



Deciphering priming-induced improvement of rapeseed (*Brassica napus* L.) germination through an integrated transcriptomic and proteomic approach



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ABSTRACT

Rape seeds primed with -1.2 MPa polyethylene glycol 6000 showed improved germination performance. To better understand the beneficial effect of osmoprimer on seed germination, a global expression profiling method was used to compare, for the first time, transcriptomic and proteomic data for osmoprimered seeds at the crucial phases of priming procedure (soaking, drying), whole priming process and subsequent germination. *Brassica napus* was used here as a model to dissect the process of osmoprimer into its essential components.

A total number of 952 genes and 75 proteins were affected during the main phases of priming and post-primer germination. Transcription was not coordinately associated with translation resulting in a limited correspondence between mRNAs level and protein abundance. Soaking, drying and final germination of primed seeds triggered distinct specific pathways since only a minority of genes and proteins were involved in all phases of osmoprimer while a vast majority was involved in only one single phase. A particular attention was paid to genes and proteins involved in the transcription, translation, reserve mobilization, water uptake, cell cycle and oxidative stress processes.

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1. Introduction

Germination represents a fundamental stage of plant's life highly responsive to environmental conditions. It begins with the uptake of water by the nondormant quiescent dry seed and finishes with the radicle protrusion through the seed coat [1]. During germination the imbibed seed undergoes transition from a maturation to a germination program of development and prepare for early seedling growth [2]. Seed vigor is a complex trait that encompasses

many biochemical and molecular processes determining its potential for rapid uniform emergence and seedling establishment. One of the methods which enhance seed vigor is seed priming, which reinforce also emergence of low-vigor seeds [3,4].

Priming procedure is based on seeds hydration below the level of natural imbibition, which allows the commencement of germination-related events but prevents radicle emergence. As a consequence, water uptake must be restricted either by imbibition in solution of low osmotic potential (osmoprimer) or by limiting the soaking time in water (hydropriming). Seed performance of various crops can also be improved after treatment with plant growth regulators during priming [5,6]. In most plant species, seeds can remain desiccation tolerant prior to radicle protrusion; therefore, after soaking seeds can be subjected to a dehydration step permitting storage, distribution and planting of the primed seeds [7]. The accelerated germination and improved germination rate and

Abbreviations: PEG, polyethylene glycol; UP_d, dry unprimed seeds; P_{nd}, primed nondried seeds (at the end of PEG soaking); P_d, primed dried seeds; UP_{7h}, unprimed seeds imbibing for 7 h on water; P_{7h}, primed seeds imbibing for 7 h on water.

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uniformity has been attributed to seeds priming [8,9]. However, cellular and biochemical mechanisms of this phenomenon remain not well understood.

Faster transition toward germinated state through priming may be considered as a result of the advanced germination metabolism [10,11], enhanced antioxidant activity [12,13] and repairing processes [14,15]. Moreover, seed priming often improves the stress tolerance of germinating seeds [11,16–18]. Priming procedure itself may generate a moderate abiotic stress during both soaking (e.g. osmotic stress, salt, and drought created by the priming agents) and dehydration steps [19]. It is tempting to propose that priming cannot simply be considered as an acceleration of germination-related processes but also involves other specific mechanisms that improve germination and allow the seeds to cope with environmental stresses during seedling establishment. Primed seeds maintain a ‘memory’ of priming-induced stress responses, which mediates greater stress-tolerance of germinating primed seeds [20]. Hence a comprehensive approach of physiological and biochemical mechanisms underlying priming process requires to separately consider metabolic status of (i) seeds at the end of the soaking period before dehydration, (ii) primed seeds after the dehydration step, (iii) primed seeds during final germination, and to compare the obtained data with the metabolic status of unprimed seeds.

The global approach such as transcriptomic analysis could be a useful tool for the determination of potential molecular markers of seed quality and germination capacity [21] but this implies that protein synthesis is coordinately regulated with corresponding gene expression. However, proteins are one of the fundamental functional components of a biological system. Metabolic processes and cellular signaling are also triggered by protein-protein interactions, posttranslational protein modifications, and enzymatic activities so transcriptional profiling alone cannot provide their accurate prediction or description [22]. Proteomic approaches offer new perspectives to analyze the complex functions of plants. DNA microarray and proteomic analysis are now available as quite powerful complementary techniques for studying the complex interplay between genes and proteins during seed germination. Although separate transcriptome and proteome analyses of seed priming and germination have proven invaluable in identifying changes occurring during priming and post-priming germination [11,21], new strategies associating a holistic transcriptomic and proteomic approach are needed to distinguish protein accumulation driven directly by transcript abundance from that post-transcriptionally regulated.

Rape (*Brassica napus*) is cultivated mainly for its oil-rich seed and provides the third most important source of vegetable oil in the world [22]. This crop belongs to the same family as the plant model species *Arabidopsis thaliana* with a completely sequenced and annotated genome. This gives the opportunity that the results obtained for rape can be directly applied to overcome problems faced in agricultural practices [10]. Recently published studies demonstrated that seed priming clearly improves the germination phase in this species under both optimal and adverse conditions [23–25].

The aim of this work was to better understand the beneficial effect of osmoprimering on rapeseed germination by using global expression profiling methods. *Brassica napus* was chosen as a model to determine some components of the molecular mechanisms associated with seed priming and post-priming germination. Thus, in the current paper, for the first time to the best of our knowledge, the comparison of proteomic and transcriptomic data for osmoprimered and germinating rape seeds at the different phases of priming procedure (soaking, dehydration), whole osmoprimering process and final germination, are described.

2. Materials and methods

2.1. Plant material, osmoprimering and germination experiments

The seeds of rape (*Brassica napus* L. cv Libomir) were kindly provided by OBROL Company. Seeds were surface sterilized and primed in PEG 6000 solution (osmotic potential –1.2 MPa) during 7 days at 25 °C in the darkness on Petri dishes lined with 3 layers of filter paper wetted with PEG. This optimal priming protocol (7 days soaking in PEG solution with –1.2 MPa osmotic potential) was selected during preliminary studies and used for all analyses. After soaking seeds were washed 3 times for 15 s with sterile deionized water to remove the osmotic agent. Washed seeds were directly frozen in liquid nitrogen and stored at –80 °C (seeds at the end of soaking, primed nondried P_{nd}) or dried 48 h at room temperature until they reach the initial moisture content (water content 5%; primed dried seeds, P_d).

Germination tests were carried out on ten replicates of 100 seeds. Seeds were incubated at 25 °C in the darkness on Petri dishes lined with three layers of filter paper wetted with 10 mL of deionized water. A seed was considered germinated when the radicle protruded through the seed coat. The parameters, such as germination curve, maximum percentage of germination (G_{max}), time to reach 50% of germination (T_{50}), uniformity of germination (U_{7525} : time interval between 25% and 75% of viable seeds to germinate) and area under the curve (AUC), were used for interpretation of germination performance using “Germinator_curve-fitting1.27.xls” Microsoft Excel script [26] and the mathematical approach described by El-Kassaby et al. [27].

Transcriptomic and proteomic analyses were conducted on seeds collected at crucial points of osmoprimering treatment, i.e. at the end of soaking (P_{nd}), after drying of osmoprimered seeds to initial moisture content (P_d), and also during germination *sensu stricto*, prior to radicle emergence (after 7 h of imbibition, P_{7h}). Analyses were also performed on dry unprimed seeds (UP $_d$) and unprimed seeds imbibing for 7 h (UP $_{7h}$).

2.2. RNA isolation and microarray analysis

Total RNA was extracted from 1 g (approx. 250 seeds) of the frozen samples. RNA was isolated as described by Asif et al. [28]. RNA digestion and quality verification were performed as described in Quinet et al. [29]. The Brassica Gene Expression Microarray 4 × 44 K plates (Agilent Technologies Santa Clara, CA, USA) was used to identify genes involved in osmoprimering and post-priming germination. The assays were replicated on two independent experiments. The microarray experiment was performed according to Quinet et al. [29]. The data discussed in this publication were deposited in NCBI's Gene Expression Omnibus [30] and are accessible through GEO Series accession number GSE61702 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61702>). To identify statistically significant differentially expressed genes, a combined criterion of fold change >3 and a P value <0.05 in the t -tests was adopted. Only common probe sets that were present in both experiments were considered significant. Arabidopsis homolog was searched for each analysed probe sets using TAIR BLAST 2.2.8 used TAIR10 data base. MIPS functional catalogue database (http://mips.helmholtz-muenchen.de/proj/funcatDB/search_main_frame.html) were used to obtain gene ontology (GO) information.

2.3. Real-time PCR

Two-step quantitative (q)PCR was used for validation of the microarray-derived gene expression profiles. The working concentration of the cDNA samples was adjusted according to the guidelines presented by Gallup and Ackermann [31]. Expression

analysis was assayed by qPCR in Rotor-GeneQ (Qiagen), 72-well rotor, using FastStart SYBR Green Master Kit (Roche) and 10 mM gene-specific primers (listed in Supplementary Table S1). The qPCR reaction was performed for 40 cycles at manufacturer-recommended thermal profile. The uniformity of the product was verified by a melting curve analysis (60–95 °C range with 0.5 °C steps) following each qPCR assay. Reaction efficiency of each assay and Ct values were calculated with Rotorgene Q analysis software. Relative gene expression was analyzed according to Pfaffl [32]. Actin 2.1 and EF1 α were used as reference genes [33,34]. Intra-assay variation was evaluated by calculating SD errors of arithmetic means of 3 sample replicates.

2.4. Protein extraction, 2D-electrophoresis and protein identification

Seeds (200 mg) were homogenized in liquid nitrogen to get a fine powder. Protein precipitation was carried out as described in Méchin et al. [35]. Then pellet was dried under vacuum for 30 min. Proteins were resuspended in buffer used in Méchin et al. [36] with vortex mixing for 120 min at room temperature followed by two centrifugations for 15 min at 15,000 \times g to remove cell debris and other insoluble materials. Protein concentration was measured using Bradford method [37].

Isoelectrofocusing was carried out using gel strips forming an immobilized pH gradient from 3 to 10 (BioRad). Strips were rehydrated for overnight at room temperature with the 300 μ L buffer as described previously by Wojtyla et al. [38]. Isoelectrofocusing was performed at 18 °C in the Multiphor II system (Amersham Pharmacia Biotech, Orsay, France) for 4 h at 300 V, 3.5 h at 1500 V and 15.5 h at 3500 V. After IEF, the immobilized pH gradient (IPG) strips were equilibrated according to Wojtyla et al. [38]. Separation of proteins according to their molecular masses was done using denaturing electrophoresis. The gels were stained with colloidal Coomassie Brilliant Blue (CBB) G-250 [39] and scanned by ImageScanner III (GE Healthcare) with LabScan 6.0 (GE Healthcare, UK).

Spot detection and image analyses (normalization, spot matching, protein accumulation analyses) were performed with Image Master 2-D Platinum 6.0 software (GE Healthcare, UK) and the abundance of each protein spot was normalized as a relative volume (% Vol) as described earlier by Kosmala et al. [40]. Six images representing three independent biological replicates for each experimental variant, were used. The differentially accumulated proteins ($P < 0.05$) between the analysed variants with a ratio of at least 2.0 in absolute value of protein abundance were applied into MS analyses and protein identification.

Protein spots were excised from the gel manually under sterile condition and analyzed by liquid chromatography coupled to the mass spectrometer in the Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences (Warsaw, Poland). Samples were concentrated and desalting on a RP-C18 pre-column (Waters), and further peptide separation was achieved on a nano-Ultra Performance Liquid Chromatography (UPLC) RP-C18 column (Waters, BEH130 C18 column, 75 μ m i.d., 250 mm long) of a nanoACQUITY UPLC system, using a 45 min linear acetonitrile gradient. Column outlet was directly coupled to the Electrospray ionization (ESI) ion source of the Orbitrap Velos type mass spectrometer (Thermo), working in the regime of data dependent MS to MS/MS switch. An electrospray voltage of 1.5 kV was used. Raw data files were pre-processed with Mascot Distiller software (version 2.4.2.0, MatrixScience). The obtained peptide masses and fragmentation spectra were matched to the National Center Biotechnology Information (NCBI) non-redundant database (23919380 sequences/8216485116 residues), with a *Viridiplantae* filter (1249273 sequences) using the Mascot search engine (Mascot Daemon v. 2.4.0, Mascot Server v. 2.4.1, MatrixScience).

The following search parameters were applied: enzyme specificity was set to trypsin, peptide mass tolerance to ± 30 ppm and fragment mass tolerance to ± 0.6 Da. The protein mass was left as unrestricted, and mass values as monoisotopic with one missed cleavage being allowed. Alkylation of cysteine by carbamidomethylation as fixed, and oxidation of methionine was set as a variable modification.

Protein identification was performed using the Mascot search engine (MatrixScience), with the probability based algorithm. The expected value threshold of 0.05 was used for analysis, which means that all peptide identifications had less than 1 in 20 chance of being a random match.

3. Results

3.1. Osmopriming improves seed germination

The osmopriming had a beneficial effect on germination speed in *Brassica napus* seeds (Fig. 1). Germination tests were carried out on dry unprimed (UP_d) and primed dried (P_d) seeds. Time needed for the first visible radical protrusion through the seed coat decreased from 11 h to 7 h in P_d seeds as compared to UP_d seeds (Fig. 1A). Moreover, at 12th hour of germination ~ 20 fold more P_d seeds than UP_d ones were germinated. The time to reach 50% germinated seeds was lower ~ 2 fold in response to osmopriming (Fig. 1C). The value of area under curve showed high difference in germination performance between P_d and UP_d . For P_d seeds this value was ~ 2 fold higher than for UP_d ones (Fig. 1D). Additionally, maximum percentage germination was $\sim 10\%$ higher in response to osmopriming (Fig. 1B). Osmopriming provides also more uniform rape seed germination. Time interval between 25% and 75% of viable seeds to germinate was 1.7 fold shorter for P_d comparatively to UP_d seeds (Fig. 1E). At the end of the soaking period, the seed water content was 50%. During final germination primed seeds exhibited a water content of 67.3% after 7 h of imbibition while it was only 59.9% for unprimed seeds.

To better understand the beneficial effect of osmopriming on rapeseed germination, we analyzed the genes and proteins involved in the different phases of osmopriming and compared them with those involved in germination process. As shown on Fig. 2, we thus investigated the transcriptomic and proteomic profile during (1) PEG soaking by comparing primed nondried (P_{nd}) and dry unprimed (UP_d) seeds, (2) drying after soaking by comparing P_{nd} and primed dried (P_d) seeds, (3) the full osmopriming process by comparing P_d and UP_d seeds and finally (4) the germination after osmopriming by comparing primed seeds imbibing for 7 h (P_{7h}) and unprimed seeds imbibing for 7 h (UP_{7h}). In this paper the authors designed an experiment in which the primed and unprimed seeds germinating on water were collected at the same time point (after 7 h of imbibition) corresponding to achievement of 1% germination of primed seeds. This experimental design gives a unique chance to identify changes linked to more advanced development of primed seeds toward complete germination (radicle protrusion) than unprimed ones.

3.2. Transcriptomic analysis

The microarray assays were replicated twice on two independent priming and germination experiments. After search for *Arabidopsis* homologs and gene function identification, 952 genes were selected for further consideration. As shown on Fig. 2, post-priming germination and the complete osmopriming process were the two steps that involved the highest number of differentially regulated genes. Regarding the different phases of priming, most of the genes which expression was affected by PEG soaking were

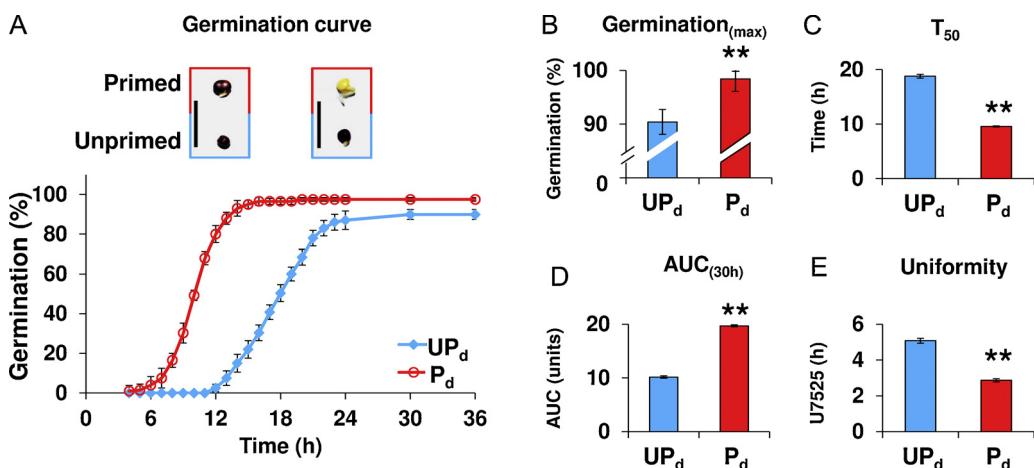


Fig. 1. Germination of dry unprimed (UP_d) and primed dried (P_d) *Brassica napus* seeds. Primed seeds were exposed to -1.2 MPa PEG soaking during 7 d. (A) Germination curve. Above the curve are the pictures of unprimed and primed seeds at 12 and 24 h of germination. (B) Maximum germination after 30 h. (C) Time to reach 50% germination, (T_{50}). (D) Area under the curve until 30 h ($AUC_{(30h)}$). (E) Uniformity of germination ($U7525$) as the time interval between 75% and 25% of viable seeds to germinate. Each value is the mean of 10 replicates (100 seeds each) and vertical bars are S.E. **: Significantly different at $P < 0.01$ according to t -test.

down-regulated while most of the genes involved in drying were up-regulated. In general, the osmoprime treatment and post-prime germination induced more up-regulations. To validate the expression profiles observed by microarray analysis in this experiment, qPCR were performed for 8 genes and the results confirmed the up- and down-regulations observed by microarray analyses (Supplementary Table S2).

Following the gene ontology analysis, the genes were categorized into 10 groups according to MIPS: metabolism and regulation of metabolism, energy, storage proteins, cell cycle and DNA processing, transcription regulation, protein synthesis and fate, protein with binding function or cofactor requirement, cellular transport and communication, stress response (interaction with the environment and cell rescue and defense), development and unclassified (Fig. 3A). Their abundance in each group and differences in their regulation were expressed in relation to osmoprime and germination steps (Fig. 3A). Genes affected during sub-phases of osmoprime treatment, osmoprime and post-prime

germination according to MIPS categories are listed in Supplementary Table S3.

In order to analyse the common regulation between the different phases of osmoprime and post-prime germination, we identified the common genes during the investigated steps. As shown on Fig. 4A, few common genes were observed. The highest proportion of common genes (Supplementary Table S4) was observed between PEG soaking and seed drying. All these common genes were regulated in an opposite way between the two phases. Less than 10% of the genes affected during seed soaking and seed drying were common with the general osmoprime and as expected most of the common genes between soaking and general osmoprime were regulated on the same direction while the opposite was true between seed drying and general osmoprime. Less than 5% of the genes were common between general osmoprime and post-prime germination and most of them were regulated in the same way. The common genes between seed soaking and post-prime germination represented ~19% of the ones affected during seed soaking. Around 12.5% of the genes affected during drying are also affected during post-prime germination and they are all regulated in an opposite way.

3.3. Proteomic analysis

For each condition, proteins were extracted from three biological replicates and two 2D gels were performed per replicate. A typical two-dimensional protein profile obtained from *Brassica napus* unprimed seeds is shown in Fig. 5A. Protein spots ranging from 12 to 119 kDa were detected in each gel following criteria: (1) all spots had to be present in all experimental variants, (2) all spots had to be present in three biological replicates. Among 300 spots reproducibly detected in 2D gels, 78 protein spots showing minimum 2 fold change abundance were selected for protein identification. Identities of a total of 75 proteins with altered accumulation were established, including 26 to 33 proteins depending on the osmoprime and germination phases (Fig. 2). Among the analyzed proteins there were more proteins which abundance decreased in response to PEG soaking and seed drying while most of the proteins analysed during complete osmoprime and post-prime germination showed an increase of their abundance during these processes. The list of identified proteins is shown in Table 1. As for microarray results, the analysed proteins were categorized into 10 groups according to MIPS (Fig. 3B).

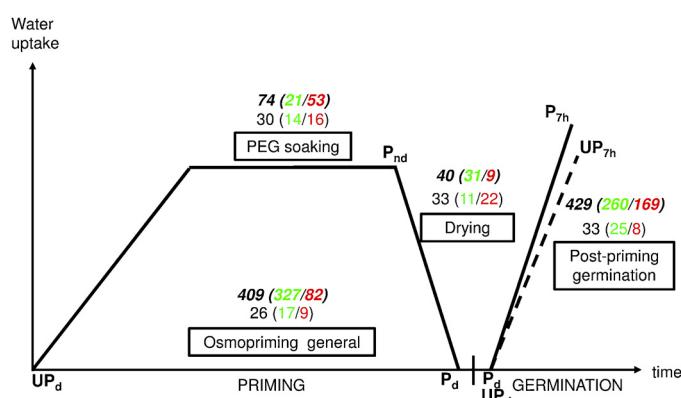


Fig. 2. Experimental design used in this study. Number of genes (bold italic) and proteins (regular) affected during the main phases of osmoprime and post-prime germination in *Brassica napus* seeds. Transcriptomic and proteomic profile were investigated during (1) PEG soaking by comparing P_{nd} and UP_d seeds, (2) drying after soaking by comparing P_{nd} and P_d seeds, (3) the full osmoprime process by comparing P_d and UP_d seeds and finally (4) the germination after osmoprime by comparing P_{7h} and UP_{7h} . Black: total number of genes or proteins; green: up-regulated genes and proteins with increased abundance; red: down-regulated genes and proteins with decreased abundance; blue: experimental variants (UP_d : dry unprimed seeds; P_{nd} : non dried primed seeds; P_d : dry primed seeds; P_{7h} : primed seeds imbibed 7 h on water; and UP_{7h} : unprimed seeds imbibed 7 h on water).

Table 1
List of proteins affected in seeds of *Brassica napus* during sub-phases of osmoprimer treatment, whole osmoprimer process and post-primer germination (P_{nd} : primed non dried; P_d : primed dried; UP_d : unprimed dried; P_{7h} : primed after 7 h of imbibition; UP_{7h} : unprimed after 7 h of imbibition).

Spot Id ^a	NCBI accession no ^b	Protein name ^c	Species ^d	MIPS functional category ^e	M_r^f theor./exp.	pI^g theor./exp.	Score ^h	SC ⁱ	MQ ^j	P_{nd} vs UP_d ^k	P_{nd} vs P_d ^l	P_d vs UP_d ^m	P_{7h} vs UP_{7h} ⁿ
191	gi 30690394	Serine hydroxymethyltransferase 2	<i>Arabidopsis thaliana</i>	Amino acid metabolism	57/54	8.81/7.04	568	27	15			-2.0	
284	gi 15239772	Aspartate aminotransferase	<i>Arabidopsis thaliana</i>	Amino acid metabolism	44/39	6.80/7.04	347	23	9		-2.3		2.6
187	gi 7433553	Glycine hydroxymethyltransferase (EC 2.1.2.1) A_IG002P16.3	<i>Arabidopsis thaliana</i>	Amino acid metabolism	59/57	8.99/7.12	824	30	17	2.2			2.4
248	gi 15237069	Phosphoserine aminotransferase	<i>Arabidopsis thaliana</i>	Amino acid metabolism	48/43	8.25/7.12	248	15	6	-2.9		-2.6	
404	gi 261343268	O-acetylserine(thiol)lyase isoform A4	<i>Brassica rapa</i> subsp. <i>chinensis</i>	Amino acid metabolism	34/30	5.50/5.63	346	25	8	2.0			2.3
213	gi 15237947	UTP-glucose-1-phosphate uridylyltransferase 1	<i>Arabidopsis thaliana</i>	Nucleotide/nucleoside/nucleobase metabolism	52/52	5.73/7.01	650	31	13	-2.3			
285	gi 15232763	Adenosine kinase 1	<i>Arabidopsis thaliana</i>	Nucleotide/nucleoside/nucleobase metabolism	38/39	5.29/5.60	655	28	8	2.4	3.1		
555	gi 297832482	4-Phosphopantetheine adenyllyltransferase	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>	Nucleotide/nucleoside/nucleobase metabolism	19/20	5.82/5.40	300	21	3			2.0	
433	gi 15232538	Putative protein phosphatase 2C 39	<i>Arabidopsis thaliana</i>	Phosphate metabolism	32/28	5.51/5.79	533	23	7				2.4
73	gi 15233349	Aconitate hydratase 1	<i>Arabidopsis thaliana</i>	C-compound and carbohydrate metabolism	99/106	5.98/6.39	4337	31	25			2.0	
90	gi 3377802	T2H3.8 SUS2	<i>Arabidopsis thaliana</i>	C-compound and carbohydrate metabolism	92/90	6.12/7.39	281	15	12			-2.5	
99	gi 5459292	Thioglucoside glucohydrolase	<i>Brassica napus</i>	C-compound and carbohydrate metabolism	61/83	6.32/6.20	715	24	10	-2.2		-2.8	-4.0
162	gi 146572852	Myrosinase	<i>Brassica napus</i>	C-compound and carbohydrate metabolism	61/65	6.27/6.5	1930	50	23	-3.1	-2.9		
186	gi 297852420	F21D18.28	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>	C-compound and carbohydrate metabolism	54/57	6.96/6.83	1493	39	19	-2.1			
208	gi 21536853	Phosphoglycerate kinase, putative	<i>Arabidopsis thaliana</i>	C-compound and carbohydrate metabolism	42/52	5.49/5.71	1977	41	12			2.2	
279	gi 297807587	Reversibly glycosylated polypeptide-3	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>	C-compound and carbohydrate metabolism	41/40	5.76/5.90	367	28	10	2.1			-2.8
318	gi 241740186	Glyceraldehyde-3-phosphate dehydrogenase 2	<i>Brassica napus</i>	C-compound and carbohydrate metabolism	37/35	7.70/7.42	872	54	16	-3.3	-2.7		-2.1
334	gi 310896467	Glyceraldehyde-3-phosphate dehydrogenase	<i>Brassica napus</i>	C-compound and carbohydrate metabolism	16/34	6.81/6.88	284	54	7	2.0	2.4		2.1
292	gi 255575381	Fructose-bisphosphate aldolase, putative	<i>Ricinus communis</i>	C-compound and carbohydrate metabolism	39/38	6.57/7.02	222	15	4				2.3
325	gi 15231715	Fructose-bisphosphate aldolase, class I	<i>Arabidopsis thaliana</i>	C-compound and carbohydrate metabolism	39/35	6.05/6.71	431	28	9				3.4
327	gi 433335660	Malate dehydrogenase	<i>Brassica oleracea</i>	C-compound and carbohydrate metabolism	36/35	6.11/6.45	678	36	12				2.4
1042	gi 297832968	UDP-glucose pyrophosphorylase	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>	C-compound and carbohydrate metabolism	52/52	5.80/7.12	360	28	11				2.7

81	gi 224814588	Chloroplast carboxyltransferase alpha suunit isoform 2	<i>Brassica napus</i>	Lipid, fatty acid and isoprenoid metabolism	85/94	5.58/4.66	1042	31	22	2.8	-2.2
106	gi 1617270	Acyl-CoA synthetase	<i>Brassica napus</i>	Lipid, fatty acid and isoprenoid metabolism	75/80	5.96/6.33	627	24	14	-2.3	
209	gi 134943	Stearoyl-ACP desaturase	<i>Brassica napus</i>	Lipid, fatty acid and isoprenoid metabolism	45/52	5.75/5.79	667	30	11	-2.7	-2.8
546	gi 196122068	Oleosin S4-3	<i>Brassica napus</i>	Lipid, fatty acid and isoprenoid metabolism	23/20	8.78/5.88	170	22	4		-2.3
466	gi 28932694	Glutathione S-transferase zeta	<i>Brassica napus</i>	Secondary metabolism	25/26	5.64/5.63	119	22	5		2.8
492	gi 31790097	Glutathione S-transferase 3	<i>Brassica juncea</i>	Secondary metabolism	24/25	6.40/7.45	359	36	11	-2.1	2.1
501	gi 145329995	Glutathione S-transferase	<i>Arabidopsis thaliana</i>	Secondary metabolism	19/24	6.06/6.74	250	37	6	-2.0	
274	gi 15225353	Succinyl-CoA ligase [GDP-forming] subunit beta	<i>Arabidopsis thaliana</i>	Tricarboxylic-acid pathway	45/40	6.30/5.79	509	26	11		2.2
677	gi 312190391	NADH-ubiquinone oxidoreductase B18	<i>Eutrema parvulum</i>	Respiration	12/12	7.66/7.72	263	31	3	-2.1	-2.5
231	gi 17805	Cruciferin cru4 subunit	<i>Brassica napus</i>	Storage facilitating proteins	46/47	8.84/7.23	734	38	12	-2.0	2.1
374	gi 461840	Cruciferin, Cru 1	<i>Brassica napus</i>	Storage facilitating proteins	56/31	7.64/7.02	562	21	8	3.2	3.3
146	gi 334185329	Translational initiation factor 4A-1	<i>Arabidopsis thaliana</i>	Translation	46/68	5.47/5.79	1900	50	22	2.1	2.1
474	gi 15228161	Translation initiation factor IF6	<i>Arabidopsis thaliana</i>	Translation	27/26	4.63/5.31	261	15	4		2.0
498	gi 15234123	Translation initiation factor 3 subunit K	<i>Arabidopsis thaliana</i>	Translation	26/24	4.92/5.14	245	11	2		2.9
267	gi 390195442	Elongation factor E1	<i>Brassica oleracea var. capitata</i>	Translation	49/40	6.11/5.98	867	32	14		-2.2
173	gi 9293959	Chaperonin, t-complex protein alpha subunit	<i>Arabidopsis thaliana</i>	Protein folding and stabilization	60/60	6.42/6.12	1073	37	15		-2.3
161	gi 15229866	TCP-1/cpn60 chaperonin family protein	<i>Arabidopsis thaliana</i>	Protein folding and stabilization	60/65	6.03/6.23	578	28	14	-2.1	
632	gi 167138	Cyclophilin, partial	<i>Brassica napus</i>	Protein folding and stabilization	18/17	8.65/8.29	360	30	4		2.0
70	gi 297799586	Hypothetical protein ARALYDRAFT_492441 (HSP 90 region)	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>	Protein folding and stabilization	94/119	4.94/5.14	2783	32	28	-2.5	
456	gi 15219257	Proteasome subunit alpha type-2-A	<i>Arabidopsis thaliana</i>	Protein/peptide degradation	26/26	5.53/5.79	186	18	4	-3.1	-2.0
441	gi 13447110	GF14 kappa	<i>Brassica napus</i>	Protein binding	20/27	5.65/4.98	111	24	4	2.1	
455	gi 224981577	1433-3	<i>Brassica napus</i>	Protein binding	29/27	4.79/5.17	133	14	4		3.1
83	gi 1655824	Myrosinase-binding protein	<i>Brassica napus</i>	Sugar binding	99/94	5.48/5.77	1122	28	22		-2.8
300	gi 124107990	Annexin 1	<i>Brassica napus</i>	Metal binding	36/37	5.34/5.96	780	58	18	-2.5	-2.8
88	gi 312282899	Unnamed protein product	<i>Thellungiella halophila</i>	Nucleotide/nucleoside/nucleobase binding	74/90	5.10/5.28	562	26	14	-2.7	-2.4
89	gi 15219109	Heat shock protein 70B	<i>Arabidopsis thaliana</i>	Nucleotide/nucleoside/nucleobase binding	71/90	5.30/5.88	968	26	16		2.7
121	gi 15219234	V-type proton ATPase catalytic subunit A	<i>Arabidopsis thaliana</i>	Nucleotide/nucleoside/nucleobase binding	69/77	5.11/5.93	952	32	17		3.4
775	gi 19570344	Nucleoside diphosphate kinase 1	<i>Brassica rapa</i>	Nucleotide/nucleoside/nucleobase binding	16/15	6.30/6.53	159	26	3	-3.4	-4.5

Table 1 (Continued)

Spot Id ^a	NCBI accession no ^b	Protein name ^c	Species ^d	MIPS fuctional category ^e	M_r^f theor./exp.	pI^g theor./exp.	Score ^h	SC ⁱ	MQ ^j	P_{nd} vs UP _d ^k	P_{nd} vs P _d ^l	P_d vs UP _d ^m	P_{7h} vs UP _{7h} ⁿ
487	gi 762785	Cysteine proteinase inhibitor	<i>Brassica rapa</i> subsp. <i>campestris</i>	Regulation of protein activity	23/25	6.18/6.47	313	33	8	-2.4	2.3		
222	gi 289526917	Chain A, structure of arabidopsis atserpin1. Native stressed conformatio	<i>Arabidopsis thaliana</i>	Regulation of protein activity	43/48	4.97/4.60	481	21	7			2.8	
570	gi 77744889	Temperature-induced lipocalin	<i>Brassica napus</i>	Transport facilities	21/20	6.18/6.20	465	47	11	-2.7	-2.1		
477	gi 87046113	Etiolation seedling like-RAN2 small Ras GTP-binding nuclear protein	<i>Brassica napus</i>	Transport routes	25/25	7.04/6.83	315	34	7		-2.1		
174 357	gi 8050828 gi 3023857	Beta-tubulin RecName: Full = guanine nucleotide-binding protein subunit beta-like protein	<i>Brassica napus</i> <i>Brassica napus</i>	Transport routes Cellular signaling	37/58 36/33	5.69/5.20 8.05/7.53	1884 356	46 32	14 9	3.0 2.3	3.7 2.7	2.0	
574	gi 297811601	Universal stress protein (USP) family protein	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i>	Stress response	20/20	6.32/6.36	274	37	8			-2.0	
283 646	gi 253762012 gi 544416	Peroxidase 12, partial RecName: Full = Glycine-rich RNA-binding protein 10	<i>Brassica rapa</i> <i>Brassica napus</i>	Oxidative stress response Oxidative stress response	36/39 16/15	6.34/7.42 5.56/5.22	579 285	30 24	8 5		2.0	2.2 -2.0	
649	gi 63259317	RNA-binding protein 10 Cu/Zn superoxide dismutase	<i>Brassica napus</i>	Oxidative stress response	15/15	5.64/6.44	280	26	4	-2.0			
551 583	gi 341872725 gi 2465461	HSP22 Low molecular weight heat-shock protein	<i>Brassica napus</i> <i>Brassica rapa</i> subsp. <i>campestris</i>	Heat shock response Heat shock response	22/20 18/19	5.58/5.44 5.55/6.64	1873 328	46 34	10 5	2.6	-2.4	2.0 2.3	2.5
614	gi 397787610	Putative 17.9 kDa class II heat shock protein	<i>Brassica napus</i>	Heat shock response	17/18	5.97/6.01	552	46	6			2.4	
615	gi 157849708	17.6 kDa class II heat shock protein	<i>Brassica rapa</i>	Heat shock response	18/18	5.82/6.20	201	22	4	2.1			
204	gi 90194338	los	<i>Brassica rapa</i> subsp. <i>chinensis</i>	Cold shock response	48/52	5.55/6.12	1344	58	17		2.4		
234	gi 14764532	Monodehydroascorbate reductase	<i>Brassica rapa</i> subsp. <i>pekinensis</i>	Oxygen and radical detoxification	46/46	5.81/5.93	1187	47	17		-2.2	2.3	
495	gi 33285914	Putative dehydroascorbate reductase	<i>Brassica rapa</i> subsp. <i>pekinensis</i>	Oxygen and radical detoxification	12/24	6.15/5.74	193	37	4	-3.0	-2.4	2.8	
479 189	gi 7381260 gi 402170145	Peroxiredoxin antioxidant Glutathione reductase	<i>Brassica napus</i> <i>Brassica rapa</i> subsp. <i>campestris</i>	Oxygen and radical detoxification Oxygen and radical detoxification	24/25 54/54	5.97/5.69 5.83/6.23	196 564	30 26	6 12		-2.6	3.0	2.0
311	gi 211905345	Epithiospecifier protein	<i>Brassica rapa</i> subsp. <i>pekinensis</i>	Cellular sensing and response to external stimulus	38/36	5.95/6.47	717	46	15	2.0	2.3		
521	gi 15231041	Em-like protein GEA1	<i>Arabidopsis thaliana</i>	Cellular sensing and response to external stimulus	17/22	5.76/6.12	159	34	5			-2.9	
210	gi 213053669	LEA protein group 3	<i>Brassica napus</i>	Embryonal development	43/52	5.64/6.23	849	35	17	3.8		2.9	

359	gi 152228768	Seed maturation protein	<i>Arabidopsis thaliana</i>	Embryonal development	27/32	4.73/4.60	690	12	3	3.1
518	gi 81022819	Late embryogenesis-abundant protein	<i>Brassica caminata</i>	Embryonal development	17/22	5.86/6.74	1161	61	12	-3.3
301	gi 300433289	Short-chain dehydrogenase reductase	<i>Brassica napus</i>	Undclassified proteins	39/37	6.05/6.45	397	26	8	2.3

^a Protein spot number according to Image Master Platinum software.

^b Accession number in NCBI database.

^c Proteins homologue with the best parameters of identification.

^d Species.

^e MIPS functional category.

^f Theoretical (theor) pI and experimental (exp) protein mass.

^g Theoretical (theor) pI and experimental (exp) pI.

^h MASCOT Score is value which determines a probability-based Ion Score for each peptide match, which indicates the statistical significance of that MS/MS spectral assignment; this value is calculated by the formula $S = -10 \log(P)$, where P is the probability that obtained result is random.

ⁱ Percentage of sequence coverage by matched peptides.

^j Number of distinct sequences.

^{k,l,m,n} Fold change of identified protein abundance after: PEG soaking (P_{nd} vs UP_{nd}); drying (P_d vs P_{nd}); osmopriming general (P_d vs UP_d); germination (P_{th} vs UP_{th}).

The common proteins between the different phases of priming and germination were also investigated (Fig. 4B). Common proteins belonged to different MIPS categories (Supplementary Table S5). Almost half of the proteins affected during PEG soaking and drying were common and they were regulated in the same way in both conditions (Fig. 4B). Respectively, 20% and 30% of the proteins affected during PEG soaking and seed drying were common with general osmopriming. As expected all common proteins between soaking and general osmopriming were regulated on the same direction while the opposite was true between seed drying and general osmopriming. Less than 20% of the proteins were common between general osmopriming and post-priming germination. Around 40% of the proteins which abundance was modified during PEG soaking and 30% of those affected during drying were also modified in response to post-priming germination.

We observed that mostly the same pathways were affected at the transcriptomic and proteomic levels during osmopriming and post-priming germination. In order to compare further the regulation at both levels, we investigated the common genes and proteins during the different phases of osmopriming and germination (Supplementary Table S6). It is noteworthy that only 12 proteins were affected both at the transcriptional and proteomic level. They were not always affected at the same experimental variants and neither in the same direction.

In next paragraphs we focus on the main affected genes and proteins involved in osmopriming and germination steps in order to highlight the most relevant genes and proteins that could explain the improvement of germination by osmopriming (Table 1, Fig. 5B, Table 2).

3.4. Transcription regulation

The expression of genes involved in transcription regulation was mainly modified during general osmopriming and post-priming germination. The microarray data revealed three main families of transcription factors regulated by priming and post-priming germination: the MYB, the ethylene responsive ERF/AP2 (mainly DREB subfamily) and the NAC domain-containing proteins (Table 2). Genes coding for MYB transcription factors were more affected during priming while genes coding for DREB and NAC transcription factors were more affected during germination. The genes *MYB59*, *MYB43* and *MYB44* were up-regulated during general osmopriming and drying while *MYB15* was down-regulated during PEG soaking and post-priming germination (Table 2). Regarding osmopriming, *DREB2D* was down-regulated during soaking and up-regulated during drying. In case of post-priming germination *DREB2A* and *DREB19* were down-regulated while *DREB26* was strongly up-regulated (Table 2). Genes coding for NAC transcription factors (*NAC6*, *NAC42*, *NAC81* and *NAC102*) were preferably down-regulated during germination of primed seeds (Table 2).

3.5. Protein synthesis potential, post-translational processing capacity and targeted proteolysis

Translation of mRNA is critical for seed germination. Transcriptome and proteome analysis of rape seeds revealed the accumulation of mRNAs and proteins associated with the translation machinery involved in different phases of priming and post-priming germination. The differentially abundant mRNAs and proteins related to the translation machinery included transcripts (*eIF-2*, *eIF4E*, *eEF1B*, *eEF2*) and proteins (*eIF4A*, *eIF3* subunit K, *eIF6*, *eEF1*) corresponding to translation initiation and elongation factors (Tables 2 and 1, respectively). Gene encoding translation initiation factor *eIF-2* was up-regulated in osmoprime seeds. During post-priming germination decrease of the transcript of *eIF4E* subunit K was observed (Table 2). Regarding the proteomic data, the *eIF6*

Table 2

List of main genes affected in seeds of *Brassica napus* during sub-phases of osmoprimer treatment, whole osmoprimer process and post-primer germination (P_{nd} : primed non dried; P_d : primed dried; UP_d : dry unprimed; P_{7h} : primed after 7 h of imbibition; UP_{7h} : unprimed after 7 h of imbibition unaffected). Fold change means relative genes expression level after: PEG soaking (P_{nd} vs UP_d); drying (P_d vs P_{nd}); osmoprimer general (P_d vs UP_d); germination (P_{7h} vs UP_{7h}).

Probe ID	Arabidopsis homologue	Gene	Gene product	FC			
				P_{nd} vs UP_d	P_{nd} vs P_d	P_d vs UP_d	P_{7h} vs UP_{7h}
MYB transcription factors							
A_46_P031221	At3g23250	MYB15	Myb domain protein 15	−4.515			−8.730
A_46_P099299	At5g59780	MYB59	Transcription factor MYB59		9.419		
A_46_P239004	At5g16600	MYB43	Myb domain protein 43		10.285		
A_46_P235894	At5g67300	MYB44	Transcription factor MYB44		3.795		
DREB transcription factors							
A_46_P011616	At1g75490	DREB2D	Dehydration-responsive element-binding protein 2D	−6.105	5.015		
A_46_P213249	At5g05410	DREB2A	Dehydration-responsive element-binding protein 2A			−14.900	
A_46_P079986	At2g38340	DREB19	Dehydration-responsive element-binding protein 19			−4.710	
A_46_P065361	At1g21910	DREB26	Dehydration-responsive element-binding protein 26			21.340	
NAC transcription factors							
A_46_P245104	At5g52380	NAC6	Vascular-related NAC domain 6 protein				−4.09
A_46_P205244	At1g32870	NAC13	NAC domain protein 13	5.055			
A_46_P256394	At2g43000	NAC42	NAC domain protein 42			−7.36	
A_46_P283723	At5g08790	NAC81	NAC domain protein 81			−3.86	
A_46_P082481	At5g63790	NAC102	NAC domain protein 102			−7.46	
ABI transcription factors							
A_46_P054006	At2g36270	ABI5	Protein ABA insensitive 5	−5.535			
Translation initiation factors							
A_46_P024806	At1g29550	eIF4E	Translation initiation factor 4E				−4.100
A_46_P123504	At1g76810	eIF-2	Translation initiation factor 2		3.698		
Translation elongation factors							
A_46_P214934	At3g18760	eEF1B	Translation elongation factor 1B			7.371	
A_46_P239544	At2g45030	eEF2	Translation elongation factor 2			3.217	
Structural constituent of ribosomes							
A_46_P269238	At5g11750	RPL19	Ribosomal L19 family protein			−5.185	
A_46_P202934	At5g57290	RPP3B	60S acidic ribosomal protein P3-2			−10.335	
A_46_P124499	At1g72370	RPSaA	40S ribosomal protein Sa-1			5.880	
A_46_P296303	At2g33370	RPL23B	60S ribosomal protein L23			4.235	
A_46_P035471	At2g33800	RPS5	30S ribosomal protein S5			4.402	
A_46_P333036	At2g39390	RPL35B	60S ribosomal protein L35-2			8.734	
A_46_P213599	At3g24830	RPL13AB	60S ribosomal protein L13a-2			6.557	
A_46_P187879	At3g28500	RPP2C	60S acidic ribosomal protein P2-3			7.884	
A_46_P211964	At4g27090	RPL14B	60S acidic ribosomal protein P2-3			4.662	
A_46_P058816	At5g02960	RPS23B	40S ribosomal S23 protein			7.303	
A_46_P242609	At3g14600	RPL18AC	60S ribosomal protein L18a-3			3.720	
A_46_P251354	At4g00810	RPP1B	60S acidic ribosomal protein P1-2			5.160	
A_46_P052406	At5g15220	RPL27	Large subunit ribosomal protein L27			18.690	
A_46_P248129	At5g23740	RPS11C	40S ribosomal protein S11-3			11.950	
A_46_P059486	At5g27820	RPL18p	Ribosomal L18p/L5e family protein			3.450	
A_46_P327205	At5g27850	RPL18C	60S ribosomal protein L18-3			12.640	
RNA helicases							
A_46_P052701	At5g62190	RH7	DEAD-box ATP-dependent RNA helicase 7			3.170	
A_46_P058236	At5g60990	RH10	DEAD-box ATP-dependent RNA helicase 10			−3.140	
Exoribonucleases							
A_46_P204469	At4g27490	RRP41L	3'-5'-Exoribonuclease			9.225	
HSPs							
A_46_P066016	At4g24190	HSP90-7	Heat shock protein 90-7				10.070
A_46_P196064	At4g16660	HSP70	Heat shock protein 70			5.480	
A_46_P343495	At3g23990	HSP60-3B	Heat shock protein 60-3B			−3.580	
A_46_P237799	At5g12030	HSP17.7	Heat shock protein 17.7			−7.240	
A_46_P237924	At3g46230	HSP17.4	Heat shock protein 17.4			−12.750	
A_46_P236464	At4g25200	HSP23.6	Heat shock protein 23.6			−7.070	
A_46_P246399	At5g12020	HSP17.6	Heat shock protein 17.6			−7.670	

Table 2 (Continued)

Probe ID	Arabidopsis homologue	Gene	Gene product	FC			
				P _{nd} vs UP _d	P _{nd} vs P _d	P _d vs UP _d	P _{7h} vs UP _{7h}
Protein disulfide isomerases							
A_46_P011681	At1g77510	PDIL1-2	Protein disulfide isomerase-like 1-2			5.149	
A_46_P189139	At2g47470	PDIL2-1	Protein disulfide-isomerase like 2-1			3.338	
Translocon complexes							
A_46_P063116	At3g16620	TOC120	Translocase of chloroplast 120		4.118		
A_46_P059301	At4g03320	TIC20-IV	Protein TIC 20-IV		3.356		
A_46_P174554	At4g24920	SEC61G1	Protein transport protein Sec61 subunit gamma-1			11.790	
A_46_P154234	At5g50460	SEC61G2	Protein transport protein Sec61 subunit gamma-2			3.390	
A_46_P197159	At5g09420	OM64	Outer envelope protein 64, mitochondrial		-4.140		
A_46_P336425	At2g29530	TIM10	Mitochondrial import inner membrane translocase subunit TIM10			4.490	
A_46_P058716	At1g17530	TIM23-1	Mitochondrial import inner membrane translocase subunit TIM23-1		4.505		
A_46_P236254	At1g55900	TIM50	Mitochondrial import inner membrane translocase subunit TIM50		-4.805		
A_46_P295233	At3g04460	PEX12	Peroxisome biogenesis protein 12		4.217		
Serine carboxypeptidases							
A_46_P299605	At4g30610	SCPL24	Serine carboxypeptidase like 24			11.800	
A_46_P057916	At5g23210	SCPL34	Serine carboxypeptidase like 34			11.890	
A_46_P243614	At5g42230	SCPL41	Serine carboxypeptidase like 41			3.800	
A_46_P002511	At2g12480	SCPL43	Serine carboxypeptidase like 43			5.720	
A_46_P081781	At1g43780	SCPL44	Serine carboxypeptidase like 44			16.460	
A_46_P011711	At3g45010	SCPL48	Serine carboxypeptidase like 48			10.030	
Proteases							
A_46_P037156	At3g47060	FTSH7	ATP-dependent zinc metalloprotease FTSH 7		3.255		
A_46_P022276	At4g16800	Clp	ATP-dependent caseinolytic (Clp) protease/crotonase family protein		6.484		
A_46_P083546	At1g19740	LON	ATP-dependent protease La domain protein		-3.225		
A_46_P105024	At5g57990	UBP23	Ubiquitin-specific protease 23		-5.115		
A_46_P029861	At1g62710	BETA-VPE	Vacuolar-processing enzyme beta-isozyme		5.488		
A_46_P036096	At4g17040	CLPR4	ATP-dependent Clp protease proteolytic subunit-related protein 4		4.319		
A_46_P242804	At5g46740	UBP21	Ubiquitin-specific protease 21			4.990	
A_46_P269823	At1g67690	F12A21.16	Zincin-like metalloprotease	-3.810			
A_46_P107894	At2g24640	UBP19	Ubiquitin-specific protease 19		4.441		
A_46_P085926	At2g47940	DEG2	DEG protease 2		15.852		
A_46_P294013	At5g65620	K21L13.14	Zincin-like metalloprotease		9.052		
A_46_P012866	At5g07030	MOJ9.20	Aspartyl protease family protein			8.610	
A_46_P271983	At5g45890	SAG12	Senescence-specific cysteine protease SAG12		-4.150		
Ubiquitin-protein ligase activity							
A_46_P105434	At5g60410	SIZ1	SUMO E3 ligase		4.166		
A_46_P252769	At1g24330	PUB6	U-box domain-containing protein 6		-3.960		
A_46_P102739	At1g71020	PUB10	U-box domain-containing protein 10		7.090		
A_46_P242884	At3g52450	PUB22	E3 ubiquitin-protein ligase PUB22		19.850		
A_46_P255229	At3g49060	PUB32	U-box domain-containing protein 32		5.900		
A_46_P085451	At1g80570	FBL14	F-box/LRR-repeat protein 14		-7.210		
A_46_P044436	At3g54650	FBL17	F-box/LRR-repeat protein 17		6.105		
A_46_P084501	At1g02860	BAH1	E3 ubiquitin-protein ligase BAH1		-4.750	-7.000	
A_46_P246384	At3g05200	ATL6	E3 ubiquitin-protein ligase ATL6		-7.940		
A_46_P006846	At5g27920	F15F15.2	F-box protein		4.031		
A_46_P216749	At1g78870	UBC13A	Ubiquitin-conjugating enzyme 13A		3.780		
Storage proteins							
A_46_P030141	At2g18540	F24H14.11	Cupin family protein	3.625			
A_46_P029091	At3g10080	T22K18.9	Germin-like protein subfamily 3 member 2	3.307			
A_46_P008496	At4g28520	CRC	Cruciferin 3, CRU3	7.647			
Lipid and fatty acid metabolism							
A_46_P274828	At3g16370	APG	GDSL-like lipase		56.750		
A_46_P219224	At4g01130	-	GDSL-like lipase		3.650		
A_46_P289878	At5g45670	-	GDSL-like lipase		21.850		
A_46_P257919	At1g09390	-	GDSL-like lipase		4.680		

Table 2 (Continued)

Probe ID	Arabidopsis homologue	Gene	Gene product	FC			
				P _{nd} vs UP _d	P _{nd} vs P _d	P _d vs UP _d	P _{7h} vs UP _{7h}
A_46_P349670	At1g28650	–	GDSL-like lipase				36.510
A_46_P257609	At1g54000	–	GDSL-like lipase				13.250
A_46_P086526	At5g55050	–	GDSL-like lipase				4.219
A_46_P256139	At5g45960	–	GDSL-like lipase				6.454
A_46_P002576	At1g20120	–	GDSL-like lipase				3.336
A_46_P365640	At1g75930	EXL6	Extracellular lipase 6				3.863
A_46_P053296	At5g56100	OLE	Oleosin				3.755
A_46_P011421	At5g40420	OLE2	Oleosin type 2				–7.850
Bifunctional inhibitor/lipid-transfer proteins							
A_46_P193939	At5g55410	MTE17.12	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein				–3.610
A_46_P228494	At1g66850	F4N21.4	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein				5.505
A_46_P211494	At5g07230	–	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein				4.466
A_46_P287388	At5g55450	MTE17.16	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein				4.068
A_46_P243849	At1g12100	F12F1.2	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein				9.810
A_46_P221339	At1g62500	T3P18.6	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein				6.910
A_46_P282238	At5g48490	MJE7.13	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein				11.320
A_46_P276788	At4g33550	T16L1.40	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin-like protein				–14.680
Glucosinolate remobilization							
A_46_P055596	At3g14210	ESM1	Epithiospecifier modifier 1				4.126
A_46_P149099	At1g54040	ESP	Epithiospecifier protein				–9.010
A_46_P130929	At3g16420	PBP1	PYK10-binding protein 1				12.150
TIPs aquaporins							
A_46_P122734	At2g25810	TIP4-1	Aquaporin TIP4-1				12.003
A_46_P121904	At3g26520	TIP1-2	Gamma-tonoplast intrinsic protein 2; aquaporin TIP1-2				3.715
Cell extension, cell wall loosening							
A_46_P284923	At1g12090	ELP	Extensin-like protein				121.130
A_46_P206264	At5g57550	XTH25	Xyloglucan endotransglucosylase/hydrolase 25				5.177
Tubulins							
A_46_P090256	At3g61650	TUBG1	Tubulin gamma-1 chain				4.170
A_46_P257054	At1g75780	TUBB1	Tubulin beta-1 chain				12.250
A_46_P131564	At5g62700	TUBB3	Tubulin beta-3 chain				6.560
Proteins with motor function							
A_46_P075616	At1g63640	F2K11.1	F2K11.1/microtubule motor activity protein KinG, kinesin family protein				4.172
A_46_P069221	At2g36200	F2H17.19	F2H17.19/microtubule motor activity protein, kinesin family protein				4.827
A_46_P294693	At3g45850	–	Microtubule motor activity protein, kinesin family protein				4.507
A_46_P234764	At4g39050	–	Microtubule motor activity protein, kinesin family protein				6.442
A_46_P093151	At5g20490	XI-K	myosin XI K				7.303
Cell division							
A_46_P078461	At3g01610	CDC48 C	Cell division control protein 48 homolog C				7.030
A_46_P002081	At5g55230	MAP65-1	Microtubule-associated protein 65-1				9.214
A_46_P128899	At1g24764	MAP70-2	Microtubule-associated protein 70-2				4.340
Histones							
A_46_P134354	At5g10400	HTR9	Histone H3.2				3.100

Table 2 (Continued)

Probe ID	Arabidopsis homologue	Gene	Gene product	FC			
				P _{nd} vs UP _d	P _{nd} vs P _d	P _d vs UP _d	P _{7h} vs UP _{7h}
LEA's proteins							
A_46_P236044	At2g21490	LEA	Dehydrin LEA				-55.740
A_46_P013936	At1g32560	LEA4-1	Late-embryogenesis abundant protein 4-1	-4.660	4.850		
A_46_P214224	At5g06760	LEA4-5	Late-embryogenesis abundant protein 4-5	-8.360	9.160		
A_46_P052136	At3g51810	EM1	EM1 protein belongs to LEA group 1				-20.43
A_46_P245044	At2g40170	EM6	EM6 protein belongs to LEA group 1				-3.250
A_46_P236639	At3g22490	SMP	Seed maturation protein, putative LEA protein	-9.120	8.650		
Oxidative stress							
A_46_P085011	At1g77100	PER13	Peroxidase 13				8.350
A_46_P023066	At2g37130	PER21	Peroxidase 21				6.036
A_46_P272908	At4g35090	CAT2	Catalase 2				3.890
A_46_P265993	At1g53670	MSRB1	Peptide methionine sulfoxide reductase B1	-4.190	4.100		
A_46_P122104	At5g01600	FER1	Ferritin-1				5.352

was accumulated during drying and post-priming germination, while eIF3 was more abundant during post-priming germination only. Translation initiation factor eIF4A was accumulated during PEG soaking and general osmopriming (Table 1, Fig. 5B). Moreover, genes encoding translation elongation factors eEF1B and eEF2 were up-regulated during complete osmopriming (Table 2). At the protein level, eEF1 was down-regulated during post-priming germination (Table 1, Fig. 5B). Initiation factors eIF4E and eIF4A are components of the EIF4F translation initiation complex in eukaryotes.

Some of the translation factors up-accumulated during priming and germination may participate in mRNA stability or favor a selective mRNA processing. Indeed, an ATP-dependent RNA helicase eIF4A accumulated during PEG soaking and general osmopriming as well as DEAD box RNA ATP-dependent helicase 7 whose gene was up-regulated during general osmopriming (Table 2) may play pivotal role in modulation of RNA metabolism. Moreover, mRNA decay is also important since impairment in the RRP41L, a putative core subunit of the exosome, delays germination and inhibits seedling growth [41]. Gene coding for RRP41L was up-regulated in osmoprimed seeds (Table 2). Apart from translation initiation and elongation factors, genes encoding proteins related to ribosome biogenesis were also affected in primed and germinating seeds. Several genes encoding structural constituent of ribosomes such as 40S and 60S ribosomal subunits were up-regulated during general osmopriming and post-priming germination, whereas no ribosomal protein genes were found during PEG soaking and drying (Table 2).

Priming and post-priming germination were accompanied by accumulation of a wide range of transcripts and proteins involved in post-translational processing such as HSP90.7, HSP70, HSP22, HSP17.6, HSP17.9, 14-3-3 chaperones and protein disulfide isomerase-like proteins. Genes encoding HSP70 and HSP90.7 were up-regulated during post-priming germination (Table 2) while the level of HSP70B (Table 1, Fig. 5B) protein increased during drying and decreased in response to general osmopriming. Proteomic results showed also accumulation of proteins spots for HSP22 and HSP17.9 (Table 1, Fig. 5B). Additionally, these two proteins were also accumulated during PEG soaking. Furthermore, the HSP22 and 14-3-3 protein were accumulated in response to general osmopriming treatment. In contrast to HSP22, the 14-3-3 protein was down-regulated during post-priming germination (Table 1, Fig. 5B). Two genes encoding protein disulfide isomerase-like (PDI-like) proteins were up-regulated in osmoprimed seeds (Table 2). PDIs catalyze disulfide bond formation and rearrangements in the

substrate proteins, facilitating the folding of nascent polypeptides in the endoplasmic reticulum.

Another important element in polypeptide fate during or after translation is their translocation into the various cell compartments. HSP/chaperones may participate in the import of proteins into plastids, mitochondria, peroxisomes and endoplasmic reticulum through their interaction with the translocon complexes. Our data disclosed the activation of the genes encoding TOC120 and TIC20-IV in osmoprimed seeds and Sec61 during post-priming germination (Table 2). These proteins are the members of plastids and endoplasmic reticulum translocon complexes. Our results revealed also the activation of TIM23 and TIM10 genes in response to general osmopriming treatment and postpriming germination, respectively (Table 2).

It is noteworthy that not only genes related to protein synthesis but also genes related to proteins/peptide degradation are activated in response to general osmopriming treatment and during post-priming germination (Supplementary Table S3). The group of six genes encoding serine carboxypeptidase-like (SCPL) proteins was up-regulated during post-priming germination while *Deg2* and *FtsH* genes were among proteases up-regulated in response to osmopriming (Table 2). SCPLs are involved in protein turnover for the mobilization of N-resources as it occurs during seed germination [42].

Interestingly, mRNA species abundant during priming and germination also involve proteins related to the ubiquitin/proteasome system. Genes encoding small ubiquitin-like modifier (SUMO) E3 ligase and F-box family proteins being components of the SCF complex were up-regulated during osmopriming while genes encoding plant U-Box 22 and ubiquitin conjugating enzyme 13A were up-regulated during germination (Table 2).

3.6. Reserve mobilization and glucosinolate remobilization

Surprisingly, transcripts and proteins for seed storage proteins (SSPs) were accumulated during osmopriming and post-priming germination of rape seeds. Protein spots corresponding to cruciferin CRU1 were up accumulated during sub-phases of osmopriming treatment and post-priming germination (Table 1, Fig. 5B). Moreover, it is noteworthy that the gene encoding vacuolar processing enzyme (VPE) involved in posttranslational processing of the precursor cruciferin forms into cruciferin subunits was up-regulated in osmoprimed rape seeds (Table 2).

Triacylglycerols (TAGs) are the major storage lipids in rape seeds. In the present work, several genes encoding enzymes playing

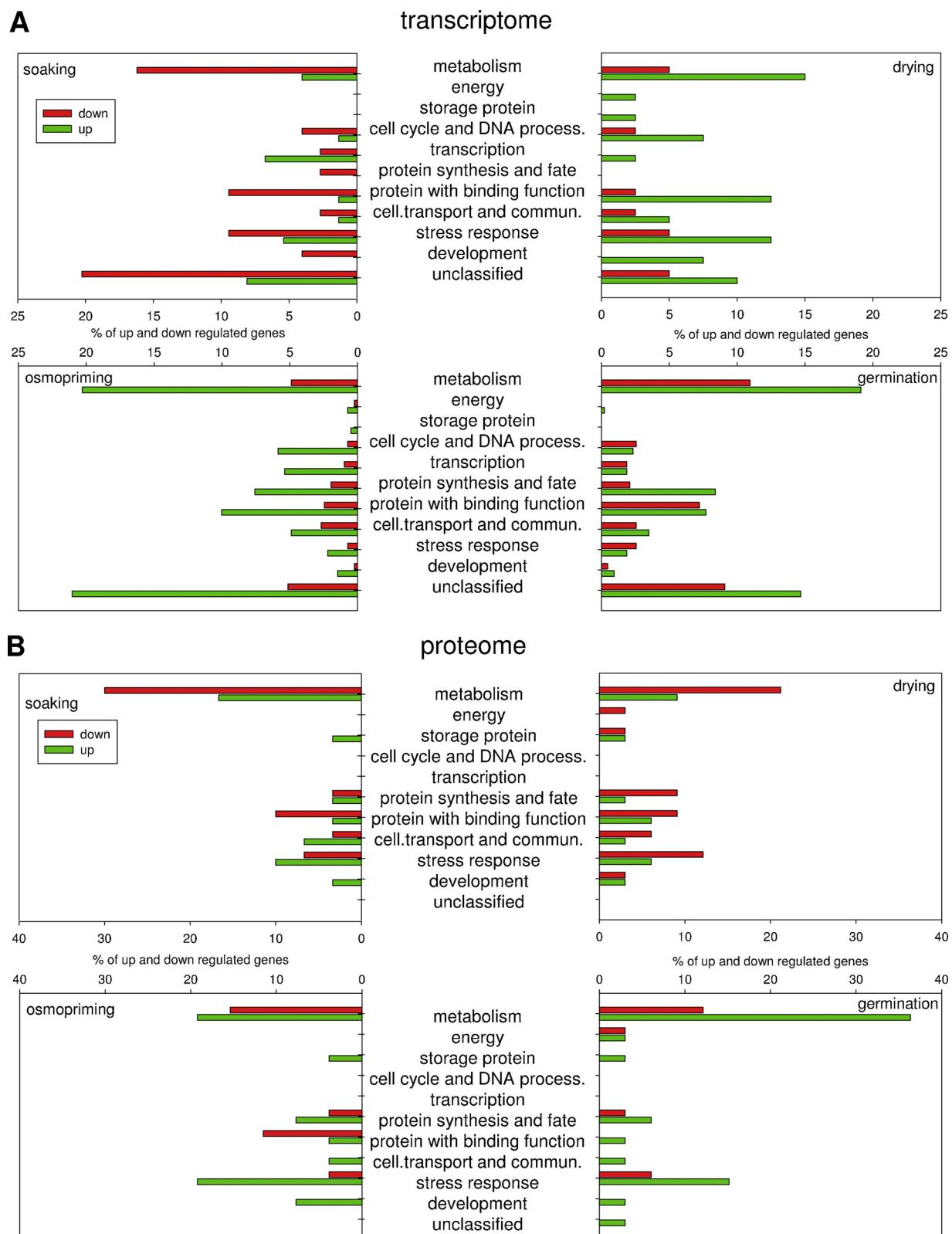


Fig. 3. Proportion of transcripts (A) and proteins (B) in osmoprime and germinating *Brassica napus* seeds. Transcripts and proteins down-regulated and up-regulated during the main phases of osmoprime (soaking, drying), whole osmoprime process and post-prime germination categorized following MIPS functional catalogue database classification. Some genes/proteins could be involved in different categories.

a role in their catabolism were identified, including genes encoding GDSL-like lipase/acylhydrolase superfamily proteins. Six genes encoding GDSL-like lipases were strongly up-regulated during post-prime germination, while other three genes for GDSL-like

lipases as well as extracellular lipase 6 were up-regulated in osmoprime seeds (Table 2). Furthermore, the activation of the genes involved in lipid catabolism was accompanied by activation of the genes involved in lipid transport with the largest group of genes

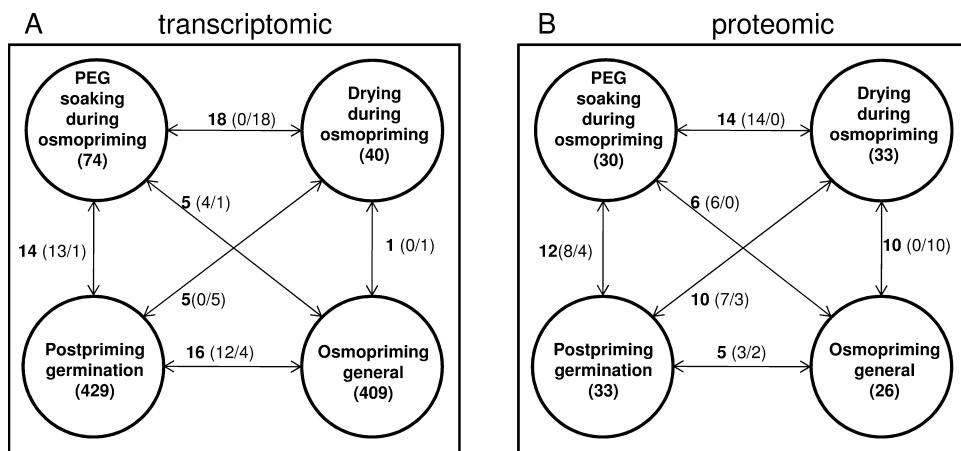


Fig. 4. Number of common transcripts (A) and proteins (B). Transcripts and proteins between the main phases of osmoprimering (soaking, drying), whole osmoprimering process and post-primering germination in *Brassica napus* seeds are shown. Under brackets are indicated the numbers of probes (A) and protein spots (B) regulated in the same way (up- or down-regulated in both conditions)/regulated in opposite ways (up-regulated in one condition and down-regulated in the other). The total number of genes or proteins affected for each phase is indicated in the circles.

encoding bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein (Table 2). Oil bodies in seeds are surrounded by oleosins, oil-body-membrane family proteins. The genes encoding oleosins were expressed in opposite way during drying and post-primering germination of rape seeds. *OLEOSIN* was up regulated during drying while *OLEOSIN2* was strongly down-regulated during post-primering germination (Table 2). Moreover, at the proteomic level, oleosin S4-3 was down accumulated in osmoprimered seeds (Table 1, Fig. 5B).

Genes and proteins involved in glucosinolate remobilization were affected during priming and post-primering germination. Glucosinaltes are sulfur-rich secondary metabolites and their overall content declines upon seed imbibition [43]. Epithiospecifier proteins (ESP) are enzymes that work in concert with myrosinases (thioglucosidases) to produce metabolites derived from glucosinaltes. The PYK10 protein was described as a root and hypocotyl specific myrosinase [44]. Gene encoding epithiospecifier protein was down-regulated during post-primering germination of rape seeds whereas gene coding for epithiospecifier modifier was up-regulated in osmoprimered seeds (Table 2). Gene encoding PYK10-binding protein was down-regulated during soaking and up-regulated during germination. The level of myrosinase and myrosinase binding protein accumulation decreased during both PEG soaking and drying while the level of epithiospecifier protein decreased during soaking and increased during drying (Table 1, Fig. 5B).

3.7. Water transport, cell wall modification, cytoskeletal organization and cell division

Seed germination is initiated by water uptake and culminates at radicle protrusion driven by embryo expansion. Although the first step of seed imbibition occurs through apoplastic water flow, seed hydration may subsequently occurs via aquaporins. In osmoprimered rape seeds two genes encoding tonoplast's aquaporins (*TIP4.1* and *TIP2*) were up-regulated (Table 2). Furthermore, expression of *TIP2* increased approx. 20 fold during post-primering germination.

Penetration of the structures surrounding the embryo is a consequence of radicle cells elongation. The genes encoding xyloglucan endotransglucosylase/hydrolase (XTH) and extensin-like protein (ELP) were up-regulated in rape seed in response to general osmoprimering and post-primering germination, respectively (Table 2). The ability of XTHs to cleave xyloglucans and rejoin the cut ends with

new partners is related to the role of these enzymes in cell wall loosening during growth and in the restructuring of the cell walls after extension [45]. Also cytoskeleton reorganization is necessary to achieve large rates of cells elongation that precedes radicle protrusion [46]. The component of microtubules β-tubulin protein showed higher level of accumulation in rape seeds during PEG soaking, drying and post-primering germination (Table 1, Fig. 5B). The up-regulation of genes encoding γ- and β-tubulins was also noticed during post-primering germination (Table 2).

Among genes related to proteins with binding function, genes encoding proteins attached to cytoskeletal were up-regulated in primed seeds. Five genes encoding proteins with motor function were identified as preferably expressed in primed seeds of rape (Table 2). Four of them encode proteins belonging to kinesin family with microtubule motor activity and one is for myosin XI K, a motor protein attached to actin microfilaments of cytoskeletal (Table 2). Many plant kinesins involved in cell division, including mitosis, are crucial components of the mitotic machinery and also display critical roles in morphogenesis, and signal transduction [47]. Also a transcript for microtubule associated protein MAP65-1 functioning mainly in cell division by stabilization of microtubule spindle and involved in interaction with cytoskeleton [48] was accumulated in primed seeds (Table 2). Moreover, a higher expression of the cell division cycle gene *CDC48C* was observed in primed seeds and accumulation of mRNA for histone 3 was stated during germination of primed seed (Table 2).

3.8. Primed seed transcriptome and proteome exhibits footprints of late maturation program

Transcripts for the late embryogenesis abundant (LEA) proteins were differentially accumulated in response to osmoprimering treatment and post-primering germination. We observed down-regulation of genes encoding LEA4-1, LEA4-5 and seed maturation protein (SMP) during PEG soaking. In contrast, these genes were up-regulated during drying (Table 2). Moreover, the accumulation of LEA3 proteins occurred during PEG soaking and in osmoprimered seeds. Following the accumulation of transcript for SMP during drying, we also observed higher accumulation of SMP protein in response to drying (Table 1, Fig. 5B). Gene encoding dehydrin belonging to group II LEAs was strongly down-regulated during post-primering germination. Furthermore, genes encoding EM1 and EM6 proteins, members of LEA group 1, were down-regulated during post-primering germination (Table 2).

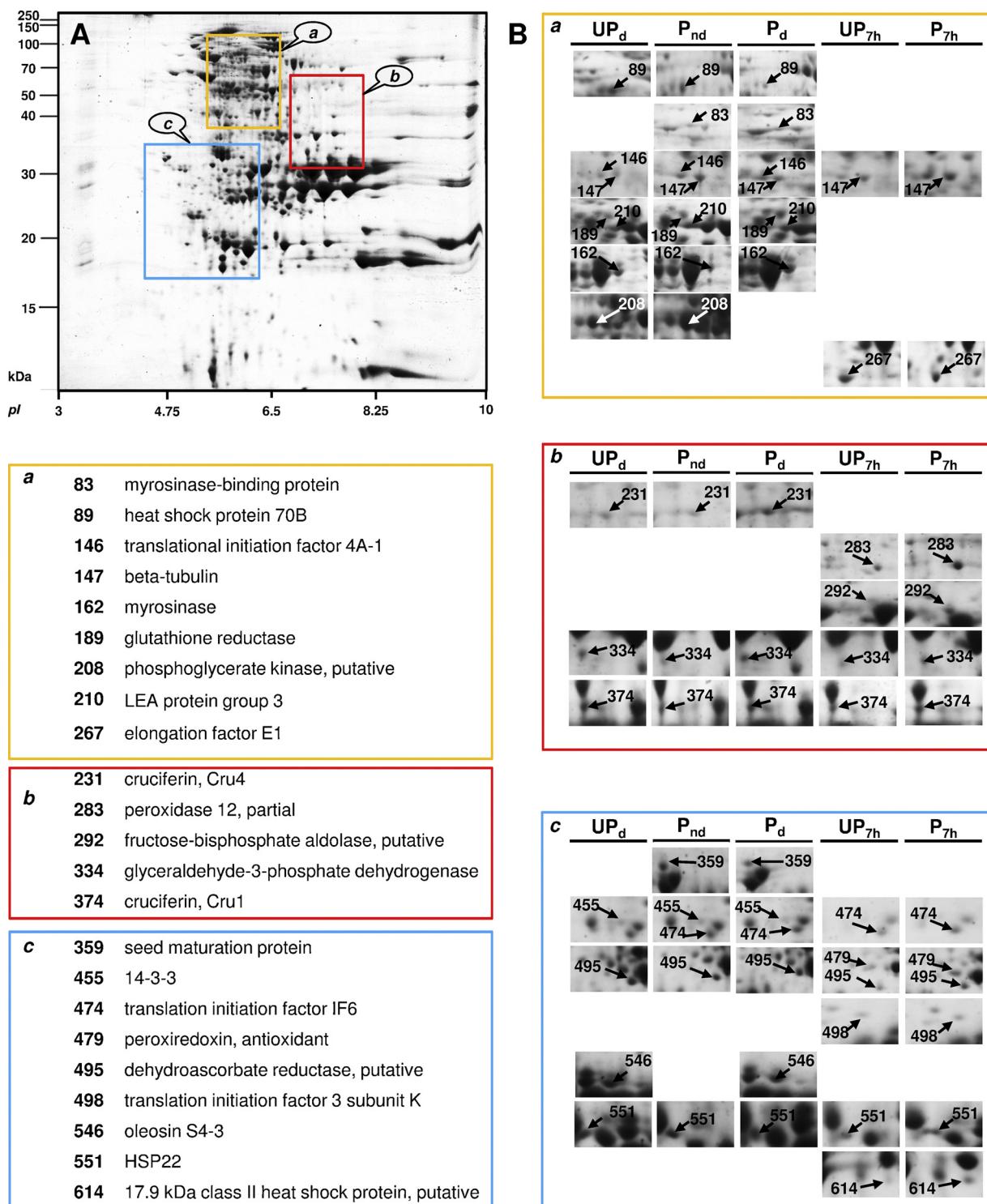


Fig. 5. Visualisation of proteins whose abundance is affected during priming and post-priming germination of *Brassica napus* seeds. (A) Typical two-dimensional protein map obtained from dry unprimed seeds. Total seed proteins were separated using 2D gel electrophoresis with IEF (pH 3–10) and detected with Coomassie Brilliant Blue staining. (B) Three representative parts of 2D-gel reproduced in enlarged **a**, **b** and **c** windows show protein spots whose abundance is affected minimum 2 fold between dry unprimed seeds (UP_d) and dry primed seeds (P_d); non dried primed seeds (P_{nd}) and P_d; UP_d and P_{nd}; unprimed seeds imbibed 7 h on water (UP_{7h}) and primed seeds imbibed 7 h on water (P_{7h}) seeds.

3.9. Management of oxidative stress and glycolytic pathway

In rape seeds the accumulation of transcripts and proteins playing an important role in response to oxidative stress was observed during osmoprimer treatment and post-priming germination. The genes encoding CAT2 and PER21 were up-regulated in response to osmoprimer. Additionally, during post-priming germination

transcript for PER13 was accumulated (Table 2). Moreover, accumulation of POX12 and peroxiredoxin proteins was observed during post-priming germination (Table 1, Fig. 5B). Our proteomic analysis showed that key enzymes catalyzing the regeneration of glutathione (glutathione reductase, GR) and ascorbic acid (dehydroascorbate reductase, DHAR) were affected during osmoprimer and post-priming germination. GR was down-regulated during

drying and up-regulated in response to general osmoprimer treatment while DHAR was down-regulated during both sub phases of osmoprimer treatment and up-regulated during post-primer germination (Table 1, Fig. 5B).

Osmoprimer treatment and post-primer germination of rape seeds were accompanied by accumulation of transcripts and proteins for key glycolytic enzymes. The mRNAs for HXK3 and GAPA1 (glyceraldehyde 3-phosphate dehydrogenase A subunit 1) were accumulated in osmoprimered seeds (Table 2). The abundance of proteins spots of glyceraldehyde 3-phosphate dehydrogenase and fructose-biphosphate aldolase increased during post-primer germination (Table 1, Fig. 5B). Additionally, proteomic analysis showed that the glyceraldehyde 3-phosphate dehydrogenase was also up accumulated during two sub phases of osmoprimer treatment. Moreover, accumulation of phosphoglycerate kinase was observed in osmoprimered seeds.

4. Discussion

The present results shed a new light on mechanisms underlying primer-induced improvement of seed germination. Schematic presentation of the main processes affected during primer and post-primer germination involved in increased germination potential is shown in Fig. 6. Mostly the same functional categories are affected at the transcriptomic and proteomic levels during primer and post-primer germination. However, comparison of the genes and proteins affected during primer and germination steps showed that primer and final germination of primed seeds trigger distinct specific pathways since only a minority of genes and proteins are involved in all phases while a vast majority is involved in only one single phase. These issues will be discussed in next paragraphs.

4.1. Are transcriptional and translational regulations during primer similar?

Differential transcriptomic and proteomic studies have led to identification of many mRNAs and proteins during germination but without distinction between stored and *de novo* synthesized counterparts [10,11,55,58]. Galland et al. [49] provided a detailed description of the dynamics of protein synthesis during the time course of germination, indicating that mRNA translation is both sequential and selective during this process. The authors stated that the stored translational machinery is already effective during the very few hours upon imbibition. In this study, we investigated the modification of mRNA and protein accumulation during the different phases of osmoprimering and germination. Although our data could not always formally establish whether the protein or transcriptional changes were '*de novo*' or resulted from changes in turnover, they suggest that '*de novo*' synthesis of mRNAs and proteins occurred during osmoprimering since several genes involved in mRNA synthesis, ribosome biosynthesis and translation initiation were up-regulated during this process. Comparing primed and germinated seeds with dry seeds let us hypothesize that at least the observed increase of mRNAs level is due to upregulation of the corresponding gene expression. Complete osmoprimering and post-primer germination were the two steps represented by highest number of differentially affected genes. Gene expression is controlled by an intricate network of numerous pathways involving transcription, post-transcriptional RNA modification and processing as well as transcript stability. The canonical players in transcriptional regulation are sequence-specific DNA-binding transcription factors (TFs) that modulate gene expression. The highest number of genes encoding TFs was identified during osmoprimering general and post-primer germination (Supplementary Table

S3). These genes encode several classes of TFs, and may be important for controlling primer-induced processes occurring during seed germination. A set of the genes functionally related and/or co-expressed often share conserved regulatory motifs, which might be responsible for coordinated expression of the set of genes. This can in part explain drastic transcriptional changes accompanying general primer and post-primer germination. Changes in gene activity and expression are also regulated by two major epigenetic modifications: DNA methylation and histone modifications. We found that genes related to histone modifications such as methylation or acetylation were specifically affected during complete osmoprimering and to a lesser extent during germination (Supplementary Table S3). The above mentioned mechanisms may ultimately regulate gene activity and expression during primer and post-primer germination. Modification at the protein level, could be due to mRNA translation as suggested by Galland et al. [49] while modifications due to protein degradation and turnover could nevertheless not be excluded.

A comparative transcriptomic and proteomic analysis was performed for the various phases of the primer process and was based on name/function of genes product and proteins. It revealed that the match between genes and proteins was limited to only 12 genes–proteins (Supplementary Table S6) involved in metabolism, transport, stress response and protein folding. The genes–proteins were divided into six groups. Groups 1 and 2 contain mostly coordinately expressed genes and proteins. In group 1 most of the proteins that accumulated during post-primer germination match the transcript whose abundance increased during both general osmoprimering and post-primer germination, suggesting that protein accumulation is primarily regulated by transcript abundance. Correspondence between expressed gene and protein was also found in group 2 (decreased level of both mRNA and protein). Groups 3 and 4 contain genes and proteins expressed in an opposite way. For other groups there is no match between transcript and protein abundance. Therefore, the regulation of mRNA translation and post-translational processing seem to have a more appreciable role than the time-lag between transcription and translation to account for the weak correspondence between proteomic data and mRNA. Further analyses characterizing the occurrence of proteins and/or transcript turnover by using radiolabeled precursors for their synthesis are needed.

It appears from our data that a total number of 952 genes were differentially regulated but under our experimental conditions, only 75 proteins were clearly related to the primer process. We did not detect any protein involved in DNA processing and transcription, despite modification in the expression of numerous genes coding for various transcription factors. Direct quantitative measurement of transcription factors on a proteome scale remains a challenge because of the low abundance of TFs. Among genes encoding TFs were DREB transcription factors which regulate the expression of many stress-inducible genes at early germination as reported by Krishnaswamy et al. [50]. Conversely, previous proteomic studies disclosed that germination-specific proteins are translated from stored mRNAs and these long-lived stored transcripts in mature dry seed were proposed to play a crucial role in seed germination emphasizing the significance of post-transcriptional and translational controls [51]. Long-lived mRNAs stored in mature seeds can remain active for long periods, even if seeds undergo severe desiccation [52]. According to Kimura and Nambara [53] and to Sano et al. [52], *de novo* protein synthesis is necessary for germination; germination-specific proteins involved in energy production and maintenance of cell structure are synthesized from long-lived mRNAs. However, the maintenance of translational capacity over several days of seeds primer and subsequent germination implies a progressive renewal of the implicated proteins, synthesized not only from long-lived mRNAs. De

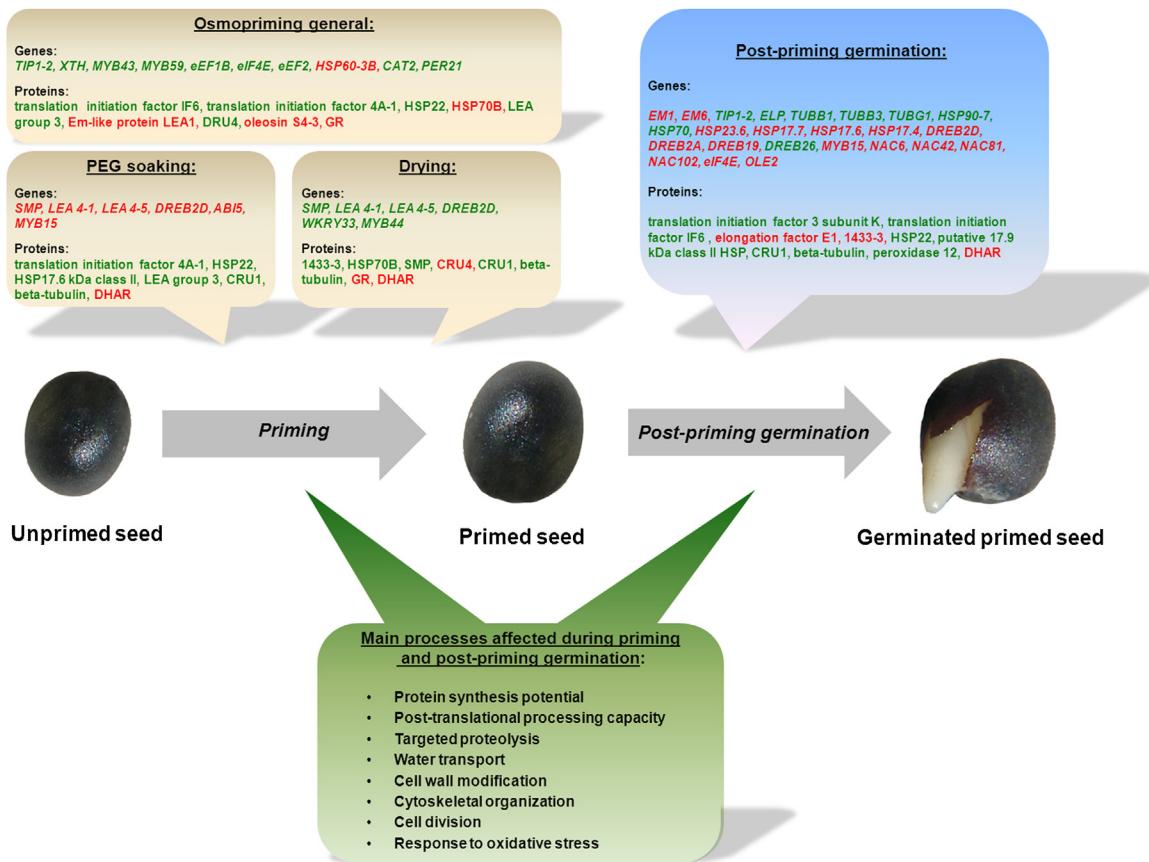


Fig. 6. Schematic presentation of the main processes affected during priming and post-priming germination involved in priming-induced improvement of seed germination. Mostly the same functional categories are affected at the transcriptomic and proteomic levels during priming and post-priming germination. However, priming and final germination of primed seeds trigger distinct specific pathways since only a minority of genes and proteins are involved in all phases while a vast majority is involved in only one single phase. Green: up-regulated genes and proteins with increased abundance; red: down-regulated genes and proteins with decreased abundance. The full names of depicted proteins and genes can be found in Tables 1 and 2, respectively.

Lespinay et al. [54] demonstrated that inhibitors of transcription (actynomicin-D) and inhibitor of translation (cycloheximide) both drastically reduced osmopriming efficiency in *Trifolium repens* and *Poa pratensis*. Transcriptional profiling of imbibing *Brassica napus* seeds allowed the identification of genes associated with imbibition and expressed in nongerminating seeds in which germination was inhibited by PEG [55].

At the beginning of the soaking period and at the end of the drying phase, the seed water content was as low as 5%. The soaking treatment allowed seed imbibitions up to 50%, which should be enough to re-initiate translation of stored mRNAs which subsequently decreased. Soeda et al. [10] showed that genes related to translational machinery, including ribosomal proteins and translation initiation factors, are activated during osmopriming. Tatematsu et al. [56] demonstrated that the ribosomal proteins are among the first to be transcribed during *Arabidopsis* seeds imbibition. Our results revealed that in *Brassica napus*, genes encoding structural constituent of ribosomes were not up-regulated during PEG soaking but that translation initiation factors, such as EIF4A, or proteins involved in post-translational processing (HSP22, HSP17.9) may accumulate during this phase to sustain further protein synthesis.

4.2. Soaking, drying and final germination of primed seeds trigger distinct specific pathways

A major aspect of the present study is that only a minority of genes and proteins were involved in all phases of osmopriming

while a vast majority was involved in only one single phase. This suggests that each phase (soaking, drying and final germination) relies on a specific array of physiological and biochemical processes.

Soaking induces a progressive re-hydration of dry seeds, but subsequent drying did not appear as a simple reversion of the physiological processes triggered by soaking: only 18 genes among 74 appeared to be regulated in an opposite way in soaking and drying. These genes were involved mainly in metabolism, transport, stress response and development. Differences between the two phases were less obvious when proteins rather than transcripts were considered since 50% of recorded proteins were involved in both phases: it is however puzzling that those proteins were regulated in a similar rather than in an opposite way during the two phases. The major exception concerns stress proteins (i.e. low molecular weight heat-shock protein, monodehydroascorbate reductase, putative dehydroascorbate reductase, glutathione reductase, LEA protein group 3 and late embryogenesis-abundant protein) which behaved in a contrasted way: surprisingly, those proteins were down-regulated during seed drying and up-regulated during PEG soaking and final germination, thus suggesting that the stress might be associated to the rehydration process and not to the dehydration phase. This, however, is not valid for all stress proteins, such as LEA. LEAs accumulate in seeds during late stages of embryo development and are associated with acquisition of desiccation tolerance in orthodox species [57]. The depletion of LEA proteins during priming and germination has been documented at transcriptomic and proteomic levels [10,17,55,58]. Some LEA genes (*LEA4-1*; *LEA4-5*) were down-regulated during soaking, while Olvera-Carrillo et al.

[59] demonstrated that the corresponding proteins were gradually reduced during *Arabidopsis* seed germination. Also *AtEM1* and *AtEM6*, genes encoding proteins, which belong into LEA group 1, were down-regulated during post-priming germination, likewise ABA inducible transcription factors *ABI5* during PEG soaking. As stated by Bensmihen et al. [60] the expression of *AtEM1* and *AtEM6* can be regulated by *ABI5*. Taken together, these results may suggest that osmoprime treatment releases germination arrest and speeds up germination of primed seeds. On the opposite, LEA3 proteins accumulated during PEG soaking. Soaking in a PEG solution with a low osmotic potential should in fact not be regarded only as a rehydration phase: water uptake may be sufficient to reinitiate physiological activity from a previous quiescent stage but a water content of 50% remained low enough to represent a water stress situation, especially when it is maintained during several days. The re-establishment of desiccation tolerance by PEG incubation in germinated seeds has been reported in *Medicago truncatula* [61] and *Arabidopsis thaliana* [62] and it was related to a programmed reversion from a metabolic active to a quiescent state similar to prior to germination. Moreover, Buitink et al. [63] demonstrated that several LEAs were up-regulated in response to prolonged PEG incubation. With these in mind, our results may suggest that osmoprime treatment could promote accumulation of SMP and LEAs akin to the maturation program. This could help to maintain desiccation tolerance in seeds up to radicle protrusion.

It is noteworthy that the number of genes up- or down-regulated during the whole priming process (409) was by far higher than the number of genes modified during soaking (74) and drying (40). This leads us to hypothesize that general priming is a more complex process than the simple sum of soaking and drying phase. It has to be mentioned that for these individual phases, comparison was established between P_{d} and P_{nd} on the one hand, and between P_{nd} and P_d on the other hand and that a factor of 3 fold change was adopted to identify up- and down-regulated genes. Screening of the raw data deposited in NCBI's Gene Expression Omnibus, including only statistically significant differentially expressed genes, confirmed this hypothesis. Genes whose expression was modified less than 3 fold during soaking, and in a similar way but still less than 3 folds during drying did not appear in our lists as regulated during the individual phases but appeared as modified during general osmoprime. This allows us to emphasize the above-mentioned statement that specific genes are regulated during specific phases of priming. Common genes may be regulated in a similar additional way, even if modification is not detected when soaking or drying are individually considered. Once again, what is valid for transcripts is not valid for proteins since only 26 proteins were identified as specific to general osmoprime.

Final germination occurred faster in primed than in unprimed seeds. However, less than 20% of recorded proteins were common between general osmoprime and post-priming germination. This suggests that only few proteins associated with a faster germination of primed seeds were already present at the end of priming and that most of them are produced during post-priming germination as a direct consequence of preliminary priming. In contrast, around 40% of the proteins which abundance was modified during PEG soaking were also modified in response to post-priming germination, suggesting once again that proteosynthesis may occur during hydration process, as suggested by Galland et al. [49], but that these proteins may be removed by the drying step. During germination, water uptake occurred faster in primed than in unprimed seeds. Hence, we could not exclude that proteins accumulated in P_{7h} will appear at a later stage in unprimed seeds when they will reach similar water content than the former. Selective proteolysis may also occur faster in germinating primed seeds, thus explaining that 25% of proteins associated with post-priming germination were down-regulated comparatively to germinating unprimed seeds.

4.3. What are the key genes/proteins explaining the improvement of germination by osmoprime?

The efficiency of germination process relies on the kinetics of imbibition and water uptake, reserve mobilization, cell elongation and division, and management of secondary stress such as oxidative stress occurring as a result of an abrupt rehydration of dry tissues [1].

Water uptake is undoubtedly facilitated in primed seeds. Our results showing up-regulation of *TIP2* in response to osmoprime followed by further increase during post-priming germination support Chen and Arora's [20] thesis that observed up-regulation of *TIPs* might be associated with enhanced germination potential of primed seeds. Although vacuolar aquaporin may contribute to generate turgor pressure required for cell elongation, no modification were noticed in the present study for water channels included in the plasma membrane. Before water loading in the symplasm, imbibitions first occur through apoplasm and Galhaut et al. [64] recently described modification occurring in the seed coat and internal structures of primed clover seeds that may directly hasten water uptake during final germination.

Reserve mobilization is a complex aspect of priming and post-priming process. In the present study, benefit resulting from osmoprime was not clearly related to improvement in reserve mobilization. Indeed, transcripts and proteins for several SSP were up-regulated during general osmoprime and remained unaffected during germination of primed seeds comparatively to unprimed ones, therefore suggesting that these proteins are not used as a source of amino acids in a more efficient way after priming. These proteins serve indeed as a nitrogen and amino acid source for seed germination and seedling establishment [49]. Only cruciferin subunit 4 was found to decrease during drying. SSPs comprise proteins accumulated mainly during seed maturation and they were highly abundant at later stages of *Brassica napus* development [22]. Since early mobilization of lipid and proteins reserves was noted during priming and germination *sensu stricto* [58,65] the observed CRU1 in rape seeds may be subunit released from storage proteins.

Oleosin S4-3 was down accumulated in osmoprime seeds. Oleosins contribute to the maintenance of individuality and integrity of oil bodies during seed maturation and drying or following seed rehydration [66]. Oleosins may also facilitate access to TAG during germination either by the simple change in surface-to-volume ratio or by a more active mechanism such as forming lipase docking sites [67]. Germination rates are positively associated with oleosin contents. Thus oleosine disappearance could hardly explain priming-induced improvement of germination in *Brassica napus*.

Glucosinolate degradation during seeds germination could represent a significant source of sulfur and nitrogen emphasizing the importance of the sulfur metabolism during seed germination. Myrosinases, such as PYK10 contribute to produce metabolites from glucosinolate. The level of myrosinase and myrosinase-binding proteins decreased during both PEG soaking and drying and did not increase during post-priming germination.

In contrast, numerous genes and proteins involved in cell wall modification, cytoskeletal organization and cell division were up-regulated during general osmoprime and post-priming germination (XTH, ELP, β -tubulin, kinesin). Tubulin subunits (α -and β -chains) were found to accumulate during *Arabidopsis* seeds priming [58]. Cytoskeletal elements involved in cytoplasmic organization are essential to restart cellular metabolism during priming and subsequently during germination. Organization of cytoskeletal is also a key component of mitotic spindle and cell division as well as it controls the orientation of deposition of cellulose in cell wall. Moreover, it is responsible for unidirectional transport of various cargos, including membranous organelles, protein complexes, and mRNAs [47].

Our data also demonstrated that priming has a positive impact on proteins involved in the management of oxidative stress during post-priming germination (CAT2, PER21, POX12, GR and DHAR). Baily et al. [12] reported that activity of detoxifying enzymes such as CAT and SOD increased in response to priming. It was also demonstrated that osmoprimeing of *Echinacea purpurea* seeds enhanced total anti-oxidative activity and activities of POX, CAT, SOD and APX [68]. Furthermore, Li et al. [55] demonstrated that expression of peroxiredoxin gene was up-regulated in seeds imbibed in PEG suggesting an osmotic stress response. Results obtained by Chen and Arora [13] and Sun et al. [69] showed that priming facilitates the metabolism of non-enzymatic antioxidants, demonstrated by the up accumulation of AsA and GSH and enhanced activity of DHAR and GR catalyzing the regeneration of these two compounds. Our results however demonstrate that DHAR and GR may be regulated during distinct phases of the priming process (general priming for GR and post-priming germination for DHAR) suggesting that components of Halliwell–Asada cycle should be differentially induced at specific phases of the whole process.

To conclude, separate transcriptomic and proteomic experiments have provided an overview of accumulation patterns of mRNAs or proteins during both priming and post-priming germination. To authors' knowledge, this study is the first one that provides a comprehensive dataset that documents changes of transcriptome and proteome and the relationship between mRNA and protein patterns at the crucial phases of priming procedure (soaking, drying), whole priming process and subsequent germination. We observed that mostly the same functional categories were affected at the transcriptomic and proteomic levels during osmoprimeing and post-primeing germination. However, each phase of osmoprimeing and post-primeing germination seems to trigger distinct specific array of physiological and biochemical processes. Progress toward germination in primed seeds was associated with an increase in protein synthesis potential, post-translational processing capacity and targeted proteolysis. Higher expression of genes and proteins involved in water transport, cell wall modification, cytoskeletal organization and cell division was also linked to the advanced germination of primed seeds. Moreover, improved germination of primed seeds was associated with higher abundance of proteins involved in the management of oxidative stress during post-primeing germination. The differences between transcriptome and proteome data set (the match between genes and proteins was limited to only 12 gene-protein pairs) emphasize the importance of the regulation of mRNA translation and post-translational processes during priming and post-primeing germination. Posttranscriptional processing such as transcript de/stabilization, translation, posttranslational modifications and protein degradation influence the quality and quantity of expressed proteins and thus affect the correspondence between transcript and protein levels.

Further research should include the dynamics of seeds proteome turnover and the selectivity of protein synthesis during the time course of priming and subsequent germination to distinguish between stored and *de novo* synthesized proteins. Moreover, coordinated studies at genes expression regulation and proteins synthesis should focus on groups of common regulators such as transcription factors, small interfering RNA or hormones responsible for final priming effect.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.plantsci.2014.11.008>.

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