The Family of MADS – Box Genes Controlling Flower Development in Crocus (*Crocus sativus* L.)

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Abstract

In order to uncover and understand the molecular mechanisms controlling flower development in cultivated *Crocus sativus* and particularly the formation of tepals, we have cloned and characterized their structures and expressions of the families of flower specific MADS-Box genes. The deduced amino acid sequence of the gene indicated high homology with members of the MADS-box family of transcription factors. The expression studies of the B-type MADs – Box genes *CsatPI* and *CsatAP3* indicated the presence of the transcripts are not restricted only in the second and third whorl of the flowers but also in the outer tepals and stigmata of the mature crocus flower parts, explaining the homeotic transformation of sepals to petals in that species.

Introduction

MADS box genes encode a family of transcription factors that are involved in different developmental processes. Genetic and molecular analysis of floral homeotic mutants in Arabidopsis and snapdragon (Yanofsky et al., 1990) led to the discovery of the first MADS box genes in plants and subsequently accelerated the molecular elucidation of the classic ABC model of flower development. Where, all of the major players in the ABC model belong to the MADS box gene family with the exception of APETALLA2.

Crocus sativus, a monocot triploid species belonging to the Iridaceae family, is cultivated for its red stigmatic lobes of the carpel that constitute saffron. It is cultivated in Southern Europe (mainly in Greece), Iran, and India. It is popular because of its delicate aroma and attractive color and can be used as

a food additive, as well as in medicine and the coloring industry. Saffron has three main chemical components that confer the bright yellow color (crocein), a bitter taste (picrocrocein) and a spicy aroma (saffronal). The flowers of crocus are bisexual. Perianth consists of 6 petaloid tepals in two whorls. Androecium consists of 3 distinct stamens and the gynoecium consists of a single compound pistil of 3 carpels, a single 3-branched style, and an inferior ovary. The flower is sterile, thus the crop is propagated asexual.*Crocus sativus* blooms only once a year and is hand harvested. After mechanical separation of tepals, the stigmas are hand separated from carpels and dried. The size and the amount of individual stigmas collected from each flower influence total yield and quality of saffron. Thus, understanding flower development in *Crocus sativus* could reveal ways to increase yield and lower production costs since flower and more specifically isolated stigmas comprise the valuable commercial part of the plant.

Towards this goal we report the cloning and characterization and studies on the expression of three homologous Apetala like (A-like) genes designated *CsatAP1a,b,c* (Tsaftaris *et al*., 2004a) two Agamous like (C-like) differentially spliced genes designated *CsatAGa,b* (Tsaftaris *et a*l., 2004b), two Apetala3 like (B-like) genes designated *CsatAP3a,b (*Tsaftaris *et a., 2005)* (AP3 and PI) and Pistillata like (B-like) designated *CsatPIA1,A2,A3,A4,A5 (*Kalivas *et al., 2005).*

Materials and methods

Crocus sativus field growing plants were collected from Kozani, Greece. Sampling was during the late flowering season in October. Tissues were separated and immediately frozen in liquid nitrogen and stored at -80^{0}C until used. Total RNA from leaves, closed flowers (3 cm in length), sepals, petals, stamens and carpels was extracted using the RNeasy plant mini kit (Qiagen). On-column digestion of DNA during RNA purification was performed using the RNase-Free DNase Set (Qiagen). For amplification of *CsAP1, CsAP3, CsatPI, CsAG* and *CsSEP* sequences, two degenerate primers, MADSF (Tsaftaris *et al*., 2004a) and MADS2F (van der Linden *et al*. 2002), which corresponding to conserved amino acid sequences of the MADS domain of MADS Box genes from other plant species and was used in 3' RACE experiments.To obtain the cDNA's 5'end, an RNA ligase-mediated rapid amplification reaction was performed on a pool from total RNA from leaves and flowers using the GeneRacer Kit (Invitrogen) according to the manufactures protocol as described in (Tsaftaris *et al*. 2004). Based on the sequence information obtained by the 3' RACE experiments, gene specific primers and degenerate, for the five family of MADS box genes, were designed from the 3-UTR and used to isolate the cDNA's 5'ends following the recommendations of the manufacturer. The expression analysis of the isolated MADS- box gene was performed with RT-PCR. One ìg of total RNA extracted from leaves, flowers, sepals, petals, stamens and carpels were used in a reverse transcription reaction as described in the RNA isolation and cDNA synthesis section of Materials and Methods.

Results

The analysis of the sequencing results using DNA Star revealed that the 5' RACE clones and the 3' RACE clones could be grouped into five populations for the gene CsatPI (*CsatPIA1, CsatPIA2, CsatPIB, CsatPIC1* and *CsatPI2*), into two populations for the gene CsAP3 (*Csap3a* and *Csap3b*), into two population for gene *CsAG* (*CsAG1a* and *CsAG1b*),into two populations for the gene *CsatSEP* (*CsSEP1* and *CsatSEP2*) and into three population for *CsatAP1* (*CsatAP1a*, *CsatAP1b* and *CsatAP1*).

Phylogeny analysis showed that *CsatAP1, CsatAP3, CsatPI CsatAG* and *CsatSEP* genes belong to AP1, AP3/DEF, PI/GLO, AG and SEP subfamilies respectively and these genes are closely related to other monocot AP1-, AP3/DEF-, PI/GLO-, AG- and SEP-like genes.

The expression pattern of the five MADS Box genes in leaves and flowers was compared by RT-PCR. Experiments revealed the presence of the transcripts for *CsatAP3, CsatSEP* and *CsatAG* only in flowers, instead for *CsatAP1*and CsatPI revealed the presence of the transcript both in flowers and in leaves. The expression pattern of these five families genes was also examined in different flower tissues. The RT-PCR experiment performed with cDNA synthesized from sepals, petals, stamens and carpels resulted in the identification of the transcript in all mature flower parts for *CsatAP1, CsatAP3, CsatPI* and *CsatSEP* and the presence of the transcript, for *CsatAG*, restricted in stamens and carpels.

Conclusions

Three AP1-, Five PI-, two AP3-, two AG- and two SEP-like MADS Box genes were isolated from *Crocus sativus* L. The sequence alignment revealed that the five CsPI proteins contain the typical domain structure of plant MADS box proteins consisted of the conserved N-terminal MADS-box, the I domain, the central K domain and the C terminal domain.

In Arabidopsis, expression of *AP1*occurs specifically in the tissues and at the developmental stage in which floral fate is assumed. In the flower, expression of *AP1* is restricted to petals and sepals. In contrast, RT-PCR experiments revealed that the three *CsatAP1* genes are expressed in leaves, as well as in all the flower organs examined. The three isolated *CsAP1* genes from crocus, are *AP1*-like MADS-box genes expressed in vegetative as well as in all floral tissues of the plant. Similar expression pattern display many monocot *AP1-like* MADS-box genes, which comprise a distinct phylogenetic clade of monocot class A MADS-box genes and may reflect a novel, yet unidentified role of their corresponding proteins in these species.

In contrast to Pistillata and AP3 studies of other plants monocots and dicots, which follow the ABC model, and the expression of AP3- and PI-like MADS Box genes is restricted in 2 and 3 whorl, the expression pattern of CsPI *CsatAP3* revealed that these genes are expressed in floral organs, especially in whorls 1, 2, 3, 4 (outer and inner tepal, stamen and carpel respectively). The expression of *CsatAP3* and *CsatPI* in whorl 1 should be a strong indication to explain the homeotic transformation of sepals to outer tepals in *Crocus sativus*.

The function of *CsatAG*, like C-class MADS-box gene, is essential for both stamen and carpel formation and in agreement with studies in other plants and the predictions of the ABC-model for floral organ identity genes, its expression studies indicated that the presence of both differentially spliced transcripts is restricted only to flowers and particularly in stamens and carpels of the mature crocus flower parts.

References

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