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ISOLATION OF microRNAs FROM ARTICHOKE AND THEIR INVOLVEMENT IN THE REGULATION OF TARGET GENES

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Artichoke, deep sequencing, microRNA, salt stress

MicroRNAs (miRNAs) are the products of endogenous non-protein-coding genes, originated from hairpin-like single stranded RNA precursors (pre-miRNAs and pri-miRNAs). miRNAs have been shown to affect gene expression at the post-transcriptional level through mRNA cleavage or translational repression. Recently, a new class of miRNAs (23–27nt long), have been demonstrated to guide cytosine DNA methylation both at their own gene loci in cis, and at their target gene loci in trans, possibly resulting in transcriptional gene silencing.

In order to characterize artichoke miRNAs, the present, we sequenced sRNA libraries obtained from artichoke leaves and roots using Illumina technology. Libraries were obtained from control tissues, and from the same tissues after saline stress treatment.

Artichoke sRNA fraction included mainly sequences in the range of 16-30 nucleotides in length, with a peak at 24 nucleotides. Bioinformatic analyses allowed to identify 83 artichoke conserved miRNAs, belonging to 25 families, and a number of novel species-specific artichoke miRNAs.

Differential expression of miRNAs was evaluated by normalizing the abundance of each miRNA in sRNA libraries as transcripts per million, and comparing stressed with control tissues, and by means of real-time PCR.

Potential miRNA precursors were identified by BLASTn searches of the mature miRNA sequences against artichoke ESTs and newly obtained Illumina genomic sequences and fold-back structure was predicted.

A bioinformatic search of miRNA targets produced over 40 putative target sequences, homologous to characterized *Arabidopsis* proteins. Most targets were transcription factors, and some of them had already proved to be involved in stress response in other plants.

Experimental validation of targets provided evidence on the mechanisms of action of artichoke miRNAs.