



The study of the E-class *SEPALLATA3*-like MADS-box genes in wild-type and mutant flowers of cultivated saffron crocus (*Crocus sativus* L.) and its putative progenitors

Athanasios Tsaftaris^{a,b,*}, Konstantinos Pasentsis^a, Antonios Makris^a, Nikos Darzentas^a, Alexios Polidoros^{a,1}, Apostolos Kalivas^{a,2}, Anagnostis Argiriou^a

^a Institute of Agrobiotechnology, Center for Research and Technology Hellas, 6th Km Charilaou Thermi Road, Thermi GR-570 01, Greece

^b Department of Genetics and Plant Breeding, Aristotle University of Thessaloniki, Thessaloniki GR-541 24, Greece

ARTICLE INFO

Article history:

Received 11 August 2010

Received in revised form 22 March 2011

Accepted 26 March 2011

Keywords:

Crocus sativus L.
MADS-box genes
Monocots
RCA-RACE
SEPALLATA

ABSTRACT

To further understand flowering and flower organ formation in the monocot crop saffron crocus (*Crocus sativus* L.), we cloned four MIKC^c type II MADS-box cDNA sequences of the E-class *SEPALLATA3* (*SEP3*) subfamily designated *CsatSEP3a/b/c/c.as* as well as the three respective genomic sequences. Sequence analysis showed that cDNA sequences of *CsatSEP3* c and c.as are the products of alternative splicing of the *CsatSEP3c* gene. Bioinformatics analysis with putative orthologous sequences from various plant species suggested that all four cDNA sequences encode for *SEP3*-like proteins with characteristic motifs and amino acids, and highlighted intriguing sequence features. Phylogenetically, the isolated sequences were closest to the *SEP3*-like genes from monocots such as *Asparagus virgatus*, *Oryza sativa*, *Zea mays*, and the dicot *Arabidopsis* *SEP3* gene. All four isolated *C. sativus* sequences were strongly expressed in flowers and in all flower organs: whorl1 tepals, whorl2 tepals, stamens and carpels, but not in leaves. Expression of *CsatSEP3a/b/c/c.as* cDNAs was compared in wild-type and mutant flowers. Expression of the isolated *CsatSEP3*-like genes in whorl1 tepals together with E-class *CsatAP1/FUL* subfamily and B-class *CsatAP3* and *CsatPI* subfamilies of genes, fits the ABCE “quartet model,” an extended form of the original ABC model proposed to explain the homeotic transformation of whorl1 sepals into whorl1 tepals in *Liliales* and *Asparagales* plants such as *C. sativus*. This conclusion was also supported by the interaction of the *CsatSEP3b* protein with *CsatAP1/FUL* and *CsatAP3* proteins. In contrast, expression of both B-class *CsatAP3* and *CsatPI* genes and the C-class *CsatAGAMOUS* genes together with E-class *CsatSEP3*-like genes in carpels, without any phenotypic effects on carpels, raises questions about the role of these gene classes in carpel formation in this non-grass monocot and requires further experimentation. Finally, taking advantage of the size and sequence differences in amplified genomic sequences of the triploid *C. sativus* and comparing them with the respective sequences from *C. tomasii*, *C. hadriaticus* and *C. cartwrightianus*, three putative wild-type diploid progenitor species, we examined the origin of *CsatSEP3a* sequence.

© 2011 Elsevier GmbH. All rights reserved.

Introduction

Most angiosperm flowers contain four distinct organ types, from the outside to the inside, namely: sepals in whorl1, petals in whorl2, stamens in whorl3, and carpels in whorl4. Depending upon the

taxa, this model can be adapted, placing species-specific homologous organs in each whorl. For example, plants such as orchids, roses, asparagus, maize, crocus (among many others) have varied and distinctive flowers. A common departure from the typical flower, observed in several monocots including saffron crocus (*Crocus sativus* L.), is that there is no clear distinction between sepals and petals, and the petaloid organs in whorls 1 and 2 are therefore referred to as tepals (Bowman, 1997).

C. sativus is a monocot triploid sterile species belonging to the *Iridaceae* family of *Asparagales*, whose whorl4 red stigmatic styles constitute saffron, a commercially very important popular food additive with delicate aroma and attractive color, also used for coloring and medical purposes. The flower of saffron is bisexual. The perianth consists of six petaloid tepals in whorls 1 and 2. The

* Corresponding author at: Institute of Agrobiotechnology, Center for Research and Technology Hellas, 6th Km Charilaou Thermi Road, Thermi GR-570 01, Greece. Tel.: +30 2310 498271; fax: +30 2310 498270.

E-mail address: tsaft@certh.gr (A. Tsaftaris).

¹ Present address: Department of Genetics and Plant Breeding, Aristotle University of Thessaloniki, Thessaloniki GR-541 24, Greece.

² Present address: Cotton and Industrial Plants Institute, National Agricultural Research Foundation, Sindos GR-574 00, Greece.

androecium consists of three distinct stamens in whorl3 and the gynoecium consists of a single compound pistil with three stigmas in whorl4. Several phenotypic flower mutants have been described, such as flowers with varying numbers of styles and stamens, as well as plants with double or even triple flowers, indicative perhaps of indeterminate inflorescences in this mutant phenotype (Grilli Caiola et al., 2004).

C. sativus blooms only once a year during mid-November and is harvested by hand. After wind separation of tepals, the red stigmas are separated from the yellow stamens by hand. Consequently, the cultivation of this crop for its flowers, and specifically for its stigmas, is very labor-intensive, leading to high costs (Tsaftaris et al., 2004, 2007). Thus, understanding flower development in crocus could help us not only to decode tepal formation in this monocot, but also reveal ways to increase yield and lower production costs.

The characteristics of the different floral organs during flower development are determined by actions of floral organ identity genes. The proposed eudicot Arabidopsis- and snapdragon-based model envisioned the action of flower-specific genes, belonging to three distinct functions termed A, B and C (hence the name of the original “ABC” model), where A-function genes alone could determine sepals, A+B function genes determine petals, B+C function genes determine stamens, and C-function genes alone determine carpels (Coen and Meyerowitz, 1991). Parallel studies found that, with the exception of the APETALA2 of A-function, all genes responsible for the A, B, and C functions involved in flower development belong to a large family of MADS-box transcription factors, which specify flowering time, floral meristem identity and space-time regulation of flower organ formation (Jack, 2001, 2004; Theissen, 2001; Immink and Angenent, 2002).

Expression of ABC genes throughout a plant does not transform leaves into floral organs and thus, the ABC functions, though necessary, are not sufficient to superimpose floral organ identity on a leaf development program (Theissen and Saedler, 2001).

An additional class of MADS-box genes identified later, the E-function *SEPALLATA* (*SEP*) genes of *Arabidopsis*, provide redundant control over the ABC system. *SEP* genes constitute one of the three phylogenetically determined clades of the E-superclade genes (the other two are the *AP1/SQUA/FUL*-like and the *AGL6*-like clades) (Becker and Theissen, 2003). Of these three subfamilies of E-class genes, only the *AGL6*-like subfamily of genes has been found in gymnosperms. *SEP* MADS-box genes are present in all angiosperms, but not in gymnosperms, and are required for the regulation of floral meristem determinacy and the specification of flower organs (Rijkema et al., 2009).

The three different *SEP* genes in *Arabidopsis* (*SEP1*, *SEP2* and *SEP3*) that were identified through their sequence similarity to *AGAMOUS* (*AG*) (Ma et al., 1991; Mandel and Yanofsky, 1998), when expressed together with the ABC genes, are sufficient for specification of petals, stamens and carpels. The ectopic expression of *SEP3*, together with BC genes, may turn leaves into floral organs (Honma and Goto, 2001). *SEP1/2/3* are still expressed in B- and C-function loss-of-function mutants, and the initial expression patterns of B- and C-function genes are not altered in the *sep1/2/3* triple mutant, indicating that *SEP1/2/3* do not act downstream of such genes and are not required for their initial activation (Pelaz et al., 2000). Moreover, *SEP4*, a fourth *SEP*-like gene discovered in *Arabidopsis*, is probably still expressed in these triple mutants and seems sufficient to confer the sepal-like structure; in quadruple *sep1/2/3/4* mutants, leaf-like structures replace flowers (Ditta et al., 2004).

Thus, *SEP* genes comprise a separate E-class subfamily of floral homeotic genes that provide an additional layer of function by encoding proteins forming higher-order complexes together with A-, B-, or C-class transcription factors, several of which are sufficient to transform leaves into floral organs (Honma and Goto, 2001).

These findings led to the suggestion of a protein-based combinatorial “floral quartet model” that could explain how the different floral organ identity genes interact at the molecular level (Theissen, 2001; Theissen and Saedler, 2001; Melzer et al., 2009). More specifically, the ABCE model postulates that sepals are specified by A-function protein (*AP1/SQUA*) activity together with E-function, petals by A+B+E, stamens by B+C+E, and carpels by C+E (Goto et al., 2001; Theissen, 2001; Theissen and Saedler, 2001). Thus, E-function activity, and consequently, *SEP* gene expression, is required for the formation of whorl1, 2, 3 and 4 organs. It should be noted that, for the ABC model, the A-function genes *AP1* or *SQUA* in *Arabidopsis* and snapdragon, respectively, together with *FRU*, constitute a clade of the E-superclade or class of genes; the other two clades of the E-family are the *SEP*-like and the *AGL6*-like subfamilies (Shan et al., 2009; Melzer et al., 2010).

Recently, Immink et al. (2009), using yeast three-hybrid and fluorescent techniques to study MADS-box protein interactions, showed strong indications that higher-order complex formation is a general and essential molecular mechanism for plant MADS-box protein functionality. The authors describe *SEP* proteins and *SEP3* in particular as the “glue” protein for higher-order complexes and flower organ formation. *SEP3*, in addition to flower organ formation and flower organ identity, was also recently found to regulate flowering (Dornelas et al., 2010) and flower organ growth (Kaufmann et al., 2009). Genes involved in organ size are targets of *SEP3* complexes. Two recent, parallel studies from the group of Theissen using *in vitro* assays demonstrated that homotetramers of *Arabidopsis* *SEP3* are sufficient to loop DNA by binding to two neighboring CArG elements and that *SEP3* forms stronger floral-quartet-like structure with the B-class proteins *AP3* and *PI* than the structure formed by *AP3* and *PI* themselves (Melzer and Theissen, 2009; Melzer et al., 2009).

SEP-like genes in grasses showing relatively heterogeneous expression patterns strongly suggest that they are also heterogeneous in function (Becker and Theissen, 2003). In maize, for example, numerous *SEP3*-like genes with distinguishable expression patterns that have been suggested to be involved in determining the alternative identity of spikelet primordial, the upper versus the lower floret within each spikelet primordium, or conferring determinacy to the spikelet or upper floret meristem have been described (Cacharron et al., 1999; Theissen et al., 2000; Theissen, 2001). Similar observations have been reported for rice plants in which *OSMADS1*, the putative ortholog of the maize *ZMM8*, plays an important role in floral meristem determination during the early development of rice florets (Agrawal et al., 2005; Cui et al., 2010; Gao et al., 2010; Kobayashi et al., 2010). Two *SEP1*-like (or *AGL2*-like) MADS-box genes, named *LMADS3* and *LMADS4* from lily (*Lilium longiflorum*), with extensive homology of *LMADS3* to the *Arabidopsis* *SEPALLATA3*, were expressed in the inflorescence meristem and in floral buds of different developmental stages. *LMADS4* mRNA is also expressed in vegetative leaf and in the inflorescence stem, where *LMADS3* expression is absent (Tzeng et al., 2003).

Previous results from our group with saffron crocus have indicated that expression of B-class paleo *AP3*-like genes (*CsatAP3*-like) is extended in whorl1 and may be important for the homeotic transformation of whorl1 sepals into tepals in this species (Tsaftaris et al., 2006) similarly to other monocot species (Kanno et al., 2003). We have also demonstrated the presence of *PI*-like genes in *C. sativus* (*CsatPI/GLO*-like), also in all four whorls of the flower, including whorl1 tepals (Kalivas et al., 2007). Among MADS-box genes, we also studied the *CsatAP1/FUL*-like genes, one of the three subfamilies of the E-superclade of MADS box genes of *C. sativus*. Three such *CsatAP1/FUL*-like genes were cloned and characterized. All three genes are expressed not only in all flower organs, as found for other *AP1/FUL/SQUA* members in other plant species, but they were

Download English Version:

<https://daneshyari.com/en/article/2057617>

Download Persian Version:

<https://daneshyari.com/article/2057617>

[Daneshyari.com](https://daneshyari.com)