Chapter

Role of Non-Coding RNAs in Plant Nutrition Through Mycorrhizal Interactions

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Abstract

In nature, many plants rely on symbiotic interaction with mycorrhizae for their nutrition and survival. For instance, nitrogen-fixing nodules and mycorrhizae are well established mutualistic biotic interactions between plants and bacterial/fungal partners under nitrogen limiting environment. Many small regulatory components of RNA like micro-RNAs play a critical role in establishment of these symbioses. These regulatory components are also crucial for balancing hormone levels, and synchronization of plant defenses and development pathways. However, functions of various sRNAs are still need to be addressed. This chapter will detailed out various important parts these regulatory components (sRNA, miRNA and siRNA) are playing during mycorrhizal interactions for plant growth, development and nutrition.

Keywords: miRNA, siRNA, mycorrhiza, nodulation, symbiosis, nutrient uptake

1. Introduction

During course of co-evolution since millions of years, plants have established symbiotic associations with the fungi and bacteria. Established mycorrhizal and rhizobia symbiosis with the plants are the best illustrated examples of such interactions. These symbiotic associations are entrenched by the molecular cross-talk including correct recognition and specific activation/repression of signaling pathways. Legume-rhizobia interactions are specific in terms of molecular cross talk, as the host plant secretes flavonoids which are perceived by compatible rhizobia for the induction, expression and activation of *Nod* genes in the bacteria, necessary for the nodule formation in the host plant. The secreted Nod factors once recognized by host specific intra-cellular kinase and extra-cellular LysM domain containing receptors, a cascade of cytoplasmic events starts within root epidermal cells. Depolarization of the membrane, alteration in calcium levels and induction of calmodulin based kinase signaling makes favorable environment for rhizobia infection thread formation and successive penetration of plant host cell through branching. Subsequently, 'Bacteroids' formation and nitrogen fixation initiates in host cell cytoplasm. In contrast, mycorrhizal interactions are not specific in terms of host range as they can colonize almost all terrestrial plants [1]. Although the signaling pathway for

mycorrhizal symbiosis activation shares some attributes of rhizobial symbiosis events, induction and reprogramming of the host cells starts after the recognition of myc-LCO (mycorrhizal lipo-chito-oligosaccharide), which leads to altered metabolic cascade in host and hyphae as well [2]. This molecular cross-talk establishes nutrients and mineral transport through specialized and branched structures called 'Arbuscules' from Arbuscular mycorrhizal (AM) partner and photo-synthetically fixed carbon sources mobilization from host plant in exchange. To bear an invasion of microorganisms, plants must have some specialized mechanisms to distinguish beneficial microbes from harmful ones. Since last few decades, we are learning about regulators of fine tuning among symbiotic associations and plant immunity [3, 4]. Contributions of non-coding RNAs (ncRNAs) in this regulation of host defenses to establish symbiosis are indispensible according to recent studies [5].

In this chapter, we have summarized the genesis of various important classes of non-coding RNAs and their role in nutrient uptake, transport, assimilation and homeostasis in plants via mycorrhizal symbiosis, and discuss the recent discoveries of cross-kingdom RNA interference (RNAi) during plant-fungus interactions. We also provide the insights and future perspectives for improved understanding of mycorrhizal associations, which would aid in the development of innovative strategies for enhancing the crop yield.

2. Genesis of non-coding RNAs and classification

Currently, a large number of endogenously formed ncRNAs involved in different regulatory functions have been discovered and functionally characterized in various plant species [6, 7]. On account of their average size, the regulatory ncRNAs can be classified into sRNAs (small RNAs of typically 18–30 nt in size), medium-sized ncRNAs (broad range of 31–200 nt), and more than 200 nt sized Long-non-coding RNAs (lncRNAs). Furthermore, depending on their morphology, theycan be classified as linear or circular (circRNA). Recently, small regulatory RNAs (sRNAs), miRNAs and small interfering (si)RNAs, have been well characterized with respect to plant immunity and symbiosis. Although thought to be small, they play vital functions in response to the biotic, abiotic stress and environmental fluctuations by regulation/modulation of target genes expression [8–11]. Similarly, lncRNAs were considered transcriptional noises, but later attracted attention for the heterogeneous groups of ncRNAs and long range [12]. Remarkably, unlike other linearly regulated ncRNAs, the newly discovered circRNAs belong to a novel class, which lacks free 5′ and 3′ end [13]. In addition, many small ncRNAs, which are derivatives of tRNAs, which are identified and characterized in plants typically comprised of 15–42 nt, termed as tsRNAs [14–16]. The tsRNAs are also classified as regulatory ncRNAs for multiple functions. Generally, the functions of certain ncRNA are similar, but some differ and overlap in silencing signaling pathways [17].

As reviewed by Chao et al. [18], the biogenesis of miRNAs is a multi-step procedure which involves transcription, processing, alterations, and then RNA-induced silencing (RISC) complex assembly. First, a pri-miRNA (primary miRNA) is transcribed from RNA Polymerase II containing a hairpin RNA secondary structure. Next, the base pri-miRNA hairpin is then cleaved by a DICER Like RNase-III family enzyme (usually DCL1). To release miRNA-miRNA* duplex, these hairpins are cleaved again and subsequently methylated (at 2′O- position) by HUA Enhancer 1 (HEN1) nuclear protein for the stability. Finally, in nucleus the mature miRNA

strand enters into AGO1 to form miRNA-AGO1 complex, which are then transported to cytoplasm leaving behind cleaved miRNA* fragment for the induction of post-transcriptional gene silencing.

Depending on their mechanism of action, siRNAs can further be classified into three major sub-categories: (1) native antisense siRNAs (nat-siRNAs), (2) heterochromatin siRNAs (hc-siRNAs), and (3) trans-acting siRNAs (ta-siRNAs). ta-siRNA is generated from the TAS gene which is transcribed from RNA Pol II into single-stranded RNA and loses its cap and poly-A tail during miRNA-AGO1 complex-controlled cleavage [8, 19]. Later, the 5′ or 3′ cleaved fragments are end protected by the suppressor of gene silencing 3 (SGS3) protein and transformed into double-stranded RNA (dsRNA) via RDRP-VI [20]. Finally, by HEN1 and DCL activities they are methylated and processed to form ta-siRNAs (21–24 nt). To participate in post-transcriptional modulation/silencing of target genes by pairing with its complementary mRNAs, these 21–24 nt sized strands are integrated with AGO1/AGO7 present in the cytoplasm, whereas a few ta-siRNAs are loaded onto AGO4/6 for assisting methylation of TAS genes via RNA Pol V.

tsRNAs, with a wide size range (15–42 nt), represent a unique ncRNA class that can be sub-categorized based on their cleavage sites: (1) tRF-1 s, (2) tRF-2 s, (3) tRF-3 s, (4) tRF-5 s, and (5) tiRNAs. However, plant research is still in its infancy and many questions remain unanswered in reference to its existence. For instance, the biosynthetic pathway for tsRNAs and their regulatory or physiological roles in plants are still very limited [21].

CircRNAs are known as RNA biomolecules which are circular, covalently closed and single-stranded [22]. They were first identified and characterized from plant viruses by Sanger and colleagues in 1976. The organization of circRNAs can be divided into three groups [23]. (1) The Exon circRNAs are generated by the circularization of lariat-derived and intron pairing, (2) the intronic circRNAs are formed by the partial intron degradation after lasso structure formation; and (3) exo-syntronic circRNAs are composed of exons as well as introns, and circularized during the splicing process.

lncRNAs biogenesis can be categorized into five major types in accordance to the sites being transcribed via RNA-Pol II. (1) The antisense lncRNA is transcribed over the complementary strand of the exon; whereas (2) sense lncRNA is transcribed on the same strand as the exon. As name indicates, (3) Intron lncRNA is transcribed into an intron. (4) The Inter-genic lncRNAs are situated between two different genes and (5) the enhancer lncRNA mostly arises from the enhancer region of the protein-encoding gene [24]. They can control target regulation in a variety of ways, including chromatin re-modeling, transcriptional repression, splicing of RNA and its transcriptional enhancers. Additionally, lncRNAs can code for certain small peptides required for various cellular processes [25]. Notably, several lncRNAs are regulated under abiotic/biotic stresses in the plants.

3. Role of sRNAs in plant nutrition

Induction of miRNAs regulates the expression of an array of genes and promotes plant nutritional homeostasis. Owing high-throughput RNASeq techniques and target prediction tools the role of ncRNAs in nutrition and stress signaling has been investigated in recent past. Majorly the role of ncRNAs in nitrogen (N), phosphate (Pi) and sulfur (S) homeostasis has been discussed below:

3.1 Nitrogen

Evidence for miRNAs controlling nitrogen responses in plants has been illustrated [26, 27]. Up-regulation of pri-miRNA156, pri-miRNA447c and down-regulation of pri-miRNA169 and pri-miR398a has been characterized in Arabidopsis under nitrogen-deficient conditions [28]. Expression of nitrogen responsive miRNA, like miRNA160, miRNA167, miRNA168 in the maize roots and miRNA164, miRNA171 in shoots whereas, miRNA169 in both are reported under nitrogen-limiting conditions [27]. Similarly, several nitrogen-responsive miRNAs have been investigated in legumes, for instance, a total of altered expressions of 168 miRNAs are reported in limiting-nitrogen-tolerant and limiting-nitrogen-sensitive genotype of soybean using RNASeq [26]. A down-regulation of miRNA2606a/b-3p and up-regulation of miRNA1512a-5p was found in limiting-nitrogen-tolerant and limiting-nitrogen-sensitive genotype respectively. Moreover, mRNA transcripts encoding Cathepsin and E3-Ubiquitin ligase protein were found to be targeted and degraded by miRNA396b/g-5p and miRNA156b/6f-5p respectively under nitrogen stress.

3.2 Phosphate

Phosphate-responsive sRNA involved in Pi-uptake, transport, assimilation and homeostasis through targeting mRNA transcripts are extensively studied and identified in plant including rice, maize, tomato, soybean and Arabidopsis [29–33]. Among these plant species, common set of plant miRNA families are characterized modulating signaling networks, including miRNA156, miRNA159, miRNA166, miRNA319, miRNA395, miRNA398, miRNA399, miRNA447, and miRNA827 are demonstrated in response to Pi-limiting environment [34, 35]. An elevated level of miRNA156, miRNA399, miRNA778, miRNA827, miRNA2111 and suppressed miRNA169, miRNA395 and miRNA398 levels are observed under Pi stress [28]. Role of miRNA2111 has been illustrated under N and Pi limiting conditions [36]. The expression of phosphate-responsive PHO2 transporter was altered by miRNA827 and miRNA399 [37]. Moreover, miRNA827 targets the Major Facility Superfamily (MFS)-XPS proteins which are involved in Pi sensing and transport [38]. Common response against nutrient starvation includes anthocyanin accumulation in plants. MYB TF regulated anthocyanin biosynthesis pathway genes are targeted by siRNAs produced by ta-siRNA4 under the regulation of Pi-responsive miRNA828, post-transcriptionally [39]. The major regulatory role in maintenance of mineral homeostasis in host plant under N, Pi and C limiting environments is performed by miRNA398a [40]. Among all the characterized Pi-responsive miRNAs, altered levels of different alleles of miRNA399 were found conserved and pre-dominant under Pi-limiting conditions [41]. miRNAs and siRNAs induction was reported upon *Candidatus liberibacter* infection in citrus plants and interestingly, miRNA399 level was found elevated in infected plants than healthy host under Pi-limiting conditions [33]. These facts demonstrate the critical role of sRNAs in post-transcription regulation of Pi-responsive transcripts enabling host adaption under nutrition stress.

3.3 Sulfate

Sulfate transporters located on root epidermal and cortical cell membrane are the key components in sulfur uptake and transport to the plants in $SO4^{2-}$ form. Based on their substrate affinity, sequence similarity and their location of expression, they

are categorized into five major groups. Group 1, group 2 and group 3 are characterized as high affinity, low affinity, and moderate affinity transporters for sulfur substrate, respectively [42–44]. Group 4 and 5 transporters are characterized as efflux transporter on tonoplast and molybdenum transporters (for being actively involved in molybdenum transport across the plant) respectively [45, 46]. S uptake from soil to the root is carried out by group1 and 2 transporters, while root to shoot transport of S is done by group 4 transporters. Under S-limiting conditions plethora of miRNAs induced including miRNA66, miRNA67 and miRNA395 while suppression of miRNA14, miRNA20 and miRNA43 is associated with regulation of post-transcription modification of S signaling. miRNA395 is a S-specific ncRNA signal and has been characterized to function as a key regulator of the sulfate depletion pathway. Under S-deprivation, miRNA395 positively regulates the expression of the low-affinity transporter AtSul2;1 [47], supporting sulfate uptake and transport of cells to shoots and leaves in *Arabidopsis thaliana*. The initial step of S assimilation into cysteine is catalyzed by ATP dependent sulphurylases (APSs), which are the target for miRNA395 in plastids [35].

4. Nutrient uptake/exchange during mutualistic plant-fungus interactions

One of the characteristics of the beneficial mycorrhizal interactions is the bidirectional nutrient exchange between both the partners and to support the growth of plant host [48, 49]. In these relationships, the fungus provides nitrogen, phosphate and sulfate nutrients to the host, whereas, in return, the host plant transfers photosynthetically fixed carbon (4–20%) to the mycorrhizal fungus [50]. In AM roots, the fungus proliferates into the root cortex intercellularly as well as intracellularly, whereas in case of ECM roots, it only covers intercellular regions, indicating the differences in the mechanism of colonization and nutrient uptake/exchange. The uptake of nutrients by plants from soil is limited by the repressed mobility of nutrients. Importantly, during AM symbiosis, the plant phosphate (P) transporters are down-regulated [51, 52]. Under these conditions, the nutrient such as P uptake is predominantly achieved through mycorrhizal pathway [1, 53, 54]. It has been observed that contribution of the mycorrhizal pathway in nutrient uptake varies with the plant and fungal partners that are involved in the interaction and also on the nutrient type [48, 52, 55–57]. To facilitate the nutrient uptake via mycorrhizal interface, the peri-arbuscular membrane (PAM) harbors high affinity transporters that are specifically induced in mycorrhizal roots. For instance, Pt4 and AMT2 are the high affinity transporters for P and ammonium ($NH4^+$) that mediate transfer of respective nutrients from fungus to plant host [58–60]. Moreover, a few mycorrhiza-inducible sulfate transporters have also been reported in AM roots [61, 62]. Recently, a sulfate transporter (SiSulT) and iron transporter (PiFtr) from *Serendipita indica* (previously known as *Piriformospora indica*) has been characterized, which transfers sulfur to the maize and iron to the rice plants, respectively and improves its growth [63, 64]. These studies highlight the importance of sulfur transport via mycorrhizal associations.

On the other hand, the plant transfers photosynthates as sucrose from source to ECM roots that serve as a carbon sink, which is then converted to simpler sugars such as glucose or fructose by invertase enzyme of host. The glucose and fructose are taken up by fungal counterparts through mycorrhizal interface. For instance, an arbuscular membrane localized monosaccharide transporter (MST2) is involved in the uptake of glucose and xylose molecules by AM fungi [65]. Intriguingly, host

carbon supply has been found to trigger the fungal gene expression, and P and N uptake during AM and ECM symbiosis [66–68]. This also leads to increased hydrolysis of polyP (an important source of P and N) and release of Pi and Arg in the fungal cytoplasm. The Arg is further broken down to $NH4^+$ and is transferred to host plant via mycorrhizal interface. Importantly, these transport processes, from host to fungus and from fungus to the host, involve diverse molecular players that mediate membrane transport for the nutrition exchange between mycorrhizal plants and fungus at the interaction interface. The membrane transporters and channels that mediate the transport of molecules such as P, N, S, K, sugar and water are collectively referred as the 'transportome' [69].

Moreover, several robust and tightly regulated signaling processes are involved in establishing the successful mycorrhizal colonization for the efficient exchange of nutrients between plant and fungus. Although, the regulatory processes of plant and fungus both are important during symbiosis, the major proportion of studies has focused on the regulation from plant's perspective. These regulators include a variety of non-coding RNAs, phytohormones, peptide signals, transcription factors such as *CYCLOPS* and *NODULATION SIGNALING PATHWAY* (NSP1 and NSP2) [70–72]. The *CYCLOPS*, NSP1 and NSP2 are conserved members of rhizobial and mycorrhizal symbiosis phenomenon. The detailed overview of transcriptional regulation of AM development has been provided by Pimprikar and Gutjahr [73]. The non-coding RNA mediated-regulation of mycorrhizal symbiosis is now gaining the scientist's attention and emerging as new area of research.

5. Plant's ncRNAs modulating interconnection networks with fungi

5.1 miRNA

In recent past decades, the focus has pushed beyond the traditional defense pathway's transcriptional control in establishing pathogenic or beneficial plant-microbe interactions to attempts to understand novel transcriptional regulators. In particular, many groups have started investigating the function of microRNAs (miRNAs) in regulating signaling processes accompanying the symbiotic interactions. Many plant miRNAs have been reported to be involved in modulating the plant-pathogenic microbe interactions. The majority of miRNAs investigated and characterized to date complements the alterations described well in the transcriptome. For example, one of the defenses against necrotrophic pathogens involves improving the physical tolerance of plant cells. *Arabidopsis* miRNA408 and miRNA160a induce physical cell reinforcement by positive regulation of lignification and callose deposition [74, 75]. In *Medicago* and *Oriza*, various miRNA targets the ET (Ethylene), JA (Jasmonic Acid), and SA (Salicylic Acid) biogenesis during pathogenesis at very early stages [76, 77]. During attack by biotrophic pathogens, many miRNAs also suppress routine cellular detoxification. One example is miRNA398, which enhances ROS generation in *Magnaporthe oryzae*-infected tissues [78]. On the other hand, in case of tomato, the necrotrophic association between plant and *Alternaria solani*, miRNAs target gene transcripts are reported to be actively involved in toxin detoxification [79]. This suggests that miRNAs have potential to modify plant cells into the toxic ecosystems, poisoning invading microbes before they spread further to strengthen host immunity.

In mutual bio-trophic interactions, a few recent investigations have shown that the a major proportion of miRNAs synthesized all through interaction establishment

modulate hormone response pathways, protein methylation, and functions of innate immunity components [78, 80]. A well-studied example includes the miRNA (annotated as E4D3Z3Y01BW0TQ) which is reported to be induced during AM symbiosis progression and interferes with GA signaling. GA signaling pathway is known to inhibit symbiotic-association [81–83]. On the other hand, miRNA172c promotes nodule formation in many plants by repressing the translation of APETALA2 TF [84, 85]. In AM-colonized roots, miRNA171b hampers with GRAS TF-responsive transcripts targeting through miRNA171, which are necessary for both nodulation and symbiosis.

5.2 siRNA

Microorganisms can have a significant impact on how plants react to colonization; they are not just background actors in the process. Since past few decades, effector proteins and siRNAs have been the two main areas of research. A variety of plant signaling pathways are altered by microbial effector proteins, which are generally tiny secreted proteins that are substantially stimulated during the colonization process. Similar to effector proteins, siRNAs target essential plant transcripts, disrupt transcripts via the ARGONAUTE (AGO) pathway, or act in a manner resembling that of miRNAs by inhibiting tRNA binding and localization. The microorganism-secreted siRNAs are taken up by the host plant, and disrupt the key transcripts of host.

Botrytis cinerea, a fungal pathogen, has been demonstrated to alter plant physiology during colonization by secreting siRNAs [86]. *B. cinerea* siRNAs initially penetrate host plant cells during the pathogenesis of tomato and *Arabidopsis*, where they diminish the host's RNAi apparatus. These relatively tiny molecules can therefore be thought of as variants of conventional effector proteins. In case of *A. thaliana*, *Bc*-siRNA3.1, *Bc*-siRNA3.2, and *Bc*-siRNA5 collectively silenced the stress-related genes, such as PRXIIF, WAK, MPK1 and MPK2 to eliminate the plant defense [87]. However, *Bc*-siRNA37 AtPMR6, AtFEI2 and AtWRKY7 are selectively silenced by *Bc*-siRNA37 [88]. While this is the only example so far in which siRNAs have been formed and released by microbes and reached to the host plant cells, there can be another common means based on genomic analysis by which microbes can modulate host responses during colonization.

5.3 lncRNA

The role of lncRNAs has been well-investigated in the context of plant defense against fungal, bacterial and viral pathogens [89–94]. Furthermore, the functions of a number of plant lncRNAs intricated in plant defenses are experimentally validated. For instance, it has been observed that the lncRNA-ACOD1 is dispensable for viral entrance but not for viral replication in the host [95]. Moreover, when turnip crinkle virus infection occurs in *Arabidopsis*, the expression of the long intergenic ncRNA LINC-AP2 gene is negatively regulated [89]. LncRNA33732 has been characterized to function as a positive regulator in tomatoes, enhancing the expression of the respiratory burst oxidase gene and raising H_2O_2 build-up, thereby increasing tomato resistance to *Phytophthora infestans* [96]. Also, lncRNA23468 in tomato can compete with endogenous RNA to regulate the NBS-LRR gene by feeding on miRNA482b, thereby controling tomato resistance to *P. infestans* pathogenesis [90]. Numerous lncRNAs have been identified as being modulated in drought, nitrogen-stress and phosphate depletion in maize [97–99]. The maize inbred line B73 tissues were subjected more than 700 high-fidelity RNA-Seq studies, which identified nearly 18,165 maize

lncRNAs [100]. Although lncRNAs are involved in the regulation of plant-microbe interactions, there are no available reports characterizing lncRNA responses to AM fungi, so far as the experimental evidence is considered.

6. miRNA in symbiosis: regulation through repression

Emphasis on existence and importance of small ncRNA, especially miRNA started with the discovery of its association with regulation of gene expression in Petunia [101, 102]. Owing genome wide studies and high-throughput sequencing efforts, till date thousands of miRNAs have been characterized throughout the kingdom of life. Most of the characterized miRNA associated with symbiosis are either involved in nutrient signaling, exchange and homeostasis or development of nodule/arbuscules or both [103, 104] are illustrated in **Figure 1**. Based on morphological analysis of host, AM Symbiosis (AMS) can be sub-categorized into four major stages: (1) pre-contact signaling, (2) contact establishment between plant root and fungal hyphae, 3) intra-radical proliferation, and 4) arbuscule formation [105]. A non-canonical form of miRNA171, which is found repressed under phosphate starvation, regulates an important transcription factor (TF) involved in common symbiotic signaling pathway, NSP1/2 (NODULE SIGNALING PATHWAY 1/2) during mycorrhizal symbiosis.

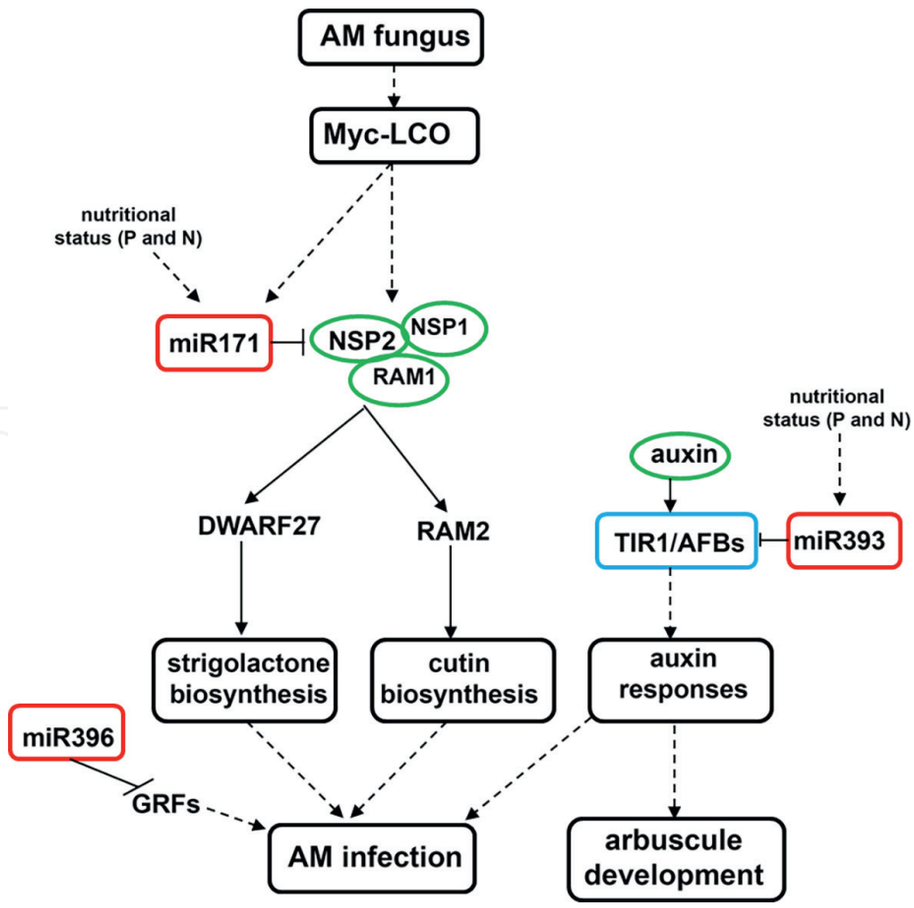

Figure 1.
Illustration of cross-talk between myc-LCO (mycorrhizal lipo-chito-oligosaccharide) factor induced common pathway signaling, miRNAs and regulatory hormones during AM symbiosis. Solid and dotted lines represents direct and indirect interconnections respectively.

The interaction of NSP2 with the mycorrhizal-specific GRAS TF, RAM1 (Required for Arbuscular Mycorrhization 1) regulates RAM2, and DWARF27 that are part of cutin and strigo-lactone biosynthetic pathway, respectively, as these two pathways facilitate AM inoculation. miRNA393, also identified as a N- and Pi-responsive miRNA, is involved in the homeostasis of auxin signaling and thus inhibits the auxin receptor TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX PROTEIN (TIR1/ABF), also mediates the repression of root growth regulatory factors (GRFs) to affect fungal colonization and arbuscule development. miRNA396b also investigated to perform a significant role in root colonization and development during mycorrhizal symbiosis by targeting six GRFs and a TF in *Medicago truncaluta* [106].

For initiating rhizobia symbiosis, interactions between miRNAs, Nod factor signaling, and hormone regulation through NSP2 is controlled by a nodule-specific miRNA171. The NUCLEAR FACTOR (NF)-YA gene, a TF necessary for the nodule initiation and maintenance of meristem, is negatively regulated by miRNA169 [107]. The combination of cytokinin-responsive miRNA172 genes, Nod factor, and various

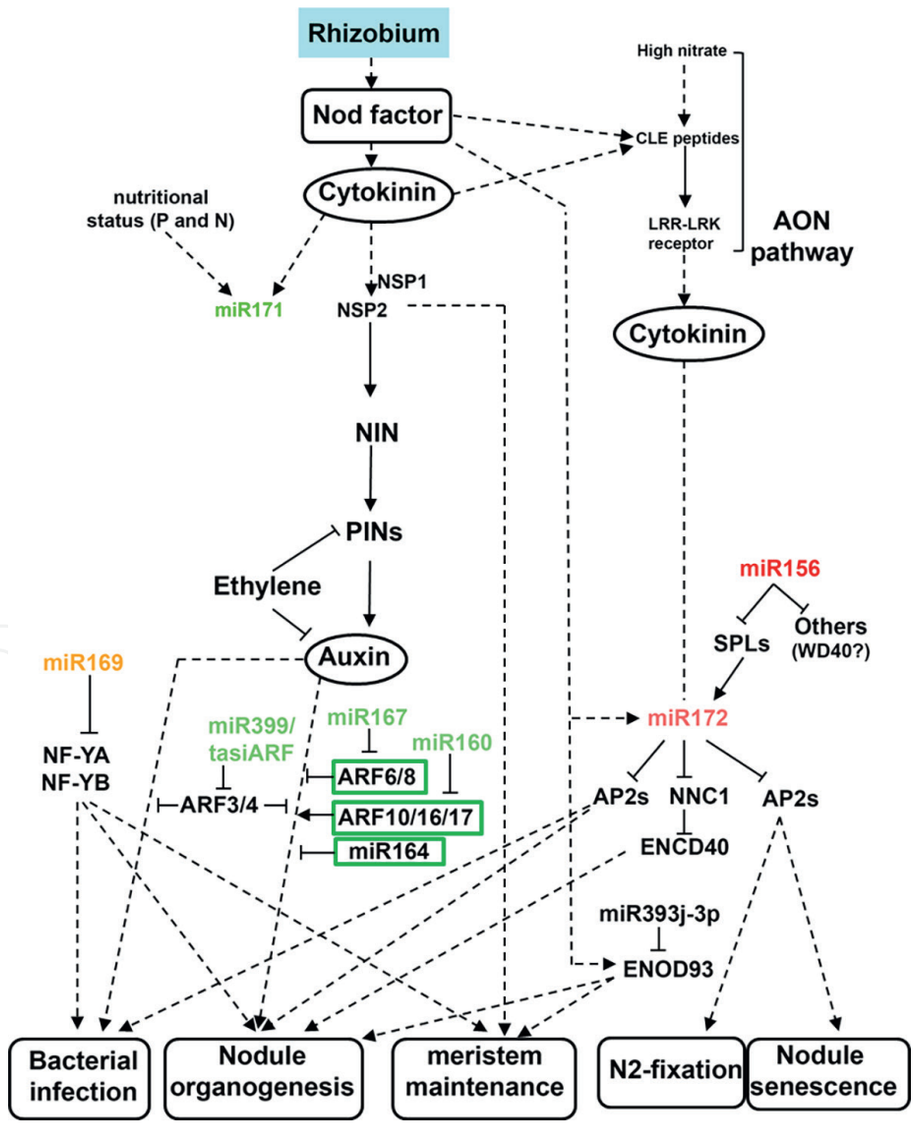

Figure 2.
Illustration of cross-talk between Nod factor induced common pathway signaling, miRNAs and regulatory hormones during rhizobial symbiosis. Solid and dotted lines represents direct and indirect interconnections respectively.

AP2 (APETALA2) targets has been implicated in rhizobia infection, stimulation of nodule organogenesis, N2 fixation, and delaying senescence in nodule cells.

The auto-regulation of the nodulation (AON) pathway, which governs the number of nodules that are formed in host plants, is one mechanism by which miRNA172 can work. In this pathway, leucine-rich repeat receptor-like kinase (LRR-LRK) receptors recognize NF- or nitrate-induced Clavata3/Embryo Surrounding Region-Related-peptides, resulting in an inhibitory signal (including CK) to cells, for establishing new nodules. By suppressing certain squamosa promoter-binding protein-like (SPL) TFs that stimulate miRNA172 production, miRNA156 antagonizes the action of miRNA172 [108].

Through the repression of nodulin gene (specifically ENOD93), miRNA393j-3p restricts nodules [109] whereas, miRNA1512 and miRNA1515 over-expression was discovered to be linked to increased nodule formation [75]. Finally, miRNA160 and miRNA167 cleave the transcripts of multiple auxin response factors (ARFs) that play key roles in the auxin response and pre-requisite for nodule initiation [110]. The detailed regulation mechanism is shown in **Figure 2**. miRNA390encourages the formation of a transacting small interfering (tasi)RNA, that represses ARF3 and ARF4during rhizobia colonization and nodule growth [78], it is known to combine auxin and ethylene signals. While, ARF10, ARF16, ARF17 and ARF6, ARF8 are directly regulated/targeted by miRNA160 and miRNA167, respectively [111] for auxin-responsive root development in both cases.

7. Mycorrhiza-derived non-coding RNAs and cross-kingdom signaling during symbiosis

Recent investigations have established non-coding RNAs as one of the central mediators of cross-kingdom communication between the plant and microbes [86, 88, 112–119]. These non-coding RNAs can move from donor organism to recipient organism, and target the specific host mRNAs for degradation. Sometimes sRNAs also trigger the production of secondary sRNA and thereby modulate the host defense and metabolic pathways [87, 120, 121]. Most of the studies focus on the plant-parasite or plant-pathogen (fungi and oomycetes) interactions [86, 88, 112, 120], however, such processes have been rarely explored in case of plant-mycorrhiza associations. Emerging body of evidences suggest that many plant miRNAs show differential expression patterns during AM symbiosis, nevertheless, their functions and cross-kingdom mobility remains unclear [80, 83, 111, 122, 123]. Mewalal et al. [124] identified several sRNAs from *Polpulus* spp. which were responsive to mutualistic/symbiotic interaction with mycorrhizal fungi like *Laccaria bicolor* and *Rhyzophagus irregularis*. Interestingly, they did not find any Populus RNAs interacting with *R. irregularis*, however, some of the miRNAs could interact with *L. bicolor*. Further the study revealed that these miRNAs can potentially target multiple host mRNAs encoding for vesicular transport and transcription regulatory proteins along with several uncharacterized proteins.

On the other hand, at present, very little information is available regarding the non-coding RNA biogenesis machinery and their functions in the development of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi or while interacting with the host plants. However, the successful application of host-induced gene silencing (HIGS) and virus-induced gene silencing (VIGS) approaches [65, 125–128] indicates that AMF, like pathogenic fungi, also possess functional RNAi machinery.

An *in silico* study identified putative RNAi machinery including a Dicer-like (DCL) gene, Argonaute-like (AGO-like) and RNA-dependent RNA polymerase (RdRp) gene families in *R. irregularis*, and validated their transcript-level expression [129]. An unsual expansion of AGO-like (5 members) and RdRp (21 members) gene families was observed in *R. irregularis.* Authors postulated that 15 out of 21RdRp genes, could be the product of a recent gene expansion event. The study also characterized the fungal sRNA and microRNA-like sequences, and predicted 237 transcripts of *Medicago truncatula* as their potential targets including a few known mRNAs that are modulated during AMF colonization. For instance, some of the *M. truncatula* mRNAs that are potentially targeted by *Rir*-sRNAs encode for the nuclear-binding leucine-rich repeat (*NBS-LRR*) type disease resistance gene, Non-specific phospholipase C4 (*NPC4*), *MtVapyrin* (Ankyrin repeat RF-like protein) and DREPP plasma membrane protein (*MtDREPP*) [129]. The homologs of NBS-LRR and NCP4 proteins from rice and arabidopsis, respectively, are involved in the plant immunity [130, 131], thus repression of these genes may allow AM colonization without triggering the robust host defense responses. MtVapyrin plays crucial role in arbuscule formation [132–134]. The down-regulation of *MtDREPP* has been reported in mycorrizal roots [135]. Though, further experimental validation is required, these findings indicate the possible existence of non-coding RNA-mediated post-transcriptional regulation and cross-kingdom gene silencing by AMF.

Another study by Silvestri et al. [136] identified the small RNA population from AMF *Gigaspora margarita* and showed their origin from different genetic sources such as endobacteria, RNA viruses and non-integrated DNA sequences from mitoviruses. Intriguingly, the extracellular vesicles (EVs), that are deployed in delivering the sRNA molecules to the other interacting partner [112, 120, 137], have also been observed in the peri-arbuscular interface of *R. irregularis* during the whole lifespan of arbuscules. This indicates the crucial role of EVs in cross-kingdom communication and nutrient exchange during AMF symbiosis [138]. More recently, a breakthrough discovery demonstrated that an ECM fungus *Pisolithus microcarpus* encodes 11 miRNAs, six of them were found induced during host colonization process. Notably, the miRNA (*Pmic_miR-8*) enters the plant cell and partakes in cross-kingdom gene silencing at some stage in symbiotic interaction with host plant *Eucalyptus grandis* [139]. The inhibition of *Pmic_miR-8* resulted in less developed Hartig nets, whereas, supplementation showed increased Hartig net depth in host tissue. Further the study showed that *Pmic_miR-8* may target the host NB-ARC (nucleotide-binding adaptor shared by APAF-1, R proteins, and CED-4) domain containing transcripts, indicating its potential role in modulating host signaling to stabilize the mutualistic association. As the CC (coiled-coil) nucleotide binding and leucine-rich repeat domain immune receptors (CC-NLR) are the largest category of NLRs, thus *Pmic_miR-8* may target several plant genes belonging to this class. Importantly, this is the first study which established the cross-kingdom gene silencing by mycorrhizal fungi and its role in beneficial interactions with host.

8. Conclusion and future prospects

In the last decade, the non-coding RNAs have emerged as one of the key regulators of diverse plant process including their development, response to abiotic/biotic stress, and nutrient uptake. A significant advancement has been made to understand the crucial roles of non-coding RNAs in plant-microbe interactions, particularly

pathogenic interactions. Nutrient uptake via mycorrizal association is an important aspect of plants lifestyle and the studies suggest the extensive involvement of non-coding RNAs in regulating the plant nutrient status via affecting symbiosis. Notably, the sRNA-mediated regulatory mechanisms during mycorrhizal symbiosis have mainly focused on plant's perspective. These studies have provided crucial insights on understanding how the mycorrizal colonization proceeds and how the host plants fine-tune the extent of fungal colonization so that it does not turns pathogenic. On the contrary, the sRNA-mediated regulation of symbiosis from the fungal perspective remains infancy. Moreover, an integrated view of both the organisms (plant and fungus) will be required to appropriately comprehend the beneficial relationships. To understand ncRNA-molecular interaction networks occurring at plant host-AM symbiosis interface, experimental evidences and rewriting of dynamics of interaction, sensing, uptake, transport, assimilation and homeostasis of nutrients regulation are required. Extensive investigations for ncRNAs mediated regulation of cross-talk between AM and host plant are also needed for teeming knowledge voids. We anticipate that the recent discovery of cross-kingdom gene silencing by ECM fungus *Pisolithus microcarpus* and AM-like model EM fungus *Serendipita indica* would pave the way for future investigations of non-coding RNA-mediated regulatory networks in mycorriza growth and development as well as during host interactions, and would trigger novel research ideas among plant scientists. The better understanding of these regulatory circuits would aid in improving the nutritional status of plants in order to combat the elevating global quality-food demand.

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References

[1] Smith SE, Smith FA. Roles of Arbuscular Mycorrhizas in plant nutrition and growth: New paradigms from cellular to ecosystem scales. Annual Review Plant Biology. 2011;**62**:227-250

[2] Zamioudis C, Pieterse CM. Modulation of host immunity by beneficial microbes. Molecular Plant-Microbe Interactions: MPMI. 2012;**25**(2):139-150

[3] López C, Szurek B, Perez-Quintero Á. Small non-coding RNAs in plant immunity. In: Dhal N, Sahu S, editors. Plant Science. London, UK: IntechOpen; 2012

[4] Plett JM, Martin FM. Know your enemy, embrace your friend: using omics to understand how plants respond differently to pathogenic and mutualistic microorganisms. The Plant Journal. 2018;**93**(4):729-746

[5] Xu L, Hu Y, Cao Y, Li J, Ma L, Li Y, et al. An expression atlas of miRNAs in Arabidopsis thaliana. Science China Life Sciences. 2018;**61**(2):178-189

[6] Bonnet E, Van de Peer Y, Rouze P. The small RNA world of plants. The New Phytologist. 2006;**171**:451-468

[7] Liu X, Hao L, Li D, Zhu L, Hu S. Long non-coding RNAs and their biological roles in plants. Genomics Proteomics Bioinformatics. 2015;**13**:137-147

[8] Chen X. Small RNAs and their roles in plant development. Annual Review of Cell and Developmental Biology. 2009;**25**:21-44

[9] D'Ario M, Griffiths-Jones S, Kim M. Small RNAs: Big impact on plant development. Trends in Plant Science. 2017;**22**:1056-1068

[10] Martinez G, Kohler C. Role of small RNAs in epigenetic reprogramming during plant sexual reproduction. Current Opinion in Plant Biology. 2017;**36**:22-28

[11] Tang J, Chu C. MicroRNAs in crop improvement: Fine-tuners for complex traits. Nat. Plants. 2017;**3**:17077

[12] Chekanova JA. Long non-coding RNAs and their functions in plants. Current Opinion in Plant Biology. 2015;**27**:207-216

[13] Sablok G, Zhao H, Sun X. Plant circular RNAs (circRNAs): Transcriptional regulation beyond miRNAs in plants. Molecular Plant. 2016;**9**:192-194

[14] Alves CS, Vicentini R, Duarte GT, Pinoti VF, Vincentz M, Nogueira FT. Genome-wide identification and characterization of tRNA-derived RNA fragments in land plants. Plant Molecular Biology. 2017;**93**:35-48

[15] Cognat V, Morelle G, Megel C, Lalande S, Molinier J, Vincent T, et al. The nuclear and organellar tRNA-derived RNA fragment population in Arabidopsis thaliana is highly dynamic. Nucleic Acids Research. 2017;**45**:3460-3472

[16] Megel C, Morelle G, Lalande S, Duchene AM, Small I, Marechal-Drouard L. Surveillance and cleavage of eukaryotic tRNAs. International Journal of Molecular Sciences. 2015;**16**:1873-1893

[17] Axtell MJ, Meyers BC. Revisiting criteria for plant microrna annotation in the era of big data. Plant Cell. 2018;**30**:272-284

[18] Chao H, Hu Y, Zhao L, Xin S, Ni Q, Zhang P, et al. Biogenesis, functions, interactions, and resources of non-coding RNAs in plants. International Journal of Molecular Sciences. 2022;**23**:3695

[19] Fei Q, Xia R, Meyers BC. Phased, secondary, small interfering RNAs in posttranscriptional regulatory networks. Plant Cell. 2013;**25**:2400-2415

[20] Peragine A, Yoshikawa M, Wu G, Albrecht HL, Poethig RS. SGS3 and SGS2/SDE1/RDR6 are required for juvenile development and the production of trans-acting siRNAs in Arabidopsis. Genes & Development. 2004;**18**:2368-2379

[21] Zhu L, Ow DW, Dong Z. Transfer RNA-derived small RNAs in plants. Science China. Life Sciences. 2018;**61**:155-161

[22] Kolakofsky D. Isolation and characterization of Sendai virus DI-RNAs. Cell. 1976;**8**:547-555

[23] Liu CX, Li X, Nan F, Jiang S, Gao X, Guo SK, et al. Structure and degradation of circular RNAs regulate PKR activation in innate immunity. Cell. 2019;**177**:865-880.e21

[24] Waititu JK, Zhang C, Liu J, Wang H. Plant non-coding RNAs: Origin, biogenesis mode of action and their roles in abiotic stress. International Journal Molecular Science. 2020;**21**:8401

[25] Matsumoto A, Nakayama KI. Hidden peptides encoded by putative noncoding RNAs. Cell Structure and Function. 2018;**43**:75-83

[26] Wang Y, Zhang C, Hao Q, Sha A, Zhou R, Zhou X, et al. Elucidation of miRNAs-mediated responses to low nitrogen stress by deep sequencing of two soybean genotypes. PLoS One. 2013;**8**:e67423

[27] Xu Z, Zhong S, Li X, Li W, Rothstein SJ, Zhang S, et al. Genome-wide identification of microRNAs in response to low nitrate availability in maize leaves and roots. PLoS One. 2011;**6**:e28009

[28] Hsieh LC, Lin SI, Shih ACC, Chen JW, Lin WY, Tseng CY, et al. Uncovering small RNA-mediated responses to phosphate deficiency in Arabidopsis by deep sequencing. Plant Physiology. 2009;**151**:2120-2132

[29] Gu M, Liu W, Meng Q, Zhang WQ, Chen AQ, Sun SB, et al. Identification of microRNAs in six solanaceous plants and their potential link with phosphate and mycorrhizal signalings. Journal of Integrative Plant Biology. 2014;**56**:1164-1178

[30] Lundmark M, Korner CJ, Nielsen TH. Global analysis of micro RNA in Arabidopsis in response to phosphate starvation as studied by locked nucleic acid-based microarrays. Physiologia Plantarum. 2010;**140**:57-68

[31] Pei L, Jin Z, Li K, Yin H, Wang J, Yang A. Identification andcomparative analysis of low phosphate tolerance associated microRNAs in two maize genotypes. Plant Physiology and Biochemistry. 2013;**70**:221-234

[32] Xu F, Liu Q, Chen L, Kuang J, Walk T, Wang J, et al. Genomewide identification of soybean microRNAs and their targets reveals their organ specificity and responses to phosphate starvation. BMC Genomics. 2013;**14**:e66

[33] Zhao X, Liu X, Guo C, Gu J, Xiao K. Identification and characterization of microRNAs from wheat (*Triticum aestivum* L.) under phosphorus

deprivation. Journal of Plant Biochemistry and Biotechnology. 2013;**22**:113-123

[34] Sun SB, Gu M, Cao Y, Huang XP, Zhang X, Ai PH, et al. A constitutive expressed phosphate transporter, OsPht1; 1, modulates phosphate uptake and translocation in phosphate-replete rice. Plant Physiology. 2012;**159**:1571-1581

[35] Sunkar R, Li YF, Jagadeeswaran G. Functions of microRNAs in plant stress responses. Trends in Plant Science. 2012;**17**:196-203

[36] Liang G, He H, Yu D. Identification of nitrogen starvation responsive miRNAs in *Arabidopsis thaliana*. PLoS One. 2012;**7**:e48951

[37] Zeng HQ, Zhu YY, Huang SQ, Yang ZM. Analysis of phosphorus-deficient responsive miRNAs and cis elements from soybean (*Glycine max* L.). Journal Plant Physiology. 2010;**167**:1289-1297

[38] Pacak A, Barciszewska-Pacak M, Swida-Barteczka A, Kruszka K, Sega P, Milanowska K, et al. Heat stress affects Pi-related genes expression and inorganic phosphate deposition/accumulation in Barley. Frontiers in Plant Science. 2016;**7**:e926

[39] Rajagopalan R, Vaucheret H, Trejo J, Bartel DP. A diverse and evolutionarily fluid set of microRNAs in *Arabidopsis thaliana*. Genes & Development. 2006;**20**:3407-3425

[40] Dugas DV, Bartel B. Sucrose induction of Arabidopsis miR398 represses two Cu/Zn superoxide dismutases. Plant Molecular Biology. 2008;**67**:403-417

[41] Kuo HF, Chiou TJ. The role of microRNAs in phosphorus deficiency signaling. Plant Physiology. 2011;**156**:1016-1024

[42] Howarth JR, Fourcroy P, Davidian JC, Smith FW, Hawkesford MJ. Cloning of two contrasting high-affinity sulfate transporters from tomato induced by low sulfate and infection by the vascular pathogen *Verticillium dahliae*. Planta. 2003;**218**:58-64

[43] Takahashi H. Regulation of sulfate transport and assimilation in plants. International Review of Cell and Molecular Biology. 2010;**281**:129-159

[44] Yoshimoto N, Inoue E, Watanabe-Takahashi A, Saito K, Takahashi H. Posttranscriptional regulation of high-affinity sulfate transporters in Arabidopsis by sulfur nutrition. Plant Physiology. 2007;**145**:378-388

[45] Shinmachi F, Buchner P, Stroud JL, Parmar S, Zhao FJ, McGrath SP, et al. Influence of sulfur deficiency on the expression of specific sulphate transporters and the distribution of sulfur, selenium and molybdenum in wheat. Plant Physiology. 2010;**153**:327-336

[46] Zuber H, Davidian JC, Wirtz M, Hell R, Belghazi M, Thompson R, et al. Sultr4;1 mutant seeds of Arabidopsis have an enhanced sulphate content and modified proteome suggesting metabolic adaptations to altered sulphate compartmentalization. BMC Plant Biology. 2010;**10**:e78

[47] Zeng H, Wang G, Hu X, Wang H, Du L, Zhu Y. Role of microRNAs in plant responses to nutrient stress. Plant and Soil. 2014;**374**:1005-1021

[48] Bucking H, Liepold E, Ambilwade P. The role of the mycorrhizal symbiosis in nutrient uptake of plants and the

regulatory mechanisms underlying these transport processes. In: Dhal NK, Sahu SC, editors. Plant Science. London, UK: IntechOpen; 2012

[49] Huey CJ, Gopinath SCB, Uda MNA, Zulhaimi HI, Jaafar MN, Kasim FH, et al. Mycorrhiza: A natural resource assists plant growth under varied soil conditions. 3 Biotech. 2020 May;**10**(5):204

[50] Wright DP, Read DJ, Scholes JD. Mycorrhizal sink strength influences whole plant carbon balance of trifolium repens L. Plant, Cell and Environment. 1998;**21**:881-891

[51] Chiou TJ, Liu H, Harrison MJ. The spatial expression patterns of a phosphate transporter (MtPT1) from Medicago truncatula indicate a role in phosphate transport at the root/soil interface. The Plant Journal. 2001;**25**(3):281-293

[52] Grunwald U, Guo WB, Fischer K, Isayenkov S, Ludwig-Müller J, Hause B, et al. Overlapping expression patterns and differential transcript levels of phosphate transporter genes in Arbuscular Mycorrhizal, Pi-Fertilised and phytohormone-treated medicago truncatula roots. Planta. 2009;**229**(5):1023-1034

[53] Li HY, Smith SE, Holloway RE, Zhu YG, Smith FA. Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. New Phytologist. 2006;**172**:536-543

[54] Smith SE, Jakobsen I, Grønlund M, Smith FA. Roles of Arbuscular Mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in Arbuscular Mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology. 2011;**156**:1050-1057

[55] Smith SE, Smith FA, Jakobsen I. Mycorrhizal Fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology. 2003;**133**(1):16-20

[56] Tanaka Y, Yano K. Nitrogen delivery to maize via Mycorrhizal hyphae depends on the form of N supplied. Plant, Cell and Environment. 2005;**28**:1247-1254

[57] Toussaint JP, St-Arnaud M, Charest C. Nitrogen transfer and assimilation between the Arbuscular Mycorrhizal Fungus Glomus intraradices Schenck and Smith and Ri T-DNA roots of *Daucus carota* L. in an vitro compartmented system. Canadian Journal of Microbiology. 2004;**50**:251-260

[58] Guether M, Neuhauser B, Balestrini R, Dynowski M, Ludewig U, Bonfante P. A Mycorrhizal-specific ammonium transporter from lotus japonicus acquires nitrogen released by Arbuscular Mycorrhizal fungi. Plant Physiology. 2009;**150**(1):73-83

[59] Harrison MJ, Dewbre GR, Liu J. A phosphate transporter from Medicago Truncatula involved in the acquisition of phosphate released by Arbuscular Mycorrhizal Fungi. The Plant Cell. 2002;**14**:2413-2429

[60] Xu GH, Chague V, Melamed-Bessudo C, Kapulnik Y, Jain A, Raghothama KG, et al. Functional characterization of LePT4: A phosphate transporter in tomato with Mycorrhiza-enhanced expression. Journal of Experimental Botany. 2007;**58**(10):2491-2501

[61] Allen JW, Shachar-Hill Y. Sulfur transfer through an Arbuscular Mycorrhiza. Plant Physiology. 2009;**149**(1):549-560

[62] Casieri L, Gallardo K, Wipf D. Transcriptional response of Medicago truncatula Sulphate Transporters to Arbuscular Mycorrhizal symbiosis with and without sulphur stress. Planta. 2012. DOI: 10.1007/s00425-012-1645-7

[63] Narayan OP, Verma N, Jogawat A, Dua M, Johri AK. Sulfur transfer from the endophytic fungus Serendipita indica improves maize growth and requires the sulfate transporter SiSulT. The Plant Cell. 2021;**33**(4):1268-1285

[64] Verma N, Narayan OP, Prasad D, Jogawat A, Panwar SL, Dua M, et al. Functional characterization of a high-affinity iron transporter (PiFTR) from the endophytic fungus Piriformospora indica and its role in plant growth and development. Environmental Microbiology. 2022;**24**(2):689-706

[65] Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N. A versatile monosaccharide transporter that operates in the Arbuscular Mycorrhizal fungus Glomus sp is crucial for the symbiotic relationship with plants. The Plant Cell. 2011;**23**:3812-3823

[66] Bücking H, Heyser W. Uptake and transfer of nutrients in ectomycorrhizal associations: interactions between photosynthesis and phosphate nutrition. Mycorrhiza. 2003;**13**:59-68

[67] Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, et al. Carbon availability triggers fungal nitrogen uptake and transport in Arbuscular Mycorrhizal symbiosis. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**(7):2666-2671

[68] Hammer EC, Pallon J, Wallander H, Olsson PA. Tit for tat? a mycorrhizal fungus accumulates phosphorus under low plant carbon availability. FEMS Microbiology Ecology. 2011;**76**:236-244

[69] Garcia K, Doidy J, Zimmermann SD, Wipf D, Courty PE. Take a trip through the plant and fungal transportome of Mycorrhiza. Trends in Plant Science. 2016;**21**(11):937-950

[70] Diédhiou I, Diouf D. Transcription factors network in root endosymbiosis establishment and development. World Journal of Microbiology and Biotechnology. 2018;**34**(3):37

[71] Middleton H, Yergeau É, Monard C, Combier JP, El Amrani A. Rhizospheric plant-microbe interactions: miRNAs as a key mediator. Trends in Plant Science. 2021;**26**(2):132-141

[72] Müller LM, Harrison MJ. Phytohormones, miRNAs, and peptide signals integrate plant phosphorus status with arbuscular mycorrhizal symbiosis. Current Opinion in Plant Biology. 2019;**50**:132-139

[73] Pimprikar P, Gutjahr C. Transcriptional regulation of arbuscular mycorrhiza development. Plant & Cell Physiology. 2018;**59**(4):673-690

[74] Feng H, Zhang Q, Wang Q, Wang X, Liu J, Li M, et al. Target of tae-miR408, a chemocyanin-like protein gene (TaCLP1), plays positive roles in wheat response to high-salinity, heavy cupric stress and stripe rust. Plant Molecular Biology. 2013;**83**:433-443

[75] Li Y, Zhang Q, Zhang J, Wu L, Qi Y, Zhou JM. Identification of microRNAs involved in pathogen-associated molecular pattern-triggered plant innate immunity. Plant Physiology. 2010;**152**:2222-2231

[76] Baldrich P, Campo S, Wu MT, Liu TT, Hsing YI, San Segundo B. MicroRNA-mediated regulation of gene expression in the response of riceplants to fungal elicitors. RNA Biology. 2015;**12**:847-863

[77] Wong J, Gao L, Yang Y, Zhai J, Arikit S, Yu Y, et al. Roles of small RNAs in soybean defense against Phytophthora sojae infection. The Plant Journal. 2014;**79**:928-940

[78] Li Y, Lu YG, Shi Y, Wu L, Xu YJ, Huang F, et al. Multiple rice microRNAs are involved in immunity against the blast fungus Magnaporthe oryzae. Plant Physiology. 2014;**164**:1077-1092

[79] Sarkar D, Maji RK, Dey S, Sarkar A, Ghosh Z, Kundu P. Integrated miRNA and mRNA expression profiling reveals the response regulators of a susceptible tomato cultivar to early blight disease. DNA Research. 2017;**24**:235-250

[80] Formey D, Sallet E, Lelandais-Briere C, Ben C, Bustos-Sanmamed P, Niebel A, et al. The small RNA diversity from *Medicago truncatula* roots under biotic interactions evidences the environmental plasticity of the miRNAome. Genome Biology. 2014;**15**:457

[81] Floss D, Levy J, Lévesque-Tremblay V, Pumplin N, Harrison M. DELLA proteins regulate arbuscule formation in arbuscular mycorrhizal symbiosis. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**:E5025-E5034

[82] Martín-Rodríguez JA, Ocampo JA, Molinero-Rosales N, Tarkowská D, Ruíz-Rivero O, García-Garrido JM. Role of gibberellins during arbuscular mycorrhizal formation in tomato: New insights revealed by endogenous quantification and genetic analysis of their metabolism in mycorrhizal roots. Physiologia Plantarum. 2015;**154**:66-81

[83] Wu P, Wu Y, Liu CC, Liu LW, Ma FF, Wu XY, et al. Identification of arbuscular mycorrhiza (AM)-responsive microRNAs in tomato. Frontiers in Plant Science. 2016;**7**:429

[84] Holt DB, Gupta V, Meyer D, Abel NB, Andersen SU, Stougaard J, et al. microRNA 172 (miR172) signals epidermal infection and is expressed in cells primed for bacterial invasion in Lotus japonicus roots and nodules. The New Phytologist. 2015;**208**:241-256

[85] Wang L, Qin L, Liu W, Zhang D, Wang Y. A novel ethylene-responsive factor from Tamarix hispida, ThERF1, is a GCC-box- and DRE-motif binding protein that negatively modulates abiotic stress tolerance in Arabidopsis. Physiologia Plantarum. 2014;**152**:84-97

[86] Wang M, Thomas N, Jin H. Cross-kingdom RNA trafficking and environmental RNAi for powerful innovative pre- and post-harvest plant protection. Current Opinion in Plant Biology. 2017a;**38**:133-141

[87] Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, et al. Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science. 2013;**342**:118-123

[88] Wang M, Weiberg A, Dellota E Jr, Yamane D, Jin H. Botrytis small RNA Bc-si R37 suppresses plant defense genes by cross-kingdom RNAi. RNA Biology. 2017b;**14**:421-428

[89] Gao R, Liu P, Irwanto N, Loh R, Wong SM. Upregulation of LINC-AP2 is negatively correlated with AP2 gene expression with Turnip crinkle virus

infection in Arabidopsis thaliana. Plant Cell Reports. 2016;**35**:2257-2267

[90] Jiang N, Cui J, Shi Y, Yang G, Zhou X, Hou X, et al. Tomato lnc RNA23468 functions as a competing endogenous RNA to modulate NBS-LRR genes by decoying miR482b in the tomato-Phytophthora infestans interaction. Horticulture Research. 2019;**6**:28

[91] Li WQ, Jia YL, Liu FQ, Wang FQ, Fan FJ, Wang J, et al. Genome-wide identification and characterization of long non-coding RNAs responsive to Dickeya zeae in rice. RSC Advances. 2018;**8**:34408-34417

[92] MuthusamyM, UmaS, SuthanthiramB, Saraswathi MS, Chandrasekar A. Genome-wide identification of novel, long non-coding RNAs responsive to Mycosphaerella eumusae and Pratylenchus co_eae infections and their differential expression patterns in disease-resistant and sensitive banana cultivars. Plant Biotechnology Report. 2019;**13**:73-83

[93] Yang Y, Liu T, Shen D, Wang J, Ling X, Hu Z, et al. Tomato yellow leaf curl virus intergenic siRNAs target a host long noncoding RNA to modulate disease symptoms. PLoS Pathogens. 2019;**15**:e1007534

[94] Zhang H, Hu W, Hao J, Lv S, Wang C, Tong W, et al. Genome-wide identification and functional prediction of novel and fungi-responsive lincRNAs in Triticum aestivum. BMC Genomics. 2016;**17**:238

[95] Runtsch MC, O'Neill LA. GOTcha: lncRNA-ACOD1 targets metabolism during viral infection. Cell Research. 2018;**28**:137-138

[96] Cui J, Jiang N, Meng J, Yang G, Liu W, Zhou X, et al. LncRNA33732-respiratory burst oxidase module associated with WRKY1 in tomato-Phytophthora infestans interactions. Plant Journal Cell Molecular Biology. 2019;**97**:933-946

[97] Du Q, Wang K, Zou C, Xu C, Li WX. The PILNCR1-miR399 regulatory module is important for low phosphate tolerance in Maize. Plant Physiology. 2018;**177**:1743-1753

[98] Lv Y, Liang Z, Ge M, Qi W, Zhang T, Lin F, et al. Genome-wide identification and functional prediction of nitrogen-responsive intergenic and intronic long non-coding RNAs in maize (*Zea mays* L.). BMC Genomics. 2016;**17**:350

[99] Pang J, Zhang X, Ma X, Zhao J. Spatio-temporal transcriptional dynamics of maize long non-coding rnas responsive to drought stress. Genes (Basel). 2019;**10**:138

[100] Han L, Mu Z, Luo Z, Pan Q, Li L. New lncRNA annotation reveals extensive functional divergence of the transcriptome in maize. Journal of Integrative Plant Biology. 2019;**61**:394-405

[101] Napoli C, Lemieux C, Jorgensen R. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. The Plant Cell. 1990;**2**(4):279-289

[102] van der Krol AR, Mur LA, Beld M, Mol JN, Stuitje AR. Flavonoid genes in petunia: Addition of a limited number of gene copies may lead to a suppression of gene expression. The Plant Cell. 1990;**2**(4):291-299

[103] Bazin J, Bustos-Sanmamed P, Hartmann C, Lelandais-Briere C, Crespi M. Complexity of miRNA-dependent regulation in rootsymbiosis.

Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences. 2012;**367**:1570-1579

[104] Simon SA, Meyers BC, Sherrier DJ. MicroRNAs in therhizobia legume symbiosis. Plant Physiology. 2009;**151**:1002-1008

[105] Gutjahr C, Parniske M. Cell and developmental biology of arbuscular mycorrhiza symbiosis. Annual Review of Cell and Developmental Biology. 2013;**29**:593-617

[106] Bazin J, Khan GA, Combier JP, Bustos-Sanmamed P, Debernardi JM, Rodriguez R, et al. miR396 affects mycorrhization and rootmeristem activity in the legume Medicago truncatula. The Plant Journal. 2013;**74**:920-934

[107] Laporte P, Lepage A, Fournier J, Catrice O, Moreau S, Jardinaud MF, et al. The CCAAT box-binding transcription factor NF-YA1 controls rhizobial infection. Journal of Experimental Botany. 2014;**65**:481-494

[108] Yan Z, Hossain MS, Wang J, Vald´es-L´opez O, Liang Y, Libault M, et al. miR172 regulates soybean nodulation. Molecular Plant-Microbe Interactions: MPMI. 2013;**26**:1371-1377

[109] Etemadi M, Gutjahr C, Couzigou JM, Zouine M, Lauressergues D, Timmers A, et al. Auxin perception is required for arbuscule development in arbuscularmycorrhizal symbiosis. Plant Physiology. 2014;**166**:281-292

[110] Khan GA, Declerck M, Sorin C, Hartmann C, Crespi M, Lelandais-Bri'ere, C. MicroRNAs as regulators of root develop-ment and architecture. Plant Molecular Biology. 2011;**77**:47-58

[111] Lelandais-Brière C, Moreau J, Hartmann C, Crespi M. Noncoding RNAs, emerging regulators in root endosymbioses. Molecular Plant-Microbe Interactions. 2016;**29**(3):170-180

[112] Cai Q, He B, Kogel K-H, Jin H. Cross-kingdom RNA trafficking and environmental RNAi — nature's blueprint for modern crop protection strategies. Current Opinion in Microbiology. 2018a;**46**:58-64

[113] Chaloner T, van Kan JAL, Grant-Downton RT. RNA "information warfare" in pathogenic and mutualistic interactions. Trends in Plant Science. 2016;**21**:738-748

[114] Knip M, Constantin ME, Thordal-Christensen H. Trans-kingdom cross-talk: small RNAs on the move. PLoS Genetics. 2014;**10**(9):e1004602

[115] Wang M, Weiberg A, Lin F-M, Thomma BPHJ, Huang H-D, Jin H. Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. Nature Plants. 2016;**2**:16151

[116] Weiberg A, Bellinger M, Jin H. Conversations between kingdoms: Small RNAs. Current Opinion in Biotechnology. 2015;**32**:207-215

[117] Weiberg A, Jin H. Small RNAs-the secret agents in the plant-pathogen interactions. Current Opinion in Plant Biology. 2015;**26**:87-94

[118] Weiberg A, Wang M, Bellinger M, Jin H. Small RNAs: A new paradigm in plant-microbe interactions. Annual Review of Phytopathology. 2014;**52**:495-516

[119] Zhou G, Zhou Y, Chen X. New insight into inter-kingdom communication: Horizontal transfer

of mobile small RNAs. Frontiers in Microbiology. 2017;**8**:1-9

[120] Cai Q, Qiao L, Wang M, He B, Lin F-M, Palmquist J. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science. 2018b;**360**(6393):1126-1129

[121] Shahid S, Kim G, Johnson NR, Wafula E, Wang F, Coruh C, et al. MicroRNAs from the parasitic plant Cuscuta campestris target host messenger RNAs. Nature. 2018;**553**:82-85

[122] Couzigou JM, Lauressergues D, André O, Gutjahr C, Guillotin B, Bécard G, et al. Positive gene regulation by a natural protective miRNA enables arbuscular mycorrhizal symbiosis. Cell Host & Microbe. 2017;**21**:106-112

[123] Devers EA, Branscheid A, May P, Krajinski F. Stars and Symbiosis: MicroRNA and microRNA*-mediated transcript cleavage involved in arbuscular mycorrhizal symbiosis. Plant Physiology. 2011;**156**:1990-2010

[124] Mewalal R, Yin H, Hu R, Jawdy S, Vion P, Tuskan GA, et al. Identification of populus small RNAs responsive to mutualistic interactions with mycorrhizal fungi laccaria bicolor and rhizophagus irregularis. Frontiers in Microbiology. 2019;**10**:515

[125] Kemppainen M, Duplessis S, Martin F, Pardo AG. RNA silencing in the model mycorrhizal fungus Laccaria bicolor: Gene knock-down of nitrate reductase results in inhibition of symbiosis with populus. Environmental Microbiology. 2009;**11**(7):1878-1896

[126] Kikuchi Y, Hijikata N, Ohtomo R, Handa Y, Kawaguchi M, Saito K, et al. Aquaporin-mediated long-distance polyphosphate translocation directed towards the host in arbuscular mycorrhizal symbiosis: application of virusinduced gene silencing. The New Phytologist. 2016;**211**:1202-1208

[127] Tsuzuki S, Handa Y, Takeda N, Kawaguchi M. Strigolactone-induced putative secreted protein 1 is required for the establishment of symbiosis by the arbuscular mycorrhizal fungus Rhizophagus irregularis. Molecular Plant-Microbe Interactions. 2016;**29**:1-59

[128] Voß S, Betz R, Heidt S, Corradi N, Requena N. RiCRN1, a crinkler effector from the arbuscular mycorrhizal fungus Rhizophagus irregularis, functions in arbuscule development. Frontiers in Microbiology. 2018;**9**:1-18

[129] Silvestri A, Fiorilli V, Miozzi L, Accotto GP, Turina M, Lanfranco L. In silico analysis of fungal small RNA accumulation reveals putative plant mRNA targets in the symbiosis between an arbuscular mycorrhizal fungus and its host plant. BMC Genomics. 2019;**20**(1):1-8

[130] García-Garrido JM, Ocampo JA. Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. Journal of Experimental Botany. 2002;**53**:1377-1386

[131] Siebers M, Brands M, Wewer V, Duan Y, Hölzl G, Dörmann P. Lipids in plant–microbe interactions. Biochimica et Biophysica Acta-Molecular Cell Biology Lipids. 2016;**1861**:1379-1395

[132] Feddermann N, Reinhardt D. Conserved residues in the ankyrin domain of VAPYRIN indicate potential protein-protein interaction surfaces. Plant Signaling & Behavior. 2011;**6**:680-684

[133] Pumplin N, Mondo SJ, Topp S, Starker CG, Gantt JS, Harrison MJ. Medicago truncatula Vapyrin

is a novel protein required for arbuscular mycorrhizal symbiosis. The Plant Journal. 2010;**61**:482-494

[134] Zhang X, Pumplin N, Ivanov S, Harrison MJ. EXO70I is required for development of a sub-domain of the periarbuscular membrane during arbuscular mycorrhizal symbiosis. Current Biology. 2015;**25**:2189-2195

[135] Aloui A, Recorbet G, Lemaître-Guillier C, Mounier A, Balliau T, Zivy M, et al. The plasma membrane proteome of *Medicago truncatula* roots as modified by Arbuscular Mycorrhizal symbiosis. Mycorrhiza. 2018;**28**:1-16

[136] Silvestri A, Turina M, Fiorilli V, Miozzi L, Venice F, Bonfante P, et al. Different genetic sources contribute to the small RNA population in the Arbuscular Mycorrhizal fungus Gigaspora margarita. Frontiers in Microbiology. 2020;**11**:395

[137] Huang CY, Wang H, Hu P, Hamby R, Jin H. Small RNAs-big players in plant-microbe interactions. Cell Host & Microbe. 2019;**26**(2):173-182

[138] Roth R, Hillmer S, Funaya C, Chiapello M, Schumacher K, Lo Presti L, et al. Arbuscular cell invasion coincides with extracellular vesicles and membrane tubules. Nature Plants. 2019;**5**:204-211

[139] Wong-Bajracharya J, Singan VR, Monti R, Plett KL, Ng V, Grigoriev IV, et al. The ectomycorrhizal fungus Pisolithus microcarpus encodes a microRNA involved in cross-kingdom gene silencing during symbiosis. Proceedings of the National Academy of Sciences of the United States of America. 2022;**119**(3):e2103527119