Supplemental Data. Graeber *et al*. (2011)**. A Guideline to Family-wide Comparative State-of-the-art qRT-PCR Analysis Exemplified with a Brassicaceae Cross-species Seed Germination Case Study**

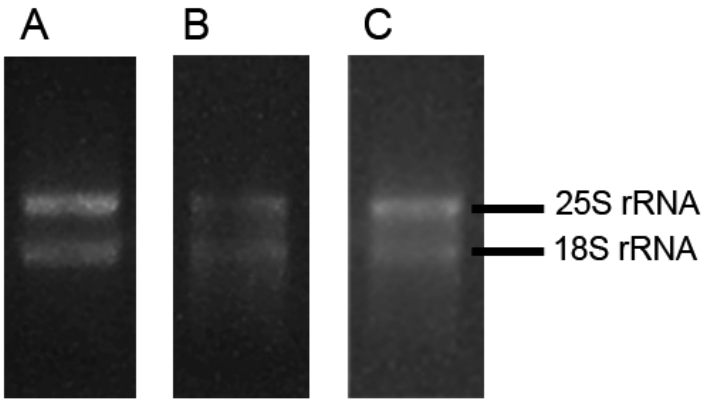
Manuscript: PLANTCELL/2011/084103

Supplemental Figure 1. Determination of RNA integrity via gel electrophoresis.

Supplemental Table 1. Sequence similarities to Arabidopsis and accession numbers of *Lepidium sativum* FR14 reference gene cDNA sequences.

Supplemental Table 2. Primer sequences used for quantitative PCR.

Supplemental Methods



Supplemental Figure 1. Determination of RNA integrity via gel electrophoresis.
(A) RNA with high integrity used for further analyses: to clear bands for the 25S and 18S rRNA, with the upper band being twice as strong as the lower one. **(B)** and **(C)** RNA with low integrity, which was not further used: showing either not the 2:1 ratio in band intensity between the 25S and the 18S rRNA **(B)** or showing RNA degradation indicated by a smear on the gel **(C)**. Between 100 and 200ng RNA were loaded on a 1% agarose gel.

Supplemental Table 1. Sequence similarities to Arabidopsis and accession numbers of *Lepidium sativum* FR14 reference gene cDNA sequences.

Lepidium sativum reference genes cDNA sequence name	GenBank accession numbers	AGI code of putative Arabidopsis ortholog	e-value	% sequence similarity between the two species
LesaG17210	HQ912755	At1G17210	0	92
LesaG04660	HQ912754	At2G04660	0	92
LesaG19980	HQ912756	At2G19980	1e-71	77
LesaG20000	HQ912757	At2G20000	4e-160	92
LesaG04320	HQ912753	At4G04320	8e-90	91
LesaCYP1	HS981851	At4G38740	7e-42	94
LesaACT7	HS981849	At5G09810	8e-32	100
LesaACT8	HS981850	At1G49240	8e-33	96
LesaEF1-α	HS981853	At5G60390	6e-20	94
LesaCYP5	HS981852	At2G29960	9e-33	93
LesaUBQ11	HS981854	At4G05050	2e-34	92

Supplemental Table 2. Primer sequences used for quantitative PCR.

Primer name	Sequence (5' to 3')
At1g17210-qF2	CTGCTTCATATGAATCACGAG
At1g17210-qR2	TCAACACTATCTGCACGTTGT
At2g04660-qF1	TTCTGGAAGCAGTGGGTGAA
At2g04660-qR1	CTCCACTTCCATCTGTAAGC
At2g19980-F1	AATATTCCACCGACGGAACG
At2g19980-R1	TAAGGCTTCTCCGTAAACCAA
At2g20000-qF1	GTATAGCTCCACCACCACTT
At2g20000-qR1	TCTTCTAGGTGCTTGAAGAGT
AT4g04320-qF1	ACTCGGTATGTGGCTTAGTC
AT4g04320-qR1	TTCTCAAGCAATGAAGCAGGA
Lesa17210-FP2	TCCGCCCTTGTATGGACGAGAAG
Lesa17210-RP2	CTCTGGCACCTGGGAAAGCCA
Lesa04660-FP1	AGCTGGGTCTATTGCACGAAGGG
Lesa04660-RP1	TCGTTTGCTCACTGCTGGTGCTT
Lesa19980-FP1	TGTAGCACAACCAAGCCTAGTCGAT
Lesa19980-RP1	GGGTCCAACCTCAGGACACAATGA
Lesa20000-FP1	TCTGGTCCACGACGGAGCTTG
Lesa20000-RP1	GCGTTGCTCACATTTCCGCTTACT
Lesa04320-FP2	AAGCAATGAAGCAGGATCATCCCAA
Lesa04320-RP2	AGGAGAAACTCGGTATGTGGCTTAGT
Ubi11-qPCR-F2	GATGCAGATCTTCGTTAAGACT
Ubi11-qPCR-R2	CCTTCCTTATCCTGGATCTTG
Ef1a-qPCR-F	TGAGCACGCTCTTCTTGCT
Ef1a-qPCR-R	GTGGCATCCATCTTGTTACA
Cyp1-qPCR-F2	GGATCCTGTCGATGGCGAAC
Cyp1-qPCR-R2	TCCACGACCTGCCCAAACAC
Cyp5-qPCR-F2	CTTCAGAGCTTTGTGCACAGG
Cyp5-qPCR-R2	AAGCTGGGAATGATTCGATG
Act7/8-qPCR-F	GGTCGTACAACCGGTATTGT
Act7-qPCR-R	GATAGCATGTGGAAGTGAGAA
Act8-qPCR-R	GAAGAGCATACCCCTCGTA
5.8S rRNA-For	CTTTGAAGCCAAGTTGCGC
5.8S rRNA-Rev	CGTCCCACACTCGTGAAAAT

Supplemental Methods

Selection procedure to identify gene-probes on a *Brassica* microarray (Xiang et al. 2008, GEO platform GPL8090) which most likely bind the putative *Brassica napus* orthologs of Arabidopsis reference genes used in our seed germination qRT-PCR analysis (Figure 4).

Genedata Expressionist Analyst 2.1 software was used to analyse the *B. napus* endosperm development dataset (GEO accession GSE14766) produced with the above mentioned microarray. First the microarray annotation file was searched for probe annotations matching Arabidopsis reference gene AGI numbers. If an annotation matched unambiguously only one probe on the array this probe was taken to represent the putative *B. napus* ortholog of the annotated Arabidopsis gene. Annotations were found for every tested reference gene except At2G04660 and At4G04320. Since not all probes on the array are annotated we checked if these genes are indeed not represented. Therefore we used BLAST (blastn, Evalue cutoff E-5) with the Arabidopsis coding sequences of these two genes against a *Brassica* EST library including all sequences present on the microarray amongst others (dataset available under www.brassicagenomics.ca/ests/). Matching ESTs were found meaning that putative orthologs of these two Arabidopsis genes are generally present in *Brassica* spp. but none of the identified ESTs had a corresponding probe on the microarray. Therefore these two genes are not included in further analysis.

Multiple probes on the *Brassica* microarray contained the same AGI annotation. Therefore we analysed which of these *Brassica* probes corresponds most likely to the annotated Arabidopsis gene. First all probes containing the same AGI annotation were checked if they bind genes that are commonly expressed in the *B. napus* endosperm development dataset (to avoid making decision between probes of which some might not yield a signal) by verifying their presence in the 'commonly expressed gene subset' (see Supplemental Table 8 in Huang et al. (2009). Only probes which were present in this subset, which means that they show at least expression signal values of 5000, were taken for further analysis. If only one probe per AGI annotation was left, this was taken to represent the putative *B. napus* otholog of the annotated Arabidopsis gene. If still multiple probes were present containing the same AGI annotation, similarity of the probes to the annotated Arabidopsis gene was analysed by pairwise alignment (using bioinformatics software suite Geneious Pro 5.0.4). Each probe was independently aligned against the Arabidopsis gene coding sequence using a global alignment algorithm (ClustalW 2.0.12, cost matrix IUB; penalties: Gap open 15, extension 6.66, free end gaps). The probe scoring the highest pairwise identity (which is the percentage of pairwise residues that are identical in the alignment which is computed by looking at a pair of bases at the same column (including gap versus non-gap residues), scoring a hit when they are identical, divided by the total number of pairs) in alignments of comparable length was considered to be the probe which most likely will bind the putative *B. napus* ortholog of a specific Arabidopsis gene.