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CHARACTERIZATION OF HACRE1, A COMPLETE *COPIA* RETROTRANSPOSON OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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Retrotransposons or their remnants constitute large portions and have largely contributed to the structure of eukaryotic genomes. In sunflower (*Helianthus annuus* L.), recent studies showed that Gypsy and Copia retrotransposons account for large portion of the genome and that retrotransposon amplification have largely contributed to the speciation of *Helianthus* genus, being strongly activated after interspecific hybridization. However, no complete retrotransposon sequences are available.

After sequencing and annotation of a sunflower BAC clone, we have obtained and analysed, for the first time, an entire sequence of a Copia retrotransposon of sunflower, named HaCRE1 (Helianthus annuus Copia RetroElement 1), which is 8,511 bp long. HaCRE1 belongs to the Superfamily Copia retrotransposons by its protein domain order and sequence similarity to other Copia elements of dicotyledons and by phylogenetic analyses.

HaCRE1 carries 5'- and 3'-long terminal repeats (LTRs) 919-bp and 931-bp in length, respectively, flanking an internal region of 4661 bp. HaCRE1 has LTRs identical in their sequence, excluding two deletions of 7 and 5 nucleotides in the 5'-LTR. Availability of both complete LTRs allows a precise estimate of insertion time of HaCRE1. Insertion time estimates are based on the occurrence of nucleotide substitutions between LTRs, that are supposed to be identical at the RE insertion, using a nucleotide substitution rate of 1.3×10^{-8} substitutions per site per year. If only one substitution had occurred in the 919 sites of LTR, the insertion of the element should date 83703 years ago. This suggests that HaCRE1 inserted in the last 83703 years. The isolated sequence contains a complete ORF, with only one complete frameshift, i.e., no additional stop codons were found beside the regular stop at the end of the pol gene. The absence of non sense mutations agrees with the nearly complete convergence between LTRs, confirming that HaCRE1 is recent.

Slot blot hybridization experiments showed that the haploid genome of sunflower (HCM inbred line) contains about 160 copies of HaCRE1. The transcription activity of HaCRE1 was analyzed by semi-quantitative reverse-transcription PCR (RT-PCR) and sequencing in different plant organs and under different culture conditions. HaCRE1 resulted constitutively expressed, probably related to the occurrence, in the LTRs, of many putative regulatory cis-elements.