Identification of aluminum-responsive sRNA in flax (*Linum usitatissimum*) by high-throughput sequencing

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Aluminum is the most abundant metal in the Earth’s crust and usually occurs in non-phytotoxic forms of aluminosilicate under most conditions. However, it is solubilized to phytotoxic Al\(^{3+}\) species in acidic soils, and becomes a major factor limiting crop production and yield in the acid soils. The involvements of sRNAs in mineral stress have been reported [1], report on systemic identification of Al\(^{3+}\)-responsive miRNAs and their targets at the global genome level by high-throughput sequencing in *Medicago truncatula* also have been reported [2]. Flax (*Linum usitatissimum* L.) is important agricultural crop. We investigated the changes of the sRNA spectrum in flax when exposed to aluminium.

Flax seedlings were collected to isolate total RNA for construction of sRNA libraries to control and after exposure to AlCl\(_3\) solution for 4 h and 24 h. Total RNA was isolated from the roots of the following three groups: control group (1), and groups treated with AlCl\(_3\) solution for 4 h (2) and 24 h (3), respectively. We constructed flax small RNA library as described in Illumina protocols. High-throughput sequencing of two cultivars (Lira, and TMP1919) was performed by genome analyzer Illumina GAIIx platform. A total of 9.2M (1), 6.1M (2), and 4.0M (3) primary reads and 5.1M (1), 10.4M (2), and 3.9M (3) primary reads were obtained from deep sequencing of TMP1919 and Lira flax cultivars, respectively.

After data analysis we selected six sRNA sequences as most interesting. Two of them are belong to known microRNA family miR166 that plays important role in stress response, UCGGACCAGGCUUCAUCCCCC and UCUCGGACCAGGCUUCAUCC mature
miRNA sequences. In case of TMP1919 cultivar for both miR166 members the expression profiles were 9.7K (1), 20.5K (2), 21.1K (3) and 9.7K (1), 31.3K (2), 26.3K (3), while in case of Lira cultivar for both miR166 members the expression profiles were 4.0K (1), 16.7K (2), 4.2K (3) and 7.3K (1), 6.5K (2), 3.9K (3) reads per million of sequenced reads. Other four identified sRNA sequences are not classified. One of the sRNA found shows about a threefold decrease at every step, (1) vs (2), and (2) vs (3) in expression under stress expose for both TMP1919 and Lira cultivars. Thus, after high-throughput sequencing and data analysis we identified six most interesting sRNA; two of them are conserved (miR166 family), while four sRNAs are not characterized yet.

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