

EVIDENCE OF GYPSY AND COPIA RETROTRANSPOSON TRANSCRIPTION IN SUNFLOWER (*HELIANTHUS ANNUUS*)

M. VUKICH, T. GIORDANI, A. CAVALLINI, L. NATALI

Department of Crop Plant Biology, Genetics Section, Via del Borghetto 80, 56124 Pisa, Italy –
mvukich@agr.unipi.it

Helianthus, retrotransposons, sunflower, transcription

Retrotransposons are an ubiquitous and highly prevalent component of plant genomes. These elements are referred to as "junk DNA", implying that they are inert as compared to the genes required for cellular metabolism. So far, for some individual retroelements transcriptional activity, stress-induced activation, translation, and integration at specific loci have been proved.

With the aim to study the activity of retrotransposon elements in sunflower (*Helianthus annuus* L.), we have analysed genetic variability, within *H. annuus* and among several species belonging to the genus *Helianthus*, using molecular markers based on retrotransposon sequences, as IRAP (Inter-Retrotransposon-Amplification-Polymorphism, Kalendar et al. 1999). The IRAP products result from two retrotransposons close enough to each other to allow amplification of a PCR fragment between them. Interestingly, the extent of variability of wild sunflower was similar or even larger than interspecific variability. This represents the first line of evidence of recent retrotransposon activity in sunflower.

In other experiments we investigated the occurrence of retrotransposons activity in sunflower embryos at four different developmental stages (7, 14, 21, 28 days after pollination). Using a partial genomic library, different subfamilies of *gypsy* and *copia* retrotransposons have been described in sunflower, based on their sequence similarity. Primers have been designed on consensus regions of the integrase gene of three different *gypsy*-like subfamilies and of the RNase-H gene of one *copia*-like subfamily.

RT-PCR experiments performed with these primers showed amplification products with the three *gypsy* and the *copia* primer pairs, indicating that these retroelements are transcribed at each stage of development.

RT-PCR products, corresponding to each retroelement investigated, were cloned and sequenced. All the sequences obtained belong to *gypsy*- or *copia*-like retroelements, and are not inactivated by mutations. Consequently they could be functionally active. Ten out of twelve sequences are different, indicating that retrotransposon transcriptional activity should be considered as usual feature during embryo development.

Retrotransposon transcriptional activity is being detected in several tissues, such as leaves, roots, and flowers to verify if it occurs also in the adult plant. Moreover, since retrotransposon life cycle involves transcription, retrotranscription and integration back into the genome, we are studying the occurrence of new insertions of retrotransposons in the embryos and plantlets, using the IRAP technique, by means of primers designed on the outward portion of the LTRs belonging to *gypsy*- and *copia*-like retroelements transcriptionally active during the embryo development.