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Identification and characterization of gene sequences expressed in wheat spikelets at the heading stage☆

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Abstract

Through differential analysis of transcripts (SDDM), 85 cDNA sequences specifically or preferentially expressed in wheat spikelets at heading time were identified and cloned; 54 of them had significant homology with genomic, cDNA and protein and 16 with EST sequences. Among these 54 clones, 44 matched genes with known functions, whereas 10 detected homology with putative genes encoding proteins whose functions have been deduced on the basis of bioinformatic comparisons. Seventeen clones corresponded to genes that had never been cloned in cereals, 5 were related to wheat genes with known functions, and the remaining 32 to genes cloned in other cereals. On the basis of their presumed functions, the 54 clones were assigned to seven groups. The first four of them contained 40 sequences likely involved in floral organ morphogenesis and gametogenesis, and precisely (i) sequences involved in the morphogenesis of floral organs; (ii) sequences expressed in pollen and/or anther tissues; (iii) sequences encoding transcription factors; (iv) sequences involved in signal perception and transduction (kinases and LRR proteins). The expression patterns of these 40 sequences have been studied by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of transcripts from different tissues and spike organs of wheat.

Keywords: Triticum; Spike; Differential analysis; RT-PCR; Flowering; Gene expression

1. Introduction

Inflorescence and flower development in higher eudicotyledonous species are controlled by a hierarchy of interacting genes (Theissen and Saedler, 1999). At the top of the hierarchy, there are the flowering time genes, which regulate the timing of transition from the vegetative to reproductive phase by a direct or indirect activation of the meristem identity genes. These direct the floral meristem formation by regulating the expression of the floral organ identity genes, which, acting as homeotic genes, activate the downstream effector genes that control the specific features of the various floral organs.

Studies on *Arabidopsis* and *Antirrhinum* have led to the definition of the ABC model of floral organ development. It proposes that the combinatorial actions of three classes of floral organ identity genes (homeotic genes), which control the A-, B-, and C-functions, specify the floral organs in the four concentric whorls

Abbreviations: *ANT*, *AINTEGUMENTA*; *AP2*, *APETALA2*; *AP3*, *APETALA3*; bp, base pair; CS, *Triticum aestivum* cv. Chinese Spring; EXS-protein, extra sporogenous cells-protein; *FT*, *FLOWERING LOCUS T*; HALF-1, HBP-1-associated leucine-zipper factor-1; HBP, histone promoter-binding protein; mRNA, messenger RNA; PEBP, phosphatidylethanolamine-binding protein; *PI*, *PISTILLATA*; RT-PCR, reverse transcriptase-polymerase chain reaction; SDDM, simple differential display method; *TFL1*, *TERMINAL FLOWER1*.

☆ Database accession numbers (See Table 1): AJ577377, AJ577375, AJ577372, AJ577374, AJ577373, AJ577378, AJ577376, AJ577379, AJ577363, AJ577364, AJ577365, AJ577366, AJ577367, AJ577368, AJ577369, AJ577370, AJ577371, AJ577380, AJ577381, AJ577382, AJ577383, AJ577384, AJ577385, AJ577387, AJ577391, AJ577388, AJ577390, AJ577392, AJ577393, AJ577389, AJ577386, AJ606033, AJ606034, AJ606035, AJ606013, AJ606014, AJ606015, AJ606016, AJ606017, AJ606018, AJ606019, AJ606020, AJ606021, AJ606022, AJ606023, AJ606024, AJ606025, AJ606026, AJ606027, AJ606028, AJ606029, AJ606030, AJ606032, AJ606031.

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(Coen and Meyerowitz, 1991). The sepals are specified by the expression of the A-function genes in the first whorl, the petals are controlled by a combination of A- and B-functions in the second whorl, the stamens by a combination of B- and C-functions in the third whorl, and the carpels by the single action of the C-function gene in the fourth whorl. The same model assumes that the A- and C-function genes exert cadastral functions through their mutual repression at the boundary between the second and third whorl. With a few exceptions, all the ABC gene sequences share a highly conserved region of about 180 base pair (bp), termed MADS-box (Theissen, 2001). On the basis of studies in *Petunia* and *Arabidopsis*, the ABC model has been extended to include two more functions, yielding an ABCDE model (Theissen, 2001), where the D function controls the ovule development, and the E function specifies for petal, stamen, and carpel identities.

Flower structures of the grass species are quite different from those of dicots. Their stamens and carpels are surrounded by glumes, lemma, palea, and lodicules, but lack the typical sepals and petals of the dicot flowers. Since studies on maize and rice MADS-box genes have suggested that lemma and palea are homologous to dicot sepals and the lodicules are modified petals, the ABCDE model can be extended to cereals (Goto et al., 2001). However, the comprehension of the molecular basis of cereal flower development can be considered just at its beginning, since most genes involved in flower induction and morphogenesis remain to be cloned and characterized. In wheat, most floral organ identity genes have yet to be identified, since only four MADS-box genes had been isolated (Murai et al., 1998; Meguro et al., 2003).

The aim of this study was the isolation and cloning of cDNA sequences differentially expressed in wheat spikelets at heading stage and the examination of their expression patterns in different wheat tissues and floral organs.

2. Materials and methods

2.1. Plant material

Triticum aestivum cv. Chinese Spring (CS) was used as experimental material. RNA was extracted from the following plant tissues, which had been collected, frozen into liquid nitrogen, and stored at −80 °C: (i) spikelets from fully emerged spikes (Feeks scale 10.5) and flag leaves; (ii) coleoptiles and young roots, 20 days old; (iii) spikes at interval of 5–7 days, starting when the spike was 1–1.5 cm long and until complete heading; (iv) single floral organs (glumes, palea, lemma, lodicules, stamens, pistil) from fully emerged spikes; (v) developing caryopses 5 and 20 days after anthesis (DAA).

2.2. Simple differential display method (SDDM)

In order to detect genes preferentially expressed in spikelets and leaves, plant tissues were analyzed by the simple differential display method (SDDM; Yoshida et al., 1994). First-strand cDNA was synthesized from 200 ng of poly(A)+messenger RNA (mRNA) by the "SUPERSCRIPT™ First-Strand Synthesis System (Invitrogen) and random hexamers as primers. PCR amplification was primed by 60 different 10-mers of the series A, B, C, D, R, S, and Z of OPERON technologies. PCR conditions were initial denaturation at 92 °C for 3 min, 35 cycles of 92 °C for 1 min, 40 °C for 1 min, and 72 °C for 3 min, followed by a final extension step at 72 °C for 5 min. PCR mixtures (10 μl) were heated at 94 °C for 2 min and separated in polyacrylamide gel (T=10% and C=2.67%) containing 0.375 M Tris–HCL pH 8.8 and 7 M urea.

2.3. Cloning of cDNAs from differentially expressed transcripts

The differential cDNA fragments were excised from gel, reamplified with the same PCR conditions used for SDDM. Reamplified products were separated in 1.5% agarose gel, purified by the Sephaglas BandPrep kit (Amersham Pharmacia) and cloned into the modified *Eco*RV site of pGEM plasmid (Promega). The size of the inserts of five plasmids for each cloned differential band was estimated by digestion with *Eco*RI or *Not*I and separation on 1.5% agarose.

2.4. Nucleotide sequencing and homology analysis

Plasmid DNA for sequencing reactions was prepared from 3 ml overnight cultures using the Plasmid miniprep kit (Quiagen). Both strands were sequenced by ABI PRISM 377 and ABI PRISM Dye Terminator sequencing kit (PE Applied Biosystem) and the vector specific primers Sp6 and T7. Similarities with sequences in nucleotide and protein databases (GenBank, PDB, EMBL, DDBJ, SwissProt, PIR, and PRF) and their EST divisions were scored using the BLAST program. The scores considered indicative of significant homology were higher or equal to 200 for searches against nr (nonredundant) nucleotide sequences (BLASTN); higher or equal to 150 for searches against dbEST (BLASTN); higher or equal to 80 for searches against nr protein sequences (BLASTX), as used in plant EST analyses by Newman et al. (1994). The analysis of the predicted protein sequences was carried out by searching for conserved motifs at the Pfam HMMs and InterPro databases (http://pfam.wustl.edu./hmmsearch.shtml and http://www.ebi.ac.uk/interpro/).

2.5. Expression analyses by RT-PCR

First-strand cDNA was synthesized from 1 μg of total RNA using the Expand™ Reverse Transcriptase (RT;