的表达水平低于非胚性组织。Long等[67]研究了珠心胚发生过程中miRNAs的种类和表达，发现了约150个miRNAs，但只有2个miRNAs的表达发生了变化，表明这2个miRNAs与多胚和单胚的形成有关。在椪柑雄性可育品种及其雄性不育突变品种的差异miRNAs研究中，共发现了82个差异表达miRNAs，并通过降解组测序技术鉴定了138个靶基因[55]。在柚子雄性不育杂交种及其雄性可育品种的miRNAs研究中，共发现了206个miRNAs和86个靶基因，其中一些靶基因是与花发育相关的转录因子[68]。

miRNAs在调控果实品质方面也有重要作用。在甜橙及其红色变异种中发现了几个差异表达的miRNAs与类胡萝卜素代谢相关[16]，进而影响果实颜色。汁胞粒化现象是影响柑橘采后贮藏品质的关键问题之一，Zhang等[69]研究发现2个miRNA家族（miR397和miR828）与木质素生物合成有关，其表

达与柑橘汁胞粒化呈负相关。

一些研究也发现，在柑橘从童期转入成年的过程中，miR156家族的表达发生下调，而miR172家族表达上调，与其他果树中关于阶段转化的研究结果一致[17]。

2.4 梨

Niu等[70]通过同源比对的方法从梨基因组中发现了186条miRNAs，这些miRNAs属于37个不同的家族。进一步研究还发现了这些miRNAs可能的326条靶基因，这些靶基因涉及多个生化进程，如蔗糖代谢、抗坏血酸代谢和淀粉代谢等。随后，Wu等[71]通过测序和生物信息学分析了188个已知的梨miRNAs的靶基因（2 216个）和184个新发现的梨miRNAs的靶基因（1 127个），结果表明大部分的靶基因与果实生长调控相关。

miRNAs与果实品质的形成也有密切关系。为研究梨果实褐皮形成与miRNAs的相关性，‘砀山酥’梨和它的褐皮变种‘锈酥’梨果皮小RNAs被测序，在2个梨品种中共获得89条已知miRNAs，属于31个不同的家族。另外在2个品种中还新发现了443条miRNAs。研究还发现miR396、miR408、miR274、miR42和miR442与木质素合成有关，而且在2个品种果皮中的表达不同[72]。Cheng等[73]将木质素和石细胞含量差异显著的2个梨品种的花粉分别授在‘砀山酥梨’柱头上，然后对杂交得到的果实进行miRNAs测序，结果发现2个miRNAs（pyr-1809和pyr-novel-miR-144-3p）的表达量截然不同，它们相应的靶基因编码2种漆酶，与木质素和石细胞的形成有关。

还有几个研究关注了miRNAs在梨花芽休眠及其解除过程中的作用。Bai等[74]分析了处于生态休眠和休眠解除2种状态下芽中的miRNAs，结果共发现了137个保守的和较保守的miRNAs和50个梨特异miRNAs。但是，这些miRNAs在2种不同状态芽中的表达没有明显差异。Niu等[75]发现秋季短期低温可以促进CBFs（C-repeat binding factors）的积累，进而启动*DAMs*（Dormancy-associated MADS-box）基因的表达；DAM随后抑制*FT*（*FLOWERING LOCUS T*）基因的表达而诱导冬芽进入休眠，但miR6390可以降解*DAM*基因而解除休眠。

2.5 桃

桃miRNAs最初是通过计算机预测大量数据库中的EST序列获得的，在这个研究中发现了很多高度保守的、非保守的和桃特异的miRNAs，这些miRNAs在桃的多个组织中表达[76]。随后，通过小RNA高通量测序和降解组（degradome sequencing）分析等手段进行miRNAs及其靶基因预测与验证日渐成熟[77-78]，PLM-RACE、PPM-RACE和qRT-PCR等方法也用于miRNAs靶基因的定性研究[79]。现在，随着测序手段的不断发展，基于新一代高通量测序技术的研究也发现了很多桃miRNAs及其靶基因[80]。

桃miRNAs也参与桃树对非生物胁迫的响应。Barakat等[19]通过小RNA测序发现桃芽中一些保守的miRNAs（miR156、miR164、miR172、miR393和miR396）和一些非保守的miRNAs（miR414和miR2275）能够响应冷胁迫。Eldem等[20]发现了几个与干旱胁迫相关的miRNAs，如表达下调的miR160、miR165/166、miR167和表达上调的miR395。Pekmezci等[81]的研究也发现了13个转座子相关的miRNAs具有干旱胁迫响应的特性。

2.6 其他果树

除了以上几种重要果树，miRNAs在其他果树中也有研究。Aryal等[21]在番木瓜叶片和花中鉴定了60条miRNAs，其中的一些miRNAs在叶片中特异表达并能够响应番木瓜环斑病毒的侵染。另一个番木瓜叶片和花器官小RNA深度测序的研究也发现了很多的miRNAs，预测其中一些miRNAs的靶基因功能涉及乙烯响应途径，表明这些miRNAs与果实生长和成熟相关[22]。Aryal等[82]还发现了14条在番木瓜不同性别类型植株中差异表达的miRNAs。大多数这些差异表达基因在雄花中表达较高，并与生长素信号途径相关，而在雌花中表达较高的miRNAs则可能调控顶端分生组织的生长。

Bi等[83]分析了乙烯或1-MCP处理后对香蕉（*Musa acuminata*）果实中miRNAs的影响，共发现了151条miRNAs，其中有82条miRNAs在乙烯或1-MCP处理后呈现不同的表达模式，这些差异表达miRNAs的靶基因共有815个，大多数编码与果实成熟相关的蛋白。Dan等[84]用乙烯处理和不加乙烯处理的香蕉果实构建了2个miRNA文库，并对其进行了序列测定。共获得了128个已知miRNA，并鉴定出12个新的miRNA。其中22个在乙烯处理后差异表达，其中6个已知的miRNAs及其靶基因经qRT-PCR鉴定，发现这些靶基因主要编码蛋白包括GA-

TA、ARF、DLC和AGO等。Sampangi-Ramaiah等[85]的研究中发现香蕉Mb-miR531和Mb-miR529的靶基因分别是*KCS11*和*KCS10*/*FDH*，而它们都是叶片蜡质形成的关键基因，表明以上2个miRNAs可以通过调控叶片蜡质的形成提高香蕉耐涝性。

Xia等[86]在草莓（*Fragaria vesca*）果实中发现了38个新miRNAs和31个已知的miRNAs，其中一个22 nt的miRNA能够从6个F-box基因中诱发阶段性小干扰RNA，这些小干扰RNA可以剪切其他的F-box基因，从而调控很多的生理进程。Šurbanovski等[87]在草莓中发现一个多组织和高丰度表达的miRNA（fve-miR1511）的靶基因是LTR反转录转座子，fve-miR1511通过精确的和甲硫氨酰基启动因子tRNA的保守引物绑定位点配对来沉默LTR反转录转座子，从而参与对多个生理进程的调控。在草莓果实品质方面，miRNAs也发挥了重要的作用，Wang等[88]发现miR399a在草莓中的过表达可改善与果实品质相关的因子，这也为培育和改良更营养优质的草莓提供思路。

Yao等[89]首次在荔枝（*Litchi chinensis*）果实中鉴定出296条miRNAs，其中11条是荔枝特有的。在这些miRNAs中，有14条与荔枝果实衰老有关，它们的靶基因涉及能量代谢、花色素代谢、激素信号和抗病防御等生理进程。Liu等[90]发现miR156a和一个新发现的miRNA（NEW41）与荔枝果实着色有关。研究还发现miR156a和NEW41q是通过反向调控靶基因LcSPL1/2和LcCHI来实现对果实着色的调控。

此外，还从桑椹（*Morus notabilis*）[91]、杏（*Prunus armeniaca*）[92]、猕猴桃（*Actinidia chinensis*）[93]、石榴（*Punica granatum*）[94]和蓝莓（*Vaccinium ashei*）[95]等果树中发现了很多miRNAs及其靶基因。

3　展　望

植物miRNAs作为一类重要的小RNA，具有调控植物生长发育、抗逆响应和新陈代谢等重要功能。因此，miRNAs作为一个崭新的研究领域，已经成为当今分子生物学研究的前沿与热点。与模式植物相比，果树miRNAs方面的研究虽然起步较晚，但随着近年来测序技术的不断发展，目前也取得了长足地进步，现在已经从柑橘、苹果、葡萄、桃和梨等具有重要经济价值的果树中获得了数百种miRNAs及其靶基因，并通过不同的试验手段验证了一部分miRNAs及其靶基因的功能和调控机制。笔者也对2010年以来的一些经试验验证的果树miRNA及其靶基因（表1）及本文中所提到的果树miRNA的生物学功能（表2）进行了一定的归纳总结。这些将为更加深入地研究果树重要性状的调控和抗逆响应等奠定很好的基础。

果树学的科学家们一直在努力发掘不同果树中的miRNAs，并试图阐述这些miRNAs的功能，但截止目前这些研究成果依然很少且不够完善。miRNAs在果树学领域的研究和应用还有很长的路要走。第一，随着测序技术日新月异地发展，通过小RNA测序获得大量miRNAs已并非难事，但由于miRNAs的长度有限，因此对这些miRNAs靶基因的精准预测和验证研究却相对滞后，以后应利用RLM-RACE、3’PPM RACE[Poly（A）polymerase-mediated 3’rapid amplification of cDNA ends]、降解组测序和遗传转化（瞬时转化和稳定转化）等技术加强这方面的研究；第二，植物miRNAs研究发展至今，在模式植物中已经形成了几种相对固定的方法来研究miRNAs的功能，其中就包括经典遗传学方法（主要是先得到功能缺失/获得的miRNAs突变体或miRNAs转基因植株，然后通过表型的变化验证目的miRNAs的功能）[96]和人工制备miRNAs结构体或靶基因模拟体并转化植物的方法[97-99]，但这些方法都建立在有稳定和高效的遗传转化体系的基础上。虽然一些果树也有稳定的遗传转化体系，但转化效率一般很低，而且有些果树还有较长的童期，因此要使用以上方法还很困难。在以后的研究中，可以通过创制果树高效转化受体种质资源、瞬时转化、病毒载体介导转化或果树品种自然变异材料等途径加强果树miRNAs及其靶基因相关研究；第三，现在的研究表明，大多数miRNA可能会作用于不同的靶基因，这些靶基因互相之间也存在着复杂的调控网络，现在果树中对于这些调控网络的研究尚不够深入。目前，各种现代先进的测序技术发展日新月异，以后可以借助各种组学和其他生物信息学研究手段，构建目的miRNA-靶基因介导的重要生物学过程的分子调控网络，并结合功能基因组学研究结果，系统深入地解析目的miRNAs及其靶基因参与的植物发育或逆境响应调控机制。第四，目前已有多种果树完成基因组测序工作，而其他植物中的研究表明miR-

表 1 2010 年以来一些经试验验证的果树 miRNA 及其靶基因

Table 1 List of a lot of experimentally validated miRNA targets of miRNA families in fruit trees since 2010

miRNA	靶基因 Target gene	参考文献 Reference
miR156	Squamosa-promoter binding-like proteins (SPLs) Acyl-CoA synthetase 5 O-fucosyltransferase family protein Phospholipid/glycerol acyltransferase family protein Branched-chain alpha-keto acid decarboxylase E1 beta subunit Plasma membrane intrinsic protein 1;4 Aluminium induced protein with YGL and LRDR motifs	[13, 33, 42, 45, 68, 70, 100]
miR159	MYB transcription factor SAUR family protein Esterase/lipase/thioesterase family protein	[13, 17, 18, 42, 45, 69, 100]
miR160	Auxin response factors Co-chaperone GrpE family protein	[20, 42, 45, 68, 75, 100, 101]
miR162	Dicer-like protein Sec14p-like phosphatidylinositol transfer protein Unknown expressed protein	[17, 18, 42, 68, 70, 100]
miR164	NAC domain proteins	[13, 17, 18, 42, 45, 68, 70, 100]
miR165	Homeobox-leucine zipper protein	[101]
miR166	HD-ZIPII transcription factor	[13, 17, 18, 68, 100, 101]
miR167	Auxin response factors (ARFs)	[13, 17, 18, 42, 45, 68, 100]
miR168	Argaunote protein	[13, 18, 100]
miR169	Nuclear factor Y (NFY) HAP2 transcription factor	[13, 17, 18, 42, 68, 100]
miR171	GRAS-family transcription factor (scarecrow-like)	[13, 17, 18, 42, 45, 68]
miR172	AP2-domain containing transcription factor Ethylene-responsive transcription factor (RAP)	[13, 17, 18, 33, 42, 45, 68, 70, 101]
miR319	TCP family transcription factor NADPH-dependent FMN and FAD containing, MYB-transcription factor GDP-D mannose 3’, 5’-epimerase	[13, 17, 18, 42, 45, 68, 70]
miR390	Ankyrin repeat protein family Leucine-rich repeat protein kinase family, nucleic acid binding, OB-fold like protein MdTAS3-1, MdTAS3-2, PpeTAS3	[13, 17, 18, 33, 100]
miR393	Auxin-signalling F-box protein Methyltransferase superfamily protein	[13, 17, 18, 33, 42, 45, 100]
miR394	F-box transcription factor	[18, 65, 70]
miR395	NAC domain proteins Sulphate transmembrane transporter Bifunctonal 3’-phosphoadenosine 5-phosphosulphate synthase	[17, 18, 33, 100]
miR396	Heat shock protein, granulin repeat cysteine protease family protein, nucleoside triphosphate Hydrolase growth regulating factor (GRF) TIR-NB LRR resistance protein, replication factor C subunit 1	[17, 18, 33, 100]
miR397	Ice1 transcription factor GDP-D mannose 3’, 5’-epimerase, acyl-coA N-acyltransferase superfamily Laccase	[13, 17, 100]
miR398	Copper/zinc superoxide dismutase Rubredoxin-like superfamily protein	[17, 18]
miR399	Isocitrate dehydrogenase (NAD) Putative ubiquitin-conjugating enzyme (PHO2)	[18, 62, 87]
miR408	RNA binding family protein Copper ion binding protein, cyclin D3	[17,100]
miR827	Major latex protein	[13]
miR472	Glutamate receptor 2.7 Disease resistance protein	[17, 68]
miR473	Phosphatidylinositolglycan-related protein	[13]
miR477	Tetratricopeptide repeat (TPR)-containing protein Thiamine pyrophosphate Ubiquitin-like superfamily protein Lateral root primordium protein-related	[17]

表1（续） Table 1（continued）

miRNA	靶基因 Target gene	参考文献 Reference
miR479	Transitional endoplasmic reticulum	[13]
miR482	Cytochrome p450 HOPZ-activated resistance 1 Alpha/beta-hydrolase superfamily protein	[13, 17, 45, 68, 101]
miR530	Lactoylglutathione lyase family protein	[17]
miR535	Squamosa-promoter binding protein, DNA primase large subunit, splicing factor Cysteine protease	[13]
miR828	MYB transcription factor Pentatricopeptide repeat-containing	[13, 18, 45, 100]
miR858	MYB transcription factor r2r3-like protein Oligopeptide transporter OPT family MATE efflux family protein, anthocyanin regulatory C1 protein 3-ketoacyl-CoA thiolase	[13, 18, 42, 45, 100]
miR1515	Transducin family protein	[17]
miR3627	Amino acid transporter	[18]
miR3951	Plant protein of unknown function	[17]
miR3954	Late embryogenesis abundant (LEA) protein family Transmembrane proteins 14C	[17]

表 2 本文所提到的果树 miRNA 的生物学功能

Table 2 Biological function of fruit tree miRNAs mentioned in this paper

生物学功能 Biological function	miRNA	参考文献 Reference
调控植物生长和发育过程 Regulating plant growth and development	miR156, miR159, miR160, miR164, miR166, miR167, miR171, miR172, miR396, miR477, miR482, miR535 miR828, miR845	[12, 17, 18, 40, 41, 42, 45, 46]
参与生物胁迫 Participation in biotic stress	miR156, miR162, miR166, miR167, miR168, miR396, miR399	[34, 50, 63,64]
参与非生物胁迫 Participation in abiotic stress	miR156, miR160, miR162, miR164, miR165/166, miR166, miR167, miR172, miR393, miR395, miR396, miR414, miR397a, miR529, miR531	[19, 20, 61, 62, 85]
调节果实品质 Regulating fruit quality	miR061, miR42, miR156, miR274, miR396, miR399a, miR408, miR442	[38, 72, 88, 90]

NAs大约占总基因的1%，这将意味着尚有许多保守的、非保守的和果树特异的miRNAs有待发现。相信随着更多果树全基因组组装的完成以及生物信息学和测序等新技术的发展，果树miRNAs的研究会更为全面和深入。第五，未来果树miRNAs的研究，除了跟踪和关注模式植物的研究方向和研究成果外，还应多关注miRNAs是如何调控一些果树特有的或有特色的性状，如花芽形成、童期转化、芽休眠、果实着色和细胞工程育种等。

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