GENE NOTE

Identification of three MADS-box genes expressed in sunflower capitulum

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Abstract
Three cDNA clones, HaPI, HaAG and HaAP3, were isolated from sunflower inflorescences at the R2 stage of development. The cDNAs share high sequence similarity with the PISTILLATA, AGAMOUS, and APETALA3 genes from Arabidopsis, respectively, which contain a MADS-box and are involved in floral organ development. Expression of the corresponding genes was analysed by northern blots and in situ hybridization. They are expressed preferentially in the R3 and R4 stages of capitulum development. HaAG accumulates in fertile flowers, mainly in stamens, while HaPI and HaAP3 are preferentially expressed in ray (sterile) flowers and more weakly in petals and stamens of fertile flowers.

Key words: Floral development, MADS-box, sunflower, transcription factor.

The MADS-box is a consensus DNA sequence that encodes a DNA binding motif found in transcription factors present in several eukaryotic organisms. The first MADS-box containing genes isolated from plants were Antirrhinum DEFICIENS (DEF) and Arabidopsis AGAMOUS (AG). These genes were isolated from homeotic mutants defective in the specification of floral organ identity. Subsequent work revealed the existence of a large family of MADS-box containing genes in plants. Although initially found in floral tissues, it was later established that they also act as regulators of various other aspects of plant development (Rounsley et al., 1995; Kim et al., 2002).

Plant MADS proteins can be divided into several families, according to sequence similarity, expression patterns and function. Based on phylogenetic criteria five major groups were revealed and these were named according to their first-sequenced member as the AGAMOUS (AG), DEFICIENS (DEF), GLOBOSA (GLO), SQUAMOSA (SQUA), and AGL2 groups (Theilén et al., 1996).

Genes from the SQUA family, which include Arabidopsis APETALA1 (AP1) and Antirrhinum SQUA, generally have dual functions: floral meristem identity and floral organ identity specification. Some members are also involved in the floral induction process. The DEF and AG family genes regulate floral organ identity and include APETALA3 (AP3), PISTILLATA (PI) and AGAMOUS (AG) from Arabidopsis. The ABC model of floral development (Weigel and Meyerowitz, 1994) predicts that three classes of homeotic genes, encoding the A, B and C functions, act alone or in combination to give rise to sepals, petals, stamens and carpels. Genes in the AG group include the C function homeotic genes, involved in stamen and carpel development. Genes in both the DEF and the GLO groups comprise the B function homeotic genes and are involved in petal and stamen development.

The authors are interested in characterizing the expression patterns of genes involved in flower development in sunflower. Sunflower belongs to the Compositae family, with a terminal inflorescence (head or capitulum) composed of hundreds of flowers of two different types: ray (sterile) flowers in the periphery, and rings of disc (fertile) flowers in the centre (actually formed by radiating arcs from the centre of the head) (Seiler, 1997). It is interesting to understand how morphologically and functionally different flowers develop from the same genetic background.

As a first step towards this goal, MADS-box containing cDNAs were cloned from sunflower R2-stage inflorescence RNA by RT-PCR using degenerated oligonucleotides deduced from conserved regions of members of the AG and DEF groups. Only one expressed gene could be identified with oligonucleotides for the AG family genes, while clones representing two different genes were recovered when oligonucleotides for the DEF family genes were used. Full-length cDNAs for two of the identified genes were obtained applying 3′- and 5′-RACE (Frohman, 1994). Sequence analysis revealed that they share significant homology with the AGAMOUS and PISTILLATA genes from Arabidopsis, respectively, and were therefore named HaAG (Helianthus annuus agamous-like) and HaPI (Helianthus annuus pistillata-like). The third gene was named HaAP3 (Helianthus annuus APETALA3-like) since it is related to Arabidopsis APETALA3. The sequences were deposited in the GenBank under the accession numbers AY157724, AY157725 and AY185363. HaAG encodes a polypeptide of 248 amino acids and shows 85% amino acid sequence identity with GAGA1 from Gerbera hybrida, another plant that has a composite inflorescence. HaPI encodes a 168-amino acid protein with 91% sequence identity with GGLO1 from Gerbera, HaAP3 is a partial clone and encodes a 67-amino acid peptide with 95% homology with GDEF2 from Gerbera (Yu et al., 1999).

The expression of HaAG and HaPI during flower development was examined by northern blot analysis and in situ hybridization as previously described (Ribichich et al., 2001). Gene-specific DNA probes for northern and riboprobes for in situ hybridizations were used.

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RNA from different stages (R1 to R5 according to the classification developed by Schneiter and Miller, 1981) was purified and analysed by RNA blots (Fig. 1). Expression of the three genes was detected at all stages, with a clear increase in transcript levels upon development from the R1 to the R4 stage and a pronounced decrease at R5, which represents an open capitulum (Fig. 1, upper panel). After pollination, HaAG transcripts were detected at very low levels, while the expression of the other two genes was not observed (not shown). A more detailed analysis using RNA prepared from isolated organs or flowers at the R5 stage (B) was fractionated in 1.5% formaldehyde-agarose gels and transferred to nylon membranes. The blots were hybridized initially with HaAG, HaPI and HaAP3 probes and then with a *Vicia faba* rRNA probe to show relative RNA loadings. After hybridization, the blots were washed three times for 15 min in 2× SSC, 0.1% SDS at 65 °C and twice for 15 min in 1× SSC, 0.1% SDS at the same temperature. R1 to R5: different stages of development according to the classification developed by Schneiter and Miller (1981); RF, ray flowers; FF, fertile flowers; ST, stamens; C, carpels.

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The expression analysis described here suggests that the sunflower homologues of *Arabidopsis AGAMOUS*, *PISTILLATA* and *APETALA3* may have functional equivalency with their counterparts, participating in the C and B functions, respectively. It is also evident that the same or very similar genes are expressed in fertile and ray flowers, although at different levels. In addition, HaAG expression is switched on in mutants that develop fertile ray flowers. Future studies will be conducted to evaluate the mechanisms involved in this process.

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