Accepted Manuscript

Jack of many trades: The multifaceted role of miR528 in monocots

Chengjie Chen, Yuanlong Liu, Rui Xia

PII: S1674-2052(19)30224-2
DOI: https://doi.org/10.1016/j.molp.2019.06.007
Reference: MOLP 803

To appear in: MOLECULAR PLANT
Accepted Date: 21 June 2019

Please cite this article as: Chen C., Liu Y., and Xia R. (2019). Jack of many trades: The multifaceted role of miR528 in monocots. Mol. Plant. doi: https://doi.org/10.1016/j.molp.2019.06.007.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

All studies published in MOLECULAR PLANT are embargoed until 3PM ET of the day they are published as corrected proofs on-line. Studies cannot be publicized as accepted manuscripts or uncorrected proofs.
Jack of many trades: The multifaceted role of miR528 in monocots

Chengjie Chen¹, Yuanlong Liu¹, Rui Xia*
State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Horticulture, South China Agricultural University, Guangzhou 510642, China

¹These authors contributed equally to this article.
*Correspondence: Rui Xia (rxia@scau.edu.cn)

MicroRNAs (miRNAs) are essential regulators, involved in almost all aspects of plant growth and development. In plants, miRNAs present in all angiosperms are regarded as conserved miRNAs; in contrast, miRNAs restricted to certain lineages (less-conserved) or a single species (species-specific) constitute the non-conserved miRNAs (Cuperus et al., 2011). Different members of a miRNA family usually target similar target genes from a gene family among different species. For instance, in most analyzed plants, the well-known miR156 family, usually consisting of a number of members in a given species, collectively target SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) genes. Generally, conserved miRNAs target genes encoding transcript factors which function in diverse biological processes. This functional diversity of miRNAs is mainly achieved by the plasticity of their target genes from the same family, such as miR156-targeted SPLs and miR167-targeted ARF (AUXIN RESPONSIVE FACTOR) genes, on regulating distinct downstream genes.

In this issue, two articles have put a spotlight on miR528 in rice. Yao et al. (2019) uncovered the transcriptional regulation of miR528 by OsSPL9 in antiviral response in rice. In combination with previous studies from the same group, miR528, upon viral infection, prefers to associate with cleavage-defective Argonaute 18 (AGO18) protein instead of the canonical partner AGO1, thereby releasing its repression on its target gene encoding L-ascorbate oxidase (also referred to as L-ascorbic acid oxidase, AAO); elevated AAO activity leads to accumulating higher basal reactive oxygen species (ROS) and enhancing antiviral defense (Wu et al., 2015; Wu et al., 2017). The other study (Yang et al., 2019) reported a novel role of miR528 in regulation of flowering time in rice. miR528 was demonstrated to target a zinc-finger transcription factor gene, RED AND FAR-RED INSENSITIVE 2 (OsRFI2), to promote heading under long-day conditions. The miR528 accumulation was fine-tuned by not only the binding of OsSPL9, but also the alternative splicing (AS) and alternative polyadenylation (APA) events of the primary MIR528 transcript (Yang et al., 2019). Intriguingly, natural variation in the MIR528 promoter, which is associated with different number of OsSPL binding elements, likely contributes to the adaption of rice to growth at different latitudes, corresponding to different photoperiod rhythms (Yang et al., 2019).

In addition to these roles, miR528 is also found to affect pollen development in rice (personal communication with Prof. Yueqin Chen at Sun-Yat Sen University). In creeping bentgrass, overexpressing osa-miR528 alters plant development and enhances resistance to salt stress and N starvation by regulating both AsAAO and COPPER ION BINDING PROTEIN1 (AsCBP1) genes (Yuan et al., 2015). In contrast, miR528 affects lodging resistance of maize by targeting two copper-containing laccase genes, ZmLACCASE3 (ZmLAC3) and ZmLACCASE5 (ZmLAC5), which
are involved in lignin biosynthesis under nitrogen-luxury conditions (Sun et al., 2018). In banana, miR528 is an essential regulator in the response to cold stress of banana fruits (generally known as peel browning), via targeting of genes encoding polyphenol oxidases (PPO) (Zhu et al., 2019).

In summary (Figure 1), miR528, unlike most miRNAs, has evolved distinct target genes that are involved in various developmental processes or biotic and abiotic stress conditions. Indeed, besides these validated target genes, miR528 has many other potential target genes, for example, there are as many as eleven predicted target genes of miR528 belonging to nine different classes in rice (Zhou et al., 2010; Yuan et al., 2015). Our comprehensive target gene profiling of miR528 in more than 20 monocot genomes confirmed the great diversity of miR528 target genes (unpublished data). Interestingly, miR528 predominantly targets a large collection of genes encoding copper-containing proteins. In addition to those which are validated (AAO, CBP, LAC, PPO), targets include many other genes encoding monocupper proteins or multicopper oxidases. All these data uncover the multifaceted role of miR528, like a performer of the ancient Chinese dramatic art “Bian Lian”, who can change vividly colored marks instantly (Figure 1).

miR528 was firstly identified in rice, and later found to be restricted in monocots (Liu et al., 2005). Due to its important roles in stress responses, miR528 is classified as a member of copper miRNAs which are usually induced by copper deficiency and direct the post-transcriptional regulation of transcripts that encoding copper-containing proteins (Pilon, 2017). Almost every monocot, except maize, has only one MIR528 locus, though several whole genome duplication events have happened after the split of monocots from its common ancestor with eudicots. Not only the miR528 sequence, but also the hairpin structure of miR528 precursor are extremely conserved among different species, ensuring the greatly consistent processing of miR528. Therefore the miR528 precursor sequence was commonly used as a backbone for the development of stable processing system of artificial miRNAs (Warthmann et al., 2008).

How does a single miRNA of almost identical sequence implement such a broad and diverse range of target capacity? One possible interpretation could be the rapid diversification rate of copper-containing proteins, which are significantly diverse due to the various composition of different copper centers. Consistent with this is the fact that miR528 has evolved a distinct preference of target genes in different monocots. Another possibility might be the quick loss-and-gain of miR528 target sites during evolution, as evidenced by that miR528 target site are always out of the functional domain region, and not at a consistent position, even for target genes from the same gene family. So there are some genes unrelated to copper-containing proteins that emerge as targets of miR528, like OsRFI2. Another question is how these diverse functions of miR528 are orchestrated with the growth and development of a monocot plant. Perhaps its proper function is accomplished by the coordinated expression of miR528 and its target genes via intertwined feedback loops (Pilon, 2017). In this regulatory network, miR528 is under the regulation of SPL proteins which is responsive to copper availability, while the copper balance is modulated by copper-containing proteins, which are target genes of miR528. Noteworthily, despite miR156 consistently targets a series of SPLs in rice, OsSPL9 is not its target gene (Xie et al., 2006; Yao et al., 2019).
Taken together, miR528 is an exceptional miRNA in plants, given its broad target capacity and distinct target preference among species. How this monocot-specific regulatory network of miR528 evolved and how each component of the network is coordinatively regulated remain open questions, worthy of further investigation.

**FUNDING**

This work was funded by the National Key Research and Developmental Program of China (#2018YFD1000104) and the National Natural Science Foundation of China (#31872063). This work was also supported by the Innovation Team Project of the Department of Education of Guangdong Province (#2016KCXTD 011), the Guangzhou Science and Technology Key Project (#201804020063).

**ACKNOWLEDGEMENTS**

The authors confirm that they have no conflict of interest. We are grateful to Prof. Yueqin Chen for sharing their unpublished data.


Figure Legend

**Figure 1 Multifaceted roles of miR528 in monocots**
Different cartoon masks, analogous to those used in the ancient Chinese dramatic art “Bian Lian”, denote the functional diversity of miR528. The function of CBP1 is inferred rather than of experimentally demonstrated, as indicated with a different arrow.