Wheat (*Triticum aestivum* L.) root proteome and identification of differentially expressed proteins between hybrid and parents

Sun Qixin, Song Xiao, Ni Zhongfu, Yao Yingyin, Peng Huiru, Zhang Yinhong
Department of Plant Genetics and Breeding, China Agricultural University, Beijing, 100094, China

INTRODUCTION

Roots of higher plants serve many important functions, including anchorage of the plant, uptake of water and nutrients, synthesis of amino acids and hormones, and secretion of organic acids, enzymes and alkaloids. Recently, genome-wide transcriptional analysis of the plant roots [1-3] demonstrated that up to 15000 genes were expressed in root tissue, indicating the complexity of the root transcriptome. In parallel, reference maps of the major soluble proteins of seedling roots in rice, maize and *Medicago truncatula* were also generated, and 1350, 81, 179 protein spots were identified, respectively [4-6]. The proteomic studies of plant roots have contributed to our understanding of root tissue differentiation and development in response to internal growth regulators as well as environmental signals. In wheat, a 2D gel map of 860 root protein spots were generated, but these proteins were not identified [7]. Moreover, to gain a better understanding of the molecular basis of wheat heterosis, we carried out a comparative proteomic analysis in seedling leaves and roots between wheat hybrid and parents.

GENERATION OF THE ROOT REFERENCE PROTEOME MAP OF WHEAT (*Triticum aestivum* L.)

Total protein was isolated from root tissues using Invitrogen's TRIZOL® Reagent according to the manufacturer's instructions. Protein concentration was determined by Bradford assay. IEF of soluble wheat root protein extracts was performed on a linear gradient: pH 4-7. After IEF, proteins were separated according to their molecular weight (Mr) in a second-dimension and stained with silver nitrate. Using Imagemaster 2D Platinum Software (GE healthcare), a total of 450 spots, with Mr varying from 10 to 110 kDa, were reproducibly detected across three replicate gels from the hexaploid wheat Line 3338 (Fig. 1), 282 spots were identified by MS or MS/MS in MASCOT database searching [8].

The 282 identified protein spots were classified into 12 groups by their functional annotation (Fig. 2). The largest group was composed of 70 unknown, hypothetical or putative proteins, followed by metabolism (20.6%), energy (14.5%), transporter (7.8%), cell structure (6.4%), protein destination & storage (5.0%), protein synthesis (4.6%), disease & defense (5.0%), signal transduction (4.3%), secondary metabolism (2.8%), cell growth & division (2.1%), transcription (2.1%). As would be expected, 35.1% (99 of 282) protein spots identified in this experiment were involved in metabolism and energy production/regulation. Proteins grouped under metabolism include those involved in the metabolism of amino acids, nitrogen and sulfur, nucleotides, phosphate, carbohydrate, lipid, fatty acid, isoprenoid, cofactors and proteins related to secondary metabolism. Forty-one identified proteins (14.5%) were associated with energy production, and play roles in glycolysis and gluconeogenesis, the glyoxylate cycle, the Entner-Doudoroff pathway, the pentose phosphate pathway, the TCA cycle, respiration, fermentation, electron transport, oxidation of fatty acids and energy conversion and photosynthesis, respectively. Proteins associated with protein synthesis and protein fate (folding, modification, destination) included ribosomal proteins, translation initiation factors 4A, 5A, 3, 6, translational elongation factors 1, Tu, chaperonin 60, 20S proteasomes and protein disulfide isomerase (PDI). Proteins related to cell structure, cell growth and division included cytoskeletal proteins such as actins, tubulins and cell cycle related proteins such as cyclin A3.1, importin, profilin. The remaining functional groups contained proteins involved in disease & defense, signal transduction, secondary metabolism and transcription.

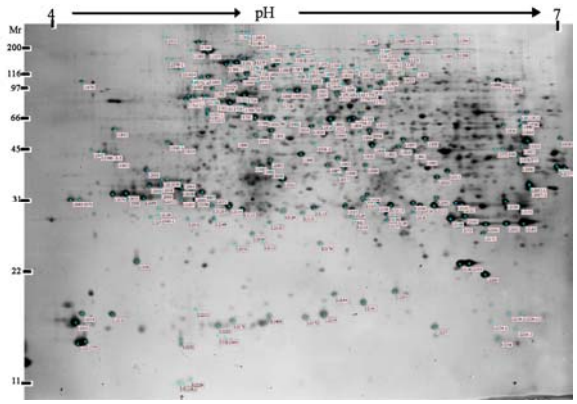

Fig.1. A wheat root proteome reference map from the hexaploid wheat (*Triticum aestivum* L.) Line 3338

The 282 proteins spots identified in this study were derived from 240 different genes or gene families. Further analysis indicated that twenty-eight proteins representing 70 protein isoforms were found in multiple spots, most of which consisted of two to four spots. All the proteins identified as multiple spots differed from each other in their p*Is* and/or *M*r. These isoforms, if

correctly identified, could represent post-translationally modified forms of the same protein.

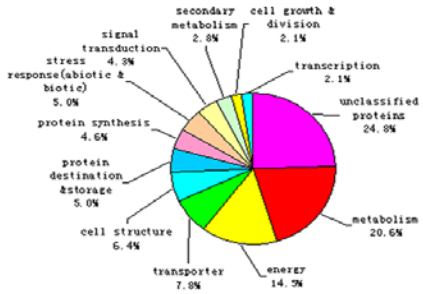

Fig. 2. Functional classification of the 282 identified protein spots of the wheat root proteome.

To further characterize the wheat root proteome map, we compared our result to the previous reference map of wheat leaf where 404 protein spots were visualized by silver staining [9]. Of the 142 identified wheat leaf proteins, proteins in categories of cell structure, growth & division (8.5% in root, 11% in leaf), protein synthesis & protein fate (9.6% in root, 9% in leaf), secondary metabolism (2.8% in root, 4% in leaf) had a similar representation in both wheat leaf and root, whereas proteins involved in metabolism (20.6% in root, 12% in leaf), transport (7.8% in root, 3% in leaf) were over-represented in root, and proteins involved in energy (14.5% in root, 24% in leaf), disease & defense (5.0% in root, 12% in leaf), transcription (2.1% in root, 5% in leaf), signal transduction (4.3% in root, 10% in leaf) were under-represented in root. Root proteins classified in metabolism were largely related to enzymes of amino acid biosynthesis, catabolism and carbohydrate metabolism and most of these enzymes showed great redundancy at the level of identified proteins. This might reflect the importance of these processes in wheat root and the fact that many of these proteins are encoded by multigene families or frequently posttranslational modifications and is encouraging for future proteomics analysis of the dissection of root functions.

DETERMINATION OF PROTEOME EXPRESSION PROFILE IN ROOTS OF WHEAT HYBRIDS AND ITS PARENTS

Attempts have been also made to characterize differentially expressed genes in roots between a hybrid and its parents, which revealed that differentially expressed genes represent diverse functional categories, such as metabolism, cell growth and maintenance, signal transduction, response to stress, transcription regulation and others [10, 11]. These results indicated that the hybridization between two parental lines can cause expression changes of different genes , which might be responsible for the observed heterosis. However, changes at the level of mRNA do not necessarily indicate changes on the protein level and/or in the hybrid phenotype, thus studies are needed to investigate differential proteomes between hybrids and its parents, and determine their functional relations to heterosis.

One highly heterotic interspecific hybrid 3338/2463 and its female parent Line 3338 (*Triticum aestivum* L, 2n=6x=42, AABBDD) and male parent Line 2463 (*Triticum spelta* L. 2n=6x=42, AABBDD) were used for this study. Heterosis analysis indicated that middle parent heterosis (MPH) of wheat hybrid 3338/2463 was significant for root fresh weight (RFW) ($P<0.05$), root dry weight (RDW) ($P<0.01$) and best parent heterosis (BPH) was also significant for RDW ($P<0.01$). Two-dimensional gel was employed to characterize the proteome expression profiles in roots of wheat hybrid 3338/2463 and its parents. The resolved protein spots on all the three replicate gels of hybrid and parents were analyzed by using Imagemaster 2D Platinum Software (GE Healthcare, USA). In total, 45 (10%) of 450 protein spots showed an accumulation difference of at least factor 1.5 between hybrid and parents and the differences of 38 protein spots were also statistically significant by Student's *t*-test at $P<5\%$. Furthermore, however, seven protein spots which showed presence/absence differences between the two parents or displayed significant position changes between hybrid and its parents were not included in the statistical analysis. When comparing the patterns of differentially expressed protein spots between hybrid and its parents, it was found that both quantitative and qualitative differences could be observed (Fig. 3). The quantitative differences can be clustered into four categories: (i) up-regulated in hybrid (URH), expression in hybrid is higher than in both female and male parents; (ii) down-regulated in hybrid (DRH), expression in hybrid is lower than in two parents; (iii) high-dominant in hybrid (HDH), expression in hybrid is equal to the highly expressed parent; and (iv) low-dominant in hybrid (LDH), expression in hybrid is equal to the lowly expressed parent. Among the 45 differentially expressed protein spots, the number of spots that showed URH, DRH, HDH and LDH expression pattern were 3, 3, 3 and 4, respectively. Interestingly, in this study, the differential expression was observed mostly in qualitative differences (31, 68.89%) and only one category was detected, that is dominant expression of uniparental genes in hybrids (UPF1), expression in hybrid of protein only expressed either paternal or maternal parents (Fig. 3).

These differentially expressed protein spots between wheat hybrid and its parental lines were eluted from representative 2-D gels for identification, and 25 spots were successfully identified. According to criteria used previously, the 25 identified differentially expressed protein spots were classified into seven functional classes, including signal transduction (8 spots, transmembrane receptor, phospholipase C2, protein kinase, calreticulin), metabolism (4 spots, methionine synthase, 3-dehydroquinate synthase, putative acyl-CoA synthetase), energy (2 spots, pyrophosphate-dependent phosphofructokinase, putative Aconitate hydratase), cell

growth & division (2 spots, alpha-tubulin), disease & defense (1 spot, disease resistance protein-like protein MsR1), secondary metabolism (1 spot, putative sesquiterpene cyclase) and seven unclassified proteins. Taken together, our observation at translational level adds circumstantial evidence that expression differences between wheat hybrid and its parents exist not only at mRNA levels but also at protein abundances.

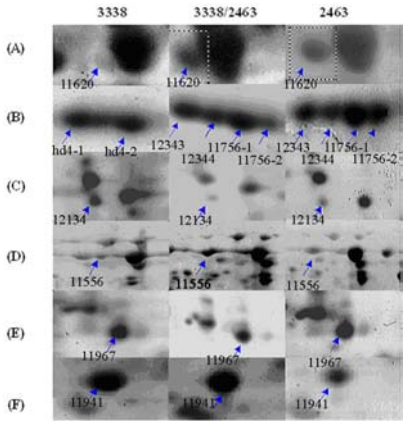

Fig. 3. Differential protein expression patterns between hybrids and its parents in wheat root.

IDENTIFICATION OF DIFFERENTIALLY ACCUMULATED PROTEINS IN SEEDLING LEAVES BETWEEN HYBRID AND PARENTS

By using same wheat hybrid and its parents, The expression patterns of the total proteins were compared in seedling leaves between hybrid and its parent by using two-dimensional gel electrophoresis with two pH ranges (pH 4-7 and pH 6-11)for the first dimension separation. Among ~900 protein spots reproducibly detected, 49 protein spots were identified as being differentially expressed between hybrid and its parental lines ($P < 0.05$) for more than 1.5 folds. When comparing the patterns of differentially expressed protein spots between hybrid and its parents, it was found that both quantitative and qualitative differences could be observed. The quantitative differences can be grouped into four categories: (i) up-regulated in hybrid (URH), expression in hybrid is higher than in both female and male parents; (ii) down-regulated in hybrid (DRH), expression in hybrid is lower than in two parents; (iii) high-dominant in hybrid (HDH), expression in hybrid is equal to the highly expressed parent; and (iv) low-dominant in hybrid (LDH), expression in hybrid is equal to the lowly expressed parent. Among the 49 differentially expressed protein spots, the number of spots that showed URH, DRH, HDH and LDH expression pattern were 2, 5, 11 and 11, respectively. The qualitative differences can be grouped into two categories, that is (i) dominant expression of uniparental proteins in hybrids (UPF_1), expression of protein in hybrid from either paternal or maternal parents, and (ii) dominant expression of uniparental proteins but not in hybrids ($UPnF_1$), expression in either of the parents but not in F_1, which were detected in 15 and 5 protein spots on the comparative proteome map, respectively. Moreover, 30 of the 49 differentially expressed protein spots were identified, which correspond to 34 proteins or protein isoforms. These 34 identified proteins or isoforms were classified into eight functional classes, including energy (9 spots), metabolism (7 spots), signal transduction (3 spots), transposable elements (4 spots), disease and defence (2 spots), cell structure (2 spots), transcription & translation (1 spot) and six unclassified proteins. These results indicated that hybridization between two parental lines can cause expression differences between wheat hybrid and its parents at the level of protein abundance and the proteins differentially accumulated between hybrids and their parents are involved in diverse physiological processes.

ACKNOWLEDGEMENTS

This work was financially supported by National Basic Research Program of China (2007CB109000), National Natural Science Foundation of China (30671297), Program for New Century Excellent Talents in University (No. NCET-05-0131) and 863 Project of China

REFERENCES

1. Fizames, C., Munis, S., Cazettes, C., Nacry, P. et al., Plant Physiol 2004, 134, 67-80.
2. Poroyko, V., Hejlek, L. G., Spollen, W. G., Springer, G. K. et al., Plant Physiol 2005, 138, 1700-1710.
3. Ekman, D. R., Lorenz, W. W., Przybyla, A. E., Wolfe, N. L., Dean, J. F. D., Plant Physiol 2003, 133, 1397-1406.
4. Koller, A., Washburn, M. P., Lange, B. M., Andon, N. L. et al., Proc. Natl. Acad. Sci. USA 2002, 99, 11969-11974.
5. Hochholdinger, F., Woll, K., Guo, L., Schnable, P. S., Proteomics 2005, 5, 4885-4893.
6. Mathesius, U., Keijzers, G., Natera, S. H., Weinman, J. J. et al., Proteomics 2001, 1, 1424-1440.
7. Bahrman, N., Gouy, A., Devienne-Barret, F., Hirel, B. et al., Plant Science 2005, 168, 81-87.
8. Song, Xiao, Zhongfu Ni, YingyinYao, Zhenxing Li, Haiyan Wu, Yinhong Zhang, Qixin Sun, 2007, Proteomics，7, 3538–3557
9. Donnelly, B. E., Madden, R. D., Ayoubi, P., Porter, D. R., Dillwith, J. W., Proteomics 2005, 5, 1624–1633.
10. Yao, Y. Y., Ni, Z. F., Zhang, Y. H., Chen, Y. et al., Plant Molecular Biology 2005, 58, 367–384.
11. Wang, Z. K., Ni, Z. F., Wu, H. L., Nie, X. L., Sun, Q. X., Theor Appl Genet 2006, 113, 1283–1294.