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Review

The MicroRNA156 system: A tool in plant biotechnology

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ABSTRACT

The discovery of small RNAs in plants has opened a new field in the study of gene regulation, and its application in plant genetic engineering and crop improvement. Of small RNAs, plant microRNAs have been used to increase crop productivity and enhance other traits, such as crop quality and stress tolerance. MicroRNA156 (miR156) and its target *SPL* genes are highly conserved in the plant kingdom, and together they form an extensive gene regulatory network that controls various aspects of plant growth and development. These include strong impacts on crop yield and quality, flowering time, root development and nodulation, reproduction capacity, secondary metabolism, and plant stress. Here, we review the most recent understanding of miR156 function in plants, and highlight the major impact we are now seeing from its application to crop germplasm improvement and its prospects for future use in plant molecular breeding.

Contents

1. Introduction

MicroRNAs (miRNAs) are small sequence-specific regulators of posttranscriptional gene expression in eukaryotes (Voinnet, 2009; Sun, 2012). Since small RNAs were first reported in *Caenorhabditis elegans* (Lee et al., 1993), miRNAs and their functions have been extensively investigated in eukaryotes (Voinnet, 2009; Sun, 2012). *MiRNA* genes are encoded mainly in intergenic loci of the plant genome (Zhang et al., 2009). Most eukaryotic *miRNA* genes are transcribed by RNA polymerase II (Pol II) transcription units, but some are also transcribed by RNA polymerase III (Lee et al., 2004; Faller and Guo, 2008; Voinnet, 2009). Plant miRNAs employ perfect or near-perfect complementary sequences to scrutinize their targets (Rhoades et al., 2002).

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Plant miRNAs target genes that predominantly encode transcription factors (Hobert, 2004, 2008), although some miRNAs are also involved in the regulation of genes encoding proteins of other functions (Jagadeeswaran et al., 2009; Li et al., 2010; Devers et al., 2011; Sun, 2012; Sunkar et al., 2012; Padmanabhan et al., 2013; Curaba et al., 2014). It is unclear why miRNAs preferentially target transcription factor genes, but these two types of gene regulators share common characteristics in regulating their target genes; such as pleiotropic effects, requirement for effector complexes, sequence specificity and degree of accessibility to their binding sites in the targets (Hobert, 2004, 2008).

Plant miRNAs and their target genes were identified and annotated using a variety of approaches, including prediction based on sequence similarity in public databases, next generation sequence technology, and 5′ RNA ligase-mediated amplification of cDNA ends (5′ RLM-RACE) (Llave et al., 2002; Zhang, 2005; Moxon et al., 2008; Voinnet, 2009; Bonnet et al., 2010; Xie and Zhang, 2010; Sun, 2012). To date more than 5400 miRNAs (including approximately 20 conserved miRNA families) have been identified in different plant species (Sun, 2012; Curaba et al., 2014). The fact that miRNAs are functionally and evolutionally conserved across the plant kingdom has sped up their study across crop species by using information from model plants, such as *Arabidopsis.*

Of the many discovered miRNAs, microRNA156 (miR156) is conserved across flowering plant species (Voinnet, 2009; Huijser and Schmid, 2011; Jones-Rhoades, 2012) and has been studied in a range of non-crop species. These include the model plant *Arabidopsis* (Voinnet, 2009; Sun, 2012), Alpine rockcress (*Arabis alpine*) (Bergonzi et al., 2013), the model legume *Lotus japonicus* (Wang et al., 2014a), English ivy (*Hedera helix*) (Poethig, 2013), and trees, including Formosa acacia (*Acacia confusa*), Cole's wattle (*Acacia colei*), blue gum (*Eucalyptus globulus*), sawtooth oak (*Quercus acutissima*), and Canadian poplar (*Populus* × *canadensis*) (Wang et al., 2011). Moreover, miR156 has been characterized in a number of crop plants, including maize (*Zea mays*) (Mica et al., 2006), tomato (*Solanum lycopersicum*) (Zhang et al., 2011); rapeseed (*Brassica napus*) (Wei et al., 2010), rice (*Oryza sativa*) (Jiao et al., 2010; Miura et al., 2010), switchgrass (*Panicum sativum*) (Fu et al., 2012), potato (*Solanum tuberosum* ssp. *andigena*) (Bhogale et al., 2014), alfalfa (*Medicago sativa*) (Aung et al., 2015), soybean (*Glycine max*) (Yoshikawa et al., 2013) and Chinese cabbage (*Brassica rapa* subspecies *pekinensis*) (Wang et al., 2014b). Plant miRNAs have been widely reviewed in recent years (Voinnet, 2009; Rubio-Somoza and Weigel, 2011; Jones-Rhoades, 2012; Macovei et al., 2012; Sun, 2012; Sunkar et al., 2012; Zhou and Luo, 2013; Curaba et al., 2014), but a comprehensive review focused on miR156 and its impact on gene regulatory networks and prospects of crop improvement is still lacking. Here, we review the latest findings on the characterization of miR156 and the exciting impact that biotechnology with miR156 has had (and should continue to have) on making dramatic improvements to important traits in crop plants.

2. MiR156-SPL regulatory networks

Currently, a number of miR156 family members are deposited in the miRBase database (http://www.mirbase.org/). These miR156 precursors were identified from different plant species, but many of them can be found within a single plant species. For instance, 10 members of miR156 (miR156a–miR156i) are found in *Arabidopsis* and 11 members are found in *Medicago truncatula*. To date, miR156 has been shown to regulate *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), WD40, TEOSINTE GLUME ARCHITECTURE1* (*TGA1*) and LIGULELESS1 (LG1) genes in plants (Chuck et al., 2007; Naya et al., 2010; Nonogaki, 2010; Jones-Rhoades, 2012; Bazin et al., 2012; Bhogale et al., 2014; Wang et al., 2012) (Table 1). Of these predicted targets, research has focused mainly on the mechanism and regulatory networks of the miR156-SPL system in plants, and much of our understanding of the mechanism of this system has been detailed in *Arabidopsis* and a few crop plants.

SPL genes encode plant-specific transcription factors (Yamasaki et al., 2004; Birkenbihl et al., 2005) that play important roles throughout different stages of plant development (Manning et al., 2006; Gandikota et al., 2007; Martin et al., 2010a,b). SPL proteins are characterized by the presence of conserved DNA binding domains that allow them to bind and regulate downstream genes containing a consensus SPL Binding Domain (SBD) element in their promoters (Yamaguchi et al., 2009; Wei et al., 2012). So far, miR156 has been shown to target 11 out of the 17 *SPL* member genes in *Arabidopsis*, 11 members of the 19 *SPLs* in rice, three in tomato and alfalfa, and two in *L. japonicus* and maize (Mica et al., 2006; Xie et al., 2006; Moxon et al., 2008; Nonogaki, 2010; Wang et al., 2014a; Aung et al., 2015).

Generally, plant miRNAs recognize their target genes by locating miRNA responsive elements (MREs), which are complementary sequences present either in the target coding regions (Bartel, 2004; Jones-Rhoades and Bartel, 2004), 5′ untranslated regions (UTR) (Allen et al., 2005; Chiou et al., 2006), or 3′ UTR (Rhoades et al., 2002; Sunkar and Zhu, 2004) (Fig. 1). In *Arabidopsis*, *SPL3*, *SPL4*, and *SPL5* share conserved MREs in the 3' UTR whereas other miR156-targeted SPLs contain MREs in the coding regions (Gandikota et al., 2007). These authors also demonstrated that the presence of MREs in the UTR region (together with an active miR156) represses the accumulation of SPL3 protein and delays flowering in *Arabidopsis*, whereas altering the MRE sequence renders miR156 ineffective.

Expression of *miR156* genes is strongly regulated by developmental changes, nutrient availability and feedback from target genes. *MiR156* genes are highly expressed during the early stages of plant development, but expression is gradually decreased as the plant ages in *Arabidopsis* (Voinnet, 2009; Sun, 2012). Availability of sugar (glucose) decreases the expression of the *miR156* gene but reduced photosynthesis increases the level of miR156 (Yang et al., 2013). In addition to repressing the expression of miR156 at the transcriptional level, sugar also reduces the abundance of miR156 at the post-transcriptional level by degrading the primary transcript (Yu et al., 2013). Wu and Poethig (2006) showed that the transcript level of *Arabidopsis* miR156 is regulated by feedback from its target *SPL3* transcript, such that overexpression of a miR156-insensitive *SPL3* transgene down-regulates its regulator miR156. Similarly, the transcript level of miR156 is reduced by feedback when the activation-tagged *Arabidopsis sk156* mutant overexpresses a miR156-insensitive *SPL15* transgene (Wei et al., 2012). In the latter plant, analysis of the miR156 promoter region revealed SPL binding sites with three repeated GTAC core sequences between −200 bp and −220 bp, suggesting that SPL15 is directly (rather than indirectly) controlling miR156 expression (Wei et al., 2012).

3. MiR156 and non-SPL targets

MiR156 targets genes that encode WD40 proteins (Naya et al., 2010; Wang et al., 2014a; Hannoufa, unpublished) but, to date, little is known about the effects of the miR156-WD40 regulatory network system in plants. WD40 proteins are required for tissue-specific anthocyanin and proanthocyanin biosynthesis in *M. truncatula* (Pang et al., 2009) and *A. thaliana* (Gou et al., 2011). In the model legume, *L. japonicus,* enhancing LjmiR156 increases shoot branching up to 3-fold through down-regulation of *SPL13* (AU089181), another *SPL* member (TC70253), and a *WD40* (TC57859) (Wang et al., 2014a).