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The identification of spatial and temporal role of lus-mir172e and lus-mir396c

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Abstract

Detection and quantification of miRNA expression is a key step in understanding their role in gene regulation. Northern blot analysis was used to confirm the expression level of lus-miR172e and lus-miR396c in selected tissues and different developmental stages of tested flax genotypes. For the study, flax genotypes with different content of alpha-linolenic acid (Amon, Libra and Raciol) and control genotype (Bethune) were used. Since flax is an interesting model of genomic research, two families of *Linum usitatissimum* micro RNAs have been selected, lus-miR172e which is incorporated in the metabolism of cell wall and lus-miR396c which is involved in controlling cell proliferation during leaflet development. Lus-miR172e which had a relatively high expression in stems of 2-week plants, moderately high in the roots and the lowest expression was shown in the leaves. Differences in expression were also noted between individual genotypes within selected plant organs. In stem and whole plant tissues (leaves and stems), the genotypes of Raciol and Bethune showed higher expression compared to the Amon and Libra genotypes. The highest expression of lus-miR396c was recorded in 1-week leaves and in 1-week plants (leaves and stems). Expression analysis by Northern Blot method confirmed accumulation of lus-miR172e in stems and lus-miR396c in whole plants and leaves depending on the age of plants.

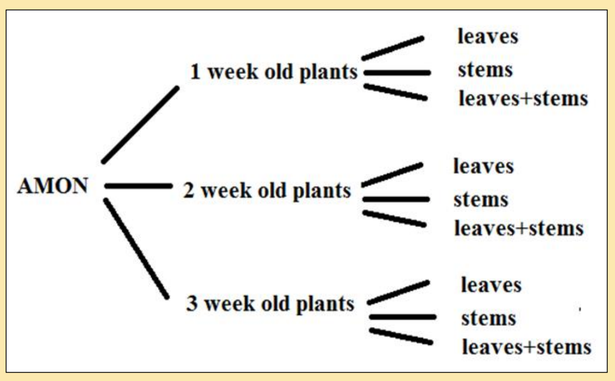
Key words: *Linum usitatissimum,* miRNA, Northerrn blot

Materials and Methods

Analyzed genotypes

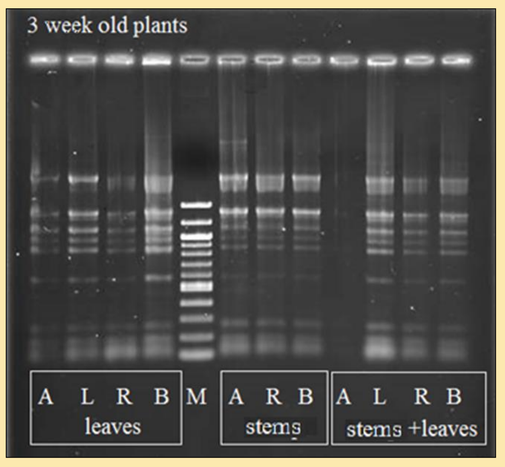
Genotype	Content of alpha-linolenic acid (%)	Type	Origin	Provided by
Amon	2.2	Intermediate	Czechia	agri tec
Raciol	32.5	Linseed	Czechia	
Libra	57.5	Linseed	Holandia	
Bethune	Unknown content Sequences of complete genome available	Intermediate	Canada	VNP

Nucleotide sequences of the mature lus-miR172e molecule which were used to design the probe.



The example of the RNA isolation scheme.

Total RNA was extracted by using TRI Reagent Solution (Ambion), the concentration and quality were evaluated using NanoDrop 2000c spectrophotometer.



RNA quantification on1.2 % agarose/EtBr gel.

Legend: A- Amon, L- Libra, R- Raciol, B- Bethune, M- marker-ladder 100 bp

Northern blot analysis

5 μg of total RNA from leaves, stems, leaves and stems and roots were denatured and loaded into denaturing 16% polyacrylamide gel. The RNA was transferred to Hybond NX (Amersham) nylon membrane through semi-dry electrophoresis transfer system (Bio-Rad). Chemical cross-linking was done at 60°C for 90 minutes by using 1-ethyl-3-(dimethylaminopropyl) carbodiimide (Sigma). The probes were generated by labelling the oligonucleotides that were reverse complementary to the sRNA of interest with γP^{32}-ATP. The membranes were hybridized with the probes at 37°C overnight.

Results

lus-miR172e - is incorporated in the metabolism of cell wall

Two-weeks old plants, lus-miR172e

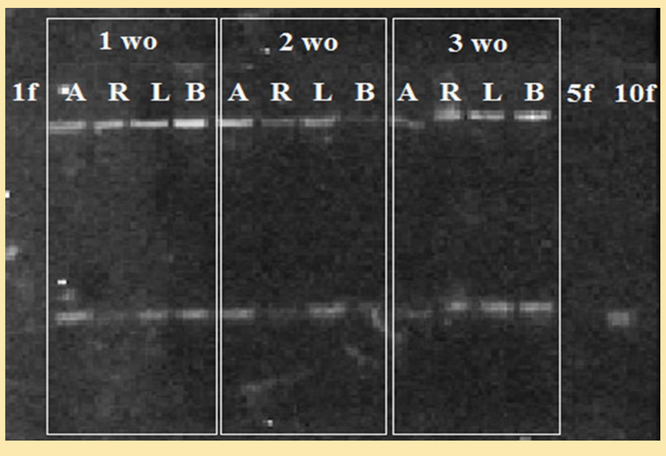
Raciol and Bethune

Amon and Libra

high expression in stems of 2-weeks old plants

the lowest expression in the leaves

lus-miR396c - is involved in controlling cell proliferation during leaflet development



Leaves, lus-miR 396c

1-week old leaves and in 1-week old plants (leaves and stems)

Legend: A- Amon, L- Libra, R- Raciol, B-Bethune, 1wo- 1 week old plants, 2wo- 2 weeks old plants, 3wo- 3 weeks old plants

Literature:

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