In Silico and in Vivo Analyses of Ests Databases Unveil Novel Mirnas from Carthamus and Cynara SPP

Catalano D, Pignone D, Finetti Sialer M
Istituto di Genetica Vegetale, Consiglio Nazionale delle Ricerche - Bari, Italy

Motivation
MicroRNAs (miRNAs) are a class of small RNAs 21-24 nucleotides in length that provide an RNA-based system of gene regulation, highly conserved in plant and animal cells. In plants, miRNAs control messenger degradation or restrain translation, affecting their development and responses to biotic and abiotic stresses. A feature of plant miRNAs is their imperfect but extensive complementarity to corresponding mRNA targets, thus making their computational prediction possible. This is a useful approach when data mining is performed, among different species, on the basis of the miRNA:mRNA targets conservation. In this study we set up a comparative approach to identify either miRNAs and their targets, evolutionarily and functionally conserved among two related species, Carthamus and Cynara spp., belonging to the Asteraceae family. This family represents one of the largest evolutive radiations of flowering plants, including more than 1.5 \times 10^3 genera and 2.3 \times 10^4 species, comprising economically important as well as ornamental crops. In this study, miRNA identification and analysis were developed through a primary tool, ‘RNAhybrid’. In a subsequent step, we looked for the homologues of ESTs in this two related species. Finally, by means a bioinformatics pipeline and a relational database, we matched the presence of single targets in homolog ESTs region in both species, validating some of the identified sequences by means of experimental assays.

Methods
Two complete expressed sequence tags (ESTs) datasets from Cynara (3.6 \times 10^5) and Carthamus spp. (4.2 \times 10^5), were analysed by means of a bioinformatic pipeline, allowing the identification of 9 potential miRNAs and their precursors, 4 from Cynara and 5 from Carthamus. Around 75% of Carthamus and Cynara ESTs shared at least a common homologous region (E-value < 10^{-4}) and about 50% of the dataset entries displayed at least 400 bp or longer aligned sequences. For each EST, through bioinformatic analyses of the Arabidopsis miRNA mature sequences as reference set we proceeded to search matching putative targets. We found that 3475 out of 42011 ESTs analyzed in Carthamus and 4775 on 36323 ESTs in Cynara showed at least one predicted miRNA target. In a subsequent analysis, 525 and 566 aligned ESTs sequences shared by Carthamus and Cynara were identified as conserved homologous/orthologous sequences (COS regions). Within them, 76 different miRNA targets were found, putatively considered
as functionally conserved. Finally, four highly significant miRNAs sequences selected from the results of the in silico analysis were experimentally validated in Cynara leaves.

Results
In conclusion the approach followed let us the identification of 10 miRNAs in both species and 149 (corresponding to 146 miRNAs + 3 miRNAs* sequences) and 109 (corresponding to 107 miRNAs + 2 miRNAs* sequences) conserved targets in Cynara and Carthamus respectively, allowing for their in vivo functional analyses. The complementarity between miRNAs, targets and sequence conservation in both species helped us to predict regulated genes, as well. The bioinformatic approach identified the ESTs whose sequences were maintained in both species as most probable targets. Most of the miRNA targets found appeared as highly or moderately conserved, highlighting an important and conserved function. Statistical assessment showed that 37 targets, found on the COS regions and belonging to 21 miRNA families, have a signal to noise ratio higher than 2, with 0.5 or higher specificity. To date, some of these miRNAs were considered specific for Arabidopsis. The putative Asteraceae miRNAs identified shared 14 families with Arabidopsis. In silico data showed that eight further miRNAs, typical of Arabidopsis, are also present in members of Asteraceae, representing the first retrieval record in Carthamus and Cynara spp.

Contact email
mariella.finetti@igv.cnr.it