

Expression of some model plant embryogenesis genes in *Phaseolus* ovules

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Introduction. Embryogenesis studies in plants reveal the importance of many genes during the embryo development. Seed development in plants requires a coordinated differentiation of the embryo proper, suspensor, endosperm tissues, and seed coat. Many genes must be expressed as the zygote divides in a regulated manner, completes morphogenesis, and differentiates into a mature embryo capable of surviving desiccation and producing a viable plant (1). These genes show temporal as well as spatial patterns of gene expression during seed development. The stage-specific cDNAs have been used as markers of cell differentiation and to follow the development of the embryos (2). Embryo development studies explored in part the analysis of embryo-defective mutants. A large number of embryo-lethal mutants was identified and analyzed in *Arabidopsis*. The number of genes that can be easily mutated to give an embryo lethal phenotype was estimated to vary between 250 to 750 (1, 3, 4). Some of these genes are heat shock proteins (HSPs), BEL, TITAN (TTN), PASTICCINO (PAS), Leafy Cotyledon (LEC) and lipid transfer proteins (LTP). The HSPs are molecular chaperonins that regulate protein homeostasis and membrane fluidity and ultimately prevent or delay cell death during heat stress (5). BEL transcription factors are essential for inflorescence and fruit development (6). The TTN genes encode chromosome scaffold proteins of the condensing and cohesion classes required for chromosome function at mitosis (7). The PAS genes, which are involved in the control of cell division, proliferation and differentiation, are required for normal organization of the apical region in the embryo (8). LEC genes are central regulators of embryogenesis that play key roles in processes that occur during both the morphogenesis and maturation phases (9). LTP genes are involved in the polar transfer of the lipids towards the peripheral layers of the cells (10). Expression of the above-mentioned genes was analyzed during *Phaseolus* seed development.

Material and methods. The genotype BAT93, a cultivated form of *P. vulgaris*, was used as plant material. Plants were grown in growth chambers under the following conditions: 27°C/23°C (day/night), 75% relative humidity and 12 hrs photoperiod. Total RNAs were extracted from 100 mg of ovules. mRNAs were purified using the "mRNA Purification Kit" from Amersham and used for RT-PCR with the "Titan One Tube RT-PCR kit" from Roche. The RT-PCR reaction was carried out using the following profile: 50°C for 30 min, 94°C for 2 min, 35 cycles of (94°C for 30 sec, Ann. Temp. for 45 sec, 68°C for 45 sec), 68°C for 5 min with Elongation Factor 1 α (EF-1 α , F, CTTCAGGATGTBTACAAGATTG & R, GCAGCCTTGTVACCTTGWCWCC) used as internal control.

Results and discussion. mRNAs extracted from ovules harvested 12 days after anthesis were used for RT-PCR. Primers were designed after alignment of species mRNA coding sequences from each gene family. Annealing temperature and MgCl₂ concentrations used are given in table 1. RT-PCR products loaded in 1.5% agarose gel showed one band for PAS, LEC, and LTP genes, two bands for BEL gene, several bands for HSP and TTN genes (Figure 1). Expected size for each gene was as follows: 270 bp for BEL gene, 350 bp for LTP genes, 440 bp for LEC gene, 500 bp for HSP gene, 550 bp for PAS gene and 590 bp for TTN gene. The presence of several

bands for some gene families could be explained by unspecific amplifications, due to some primers degeneracy. High intensity band for LEC and LTP genes was obtained after 35 cycles of PCR. Band intensities are intermediate for HSP and PAS genes, and low for BEL and TTN genes. These results mean that transcripts of LEC and LTP genes accumulate more than the others; these genes are strongly expressed in the *Phaseolus* ovules. Amplified fragments will now be sequenced. Sequence homology analyses in gene banks will be performed by using the National Center for Biotechnology Information (NCBI) BLAST network service. The gene sequences with high homologies in genes banks and unknown in *Phaseolus* data base (BEL, TTN, PAS, etc.) will be submitted to gene banks such as NCBI.

Table 1. Oligonucleotides, annealing temperatures and MgCl₂ concentrations used for RT-PCR

Gene families	Annealing temperature & MgCl ₂ concentrations
BEL	45°C, 1.5 mM
HSP	45°C, 2.5 mM
LTP	56°C, 1.5 mM
PAS	50°C, 1.5 mM
LEC	50°C, 1.5 mM
TTN	45°C, 2.5 mM

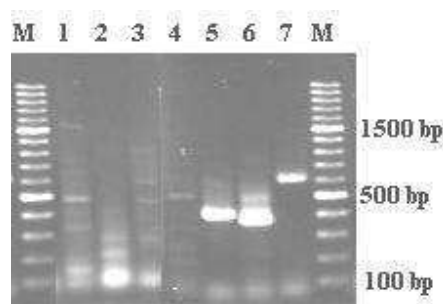


Figure 1. RT-PCR products separated on agarose gel, with one band for PAS (4), LEC (5), and LTP (6) genes, two bands for BEL (2) gene, several bands for HSP (1) and TTN (3) genes. EF-1 α (7) used as internal control.

References

1. McElver *et al.* (2001). *Genetics* **159**, 1751 - 1763
2. Bommert & Werr (2001). *Gene* **271**, 131 - 124
3. Franzmann *et al.* (1995). *Plant J.* **7**, 341 - 350
4. Tzafrir *et al.* (2004). *Plant Physiol.* **135**, 1206 - 1220
5. Fu *et al.* (2002). *Plant Cell* **14**, 3119 - 3132
6. Smith *et al.* (2004) *Curr Biol* **14**, 812 - 817
7. Liu & Makaroff (2006). *Plant Cell* **18**, 1213 - 1225
8. Bellec *et al.* (2002). *Plant J.* **32** (5), 713 - 722
9. Baumbusch *et al.* (2004). *Journal of Experimental Botany* **55** (394), 77 - 87
10. Kader (1996). *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 627 - 654