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Comparative proteomic identification of embryo proteins associated with hydropriming induced rapid-germination of maize seeds

Fangping Gong, Xiaolin Wu, Wei Wang*

Synergetic Innovation Center of Henan Grain Crops, College of Life Sciences, Henan Agricultural University, Zhengzhou, China

***Corresponding author: wangwei@henau.edu.cn**

Abstract

Maize (*Zea mays*) is one of the most important crops and widely planted in the world. In the arid and semi-arid maize production regions, hydropriming is a simple technique to improve seed germination and seedling establishment. However, the mechanism underlying hydropriming is largely unclear at the molecular level. This study aimed to analyze the priming-induced changes in maize embryo proteome and to identify priming-associated proteins. Mature maize seeds of Zhengdan 958, the most sown cultivar in China currently, were used for hydropriming. Hydropriming was performed by soaking seeds in water for 12 h (25℃) and air-drying them to original moisture contents. Primed and unprimed seeds were subjected to germination test and comparative proteomic analysis. The results showed that hydropriming significantly improved seed germination and overwhelming embryo proteins were unchanged in abundance after priming. At least 8 protein spots changed greatly, of which 5 increased and 3 decreased in the primed seeds. These proteins were identified as peroxiredoxin-5, 1-Cys peroxiredoxin, embryonic protein DC-8, cupin, globulin-1 and late embryogenesis abundant protein. DC-8 and globulin-1 may be used for the molecular markers of priming effect and seed vigor. Our results would contribute to the understanding of hydropriming mechanism at the proteome level.

Keywords: Proteome profiling; maize seed priming; two dimensional electrophoresis; mass spectrometry; hydropriming-induced embryo proteins.

Abbreviations: 2-DE_two-dimensional electrophoresis; CBB_Coomassie brilliant blue; DTT_dithiothreitol; IEF_isoelectric focusing; LEA_late embryogenesis abundant; MALDI-TOF_matrix-assisted laser desorption/ionization time of flight; Mr_molecular weight; Prx_peroxiredoxin.

Introduction

Maize (*Zea mays* L.) is one of the most important crops and widely planted in various regions of the world. In recent years, abiotic stress caused by global climate change together with biotic stresses, negatively affects crop growth and productivity throughout the world, reducing crop yields by more than 50% (Swindell et al., 2007). Seed priming in crop (including maize) has become a common practice to obtain optimal seed performance and ultimate yield notably under adverse germination conditions and in the arid and semi-arid regions (e.g., India, Pakistan, Zimbabwe, and northwest China) (Harris, 1996; Mabhaudhi and Modi, 2011; Lu, et al., 2012; Jin, et al., 2012). Seed priming is defined as pre-sowing treatments in water or in an osmotic solution that allows seeds to imbibe but prevents the emergence of radicle (Bradford and Bewley, 2002). It can accelerate seed germination, improve seedling uniformity and increase yields in various crops (Adebisi et al., 2011; Bhardwaj et al., 2012; Nawaz et al., 2013). Seed priming techniques are classified as 1iquid priming (e.g., hyrdopriming), solid matrix priming, bio-priming, membrane priming, etc., based on the matrixs used. Among them, hydropriming is simple and low-cost and extremely welcomed by farmers in the developing countries (Mabhaudhi and Modi, 2011). The physiological effects of seed priming have been extensively studied in a wide range of crops. These studies have showed that the activity of protective enzymes (e.g., peroxidase, catalase and superoxide dismutase) and the contents of compatible solutes (e.g., malondialdehyde, proline and soluble sugar) are important indicators during priming and germination process (Ruan et al., 2003; Sun et al., 2010; Wattanakulpakin et al., 2012). However, the performance of primed seeds rests on subsequent germination assays, which only provide retrospective indication of the priming conditions (Gallardo et al., 2001). So, molecular markers for seed priming are required to develop and used to monitor priming effect and seed vigor of commercial seed lots. As a novel tool for protein characterization and function analysis, proteomics become widely applicable in seed research (Gallardo et al., 2001; Job et al., 2005; Rajjou et al., 2006, 2008). A proteomic analysis of Arabidopsis seed has identified three priming-associated polypeptides, which may serve as protein markers for seed vigor and priming effect (Gallardo et al., 2001). Seeds develop differently in dicots and monocots, especially the changes induced by priming treatment. So, the results obtained from Arabidopsis may be not applicable in monocot crops (e.g. maize). Up to date, there still lacks of the proteomic analysis of protein profiles of seed priming in maize. This study aimed to analyze the priming-induced changes in maize embryo proteome and to identify priming-associated proteins in maize seeds. A widely planted maize hybrid (cv. Zhengdan 958) in China was used as experimental material. Comparative proteomic analysis was used to differentially expressed proteins between the hydroprimed and unprimed seeds. The possible roles of these proteins in maize seed priming were discussed.

Table 1. Effect of hydropriming on the germination of maize seeds.

Treatment	Germination vigor (%)	Germination rate (%)	Germination index
Hydropriming	73±9.59a	91.5±5.51a	29.43±2.65a
Control	32.8±12.96b	70.5±7.00b	11.36±2.33b

Values represent means ± SD, and the different letters indicate significant difference D (P = 0.05).

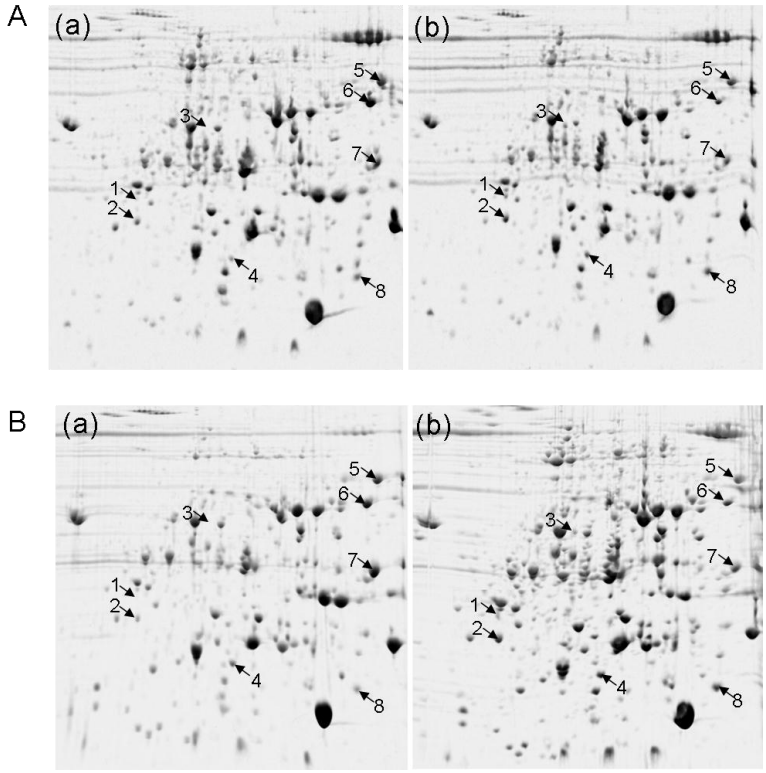

Fig 1. 2-DE comparison of protein profiles between primed seeds and unprimed seeds of maize cultivar Zhengdan 958. Proteins (750μg) were separated by IEF with 11 cm IPG dry strips (pH 4–7) and then by SDS–PAGE (15% gel). More than 650 protein spots were detected in CBB-stained 2-DE gel of each embryo sample. Differentially expressed proteins are indicated by arrows. Panel A and B represent two independent 2-DE gels. (a), unprimed embryos; (b), primed embryos. In our study, discrete protein spots in each gel were detected upon 2-DE with good reproducibility. The landmark protein spots (approximately 48), were constantly present throughout the gel, indicating good reproducible 2-DE analyses.

Results

Compared with the control, the fresh weight of the primed seeds grew by 35.6%, the germination vigor by 41.8% and the final germination rate by 21.0%. Germination index of hydroprimed seeds was also higher than unprimed seeds (Table 1). So, maize seed germination was significantly improved by hydropriming. Two dimensional electrophoresis (2-DE) was carried out to detect differentially expressed embryo proteins between hydroprimed and unprimed seeds (Fig. 1). More than 650 protein spots (10 kDa to 60 kDa) were visualized by Coomassie brilliant blue (CBB)-stained in a typical 2-DE gel (protein loading 750 μg). Spot-to-spot comparison using PDQUEST SOFTWARE indicated that the abundance of approximately 98% spots was almost the same between hydroprimed and unprimed embryos in all 3 biological replicates (Supplementary material, Fig. 1). However, 8 spots were markedly changed in abundance between hydroprimed and unprimed embryos (arrows, Fig. 1). These 8 protein spots were identified via MALDI-TOF analysis. After searching the NCBInr databases for proteins using mass data, 7 were matched to known maize proteins, only 1 (spot 5) unknown. Spot 5 was manually matched to a hypothetical protein (K7VM99) in UniProtKB (http://www.uniprot.org/). Using the sequence of spot 5 as a query, BLAST search of NCBI data base showed spot 5 shares 70% similarity with LEA protein. Additionally, it shares several same conserved domains with LEA_4 protein super family, which is expressed at late embryogenesis in seeds under dehydration stresses. Thus, spot 5 should be a LEA_4 like protein. Among the 8 changed spots, 5 spots (spot 1-4 and 8) were more abundant and the other 3 spots (spot 5-7) were less abundant in the primed seeds (Fig. 1C, Table 2). In particular, the increase of peroxiredoxin-5 (Prx-5, spot 1) and cupin protein (spot 3) was obvious, both of which were hardly detected in the unprimed seeds. Moreover, Prx-5 (spot 2) increased by approximately 3 folds in abundance, while embryonic protein DC-8 (spot 6) decreased by 6 folds in abundance in the primed embryos.

Discussion

Hydropriming indeed induced the subtle change in protein profile of maize embryos

Seed priming is a common technique used to improve seed performance, especially under stress conditions (Harris et al., 2002; Li et al., 2011; Wattanakulpakin et al., 2012). Our results here also indicated that after hydropriming maize seeds (cv. Zhengdan 958) germinated faster than the control. It was expected that hydropriming effect would reflect in the

proteome level of maize embryo. Comparative proteomic analysis identified 8 protein spots which markedly changed in abundance between the primed and unprimed seeds. However, there was a contrary report (Sathish et al., 2012) that no difference was observed in the protein profiles between the primed and unprimed maize seeds. This discrepancy obviously resulted from the less powerful SDS-PAGE rather 2-DE used in protein profiling in the mentioned-above study.In this study, the change in protein profile induced by hydropriming was subtle in maize embryo proteome. Between the hydroprimed and unprimed maize seeds, only 8 embryo protein spots were identified to exist in a substantial different abundance, while the abundances of approximately 98% spots were almost the same. This was reasonable, which may result from two main causes. First, after priming in water for 12 h, maize seed moisture content increased by 35.6%, less than the lowest moisture content (39.8%) for maize seed germination (Ye and Dai, 1994). Thus, during the 12 h priming process, metabolic activities (such as protein synthesis and degradation) are quite low. Second, we used the relatively insensitive Coomassie brilliant blue (CBB) staining to visualize proteins in 2-DE gels; thereby the changes of low-abundance proteins may not be detected. Similarly, only three hydropriming-associated proteins (i.e., β-2 tubulin and two β-subunits of 12S-cruciferin) were identified in Arabidopsis via 2-DE-based proteomic analysis (Gallardo et al., 2001).

Proteins whose abundance increase in primed maize seeds

Two isoforms (spot 1 and 2) of Prx-5 increased highly in abundance in the primed maize seeds in this study. Prx-5 belongs to atypical 2-Cys Prxs that are a ubiquitous family of antioxidant enzymes. Prxs can reduce H_2O_2, alkyl hydroperoxides and hydroxyl radicals (Baier and Dietz, 2003) and protect lipids, enzymes, and DNA against radical attack. It is well recognized that these antioxidant/scavenging enzymes protect the plant cell from oxidative damage (Mittler, 2002; Campo et al., 2004). Hydropriming initiates an oxidative stress, generating reactive oxygen species (Schopfer et al., 2001; Fu et al., 2011). Therefore, the accumulation of Prx-5 in primed seeds may cope with oxidative stress during hydropriming. Spot 3 was the degraded product of cupin protein, estimated on its smaller size (38.2 kDa) tan the theoretical size (56.8 kDa). A recent report (Tnani et al., 2012) also found the discordance between the theoretical and experimental sizes of cupin in imbibed maize seeds, resulting from cupin degradation during early germination. Cupin proteins belong to a superfamily of functionally diverse proteins including enzymes, transcription factors, seed storage proteins, auxin binding proteins and stress-related proteins etc, and are involved in seed growth and development (Dunwell et al., 2004). Cupin proteins such as native germin and germin-like proteins protected plant cells from the oxidative stress during seed germination (Pandey et al., 2006; Fu et al., 2011). In Arabidopsis, cupin-domain protein (AtPirin1) along with G protein α-subunit plays a defined role in regulating seed germination and early seedling development (Lapik and Kaufman, 2003). In this study, the degradation of cupin protein in primed maize seeds suggested it involving in seed germination. Similar to cupin protein (spot 3), spot 8 was the degraded product of globulin-1. In sugar beet seeds (Job et al., 1997) and Arabidopsis seeds (Gallardo et al., 2001), globulin degradation resulted in the increase of its degraded products during priming. As the most widely distributed storage proteins in seeds (Kriz, 1989; Dunwell et al., 2004), globulins provide energy during germination for many metabolic pathways and signal transduction processes (Tnani et al., 2012). Our results here indicated that globulin-1 was early mobilized in primed maize seeds. Embryonic protein DC-8 belongs to the class of abscisic acid-regulated LEA proteins (Franz et al., 1989; Hatzopoulos et al., 1990). It is expressed as early as the globular and heart embryo stages (Borkird et al., 1988) and in mature seeds (including embryos and endosperm) (Franz et al., 1989). Recently, we found that DC-8 proteins (spot 4 and 6) are heat-stable (Wang et al., 2012). However, the molecular function of DC-8 remains unclear, and there lacks of the functional annotation of DC-8 in UniProtKB. A recent study implicated the role of DC-8 in the protection of embryo cellular structures during drought stress and thus in the establishment of tolerance to desiccation (Sghaier-Hammami et al., 2010). Therefore, the increased abundance of DC-8 (spot 4, gi|226507683) observed here suggested that DC-8 may play a role (protective or regulative) in the process of priming.

Proteins whose abundance decrease in primed maize seeds

Different from spot 4, another embryonic protein DC-8 (spot 6, gi|226497424) was found to exist in greatly decreased abundance in the primed seeds in this study. DC-8 (gi|226497424) in the scutellum of germinating maize seeds is oil body-associated protein, reflecting organelle interaction between endoplasmic reticulum and oil body (Tnani et al., 2011). And, its level decreased during the first steps of imbibition of maize seeds (Tnani et al., 2012). Several ABA-regulated genes are involved in root elongation and branching (De Smet et al., 2006), so ABA-inducible DC-8 protein may involve in the initiation of seminal and shoot-borne roots (Muthreich et al., 2010; Takáč et al., 2011). 1-Cys Prx (spot 7) was present in low abundance in the primed seeds. Its accumulation was different with the increase of Prx-5. Similar to this situation, Prx-1 and Prx-2 in *Vibrio vulnificus* were found to differentially accumulate (increase or decrease) in response to H_2O_2 level (Bang et al., 2012). The antioxidant activities of 1-Cys Prx have been demonstrated in barley (Stacy et al., 1996), rice (Lee et al., 2000) and *Arabidopsis thaliana* (Haslekås et al., 2003). 1-Cys Prx is not involved in seed dormancy, but play a role in the inhibition of seed germination under unfavorable conditions (Haslekås et al., 2003). In addition, LEA_4 like protein (spot 5) was decreased in abundance in the primed seeds here. Currently, there is still no annotation on the molecular function of LEA_4 proteins in UniProtKB. The cause for the decreased abundance of these proteins (i.e., DC-8, Prx-5 and LEA_4 like) here and their possible roles during priming are unclear.

Materials and methods

Seed materials

Mature seeds of maize (*Zea mays* L.) cultivar Zhengdan 958, a widely grown high-yield hybrid in China, were used as the experimental material.

Seed priming

Seeds were disinfected with 75% ethanol for 15 min, rinsed thoroughly to remove ethanol and soaked in water (3: 1, ml: g) for 12 h priming at 25°C. Then, the primed seeds were air-dried to their original moistures (assessed by weight). The primed and unprimed seeds were subjected to germination test and comparative proteomic analysis (Table S1).

Table 2. MALDI-TOF identification of the proteins whose abundance changed between hydroprimed and unprimed seeds.

Spot	Relative abundance ratio unprimed/primed	Protein name	NCBI accession	Mascot score	Matched peptide sequences	Coverage (%)	Molecular function
Protein spots whose abundance increase in primed seeds							
1	-/100	Peroxiredoxin-5 (Prx-5)	gi|226505300	201	KFLADGSGAYTKA, KALDLELDLTDKGLGVRS, KGVDEILLISVNDPFVMKA (2) KVILFGVPGAFTPTCSNQHVPGFITQAEQLKA	45	Oxidoreductase activity
2	26.7/100	Peroxiredoxin-5 (Prx-5)	gi|226505300	236	KFLADGSGAYTKA, KALDLELDLTDKG KALDLELDLTDKGLGVRS, KGVDEILLISVNDPFVMKA (2) K.KVILFGVPGAFTPTCSNQHVPGFITQAEQLKA	46	Oxidoreductase activity
3	-/100	Degraded cupin protein	gi|226509468	311	RGLFLLHRV, KVVESEGGQVRV, RGQPWPPASFACRE KVGYIHKDELVERK, REGLMHIGFITMEPKT (2) KTLFVPQYLDSSITLFVQRG, KMGDVLHIDAGSTFYMVNPGKG RTGGPIVYYTADAEPESGAAEEERG	22	Nutrient reservoir activity
4	49.8/100	Embryonic protein DC-8	gi|226507683	319	KAADELHASRQ, RGGGGILGSLQEGKA, KAGAAESEEDVMLRV, KAGAAESEEDVMLRVKA (2) RTGNNKAGAAESEEDVMLRV, KAADQMTGQAFNDVGTMGEEGTGVPR.R (3) RVKAADQMTGQAFNDVGTMGEEGTGVPRR (2)	48	Unknown
8	43.3/100	Degraded globulin-1	gi|195658011	391	REEEEERE, REEEQGGGGGQKA, RGHGREEEEERE KVFLAGTNSALQKM, RVIGAQKDAVFLRG, KDAVFLRGPQSHRV, KLLAFGADEEQQVDRV REEEEEREEEQGGGGGQKA,REGSVIVIPAGHPTALVAGEDKN KLLAFGADEEQQVDRVIGAQKD, RIREGSVIVIPAGHPTALVAGEDKN	19	Nutrient reservoir activity
Protein spots whose abundance decrease in primed seeds							
5	100/60.7	LEA_4 like protein	gi|413955864	632	RSAADEIARS, RRAVEELARA, KAGEYTDASRE RQKAGEYTDASRE, RAAERVDQCVEKC, KAASVYFEEKDRL, KLTLGQGQHVDVRL KCVEGCAGSSCVHRQ, RGAAGGGGILESVQEGARS, KEKLTLGQGQHVDVRL RADRGAAGGGGILESVQEGARS, RETASTHDTDREQGQGLLGALGNVTGAIKE	35	Unknown
6	100/13.3	Embryonic protein DC-8	gi|226497424	191	KEKAGEGYERV, KHHADVDEEETARK, KDAAWETVEAAKEKA, KHHADVDEEETARKA	10	Unknown
7	100/45.3	1-Cys peroxiredoxin (1-Cys Prx)	gi|162460575	436	RNMDEVLRA (2), KVTFPILADPARD, KLSFLYPATTGRN, KLLGISCDDVESHRQ KMFPQGFETADLPSKK, RQLNMVDPDEKDAAGRS (2) KMFPQGFETADLPSKKG (2), KDVEAYGGKQQQQQATTTKV KLLGISCDDVESHRQWTKD, MPGLTIGDTVPNLELDSTHGKI MPGLTIGDTVPNLELDSTHGKIRI, KVATPANWKPGECAVIAPGVSDEEARK (2)	61	Peroxidase and peroxiredoxin activity

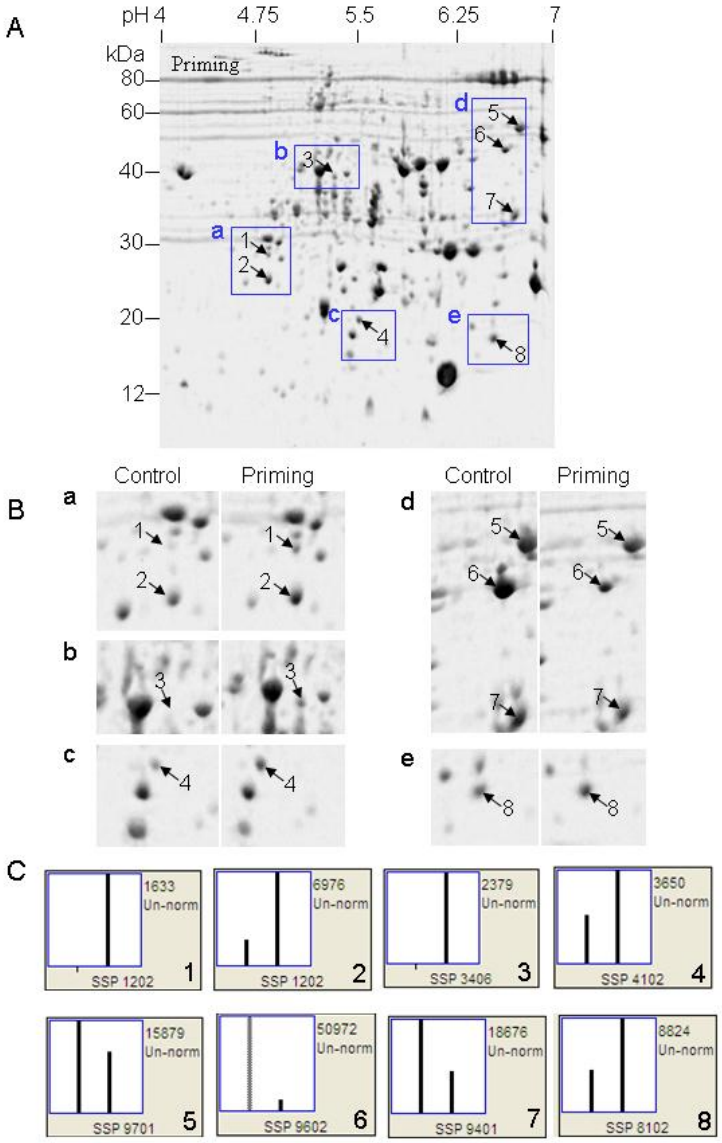

Fig 2. 2-DE comparison of protein profiles between the primed seeds and the control. Proteins (750 μg) were separated by IEF with 11 cm IPG dry strips (pH 4–7) and then by SDS–PAGE (15% gel). Gels were visualized with colloidal CBB G. A, a representative 2-DE gel of the embryo proteins from primed Zhengdan 958 seeds from three replicates. B, the magnified regions, indicating the spots which markedly changed in abundance between the primed seeds and the control. C, relative abundance of protein spots; left, unprimed seeds; right, primed seeds. Spot number was indicated in blade in each frame.

Germination test

Germination test was carried out in a growth chamber (25°C, in darkness) on four replicates (50 seeds each). Seeds were soaked in water for 12 h, and then sown on moistened filter papers for germination. A seed was regarded as germination when its radicle protruded through seed coat. The number of germinating seeds was counted every day. At 7 d, the germination vigor, germination rate and germination index were calculated (Sun et al., 2010).

Protein extraction

Maize embryos were used for protein extraction and proteomic analysis. The primed and unprimed embryos (25 each) were homogenized in a mortar in cold acetone plus 5 mM dithiothreitol (DTT), respectively. The homogenate was centrifuged at 15,000 g for 10 min (4°C). The resultant tissue pellet was rinsed twice with cold acetone plus 5 mM DTT and centrifuged as above. The final powered tissue was air-dried and used as for protein extraction. The powered embryo tissue (0.04 g) was homogenized in a mortar and pestle in 5 ml of the buffer containing 50 mM Tris-HCl, pH 6.8, 0.1％SDS, 10 mM DTT. The extract was transferred into microtubes and heated at 70°C for 15 min to facilitate protein extraction. After cool, the extract was centrifuged at 12,000 g for 5 min and the resultant supernatant was precipitated with 5 volumes of 10% trichloroacetic acid /acetone on ice for 30 min. Protein pellets were recovered by centrifugation at 15,000 g for 5 min (4°C), washed with cold acetone twice, air-dried and dissolved in 2-DE rehydration buffer (2 M thiourea, 7 M urea, 4% CHAPS, 2% IPG buffer, 20 mM DTT). Protein extract was clarified by centrifugation prior to 2-DE. Protein was quantified by Bradford protein assay (Bradford MM, 1976) with bovine

serum albumin as a standard.

Two-dimensional electrophoresis (2-DE)

Isoelectric focusing (IEF) of proteins was performed using 11-cm linear pH 4-7 strips. Rehydration was performed at 20°C overnight (Tai et al., 2007). The voltage settings of IEF with the Ettan III system (GE Healthcare, USA) were 250 V for 1 h, increasing voltage to 1,000 V in 4 h, finally increasing to 8,000 V in 4 h and holding for 16 h (20°C). Focused strips were equilibrated in Buffer I (0.1 M Tris–HCl, pH 8.8, 4% SDS, 6 M urea, 30% glycerol, 0.1 M DTT) and then in Buffer II (its composition was the same as Buffer I, but with 0.25 M iodoacetamide replacement of DTT) for 15 min, respectively. SDS-PAGE was run in 15% gel (20×15×0.1 cm), with 0.2% SDS in the gel and the running buffer.

Gel staining, image, and statistical analysis

Gels were stained with 0.1% (w/v) CBB G250 and destained in 10% (v/v) acetic acid. Digital images of the gels were processed using PDQUEST software (version 7.0, Bio-Rad). Quantitative analyses were carried out after normalizing the spot volumes in all gels, in order to compensate for abundance related variations. The selection of differentially expressed protein spots for MS/MS analysis was based on fold change >2.0 in abundance, with a consistent change in the replicate gels of the three biological replicates. All data were analysed by SAS v8.0. Analysis of variance (ANOVA) was used to compare treatment effects and the significance differences of the means using least significance difference test at 5% level of probability.

Mass spectrometry and protein identification

Differentially expressed protein spots between hydroprimed and unprimed seeds were excised from the gels and digested using trypsin (Wu et al., 2011). Proteins were reduced (10 mM DTT), alkylated (50 mM iodoacetic acid) and then digested with 10 mg/ml trypsin for 16 h at 37°C in 50 mM ammonium bicarbonate. The supernatants were vacuum-dried and dissolved in 10 µL 0.1% trifluoroacetic acid and 0.5 µL added onto a matrix consisting of 0.5 µL of 5 mg/ml 2, 5-dihydroxybenzoic acid in water: acetonitrile (2:1). The digested fragments were analyzed using a MALDI-TOF/TOF analyzer (ultraflex III, Bruker, Germany). MALDI-TOF/TOF spectra were acquired in the positiveion mode and automatically submitted to Mascot 2.2 (http://www.matrixscience.com, Matrix Science) for peptide mass finger printings against the NCBInr 20120922 database (20,543,454 sequences, http://www.ncbi.nlm.nih.gov/). The taxonomy was Viridiplantae (green plants) (1,093,036 sequences). The search parameters were as follows: type of search: MALDI-TOF ion search; enzyme: trypsin; fixed modifications: carbamidomethyl (C); variable modifications: acetyl (protein N-terminal) and oxidation (M); mass values: monoisotopic; protein mass: unrestricted; peptide mass tolerance: ±50 ppm; fragment mass tolerance: ±0.2 Da; max missed cleavages: 1; instrument type: MALDI-TOF. Only significant scores defined by Mascot probability analysis greater than ‘‘identity’’ were considered for assigning protein identity. All of the positive protein identification scores were significant (P<0.05, score>60). Theoretical Mr and Isoelectric point of identified proteins were predicted at http://www.expasy.ch/ tools/pI_tools.html.

Conclusions

Our results showed that hydropriming for 12 h accelerates maize seeds germination and indeed induces changes in the protein profiles of maize embryos. At least 8 protein spots are highly different in abundance between the primed and unprimed seeds, especially embryonic protein DC-8 and globulin-1. These two proteins may be the candidates for the protein markers for priming effect and seed vigor. Further analyses are needed to explore the function of these identified embryo proteins in hydropriming in other maize cultivars or lines. Our results would contribute to the understanding of hydropriming mechanism at the proteome level.

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References

Adebisi MA, Akintoye EA, Kehinde TO, Adekunle MF (2011) Seed priming for improved seedling emergence and vigour of cordia (*Cordia millennii*) seed. Res J Seed Sci. 4:137-147

Baier M, Dietz KJ (2003) The plant 2-Cys peroxiredoxin BAS1 is a nuclear-encoded chloroplast protein: its expressional regulation, phylogenetic origin, and implications for its specific physiological function in plants. Plant J. 12:179-190

Bang YJ, Oh MH, Choi SH (2012) Distinct characteristics of two 2-Cys peroxiredoxins of *Vibrio vulnificus* suggesting differential roles in detoxifying oxidative stress. J Biol Chem. 287:42516-42524

Bhardwaj J, Anand A, Nagarajan S (2012) Biochemical and biophysical changes associated with magnetopriming in germinating cucumber seeds. Plant Physiol Biochem. 57:67-73

Borkird C, Choi J, Jin Z, Franz G, Hatzopoulos P, Chorneau R, Bonas U, Pelegri F, Sung ZR (1988) Developmental regulation of embryonic genes in plants. Proc Natl Acad Sci USA. 85:6399-6403

Bradford KJ, Bewley JD (2002) Seeds: Biology, technology and role in agriculture. In MJ Chrispeels and DE Sadava (eds). Plants, genes and crop biotechnology, 2nd ed. Jones and Bartlett, Boston, pp.210-239

Bradford MM (1976) A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248-254

Campo S, Carrascal M, Coca M, Abian J, San Segundo B (2004) The defense response of germinating maize embryos against fungal infection: a proteomics approach. Proteomics. 4:383-396

De Smet I, Zhang H, Inze D, Beeckman T (2006) A novel role for abscisic acid emerges from underground. Trends Plant Sci. 11:434-439

Dunwell JM, Purvis A, Khuri S (2004) Cupins: the most functionally diverse protein superfamily? Phytochem. 65:7-17

Franz G, Hatzopoulos P, Jones TJ, Krauss M, Sung ZR (1989) Molecular and genetic analysis of an embryonic gene, DC 8, from *Daucus carota* L. Mol Gen Genet. 218:143-151

Fu Z, Jin X, Ding D, Li Y, Fu Z, Tang J (2011) Proteomic analysis of heterosis during maize seed germination. Proteomics. 11:1462-1472

Gallardo K, Job C, Groot SP, Puype M, Demol H, Vandekerckhove J, Job D (2001) Proteomic analysis of

Arabidopsis seed germination and priming. Plant Physiol. 126:835-848

Harris D (1996) The effects of manure, genotype, seed priming, depth and date of sowing on the emergence and early growth of *Sorghum bicolor* (L.) Moench in semi-arid Botswana. Soil Till Res. 40:73-88

Harris D, Rashid A, Hollington PA, Jasi L, Riches C (2002) Prospects of improving maize yields with 'on-farm' seed priming. Pp 180-185 in: N.P. Rajbhandari, J.K. Ransom, K. Adikhari and A.F.E. Palmer (eds) 'Sustainable Maize Production Systems for Nepal': Proceedings of a Maize Symposium, December 3-5, 2001, Kathmandu, Nepal

Haslekås C, Viken MK, Grini PE, Nygaard V, Nordgard SH, Meza TJ, Aalen RB (2003) Seed 1-cysteine peroxiredoxin antioxidants are not involved in dormancy, but contribute to inhibition of germination during stress. Plant Physiol. 133:1148-1157

Hatzopoulos P, Fong F, Sung Z (1990) Abscisic-acid regulation of DC-8, a carrot embryonic gene. Plant Physiol. 94:690-695

Jin YH, Zhou DW, Qin LJ (2012) Sowing date of corn in semiarid region of Jilin Province , Northeast China in adapting to climate change. Chin J Appl Ecol. 23(10): 2795-2802

Job C, Kersulec A, Ravasio L, Chareyre S, Pe´pin R, Job D (1997) The solubilization of the basic subunit of sugarbeet seed 11-S globulin during priming and early germination. Seed Sci Res. 7:225-243

Job CL, Lovigny Y, Belghazi M, Belghazi M, Job D (2005) Patterns of protein oxidation in Arabidopsis seeds and during germination. Plant Physiol. 138:790-802

Kriz AL (1989) Characterization of embryo globulins encoded by the maize Gib genes. Biochem Genet. 27:239-251

Lapik YR, Kaufman LS (2003) The Arabidopsis cupin domain protein AtPirin1 interacts with the G protein alpha-subunit GPA1 and regulates seed germination and early seedling development. Plant Cell. 15:1578-1590

Lee KO, Jang HH, Jung BG, Chi YH, Lee JY, Choi YO, Lee JR, Lim CO, Cho MJ, Lee SY (2000) Rice 1Cys-peroxiredoxin over-expressed in transgenic tobacco does not maintain dormancy but enhances antioxidant activity. FEBS Lett. 486:103-106

Li J, Yin LY, Jongsma MA, Wang CY (2011) Effects of light, hydropriming and abiotic stress on seed germination, and shoot and root growth of pyrethrum (*Tanacetum cinerariifolium*). Ind Crop Prod. 34:1543-1549

Lu YX, Cui XW, Zhang D, Huang Y, Huang JS, Hu WJ, Xiao SL (2012) Effects of drought-resistant seed dressing agents on seedling growth and yield of maize. J South Agric. 43:991-994

Mabhaudhi T, Modi AT (2011) Can hydro-priming improve germination speed, vigour and emergence of maize landraces under water stress? J Agric Sci Technol. B 1:20-28

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410

Muthreich N, Schützenmeister A, Schütz W, Madlung J, Krug K, Nordheim A, Hochholdinger F (2010) Regulation of the maize (*Zea mays* L.) embryo proteome by RTCS which controls seminal root initiation. Eur J Cell Biol. 89:242-249

Nawaz F, Ashraf MY, Ahmad R, Waraich EA (2013) Selenium (Se) seed priming induced growth and biochemical changes in wheat under water deficit conditions. Biol Trace Elem Res. 151:284-293

Pandey S, Chen JG, Jones AM, Assmann, SM (2006) G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and postgermination development. Plant Physiol. 141:243-256

Rajjou L, Belghazi M, Huguet R, Robin C, Moreau A, Job C (2006) Proteomic investigation of the effect of salicylic acid on *Arabidopsis* seed germination and establishment of early defense mechanisms. Plant Physiol. 141:910-923

Rajjou L, Lovigny Y, Groot SPC, Belghazi M, Job C, Job D (2008) Proteome-wide characterization of seed aging in *Arabidopsis*: a comparison between artificial and natural aging protocols. Plant Physiol. 148:620-641

Ruan SL, Xue QZ, Wang QH (2003) Physiological effects of seed priming on salt-tolerance of seedlings in hybrid rice (*Oryza sativa* L.). Sci Agric Sin. 36:463-468

Sathish S, Sundareswaran S, Senthil N, Ganesan KN (2012) Biochemical changes due to seed priming in maize hybrid COH(M) 5. Res J Seed Sci. 5:71-83

Schopfer P, Plachy C, Frahry G (2001) Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide and hydroxyl radicals) and peroxidase in germination radish seeds controlled by light, gibberellins and abscisic acid. Plant Physiol. 125:1591-1602

Sghaier-Hammami B, Jorrín-Novo JV, Gargouri-Bouzid R, Drira N (2010) Abscisic acid and sucrose increase the protein content in date palm somatic embryos, causing changes in 2-DE profile. Phytochemistry. 71:1223-1236

Stacy RAP, Munthe E, Steinum T, Sharma B, Aalen RB (1996) A peroxiredoxin antioxidant is encoded by a dormancy-related gene, *Per1*, expressed during late development in the aleurone and embryo of barley grains. Plant Mol Biol. 31:1205-1216

Sun YY, Sun YJ, Wang MT, Li XY, Guo X, Hu R, Ma J (2010) Effects of seed priming on germination and seedling growth under water stress in rice. Acta Agron Sin. 36:1931-1940

Swindell WR, Huebner M, Weber AP (2007) Transcriptional profiling of Arabidopsis heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. BMC Genomics. 8:125

Tai FJ, Yuan ZL, Yuan ZL, Wu XL, Zhao PF, Hu XL, Wang W (2007) Identification of membrane proteins in maize leaves, altered in expression under drought stress through polyethylene glycol treatment. Plant Omics. 4:250-256

Takáč T, Pechan T, Šamaj J (2011) Differential proteomics of plant development. J Proteomics. 74:577-588

Tnani H, López I, Jouenne T, Vicient CM (2011) Protein composition analysis of oil bodies from maize embryos during germination. J Plant Physiol. 168:510-513

Tnani H, López I, Jouenne T, Vicient CM (2012) Quantitative subproteomic analysis of germinating related changes in the scutellum oil bodies of *Zea mays*. Plant Sci. 191:1-7

Wang W, Wu X, Xiong E, Tai F (2012) Improving gel-based proteome analysis of soluble protein extracts by heat prefractionation. Proteomics. 12:938-943

Wattanakulpakin P, Photchanachai S, Ratanakhanokchai K, Kyu KL, Ritthichai P, Miyagawa S (2012) Hydropriming effects on carbohydrate metabolism, antioxidant enzyme activity and seed vigor of maize (*Zea mays* L.). Afr J Biotechnol. 11:3537-3547

Wu X, Liu H, Wang W, Chen S, Hu X, Li C (2011) Proteomic analysis of seed viability in maize. Acta Physiol Plant. 33:181-191

Ye CF, Dai XD (1994) Seed germination. In: Spermology. Agriculture Press, China