High-throughput sequencing identifies novel and conserved salt-stress-regulated miRNAs from roots of *Medicago sativa* and *Medcago truncatula*

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Small ribonucleic acids (21-24 nt), notably the microRNA, are emerging as a posttranscriptional regulation mechanism. Salt stress is one of the primary abiotic stresses that cause the crop losses worldwide. Plant root growth and function are determined by the action of environmental salt stress through specific genes that adapt root development to the restrictive condition. Small RNAs are known to silence genes post-transcriptionally by guiding target mRNAs for degradation or by repressing translation. To elucidate the role of miRNAs in salt stress regulation in *Medicago*, we used a high-throughput sequencing approach to analyze four small RNA libraries from roots of *Medicago sativa* and *Medicago truncatula*, which were treated with 300 mM NaCl. Each library generated about 20 million short sequences and contained predominantly small RNAs of 24-nt length, followed by 21-nt and 22-nt small RNAs. We identified 385 conserved miRNAs from 96 families, along with 68 novel candidate miRNAs. Fifteen of all the 68 predicted novel miRNAs were identified to have miRNA*. Statistical analysis on abundance of sequencing read revealed specific miRNA showing contrasting expression patterns between *M. sativa* and *M. truncatula* roots, as well as between roots treated for 0 and 8 h (Fig. 1). The expression of some conserved and novel miRNAs was also quantified by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). The miRNA precursor and target genes were predicted by bioinformatics analysis. We concluded that the salt stress related conserved and novel miRNAs may have a large variety of target mRNAs, some of which might play key roles in salt stress regulation of *Medicago*.

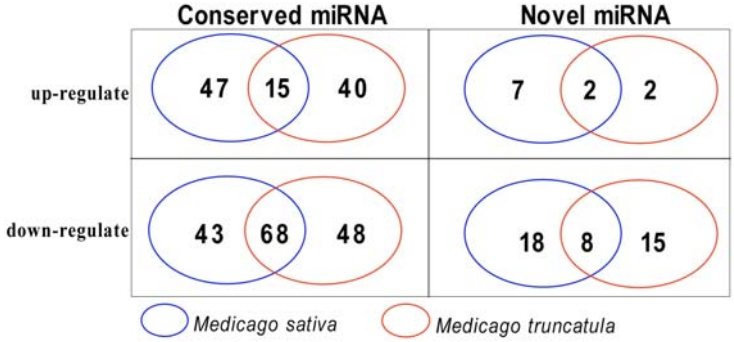

Fig. 1 The expression level diversity of the novel and conserved salt-stress-regulated miRNAs in *Medicago sativa* and *Medicago truncatula* roots.