MADS-BOX GENES OF MIKC TYPE IN WHEAT (*TRITICUM AESTIVUM* L.): MOLECULAR AND PHYLOGENETIC ANALYSIS

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

Dipartimento di Agrobiologia e Agrochimica, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo (Italy) – ciaffi@unitus.it

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In higher eukaryotes MADS-box genes encode a family of highly conserved transcription factors, which are involved in several developmental processes and in signal transduction. The MADS family includes two main lineages, type I and type II, both represented in plants, animals and fungi. In plants type II genes, or MIKC-type, have extensively been studied, much less is known on type I genes. MIKC genes control flowering induction and morphogenesis of the different flower organs. The interaction of the floral-specific MIKC-type MADS-box genes has been summarised in the ABCDE model of flower development. Moreover, the involvement of MIKC-type genes has been reported in a number of other metabolic processes, suggesting their participation in most aspects of plant development. The aim of the present research is the study of the complexity and diversity of this gene family in wheat. The knowledge of the structural and functional characteristics of these genes will allow the fine-tuning of plant growth and development to specific environments; this will be possible by modulating the extent of the life cycle phases. Moreover, the increase of wheat productivity will be made possible by modifying the spike and flower morphology.

The available sequences of MIKC-type genes of rice (34 sequences) and *Arabidopsis* (37 sequences) were exploited to BLAST search the public databases of wheat ESTs (Expressed Sequence Tags): TIGR wheat gene index database (TaGI, version 10), HarvEST wheat (version 1.13) and NCBI. BLAST searches identified 29 non-redundant MIKC-type consensus sequences, which were used as templates for 5’ and 3’ RACE (Rapid Amplification of cDNA Ends) extensions. Full-length cDNAs of the 29 putative wheat MIKC-type genes were cloned by RT-PCR of mRNA from various plant tissues using specific primer pairs designed in the 5’ and 3’ untranslated regions. For each of the 29 primer pairs, five independent RT-PCR reactions amplified products with the same electrophoretic mobility, which were cloned and sequenced. All five full-length cDNAs cloned from RT-PCR products exhibited the same sequence for 15 primer pairs, whereas the five clones obtained by each of the remaining 14 primer pairs showed either two or three similar but not identical sequences. Multiple cDNAs cloned from independent amplifications with the same primer pair showed high identity (over 90%) at both nucleotide and amino acid levels, most probably because they derived from transcripts of MADS box genes located in homoeologous chromosomes. Southern analyses showed that in hexaploid wheat there are three homoeologous copies for each of the 29 identified MIKC-type sequence, indicating that the genome of *T. aestivum* contains at least 87 (29x3) type II MIKC MADS-box genes. This genome organization was further confirmed by aneuploid analysis of six genes assigned to the *SEP*-subfamily, each showing three copies located in different homoeologous chromosomes. Phylogenetic analysis included the wheat MIKC cDNAs into 11 of the 13 MIKC subclasses identified in plants and corresponding to most genes controlling
the floral homeotic functions. The expression patterns of the cDNAs corresponding to different homoeotic classes was analysed in 18 wheat tissues and floral organs by RT-PCR, real time RT-PCR and northern hybridisation and compared with those of functionally characterized MADS-box genes from *Arabidopsis* and monocot species. Sequence similarity and comparable expression patterns were the parameters used for a preliminary prediction of the potential functions of the genes corresponding to the isolated wheat MADS-box sequences.