

SEPALLATA (SEP) GENES IN WHEAT: PHYLOGENETIC AND EXPRESSION ANALYSES

A.R. PAOLACCI, O.A. TANZARELLA, E. PORCEDDU, M. CIAFFI

Department of Agrobiological and Agrochemistry, University of Tuscia, Via S. Camillo de Lellis, Viterbo, Italy - ciaffi@unitus.it

MADS-box genes, flower development, Triticum, gene expression, phylogenesis

Molecular and genetic analyses of floral homeotic mutants in *Arabidopsis* resulted in the formulation of the ABCDE model, which explains how the combined functions of five classes of genes (A, B, C, D and E) determine the identity of the organs (sepals, petals, stamens and carpels) of the four concentric floral whorls and ovules. A number of genes controlling these functions have been cloned and most of them are members of the MADS-box gene family. The four E class MADS-box genes *SEPALLATA (SEP1-4)* of *Arabidopsis*, which play an essential role in floral development and meristem determinacy, accomplish largely redundant functions, in fact mutants for single *sep* genes have very subtle phenotypes. On the contrary, flowers of *sep1/2/3* triple mutants consist entirely of sepal-like organs, whereas those of *sep1/2/3/4* quadruple mutants show leaf-like structures only. Within the MADS box gene phylogenetic tree *SEP* genes form a well-represented subfamily, which can be further subdivided into two major clades: *SEP3* and *SEP1/2/4*, wherein several gene duplications have been detected in monocot and eudicot lineages. At least five subclades have been detected in grasses, three in the *SEP1/2/4* clade and two in the *SEP3* clade, suggesting that they may have experienced at least three gene duplications. The orthologous *SEP* genes of rice and maize show different expression patterns, this heterogeneity suggests that *SEP* genes in grasses are not functionally homogeneous and might have been important in the evolution of their complex inflorescence. Much less is known on the *SEP* genes of wheat, wherein only seven MADS box genes of six different subfamilies had been isolated. A search in wheat EST databases detected many sequences homologous to *SEP* genes of rice and maize and 70 of them were cloned by RT-PCR from mRNA of fully emerged wheat spikes. On the basis of their sequences the 70 clones formed six groups and full-length cDNAs of six clones (WM4, WM5, WM10, WM11, WM19 and WM20), one for each group, were obtained by RACE extension. Phylogenetic analysis of several monocot and eudicot *SEP* genes indicated that the six wheat full-length sequences could be assigned to the five grass subclades of the *SEP* subfamily: WM11 and WM10 to the *OsMADS7* and *OsMADS8* subclades (*SEP3* major clade), respectively, WM4 and WM5 to the *LHS1* subclade and WM19 and WM20 to the *OsMADS34* and *OsMADS5* subclades (*SEP1/2/4* major clade), respectively. The expression patterns of the cloned sequences were analysed by northern and RT-PCR in different wheat plant and floral tissues. The six wheat cDNA sequences were expressed during all the stages of spike development and in developing caryopses. However, in spikelets at heading time the expression of WM10 and WM11 was restricted to lodicules, stamens and pistils, whereas lower amount of transcripts of WM4, WM5, WM19 and WM20 were also detected in lemmas and paleas. The expression of WM10, WM11 and WM20 was restricted to inflorescence tissues and caryopses, but a low amount of WM4, WM5 and WM19 transcripts was also present in vegetative tissues, such as coleoptiles and leaves. Further analyses of the six cloned *SEP* cDNA

sequences will include: I) evaluation of their copy number per genome and chromosomal location by Southern analysis; II) quantitative expression analysis by real-time RT-PCR in different plant and floral tissues at heading time; III) *in situ* hybridisation to floral organs of spikes at different developmental stage; IV) quantitative expression analysis of spring and winter cultivars of wheat grown at different conditions of thermo- and photo-period collected at the stage of floral transition.