

## **Identification and characterization of microRNA in *Linum usitatissimum***

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Flax (*Linum usitatissimum* L.) is an economically important agricultural crop, and its fiber and oil have multiple industrial applications. Small non-coding RNAs (sRNAs) are a class of regulatory elements, which control a variety of biological processes and are found in diverse organisms [1]. Small RNAs consist of 18-30 nt and negatively regulate gene expression at both the transcriptional and post-transcriptional level by mRNA translation inhibition or mRNA cleavage. Small RNAs regulate growth and development, and are also important components in plant stress responses. Among sRNA, microRNAs (miRNAs) and small interfering RNAs (siRNAs) were found to play very important regulatory roles [1]. To date, there is only computational data concerning flax miRNA prediction, and only limited experimental data were obtained [2]. In order to have a complete understanding of flax gene regulation, more information about miRNAs is needed. In this study, for the first time, we sequenced flax (cultivar 'Stormont Cirrus') sRNA by high-throughput sequencing using Illumina GAIIx and identified conserved and novel miRNAs in flax. A sample of upper leaves was collected from individual plants after 6 weeks of growth. Total RNA was extracted using ZR RNA MicroPrep reagents. We constructed flax small RNA library as described in Illumina protocols. A total of 7.2M raw reads were generated during sequencing, low-quality reads and adapters were removed. For the next processing, we used cleaned reads with abundance six or more times. To identify conserved known miRNA flax, sRNA were aligned with known matured miRNA sequences found in miRBase19.0. Other known non-miRNA sRNA sequences were filtered using Rfam database. We identified 76 conserved miRNAs,

which belong to 20 distinct miRNAs families. The largest and the most abundant miRNA family in flax was miR165/166, which includes 18 members. This miRNAs family plays an important role in the regulation of HD-ZIP III gene expression, tissue identity and stress response. 156 potential novel miRNAs in the flax were predicted. One of the predicted miRNAs targets the E1 activating enzyme. This enzyme catalyzes the first step in the ubiquitination reaction, and predicted miRNA in this way could regulate phosphate starvation response in flax. Bioinformatics analysis showed that miRNAs target genes are involved in plant growth and development, phytohormone signaling, flowering, stress response, DNA damage repair, and phosphate homeostasis regulation. These results give us new information about the miRNA regulation of gene expression in flax.

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