J Biosci (2020)45:62
DOI: 10.1007/s12038-020-00034-5

Brief communication

Identification of miRNAs linked to peanut nodule functional processes

María Soledad Figueredo[1], Damien Formey[2], Johan Rodríguez[1], Fernando Ibáñez[1], Georgina Hernández[2] and Adriana Fabra[1]*

[1]*Instituto de Investigaciones Agrobiotecnológicas (INIAB)-Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-químicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, 5800 Córdoba, Argentina*

[2]*Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, 62210 Cuernavaca, Morelos, Mexico*

**Corresponding author (Email, afabra@exa.unrc.edu.ar)*

MS received 12 July 2019; accepted 5 April 2020

microRNAs (miRNAs) are non-coding small RNAs that regulate gene expression at post-transcriptional level. Thousands of miRNAs have been identified in legumes, but studies about miRNAs linked to peanut nodule functionality are scarce. In this work we analyzed transcriptional changes in peanut nodules to identify miRNAs involved in functional processes of these organs. We found 32 miRNAs precursors differentially expressed in nodules compared with roots, and predicted the potential targets of their corresponding mature miRNAs. Among them, 20 belong to 14 conserved miRNAs families and 12 are *Arachis hypogaea*-specific miRNAs. Expression levels of 3 miRNAs (ahy-miR399, ahy-miR159 and ahy-miR3508) were confirmed experimentally by qPCR. We also demonstrated that the expression of these miRNAs was not affected by inoculation of a biocontrol bacterium or a fungal pathogen. The catalogue of differentially expressed miRNA precursors and the expression of the corresponding mature miRNA potential targets in the nodules of *A. hypogaea* obtained in this work is a database of strong candidates, including *A. hypogaea*-specific miRNAs, for the regulation of the nodule functionality. The analysis of their role in this process will certainly lead to the characterization of essential regulators in these particular aeschynomenoid nodules.

Keywords. *Arachis hypogaea* L.; microRNAs; nodulation; symbiosis

1. Introduction

Rhizobial bacteria and legumes establish a symbiotic relationship that leads the formation of new organs called nodules, where bacteria reduce atmospheric nitrogen into ammonia, used by the host plant. Nodule development begins with rhizobial root infection. Two extreme routes of rhizobial infection have been described in legume roots: intracellular entry at root hair and infection thread (IT) development (which occurs in most legumes, e.g. *Phaseolus vulgaris, Medicago truncatula*), or intercellular entry without IT formation. In *Arachis hypogaea* L. (peanut), bradyrhizobia enter the root intercellularly through a space between two adjacent root hair cells and spread also intercellularly by separating cortical cells at the middle lamellae (Van Rossum *et al.* 1995). Nodules formed in peanut are determinate and aeschynomenoid, as they have no interstitial cells (Stalker 1997).

In model legumes such as *Lotus japonicus*, *M. truncatula, Glycine max* and *P. vulgaris*, miRNAs are involved in the nodule development (De Luis *et al.*

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s12038-020-00034-5) contains supplementary material, which is available to authorized users.

http://www.ias.ac.in/jbiosci
Published online: 24 April 2020

2012; Formey *et al.* 2015; Wang *et al.* 2015). Although studies have been done to identify miRNAs expressed in different peanuts tissues (Zhang *et al.* 2017; Zhao *et al.* 2010; Jin *et al.* 2018; Rajendiran et al. 2019), to our knowledge, their role in the interaction with bradyrhizobia has not been reported yet.

Accumulating evidences indicate that miRNAs are hypersensitive to abiotic and biotic stress, and that they are key regulators of plant defense responses against pathogens (Gupta *et al.* 2014; Shriram *et al.* 2016). Peanut is susceptible to the attack of S*clerotium rolfsii,* the causal agent of stem wilt disease. We have demonstrated that the stem colonization of this pathogen diminishes not only the number but also the percentage of efficient nodules induced in peanut root by *Bradyrhizobium* sp. SEMIA6144. On the other hand, simultaneous inoculation of peanut with *Bradyrhizobium* sp. SEMIA6144 and the beneficial bacteria *Bacillus* sp. CHEP5 increase the plant nitrogen content, evidencing the functioning of a more effective symbiosis (Figueredo *et al.* 2017).

The aim of this study was to identify miRNAs involved in peanut nodule functions. Using RNA-seq libraries, we searched for miRNA precursors and its associated target transcripts differentially regulated in peanut mature nodules. We experimentally validated, by qPCR, the expression level of three miRNAs in peanut nodules. Furthermore, we evaluated changes in the expression levels of mature miRNAs in nodules from plants co-inoculated with *Bacillus* sp. CHEP5 or challenged with *S. rolfsii.* miRNAs and their corresponding predicted targets related to symbiosis in peanut were identified for the first time. The regulatory ahy-miR3508-pectinesterase node seems to be involved in the rhizobial infection of peanut roots.

2. Materials and methods

2.1 *Identification of peanut nodules miRNAs and their target genes*

The paired-end RNA-seq libraries from roots and nodules (inoculated with *Bradyrhizobium* (Peanut Special; EMD Crop Bioscience) (Clevenger *et al.* 2016) of *A. hypogaea* L. were obtained from the National Center for Biotechnology Information (NCBI) (*http://www.ncbi.nlm.nih.gov/*) Sequence Read Archive (SRA) (Accession numbers: root RNA-seq libraries (SRX1125177, SRX1125179, SRX1125205) and nodule RNA-seq libraries (SRX1125208, SRX1125213, SRX1125215)).

In order to identify miRNA precursors, we performed a *de novo* assembly of the 6 RNA-seq libraries with Trinity 2.4 (Grabherr *et al.* 2011) and the default parameters except for the contig length set on 110 nt. Expression counts were calculated using RSEM 1.3 with the default parameters (Li and Dewey 2011). Normalization and expression comparison between three root libraries and three nodule libraries were performed with DESeq2 following the default protocol, using as input the rounded RSEM "expected count" (Love *et al.* 2014). miRNA precursors were annotated using BLASTn comparison (minimum of 80% of identity and 80% of subject precursor coverage, containing the corresponding mature miRNA, and a p-value lower than 0.05) between the assembled transcriptome and all the Viridiplantae miRNA precursors referenced in miRBase 21 (Johnson *et al.* 2008; Kozomara and Griffiths-Jones 2013). The targets of the identified miRNAs were predicted using psRNAtarget (Dai and Zhao 2011) and the whole *A. hypogaea* transcriptome as reference, allowing a maximum expectation score of 5.

The gene ontology analysis was performed by using Blast2Go 5.2 with default parameters (Götz *et al.* 2008).

2.2 *Microorganisms and culture conditions*

Bradyrhizobium sp. SEMIA6144 (reference strain recommended as inoculant by Microbiological Resource Center, Porto Alegre, Brasil), a native biocontrol agent *Bacillus* sp. CHEP5 and the fungal pathogen *S. rolfsii* were used in this study. They were cultivated and inoculated to plants as previously described (Figueredo *et al.* 2017).

2.3 *Peanut growth condition*

Seeds of *A. hypogaea* L. (peanut) var. Runner cultivar Granoleico, were surface disinfected as described by Vincent (1970), and germinated and sowed as previously described (Figueredo *et al.* 2017). Two days after sown, radicles of peanut seedlings were inoculated with 4 ml of a *Bradyrhizobium* sp. SEMIA6144 culture, or with a 1:1 mixture of both *Bradyrhizobium* sp. SEMIA6144 and *Bacillus* sp. CHEP5. Seven days after *Bradyrhizobium* sp. SEMIA6144 inoculation, another group of plants were challenged with *S. rolfsii* by adding one wheat seed infected with mycelium. Plants

were grown and maintained under controlled environment (Figueredo *et al.* 2017).

At 40 days post-inoculation (dpi) of *Bradyrhizobium* sp. SEMIA6144, nodules and radical tissues of inoculated plants were collected in liquid nitrogen and stored at −80°C.

2.4 *qPCR analysis of miRNAs and miRNA targets*

For RNA extraction, samples from 4 plants per treatment were pooled. RNA from nodules and roots was extracted using the *mir*Vana™ miRNA Isolation Kit (Ambion, Foster City, CA, USA) according to the manufacturer's protocol. For the quantification of mature miRNA levels, cDNA was synthesized using the NCode miRNA First-Strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA) and quantitative reverse-transcriptase polymerase chain reaction (qPCR) assays using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was done following the manufacturer's instructions. NCode universal reverse primer and a sequence-specific forward primer (supplementary table 1) were used. Reactions were performed in a real-time thermocycler (Eco Illumina Real-Time PCR System; Illumina, San Diego, CA, USA) with settings of 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 57°C for 60 s. Technical duplicates on three biological replicates were performed for each gene. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Melting curves were recorded after cycle 40 by heating from 55 to 95°C. The Ct value obtained after each reaction was normalized with the Ct value of snU6 for expression levels of miRNAs.

Data were subjected to analysis of variance (ANOVA). Comparison between treatments was done by Student's *t*-test. Infostat statistical software (1.0, FCA, UNC, Argentina) was used.

3. Results

3.1 *Identification of miRNAs and their targets in nodule transcriptome*

A total of 354,535 contigs were assembled based on the 6 available transcriptome libraries. Among these assembled contigs, 178 corresponded to known miRNA precursors (supplementary table 2), producing mature miRNAs belonging to 52 families. From the 14 documented *A. hypogaea*-specific miRNA families (miRBase 21), we retrieved 10 in our transcriptome analysis being ahy-miR3509 the most represented family with 76 transcripts.

The 14 families of *A. hypogaea*-specific miRNA precursor generate 19 different mature miRNAs (5p and 3p, miRBase 21). Based on the transcriptome, we predicted 399 new targets for these mature miRNAs, representing a mean of 15 possible targets per miRNA if we consider only predicted targets with an expectation value of 3 or less (supplementary table 3).

3.2 *Differential expression of miRNA precursors and targets in nodules vs. roots*

From the 354,535 contigs, the expression level in nodules of 19,592 contigs (5.5%) was statistically different from root (non-zero log2-fold change, p-value lowers than 0.05): 9,327 were up-regulated and 10,265 were down-regulated. From this set of DECs (Differentially Expressed Contigs), 32 were identified as miRNA precursors. Twenty of them generate the following miRNAs matures previously identified in other plants: 4 members of the miR156 family, 4 of the miR166 family and 1 of the miR159, miR160, miR162, miR164, miR167, miR172, miR390, miR396, miR398, miR399, miR408 and miR1511 families. The other 12 DEPs (Differentially Expressed Precursors) are *A. hypogaea*-specifics and belong to 3 different families: 2 to the ahy-miR3508, 9 to ahy-miR3509 and 1 to ahy-miR3516 family. For each mature miRNA potentially produced by the selected precursors, we investigated the top 3 predicted target whose expression levels were different in nodule versus root transcriptomes. For the 76% of the miRNA candidates we found one or more predicted target transcript showing a significant inverse correlation with that of the miRNA precursor (supplementary table 4).

To understand the biological significance of the miRNA regulation network, the functional categories of the differentially expressed target genes were analyzed. About 10% is involved in transport activity, 15% in protein binding, 10% showed kinase activity, among other molecular functions. On the other hand, about 22% of the differentially expressed target genes participate in biological regulation, 36% in biosynthetic process, 30% in response to stimulus, among other functional categories. Genes within this last category could be important to maintaining rhizobia alive inside the nodule. Interestingly, we also found that 23% of the differentially expressed target genes are involved in the nitrogen compounds metabolic process (figure 1). These could be relevant for the optimal peanut nodules functioning.

3.3 *Experimental validation of bioinformatic predictions*

To experimentally evaluate expression levels of miRNAs differentially expressed in mature peanut nodules, among those families related with nodulation in other legumes we randomly selected ahy-miR399 and ahy-miR159. Furthermore, ahy-miR3508 was selected since it is one of the specific peanut miRNAs and one of their potential targets is involved in nodulation in other legumes. They were amplified by qPCR. Data obtained were consistent with the bioinformatic up-regulation of precursor genes prediction (approximately 2-fold each one) (figure 2).

3.4 *Expression of ahy-mi399, ahy-miR159 and ahy-miR3508 in nodules from peanut roots inoculated with Bacillus sp. CHEP5 or challenged with S. rolfsii*

To know whether the symbiotic phenotype of plants treated with *Bacillus* sp. CHEP5 or *S. rolfsii* is associated with changes in the miRNAs expression levels, we used qPCR. No difference was found between abundance of these miRNAs in nodules from plants inoculated only with the microsymbiont and those from plants co-inoculated with *Bacillus* sp. CHEP5 or challenged with *S. rolfsii* (figure 3), indicating that these miRNAs are not associated with the changes in the symbiotic phenotype we have previously demonstrated in these plants (Figueredo *et al.* 2017).

4. Discussion

Many studies have successfully employed comparative expression analysis from mature nodules and roots to predict genes related to symbiosis (Høgslund *et al.* 2009; Lelandais-Brière *et al.* 2009). In mature nodules, a high accumulation of miRNA could be linked to regulatory networks associated with nitrogen fixation or, alternatively, with nodule senescence.

In peanut plants it has been demonstrated that miRNAs play roles in growth, development, and

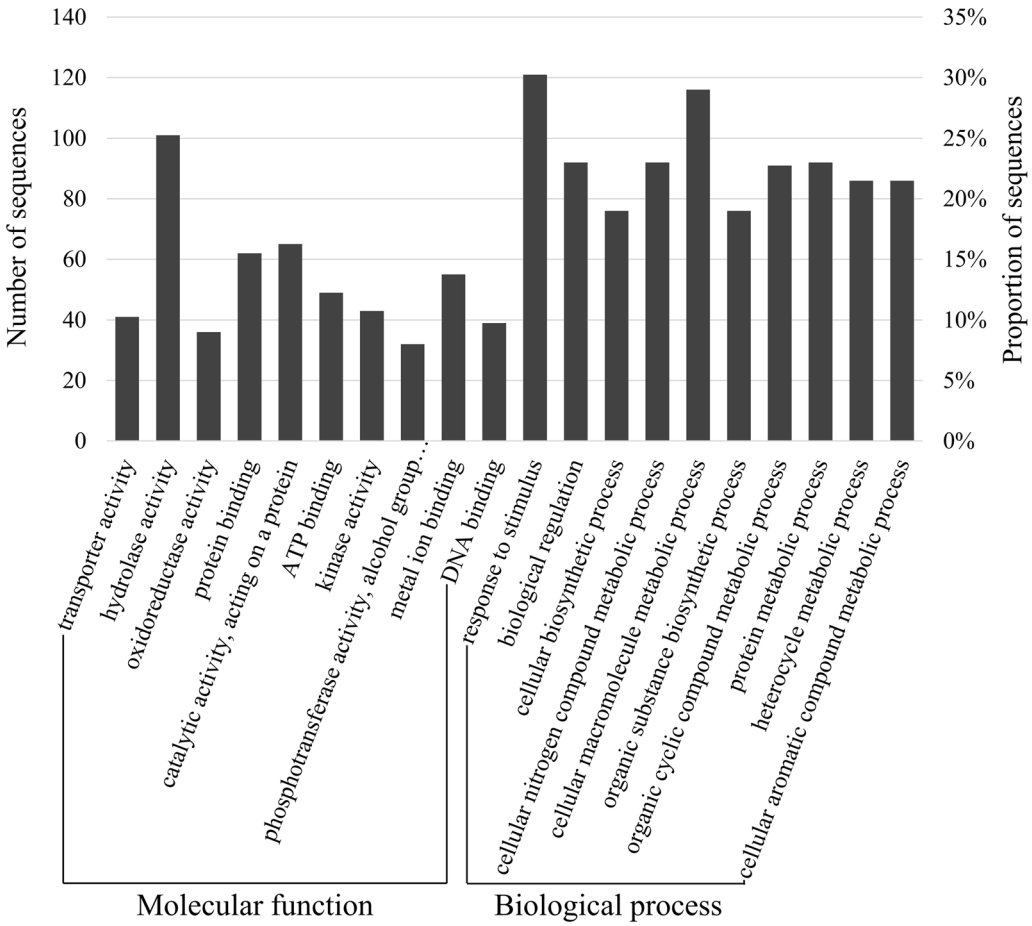

Figure 1. Functional categories of the differentially expressed target genes in peanut plants.

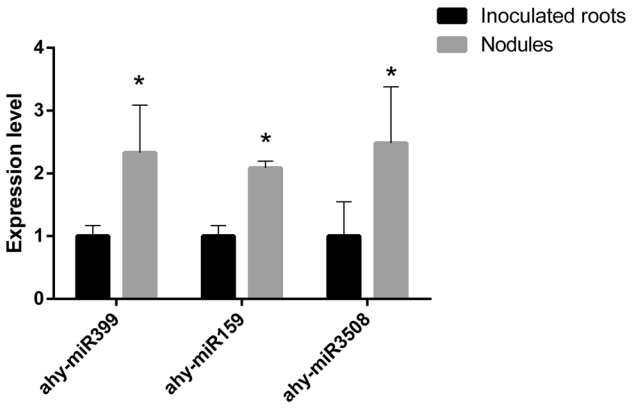

Figure 2. Expression analysis of ahy-miR399, ahy-miR159 and ahy-miR3508 in nodules and peanuts roots inoculated with *Bradyrhizobium* sp. SEMIA6144 at 40 dpi. Values are the relative expression level means ± SE. The inoculated roots expression levels were normalized to 1. Asterisks indicate significant differences according to the Student's *t*-test ($p < 0.05$). The experiment was repeated twice.

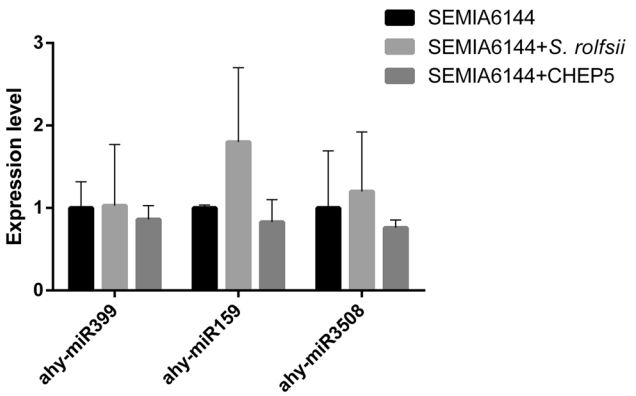

Figure 3. Expression analysis of ahy-miR399, ahy-miR159 and ahy-miR3508 in peanuts nodules from plant inoculated with *Bradyrhizobium* sp. SEMIA6144 and *S. rolfsii* or *Bacillus* sp. CHEP5. Values are the relative expression level means ± SE. The expression levels from nodules inoculated only with *Bradyrhizobium* sp. SEMIA6144 were normalized to 1. The experiment was repeated twice.

environmental responses (Chi *et al.* 2011; Zhang *et al.* 2017; Zhao *et al.* 2010; Jin *et al.* 2018; Rajendiran *et al.* 2019). However, to our knowledge, there is no report about participation of miRNAs in peanut nodule functions.

In this work we identified 14 families of conserved miRNA potentially associated with peanut nodule functions. Seven of these families (miR156, miR166, miR167, miR172, miR396, miR398 and miR399) have already been identified in nodules from other legumes (De Luis *et al.* 2012; Boualem *et al.* 2008; Wang *et al.* 2009; Yan *et al.* 2015) while the other seven have been associated with early stages of the interaction between legumes and rhizobia (Subramanian *et al.* 2008).

On the other hand, among differentially expressed miRNA identified in our analysis, we found three *A. hypogaea*-specific miRNAs (ahy-miR3508, ahy-miR3509 and ahy-miR3516). One potential target of ahy-miR3508 is a pectinesterase. The up-regulation of pectinesterase gene in *M. truncatula* and *L. japonicus* has been associated with the role of this enzyme in the cell wall softening required for rhizobial root infection (El Yahyaoui *et al.* 2004; Colebatch et al. 2004). The *in silico* lower expression level of this gene and the higher ahy-miR3508 expression in peanut mature nodules than in roots could be related with the absence of rhizobial infection at this stage of the symbiotic interaction. Nevertheless, considering that ahy-miR3508 is peanut specific and that this legume is infected intercellularly by bradyrhizobium, it is possible to hypothesize that the regulatory ahy-miR3508-pectinesterase node is involved in the rhizobial infection of peanut roots at the beginning of this symbiotic interaction.

It has been reported that there is an overlap in some miRNAs identified in the rhizobia-legume symbiosis and those involved in plant–pathogen interaction (Formey *et al.* 2014). In mature nodules from peanut, we found that the inoculation of *Bacillus* sp. CHEP5 or *S. rolfsii* does not change the expression of ahy-miR399, ahy-miR159 and ahy-miR3508. However, we have previously reported that nodules from *S. rolfsii*-challenged plants are deficient in nitrogen fixation while plants co-inoculated with *Bacillus* sp CHEP5 showed an increase in the nitrogen content compared with control plants (Figueredo *et al.* 2017). It is possible that these phenotypic changes are related with an effect of these microorganisms on the early stages of symbiosis establishment. Therefore, it will be interesting to analyze the expression of miRNAs at the beginning of the symbiotic process.

In this work we obtained a catalogue of differentially expressed miRNA precursors and the expression of the corresponding mature miRNA potential targets in the nodules of *A. hypogaea*. This is a database of strong candidates, including *A. hypogaea*-specific miRNAs, involved in the regulation of peanut nodule functionality. The analysis of their role will lead to the characterization of essential regulators in these particular aeschynomenoid nodules.

Acknowledgements

MSF and JR hold a scholarship granted by CONICET and ANPCyT, respectively. FI and AF are members of the Research Career from CONICET. DF and GH are members of the National System of Researchers from CONACYT.

References

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

Chi X, Yang Q, Chen X, Wang J, Pan L, Chen M, *et al.* 2011 Identification and characterization of microRNAs from peanut (*Arachis hypogaea* L.) by high-throughput sequencing. *PloS one* **6** e27530

Clevenger J, Chu Y, Scheffler B and Ozias-Akins P 2016 A developmental transcriptome map for allotetraploid *Arachis hypogaea*. *Front. Plant Sci.* **7** 1446

Colebatch G, Desbrosses G, Ott T, Krusell L, Montanari O, Kloska S, *et al.* 2004 Global changes in transcription orchestrate metabolic differentiation during symbiotic nitrogen fixation in *Lotus japonicus*. *Plant J.* **39** 487–512

Dai X and Zhao P 2011 psRNATarget: a plant small RNA target analysis server. *Nucleic Acids Res*. **39** W155–W159

De Luis A, Markmann K, Cognat V, Holt D, Charpentier M, Parniske M, Stougaard J and Voinnet O 2012 Two microRNAs linked to nodule infection and nitrogen-fixing ability in the legume *Lotus japonicus*. *Plant Physiol.* **160** 2137–2154

El Yahyaoui F, Kuster H, Ben Amor B, Hohnjec N, Puhler A, Becker A, Gouzy J, Vernié T, Gough C, Niebel A, Godiard L and Gamas P 2004 Expression profiling in *Medicago truncatula* identifies more than 750 genes differentially expressed during nodulation, including many potential regulators of the symbiotic program. *Plant Physiol*. **136** 3159–3176

Figueredo MS, Tonelli ML, Ibáñez F, Morla F, Cerioni G, Tordable M and Fabra A 2017 Induced systemic resistance and symbiotic performance of peanut plants challenged with fungal pathogens and co-inoculated with the biocontrol agent *Bacillus* sp. CHEP5 and *Bradyrhizobium* sp. SEMIA6144. *Microbiol. Res*. **197** 65–73

Formey D, Iñiguez LP, Peláez P, Li YF, Sunkar R, Sánchez F, *et al.* 2015 Genome-wide identification of the *Phaseolus vulgaris* sRNAome using small RNA and degradome sequencing. *BMC Genomics* **16** 423–440

Formey D, Sallet E, Lelandais-Brière C, Ben C, Bustos-Sanmamed P, Niebel A and Poulain J 2014 The small RNA diversity from *Medicago truncatula* roots under biotic interactions evidences the environmental plasticity of the miRNAome. *Genome Biol*. **15** 457–478

Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, *et al.* 2008 High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acid Res*. **36** 3420–3435

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, *et al.* 2011 Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nat. Biotechnol*. **29** 644–652

Gupta OP, Sharma P, Gupta RK and Sharma I 2014 MicroRNA mediated regulation of metal toxicity in plants: present status and future perspectives. *Plant Mol. Biol.* **84** 1–18

Høgslund N, Radutoiu S, Krusell L, Voroshilova V, Hannah MA, Goffard N, Sanchez DH, Lippold F, *et al.* 2009 Dissection of symbiosis and organ development by integrated transcriptome analysis of *Lotus japonicus* mutant and wild-type plants. *PLoS One* **4** e6556

Jin D, Meng X, Wang Y, Wang J, Zhao Y and Chen M 2018 Computational investigation of small RNAs in the establishment of root nodules and arbuscular mycorrhiza in leguminous plants. *Sci. China Life Sci.* **61** 706–717

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S and Madden TL 2008 NCBI BLAST: a better web interface. *Nucleic Acids Res*. **36** W5–W9

Kozomara A and Griffiths-Jones S 2013 miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res*. **42** D68–D73

Lelandais-Brière C, Naya L, Sallet E, Calenge F, Frugier F, Hartmann C, Gouzy J and Crespi M 2009 Genome-wide *Medicago truncatula* small RNA analysis revealed novel microRNAs and isoforms differentially regulated in roots and nodules. *Plant Cell* **21** 780–796

Li B and Dewey CN 2011 RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12** 323

Livak KJ and Schmittgen TD 2001 Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25** 402–408

Love MI, Huber W, Anders S 2014 Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. **15** 550

Rajendiran A, Vijayakumar S and Pan A 2019 Exploring microRNAs, target mRNAs and their functions in leguminous plant *Arachis hypogaea*. *MicroRNA* **8** 135–146

Shriram V, Kumar V, Devarumath RM, Khare TS and Wani SH 2016 MicroRNAs as potential target for abiotic stress tolerance in plants. *Front. Plant Sci.* **7** 817–835

Stalker HT 1997 Peanut (*Arachis hypogaea* L.). Field Crops Res. **53** 205–217

Subramanian S, Fu Y, Sunkar R, Barbazuk WB, Zhu JK and Yu O 2008 Novel and nodulation-regulated microRNAs in soybean roots. *BMC Genomics* **9** 160–174

Van Rossum D, Schuumans F, Gillis M, Muyotcha A, Van Verseveld H, Stouthamer A and Boogerd F 1995 Genetic and phenetic analyses of *Bradyrhizobium* strains nodulating peanut (*Arachis hypogaea* L.) roots. *Appl. Environ. Microbiol*. **6** 1599–1609

Vincent JM 1970 A manual for the practical study of the root-nodule bacteria. *A manual for the practical study of the root-nodule bacteria.* IBP Handbook. Oxford: Blackwell Scientific Publications Ltd, pp 73–97

Wang Y, Li K, Chen L, Zou Y, Liu H, Tian Y, Li D, Wang R, *et al.* 2015 MicroRNA167-directed regulation of the Auxin response factors *GmARF8a* and *GmARF8b* is required for soybean nodulation and lateral root development. *Plant Physiol.* **168** 984–999

Wang Y, Li P, Cao X, Wang X, Zhang A and Li X 2009 Identification and expression analysis of miRNAs from nitrogen-fixing soybean nodules. *Biochem. Biophys. Res. Commun*. **378** 799–803

Yan Z, Hossain MS, Arikit S, Valdés-López O, Zhai J, Wang J and Stacey G 2015 Identification of microRNAs and their mRNA targets during soybean nodule development: functional analysis of the role of miR393j-3p in soybean nodulation. *New Phytol*. **207** 748–759

Zhang T, Hu S, Yan C, Li C, Zhao X, Wan S and Shan S 2017 Mining, identification and function analysis of microRNAs and target genes in peanut (*Arachis hypogaea* L.). *Plant Physiol. Biochem*. **111** 85–96

Zhao CZ, Xia H, Frazier TP, Yao YY, Bi YP, Li AQ, Li MJ, Li CS, *et al.* 2010 Deep sequencing identifies novel and conserved microRNAs in peanuts (*Arachis hypogaea* L.). *BMC Plant Biol*. **10** 3–15

Corresponding editor: BJ RAO