IDENTIFICATION OF ARTICHOKE MICRORNA HOMOLOGS BY MEANS OF BIOINFORMATIC TOOLS

CATALANO D., PIGNONE D., DE VIRGILIO M., FINETTI-SIALER M.M.

Istituto di Genetica Vegetale, CNR,Via Amendola 165/A, 70126 Bari, Italy

MicroRNA, database, gene expression

MicroRNAs (miRNAs) are a class of non coding RNAs that regulate gene expression, specifically at the post-transcriptional level in plants and animals, performing important regulatory roles for plant growth and development, as well as plant stress responses. MiRNA are highly conserved families of small RNAs of 20-24 nucleotides originating from endogenous long self-complementary precursors (pri-miRNAs). MiRNA identification has been carried out mostly by specific cloning of small molecules and/or computational methods. We developed a bioinformatic approach that allowed us to identify candidate miRNAs in the publicly available Cynara scolymus EST dataset. The complete EST dataset (36000 sequences) was analyzed by means of a Blast procedure, using the miRBase sequences (9359 entries) as query. The comparative data analysis showed 110 EST from Cynara (304 different regions) matching with 199 sequences of the miRBase sequence database. In all cases we obtained a high value of identity, 209 out of 304 sequences showing a 100% match. Furthermore, 250 out of 304 sequences showed a score higher than 95%. The Cynara EST dataset, the miRBase sequences and the best Blast hit results were stored in a local relational MySQL database. The biological targets of the miRNAs were obtained by means of specific MySQL queries, that allowed the identification of about 127 Cynara sequences, to be validated through expression assays and comparative in silico analyses. The method takes advantage of the sequences conservation level, typical of miRNA genes, since mature miRNAs are much more conserved and are required in the mRNA:miRNA interaction, allowing predictions about potential artichoke miRNAs and mRNA targets. Successful approaches for plant miRNA homologs (orthologs/paralogs) identification were previously described, however, this is the first application of this tool for artichoke miRNA identification. In order to assess the reliability of the putative miRNAs, a validation analysis of expression assay will be carried out through Northern blot hybridisation, as well as real time PCR.