



木本植物中microRNA的研究进展

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摘要: MicroRNAs (miRNAs)是一类非编码小RNA, 长度为20~24 nt, 通过降解靶基因的mRNA和抑制翻译过程在转录后水平调控植物的生理过程。miRNAs已被证明在植物的生长发育中起重要调控作用, 但在木本植物中的研究较少。本文将对非编码RNA的分类、miRNAs合成机制以及其对木本植物花、果实、种子、根生长发育和胁迫响应的调控功能进行总结, 旨在为研究miRNAs调控木本植物生长发育提供一些参考。

关键词: miRNAs; 功能; 植物生长; 木本植物

Research progress of microRNA in woody plants

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Abstract: MicroRNAs (miRNAs) are non-coding small RNAs, 20–24 nt in length, and regulate the physiological processes of plants by degrading target gene mRNA and inhibiting translation at post-transcriptional level. miRNAs have been shown to play an important regulatory role in plant growth and development, but there are few studies in woody plants. This paper summarized the classification of non-coding RNAs, its synthesis mechanism and its regulation function on flower, fruit, seed and root growth and stress response, aiming to provide some reference for the study of miRNAs regulation on woody plant growth and development.

Key words: miRNAs; function; plant growth; woody plants

miRNAs是一类长度为20~24 nt的小RNA (Kurihara和Watanabe 2004)。miRNAs基因可以在生物体中独立的转录, 通过蛋白酶剪切为成熟的miRNAs来调控基因的表达(Yang等2006)。miRNAs最早发现于秀丽隐杆线虫(*Caenorhabditis elegans*), 后来的研究证明在生物体中普遍存在(李瑞雪等2020)。miRNAs主要在转录后水平调控生命过程, 通过降解或抑制靶基因蛋白质翻译过程实现(Arribas-Hernández等2016; Singh 2017; Yu等2020)。在植物生长

发育的每个阶段都有miRNAs的参与。miRNAs可以通过调控植物生长发育相关转录因子来调节植物胚胎发生、种子萌发、营养生长、生殖生长、开花和结果、衰老和死亡的过程。木本植物优良的抗性基因, 具有极高的生态价值和药用价值。在木本植物中, 已经发现了大量且快速增加的miR-

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NAs, 但其生物学功能在很大程度上是未知的。目前为止, miRNAs的功能研究主要集中在拟南芥和杨树中(Fang和Wang 2021)。本文将综述近年来miRNAs对木本植物不定根、果实、种子和花生长发育的调控和胁迫响应。

1 非编码RNA分类

非编码RNA (non-coding RNAs, ncRNAs)在基因表达中发挥着重要的调控作用。按照序列长度划分, ncRNAs主要分为长链非编码RNA (long-non-coding RNAs, lncRNAs)和小RNA (small RNA) (Yu等2019)。根据Axtell (2013)的分类系统, 小RNA主要分别为两大类。一类是前体是由两个互补RNA链形成的双链结构; 另一类是前体由自身分子互补形成的单链“发夹”结构。由双链RNA (double-stranded RNAs, dsRNAs)前体衍生而来的小RNA被称为小干扰RNA (small-interferingRNAs, siRNAs); 来自单链发夹结构的小RNA称为发夹RNA (hairpinRNAs,

hpRNAs)。如图1所示, siRNAs和hpRNAs又可以分为几个次级分类。其中siRNAs次级分类中的异染色质siRNAs (heterochromatic siRNAs)主要来源于基因间或基因组重复区域; 通常长度为23~24 nt, 与抑制性染色质标记的从头沉积有关; 次级siRNA (secondary siRNAs)可响应上游小RNA的活性; 除此之外, siRNAs还有天然反义转录本siRNA (nat-siRNA), 其前体是由2个独立转录的RNA杂交形成的。最近发表的sRNAanno数据库包含了一种新的小RNA——阶段性siRNA (phasiRNA) (Chen等2021a)。phasiRNA可以使RNA调控网络精细级联(Jyothsna和Alagu 2022)。hpRNAs可以分为miRNAs (microRNAs)和其他hpRNAs, miRNAs可以是谱系特异性的, 也可以是长链的。谱系特异性miRNAs (lineage-specific miRNAs)是仅在一个物种或少数近缘物种中发现的miRNAs; 而长链miRNAs (long miRNAs)其功能类似于异染色质siRNAs, 可沉积抑制性染色质标记。下文内容主要围绕miRNAs展开。

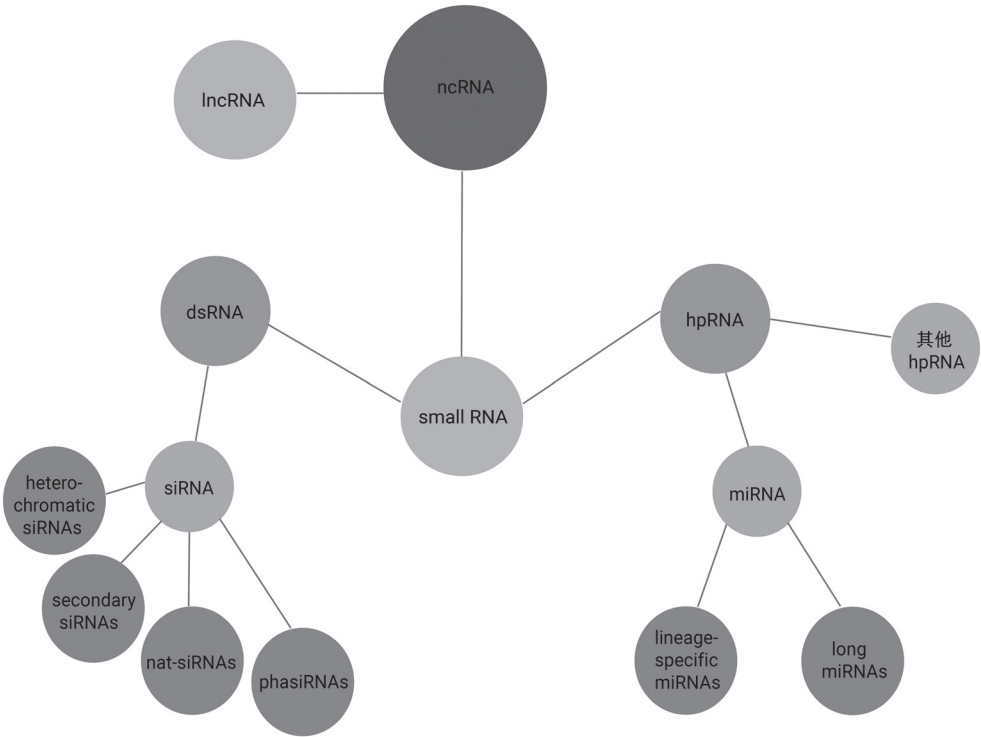


图1 非编码RNA分类
Fig. 1 Classification of non-coding RNA

2 miRNAs概述

2.1 miRNAs位置分布

miRNAs的位置大体分为三类。

第一类*miRNA*基因有独立的启动子,可以在生命活动中独立转录。这类*miRNA*与编码蛋白质的基因序列距离较远。彼此的复制转录几乎互不干扰;第二类*miRNA*基因存在于蛋白质编码基因的内含子序列中,在蛋白质基因转录时,*miRNA*基因与其同时同方向转录,之后在特殊酶的切割下分离。并且研究者们发现,寄存于同一类的蛋白质基因中的*miRNA*基因也往往是同类型的,这说明两者是共同进化并且保守的;第三类*miRNA*基因家族成簇的分布于染色体上,转录时共用一个启动子(Lau等2001)。

2.2 miRNAs生物合成机制

内源性*miRNA*基因在RNA聚合酶II和RNA聚合酶III的作用下转录生成初级转录本pri-miRNA, pri-miRNA折叠形成一个发夹结构,并且有一个5'端的帽子和一个3'多聚腺苷酸尾巴(Achkar等2016)。一种双链RNA结合蛋白(dicer-like 1, DCL1)切割pri-miRNA,并且在RNA结合蛋白HYL1 (hyponastie Leavas 1, HYL1)和C2H2型锌指结构域的蛋白SE (serrate)的协助下将其加工为70~90 nt的前体miRNA (pre-miRNA) (Kurihara等2006)。此外, DCL1进一步切割pre-miRNA为miRNA/miRNA*双链结构。通过甲基转移酶HEN1在3'端核糖上添加一个甲基以保护miRNA。输出蛋白HASTY将miRNA双链输出到细胞质中,其中一条单链miRNA*被降解,另一条单链与AGO (argonaute)等蛋白一起被纳入RNA诱导沉默复合物(RNA induced silencing complex, RTSC)中行使其调控功能(Brodersen等2008; Bollman等2003; Li等2013a)。

3 miRNAs与木本植物的生长发育

miRNAs是基因表达的常见调控因子,参与了大量的细胞过程。近年来的研究表明,在生物发育过程中, miRNAs形成了一个调控基因表达的时空网络。miRNAs主要通过和靶基因mRNA的互补配对来降解或抑制蛋白质翻译过程,在转录后水平

调控植物生长发育。靶基因的mRNA与miRNA序列在完全互补时就会被AGO蛋白切割从而被降解;若两者的碱基序列互补配对的适配度不高,则mRNA的翻译过程就会被抑制(Arribas-Hernández等2016; Singh 2017)。大多数miRNAs的靶基因编码植物生长发育相关的转录因子和其他调控的调节蛋白。木本植物生长发育过程中关于逆境响应、根系发育、开花结果等过程的调控网络均有miRNAs的出现。

3.1 miRNAs调控木质素的合成与次生生

木质素作为陆地生物圈中第二丰富的生物聚合物,是维管植物次生细胞壁的主要结构成分之一,主要由紫丁香基木质素(S-木质素)、愈创木基木质素(G-木质素)、对-羟基苯基木质素(H-木质素)构成(Freudenberg 1965; Boerjan等2003)。木质素在维持植物细胞壁的完整性、茎的机械强度、长距离的水运输和植物对害虫和病原体的防御方面发挥着关键作用(Bonawitz和Chapple 2010)。大量研究表明,在次生细胞壁形成过程中,许多转录因子在调节木质素的生物合成中发挥重要作用(Xie等2018)。例如, R2R3-MYB转录因子、bHLH (basic helix-loop-helix)转录因子和WDR (wd repeat)蛋白已被证明可直接靶向并激活木质素合成途径中的结构酶基因启动子的次生壁MYB响应元件(Zhong等2019)。在拟南芥(*Arabidopsis thaliana*)的研究中发现, MYB46和MYB83可编码两个R2R3-MYB蛋白,促进木质素沉积(Ko等2012)。另外,它们在桉树(*Eucalyptus robusta*)和杨树(*Populus*)中的同源蛋白EgMYB2、PtrMYB002、PtrMYB003、PtrMYB020和PtrMYB021也被证明可以调节细胞壁的木质化(Goicoechea等2005; Zhong等2013)。

研究表明, miR858a直接调控多个MYB基因,导致拟南芥木质素异位沉积(Sharma等2016)。在杨树中过表达miR397a和miR397b,会下调漆酶基因(laccases, LAC)并导致木质素的沉积(Lu等2013; Wang等2014)。在白梨(*Pyrus bretschneideri*)中, miR397a可调控石细胞,使其细胞壁木质化(Xue等2019)。上述内容表明, miRNAs是木质素生物合成的重要调控因子。然而,在木本植物中, miRNAs协调转录因子定向调控木质素合成的分子通路尚未建立。

阿魏酸-5-羟基化酶(ferulate 5-hydroxylase, F5H)是一种单加氧酶,参与S-木质素的生物合成(Meyer等1998)。迄今为止,在维管植物,特别是木本植物中,关于次生壁形成过程中F5H的调控信息较少。Fan等(2020a)通过高通量测序发现F5H2是miR6443的直接靶点,被miR6443定向切割。miR6443优先表达于维管组织,在韧皮部和叶柄中大量积累。在毛白杨(*Populus tomentosa*)中过表达miR6443, F5H2的表达量和S-木质素的含量明显降低,但植物的形态表型和细胞壁解剖没有明显变化。表明miR6443以一种组织特异性的方式下调F5H2来对杨树的S-木质素含量进行调控。然而,miR6443在木质部和韧皮部中调控S-木质素生物合成的机制是否保守,仍需进一步研究。

miR828被发现于拟南芥中,负调控拟南芥花青素的合成途径(Luo等2012)。此外,miR828还以葡萄MYB114为靶点,控制毛状体和棉纤维的形成(Guan等2014; Chen等2021b)。之后miR828又被发现可通过调控MYB来促进金叶甘薯(*Ipomoea batatas*)木质素生物合成(Lin等2012)。在最新的研究中,Wang等(2022)通过毛白杨的遗传转化、基因克隆、荧光定量PCR、组织化学染色等方法探究发现miR828-MYBs模块在协调杨树茎木质素生物合成方面具有重要作用。研究发现,过表达miR828会下调木质素生物合成基因的转录水平,比如: *PAL1*、*C4H2*、*HCT1*、*C3'H*、*CCR2*、*CAD1*、*CCoAOMT1*、*COMT2*、*F5H4*。MYB171的转录本主要集中在茎的木质部,过表达MYB171会导致杨树木质素沉积增加,细胞壁厚度上调,茎中木质部发育改善等。miR828可直接靶向MYB171,导致其裂解,从而在茎发育过程中对细胞壁木质化产生负面影响。为了验证miR828-MYBs模块的作用机制,研究者还发现,MYB171可特异性激活*PAL1*和*CCR2*的转录来调控茎的木质化。

TCP (TEOSINTE BRANCHED1/CYCLOIDEA/PCF)转录因子在拟南芥家族中受到了广泛的研究,并发现其许多的生物过程,比如次生细胞壁的生物合成(Fan等2020b)。在基因筛选中发现,miR319a的靶基因中包括TCP。为了探究miR319a和TCP20的关系,Hou等(2020)构建了miR319a的转基因毛白

杨植株。研究显示,在毛白杨中,miR319a也靶向切割TCP20。并且于野生型相比,miR319a过表达植株的节间高度明显缩短,维管形成层的层数也减少了32.4%。抑制miR319a的样本中,次生木质部宽度和木质部纤维细胞壁厚度显著增加,过表达植株中降低,木质部纤维细胞直径减小,TCP20的表达量也随miR319a动态变化。

HD-ZIP III转录因子在木质部发育过程中起着重要的作用。miR166可通过RNAi和表观遗传沉默下调HD-ZIP III转录本,以此来控制马占相思木(*Acacia mangium*)次生壁的形成(Ong和Wickneswari 2012)。

3.2 种子发育

miRNAs在种子发育、休眠和萌发过程中起着重要作用。在火炬松(*Pinus taeda*)中,miR166被证明在合子胚胎和雌性配子体中表达,miR167则仅仅在合子胚胎中检测到,这是miRNAs在种子组织中的首次报道(Oh等2008)。之后,研究者证明miR166a、miR397a和miR398a在椰子(*Cocos nucifera*)未成熟的胚乳中高量表达(Li等2009)。但是,miR166的过表达却抑制体细胞胚胎发生过程中子叶的形成,导致顶端胚胎的异常发育(Li等2016)。miR156b、miR414、miR2673b和miR7826可以通过调控牡丹(*Paeonia suffruticosa*)中脂肪酸生物合成和脂质代谢所需基因的表达来控制种子发育(Yin等2018)。此外,许多miRNAs在龙眼(*Dimocarpus longan*)体细胞发育的不同阶段优先表达。例如,miR156在早期胚胎培养中表达;miR160、miR390在心形胚和鱼雷形胚阶段高表达;miR167、miR397、miR808则主要分布在子叶中;在成熟胚胎阶段,检测到的miRNAs主要为miR808和miR5077 (Lin和Lai 2013)。miR159通过负调控其靶点MYB33来维持体细胞胚胎的成熟(Li等2013b)。另外,MYB33还可以促进miR156的表达来影响营养阶段的转变(Guo等2021)。miR397通过调节细胞壁的厚度,参与了将休眠胚胎转化为萌发胚胎的过程。在这一过程中,脱落酸通过调节miRNAs生物发生基因的表达水平来改变miRNAs的表达(Zhang等2013)。

3.3 果实发育

多种miRNAs,比如miR156、miR160、miR167、

miR172、miR390、miR393、miR397、miR828和miR858对果实发育非常重要(Damodharan等2016; Chen等2018)。其中, 研究发现miR172在协调果实的生长中起着主要作用, 因为当miR172的活性受损时, 果实的生长就会受到阻碍。在拟南芥的研究中发现, miR172在果实形态发生中起着关键作用, 通过抑制靶向转录因子AP2 (APETELA2), 促进受精后的果实生长(Ripoll等2015)。内源激素乙烯可以促进果实成熟, 在番茄(*Solanum lycopersicum*) miR172过表达植株中, 乙烯的含量是野生型植株的3~4倍, 并在果实破碎7 d后保持不变, 因此, miR172也可以在激素水平调控果实发育(Chung等2020)。

在荔枝(*Litchi chinensis*)中, miR156靶向基因SPL1/2编码转录因子, 与花青素生成相关基因MYB1相互作用, 另外, 一个新的miRNA——NEW41以荔枝查尔酮异构酶(chalcone isomerase, CHI)基因为靶点, 与miR156一起通过调节花青素的合成控制果实着色(Liu等2017a)。有的研究也报道, miR159、miR828、miR858和miR5072可以调控苹果皮中花青素的积累(Xia等2012; Qu等2016)。在油茶(*Camellia oleifera*)中, miR156、miR390和miR395参与了调节果实生长发育过程中碳水化合物的积累(Liu等2019)。miR164以 β -果糖苷酶基因为靶点, 控制果实的质量和营养价值。对梨不同发育阶段miRNAs的表达进行分析发现, 许多miRNAs通过调节木质素合成、糖代谢、酸代谢和激素信号转导相关基因的表达, 在果实发育中发挥重要作用(Wu等2014)。LAC基因可控制木质素的合成, miR397以LAC基因为靶点, 控制中国梨果实细胞的木质化。此外, 生长素响应因子基因ARFs是miR160和miR167的潜在靶点, 可在miRNAs的调控下抑制果实脱落(Zhang等2019)。

3.4 开花及花器官发育

花的状态转变是植物发育过程中最重要的阶段, 这能确保植物生殖生长的正常进行。花的生长起始于叶片和茎尖分生组织(shoot apical meristem, SAM)中, 叶片接收到环境线索的诱导后产生FT (FLOWERING LOCUS T)蛋白, FT蛋白又被称为植物的成花素, 它的产生起始了开花时间的调控网络, 最终导致SAM分化为花分生组织(floral mer-

istem, FM) (Barton 2010)。miRNAs在花器官的形成中和开花时间的调控中发挥了不可替代的作用。拟南芥和其他植物的遗传分子分析已经发现了多种相互依赖的基因途径控制花转变的时间。一些关键组件被认为在开花的调控网络中非常重要。其中一类关键组成部分是花分生组织的身份基因, 如LFY, 可以促进SAM原基分化为FM (Schmid等2003; Wigge 2006; Amasino 2010)。

在SAM中, miR156、miR172和miR159的靶点参与了花分生组织识别基因的激活。SPLs基因家族早期就被证明参与植物开花过程(Klein等1996)。miR156靶向调控SPLs基因家族的部分成员, 致使SPLs蛋白含量降低, 不利于花分生组织基因的激活。麻栎(*Quercus acutissima*)和加拿大杨树(*Populus × canadensis*)的miR156在幼苗阶段表达量较高, 并且在幼成年过渡过程中表达量下降。miR156-SPLs模块似乎定义了一个年龄依赖的诱导开花的途径, 独立于已知的花调节因子, 如温度、春化, 而有条不紊地进行(Wang等2011)。

AP2转录因子基因可介导光周期诱导开花, 所有AP2类似基因的敲除会导致开花极早, 与过表达miR172引起的表型类似(Yant等2010), 研究也表明, miR172通过抑制AP2的表达来促进开花。有趣的是, miR172的表达模式与miR156的表达模式相关, 研究显示miR156可通过下调SPL9来促进miR172的表达(Wang等2009; Wu等2009)。

拟南芥MYB33是一种赤霉素(gibberellins, GA)特异性的转录调控因子, 通过激活GA信号通路中的LFY来调控花的起始, 在此过程中, MYB33的表达受到miR159的强烈抑制(Blazquez和Weigel 2000; Gocal等2001)。miR159是正常花药发育所必需的, 它的过表达会导致短日光周期的开花延迟, 这与MYB33和LFY水平的降低相关(Achard等2004)。

最近的研究也为上述通路提供了证据。miR-172-AP2通路被证明刺激木兰花(*Magnolia × soulangeana*)从营养生长转变为生殖生长的阶段(Sun等2021)。miR172a的过表达导致麻疯树(*Jatropha curcas*)的早期开花早、花形成异常和叶片发育异常(Tang等2018)。同样, miR156在海棠(*Malus hupehensis*)叶片中的表达水平较高, 而miR172的表达

水平较低(Xing等2014)。在中国山核桃(*Carya cathayensis*)中, miR156、miR172在花发育过程中表达上调(Wang等2012)。同样, miR156及其靶基因差异表达, 进一步证明miRNAs在雄雌花发育中的关键作用(Sun等2017)。miR172在火炬松成熟或萌发的花粉中表达, 表明它在花粉萌发和花粉管生长发育中的潜在功能(Quinn等2015)。

除上述miRNAs之外, 在烟草(*Nicotiana tabacum*)中表达毛果杨(*Populus trichocarpa*)的miR396c前体, 改变了雄蕊和心皮的特性, 表明miR396对花器官的发育至关重要(Bauchet等2013)。miR164参与花瓣和雌蕊器官的发生(Mallory等2004)。

3.5 不定根的发育

不定根(adventitious root, ARs)是在胚胎后由非根植物组织发育而来, 包括下胚轴、叶片、芽和生殖器官。遗传学和分子研究表明, 不定根需要转录因子(transcription factors, TFs)的动态调控(Bellini等2014)。对于植物ARs的分子调控在拟南芥中已得到了较广泛的研究, 然而在木本植物中ARs形态发生的分子理解仍然有限(Legue等2014)。对杨树miR167及其靶基因*ARF6*和*ARF8*的功能验证中发现, 过表达miR167a抑制其靶点的转录本, 促进了侧根(lateral roots, LRs)发育, 但过表达ARF8会增加ARs的数量, 并抑制LRs发育(Cai等2019)。线粒体功能的缺失会导致根系形态缺陷(Ohbayashi等2019)。毛白杨中, miR476a在ARs形成过程中表现出可诱导表达, 并直接靶向编码线粒体定位五肽重复(pentatricopeptide repeat protein, PPR)蛋白的发育恢复类(*RFL*)基因。miR476a-*RFL*表达的基因修饰表明, miR476a-*RFL*介导的线粒体稳态动态调控影响杨树ARs的形成(Xu等2021)。在刺槐(*Robinia pseudoacacia*)不定根生根阶段, miRNAs的表达差异显著(Uddin等2022)。

4 胁迫响应

4.1 非生物胁迫

4.1.1 盐胁迫

盐分胁迫是一个日益严重的全球农业问题。它会影响植物根系的生长, 降低作物的质量。在盐处理后的沙漠胡杨中, miR156的表达水平优先显著

升高, miR166、miR167、miR169、miR172、miR-827、miR2119和miR5020的表达也随之增强。相反的是, miR393、miR645、miR860和miR1444的表达下降(Si等2014)。在毛白杨中, 盐胁迫下调了19个保守的miRNAs, 上调了2个保守的miRNAs; 并且有5个非保守miRNAs下调, 2个非保守miRNAs上调(Ren等2013)。盐胁迫还会使miR390的表达增加, 这促进了反式作用小干扰RNA的增加和ARF4转录本的降解。ARF4表达的降低会促进释放生长素信号, 促进侧根生长和提高耐盐性(He等2018)。盐处理降低了miR474c和miR398b在青杨(*Populus cathayana*)的表达, 但增加了它们在旱柳(*Salix matsudana*)中的表达, 这表明miRNAs在不同植物生态型对盐胁迫的差异响应中发挥关键作用, 通过不同的调节机制增强植物对盐碱环境的适应(Zhou等2012)。对湖北海棠(*Malus hupehensis*)砧木进行研究发现硫化氢可以缓解碱盐胁迫, 并且特异性诱导miR408a的下调、miR477a和miR827的上调。此外, 通过调控海棠miR159c和miR169及其靶基因的表达, 还可以改善根系结构来响应盐分胁迫(Li等2021)。

4.1.2 干旱胁迫

干旱对农林产业十分不利, miRNAs在树木耐旱中的作用也开始被研究。miR159、miR164、miR-166、miR167、miR168、miR169、miR171和miR-396被证明通过下调靶蛋白参与干旱胁迫反应(Eveno等2008; Ding等2013)。葡萄(*Vitis vinifera*)中, miR166、miR319、miR396、miR3631、miR3633和miR3639在严重的水分胁迫下被下调(Pantaleo等2016)。此外, 在蒙古沙冬青(*Ammopiptanthus mongolicus*)中, miR2118和miR858上调了控制黄酮醇产生的*OZFI*和各种*MYB*基因的表达。miR156的表达也被上调来响应干旱; miR164被短暂的干旱胁迫上调, 但miR164是否影响根发育尚不清楚(Liu等2017b)。在巴西橡胶树(*Hevea brasiliensis*)中, miR168和miR160被证明与植物耐旱性相关(Kuru-villa等2019)。在桑树(*Morus alba*)中, miR156、miR172和miR396及其靶基因可能参与了对干旱胁迫的响应(Li等2017)。毛白杨中的miR394、miR160f、miR472、miR1448及其靶基因在干旱和洪涝胁迫下出现相反的表达模式(Ren等2012)。之后的一项

研究表明, miR172d可通过抑制其靶基因*GTL1*来降低杨树的气孔密度、净光合速率、蒸腾作用来应对干旱(Liu等2021)。

4.1.3 高温胁迫

热胁迫影响树木的生长发育, miRNAs响应热应激调节植物生长也已在各种植物中得到鉴定, 但是木本植物中的研究较少。毛果杨中, miR156、miR159、miR164、miR171和miR408受到热应激的调控(Lu等2005)。毛白杨中, 有52个miRNAs对热应激有反应, 其中大部分是被下调的(Chen等2012a)。分别下调亮叶桦(*Betula luminifera*)中miR162和miR403的靶基因*DCL1*和*AGO2*会影响miRNAs的生物合成, 从而改变miRNAs的丰度及其对热应激的反应(Pan等2017)。此外, 在木薯(*Manihot esculenta*)中, miR156a、miR159、miR160、miR397和miR408被热应激下调, 从而影响miRNAs下游的生命活动(Ballen-Taborda等2013)。

4.1.4 低温胁迫

与其他非生物胁迫一样, 冷胁迫对树木生长发育的负面影响也非常严重, 会限制树木的空间分布。甜杨(*Populus suaveolens*) miR475b被报道响应冷冻胁迫(Niu等2016)。miR319a-c和miR395b-k在毛白杨中受到冷胁迫而下调, 通过调控其靶基因*MYB*和*APS*来增强对冷胁迫的抗性(Chen等2012b)。在李属植物扁桃(*Prunus dulcis*)中, 冷胁迫增加了miR159、miR1649、miR162c、miR171a/c/g/f、miR394a/b、miR482f和miR7122a-5p的表达, 但降低了miR156a/b、miR162、miR166a-e、miR396b、miR398a-3p、miR6262和miR8123-5p的表达(Karimi等2016)。miR396b通过负调控1-氨基环丙烷-1-羧酸氧化酶(*ACO*)基因的表达来增强枳(*Poncirus trifoliata*)的耐寒性, 从而影响乙烯和多胺的合成(Zhang等2016)。进一步的研究发现, 小黑杨(*Populus simonii*×*P. nigra*) miR319、miR159、miR167、miR395、miR390和miR172在冷处理下存在差异表达, 并负调控其靶基因, 包括*ARF*、*MYB*、*SBP*、*LHW*和*bZIP*。这些靶基因在抗寒性中发挥了重要作用(Zhou等2019)。

4.2 生物胁迫

除去外界非生物环境外, 疾病也会影响miRNAs

对树木生长发育的调控。miR156、miR159、miR160、miR164、miR166、miR168、miR172、miR319、miR398、miR408、miR1448和miR1450在杨树感染茎溃疡病原菌后表达上调(Zhao等2012)。此外, 在来檬(*Citrus aurantifolia*)中, miR157可能通过改变树木形态和叶片结构来响应病原体的侵袭(Ehya等2013)。在葡萄中, 植物病原体感染诱导了各种保守和新的miRNA的差异表达, 这些miRNAs可能靶向涉及形态、激素信号传导、营养稳态和胁迫的基因(Snyman等2017)。桑树被植物原体感染后, 韧皮部汁液中miR482a-5p的表达增加。嫁接实验表明, 桑树miR482a-5p可以从接穗上运输到砧木上。因此, 韧皮部汁液中的miR482a-5p可能调控其在根中的靶基因(Gai等2018)。

5 总结和展望

本文主要总结了近几年miRNAs在木本植物调控中的作用, 如图2所示。随着下一代测序技术的发展, 已经在木本植物中发现了许多保守的miRNAs和物种特异性的miRNAs, 其部分靶基因序列也得到了预测, 这为今后的研究奠定了坚实的基础(Du等2011)。目前, 在木本植物中鉴定出的miRNAs的一些功能可以通过模式植物进行验证。然而, 由于多年生木本植物的世代周期较长, 这些植物的遗传系统的建立受到了限制, 很难调查木本植物中树木发育的具体方面, 如木材的形成、季节变化和多年生植物的生长特征等。病毒诱导基因沉默(VIGS)技术可用于对缺乏稳定遗传系统的木本植物中miRNAs的功能验证, CRISPR/Cas9可用于产生大量的定点突变体, 丰富实验资源。VIGS和CRISPR/Cas9技术的联合应用为未来木本植物的功能分析提供了广阔的前景(Fang和Wang 2021)。另外, 木本植物的转基因实验较难成功, 效率低, 对于木本植物miRNAs的功能研究较困难, 应加强适用于木本植物及其靶基因的高效率研究系统。目前的研究也主要集中于单个miRNA, 缺乏一个miRNAs网络的系统研究。虽然对于木本植物miRNAs的研究还处于初级阶段, 但随着测序技术的发展、新技术的应用, miRNAs在木本植物中的调控网络会逐渐清晰, 为后续研究木本植物新品种提供经验。

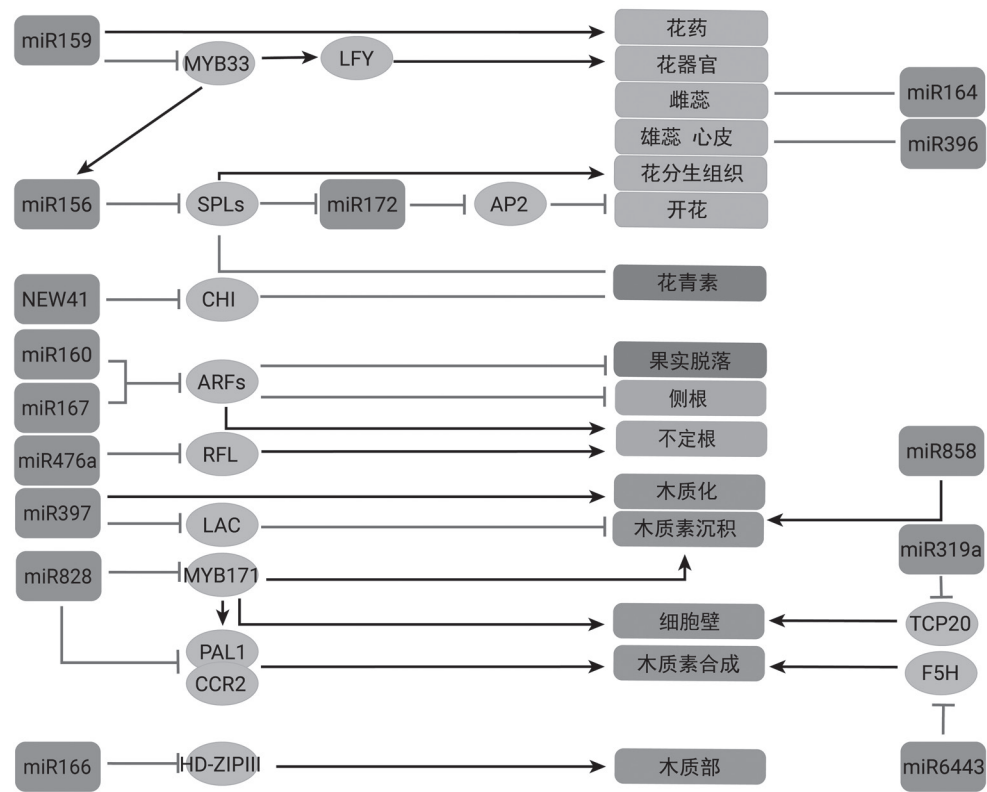


图2 miRNAs参与的分子调控网络

Fig. 2 The miRNAs-mediated molecular regulation networks

→: 促进作用; ⊥: 抑制作用; —: 调控作用。

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