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RESEARCH ARTICLE

Dynamic of Proteomic Responses on Wheat Seed before Germination

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Abstract

As protein metabolism plays an important role in plant adaptation to growth, this study was designed to identify protein expression in wheat seed at 0h,3h,17h,25h before germination. Proteins were extracted and separated by running two-dimensional gel electrophoresis (2-DE), which allowed the identification of some significantly different gel spots. The spots were further verified by matrix-assisted laser desorption/ionization-time of flightmass spectrometry, in which they were confirmed to be wheat seeds proteins. The results showed: (i) On 0h,3h,17h,25h, the number of protein spots which could be identified were 231,233,232,231 respectively. (ii) 4 protein spots were identified using mass spectrometry They were further verified as nucleoside diphosphate kinase Ⅰ(NDKP Ⅰ), 17.5ku heat shock protein (HSP), ATP synthase and LEA protein 27. These data support the assumption that outer environment may have a regulatory role on protein metabolism of wheat seed and make it to regulatory protein expression to adapt the changed environment

INTRODUCTION

In the world's grain output, wheat is ranked first .The germination of seeds is the most critical period of plant in the growth of plant. In the germination under certain condition of temperature, moisture, humidity, germination, the change of protein reflects the first activated gene expression in plant genome that is a genome from close to open [1].In higher level plants, seeds occupyies the important status in the entire life cycle, different type and number of proteins will be synthesised and decomposed in different developmental stages of an organism, the dynamic change of protein form the basis of life characteristic activity the biology at a certain moment,it is an appropriate and direct way to understand the nature of life activity [2]. It causes the metabolic changes in the seed. It isn't clear how a series of complicated physical process coordinate with each other, so differences in protein research at different time on wheat seed germination differences, is of great significance to explore mechanism of wheat seed germination.

Proteomic techniques provides important technical support for studying the dynamic changes of the protein. The core technology includes two-dimensional electrophoresis technology and mass spectrometry [3].The technology and methods currently have been relevant reported only in arabidopsis seeds, barley, rubber and so on a few plants [4].But it have not yet seen on wheat seed germination periods different differences proteomics and mass spectrometry. Based on "*jinmai - 47*" as the test material, to compare the early stage of the wheat seed germination, protein expression differences and mass spectrum identification of proteins, which provide a reference for the regulatory mechanism study of wheat seed germination.

2 MATERIALS AND METHODS

2.1 Plant Culture

Wheat seeds (*Triticum aestivum*, *jinmai-47*)were procured from Shanxi Wheat Research Institute of Agricultural Sciences. Selected healthy seeds were sterilized for 10 min with 0.1% $HgCl_2$ and washed in running tap water for 50

min. This was followed by washing twice with distilled water and leaving the seeds overnight for incubation at 4℃. After 10h the seeds were kept in Petri dishes (diameter 18 cm), 50 seeds per dish, with distilled water and left in the dark for germination.

2.2 Experimental design

The seeds of random imbibitions after 0 h, 3 h, 17 h,25 h, at 25h to take the seeds whose embryo root break the seed coat for 2 ~ 3 mm. In order to determine the fresh weight and dry weight of seeds, in 0 h, 0.5 h, 1 h, 2 h, 3 h, 5 h, 7 h, 10 h and 13 h random take 20 grains, the once every 3 h, until no longer increases germination of seeds. According to samples to fresh weight and dry weight, statistics of germination of seeds at the same time, repeat 3 times.

2.3 Protein Extraction

Urea/thiourea method was used to extract the wheat seed protein, remove the in different periods of samples of wheat powder in centrifuge tube, add urea/thiourea protein extract oscillation 30 s, after centrifugal 15 min, Take that repeat centrifugal supernatant fluid, collecting supernatant, add 3 times the volume of cold acetone, and arrange in the refrigerator overnight - 20 ℃, the next day collecting precipitation (according to specific situation can add once again, the centrifugal ditto), 4 ℃ placed, make the acetone fully evaporate, Stay dry precipitation into 400 ul sample cracking liquid, 4 ℃ refrigerator overnight, the second day take on centrifugal clear liquid, liquid get protein samples, 4 ℃ placed aside.

2.4 Two-dimensional PAGE

Remove has good hydration worthy of sample liquid, add 0.001 g DTT, 40 % Bio - 5 ul Lyte (pH3-10), dissolved in full, take sample solution of homogeneous sample liquid on hydration and protein to 4:1 blending, is the sample liquid. By using linear interpublic precast pH3 ~ 10, 7 cm strip.Active hydration after 12 h under 50 v voltage, after 250 v 0. 5 h, 500 v 0. 5 h, 4000 v3h, finally is stable under 4000 v 20000 vh, 500 v quick time as the case may be.After focusing, glue in balance fluid first Ⅰ（urea 36 g, SDS2g, Tris - HCl (pH8.8), 25 ml, glycerin 20 ml, DTT0.2 g), the balance on shaking table 15 min, then in balance fluid Ⅱ(gIAM instead of 0.25 0.25 gDTT, the rest of the components with balancing Ⅰ) balance in 15 min.Then the second to the SDS polyacrylamide gel electrophoresis, separating gel concentration of 12 % .

2.5 Coomassie brilliant blue staining

To stripping gel and put it instantly into fixed fluid (12%TCA) to get fixed for 2 h, after fully fixed with a steam bath 3 times, each time 5 min, put it into coomassie brilliant blue dye solution (20 % of methanol, 1.8 % phosphoric acid, 8 % ammonium sulfate, 0.08 % of coomassie brilliant blue G250) staining at least 2 h, decoloring liquid (7%, 5% methanol) decolorization in after 1 h with double steaming water decoloring, until protein points clear.

2.6 2-DE gel image analysis

To scan the gel with the scanner (UMAX) to get protein image and then analysis with PDQest software background decrease, spot detection**,** including background matching, obtain spot location coordinates and protein standardization analysis, etc.

3 RESULTS

3.1 Determination of time period of proteome study before wheat seeds' germination.

According to different moisture content of wheat seed in its germination process, there are three periods, respectively the first time of slow water phase, the second period of fast absorbing water, and the third period of imbibition water absorption.As the radicle break through seed coat 2-3 mm,respectively, 13 periods were determined the seed dry weight and wet weight of multiple periods, therelationship between water moisture content of "jinmai - 47" seed (figure 1), at the same time to determine the sampling period for the early stage of the wheat seed germination proteome research.

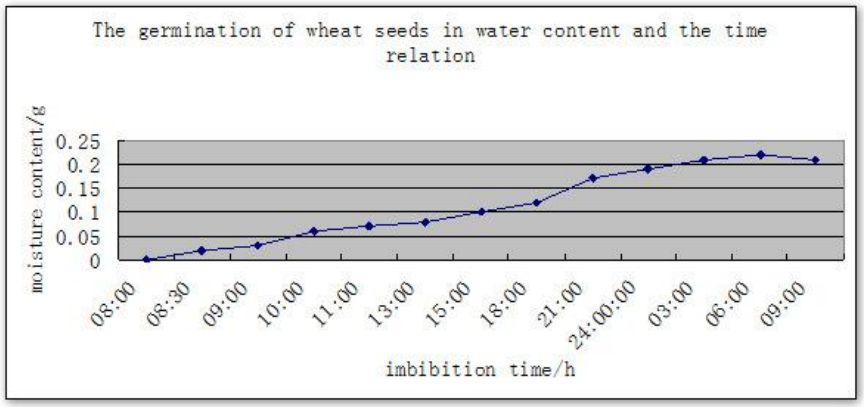
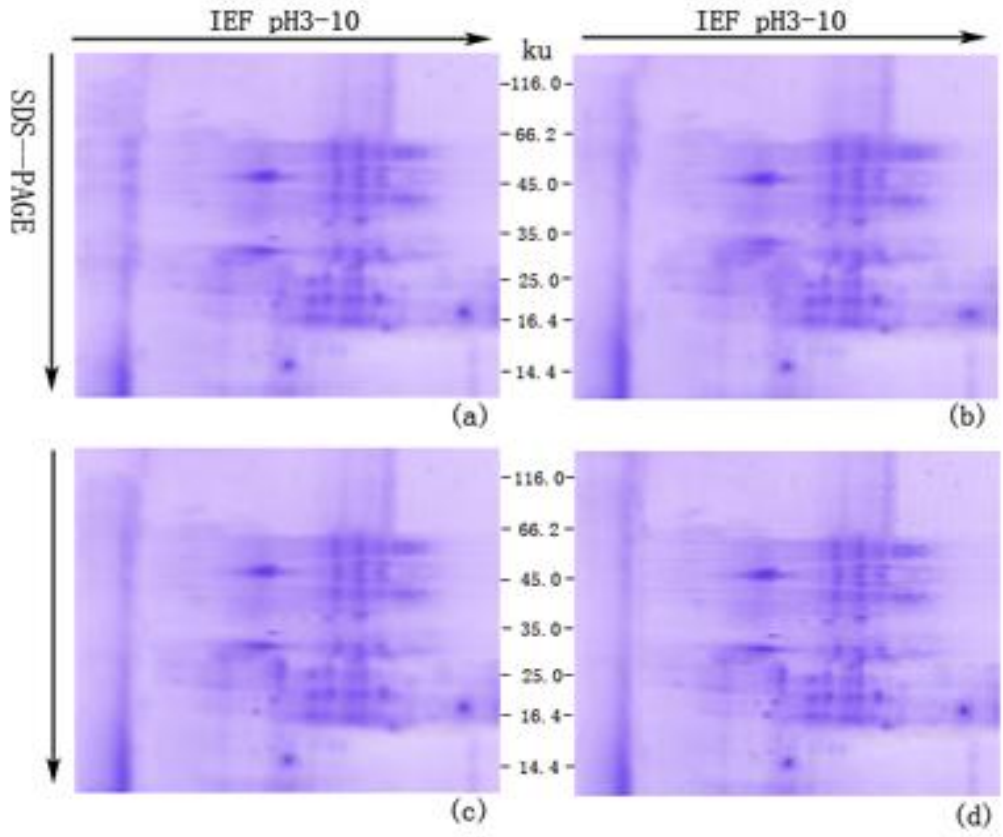


Fig.1 change of moisture content during wheat seed germination

In order to optimize the early stage of the seed germination of protenomics research sampling work, researcher has measured the water content of 13 periods change, the more groups of repetition, the dry and wet weight of statistics. Achieved the relations between the water content and time of the early stage of "jinmai 47"seed germination. You can see in figure 1,the first three phrases of wheat seed germination, respectively (dry seeds) from 0 h to 3 h, slow seed moisture content increases from 0 g to 0.06 g. in the process of 3 h to 17 h, seed moisture content, increases rapidly,from 0.06 g to 0.19 g, when water content was bigger. seed moisture content increases slowly from 17 to 25 h h (radicle break through seed coat 2-3 mm), only 0.19 g to 0.24 g.87% of seed thief occurred in the third stage,which shows that wheat seed budding time is more concentrated.So it will soon be in the early stage of the seed germination process of sampling time set at 0 h, 3 h, 17 h and 25 h is appropriate.

3.2 2-DE **analysis of wheat seed protein of different germination.**

Through strictly consistent repelition of 3 times' dielectrophoresis operation, get 0 h, 3 h, 17h, 25 h high repeatability in the four periods of 2 - DE electrophoresis (figure 2), using PDQuest software analysis, in pH3-10, molecular mass within the scope of 10-120 ku identified 230 proteins points on average, PDQuest software analysis 0 h and 3 h protein correlation coefficient is 86.3%, with 17 h is 84.9%, with 25 h is 83.7%, which showed that protein gradual changes.



"Jinmai - 47" seed germination early different period 2 - DE protein electrophoresis
a: not germination b: 3h c: 17h d: 25h

3.3 MALDI - TOF - MS analysis of differentially expressed proteins and database retrieval.

Above 2-DE gel protein relative volume changes with emergence of wheat seed germination early development process or not and expression quantity increase or decrease. Through the analysis of the PDQuest software, in 17 of proteins which analyses repetability and express the amount of more than 2.5 times for MALDI - TOF - MS analysis, a substrate peak and enzyme degradation fragment peaks automatically for correction, remove keratin peak and pancreatic enzyme cut since the peak, accurate calibration for the substrate peak strength more than 2 times of peak, 17 protein points all obtained peptide mass fingerprint (PMF), figure (3) is spot11 peptides tandem mass spectra,appraisal for four differences proteins is a nucleoside diphosphate kinase Ⅰ, 17.5 ku heat shock protein, ATP synthase, LEA protein 27 respectively.

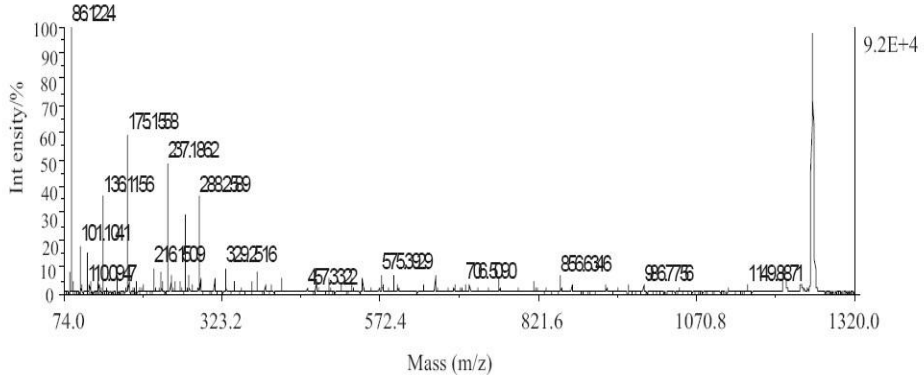


Fig.3 Representative peptide mass

Table1 Differentially-expressed proteins identified by PMF query

Spot No.	Protein name	Theoretical Mr/PI	Experimental Mr/PI	Sequence coverage(%)
3	NDPK Ⅰ	16.3/6.9	16.0/6.9	41
7	HSP 18.5ku	18.5/5.8	18.3/6.2	37
11	ATP Synthase	27.3/6.6	26.5/6.2	21
12	LEA protein12ku	11.9/15.4	12.3/5.8	31

4 DISCUSSION

For plants in their growth period, their dynamic change of protein, protein of different types and the change of their number all together form the basis of cell life in different historical periods. Changes of protein of wheat seed in the germination period reflect the wheat genome expression first activated is genome from close to open and then to the expression. Therefore, understanding the change of protein provides a direct way for understanding the nature of life activity [5, 6].The further development of the proteomics research technology provides a new strategy for the study of dynamic change of wheat seed germination .

Spot3, identified as nucleoside diphosphate kinase Ⅰ (NDPK Ⅰ), is a kind of low molecular weight (16 kd). Acidic protein belongs to a class of NDPKs (NDP kinase; EC2.7.4.6) protein kinase family, accounting for more than 70% of the total active NDPKs. As a kind of nucleotide in the cell, NDPKs ,the main function is to maintain the balance of metabolism of NDP in the nuclei and NTP (NTP + NDPK<=>NDP + NDPK - P; N = G, T, C, U). Phosphorylation and take off the phosphorylation reaction, catalytic high-energy compounds (ATP) to phosphate group transfer to another molecule, thereby activation and inhibition of related protein molecules provides new materials for the synthesis of nucleic acid.Different NDPKs in purines and pyrimidines, RNA and DNA as the substrate.NDPK1 as a kind of "housekeeping enzyme" of nucleic acid metabolism in cells,is a multifunctional protein involved in an important role in cell proliferation, transcription regulation and so on . In wheat seed germination 17 h the protein expression quantity increase, until 25 h maintained a high expression level,which indicates that the active nucleic acid synthesis is conducive to the growth of the seed.

Spot7 identified as 17.5 ku heat shock protein (HSP), its moleculat weight is 16 kd.HSP is one of the most conservative protein in biology, mainly existing in the cytoplasm and membrane systems.The HSP has the very high homology between different species, which shows that has a very important role in life activities.According to the size of the molecular weight of the divided into HMW HSPs and LMW HSPs two kinds big.Many studies have shown that the formation of HSPs, associated with the biological heat resistance,during the heat shock HSP105 can be quickly moved to the core and accumulated in the nucleoli and nuclear transfer, when lifting the heat shock, it can be moved to the cytoplasm, with function of protect body cells from damage, and some small molecular mass HSPmRNA combine with specificity of EMPR-C6 in meiosis.[7].HSP17.5 is detected in the dry seeds, and large numbers of expression in 25 h, may be after breaking the dormancy to protection germination in the process of cell membrane structure system and antioxidant system from harm, guarantee the smooth wheat seed germination.

Spot11 is the ATP Synthase, ATP Synthase (F1F0 compound enzyme) is the key to the energy metabolism enzymes involved in the phosphorylation and photophosphorylation. ATP synthase is widespread in the chloroplasts, mitochondria, and bacteria. Through oxidative phosphorylation and photophosphorylation ATP synthesis are the most frequently occurring in biological energy conversion reaction. Potential energy to synthesize ATP on proton power also can form important proton gradient of the boby of the hydrolysis of ATP.Main function is to synthesize ATP [8], in this study the enzyme express quantity highest germination in 25 h, suggests the radicle break through the seed coat to spend a lot of energy.

Spot12 for 27 ku seed mature protein, LEA protein (late embryonic development is rich in protein) widely exists in higher plants, not limited in the seeds of growth [9].The water deficit , salt stress and osmotic stress etc have existed in the plant tissue.LEA proteins are characterized with hydrophilic and thermal stability.During drought stress, part of LEA protein instead of water molecules, so as to avoid cellular structure collapse, the stability of cell membrane structure [10].27 ku seeds mature protein appeared in 25 h, on the seed germination and seedling growth of normal protection, ensure the normal order of the wheat seed germination.

5. Conclusion

In conclusion, outer environment may have a regulatory role on protein metabolism of wheat seed and make it to regulatory protein expression to adapt the changed environment.

ACKNOWLEDGEMENTS

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