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Identification and characterization of transcripts differentially expressed during embryogenesis in *Capsella bursa-pastoris*

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Abstract

Large-scale gene expression analyses were conducted during embryonic development of *Capsella bursa-pastoris* using the cDNA-AFLP technique, and 231 differentially expressed genes were identified. Most of the transcript-derived fragments (TDFs) were identified against *Arabidopsis* sequences and a minority against other plant sequences by searching in *NCBI* database. A total of 128 TDFs, homologous to genes with known functions, were classified into 13 functional categories, and many of them were involved in transcription, metabolism, disease defense and protein binding. Expression profiles of 90 *C. bursa-pastoris* genes were compared with those of the corresponding *Arabidopsis* genes and 64 genes showed similar expression profiles and the expression profiles of the rest 26 TDFs were different from those of the relevant *Arabidopsis* genes. Besides, some genes related to oxidative stress and ABA response were fiercely up-regulated during embryogenesis of *C. bursa-pastoris* according to RT-PCR.

Additional key words: cDNA-AFLP, gene expression, *in vitro* culture, transcript-derived fragments.

Introduction

Capsella is a small genus within the family *Brassicaceae* containing only three species that show differences in ploidy level and habitat range (Hurka and Neuffer 1997). Two of the *Capsella* species, *C. grandiflora* and *C. rubella*, are diploid, while *C. bursa-pastoris* is tetraploid. *C. bursa-pastoris* is an abundant weed in early spring and its seeds germinate in autumn with seedling rosettes overwintering, or germinate soon after the ground thaws in spring. Flowering usually occurs in late spring, and the plant matures and dies in the heat of summer. However, germination and flowering can continue over a longer period of the year in cooler climates (Baskin and Baskin 1989). *C. bursa-pastoris* is a good model plant for analyzing development, flowering, adaptation and evolution of plants (Nutt *et al*. 2006, Slotte *et al*. 2007). To date, the embryonic morphology of *C. bursa-pastoris* is well known as a very typical course from the globular, heart, torpedo and mature embryonic stages. However, molecular studies have not been reported. In related *Arabidopsis thaliana*, different processes during zygotic and somatic embryogenesis on molecular levels are almost fully understood (Girke *et al*. 2000, Casson *et al*. 2005, Schmid *et al*. 2005, Spencer *et al*. 2007, Le *et al*. 2010). In fact, analyzing gene expression is important to understand physiological processes occurring during embryogenesis of *C. bursa-pastoris*. In the present study, we analyzed gene expression in six zygotic embryo stages distinguished on the base of the shape and colour of the developing *C. bursa-pastoris* embryo. During embryogenesis, multiple TDFs were collected based on cDNA-AFLP results, and differentially expressed TDFs from various embryo developmental stages were sequenced and analyzed using *BLASTN* and *BLASTX* in *NCBI*.

Materials and methods

Capsella bursa-pastoris (L.) Medik. siliques were collected from vegetable field in Hubei Academy of Agricultural Sciences in China. The zygotic embryos were separated by manual operation and assorted into the six

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Abbreviations: AFLP - amplified fragment length polymorphism; RT - reverse transcription; TDF - transcript-derived fragment.

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developmental stages including globular, heart, torpedo, cotyledon, premature and mature stages distinguished by the shape and color of the embryos. Each of the six embryonic materials was collected twice as biological duplicates.

Total RNA was isolated using *TRIzol* reagent according to the manufacturer's instructions (*Invitrogen*, USA). DNaseI-treated RNA was primed with oligo-(dT)$_{18\sim25}$ and converted into cDNA using reverse transcriptase (RT) *SuperScriptIII* (*Invitrogen*). Second-strand cDNA synthesis was performed using 10 U DNA polymerase I and 3 U RNase H (*Takara*, Otsu, Japan) according to standard protocols (Sambrook *et al.* 2001). Hereafter, the resulted double-stranded cDNA purification was carried out by phenol chloroform extraction and ethanol precipitation, followed by resuspending in a final volume of 30 mm^3 ddH$_2$O. A quarter of this volume was loaded on an agarose gel to check the size of the cDNA sample. If the size mostly ranged between 100 and 2 000 bp, the rest of the cDNA would be used for cDNA-AFLP analyses.

The cDNA-AFLP reactions were performed according to Bachem *et al.* (1996). Mixed cDNA (500 ng) was digested by *Mse*I/*Eco*RI enzyme combination (*New England Biolabs*, Beijing, China) at 37 °C overnight. Reactions were terminated by heating at 70 °C for 15 min. After digestion, the restriction fragments were incubated with 5 pmol of *Eco*RI adaptor and 50 pmol of *Mse*I adaptor by using 3 U T4 DNA ligase (*Promega*, USA) at 16 °C for 4 h. The ligated products were pre-amplified with *Eco*RI and *Mse*I pre-selection primers. The PCR program was as follows: 3 min at 94 °C, 24 cycles consisting of 30 s at 94 °C, 1 min at 56 °C, 1 min at 72 °C, and a final extension step of 10 min at 72 °C. The pre-amplification product was loaded on an agarose gel, and the expected smear mostly between 100 and 1 000 bp was observed. The pre-amplification PCR product was diluted 10-fold with ddH$_2$O, and 1 mm^3 was used as template for selective PCR amplification with selective primers containing 2 - 3 selective bases. The temperature profile for the selective PCR reaction was as follows: 94 °C for 3 min, 36 cycles of 30 s denaturing at 94 °C, 30 s annealing and 60 s extension at 72°C, ending with 10 min at 72 °C to complete extension. Annealing was initiated at a temperature of 65 °C, which was then reduced by 0.7 °C for the next 12 cycles, and maintained at 56 °C for the remaining 23 cycles. The sequences of adaptors and primers were listed in Table 1.

After selective PCR amplification, the PCR products were separated by electrophoresis on 6 % denaturing polyacrylamide gels. The gels were pre-run at 100 W for about 30 min before 4.5 mm^3 of the mix was loaded, then run at 65 W until the bromophenol blue reached the bottom. DNA fragments were visualized by silver-staining according to the DNA silver staining system procedure (*Promega*). The replicate gels from different preparations of the same embryo stage were run to minimize the variations during processing. The TDFs of cDNA-AFLP gels were scored for data analyses and were transformed into a binary character matrix using "1" and "0" to indicate the presence and absence, respectively, of a TDF at a particular position.

Table 1. Sequences of adaptors and primers used for pre-amplification and selective amplification in cDNA-AFLP analysis.

Adapters/primers	*Sequence (5'-3')*
*Eco*RI adaptor1	CTCGTAGACTGCGTACC
*Eco*RI adaptor2	AATTGGTACGCATAC
*Mse*I adaptor1	GACGATGAGTCCTGAG
*Mse*I adaptor2	TACTCAGGACTCAT
*Eco*RI pre-selective primer	GACTGCGTACCAATTC
*Mse*I pres-selective primer	GATGAGTCCTGAGTAA
*Eco*RI selective primer 1-AA	GACTGCGTACCAATTCAA
*Eco*RI selective primer 2-AC	GACTGCGTACCAATTCAC
*Eco*RI selective primer 3-AG	GACTGCGTACCAATTCAG
*Eco*RI selective primer 4-AT	GACTGCGTACCAATTCAT
*Eco*RI selective primer 5-TA	GACTGCGTACCAATTCTA
*Eco*RI selective primer 6-TT	GACTGCGTACCAATTCTT
*Eco*RI selective primer 7-TC	GACTGCGTACCAATTCTC
*Eco*RI selective primer 8-TG	GACTGCGTACCAATTCTG
*Eco*RI selective primer 9-ACC	GACTGCGTACCAATTCACC
*Eco*RI selective primer 10-ACG	GACTGCGTACCAATTCACG
*Eco*RI selective primer 11-AAG	GACTGCGTACCAATTCAAG
*Mse*I selective primer 1-CAA	GATGAGTCCTGAGTAACAA
*Mse*I selective primer 2-CAC	GATGAGTCCTGAGTAACAC
*Mse*I selective primer 3-CAG	GATGAGTCCTGAGTAACAG
*Mse*I selective primer 4-CAT	GATGAGTCCTGAGTAACAT
*Mse*I selective primer 5-CTA	GATGAGTCCTGAGTAACTA
*Mse*I selective primer 6-CTC	GATGAGTCCTGAGTAACTC
*Mse*I selective primer 7-CTG	GATGAGTCCTGAGTAACTG
*Mse*I selective primer 8-CTT	GATGAGTCCTGAGTAACTT
*Mse*I selective primer 9 -CGA	GATGAGTCCTGAGTAACGA
*Mse*I selective primer 10-CGT	GATGAGTCCTGAGTAACGT
*Mse*I selective primer 11-CGC	GATGAGTCCTGAGTAACGC
*Mse*I selective primer 12-CGG	GATGAGTCCTGAGTAACGG

TDFs, showing differential expression in embryonic stages, were cut from cDNA-AFLP gels and dissolved out of the polyacrylamide by incubation in 50 mm^3 of sterile water for 10 min at 100 °C. After cooling slowly to room temperature, the eluted TDF was amplified by PCR using the same primers as those used to generate the cDNA-AFLP profile. The PCR condition was as follows: 3 min at 94 °C, 30 s at 94 °C, 30 s at 56 °C, 1 min at 72 °C, followed by 30 cycles, and a final extension step of 10 min at 72 °C. The amplified product was extracted and cloned with the *Dual Promoter TA Cloning®* kit (*Invitrogen*), and sequenced. Sequencing results were analyzed using *BLASTN* and *BLASTX* algorithms (http://www.ncbi.nlm.nih.gov/ BLAST/).

For qRT-PCR and RT-PCR, total RNA was extracted using *TRIzol* reagent (*Invitrogen*) and was treated with RNase-free DNase I (*Fermentas*). First-strand cDNA was synthesized from total RNA using oligo-(dT)$_{18\sim25}$ and moloney murine leukemia virus reverse transcriptase (*Promega*). Primer sets were designed with the *Primer5* software for future qRT-PCR amplification experiments

(Table 2). PCR was carried out in a reaction volume of 20 mm^3 including 1 mm^3 of diluted cDNA, 0.6 mm^3 of reverse and forward primers, 7.4 mm^3 of ddH$_2$O, 0.4 mm^3 of ROX and 10 mm^3 of the PCR master-mixture (*Thunderbird SYBR* qPCR mix, Toyobo, Japan). The following PCR program was used: 95 ºC for 10 min, 95 ºC for 15 s, 60 ºC for 1min; steps 2 and 3 were repeated 40 times. The specificity of the PCR amplification was checked with a heat dissociation curve (60 - 95 °C) following the final cycle of the PCR. The relative quantification analysis was performed by relative standard curve according to threshold values (CT) generated from the *ABI StepOne*$^{\text{TM}}$ real-time PCR system (*Applied Biosystems*, USA). The results were standardized using *ACTIN7* (*ACT7*) as internal control. Two biological replicates were programmed and each cDNA sample was run in duplicate.

Five TDFs were selected for carrying out RT-PCR amplification. Primer sets were designed and shown in Table 2. As a result of a steady-state expression at all six developmental stages, *ACT7* was used as an endogenous standard, and then cDNA samples of six embryonic stages were amplified using the following PCR condition: 1 min at 94 °C, 15 s at 94 °C, 15 s at 60 °C, 45 s at 72 °C, followed by 36 cycles, and a final extension step of 10 min at 72 °C. Finally, 5 mm^3 of the PCR product was checked on an agarose gel. Relative expression of five TDFs was obtained based on the result of RT-PCR. Two biological replicates were run to minimize the variations during processing.

Table 2. Primer sets for RT-PCR and qRT-PCR of target genes.

TDF	Forward primer (5'-3')	Reverse primer (5'-3')	Size of amplicon [bp]
TDF8	ATTCTTGCGTTCCCTTGTAA	CCACTTGATGCCGTCTCC	198
TDF25	GCTCCTTGGTTTGTCTTGTG	ATCTCTTTGTTCGGGTCTGC	120
TDF29	ATTGTTTCCGCTCTTGTTG	ACTGTCTTCACTTTCCCCTC	84
TDF33	AAGGCAGGTGGTATGATGGA	AGGAAGTTGGAAGGGATTAGTG	163
TDF97	CAAATGCGACTGCGTTACA	GAAGAAGGGGATGATGGACA	125
TDF4	TATCCATAAGATTCTCAGC	GCATCTATTATTGTCCAG	74
TDF105	CATCAGGTGGTGGAAGCT	GATTACAGCCATCGGAACT	119
TDF341	GGGAATCGGGAGGAGTGAAG	CCCAGTCGTCGGAGACATAGC	205
TDF521	TTATCAGCAGAAAACGAG	CAATACATGGGGAGAAGC	120
TDF565	GCACAGAAAGTGCCTGAA	TAACGCCTTACCTGGATC	101
TDF623	CTAAACCCAACTCACGTACCACT	CATCTGCGTCTCGAAGCAAT	180
ACT7	GAGCGATGGCTGGAATAGAA	GAGCGAGAAATTGTCCGTGA	186

Results and discussion

Changes in TDFs during embryo development: Ninety-eight primer combinations were used to screen differences in gene expression among embryos of the six different stages. Some examples of the cDNA-AFLP comparative expression profile after polyacrylamide gel electrophoresis and staining are shown in Fig. 1. Each primer combination generated about 80 products on polyacrylamide gels. In total, 8061 TDFs were generated using 98 primer sets, and the number of TDFs was different among the six developmental stages. The largest number of TDFs (4693) was found in the cotyledon embryo stage following heart embryo stage (3519), torpedo stage (3393), mature embryo (3145) and the least (2685) was found in the globular embryo stage. As to stage-specific TDFs, *i.e.* expressed only in one of the six stages, the most (540) were expressed in the heart embryo stage and the least (279) were expressed in the mature embryo stage. We also calculated the number of TDFs expressed in two or more different stages. In this study, 133 TDFs were present simultaneously in the globular, heart and torpedo embryos; 206 TDFs in the cotyledon, premature and mature embryos; 160 TDFs in the globular, heart, torpedo and cotyledon embryos; 243 TDFs in the

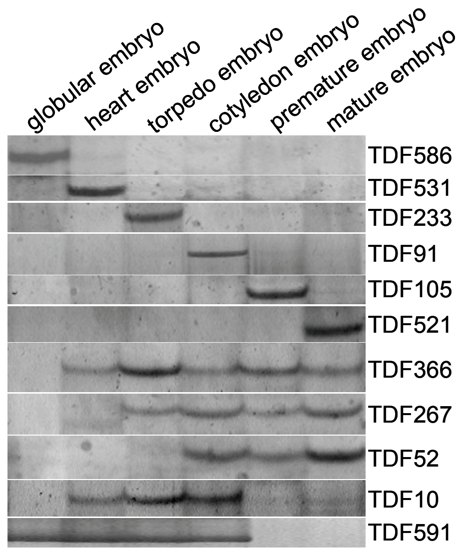

Fig. 1. Eleven selected TDFs showing differential expression in different embryo stages by cDNA-AFLP analysis.

torpedo, cotyledon, premature and mature embryos; 64 TDFs in the globular, heart, torpedo, cotyledon and premature embryos; and 224 TDFs in the heart, torpedo, cotyledon, premature and mature embryos. The number of TDFs simultaneously expressed in later embryonic stages was distinctly higher than that expressed during earlier embryonic stages.

Identification and functional classification of 231 sequenced TDFs during embryonic development: A total of 231 TDFs were isolated from the silver-stained cDNA-AFLP gels based on differential expression, then cloned and sequenced after purification. We searched and analyzed the TDFs using *BLASTN* and *BLASTX* in *NCBI* to obtain information about potential gene function for these TDFs (data not shown). As a result, 24 TDFs did not match with plant genes and were defined as the no-match group, suggesting that they could correspond to novel embryonic genes. A sequence comparison of the remaining 207 cloned TDFs against the database revealed that 79 TDFs (34.2 %) belonged to unknown genes, whereas 128 TDFs were homologous to genes with known functions (Table 3). The largest group of the genes was involved in disease-defense responses, whereas the least numbers of genes were involved in the energy pathways. To further verify the differential expression of genes identified by cDNA-AFLP, six genes were selected for qRT-PCR analysis (Table 3). Expression level of the transcripts was normalized by the *ACT7* gene, which was isolated and sequenced in this study. The expression patterns for all six genes agreed with the cDNA-AFLP results. Based on the comparison, the qRT-PCR results were more accurate than those measured by cDNA-AFLP. For example, TDF105 was expressed only in premature embryos according to cDNA-AFLP, but a trace number of transcripts were found in the torpedo and cotyledon embryonic stages using qRT-PCR. Taken together, the qRT-PCR validation indicated that the differential expression detected by cDNA-AFLP was highly reliable.

We used the *Arabidopsis eFP Browser*, available at http://www.bar.utoronto.ca/, to obtain the relevant *Arabidopsis* gene expression data from the *AtGenExpress Consortium* (Winter *et al.* 2007). This approach allowed us to compare gene expression between *C. bursa-pastoris* and *Arabidopsis*. In total, 90 differentially expressed TDFs were compared with the relevant *Arabidopsis* gene expression data (Casson *et al.* 2005, Schmid *et al.* 2005, Le *et al.* 2010). As a result, expression profiles of 64 TDFs were found similar to those of the corresponding *Arabidopsis* genes. Many of these TDFs were homologous to genes already reported to have a function in embryogenesis. TDF292 was related to *TOPLESS-RELATED 2* (*TPR2*) in *Arabidopsis*. In recent research, TPR2 shares extensive amino acid similarity with TOPLESS, and both of them are involved in determining the apical shoot pole and a basal root pole in globular embryos of *Arabidopsis* (Long *et al.* 2006). TDF22 matched with *NOV*, and *nov* mutants exhibit an embryo-defective phenotype characterized by size reduction and frequent fusion of cotyledons and abnormalities in the early steps of vascular development (Tsugeki *et al.* 2009). TDF180, corresponding to *SOMNUS* (*SOM*), was detected in both premature and mature embryos of *C. bursa-pastoris*. Kim *et al.* (2008) have found the *SOM* is expressed during seed maturation and inhibits seed germination by regulating the expression of gibberellic acid and abscisic acid (ABA) metabolic genes. TDF507, matched with *NADP-MALIC ENZYME 1* (*NADP-ME1*), was expressed during the later stages of embryogenesis. Previous study has indicated *NADP-ME1* might play specific roles at particular developmental stages rather than being involved in primary metabolism in *Arabidopsis* (Wheeler *et al.* 2005). Besides, the transcription of the *NADP-ME1* gene decreases in response to many of the stresses in the leaves of hexaploid wheat (Fu *et al.* 2011). TDF486, TDF488, TDF533 and TDF538, all related to seed storage protein genes, were detected both in premature and mature *C. bursa-pastoris* embryos. In soybean, seed storage proteins are the major contributors to protein content at later stages of seed filling (Hajduch *et al.* 2005). TDF535 was homologous to *HSP17.6A-CI* of *Arabidopsis.* HSP17.6A-CI was characterized as a small HSP and might play a role in desiccation tolerance or dormancy for protecting embryos during drying (Wehmeyer *et al.* 1996). Small HSPs have the remarkable ability to sense increase in temperature directly and can switch from an inactive to a chaperone-active state (Xue *et al.* 2010). Two TDFs (TDF103 and TDF198) showed similarity to *PROTEIN KINASE FAMILY PROTEIN* and *RECEPTOR LECTIN KINASE*, respectively. In the past few years, they have been proved to be involved in ABA-dependent processes in *Arabidopsis* seed development (Osakabe *et al.* 2005) and seed germination (Xin *et al.* 2009), respectively. Taken together, expression profiles of most TDFs resembled those of the homologous *Arabidopsis genes*, suggesting that two closely related species showed

Table 3. Functional classification of 231 TDFs differentially expressed in the six embryo stages according to their homology.

Functional classification	Number of TDFs	[%]
Transcription	13	5.6
Protein with binding function	17	7.4
Signal transduction	3	1.3
Metabolism	17	7.4
Transporter	8	3.5
Protein synthesis	4	1.7
Protein fate	13	5.6
Storage protein	4	1.7
Development	4	1.7
Disease/defense	20	8.7
Energy	2	0.8
Transposable elements	9	3.9
Unclassified gene	14	6.1
Unknown gene	79	34.2
No match	24	10.4

similarity each other not only in embryonic morphology but also in gene expression of embryogenesis.

Expression of some *C. bursa-pastoris* genes was distinctly different from that of the relevant *Arabidopsis* genes. TDF341 was similar to *CRCK2* (AT4G00330) and expressed in premature and mature embryos in our study, and the result was further confirmed by qRT-PCR (Table 4). However, rare transcripts of *CRCK2* were detected in embryo development of *Arabidopsis* according to data obtained from *Arabidopsis eFP Browser*. TDF348 showed the highest expression in premature embryonic stages of *C. bursa-pastoris* according to cDNA-AFLP results. On the contrary, the relevant stress-inducible protein gene (AT4G12400) was expressed rarely in premature embryos of *Arabidopsis* (data from the *AtGenExpress Consortium*). *PWD* (AT4G24450) was detected rarely during the whole embryo development of *Arabidopsis*. However, TDF127 similar to *PWD* was detected in premature and mature embryonic stages in this study. Altogether, due to obvious differences in ploidy level and genetic background of two closely related species, some discrepancies of gene expression existed between *Arabidopsis* and *C. bursa-pastoris*.

Table 4. qRT-PCR showing relative expression of six selected TDFs at the five stages of embryo development. G, H - mixes of globular and heart embryos; T - torpedo embryos; C - cotyledon embryos; P -premature embryos; M - mature embryos. *ACT7* was used as an internal control. Data represent means ±SD of two biological replicates.

TDF	G-H	T	C	P	M
TDF4	0	1.0 ± 0.9	0	1.4 ± 0.5	54.1 ± 0.8
TDF105	1.0 ± 0.4	10.2 ± 6.2	49.6 ± 4.4	212.3 ± 31.4	1.4 ± 0.9
TDF341	1.1 ± 0.3	1.0 ± 0.3	1.1 ± 0.2	6.5 ± 0.3	9.6 ± 2.8
TDF521	0	0	0	1.0 ± 0.5	40.6 ± 7.3
TDF565	1.0 ± 0.3	6.9 ± 1.0	9.9 ± 0.8	51.5 ± 7.2	3.0 ± 1.7
TDF623	74.1 ± 23.8	0	0	0	1.0 ± 0.7

Up-regulation of TDFs related to oxidative stress and ABA response in embryogenesis: In developing seeds, endogenous ABA levels peak during the late phase of seed maturation, and ABA is involved in the onset of seed dormancy and the acquisition of desiccation tolerance (Seo and Koshiba 2002). Besides, the generation of free radicals and reactive oxygen species is a major threat in maturing seeds (Leprince *et al*. 1996). Some TDFs related to oxidative stress and ABA response were also found during embryogenesis of *C. bursa-pastoris*, and 5 TDFs (TDF8, TDF25, TDF29, TDF33 and TDF97) were selected for assessing the relative abundance of the transcripts by RT-PCR. We found the same expression patterns as with cDNA-AFLP. Although not strictly quantitative, this RT-PCR technique provided sufficient related information to demonstrate the up-regulation of the five TDFs related to oxidative stress and ABA response during embryogenesis in six embryonic samples (Fig. 2).

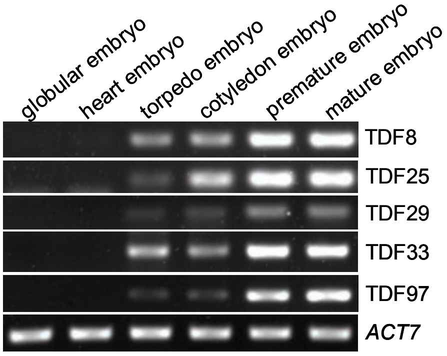

Fig. 2. RT-PCR showing up-regulations of 5 TDFs related to stress and ABA response in embryogenesis. Two biological replicates were performed for RT-PCR.

Three TDFs (TDF8, TDF25 and TDF29), showing homology to glutathione peroxidase 6 (*ATGPX6*), antioxidant/thioredoxin peroxidase (*ATPER1*) and zinc-binding dehydrogenase family protein, respectively, were related to oxidative stress-responsive genes. These TDFs were distinctly up-regulated during *C. bursa-pastoris* embryogenesis (Fig. 2). Rodriguez-Milla *et al.* (2003) have indicated that *ATGPX6* shows the strongest responses under most abiotic stresses tested and is highly expressed throughout development in most tissues, particularly in seeds, thus supporting an important role for this gene in oxidative damage protection. TDF25 showed identity to *ATPER1*. Haslekas *et al*. (1998) have suggested that ATPER1 may protect tissues from reactive oxygen species during desiccation and early imbibition and/or is involved in maintenance of seed dormancy or seed protection during dormancy. Some genes of specific peroxidases were also identified in radicles and endosperm of dry seed of *Lepidium sativum*, and earlier germination phenotypes were displayed in the case of some mutants when compared with wild type (Linkies *et al.* 2010). The peroxidase activity is maximum in white fruits of strawberry and decreases during the ripening stages (López *et al.* 2010). TDF29 (*zinc-binding dehydrogenase family protein* gene) was also distinctly up-regulated during embryogenesis. ABA plays a crucial role in seed maturation and induces dormancy during the late phase of seed maturation (Seo and Koshiba 2002). Two TDFs (TDF33 and TDF97) showed conspicuous similarity to *ABA-responsive protein-related* and *RAB18* genes related to ABA response. *ABA-responsive protein-related* has been identified in *Arabidopsis* under progressive soil drought using whole-genome oligonucleotide microarrays (Huang *et al*. 2008). RAB18, which belongs to the dehydrin protein family, was strongly induced both in water-stressed and ABA-treated plants but was only slightly responsive to cold. Moreover, transgenic plants overexpressing *RAB18* together with *COR47* are cold-tolerant (Welin *et al*. 1994, Puhakainen *et al*. 2004, Kosová *et al*. 2007). The expression patterns of the five TDFs, related to oxidative stress and ABA response, suggested that more genes could play essential roles in protecting tissues from reactive oxygen species and desiccation tolerance during embryonic development, especially at later embryo stages. Recently the genes related to oxidative stress and ABA response were also found involved in early stages of seed germination (Weitbrecht *et al*. 2011).

Conclusions: Numerous gene expression patterns are showed in this study, and many differentially expressed genes are identified during embryogenesis of *C. bursa-pastoris*. Later embryo developmental stages share more TDFs than those in earlier stages. Many genes of early embryo stage are related to establishment of the embryonic pattern. While the genes related to storage proteins, antioxidant systems, ABA response and heat shock proteins are expressed in later embryos or up-regulated during embryogenesis. Gene expression in embryogenesis of *C. bursa-pastoris* is similar to that in *Arabidopsis*.

References

Bachem, C.W.B., Van der Hoeven, R.S., De Bruijn, S.M., Vreugdenhil, D., Zabeau, M., Visser, R.G.F.: Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: analysis of gene expression during potato tuber development. - Plant J. **9**: 745-753, 1996.

Baskin, J.M., Baskin, C.C.: Germination responses of buried seeds of *Capsella bursa-pastoris* exposed to seasonal temperature changes. - Weed Res. **29**: 205-212, 1989.

Casson, S., Spencer, M., Walker, K., Lindsey, K.: Laser capture microdissection for the analysis of gene expression during embryogenesis of *Arabidopsis*. - Plant J. **42**: 111-123, 2005.

Fu, Z.Y., Zhang, Z.B., Liu, Z.H., Hu, X.J., Xu, P.: The effects of abiotic stresses on the NADP-dependent malic enzyme in the leaves of the hexaploid wheat. - Biol. Plant. **55**: 196-200, 2011.

Girke, T., Todd, J., Ruuska, S., Benning, C., Ohlrogge, J.: Microarray analysis of developing *Arabidopsis* seeds. - Plant Physiol. **124**: 1570-1581, 2000.

Hajduch, M., Ganapathy, A., Stein, J.W., Thelen, J.J.: A systematic proteomic study of seed filling in soybean. Establishment of high-resolution two-dimensional reference maps, expression profiles, and an interactive proteome database. - Plant Physiol. **137**: 1397-1419, 2005.

Haslekas, C., Stacy, R.A.P., Nygaard, V., Culianez-Macia, F.A., Aalen, R.B.: The expression of a peroxiredoxin antioxidant gene, *AtPer1*, in *Arabidopsis thaliana* is seed specific and related to dormancy. - Plant mol. Biol. **36**: 833-845, 1998.

Huang, D., Wu, W., Abrams, S.R., Cutler, A.J.: The relationship of drought-related gene expression in *Arabidopsis thaliana* to hormonal and environmental factors. - J. exp. Bot. **59**: 2991-3007, 2008.

Hurka, H., Neuffer, B.: Evolutionary processes in the genus *Capsella* (*Brassicaceae*). - Plant Syst. Evol. **206**: 295-316, 1997.

Kim, D.H., Yamaguchi, S., Lim, S., Oh, E., Park, J., Hanada, A., Kamiya, Y., Choi, G.: SOMNUS, a CCCH-type zinc finger protein in *Arabidopsis*, negatively regulates light-dependent seed germination downstream of PIL5. - Plant Cell **20**: 1260-1277, 2008.

Kosová, K., Vítámvás, P., Prášil, I.T.: The role of dehydrins in plant response to cold. - Biol. Plant. **51**: 601-617, 2007.

Le, B.H., Cheng, C., Bui, A.Q., Wameister, J.A., Henry, K.F., Pelletier, J., Kwong, L., Belmonte, M., Kirkbride, R., Horvath, S., Drews, G.N., Fischer, R.L., Okamuro, J.K., Harada, J.J., Goldberg, R.B.: Global analysis of gene activity during *Arabidopsis* seed development and identification of seed-specific transcription factors. - Proc. nat. Acad. Sci. USA **170**: 8063-8070, 2010.

Leprince, O., Hendry, G.A.F., Atherton, N.M., Walters-Vertucci, C.: Free radicals and metabolism associated with the acquisition and loss of desiccation tolerance in developing seeds. - Biochem. Soc. **24**: 451-455, 1996.

Linkies, A., Schuster-Sherpa, U., Tintelnot, S., Leubner-Metzger, G., Müller, K.: Peroxidases identified in a subtractive cDNA library approach show tissue-specific transcript abundance and enzyme activity during seed germination of *Lepidium sativum*. - J. exp. Bot. **61**: 491-502, 2010.

Long, J.A., Ohno, C., Smith, Z.R., Meyerowitz, E.M.: TOPLESS regulates apical embryonic fate in *Arabidopsis*. - Science **312**: 1520-1523, 2006.

López, A.P., Gochicoa, M.T.N., Franco, A.R.: Activities of antioxidant enzymes during strawberry fruit development and ripening. - Biol. Plant. **54**: 349-352, 2010.

Nutt, P., Ziermann, J., Hintz, M., Neuffer, B., Theisen, G.: *Capsella* as a model system to study the evolutionary relevance of floral homeotic mutants. - Plant Syst. Evol. **259**: 217-235, 2006.

Osakabe, Y., Maruyama, K., Seki, M., Satou, M., Shinozaki, K., Yamaguchi-Shinozaki, K.: Leucine-rich repeat receptor-like kinase1 is a key membrane-bound regulator of abscisic acid early signaling in *Arabidopsis*. - Plant Cell **17**: 1105-1119, 2005.

Puhakainen, T., Hess, M.W., Mäkelä, P., Svensson, J., Heino, P., Palva, E.T.: Overexpression of multiple dehydrin genes enhances tolerance to freezing stress in *Arabidopsis*. - Plant mol. Biol. **54**: 743-753, 2004.

Rodriguez-Milla, M.A., Maurer, A., Rodriguez-Huete, A., Gustafson, J.P.: Glutathione peroxidase genes in *Arabidopsis* are ubiquitous and regulated by abiotic stresses through diverse signaling pathways. - Plant J. **36**: 602-615, 2003.

Sambrook, J., Fritsch, E.F., Maniatis, T.: Molecular Cloning: A Laboratory Manual. 3rd Ed. - Cold Spring Harbor Laboratory Press, Cold Spring Harbor 2001.

Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Scholkopf, B., Weigel, D., Lohmann, J.U.: A gene expression map of *Arabidopsis thaliana* development. -

Nat. Genet. **37**: 501-506, 2005.

Seo, M., Koshiba, T.: The complex regulation of ABA biosynthesis in plants. - Trends Plant Sci. **7**: 41-48, 2002.

Slotte, T., Holm, K., McIntyre, L.M., Lagercrantz, U., Lascoux, M.: Differential expression of genes important for adaptation in *Capsella bursa-pastoris* (*Brassicaceae*). - Plant Physiol. **145**: 160-173, 2007.

Spencer, M.W.B., Casson, S.A., Lindsey, K.: Transcriptional profiling of the *Arabidopsis* embryo. - Plant Physiol. **143**: 924-940, 2007.

Tsugeki, R., Ditengou, F.A., Sumi, Y., Teale, W., Palme, K., Okada, K.: NO VEIN mediates auxin-dependent specification and patterning in the *Arabidopsis* embryo, shoot, and root. - Plant Cell **21**: 3133-3151, 2009.

Wehmeyer, N., Hernandez, L., Finkelstein, R., Vierling, E.: Synthesis of small heat-shock proteins is part of the developmental program of late seed maturation. - Plant Physiol. **112**: 747-757, 1996.

Weitbrecht, K., Müller, K., Leubner-Metzger G.: First off the mark: early seed germination. - J. exp. Bot. **62**: 3289-3309, 2011.

Welin, B.V., Olson, A., Nylander, M., Palva, E.T.: Characterization and differential expression of DHN/LEA/RAB-like genes during cold-acclimation and drought stress in *Arabidopsis thaliana*. - Plant mol. Biol. **26**: 131-144, 1994.

Wheeler, M.C., Tronconi, M.A., Drincovic, M.F.h, Andreo, C.S., Flugge, U.I., Maurino, V.G.: A comprehensive analysis of the NADP-malic enzyme gene family of *Arabidopsis*. - Plant Physiol. **139**: 39-51, 2005.

Winter, D., Vinegar, B., Nahal, H., Ammar, R., Wilson, G.V., Provart, N.J.: An ‘electronic fluorescent pictograph’ browser for exploring and analyzing large-scale biological data sets. - PLoS ONE **2**: e718, 2007.

Xin, Z., Wang, A., Yang, G., Gao, P., Zheng, Z.L.: The *Arabidopsis* A4 subfamily of lectin receptor kinases negatively regulates abscisic acid response in seed germination. - Plant Physiol. **149**: 434-444, 2009.

Xue, Y., Peng, R., Xiong, A., Li, X., Zha, D., Yao, Q.: Over-expression of heat shock protein gene hsp26 in *Arabidopsis thaliana* enhances heat tolerance. - Biol. Plant. **54**: 105-111, 2010.