Cloning & Transgenesis

Wang TZ, Clon Transgen 2014, 3:2
DOI: 10.4172/2168-9849.1000124

Review Article Open Access

Recent Research Progress on MicroRNA's in *Medicago Truncatula*

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Rec date: Jan 02, 2014, **Acc date:** Mar 16, 2014, **Pub date:** Mar 18, 2014

Abstract

MicroRNAs (miRNAs) are small, endogenous RNAs that play important regulatory roles in development and stress response by down-regulating gene expression post-transcriptionally. MiRNA study in *Medicago truncatula* is advancing quickly. This paper will introduce this field to readers from five aspects: nodulation, development of roots, response to drought, metal ions and phytohormone.

Keywords MicroRNA; *Medicago truncatula;* Research progress

Introduction

In plants, there are two kinds of small RNAs, endogenous small interfering RNAs (siRNAs) and microRNAs (miRNAs). Of them, miRNAs have an approximate length of 21 nt, and play key roles in negatively regulating gene expression by targeting corresponding messenger RNAs (mRNAs) [1,2]. It has been reported that miRNAs play a regulatory role in many processes in plants. In the field of growth and development, they are involved in seed germination [3], leaf morphogenesis [4,5], floral organ and root development [6-8]. In addition, they participated in responses of plants to various abiotic stresses, such as drought [9,10], cold [11], salinity [12], oxidative stress [13], metal ion toxicity [14,15], and nutrition starvation [16,17].

As the third model plant after Arabidopsis and rice, the miRNA study in *Medicago truncatula* is not so much detailed in contrast to Arabidopsis and rice [18-20]. However, the research development is so rapid. Here, the recent research progress on microRNAs will be introduced from several areas in *M. truncatula.*

Nodulation

As leguminous species, the roots of *M. truncatula* have the ability to interact with rhizobia to develop nitrogen-fixing root nodules. miR164 may be the first miRNA found to be involved in nodule formation, which can target *MtHAP2-1* encoding a transcription factor of the CCAAT-binding family with an ability of controlling nodule meristem function. Overexpression of miR164 leads to block of nodule development by down-regulating the expression of *MtHAP2-1* [21]. mR166 is another miRNA which affects nodule formation by negatively regulating several class-III HD-ZIP genes, and overexpression it makes number of symbiotic nodules decrease [8]. Lelandais-Briere et al. have identified many miRNAs in root nodules of *M. truncatula* by the method of high-throughput sequencing technology, which supplies abundant message for researchers about miRNA in nodules development [22].

Development of Roots

The root system is crucial for resource acquisition from soil. miRNAs involved in root development in *M. truncatula* have been identified [23]. Of them, overexpression of miR166 can reduce the number of lateral roots and induce ectopic development of vascular bundles in roots [8]. In addition, miR396 can regulate the architecture of the root system by targeting *MtGRF5* [24].

Drought

Thirty-two known members of 10 miRNA families and 8 new miRNAs/new members of known miRNA families were identified to be responsive to drought stress by high-throughput sequencing of sRNA libraries from *M. truncatula.* The targets of these miRNAs were involved in development, transcription, protein degradation, detoxification, nutrient status and cross adaptation [25]. An interesting discovery is miR398 targeting two Cu/Zn superoxide dismutases was found to be down-regulated transcriptionally in response to drought, different from the results reported by Trindale et al. [10]. This difference may results from the different extent and duration of drought stress, and it is needed to be studied sequentially.

Metal Ions

The expression response of miRNAs to aluminum and mercury toxicity was also been analyzed. Twenty-three miRNAs were responsive to Al^{3+}. By analysis the expression pattern of these miRNAs, it's found that the majority of these mRNAs was down-regulated and responded rapidly to Al^{3+} [15]. miRNAs and their targets response to Hg^{2+} were identified by high-throughput sequencing, too [14]. From these two articles, we found miR390 and miR2199 were all down-regulated by Al^{3+} and Hg^{2+}.

Phytohormone

The target of miR164, *MtNAC1* can be down-regulated by auxin homologous naphthaleneacetic acid (NAA), whose homolog *AtNAC1* plays a key role in auxin signal transport in Arabidopsis, but the function of *MtNAC1* is different from *AtNAC1* [7,26]. In addition, ethylene-responsive miRNAs in roots of *M. truncatula* have been identified by high-throughput sequencing. Eight miRNAs were down-regulated after exposure to ethylene, and the potential role of these miRNAs in the ethylene-induced inhibition of root elongation was discussed [27].

Forecast

From the Web of Science, it's known that there are many papers about genome-wide identification of microRNAs from different treatments of *Medicago truncatula.* According to the newest miRNA database (miRBase 20, released in Jun. 2013), M. truncatula has the most amount of precursors and mature miRNAs in plants, even more than Arabidopsis and rice. But the functional study level of miRNAs in *M. truncatula* is not as high as Arabidopsis and rice. For example, two Cu/Zn superoxide dismutase genes (*CSD1* and *CSD2*), a copper chaperone gene (*CCS1*) and a subunit gene of cytochrome c oxidase (*COX5b-1*) were all identified as target genes of miR398 in Arabidopsis. These genes were reported to be involved in response to diverse abiotic and biotic stresses, such as oxidative stress, salt stress and heat stress, Cu and phosphate deficiency, and addition of sucrose or plant pathogens, forming a detailed regulatory net of miR398 in Arabidopsis [13,28-32]. Because most of miRNAs have been identified and to find novel miRNAs from M. truncatula is more and more difficult, researchers are beginning to pay more attention to the functional study of important miRNAs in diverse biochemical and physiological processes, which will become the trend of miRNAs study in *M. truncatula.*

Acknowledgements

This work was supported by No. 31300231 of the National Nature Science Fund of China.

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