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Wheat seed proteins regulated by imbibition independent of dormancy status

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Seed dormancy is an important trait in wheat (*Trticum aestivum* L.) and it can be released by germination-stimulating treatments such as after-ripening. Previously, we identified proteins specifically associated with after-ripening mediated developmental switches of wheat seeds from the state of dormancy to germination. Here, we report seed proteins that exhibit imbibition induced co-regulation in both dormant and after-ripened seeds of wheat, suggesting that the expression of these specific proteins/protein isoforms is not associated with the maintenance or release of seed dormancy in wheat.

Dormancy is an adaptive trait that inhibits the germination of viable seeds under optimal conditions.[1] It is desirable for seeds of crop species to have a certain degree of dormancy to prevent sprouting before harvest. However, most of the commercial cultivars of cereal crops such as wheat have been selected against dormancy to achieve quick and uniform germination, and thereby good stand establishment. Elucidating the molecular mechanisms underlying the maintenance and release of seed dormancy forms the foundation to facilitate wheat breeding for improved tolerance against field sprouting. It has been shown that 38% of mRNAs represented on the GeneChip Wheat Genome Array[2] and a number of proteins are stored in mature dormant seeds of wheat.[3] After-ripening, a period of dry storage that releases seeds from a dormant state, is associated with the accumulation of reactive oxygen species (ROS).[4] The resulting ROS ultimately causes non-enzymatic oxidative reactions, including selective oxidation of seed stored mRNAs and proteins, which are proposed to induce change in seed water status.[5] While oxidation of seed stored transcripts leads to a decrease in the synthesis of the corresponding proteins,[6] the change in seed water status might activate post-transcriptional changes that contribute to seed dormancy decay and subsequent germination. Previously, we showed dry after-ripening induced repression of specific seed stored proteins identified as the antioxidative superoxide dismutase, α-amylase/trypsin inhibitor, a protease inhibitor cystatin, and 14-3-3 proteins that control abscisic acid (ABA) action in seeds.[3] Consistently, dormancy release has been shown to be associated with seed ROS level[4] and sensitivity to ABA.[7] Moreover, after-ripening induces alterations in enzymatic post-transcriptional processes that occur during imbibition of wheat seeds, including the repression of granule-bound starch synthase (GBSS), protease inhibitor serpins, eukaryotic translation initiation factors (eIF) 5A1 and eIF6.[3] These results suggest that the oxidized mRNAs and the specific proteins differentially regulated by after-ripening form an integral part of the mechanisms regulating seed dormancy release and germination in wheat.

In this study, we used a dormant wheat cultivar, AC Domain. In order to maintain dormancy, freshly harvested seed samples were stored at −80 °C while a corresponding seed sample was stored at room temperature and ambient relative humidity for 10 months to release seeds from dormancy. Seed germination was assayed using Petri-plates and layers of Whatman #1 paper under darkness at room temperature as previously described.[2] Seeds of both dormant and after-ripened samples imbibed for 24 h were subjected to protein extraction and subsequent proteomic analysis as described before.[3] Our comparative analysis between dormant and after-ripened wheat seeds revealed specific proteins that exhibit co-downregulation in both dormant and after-ripened seeds during imbibition, including isoforms of soluble starch synthase (SS) and GBSS, β-amylase, heat shock protein (HSP) 70, elongation factor 1 β (eF-1β), vicilin-like protein, an isoform of serpin 3, and glyoxalase (GLX) II (**Table 1**; **Fig. S1**). Given that 95% of after-ripened but none of the dormant seeds of cultivar AC Domain germinated by 24 h after imbibition,[2] our results imply that the regulation of these specific proteins/isoforms by imbibition is not associated with the acquisition of germination potential in the dormant wheat seeds.

The transcription of genes encoding SSs and activity of the corresponding enzymes are high during grain filling.[8,9] Thus, the downregulation of specific isoforms of SSs and GBSSs (**Table 1**) may indicate imbibition mediated repression of starch synthesis in wheat seeds. Consistently, the expression of 6 probesets corresponding to *SS* was suppressed in imbibing seeds of both dormant and after-ripened samples (**Table S1**). The abundance of

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Table 1. Proteins downregulated in both after-ripened and dormant seeds in response to imbibition*

Spot[a]	Identification	ID number[b]	MS/MS MASCOT peptides (score)[c]	AR-0/AR-24[d]	P-value[e]	D-0/D-24	*P*-value
c1	starch synthase I	AAD54661.1	R.EDVPLIGFIGR.L (101) R.SIVFVTGEAAPYAK.S (99)	9.76	0.035	6.67	0.020
c2	GBSSI	AAB26860.1	K.SSFDFIDGYDKPVEGR.K (77) R.KVPLVAFIGR.L (43)	P/N	NA	P/N	NA
c3	β-amylase	BAA04815	R.EGLNVACENALPR.Y (88) K.VPSHAAELTAGYYNLHDR.D (71)	1.99	0.007	2.40	0.015
c4	β amylase	AAP806.1	R.FFLAWYSNNLIK.H (82) K.AAAAMVGHPEWEFPR.D + Oxidation (M) (77)	2.48	0.036	4.17	0.013
c5	β-amylase	BAA04815.1	K.VPSHAAEITAGYYNLHDR.D (88) K.AAAAMVGHPEWEFPR.D + Oxidation (M) (76)	2.54	0.037	3.25	0.008
c6	HSP70	AAB99745.1	K.NQVAMNPTNTVFDAK.R + Oxidation (M) (1) K.EQVFSTYSDNQPGVLIQVYEGER.A (93)	2.08	0.046	2.20	0.041
c7	putative eF-1β	CAB902.1	R.SYISGYQASK.D (89) K.SSVLLDVKPWDDETDMVK.L + Oxidation (M) (80)	3.83	0.002	4.32	0.004
c8	eF-1β	BAA02436.1	M.AVTFSDLHTADGLK.A (92) R.SVQMEGLTWGASK.L + Oxidation (M) (87)	3.	0.006	3.92	0.004
c9	putative eF-1β	CAB902.1	R.SYISGYQASK.D (89) K.SSVLLDVKPWDDETDMVK.L + Oxidation (M) (76)	3.35	0.012	6.16	0.013
c10	vicilin-like	CD925597	R.VKEGDVFVVPR.F (63) K.EIVALALGQK.N (55)	P/N	NA	P/N	NA
c11	serpin 3	ACN59485.1	K.AAEVTTQVNSWVEK.V (115) K.ISFGIEASDLLK.C (97)	2.21	0.034	2.74	0.032
c12	glyoxalase II-like	CL1Contig12535	R.VDLPEIQAK.F (74) K.AAAAVDPVEPEK.V (70)	P/N	NA	P/N	NA

*Mass spectrometry analyses of the peptide samples were performed as described before[3] and all experiments were performed with 3 independent biological replicates; [a]Protein spot names correspond to the 2D gels in Fig. S1; [b]GenBank IDs of the matching proteins. The ID names starting with "CL" are from the local wheat EST database; [c]Amino acid sequences of the top 2 peptides matching the MS/MS spectra. Ions score is -10*Log (*P*), where *P* is the probability that the observed match is a random event. Individual ions scores > 50 indicate identity or extensive homology ($P < 0.05$); [d]Normalized protein spot volumes in dry seeds divided by spot volumes in the corresponding imbibed seeds; [e]Significant differences between samples was determined using Student *t*-test at *P*-value of < 0.05; GBSSI = granule-bound starch synthase I; HSP70 = heat shock protein 70; eF-1β = elongation factor 1 β; p = protein spot present in the 2-dimensional gel; n = protein spot absent in the 2-dimensional gel; NA = not available.

starch degrading enzyme β-amylase also showed downregulation during imbibition irrespective of seed dormancy status (**Table 1**). Germination of cereal seeds involves the breakdown of storage starch by hydrolytic enzymes; the repression of β-amylase during imbibition might reflect the tight regulation starch catabolism in imbibing wheat seeds. Alternatively, our data may suggest the minimal role of β-amylase in degrading native starch granule,[10] as this enzyme is subjected to redox regulation.[11] Ten probesets of β-amylase were also downregulated by imbibition in both seed samples (**Table S1**). It is apparent from our data that one of the seed desiccation related proteins; HSP70 is downregulated by imbibition in both seed samples (**Table 1**). The HSPs play important roles in cellular processes by acting as molecular chaperones in stabilizing enzyme structure and activity during the later phases of seed maturation.[12] Therefore, the decline in HSP70 abundance in imbibing dormant and after-ripened seeds may suggest a loss in desiccation tolerance. Consistent with the protein data, 2 *HSP70* probesets in dormant and a probeset in after-ripened seeds showed downregulation during imbibition (**Table S1**). It has been reported previously that late seed maturation programs are re-induced during imbibition with a potential role of enhancing seed transition from quiescent to metabolically active state.[13] Thus, it is likely that such functionality in wheat is catalyzed by other seed maturation proteins such as other isoforms of HSPs whose abundance remained constant during wheat seed imbibition.

Selective translation of specific seed stored mRNAs is an important mechanism underlying the regulation of seed dormancy.[15] Our analysis showed repression of eF-1β, a protein comprising translation machinery, in response to imbibition irrespective of seed dormancy status (**Table 1**), suggesting that imbibition induces repression of the de novo synthesis of specific wheat seed proteins. Although the types of mRNAs translated by these factors are unknown, it is unlikely that the corresponding proteins are required for acquisition of germination potential in dormant wheat seeds. Contrary to the protein data, similar expression of probesets representing *eF-1*β was evident between the dormant and after-ripened seeds (**Table S1**). Among the proteins downregulated by imbibition in both samples are vicilin (one of seed storage proteins)-like protein and an isoform of

serpin (**Table 1**). A decrease in the abundance of a vicilin-like protein, and an isoform of serpin 3 that appear to be involved in irreversible inhibition of endogenous and exogenous proteases,[16] might reflect imbibition induced proteolytic degradation of seed storage proteins. Consistently, most of the serpin 3 probesets were downregulated by imbibition in both seed samples (**Table S1**). Our data are suggestive of the requirement of other associated essential molecular events, in addition to those involved in proteolysis, for the seed to break its dormancy and germinate. Another protein downregulated by imbibition in both seeds is GLXII (**Table 1**). Plants detoxify methylglyoxal (MG), a toxic byproduct of glycolysis, through the glyoxalase pathway that involves GLXI and GLXII. The GLXI enzyme converts MG to *S*-D lactoylglutathione by using glutathione (GSH) as a cofactor; and GLXII converts *S*-D lactoylglutathione to D-lactate and GSH.[17] Thus, the repression of GLXII by imbibition may indicate that decreased regeneration of GSH partly contributes to the accumulation of ROS, which acts as a signal to induce germination.[4] Most of the probesets representing *GLXII* also showed imbibition induced downregulation in both seed samples (**Table S1**). As both the non-germinable dormant and germinable after-ripened seeds exhibit comparable downregulation of GLXII during their imbibition, it is, however, likely that this mechanism does not play regulatory role in the modulation of seed ROS level. In summary, the findings of this study contribute to further our understanding of the molecular features regulating seed dormancy in wheat.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplementary Material

Supplementary material may be found here:
https://www.landesbioscience.com/journals/psb/article/26601/

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