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REVIEW ARTICLE

Non-conserved microRNAs and their roles in plants: the case for legumes

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ABSTRACT

Several classes of small RNAs function to regulate stress and development pathways in all kingdoms of life. In animals and plants, microRNAs have been widely studied as important regulators of gene expression. However, non-conserved microRNAs have proven more difficult to study, raising questions as to their functionality. Using the legume family of plants as reference, we discuss this concept and provide examples where miRNAs functions have been described, highlighting their potential role in regulating important processes in these plants, such as stress responses and communication with other organisms, including bacteria and fungi. These examples suggest that non-conserved miRNAs are likely to contribute to more gene regulation circuits than currently appreciated, and in a wider range of plant species.

SMALL RNAS ARE PRESENT IN THE THREE DOMAINS OF LIFE

It has been widely documented that small RNAs (sRNAs) play a significant role in regulating various cellular processes across a wide range of organisms, from bacteria to multicellular eukaryotes (Fig. 1). In bacteria, several sRNAs play relevant roles in stress responses and in adapting to fluctuations in nutrient availability (Thomason *et al.* 2012; Holmqvist & Wagner 2017). In multicellular eukaryotes, a particular kind of sRNAs, known as microRNAs (miRNAs), has been extensively studied, along with other sRNAs involved in epigenetic regulation (Yu *et al.* 2019; Shi *et al.* 2022). Interestingly, miRNA-like sRNAs have also been predicted in Archaea, the third domain of life (Wang *et al.* 2014a), and numerous investigations have experimentally validated the existence of small non-coding RNAs. These archaeal sRNAs are generally found to act *in trans* with respect to their target mRNAs, some of them are clearly related to eukaryotic snoRNAs dedicated to rRNA modification, while others show a base-pairing preference for mRNAs, mediated by either short or imperfect base-pairing, akin to miRNA function (Busch *et al.* 2008; Bernick *et al.* 2012; Prasse *et al.* 2013). Hence, translational repression or activation guided by sRNAs in Archaea can be contrasted to their counterparts in Eukarya. Based on current evidence, it has been proposed that sRNA-mediated regulation in Archaea might be a primitive mechanism by which members of this domain are prompted to respond to adverse environmental conditions (Xu *et al.* 2012), thus offering a comparative viewpoint for phenomena observed with eukaryotic miRNAs.

Unlike sRNAs in Eukarya, most of the sRNAs present in Archaea and Bacteria involved in posttranscriptional regulation show limited conservation across species (Vogel 2009; Gómez-Lozano *et al.* 2012). For instance, the response to nitrogen limitation mediated by sRNAs seems to enhance the fitness to this condition exclusively in the Archaea family Methanosarcinaceae (Jäger *et al.* 2009; Prasse *et al.* 2017; Buddeweg *et al.* 2018). Moreover, sRNAs identified in *Haloferax volcanii* were not found even in other closely related species (Jaschinski *et al.* 2014). Cyanobacteria, one of the most diverse prokaryotic phyla, has a large number of late-emerging sRNAs (Hu & Wang 2018). The biogenesis of sRNAs in the three kingdoms of life differs significantly, and only limited comparisons can be drawn. In eukaryotes, miRNAs are first processed from longer precursor RNAs by RNAse III-type enzymes, and later are recruited to effector complexes where Argonaute proteins target complementary RNAs for regulation. In contrast, sRNAs in prokaryotes are generally not processed post-transcription and primarily utilize the RNA chaperone Hfq (an ancient homologue of eukaryotic Sm proteins) for stabilization of sRNA:target RNA interactions (Kavita *et al.* 2018). Interestingly, ancient homologues of Argonaute proteins exist in both bacteria and archaea. While most of them are involved in DNA interactions, an RNA-binding Argonaute protein was recently identified in an Asgard archaeon, demonstrating RNA silencing functionality (Bastiaanssen *et al.* 2024).

In Eukarya, mainly in animals and plants, miRNAs have been widely characterized. In contrast to the lack of conservation observed in Archaea and Bacteria, miRNA sequences of very ancient origin have been identified separately in animals

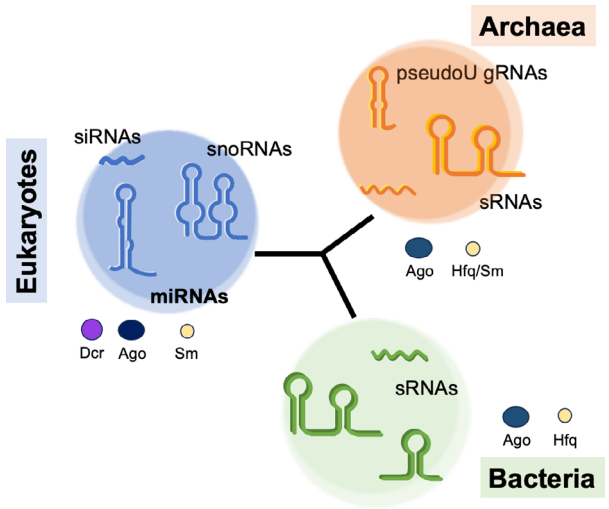

Fig. 1. Small RNAs are present in all three kingdoms of life. Archaea possess pseudoU-gRNAs, sRNAs similar to snoRNAs, and other sRNAs with complementarity to potential target mRNAs, which could be regulated by an Hfq/Sm factor. Bacteria carry a battery of sRNAs with different degrees of secondary structure that employ base-pairing interactions to regulate partially complementary RNAs, mostly mediated by Hfq. Eukaryotes display several types, including snoRNAs, siRNAs, miRNAs, in addition to piwi-RNAs in animals and phasiRNAs in plants. Processing and function of miRNAs requires Dicer and Argonaute proteins, respectively. Remarkably, Argonaute homologue proteins exist in the three domains of life. Other sRNAs include the ubiquitous tRNAs (not shown).

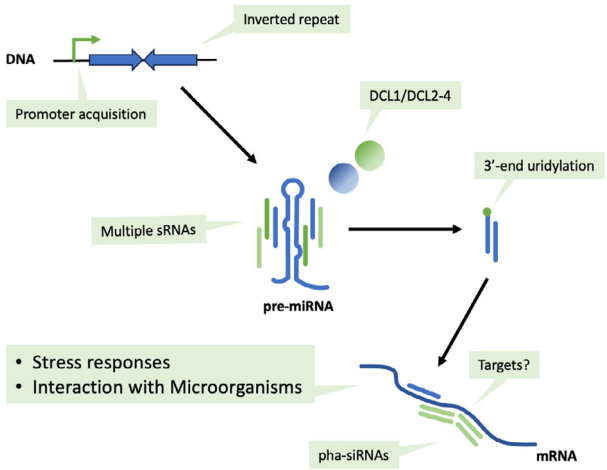

Fig. 2. Unique features present in the biogenesis and functions of plant non-conserved miRNAs. The canonical biogenesis and action of miRNAs proceeds through the transcription of precursor miRNAs, the formation of a hairpin structure and its processing by DCL1 and associated factors (not shown). From the resulting RNA duplex, one strand is selected as the mature miRNA, which will find a target mRNA by sequence complementarity. The specific features of non-conserved miRNAs are highlighted here in green and discussed in the main text. These features include the relevance of promoter acquisition by inverted repeat sequences to form a new *MIR* gene; the subsequent generation of multiple sRNAs from a single pre-miRNA, and processing by different DCL proteins. In the case of the legume-specific miR1510, modification of the 3′-end by uridylation is shown. Newly emerged miRNAs may produce secondary sRNAs known as phasiRNAs, and examples are given in the text of how miRNAs contribute to regulation of gene expression in response to biotic and abiotic cues.

(such as *let7*) and plants (like miR390, present from the moss *Physcomitrium patens* to *Arabidopsis*), while shared miRNA sequences do not seem to exist between these domains (Pasquinelli *et al.* 2000; Axtell *et al.* 2011; Xia *et al.* 2017). In both cases, conserved miRNAs are important components of pathways involved in development and responses to external stimuli. In contrast to those deeply conserved miRNAs, several unique miRNAs have evolved independently and more recently in those lineages where they have been sought. Their lack of conservation suggests that they may have arisen as recent evolutionary novelties. Often, a specific target mRNA has not been identified for those miRNAs, and coupled with their relatively low expression levels, this has led to the hypothesis that they may not have been integrated into molecular pathways and, as a result, might lack a defined biological function. In the case of plants, accumulated evidence in recent years, however, points to a different picture, where non-conserved miRNAs play important roles in specific cellular processes. Thus, in this review we'll focus on the origin of these unique miRNAs, their biogenesis, and the roles they play in legumes, a plant family of economic importance and with a wide repertoire of biotic interactions (Fig. 2).

HOW ARE NEW MIRNAS BORN?

There are different hypotheses describing the origin of new miRNAs. Among these, inverted gene duplication (Allen *et al.* 2004), spontaneous evolution (Felippes *et al.* 2008), and miniature inverted-repeat transposon elements (Piriyapongsa & Jordan 2008) have been proposed. These models have been documented in previous reports (Voinnet 2009; Cuperus *et al.* 2011; Cui *et al.* 2017). However, how miRNAs arise *de novo* is still of interest to researchers attempting to uncover functions for these newly emerged molecules. Recently, two hypotheses have offered novel options on other possible events that could give rise to a new miRNA.

Partial inverted duplication of antisense-transcribed sequences

Gramzow *et al.* (2020) recently proposed that miRNA (*MIR*) genes may emerge when its future target has been transcribed in an antisense orientation, following the acquisition of a promoter. Subsequently, a partially inverted duplication at the future target site may lead to an antisense transcript that folds into a stem-loop structure, which is then processed by the miRNA biogenesis machinery to produce a mature miRNA. As a result, the *MIR* and target genes are naturally arranged in an antisense orientation. However, the natural antisense organization could be lost if the target gene is duplicated, with the new copy becoming a new target of the emerging miRNA (Gramzow *et al.* 2020).

Short inverted repeats generation

This model hypothesized that promoter activity is gained before the *de novo* origination of a miRNA, due to the insertion of a transposon element into the 3′UTR region of an ancient

target gene (Lu 2019). Subsequently, short, inverted repeats are generated from palindromic-like sequences or from imperfect inverted repeat sequences present in the ancient target. This process eventually leads to the formation of miRNA precursors and their characteristic hairpin structures, giving rise to new miRNAs (Lu 2019).

All models proposed to date attempt to elucidate the origin and evolution of miRNA secondary structures. However, unlike previous models, recent proposals highlight the importance of acquiring a functional promoter prior to the origin of a new *MIR* gene. In addition, it has been suggested that insertion of transposons with significant ci-regulatory elements may have contributed to promoter gain (Lu 2019). This may be a crucial step, as promoter activity will determine the accurate regulation of the *MIR* gene transcription and, to some extent, its integration into regulatory networks (Schlötterer 2015). Thus, the promoter might have a relevant effect in the evolutionary fate of the *MIR* gene (Li & Mao 2007). Although, different origin mechanisms are observed in the evolution of miRNA genes and their targets, these new findings support and enrich our existing knowledge. However, there is still much to discover, particularly how hairpin-forming sequences are generated remains unclear (Allen *et al.* 2004; Lu 2019), as well as other processes leading to hairpin formation that may arise from the characterization of other precursor sequences.

FEATURES OF YOUNG microRNA BIOGENESIS

Plant *MIR* gene expression follows a sequence of steps that closely resemble those found in animal systems, with some distinct differences unique to plants. These steps ultimately result in the processing of a single-stranded mature miRNA of 20–22 nucleotides in length. In general, non-conserved miRNAs follow the same general path with some peculiarities that will be highlighted below. In plants, the biogenesis of miRNAs is compartmentalized between the nucleus and cytoplasm. *MIR* genes are located mostly in intergenic regions of the genome (Naqvi *et al.* 2012) and transcribed as independent transcription units (Ramalingam *et al.* 2014). In the nucleus, *MIR* loci are transcribed by the RNA Polymerase II, generating primary miRNA (pri-miRNA) transcripts that are 5′ capped and 3′ polyadenylated (Xie *et al.* 2005). This initial pri-miRNA is cleaved to form a precursor miRNA (pre-miRNA), which is processed to produce sRNA duplex, where one strand becomes the mature miRNA (Park *et al.* 2002; Kurihara *et al.* 2006). Several protein factors participate in the excision of the hairpin structure, in particular, members of the DICER-LIKE (DCL) protein family. DCL enzymes cleave dsRNA to release small RNA duplexes containing a 5′-phosphate group and two overhanging nucleotides at the 3′-ends (Wu *et al.* 2010; Axtell *et al.* 2011; Qin *et al.* 2014). DCL1 is exclusively responsible for orrect processing of conserved miRNAs (Bologna *et al.* 2013), whereas those of recent origin are generated by DCL1 and can also be processed by different members of this RNAse III family of enzymes (Rajagopalan *et al.* 2006; Vazquez *et al.* 2008).

In addition to DCL1, DCL2, DCL3, and DCL4 have been very well described in *Arabidopsis* and *Oryza sativa* (Liu *et al.* 2005; Vazquez *et al.* 2008). Pre-miRNAs processed by these enzymes result in miRNAs and/or other sRNA variants (Axtell *et al.* 2011; Qin *et al.* 2014). For instance, DCL1 can generate 22-nt isoforms if the pre-miRNA has an asymmetric internal bulge (Manavella *et al.* 2012). Likewise, DCL4 produces ∼21-nt RNAs when processing dsRNA precursors to generate trans-acting siRNAs (ta-siRNAs) and phased siRNAs (phasiRNAs) (Liu *et al.* 2007). In turn, DCL2 and DCL3 produce ∼22-nt natural antisense siRNAs (nat-siRNAs) and ∼24-nt phasiRNA or heterochromatic siRNAs (hc-siRNAs), respectively (Xie *et al.* 2005; Bouché *et al.* 2006; Xia *et al.* 2019). In general, inverted repeat transcripts are processed by non-canonical biogenesis pathways (Axtell *et al.* 2011). Different studies have demostrated that young miRNAs can mediate the formation of specific siRNAs, among them, phasiRNAs (Liu *et al.* 2017; Sosa-Valencia *et al.* 2017; Guo *et al.* 2018; Wang *et al.* 2018). For example, the legume-specific miR1514 regulates NAC gene mRNAs by cleaving them, which leads to the subsequent production of phasiRNAs (Sosa-Valencia, Palomar, *et al.* 2017). Likewise, the lncRNA termed WSGAR in wheat (*Triticum aestivum* L.) is cleaved by tae-miR9678, triggering the biosynthesis of phasiRNAs (Guo *et al.* 2018), which might be processed by DCL4 as they derive from a long hairpin precursor. On the other hand, although lineage-specific miRNAs are of recent origin, their biogenesis is generally processed by DCL1 (Qin *et al.* 2014). Interestingly, it has been revealed that miR6026, a tomato (*Solanum lycopersicum* L.) specific miRNA, is processed by DCL2 (Wang, Hardcastle, *et al.* 2018). In turn, the antiviral factor DCL2 mRNA is targeted by miR6026, yielding DCL2-derived phasiRNAs. This is a peculiar example where the absence of bulges in the pre-miR6026 makes it dependent on DCL2 rather than DCL1 (Wang, Hardcastle, *et al.* 2018). Thus, different sRNAs can be generated by the action of distinct DCL enzymes. In addition, imperfect processing by DCL1 can generate different sRNA sequences from the same precursor, a feature observed more frequently for non-conserved miRNAs. While several isoforms result in one- or two-nucleotide variants that do not affect target specificity, others can show larger variations that have not been characterized thus far, hence their regulatory effects, if any, remain unknown. In addition, conserved miRNAs exhibit a marked preference for carrying a uridine (U) at the 5′-end, aligning with AGO1 known binding affinity for sRNAs that possess this feature. Consequently, if non-conserved miRNAs are indeed functional, there may be evolutionary pressure for them to include a 5′-U as well. However, this issue has not been directly addressed in published reports.

Following the precursor cleavage events, the miRNA/miRNA* duplex is methylated by HEN1 at the 2'OH position, contributing to its stability (Yang *et al.* 2007). Alternatively, an interesting mechanism of miRNA uridylation has been reported in the Phaseoleae tribe of legume species. HEN1 can only partially methylate the legume-specific miR1510 duplex due to a 3′-end mismatch in the dsRNA region. This defect is counteracted by 3′-uridylation of this miRNA isoform via HESO1. Whether AGO-bound uridylated 22-nt miR1510 is methylated by HEN1 is not known yet, but what is clear is that this isoform produced during biogenesis safeguards the generation, fate, and function of miR1510 in Phaseoleae (Fei *et al.* 2018).

Once the duplex is methylated (or monouridylated), it is exported to the cytoplasm. Until recently, the exportin HASTY was thought to be solely responsible for this task (Park *et al.* 2005). Two research groups have linked the function of HASTY to pri-miRNA transcription and processing as well as regulation of miRNA movement (cell-to-cell and long distances) (Brioudes *et al.* 2021; Cambiagno *et al.* 2021). Recently,

a study revealed that nuclear export of miRNAs can be performed by ARGONAUTE (AGO) proteins, which associate with the miRNA duplex inside the nucleus and transport it to the cytosol via EXPORTIN1 in a pathway dependent on a nuclear-export signal present in AGO1 (Bologna *et al.* 2018). Moreover, a recent study by Zhang *et al.* (2020) revealed that the TREX-2 complex plays a key role in coordinating the export of miRNA/AGO complexes through the nuclear pore (Zhang *et al.* 2020). Once in the cytosol, the RNA-induced silencing complex (RISC) regulates the miRNA-directed cleavage, degradation, or translational repression of target mRNAs (Llave *et al.* 2002; Chen 2004). Alternatively, 22-nt miRNAs initiate secondary siRNA formation upon target cleavage (Sosa-Valencia, Palomar, *et al.* 2017; Wang, Hardcastle, *et al.* 2018); nevertheless, their biogenesis has been reviewed recently (Liu *et al.* 2020; Sanan-Mishra *et al.* 2021) and will not be covered here.

ARE NEWLY EMERGED MIRNAS FUNCTIONAL?

Non-conserved miRNAs have been generally defined as recently evolved molecules that are often weakly expressed, imprecisely processed, apparently lack functional targets, and thus they may represent a burden of ineffective products for the plant genome, potentially on a path to elimination. Because miRNA levels have been shown to modulate the degree to which a target transcript is silenced (Denzler *et al.* 2016), studies on miRNAs have focused on those that display relatively high expression levels. Nevertheless, a study performed by Persson *et al.* (2006) on small vault RNAs with miRNA like activity (or svRNAs), demonstrated that these molecules can efficiently repress CYP3A4 expression in human cells, even when present at relatively low levels. Moreover, target gene repression mediated by miRNAs can occur in a dose-dependent manner of miRNA accumulation, as has been shown for miR-17~92 in mice (Jin *et al.* 2017). In *Arabidopsis*, miR159 regulates the expression of a subgroup of MYB genes, while the lower expression of the sequence-related miR319 prevents it from recognizing those same transcripts and instead it targets TCP mRNAs for cleavage (Palatnik *et al.* 2007). Thus, to ensure appropriate target gene regulation, the functional concentration of miRNA will be defined by a minimum and a maximum threshold level with respect to the abundance of a given target transcript (Jin *et al.* 2017). Consequently, differential expression of the target transcripts may require variable miRNA concentrations, leading to significant fluctuations in their functional levels, depending on the specific miRNA:target pair being studied. Therefore, using conserved miRNAs as a reference to define low expression levels in non-conserved miRNAs may introduce a biased framework that could obscure our understanding of the roles of other miRNAs.

A second argument to dismiss recently emerged miRNAs as relevant regulators is their imprecise precursor processing, which produces a cohort of small RNAs. While some of these may be functional, others could be harmful if they target unintended transcripts. Given that the newly emerged miRNAs have relatively low accumulation levels, in some cases not reaching a functional concentration (see above discussion), the contribution of alternate sRNAs to targeting certain mRNAs may be minimal. In addition, RNA:target recognition in plants relies on extensive base-pairing and therefore the presence of complementary sequences in transcripts may also be limited. Thus, miRNA regulation of only a few selected sequences that have an impact on regulatory pathways may undergo positive selection, while other sRNAs may not persist over evolutionary time.

Interestingly, one of the pathways to generate phasiRNAs (discussed above) involves cleavage of a target RNA directed by the activity of 22-nt miRNAs, followed by double-stranded RNA formation and phased-sRNA generation by a DCL enzyme. Known examples of 22-nt miRNAs are predominantly found among non-conserved miRNAs (Liu *et al.* 2020), indirectly suggesting that non-conserved miRNAs may have a larger contribution to phasiRNA formation and, consequently, a distinct functional impact than conserved miRNAs.

TARGETS OF NON-CONSERVED MICRORNAS

Do non-conserved miRNAs lack functional targets? Gene gains and losses have been observed throughout the evolutionary history of biological systems, leaving as a major legacy, the great diversity that exists today. Newly emerged miRNAs are not exempt from these evolutionary processes. The accelerated evolutionary rate of sRNA/miRNA observed in different animal species (Mao & Cao 2016) may result in sequence changes that cause the loss of base-pairing to its original target genes. However, other potential binding sites exist on the immense pool of mRNA sequences (Jin *et al.* 2017), which may allow young miRNAs to acquire new functions through their new targets. Those new miRNAs that succeed to integrate into pre-existing regulatory networks become functionally relevant and are retained by positive selection pressure, as observed for *mir-972c* in *Drosophila* (Lyu *et al.* 2021). In this way, new miRNAs could recruit additional targets over time, expanding their contribution to different gene regulatory pathways and gradually modulating their expression levels to accommodate cellular needs (Mao & Cao 2016).

Unlike animals, clustering of *MIR* genes is not as common in plants. However, polycistronic miRNA clusters have been identified in *A. thaliana*, *Manihot esculenta*, *O. sativa* and *T. aestivum* (Rajagopalan *et al.* 2006; Patanun *et al.* 2013; Baldrich *et al.* 2016; Singh *et al.* 2020). To the best of our knowledge, de la Rosa *et al.* (2019) performed the first study of a polycistron containing a conserved and a non-conserved miRNA in plants. Both miRNAs, the legume-specific miR2119 and miR398a, are co-expressed in a single transcript to regulate their respective targets in response to water deficit in *Phaseolus vulgaris* (de la Rosa *et al.* 2019). Thus, the emergence of species-specific miRNAs might have a relevant role in modulating diverse gene regulatory networks, potentially serving as a source of genetic and functional innovation. Considering that the new miRNAs are energy wasting elements, is like involuting to the moment in which non-coding DNA were considered as junk DNA (Ohno 1972; Kuska 1998). We are at a point where we should turn our attention to the study of these miRNAs to reveal how they originated, their evolutionary history, and present functions.

FUNCTIONS OF LINEAGE-SPECIFIC MICRORNAS

What role do miRNAs play in determining lineage identity and regulating cellular functions in plants? Answering this question will help us to better understand the emerging functions of miRNAs and their influence on plant evolutionary history.

An interesting example of a lineage-specific miRNA is miR2275, which regulates reproduction in some angiosperms (Xia *et al.* 2019). Initially miR2275-24-nucleotide phasiRNAs pathway was thought to be unique to monocots (Zhai *et al.* 2015). However, its recent discovery in some eudicots changed this view; in addition, the absence of miR2275/DCL5 in some eudicot families such as Solanaceae and the contrasting presence of phasiRNA revealed that not everything is known about the 24-nucleotide phasiRNAs pathway (Xia *et al.* 2019). Hence, the study of this mysterious route might reveal new aspects of miRNA/PHAS-phasiRNA regulatory cascades. Another miRNA that shows complex dynamics of gene regulation associated with seed germination is miR9678. This miRNA delays seed germination in wheat by targeting a lncRNA and triggers phasiRNA production that modulates GA/ABA signalling (Guo *et al.* 2018). The monocot-specific miR528 targets genes involved in the regulation of drought stress and nitrogen starvation in *O. sativa*. It plays a significant role in rice's ability to withstand water and nutrient scarcity (Yuan *et al.* 2015), a crucial trait given its importance as a crop in different environments. In addition, miR528 contributes to the process of somatic embryogenesis and responses to auxins and nitrogen availability in maize (Luján-Soto *et al.* 2021, 2022). On the other hand, the species-specific miR6026 may negatively regulate the resistance to potato virus X and tobacco mosaic virus by targeting DCL2 mRNA and triggers secondary sRNA production in tomato (Wang *et al.* 2018). However, transgenic plants overexpressing a miR6026 target mimicry construct showed decrease abundance of the cognate miRNA, upregulation of DCL2, and improved antivirus resistance (Wang, Deng, *et al.* 2018). This approach not only provides a means to protect crops from viral infections but also showcases the potential of biotechnology to engineer plants with specific and beneficial traits, contributing to agricultural sustainability and food security. The functions identified for these non-conserved miRNAs show that they contribute to diverse regulatory networks in different plant species. In the case of legumes, non-conserved miRNAs have been characterized as playing essential roles in the regulation of genes involved in processes like symbiotic relationships and stress responses. These functions have revealed unique pathways in legumes, with the most recent advances on this subject presented in the following sections.

THE ROLE OF NON-CONSERVED MICRORNAS IN LEGUMES

Why legumes?

Legume domestication started during the Neolithic period ca. 10,000 years ago (Koenen *et al.* 2021). Since then, trait selection and production have transcended geographic and cultural boundaries. Consequently, pulse crops have adopted wide environmental resilience, and the organoleptic characteristics of their grains reflect their wide phenotypic diversity. Currently, legumes are one of the most produced crops worldwide. Nevertheless, among the economically important pulses, soybean, peas, lentil, and those known as dried beans (*P. vulgaris*), are the most demanded as staple food and valuable source of fibre, vitamins, and vegetal protein (Medendorp *et al.* 2022). In recent years, legume consumption has gained relevant importance as part of free-gluten and/or vegan diets (Messina 2014; Gomaa & Elhadidy 2020), becoming a key component to safeguard human health. Legumes also play an essential role in food diversification and sustainability.

The legume family, also called Leguminosae or Fabaceae, is one of the largest in the plant kingdom, possibly due to its geographical distribution and colonization of different ecological niches. The basis for this success can be found in its anatomical and physiological diversity, and significantly, in the symbiotic associations they form with some bacteria of the genus Rhizobiaceae and with mycorrhizal fungi. Both plant–microorganism collaborations are important because of the nutrient exchange occurring during symbiosis. Some members of this family (e.g. *P. vulgaris* and *Glycine max*) are important as species for human consumption and foraging in different parts of the world, while model species such as *L. japonicus* and *M. truncatula* have been adopted for the ease of gene transformation, availability of mutant banks, and extensive omics databases available. Small RNA sequencing (sRNA-seq) has offered a powerful tool for the analysis of thousands of miRNA sequences, enabling researchers to detect low-abundance miRNAs and to explore their regulatory roles across various biological processes, including but not limited to, those related to abiotic stress responses and the regulation of symbiotic interactions, providing the research field with a vast volume of data to explore. Given the significance of legumes, several studies have explored the contribution of non-conserved miRNAs to the regulation of these processes, which will be the focus of the following sections (summarized in Table 1).

Legume miRNAs in response to abiotic stress

Plants constantly cope with environmental challenges, such as drought, salinity, temperature extremes, heavy metal exposure, and nutrient deficiencies. These abiotic stresses modulate and alter their development, impacting physiological processes, homeostasis, and ultimately, yield. This is often reflected in a significant reduction in the number and size of pods and seeds, which are key productivity traits in Fabaceae species. Responses to abiotic stress can be species-dependent and may fluctuate among crop varieties. The ability of plants to adapt to these stressors involves complex regulatory networks, including hormonal signalling, gene expression changes, and the activation of stress-responsive pathways, all of which are essential for their survival and productivity in shifting environments. At the basis of these processes, miRNAs participate in the regulation of expression profiles (Zhang *et al.* 2022; Samynathan *et al.* 2023). For this reason, an exploration into legume stress response pathways modulated by legume-specific miRNAs is indeed necessary.

Recent studies have shown that diverse conserved plant miRNAs confer abiotic stress resistance, thereby ensuring sustainable agricultural production (Samynathan *et al.* 2023). However, there are also documented examples of novel legume miRNAs that specifically respond to abiotic stress. Most of these have been identified through sRNA-seq approaches (Barrera-Figueroa *et al.* 2011; Ruan *et al.* 2024), but only a few have been fully validated.

Originally described in *G. max*, miR1511 is present in Fabaceae and at least in some Rosaceae species. In *Phaseolus vulgaris*, miR1511 responds to metal toxicity, while the ALS3 (Aluminium Sensitive Protein 3) gene, known to play a critical

Table 1. Non-conserved miRNAs in legumes and other plants and their known biological functions.

miRNA	lineage/species	function	references
miRNAs involved in stress responses			
miR528	Specific to monocots	Response to water and nutrient scarcity	Yuan *et al*. (2015) Luján-Soto *et al*. (2021, 2022)
miR1510	Legume-specific	Disease resistance	Fei *et al*. (2018)
miR1511	Specific to Fabaceae and Rosaceae	Aluminium detoxification	Martín-Rodríguez *et al*. (2021)
miR1514	Legume-specific	Response to stress	Sosa-Valencia, Palomar, *et al*. (2017)
miR2109	Legume-specific	Regulation of plant immunity	Sós-Hegedűs *et al*. (2020)
miR2119	Legume-specific	Response to water deficit	de la Rosa *et al*. (2019)
miR6026	*Solanum lycopersicum* L.	Defence against PSTVd infection	Wang, Hardcastle, *et al*. (2018)
miR9678	*Triticum aestivum* L.	Affects seed germination and modulating ABA/Gibberellin signalling	Guo *et al*. (2018)
miRNAs involved in nodulation			
miR1507	Legume-specific	Regulation of plant immunity and symbiotic interactions	Sós-Hegedűs *et al*. (2020)
miR1509	Legume-specific	Control of nodulation	Tiwari *et al*. (2021)
miR2111	Specific to dicots	Controlling nodulation	Sexauer *et al*. (2023)

role in aluminium detoxification in several plant species, was experimentally validated as a target of miR1511 (Martín-Rodríguez *et al*. 2021). Interestingly, *MIR1511* gene sequence analysis in diverse genotypes revealed that many displayed a truncated version of the gene (Martín-Rodríguez *et al*. 2021). The absence of miR1511 due to a 58-bp deletion in the gene in certain *P. vulgaris* genotypes, resulting in reduced degradation of ALS3 transcripts, and appears to confer an evolutionary advantage to high aluminium levels in soils with increased drought conditions (Martín-Rodríguez *et al*. 2021).

More than a decade has passed since the first report of novel miRNAs involved in responses to water deficit in legumes (Arenas-Huertero *et al*. 2009). Based on these initial data, subsequent functional analysis of miR1514a revealed its role in controlling the expression of a NAC transcription factor gene by inducing the production of phasiRNAs, which accumulate under water deficit conditions in *P. vulgaris* (Sosa-Valencia, Palomar, *et al*. 2017) and similarly in *M. truncatula* (Sosa-Valencia *et al*. 2017). A second miRNA analysed more recently is miR2119, which is encoded as a bicistronic miRNA along with miR398a. Together, miR2119 and miR398a have been linked to water scarcity responses through the regulation of ALCOHOL DEHYDROGENASE 1 (ADH1) and COPPER-ZINC SUPEROXIDE DISMUTASE 1 (CSD1) transcripts, respectively (de la Rosa *et al*. 2019). These examples are some of the few miRNAs exclusively found in legumes that have been characterized by experimental validation to uncover their biological relevance. Clearly, many non-conserved miRNAs remain undiscovered and/or uncharacterized, representing missed opportunities for crop improvement and underutilization of genetic resources. Further research into these miRNAs is essential to unlock the full potential of legume crops and address the challenges posed by changing environmental conditions.

microRNAs in nodulation, what we know and what remains to be deciphered

When soil nitrogen levels are low, legume plants establish a symbiotic relationship with rhizobia, free-living nitrogen-fixing bacteria in the soil. This interaction leads to the formation of root nodules, specialized structures on the plant roots where the bacteria reside. Inside, rhizobia convert atmospheric nitrogen (N_2) into ammonia (NH_3), which the host plant can utilize. In return, legumes supply the bacteria with photosynthetically derived carbon, supporting their growth (Oldroyd *et al*. 2011).

miRNAs have been identified as crucial regulators of this endosymbiosis. Coupled to *in silico* approaches, sRNA-seq has facilitated the identification of hundreds of both conserved and novel miRNAs potentially involved in different stages of nodule ontogeny in model legumes such as *L. japonicus*, *G. max*, *P. vulgaris*, and *M. truncatula* (Subramanian *et al*. 2008; Wang *et al*. 2009; Joshi *et al*. 2010; De Luis *et al*. 2012; Turner *et al*. 2012; Formey *et al*. 2016; Yan *et al*. 2016). Functional evidence for their contribution in this process has been obtained for a selected number of candidates. Several miRNA families that, although deeply conserved in plants, modulate the expression of transcription factors that have been coopted to control nodule development. In *M. truncatula*, miR166 modulates meristem activity and vascular differentiation in both roots and nodules by targeting HD-ZIP III transcription factor mRNAs (Boualem *et al*. 2008; Carlsbecker *et al*. 2010). Meanwhile, miR169 controls the spatial distribution of the transcription factor HAP2-1, which is involved in meristem maintenance and bacterial release in nodules (Combier *et al*. 2006). miR171 targets members of the scarecrow-like (GRAS domain) family of transcription factors, which are primarily expressed in inflorescence and floral tissues (Pei *et al*. 2023). However, in legume lineages, other isoforms of this family, such as miR171c in *L. japonicus*, miR171h in *M. truncatula*, or miR171o and miR171q in soybean, specifically target NSP2 transcripts, encoding a transcription factor essential for nodulation (De Luis *et al*. 2012; Hossain *et al*. 2019). The miR172 family targets APETALA2 (AP2) and AP2-like genes, playing a significant role in promoting flowering (Aukerman & Sakai 2003; Chen 2004) and modulating the juvenile-to-adult phase transition during shoot development (Wu *et al*. 2009). In legumes, however, miR172c also acts as a crucial promoter of nodulation (Wang *et al*. 2014b). Soybean miR172c,

produced in root nodules, serves as a long-distance mobile signal, travelling from the nodules to the leaves, while nitrogen fixed in the nodules stimulates the production of miR172c in the leaves (Yun *et al.* 2023). Interestingly, the combined action of symbiotic and locally expressed miR172c activates florigen-encoding FLOWERING LOCUS T (FT) homologues by repressing TARGET OF EAT1-like 4a, allowing legumes to reproduce under low-nitrogen conditions (Yun *et al.* 2023). MiR172c has been also found in *L. japonicus* (Holt *et al.* 2015), *P. vulgaris*, and *M. truncatula* (Nova-Franco *et al.* 2015).

Additionally, the miR397 family, which contains several conserved members that target laccase genes involved in lignin synthesis, are also essential during legume symbiosis (De Luis *et al.* 2012). miRNAs such as miR393, miR164, miR167, and miR160, which regulate auxin balance and are essential for plant growth and development, have also been proven to be crucial for nodule ontogeny (Navarro *et al.* 2006; Subramanian *et al.* 2008; Wang *et al.* 2015; Cai *et al.* 2017).

Perhaps the most remarkable example of how conserved miRNAs acquire novel functions during endosymbiosis is miR2111. Exclusive to eudicotyledon plants, miR2111 participates in the regulation of lateral root formation (Sexauer *et al.* 2023). However, in nodulating lineages, miR2111 controls the number of nodules on the roots of the host plant. Initially, rhizobia infection stimulates the production of CLE peptide hormones in the roots, which are transported to the shoot to induce the expression of miR2111. miR2111 is then transported back to the roots through the phloem, where it targets transcripts of TOO MUCH LOVE (TML), a negative regulator of nodulation, allowing nodulation to progress. Shoot perception of rhizobia-induced CLE peptides suppresses miR2111 expression, resulting in TML accumulation in roots and subsequent inhibition of nodule organogenesis (Gautrat *et al.* 2020). The general mechanism of miR2111 controlling nodulation was first demonstrated in *M. truncatula*, with subsequent studies in *L. japonicus* and soybean indicating that this regulatory circuit is a common feature in legumes (Okuma *et al.* 2020; Zhang *et al.* 2021).

The legume–rhizobia interaction and the subsequent root nodulation process require the suppression of host defences to ensure successful symbiosis and prevent immune responses from the host plant (Yang *et al.* 2010). In *M. truncatula*, a specific subset of miRNAs, including miR1507, miR1509, miR2109 (also known as miR5213), and miR2118 (a member of the miR482 superfamily, which is closely related to soybean miR482-3p and common bean miR482), target resistance (R) genes with nucleotide-binding (NB) and leucine-rich repeat (LRR) domains (NB-LRR) to regulate plant immunity and facilitate the establishment of symbiotic interactions (Bazin *et al.* 2012; Sós-Hegedűs *et al.* 2020). In soybean, the overexpression of miR482, miR1512, and miR1515, which also target NB-LRR genes, has been shown to increase nodule numbers (Li *et al.* 2010). Collectively, these miRNAs may play a role in regulating the crosstalk between nodulation and pathogenesis to facilitate the onset of nodulation.

While significant progress has been made in uncovering the role of miRNAs in legume nodulation, several crucial aspects remain unresolved. Both conserved and novel miRNAs have provided valuable insights into the regulatory networks governing legume–rhizobia symbiosis.

The role of miRNAs in the mycorrhizal colonization of legume plants

Most land plants, including legumes, establish endosymbiosis with mycorrhizal fungi. Arbuscular mycorrhiza (AM) is a root endosymbiosis between fungi from the ancient phylum Glomeromycota and terrestrial plants. This symbiosis occurs in 70–90% of land plant species, enhancing their ability to absorb water and mineral nutrients. Despite its vital ecological role, the mechanisms behind the formation of this symbiosis are not well understood, primarily due to the obligate biotrophic lifestyle and multinucleate nature of AM fungi. Like the rhizobia–legume symbiosis, the partnership between the host plant and the fungi begins with a molecular dialogue, leading to mutual recognition and the penetration of the outer cells of the plant root by the fungal hyphae. This allows the fungi to colonize the inner cortical cells, where they form intricately crafted structures called arbuscules. Mycorrhizal endosymbiosis enhances the supply of water and uptake of immobile or low-availability nutrients from the soil, mainly phosphate, to the host plant in return for sugars produced through photosynthesis by the plant (Parniske 2008; Luginbuehl & Oldroyd 2017). Additionally, arbuscular mycorrhizal (AM) symbiosis enhances plant resistance to biotic and abiotic stresses (Lenoir *et al.* 2016).

Small-RNA seq coupled with degradome studies of non-mycorrhizal and mycorrhizal roots of *M. truncatula* identified 20 distinct miRNAs that are differentially expressed (Devers *et al.* 2011). However, few studies have functionally linked these miRNAs to AM symbiosis. The contribution of conserved miRNAs has been recently reviewed (Ledford *et al.* 2024) and will be only briefly recounted here.

One notable example is miR399, previously identified in *A. thaliana* as a systemic signal for phosphate starvation (Bari *et al.* 2006). In *M. truncatula*, miR399 accumulates to higher levels in mycorrhizal roots compared to non-mycorrhizal roots under low phosphate (Pi) conditions (Branscheid *et al.* 2010). This miRNA suppresses its target transcript, MtPho2, which would otherwise increase in response to symbiotic Pi uptake (Branscheid *et al.* 2010). Since Pho2 is a ubiquitin-conjugating enzyme that negatively regulates a group of Pi-starvation inducible genes, including some Pi-transporters (Bari *et al.* 2006), it is believed that miR399 regulates arbuscular mycorrhizal symbiosis both locally and systemically, linking it to phosphorus status.

Another example is the regulatory role of miR171h, which is also activated in *M. truncatula* in response to AM colonization (Lauressergues *et al.* 2012). This miRNA targets MtNSP2 (Branscheid *et al.* 2011; Devers *et al.* 2011; Lauressergues *et al.* 2012), a GRAS transcription factor involved in both the biosynthesis of strigolactone and the mycorrhizal signalling pathway (Liu *et al.* 2011; Lauressergues *et al.* 2012). Strigolactones have been demonstrated to activate spore germination and hyphal proliferation of AM fungi (Akiyama *et al.* 2005; Besserer *et al.* 2006). Therefore, it was proposed that in roots invaded by the fungus, the induction of miR171h, which downregulates NSP2, may decrease strigolactone content and thus control fungal colonization (Lauressergues *et al.* 2012).

Remarkably, there are no reports of legume–AM symbiosis involving non-conserved miRNAs, perhaps this is evidence that the interaction is so ancient that it recruited miRNAs

regulatory networks from its origins. Continued research may deepen our understanding about the role of miRNAs, particularly those of more recent emergence, in this ancestral yet unique symbiosis.

CONCLUDING REMARKS

Several classes of small RNAs are present in all kingdoms of life, however, research has focused on a few of those families, in particular miRNAs, present in plants and animals. The study of these organisms has revealed their contribution to the regulation of growth, development, and environment interactions in several biological contexts.

The emergence of new miRNA-encoding genes is a phenomenon that threats to imbalance existing gene regulatory pathways, as they can alter the balance of transcripts within them. Thus, this has been a focus of discussion: how novel miRNAs are selected over evolutionary time and whether they are functional. While some of them may disappear into oblivion, others have gained regulatory functions. As is evident from the examples described here for the legume family of plants, at least some of them have an important influence in major biological processes, including symbiosis with rhizobia and the responses to adverse conditions in their different forms. Clearly, other processes – such as mycorrhizal symbiosis – need to be further explored to identify other players, including miRNAs, not only in legumes, but surely present in most, if not all, land plant families.

AUTHOR CONTRIBUTIONS

CAS-S conceived the original idea for this work; YH, CAS-S, CD-C contributed with literature mining for different sections; YH wrote the initial draft manuscript; JLR designed and elaborated figures, and all authors contributed to text writing, editing, and correction to the final version of the manuscript.

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REFERENCES

Akiyama K., Matsuzaki K., Hayashi H. (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature*, **435**, 824–827.

Allen E., Xie Z., Gustafson A.M., Sung G.H., Spatafora J.W., Carrington J.C. (2004) Evolution of microRNA genes by inverted duplication of target gene sequences in Arabidopsis thaliana. *Nature Genetics*, **36**, 1282–1290.

Arenas-Huertero C., Pérez B., Rabanal F., Blanco-Melo D., De la Rosa C., Estrada-Navarrete G., Sanchez F., Covarrubias A.A., Reyes J.L. (2009) Conserved and novel miRNAs in the legume *Phaseolus vulgaris* in response to stress. *Plant Molecular Biology*, **70**, 385–401.

Aukerman M.J., Sakai H. (2003) Regulation of flowering time and floral organ identity by a MicroRNA and its APETALA2-like target genes. *The Plant Cell*, **15**, 2730–2741.

Axtell M.J., Westholm J.O., Lai E.C. (2011) Vive la différence: Biogenesis and evolution of microRNAs in plants and animals. *Genome Biology*, **12**, 221–233.

Baldrich P., Hsing Y.I., San Segundo B. (2016) Genome-wide analysis of polycistronic microRNAs in cultivated and wild rice. *Genome Biology and Evolution*, **8**, 1104–1114.

Bari R., Datt P.B., Stitt M., Scheible W.R. (2006) PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiology*, **141**, 988–999.

Barrera-Figueroa B.E., Gao L., Diop N.N., Wu Z., Ehlers J.D., Roberts P.A., Close T.J., Zhu J.-K., Liu R. (2011) Identification and comparative analysis of drought-associated microRNAs in two cowpea genotypes. *BMC Plant Biology*, **11**, 127–138. https://doi.org/10.1186/1471-2229-11-127

Bastiaanssen C., Bobadilla Ugarte P., Kim K., Finocchio G., Feng Y., Anzelon T.A., Köstlbacher S., Tamarit D., Ettema T.J.G., Jinek M., MacRae I.J., Joo C., Swarts D.C., Wu F. (2024) RNA-guided RNA silencing by an Asgard archaeal Argonaute. *Nature Communications*, **15**, 5499.

Bazin J., Bustos-Sanmamed P., Hartmann C., Lelandais-Brière C., Crespi M. (2012) Complexity of miRNA-dependent regulation in root symbiosis. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **367**, 1570–1579.

Bernick D.L., Dennis P.P., Höchsmann M., Lowe T.M. (2012) Discovery of Pyrobaculum small RNA families with atypical pseudouridine guide RNA features. *RNA*, **18**, 402–411.

Besserer A., Puech-Pagès V., Kiefer P., Gomez-Roldan V., Jauneau A., Roy S., Portais J.C., Roux C., Bécard G., Séjalon-Delmas N. (2006) Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biology*, **4**, e226.

Bologna N.G., Iselin R., Abriata L.A., Sarazin A., Pumplin N., Jay F., Grentzinger T., Dal Peraro M., Voinnet O. (2018) Nucleo-cytosolic shuttling of ARGONAUTE1 prompts a revised model of the plant MicroRNA pathway. *Molecular Cell*, **69**, 709–719.e705.

Bologna N.G., Schapire A.L., Zhai J., Chorostecki U., Boisbouvier J., Meyers B.C., Palatnik J.F. (2013) Multiple RNA recognition patterns during microRNA biogenesis in plants. *Genome Research*, **23**, 1675–1689.

Boualem A., Laporte P., Jovanovic M., Laffont C., Plet J., Combier J.P., Niebel A., Crespi M., Frugier F. (2008) MicroRNA166 controls root and nodule development in Medicago truncatula. *The Plant Journal*, **54**, 876–887.

Bouché N., Lauressergues D., Gasciolli V., Vaucheret H. (2006) An antagonistic function for Arabidopsis DCL2 in development and a new function for DCL4 in generating viral siRNAs. *EMBO Journal*, **25**, 3347–3356.

Branscheid A., Devers E.A., May P., Krajinski F. (2011) Distribution pattern of small RNA and degradome reads provides information on miRNA gene structure and regulation. *Plant Signaling & Behavior*, **6**, 1609–1611.

Branscheid A., Sieh D., Pant B.D., May P., Devers E.A., Elkrog A., Schauser L., Scheible W.R., Krajinski F. (2010) Expression pattern suggests a role of MiR399 in the regulation of the cellular response to local pi increase during arbuscular mycorrhizal symbiosis. *Molecular Plant-Microbe Interactions*, **23**, 915–926.

Brioudes F., Jay F., Sarazin A., Grentzinger T., Devers E.A., Voinnet O. (2021) HASTY, the Arabidopsis EXPORTIN5 ortholog, regulates cell-to-cell and vascular microRNA movement. *EMBO Journal*, **40**, e107455.

Buddeweg A., Sharma K., Urlaub H., Schmitz R.A. (2018) sRNA(41) affects ribosome binding sites within polycistronic mRNAs in Methanosarcina mazei Gö1. *Molecular Microbiology*, **107**, 595–609.

Busch A., Richter A.S., Backofen R. (2008) IntaRNA: Efficient prediction of bacterial sRNA targets incorporating target site accessibility and seed regions. *Bioinformatics*, **24**, 2849–2856.

Cai Z., Wang Y., Zhu L., Tian Y., Chen L., Sun Z., Ullah I., Li X. (2017) GmTIR1/GmAFB3-based auxin perception regulated by miR393 modulates soybean nodulation. *New Phytologist*, **215**, 672–686.

Cambiagno D.A., Giudicatti A.J., Arce A.L., Gagliardi D., Li L., Yuan W., Lundberg D.S., Weigel D., Manavella P.A. (2021) HASTY modulates miRNA biogenesis by linking pri-miRNA transcription and processing. *Molecular Plant*, **14**, 426–439. https://doi.org/10.1016/j.molp.2020.12.019

Carlsbecker A., Lee J.Y., Roberts C.J., Dettmer J., Lehesranta S., Zhou J., Lindgren O., Moreno-Risueno M.A., Vatén A., Thitamadee S., Campilho A., Sebastian J., Bowman J.L., Helariutta Y., Benfey P.N. (2010) Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature*, **465**, 316–321.

Chen X. (2004) A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science*, **303**, 2022–2025.

Combier J.P., Frugier F., de Billy F., Boualem A., El-Yahyaoui F., Moreau S., Vernié T., Ott T., Gamas P., Crespi M., Niebel A. (2006) MtHAP2-1 is a key transcriptional regulator of symbiotic nodule development regulated by microRNA169 in Medicago truncatula. *Genes & Development*, **20**, 3084–3088.

Cui J., You C., Chen X. (2017) The evolution of microRNAs in plants. *Current Opinion in Plant Biology*, **35**, 61–67.

Cuperus J.T., Fahlgren N., Carrington J.C. (2011) Evolution and functional diversification of MIRNA genes. *The Plant Cell*, **23**, 431–442.

de la Rosa C., Covarrubias A.A., Reyes J.L. (2019) A dicistronic precursor encoding miR398 and the legume-specific miR2119 coregulates CSD1 and

ADH1 mRNAs in response to water deficit. *Plant, Cell & Environment*, **42**, 133–144.
De Luis A., Markmann K., Cognat V., Holt D.B., Charpentier M., Parniske M., Stougaard J., Voinnet O. (2012) Two microRNAs linked to nodule infection and nitrogen-fixing ability in the legume Lotus japonicus. *Plant Physiology*, **160**, 2137–2154.
Denzler R., McGeary S.E., Title A.C., Agarwal V., Bartel D.P., Stoffel M. (2016) Impact of MicroRNA levels, target-site complementarity, and cooperativity on competing endogenous RNA-regulated gene expression. *Molecular Cell*, **64**, 565–579.
Devers E.A., Branscheid A., May P., Krajinski F. (2011) Stars and Symbiosis: MicroRNA- and MicroRNA*-mediated transcript cleavage involved in arbuscular mycorrhizal Symbiosis. *Plant Physiology*, **156**, 1990–2010.
Fei Q., Yu Y., Liu L., Zhang Y., Baldrich P., Dai Q., Chen X., Meyers B.C. (2018) Biogenesis of a 22-nt microRNA in Phaseoleae species by precursor-programmed uridylation. *Proceedings of the National Academy of Sciences*, **115**, 8037–8042. https://doi.org/10.1073/pnas.1807403115
Felippes F.F., Schneeberger K., Dezulian T., Huson D.H., Weigel D. (2008) Evolution of Arabidopsis thaliana microRNAs from random sequences. *RNA*, **14**, 2455–2459. https://doi.org/10.1261/rna.1149408
Formey D., Martín-Rodríguez J., Leija A., Santana O., Quinto C., Cárdenas L., Hernández G. (2016) Regulation of small RNAs and corresponding targets in nod factor-induced *Phaseolus vulgaris* root hair cells. *International Journal of Molecular Sciences*, **17**, 887–901. https://doi.org/10.3390/ijms17060887
Gautrat P., Laffont C., Frugier F. (2020) Compact root architecture 2 promotes root competence for nodulation through the miR2111 systemic effector. *Current Biology*, **30**, 1339–1345.
Gomaa E., Elhadidy G. (2020) Preparation of functional foods free of gluten for celiac disease patients. *Journal of Sustainable Agricultural Sciences*, **46**, 13–24.
Gómez-Lozano M., Marvig R.L., Molin S., Long K.S. (2012) Genome-wide identification of novel small RNAs in Pseudomonas aeruginosa. *Environmental Microbiology*, **14**, 2006–2016.
Gramzow L., Lobbes D., Innard N., Theißen G. (2020) Independent origin of MIRNA genes controlling homologous target genes by partial inverted duplication of antisense-transcribed sequences. *The Plant Journal*, **101**, 401–419.
Guo G., Liu X., Sun F., Cao J., Huo N., Wuda B., Xin M., Hu Z., du J., Xia R., Rossi V., Peng H., Ni Z., Sun Q., Yao Y. (2018) Wheat miR9678 affects seed germination by generating phased siRNAs and modulating abscisic acid/gibberellin signaling. *The Plant Cell*, **30**, 796–814.
Holmqvist E., Wagner E.G.H. (2017) Impact of bacterial sRNAs in stress responses. *Biochemical Society Transactions*, **45**, 1203–1212.
Holt D.B., Gupta V., Meyer D., Abel N.B., Andersen S.U., Stougaard J., Markmann K. (2015) Micro RNA 172 (miR172) signals epidermal infection and is expressed in cells primed for bacterial invasion in Lotus japonicus roots and nodules. *New Phytologist*, **208**, 241–256.
Hossain M.S., Hoang N.T., Yan Z., Tóth K., Meyers B.C., Stacey G. (2019) Characterization of the spatial and temporal expression of two soybean miRNAs identifies SCL6 as a novel regulator of soybean nodulation. *Frontiers in Plant Science*, **10**, 1–14.
Hu J., Wang Q. (2018) Regulatory sRNAs in cyanobacteria. *Frontiers in Microbiology*, **9**, 2399.
Jäger D., Sharma C.M., Thomsen J., Ehlers C., Vogel J., Schmitz R.A. (2009) Deep sequencing analysis of the Methanosarcina mazei Gö1 transcriptome in response to nitrogen availability. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 21878–21882.
Jaschinski K., Babski J., Lehr M., Burmester A., Benz J., Heyer R., Dörr M., Marchfelder A., Soppa J. (2014) Generation and phenotyping of a collection of sRNA gene deletion mutants of the haloarchaeon Haloferax volcanii. *PLoS One*, **9**, e90763.
Jin H.Y., Oda H., Chen P., Yang C., Zhou X., Kang S.G., Valentine E., Kefauver J.M., Liao L., Zhang Y., Gonzalez-Martin A., Shepherd J., Morgan G.J., Mondala T.S., Head S.R., Kim P.H., Xiao N., Fu G., Liu W.H., Han J., Williamson J.R., Xiao C. (2017) Differential sensitivity of target genes to translational repression by miR-17~92. *PLoS Genetics*, **13**, e1006623.
Joshi T., Yan Z., Libault M., Jeong D.H., Park S., Green P.J., Sherrier D.J., Farmer A., May G., Meyers B.C., Xu D., Stacey G. (2010) Prediction of novel miRNAs and associated target genes in *Glycine max*. *BMC Bioinformatics*, **11**(Suppl 1), S14. https://doi.org/10.1186/1471-2105-11-S1-S14
Kavita K., de Mets F., Gottesman S. (2018) New aspects of RNA-based regulation by Hfq and its partner sRNAs. *Current Opininon in Microbiology*, **42**, 53–61.
Koenen E.J.M., Ojeda D.I., Bakker F.T., Wieringa J.J., Kidner C., Hardy O.J., Pennington R.T., Herendeen P.S., Bruneau A., Hughes C.E. (2021) The origin of the legumes is a complex paleopolyploid phylogenomic tangle closely associated with the cretaceous-Paleogene (K-Pg) mass extinction event. *Systematic Biology*, **70**, 508–526.
Kurihara Y., Takashi Y., Watanabe Y. (2006) The interaction between DCL1 and HYL1 is important for efficient and precise processing of pri-miRNA in plant microRNA biogenesis. *RNA*, **12**, 206–212.
Kuska B. (1998) Should scientists scrap the notion of junk DNA? *Journal of the National Cancer Institute*, **90**, 1032–1033.
Lauressergues D., Delaux P.M., Formey D., Lelandais-Brière C., Fort S., Cottaz S., Bécard G., Niebel A., Roux C., Combier J.P. (2012) The microRNA miR171h modulates arbuscular mycorrhizal colonization of Medicago truncatula by targeting NSP2. *Plant Journal*, **72**, 512–522. https://doi.org/10.1111/j.1365-313x.2012.05099.x
Ledford W.C., Silvestri A., Fiorilli V., Roth R., Rubio-Somoza I., Lanfranco L. (2024) A journey into the world of small RNAs in the arbuscular mycorrhizal symbiosis. *New Phytologist*, **242**, 1534–1544.
Lenoir I., Fontaine J., Lounès-Hadj Sahraoui A. (2016) Arbuscular mycorrhizal fungal responses to abiotic stresses: A review. *Phytochemistry*, **123**, 4–15.
Li A., Mao L. (2007) Evolution of plant microRNA gene families. *Cell Research*, **17**, 212–218.
Li H., Deng Y., Wu T., Subramanian S., Yu O. (2010) Misexpression of miR482, miR1512, and miR1515 increases soybean nodulation. *Plant Physiology*, **153**, 1759–1770.
Liu B., Chen Z., Song X., Liu C., Cui X., Zhao X., Fang J., Xu W., Zhang H., Wang X., Chu C., Deng X., Xue Y., Cao X. (2007) Oryza sativa dicer-like4 reveals a key role for small interfering RNA silencing in plant development. *The Plant Cell*, **19**, 2705–2718.
Liu B., Li P., Li X., Liu C., Cao S., Chu C., Cao X. (2005) Loss of function of OsDCL1 affects microRNA accumulation and causes developmental defects in rice. *Plant Physiology*, **139**, 296–305. https://doi.org/10.1104/pp.105.063420
Liu W., Kohlen W., Lillo A., Op den Camp R., Ivanov S., Hartog M., Limpens E., Jamil M., Smaczniak C., Kaufmann K., Yang W.C., Hooiveld G.J., Charnikhova T., Bouwmeester H.J., Bisseling T., Geurts R. (2011) Strigolactone biosynthesis in Medicago truncatula and rice requires the symbiotic GRAS-type transcription factors NSP1 and NSP2. *The Plant Cell*, **23**, 3853–3865.
Liu Y., Ke L., Wu G., Xu Y., Wu X., Xia R., Deng X., Xu Q. (2017) miR3954 is a trigger of phasiRNAs that affects flowering time in citrus. *The Plant Journal*, **92**, 263–275.
Liu Y., Teng C., Xia R., Meyers B.C. (2020) PhasiRNAs in plants: Their biogenesis, genic sources, and roles in stress responses, development, and reproduction. *The Plant Cell*, **32**, 3059–3080.
Llave C., Xie Z., Kasschau K.D., Carrington J.C. (2002) Cleavage of scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. *Science*, **297**, 2053–2056.
Lu S. (2019) De novo origination of MIRNAs through generation of short inverted repeats in target genes. *RNA Biology*, **16**, 846–859.
Luginbuehl L.H., Oldroyd G.E.D. (2017) Understanding the arbuscule at the heart of endomycorrhizal symbioses in plants. *Current Biology*, **27**, R952–R963.
Luján-Soto E., de la Aguirre Cruz P.I., Juárez-González V.T., Reyes J.L., Sanchez M.P., Dinkova T.D. (2022) Transcriptional regulation of zma-MIR528a by action of nitrate and auxin in maize. *International Journal of Molecular Sciences*, **23**, 15718–15738.
Luján-Soto E., Juárez-González V.T., Reyes J.L., Dinkova T.D. (2021) MicroRNA Zma-miR528 versatile regulation on target mRNAs during maize somatic embryogenesis. *International Journal of Molecular Sciences*, **22**, 5310–5325.
Lyu Y., Liufu Z., Xiao J., Tang T. (2021) A rapid evolving microRNA cluster rewires its target regulatory networks in drosophila. *Frontiers in Genetics*, **12**, 760530–760541.
Manavella P.A., Koenig D., Weigel D. (2012) Plant secondary siRNA production determined by microRNA-duplex structure. *Proceedings of the National Academy of Sciences*, **109**, 2461–2466.
Mao X.F., Cao Y.C. (2016) MicroRNA analysis in model species based on evolutionary rates. *Genetics and Molecular Research: GMR*, **15**, 1–14. https://doi.org/10.4238/gmr.15017216
Martín-Rodríguez J.A., Ariani A., Leija A., Elizondo A., Fuentes S.I., Ramirez M., Ángel Martín-Rodríguez J., Gepts P., Hernández G., Formey D. (2021) Phaseolus vulgaris MIR1511 genotypic variations differentially regulate plant tolerance to aluminum toxicity. *The Plant Journal*, **105**, 1521–1533. https://doi.org/10.1111/tpj.15129
Medendorp J., DeYoung D., Thiagarajan D.G., Duckworth R., Pittendrigh B. (2022). In: Siddiq M., Uebersax M.A. (Eds), *Dry beans and pulses: Production, processing, and nutrition*. Wiley, Hoboken, NJ, pp 505–550.
Messina V. (2014) Nutritional and health benefits of dried beans. *The American Journal of Clinical Nutrition*, **100**, 437–442.
Naqvi A.R., Sarwat M., Hasan S., Roychodhury N. (2012) Biogenesis, functions and fate of plant microRNAs. *Journal of Cellular Physiology*, **227**, 3163–3168.

Navarro L., Dunoyer P., Jay F., Arnold B., Dharmasiri N., Estelle M., Voinnet O., Jones J.D. (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science*, **312**, 436–439.

Nova-Franco B., Íñiguez L.P., Valdés-López O., Alvarado-Affantranger X., Leija A., Fuentes S.I., Ramírez M., Paul S., Reyes J.L., Girard L., Hernández G. (2015) The micro-RNA72c-APETALA2-1 node as a key regulator of the common bean-rhizobium *etli* nitrogen fixation symbiosis. *Plant Physiology*, **168**, 273–291.

Ohno S. (1972) So much "junk" DNA in our genome. *Brookhaven Symposia in Biology*, **23**, 366–370.

Okuma N., Soyano T., Suzaki T., Kawaguchi M. (2020) MIR2111-5 locus and shoot-accumulated mature miR2111 systemically enhance nodulation depending on HAR1 in Lotus japonicus. *Nature Communications*, **11**, 5192–5205.

Oldroyd G.E., Murray J.D., Poole P.S., Downie J.A. (2011) The rules of engagement in the legume-rhizobial symbiosis. *Annual Review of Genetics*, **45**, 119–144.

Palatnik J.F., Wollmann H., Schommer C., Schwab R., Boisbouvier J., Rodriguez R., Warthmann N., Allen E., Dezulian T., Huson D., Carrington J.C., Weigel D. (2007) Sequence and expression differences underlie functional specialization of Arabidopsis microRNAs miR159 and miR319. *Developmental Cell*, **13**, 115–125.

Park M.Y., Wu G., Gonzalez-Sulser A., Vaucheret H., Poethig R.S. (2005) Nuclear processing and export of microRNAs in Arabidopsis. *Proceedings of the National Academy of Sciences*, **102**, 3691–3696.

Park W., Li J., Song R., Messing J., Chen X. (2002) CARPEL FACTORY, a dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in Arabidopsis thaliana. *Current Biology*, **12**, 1484–1495.

Parniske M. (2008) Arbuscular mycorrhiza: The mother of plant root endosymbioses. *Nature Reviews Microbiology*, **6**, 763–775.

Pasquinelli A.E., Reinhart B.J., Slack F., Martindale M.Q., Kuroda M.I., Maller B., Hayward D.C., Ball E.E., Degnan B., Müller P., Spring J., Srinivasan A., Fishman M., Finnerty J., Corbo J., Levine M., Leahy P., Davidson E., Ruvkun G. (2000) Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature*, **408**, 86–89.

Patanun O., Lertpanyasampatha M., Sojikul P., Viboonjun U., Narangajavana J. (2013) Computational identification of microRNAs and their targets in cassava (Manihot esculenta Crantz.). *Molecular Biotechnology*, **53**, 257–269.

Pei L.L., Zhang L.L., Liu X., Jiang J. (2023) Role of microRNA miR171 in plant development. *PeerJ*, **11**, e15632.

Persson K.P., Ekehed S., Otter C., Lutz E.S., McPheat J., Masimirembwa C.M., Lutz E.S.M., Andersson T.B. (2006) Evaluation of human liver slices and reporter gene assays as systems for predicting the cytochrome p450 induction potential of drugs in vivo in humans. *Pharmaceutical Research*, **23**, 56–69. https://doi.org/10.1007/s11095-005-8812-5

Piriyapongsa J., Jordan I.K. (2008) Dual coding of siRNAs and miRNAs by plant transposable elements. *RNA*, **14**, 814–821.

Prasse D., Ehlers C., Backofen R., Schmitz R.A. (2013) Regulatory RNAs in archaea: First target identification in Methanoarchaea. *Biochemical Society Transactions*, **41**, 344–349.

Prasse D., Förstner K.U., Jäger D., Backofen R., Schmitz R.A. (2017) sRNA(154) a newly identified regulator of nitrogen fixation in Methanosarcina mazei strain Gö1. *RNA Biology*, **14**, 1544–1558.

Qin Z., Li C., Mao L., Wu L. (2014) Novel insights from non-conserved microRNAs in plants. *Frontiers in Plant Science*, **5**, 586.

Rajagopalan R., Vaucheret H., Trejo J., Bartel D.P. (2006) A diverse and evolutionarily fluid set of microRNAs in Arabidopsis thaliana. *Genes & Development*, **20**, 3407–3425.

Ramalingam P., Palanichamy J.K., Singh A., Das P., Bhagat M., Kassab M.A., Sinha S., Chattopadhyay P. (2014) Biogenesis of intronic miRNAs located in clusters by independent transcription and alternative splicing. *RNA*, **20**, 76–87.

Ruan Q., Bai X., Wang Y., Zhang X., Wang B., Zhao Y., Zhu X., Wei X. (2024) Regulation of endogenous hormone and miRNA in leaves of alfalfa (*Medicago sativa* L.) seedlings under drought stress by endogenous nitric oxide. *BMC Genomics*, **25**, 229–248.

Samynathan R., Venkidasamy B., Shanmugam A., Ramalingam S., Thiruvengadam M. (2023) Functional role of microRNA in the regulation of biotic and abiotic stress in agronomic plants. *Frontiers in Genetics*, **14**, 1272446.

Sanan-Mishra N., Abdul Kader Jailani A., Mandal B., Mukherjee S.K. (2021) Secondary siRNAs in plants: Biosynthesis, various functions, and applications in virology. *Frontiers in Plant Science*, **12**, 610283–610345.

Schlötterer C. (2015) Genes from scratch the evolutionary fate of de novo genes. *Trends in Genetics: TIG*, **31**, 215–219.

Sexauer M., Bhasin H., Schön M., Roitsch E., Wall C., Herzog U., Markmann K. (2023) A micro RNA mediates shoot control of root branching. *Nature Communications*, **14**, 8083–8092.

Shi J., Zhou T., Chen Q. (2022) Exploring the expanding universe of small RNAs. *Nature Cell Biology*, **24**, 415–423.

Singh A.K., Singh N., Kumar S., Kumari J., Singh R., Gaba S., Yadav M.C., Grover M., Chaurasia S., Kumar R. (2020) Identification and evolutionary analysis of polycistronic miRNA clusters in domesticated and wild wheat. *Genomics*, **112**, 2334–2348.

Sosa-Valencia G., Palomar M., Covarrubias A.A., Reyes J.L. (2017) The legume miR1514a modulates a NAC transcription factor transcript to trigger phasiRNA formation in response to drought. *Journal of Experimental Botany*, **68**, 2013–2026. https://doi.org/10.1093/jxb/erw380

Sosa-Valencia G., Romero-Pérez P.S., Palomar V.M., Covarrubias A.A., Reyes J.L. (2017) Insights into the function of the phasiRNA-triggering miR1514 in response to stress in legumes. *Plant Signaling & Behavior*, **12**, e1284724.

Sós-Hegedűs A., Domonkos Á., Tóth T., Gyula P., Kaló P., Szittya G. (2020) Suppression of NB-LRR genes by miRNAs promotes nitrogen-fixing nodule development in Medicago truncatula. *Plant, Cell & Environment*, **43**, 1117–1129.

Subramanian S., Fu Y., Sunkar R., Barbazuk W.B., Zhu J.K., Yu O. (2008) Novel and nodulation-regulated microRNAs in soybean roots. *BMC Genomics*, **9**, 160–174.

Thomason M.K., Fontaine F., De Lay N., Storz G. (2012) A small RNA that regulates motility and biofilm formation in response to changes in nutrient availability in Escherichia coli. *Molecular Microbiology*, **84**, 17–35. https://doi.org/10.1111/j.1365-2958.2012.07965.x

Tiwari M., Singh B., Yadav M., Pandey V., Bhatia S. (2021) High throughput identification of miRNAs reveal novel interacting targets regulating chickpea–hizobia symbiosis. *Environmental and Experimental Botany*, **186**, 104469.

Turner M., Yu O., Subramanian S. (2012) Genome organization and characteristics of soybean microRNAs. *BMC Genomics*, **13**, 169–185.

Vazquez F., Blevins T., Ailhas J., Boller T., Meins F., Jr. (2008) Evolution of Arabidopsis MIR genes generates novel microRNA classes. *Nucleic Acids Research*, **36**, 6429–6438. https://doi.org/10.1093/nar/gkn670

Vogel J. (2009) A rough guide to the non-coding RNA world of salmonella. *Molecular Microbiology*, **71**, 1–11.

Voinnet O. (2009) Origin, biogenesis, and activity of plant microRNAs. *Cell*, **136**, 669–687.

Wang S., Xu Y., Lu Z. (2014a) Genome-wide miRNA seeds prediction in Archaea. *Archaea*, **2014**, 671059–671065.

Wang T., Deng Z., Zhang X., Wang H., Wang Y., Liu X., Liu S., Xu F., Li T., Fu D., Zhu B., Luo Y., Zhu H. (2018) Tomato DCL2b is required for the biosynthesis of 22-nt small RNAs, the resulting secondary siRNAs, and the host defense against ToMV. *Horticulture Research*, **5**, 62–76. https://doi.org/10.1038/s41438-018-0073-7

Wang Y., Li K., Chen L., Zou Y., Liu H., Tian Y., Li D., Wang R., Zhao F., Ferguson B.J., Gresshoff P.M., Li X. (2015) MicroRNA167-directed regulation of the auxin response factors GmARF8a and GmARF8b is required for soybean nodulation and lateral root development. *Plant Physiology*, **168**, 984–999.

Wang Y., Li P., Cao X., Wang X., Zhang A., Li X. (2009) Identification and expression analysis of miRNAs from nitrogen-fixing soybean nodules. *Biochemical and Biophysical Research Communications*, **378**, 799–803.

Wang Y., Wang L., Zou Y., Chen L., Cai Z., Zhang S., Zhao F., Tian Y., Jiang Q., Ferguson B.J., Gresshoff P.M. (2014b) Soybean miR172c targets the repressive AP2 transcription factor NNC1 to activate ENOD40 expression and regulate nodule initiation. *Plant Cell*, **26**, 4782–4801.

Wang Z., Hardcastle T.J., Canto Pastor A., Yip W.H., Tang S., Baulcombe D.C. (2018) A novel DCL2-dependent miRNA pathway in tomato affects susceptibility to RNA viruses. *Genes & Development*, **32**, 1155–1160.

Wu G., Park M.Y., Conway S.R., Wang J.W., Weigel D., Poethig R.S. (2009) The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. *Cell*, **138**, 750–759.

Wu L., Zhou H., Zhang Q., Zhang J., Ni F., Liu C., Qi Y. (2010) DNA methylation mediated by a microRNA pathway. *Molecular Cell*, **38**, 465–475.

Xia R., Chen C., Pokhrel S., Ma W., Huang K., Patel P., Wang F., Xu J., Liu Z., Li J., Meyers B.C. (2019) 24-nt reproductive phasiRNAs are broadly present in angiosperms. *Nature Communications*, **10**, 627–635. https://doi.org/10.1038/s41467-019-08543-0

Xia R., Xu J., Meyers B.C. (2017) The emergence, evolution, and diversification of the miR390-TAS3-ARF pathway in land plants. *The Plant Cell*, **29**, 1232–1247.

Xie Z., Allen E., Fahlgren N., Calamar A., Givan S.A., Carrington J.C. (2005) Expression of Arabidopsis MIRNA genes. *Plant Physiology*, **138**, 2145–2154.

Xie Z., Allen E., Wilken A., Carrington J.C. (2005) DICER-LIKE 4 functions in trans-acting small

interfering RNA biogenesis and vegetative phase change in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences*, **102**, 12984–12989.

Xu N., Li Y., Zhao Y.T., Guo L., Fang Y.Y., Zhao J.H., Wang X.J., Huang L., Guo H.S. (2012) Identification and characterization of small RNAs in the hyperthermophilic archaeon Sulfolobus solfataricus. *PLoS One*, **7**, e35306. https://doi.org/10.1371/journal.pone.0035306

Yan Z., Hossain M.S., Valdés-López O., Hoang N.T., Zhai J., Wang J., Libault M., Brechenmacher L., Findley S., Joshi T., Qiu L., Sherrier D.J., Ji T., Meyers B.C., Xu D., Stacey G. (2016) Identification and functional characterization of soybean root hair microRNAs expressed in response to Bradyrhizobium japonicum infection. *Plant Biotechnology Journal*, **14**, 332–341.

Yang S., Tang F., Gao M., Krishnan H.B., Zhu H. (2010) R gene-controlled host specificity in the legume-rhizobia symbiosis. *Proceedings of the National Academy of Sciences*, **107**, 18735–18740.

Yang Z., Vilkaitis G., Yu B., Klimašauskas S., Chen X. (2007) Approaches for studying microRNA and small interfering RNA methylation in vitro and in vivo, *Methods in enzymology 427*. Academic Press, California, USA, pp 304.

Yu Y., Zhang Y., Chen X., Chen Y. (2019) Plant noncoding RNAs: Hidden players in development and stress responses. *Annual Review of Cell and Developmental Biology*, **35**, 407–431.

Yuan S., Li Z., Li D., Yuan N., Hu Q., Luo H. (2015) Constitutive expression of Rice MicroRNA528 alters plant development and enhances tolerance to salinity stress and nitrogen starvation in creeping Bentgrass. *Plant Physiology*, **169**, 576–593.

Yun J., Wang C., Zhang F., Chen L., Sun Z., Cai Y., Luo Y., Liao J., Wang Y., Cha Y., Zhang X., Ren Y., Wu J., Hasegawa P.M., Tian C., Su H., Ferguson B.J., Gresshoff P.M., Hou W., Han T., Li X. (2023) A nitrogen fixing symbiosis-specific pathway required for legume flowering. *Science Advances*, **9**, eade1150-1164. https://doi.org/10.1126/sciadv.ade1150

Zhai J., Zhang H., Arikit S., Huang K., Nan G.L., Walbot V., Meyers B.C. (2015) Spatiotemporally dynamic, cell-type-dependent premeiotic and meiotic phasiRNAs in maize anthers. *Proceedings of the National Academy of Sciences*, **112**, 3146–3151. https://doi.org/10.1073/pnas.1418918112

Zhang B., You C., Zhang Y., Zeng L., Hu J., Zhao M., Chen X. (2020) Linking key steps of microRNA biogenesis by TREX-2 and the nuclear pore complex in Arabidopsis. *Nature Plants*, **6**, 957–969.

Zhang F., Yang J., Zhang N., Wu J., Si H. (2022) Roles of microRNAs in abiotic stress response and characteristics regulation of plant. *Frontiers in Plant Science*, **13**, 1–21.

Zhang M., Su H., Gresshoff P.M., Ferguson B.J. (2021) Shoot-derived miR2111 controls legume root and nodule development. *Plant, Cell & Environment*, **44**, 1627–1641.