



Proteomic Responses of Seed Embryo in Wheat Germination as Influenced by NaCl Stress

Duan Jiang-Yan, Chen Yan-Yun, Wang Hui & Jia Zhen-Hu

Abstract – High extracellular NaCl can perturb and even kill cells, Many high-NaCl-induced perturbations responses are known. As protein metabolism plays an important role in plant growth, this study was designed to identify NaCl - responsive proteins in wheat seed. Wheat seed (*Jinmai-47*) was treated with NaCl at 0mol/L, 0.1mol/L, 0.2mol/L. Seed embryo proteins were extracted and separated by two-dimensional gel electrophoresis (2-DE) in wheat germination, which allowed the identification of some significantly different gel spots. The spots were further verified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry, in which they were confirmed to be wheat seeds embryo proteins. The results showed: (1) NaCl at 0.1mol/L, 21 protein spots which could be identified were up-regulated and 32 protein spots were down-regulated. 3 new proteins were expressed. (2) NaCl at 0.2mol/L, 16 protein spots which could be identified were up-regulated and 46 protein spots were down-regulated. 2 new proteins were expressed. These data support the assumption that NaCl stress may have a regulatory role on protein metabolism of wheat seed and make it to regulatory protein expression to adapt the changed environment.

Keywords – Wheat Embryo, NaCl Stress, Proteomic Responses.

I. INTRODUCTION

Soil salinization affects the agricultural production, the ecological environment is also facing serious challenges. The United Nations Food and Agriculture Organization (FAO) and the UNESCO analyzed the soil of the Earth, shown the world's arable land about 150 million hm^2 , 0.77 million hm^2 by salinization^[1]. China holds nearly 20 million hm^2 salinization and the secondary soil salinization of about 6.7 million hm^2 , accounting for about 14% of the total area of arable land. simultaneously, an area of secondary salinization soil's development at an annual rate of 3%^[2]. In the world of food production, Wheat ranked first. Seed germination is the most critical period of plant growth. Germination, a certain temperature, moisture, humidity, reflects changes in the protein of the plant genome. It is the first activated gene expression of the whole genome, and the process from inactivity to activity^[3]. Seeds are composed of embryo, endosperm, aleurone layer. The endosperm, the aleurone layer cells apoptosis with metabolism procedural and the embryo eventually develop into a complete plant. In recent years there has been a lot of literatures about the seed germination period proteomics research in *Arabidopsis thaliana* seeds as the research object for the study of seed^[4]. Proteomic analysis of the seed, more than 1300 kinds of proteins were isolated and some seed germination of new proteins related^[5]. do further analysis of *Arabidopsis*, Identified 355 independent genes encoding

proteins of 437 points, and located the different metabolic pathways^[6]. On rice seed germination Proteome Research found 63 points of protein down, 69 kinds of protein upregulated (which contains inducible protein 20). These studies are more concentrated in the *Arabidopsis*, rice and other model plants, and in the important food crops but few reports. Studies have shown that: Wheat has similar regulatory pathways and salt factor with model plants, especially has Salt gene^[7]. The research of seed protein content in wheat embryo germination process after NaCl stress, Whether in the production practice or in theory have an important significance.

2-DE technique for the study of dynamic changes of proteins provides important technical support, quantitative analysis can be performed on protein expression of biological cells or tissues and can be used to reveal the changes in protein expression under different conditions. This technique has high-throughput, high sensitivity, high resolution, good reproducibility merits and so on^[8]. Proteomics technologies identified in the seed germination process of protein, mostly downstream components of the process, rather than the upstream component^[9]. Meanwhile in related proteins to identify on the number, species cannot be formed into a detailed kinetic metabolism pathway. The lack of informations of seed germination mechanism to our human beings further understanding is deficient remotely. Therefore, the study about protein content of wheat seed germination stage by identifying changes in NaCl stress, at the protein level the real seed germination, has important implication.

In this paper, "Jinmai -47" for the research material, compared the inhibition during germination of wheat seeds in NaCl solutions in different concentrations. Then determined the NaCl concentration of wheat seed germination's survival threshold and damage threshold. Wheat germination 27h (radicle breakthrough testa 2 ~ 3mm), separated the embryo and endosperm (include vague powder layer), used the embryo to 2-DE electrophoresis, Coomassie blue staining, studied it through the PDQuest software. Help us further understand seed germination under NaCl stress and adaptation mechanisms or injury mechanisms from the temporal and spatial variation of the angle.

II. MATERIALS AND METHODS

2.1 Plant Culture

Wheat seeds (*Triticum aestivum*, *jinmai-47*) are procured from Shanxi Wheat Research Institute of Agricultural Sciences. Select dryness consistent, full, seed coat color normal seed 540. (By 0.1% HgCl_2 solution immersion disinfection 2min after rinsing with pure water

five times), placed in 25ml water, covered with a double qualitative filter glass petri dishes, each dish covered with lid 60. Germinate in the dark at 25 °C treatment. Within 10 petri dishes were added 0mol / L, 0.05mol / L, 0.1mol / L, 0.15mol / L, 0.2mol / L, 0.3mol / L, 0.4mol / L NaCl solution L concentration, real-time dish weighing supplement evaporation of water, to ensure that the same concentration of each treatment group. To break through the seed coat radicle 27h 2 ~ 3mm standard seed. In 7h, 10h, 13h, 14h, 17h, 20h, 23h, 27h 20 randomly selected, while the germination rate statistics for each group, each group were repeated three times. According to NaCl concentration on wheat seed germination, three NaCl concentrations were chosen.

2.2 Protein Extraction

According to the damage threshold, during the wheat survival threshold period by NaCl treatment, then proceed to extraction of wheat seed embryo protein with urea / thiourea. The embryos samples were removed in liquid nitrogen and ground to a powder, then added urea / thiourea protein extracts centrifuged 30s in the centrifuge tube. After centrifugation 15min, the supernatant was repeated centrifugation, the supernatant was collected. Adding 3 volumes of cold acetone, placed in the refrigerator of -20 °C overnight, precipitate was collected the next day (as the case may be together again, centrifuged as above), put at 4 °C. Sufficiently volatile acetone, the precipitate was dried thoroughly and dissolved in 400ul sample lysate, 4 °C refrigerator overnight, the supernatant was centrifuged to obtain the protein sample solution the next day, 4 °C put aside.

2.3 Two-dimensional PAGE

Fetch the sample solution with hydration, adding 0.001gDTT, 5ul of 40% Bio-Lyte (pH3-10), fully dissolved, take homogeneous sample rehydration fluid and protein sample solution in 4: 1 mix, that is, the sample solution. Using the PH3 ~ 10 and the length 7cm linear IPG strips which are prefabricated. Active Hydration 12h at 50V voltage, after 250V0.5h, 500V0.5h, 4000V3h, finally stabilizing at 4000V 20000Vh, keep 500V fast, time depends. After completing the focus, Firstly the strip in fluid balance I (urea 36g, SDS2g, Tris-HCl (pH8.8) 25ml, glycerin 20ml, DTT0.2g), balance 15min in Shaker. Secondly in the balanced solution II (0.25gIAM instead 0.2gDTT, with the balance remaining components I) equilibrated 15min. Followed by a second to SDS-polyacrylamide gel electrophoresis separation gel concentration of 12%.

2.4 Coomassie brilliant blue staining

Peeling gel immediately placed in fixative (12% TCA) fixed 2h, After sufficiently fixed with double distilled water 3 times, each time 5min, out into the Coomassie blue dye solution (20% methanol, 1.8% phosphoric acid, 8% ammonium sulfate, 0.08% of Coomassie Brilliant Blue G250) staining at least 2h, then in destaining solution (7% after 5% methanol) 1h. Change decolorization with double distilled water until the spots clear up.

2.5 2-DE gel image analysis

The gel was scanned by scanner (UMAX), protein images obtained, then analyzed by PDQest software including background subtraction, blob detection, matching, protein expression analysis.

III. RESULTS

3.1 The concentration of NaCl stress germination of wheat seeds to determine

Figure 1 shows that the decline of wheat germination rate after treatment with different concentrations of NaCl. The higher the concentration, the greater the degree of decline, and the greater the degree of harm to seed. It also suggests that NaCl stress does reduce the ability of wheat seed germination. Between the concentration of 0 mol / L and 0.05 mol / L, the germination rates have few differences. 0.05 mol / L of NaCl on germination of wheat is not obvious, we can put 0.05 mol / L as Salt threshold of the process of wheat seeds germination. Between 0.1 mol / L and 0.15 mol / L, the germination differences are unobvious, but compared with 0 mol / L germination significantly different, indicating that this time the concentration has reached or exceeded the wheat seeds' limits of anti-harm, 0.1 mol / L NaCl solution can be viewed as the damage threshold of wheat seeds; 0.2 mol / L, 0.3 mol / L concentration of these two sharp decline in germination rate, 0.2 mol / L NaCl solution can be seen as a threshold for wheat seed survival; under the 0.4 mol / L concentration the germination rate is very low, we can be considered 0.4 mol / L for lethal concentration. Depending on the concentration of NaCl impact on germination of wheat seeds, selected representative three concentrations: 0 mol / L (control group), 0.1 mol / L (damage threshold), 0.2 mol / L (survival threshold) as the following NaCl concentration of research. This study discuss changes in wheat seeds under NaCl stress protein content during embryo germination, therefore, the range lethal concentration of 0.4 mol / L is not be thought in the current study.

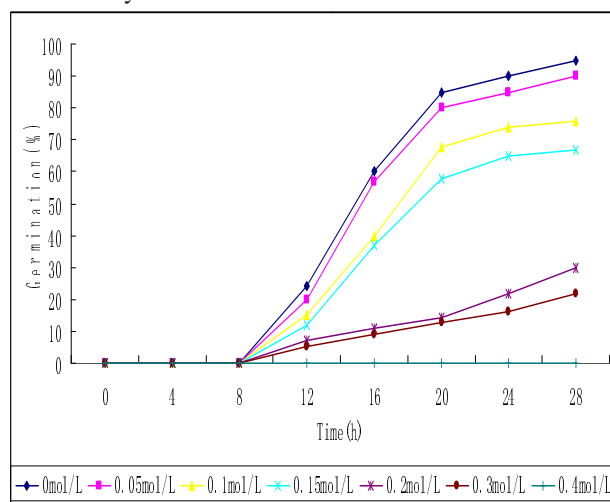
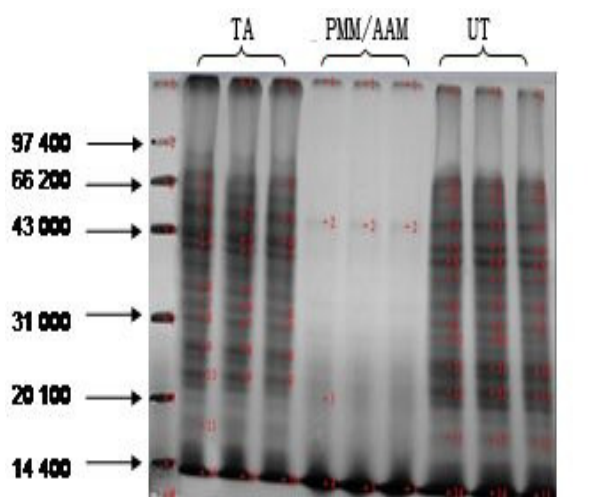


Fig. 1. Influence the process of germination of different concentrations of NaCl concentration on wheat seed germination

3.2 Comparison of protein extracts of wheat seeds

This experiment was used phenol mention - methanol / ammonium acetate precipitation, TCA / acetone precipitation, thiourea and urea extracted protein of wheat seeds, After detection by SDS-PAGE. Its analysed and processed with Jetta gel analysis software, you can get figure3, As can be seen from FIG, Both in the number of protein bands or in terms of depth stained, protein sample obtained whether from the type or quantity by comparing three methods are different. Phenol mention - methanol / ammonium acetate precipitation extraction of the root protein was significantly weaker than the TCA / acetone precipitation and urea thiourea methods. we can also be seen from the figure, In the high molecular weight area, extraction TCA / acetone method is not as urea thiourea method because you can clearly see that the method will be lost parts of protein in this range, In comparison, protein extraction urea thiourea more complete.



TA: TCAacetone PMM/AAM: Phenol mention methanol/Ammonium acetate method UT: Urea thiourea
 Fig.2. SDS-PAGE of protein in wheat seed after NaCl stress. M: Marker;

3.3 Expressed proteins of wheat seeds with different NaCl treatment

Wheat seeds in 0mol / L, 0.1mol L, 0.2mol L concentration of NaCl respectively, wheat germ protein extraction in grin period, Selecting 10cm PH3-10 nonlinear strips to material above by two-dimensional electrophoresis. In order to ensure the credibility of the results, the protein concentration in the sample are three kinds Repeat 3 times. Then the seed embryo 0mol / L NaCl treated as a reference gel (Fig. 3) and the other two pieces of gel are compared by analysis PDQuest8.0, Grayscale and position changes on the 2-DE gel can be understood as the degradation of proteins, the post-synthesis or post-translational modification of these changed points can be used to understand the changes of protein in different concentration of germination wheat seeds. In Seed embryos detected 113 points, There are 32 spots lower abundance under the stress of 0.1mol / L NaCl (Fig. 4), including 7 expression disappeared, 21

protein spots abundance increases which are three newly expressed protein spots included ; In 0.2 mol / L NaCl stress (Fig. 5), 46 protein spots are detected in abundance down, including 13 protein spots disappeared, the abundance of 16 proteins up-regulated, including two newly expressed protein spots.(Fig. 6) In order to make the difference more clearly observe proteins, some protein points are amplified.

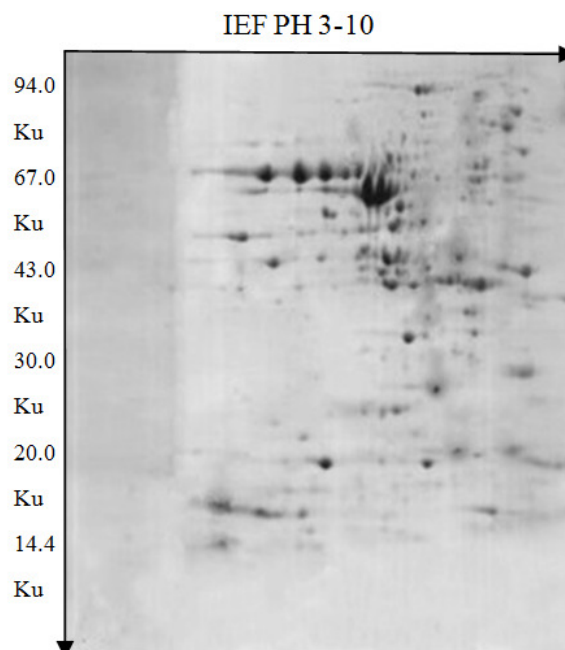


Fig.3. 0mol / L NaCl stress on wheat seeds 2-DE map

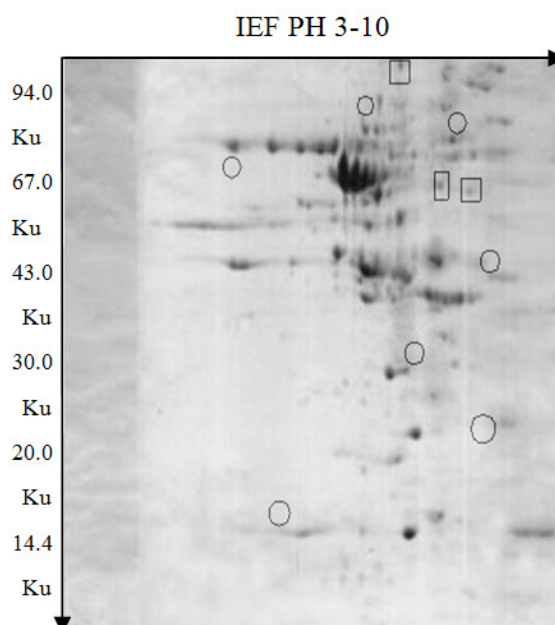


Fig.4. 0.1mol / L NaCl stress on wheat seeds 2-DE map
 (○Expressed protein spots disappear □ Represents a new expression of proteins)

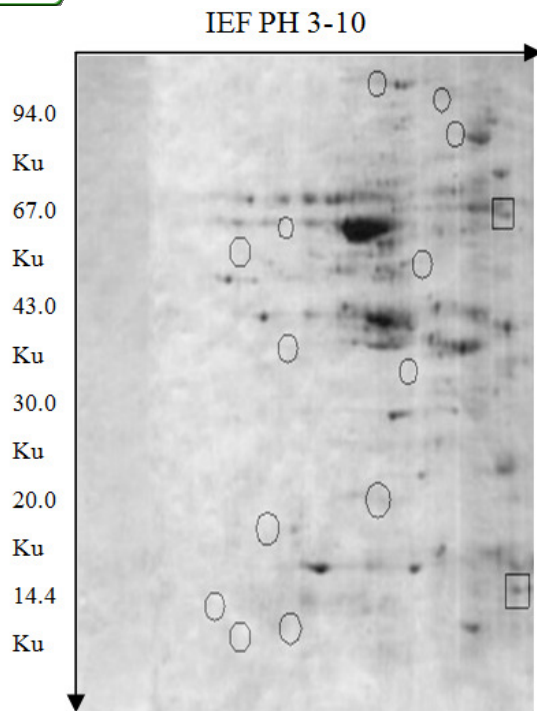


Fig.5. 0.2mol/L NaCl stress on wheat seeds 2-DE map (○Expressed protein spots disappear□Represents a new expression of proteins)

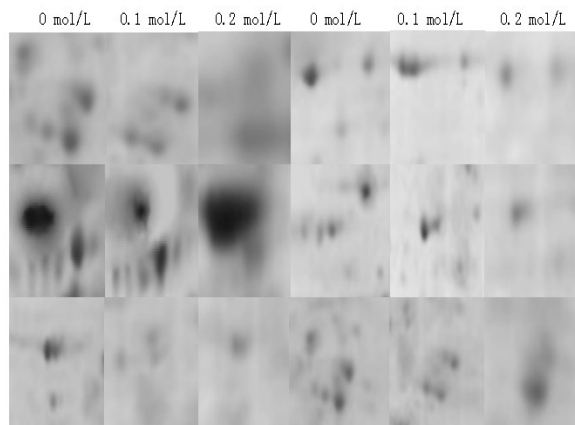


Fig.6. Different concentrations of wheat seeds under salt stress protein portion of the embryo during the amplification of differentially expressed proteins germination(0 mol / L indicates a normal germination of seeds of wheat germ proteins, 0.1 mol / L, 0.2mol / L represents the concentration at this time is the difference between proteins found by the software analysis)

IV. DISCUSSION

4.1 Under salt stress conditions NaCl concentration choice

Plant proteins in the timing and space exist a series of dynamic processes in the process of growth and development, the protein of the same organization in space and time are not the same expression. Wheat seed in germination process, the embryo of the protein in a dynamic process of change. In order to select the exact

impact of the protein under appropriate concentration of salt stress during seed germination. According to this study, as the increases of concentration, the number of seeds ungerminated trend is enhanced. Elect three representative NaCl concentrations of different salt stress, 0 mol / L (control group), 0.1 mol / L (damage threshold), 0.2 mol / L (survival threshold), As the influence of the concentration of protein in wheat seeds under salt stress.

4.2 Analysis of differentially expressed proteins

Throughout the life cycle of plants, seed germination is a heterotrophic process. At this stage of seed germination lacks of absorption of minerals and photosynthesis, nutrients and energy required in the process by the plant seeds own. From wheat seeds treated with different concentrations, clearly found that salt stress delayed germination of wheat seeds. When plants suffer abiotic stress, some protein abundance raised in the germination process, which reflects the plants will induce some new proteins or the original protein content will be significantly increased in order to adapt to Stress. The reducing of expression abundance indicates that in the case of stress prevents the expression of certain proteins or protein degradation during germination. This is consistent with results of previous studies^[10-11]. Under stress of salt stress, ozone and other external factors, enhanced gene expression SAM^[10]. The product of SAM gene is ethylene and polyamines. These substances are considered to be defense system after the plant subjected to stress, resulting in up-regulation of the expression of some proteins. According to the literature, In the seeds of barley, corn, minimize, arabidopsis, the degree of seed LEA protein abundance's reduced related the plant in the cold or drought stress^[11]. The LEA proteins able to protect the germination of active tissue by binding water molecules, and thus as a stabilizing protein. In this study, the protein content in 0.1 mol / L NaCl stress have a significant change compared with the control group, indicating that the concentration impacts protein content and composition of wheat seed during germination. This is consistent with previous studies^[12-13]. In rice, Arabidopsis, PSCS gene transcription levels increased five-fold under salt stress^[12], demonstrating that PSCS gene expression can be induced under salt stress. Sorbitol and trehalose etc are also important soluble substances produced during cell osmotic adjustment and conducive to enhancing the salt tolerance of plants^[13], Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) etc play an important role in clear other harmful substances arising under salt stress. Meanwhile, Expression in transgenic plants were also found in the protection of plant tissue associated protein increases the salt tolerance of plants^[14, 15, 16]. Through this study, different concentrations of salt stress 2-DE analysis, found there is protein content decreased and increased in 0.1 mol / L concentration, and will be some new proteins. The function expression take effect under the enhanced salt stress, we hypothesized that these changes are the plants adjust their physical and chemical reactions, thereby slowing under salt stress conditions caused by damage to the body.

V. CONCLUSION

In the research of the basis on proteins of wheat germ seed protein content under salt stress, needing for further study of protein points changes in the expression of mass spectrometry analysis and identification in future, studying the types and functions of these differentially expressed proteins, and then establishing the interaction between the protein network Atlas. Fully understand the role of individual differences in the expression of proteins in plants and plants own adaptation mechanism of stress conditions able to provide a theoretical basis for wheat under salt stress.

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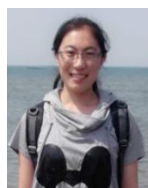
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