Theor Appl Genet (2009) 118:213–225
DOI 10.1007/s00122-008-0890-4

ORIGINAL PAPER

Identification of differentially expressed proteins between hybrid and parents in wheat (*Triticum aestivum* L.) seedling leaves

Xiao Song · Zhongfu Ni · Yingyin Yao · Yinhong Zhang · Qixin Sun

Received: 20 May 2008 / Accepted: 6 September 2008 / Published online: 25 September 2008

Abstract In spite of commercial use of heterosis in agriculture, the molecular basis of heterosis is poorly understood. To gain a better understanding of the molecular basis of wheat heterosis, we carried out a comparative proteomic analysis in seedling leaves between wheat hybrid and parents. Common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) Line 3338 and spelt wheat (*Triticum spelta* L., $2n = 6x = 42$, AABBDD) Line 2463 were used to produce a heterotic F_1 hybrid. The expression patterns of the total proteins were compared in seedling leaves between hybrid and its parents by using two-dimensional gel electrophoresis with two pH ranges 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. Six possible modes of differential expression were observed, including high- and low-parent dominance, underdominance, and overdominance, uniparent silencing and uniparent dominance. Moreover, 30 of the 49 differentially expressed protein spots were identified, which were involved in metabolism, signal transduction, energy, cell growth and division, disease and defense, secondary metabolism. These results indicated that wheat hybridization can cause protein expression differences between hybrid and its parents; these proteins were involved in diverse physiological process pathways, which might be responsible for the observed heterosis.

X. Song and Z. Ni have equally contributed to this work.

Communicated by M. Kearsey.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-008-0890-4) contains supplementary material, which is available to authorized users.

X. Song · Z. Ni (✉) · Y. Yao · Y. Zhang · Q. Sun (✉)
Key Laboratory of Crop Heterosis and Utilization (MOE), China Agricultural University, Yuanmingyuan Xi Road No. 2, Haidian district, 100193 Beijing, China
e-mail: wheat3392@cau.edu.cn

Q. Sun
e-mail: qxsun@cau.edu.cn; qxsun62@public.bta.net.cn

X. Song · Z. Ni · Y. Yao · Y. Zhang · Q. Sun
State Key Laboratory for Agrobiotechnology, China Agricultural University, 100193 Beijing, China

X. Song · Z. Ni · Y. Yao · Y. Zhang · Q. Sun
Key Laboratory of Crop Genomics and Genetic Improvement (MOA), China Agricultural University, 100193 Beijing, China

X. Song · Z. Ni · Y. Yao · Y. Zhang · Q. Sun
Beijing Key Laboratory of Crop Genetic Improvement, China Agricultural University, 100193 Beijing, China

X. Song · Z. Ni · Y. Yao · Y. Zhang · Q. Sun
National Plant Gene Research Centre (Beijing), 100193 Beijing, China

Abbreviations

ACN	Acetonitrile
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BPH	Best-parent heterosis
CHA	Chemical hybridizing agent
CHAPS	3-[(3-Cholamidopropyl)-dimethylammonio]-1-propane sulfonate
CHCA	A-cyano-4-hydroxycinnamic acid
DDRT	Differential display reverse transcript
DRH	Down-regulated in hybrid
DTT	1,4-Dithio-DL-threitol
FBA	Fructose-1,6-bisphosphate aldolase
GO	Gene ontology
HDH	High-dominant in hybrid

IEF	Isoelectric focusing
LDH	Low-dominant in hybrid
LDW	Leaf dry weight
LFW	Leaf fresh weight
LRR	Leucine-rich repeat protein
MPH	Mid-parent heterosis
MS	Mass spectrometry
PMF	Peptide map fingerprinting
PTM	Post translational modification
RcbA	Rubisco activase
RcbL	Rubisco large subunit
SSH	Suppression subtractive hybridization
2DE	Two-dimensional gel electrophoresis
TFA	Trifluoroacetic acid
TIR	Toll/interleukin-1 receptor
TLN	Total leaf number
TTN	Total tiller number
UPF1	Dominant expression of uniparental proteins in hybrids
UPnF1	Dominant expression of uniparental proteins but not in hybrids
URH	Up-regulated in hybrid

Introduction

Heterosis or hybrid vigor is defined as the advantage of hybrid performance over its parents in terms of viability, growth, and productivity. Hybrid wheat was first commercialized in the United States in the 1970s. Today, it is cultivated in Australia, China, South Africa and India (Matuschke et al. 2007). In India, the reported adoption of hybrid wheat was 60,000 acres in 2005 (Matuschke et al. 2007). In China, more than ten hybrid wheat cultivars, mainly produced by CHA and two-line system, were registered by 2007, and hybrid wheat is planted in more than 10,000 hectares annually, with yield advantage of 20%. Previous studies detected significant difference in mRNA quantity and expression patterns between hybrids and their parental inbreds (Sun et al. 1999; Ni et al. 2002). Further analysis indicated that differential gene expression patterns in leaf tissue of rice and wheat were correlated with heterosis (Xiong et al. 1998; Sun et al. 2004). Attempts have also been made to characterize differentially expressed genes in leaves 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, photosynthesis and others (Yao et al. 2005; Wang et al. 2006; Zhang et al. 2006). These results indicated that the hybridization between two parental inbred lines can cause expression changes of different genes, which might be responsible for the observed heterosis (Ni et al. 2000; Wu et al. 2003; Sun et al. 2004). Although transcriptome analyses of gene expression have contributed greatly to our understanding of the heterosis in rice, maize and wheat (Yao et al. 2005; Zhang et al. 2006; Guo et al. 2004; Swanson-Wagner et al. 2006; Huang et al. 2006), changes on the level of mRNA do not necessarily indicate changes on the protein level. Therefore, differential protein expression between hybrid and its parental lines is still an area to be elucidated. In fact, as early as 1970s, several investigators estimated the correlations between isozyme allelic diversity and grain yield of single-cross maize hybrid (Hunter and Kannenberg 1971; Heidrich-Sobrinho and Cordeiro 1975; Gonella and Peterson 1978; Hadjinov et al. 1982; Tsaftaris 1987). Two-dimensional gel electrophoresis was then employed to determine correlations between polymorphism of individual protein amounts indices and hybrid vigor for agronomic traits (Damerval et al. 1987; Leonardi et al. 1987), and report also demonstrated that some proteins are differentially synthesized and expressed in root tips between a maize hybrid and its parents (Romagnoli et al. 1990). Recently, in the studies on wheat root proteome, the abundance of 45 protein spots was found to be differentially accumulated between a wheat hybrid and its parents, which revealed that differentially expressed proteins were involved in metabolism, cell growth and maintenance, signal transduction, stress response, transcription regulation and others (Song et al. 2007).

Plant leaves serve as the site of important biological functions, such as photosynthesis, respiration, transpiration, and guttation (Swanson-Wagner et al. 2006). In wheat, previous investigations demonstrated that the heterotic hybrids had larger aerial parts than the parental lines at seedling stages, and significant difference in mRNA quantity and expression patterns were detected in leaves of wheat hybrids and their parental inbreds (Yao et al. 2005). In the present study, in order to elucidate the relationship between protein expression and heterosis, differentially expressed protein profiles in seedling leaves of wheat hybrid and its parents were studied by using high-throughput 2D gel electrophoresis (2DE).

Materials and methods

Plant materials

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. Pre-germinated seeds were placed at 4°C for 3 days, and planted in vermiculite. For measurement of the heterosis in seedling stage, pre-germinated seeds of hybrid and its parents were grown with seven replicates (one seedling for each replicate) in vermiculite that was watered with

nutrition solution containing: $CaCl_2$, 1 mM; KH_2PO_4, 0.5 mM; ferric citrate, 10 μM; $MgSO_4$, 0.25 mM; K_2SO_4, 0.25 mM; $MnSO_4$, 1 μM; H_3BO_3, 2 μM; $ZnSO_4$, 0.5 μM; $CuSO_4$, 0.2 μM; $CoSO_4$, 0.1 μM; Na_2MoO_4, 0.1 μM (Broughton and Dilworth 1971). The seedlings were grown in a growth chamber at a relative humidity of 75% and 26/20°C day and night temperature, a 16/8 h (day/night) photoperiod with light supplementation to reach at least 3,000 lx. For proteomics analyses, the 20-day seedling leaf samples of the hybrid and its parents from nine seedlings were harvested and frozen in liquid nitrogen and stored at −80°C before use. The first four fully unfolded leafs of main stems from three individual plants were pooled as one biological replicate for protein extraction, and three replicates were harvested for each genotype, and then run on three separated gels.

Leaf heterosis measurement

Aerial parts of hybrids and parents were harvested and dried in an oven at 120°C for 0.5 h, then 80°C for 24 h for dry weight determination. Four aerial parts traits were characterized including leaf fresh weight (LFW), leaf dry weight (LDW), total leaf number (TLN), total tiller number (TTN). The mid-parent heterosis (MPH) and best-parent heterosis (BPH) were calculated using following formula: MPH = (F_1-mid-parental value)/mid-parental value in %; BPH = (F_1-best parental value)/best parental value in %. Statistical analysis of the differences in aerial part traits was performed by using *t*-test.

Total protein extraction and quantitation

Total protein was isolated from leaf tissues using Invitrogen's TRIZOL® Reagent according to the manufacturer's instruction. Protein concentration was determined by Bradford assay (Ramagli 1999).

DE and image analysis

The wheat leaf proteins in the dried powder were solubilized in 7 M urea, 2 M thiourea, 2% CHAPS (powder to solution, w/v), 0.5% IPG buffer (v/v) (pH 4–7 and pH 6–11) (GE Healthcare, USA) and 36 mM DTT (5.6 mg/ml) via incubation at room temperature for 1 h, vortexing every 10 min, the mixture was then centrifuged (15,000 rpm) for 15 min, and the supernatant was collected. Total protein extract (500 μg) was loaded onto GE Healthcare 24 cm IPG gel strips (pH 4–7 and pH 6–11) during strip rehydration overnight. IEF of the acidic range IPG strips (pH 4–7) and basic range IPG strips (pH 6–11) were conducted using IPGPhorII (GE Healthcare, USA) at 20°C for a total of 65 and 110 kVh, respectively. The IPG strips were equilibrated according to the manufacturer (GE Healthcare, USA). The second dimension SDS-PAGE gels (12.5% linear gradient) were run on an Ettan Dalt six (GE Healthcare, USA), 0.5 h at 2.5 W per gel, then at 15 W per gel until the dye front reached the gel bottom. Upon electrophoresis, the protein spots were stained with silver nitrate according to the instruction of protein PlusOne™ Silver Staining Kit (GE Healthcare, USA), which offered improved compatibility with subsequent mass spectrometric analysis. Briefly, gels were fixed in 40% ethanol and 10% acetic acid for 30 min, and then sensitized with 30% ethanol, 0.2% sodium thiosulfate (w/v) and 6.8% sodium acetate (w/v) for 30 min. Then gels were rinsed with distilled water three times, 5 min for each time, then incubated in silver nitrate (2.5 g/l) for 20 min. Incubated gels were rinsed with distilled water and developed in a solution of sodium carbonate (25 g/l) with formaldehyde (37%, w/v) added (300 μl/l) before use. Development was stopped with 1.46% EDTA-$Na_2{\cdot}2H_2O$ (w/v), and gels were stored in distilled water until they could be processed and the reproducible spots removed from them. Gel images were acquired using Labscan (GE Healthcare, USA). Image analysis was carried out with Imagemaster 2D Platinum Software Version 5.0 (GE Healthcare, USA). Spot detection was performed with the parameters smooth, minimum area and saliency set to 2, 15 and 8, respectively, and was done automated by the software used, followed by manual spot editing, such as artificial spot deletion, spot splitting and merging. All the gels were matched to the reference gel selected in automated mode followed by manual pair correction. The volume of each spot from three replicate gels was normalized against total spot volume, quantified, and subjected to ANOVA test ($P < 0.05$). In the software we used, spot normalization was performed by the use of the relative intensity (%Intensity) or relative volume (%Vol) to quantify and compare the gel spots. These measures take into account of variations due to protein loading and staining, by considering the total intensity or volume over all the spots in the gel.

In gel digestion

Spots of varied intensities were excised manually and transferred to 1.5 ml microcentrifuge tubes. For proteins of lower abundance, protein spots were removed from all the replicate gels, pooled and digested in a single tube. Protein spots were destained twice with 30 mM potassium ferricyanide and 100 mM sodium thiosulfate, and then rinsed with 25 mM ammonium bicarbonate in 50% acetonitrile. Gel pieces were dehydrated with 100% acetonitrile, dried under vacuum and incubated for 16 h at 37°C with 10 μl of 10 ng/μl trypsin in 25 mM ammonium bicarbonate. The resulting tryptic fragments were eluted by

diffusion into 50% v/v acetonitrile and 0.5% v/v trifluoroacetic acid.

Mass spectrometry (MS)

Protein MS was conducted using a AUTOFLEX II TOF-TOF (Bruker Daltonics, Germany). Digested protein samples (70% v/v acetonitrile and 0.5% v/v trifluoroacetic) were spotted on an AnchorChipTM plate (1.0 µl) twice and recrystallized CHCA matrix (Bruker Daltonics, Germany) dissolved in 0.1% TFA/70% ACN (0.5 µl) once. External standards from the manufacturer were dissolved in the same matrix solution and spotted on the fixed positions labeled on the plate. Each sample spot was desalted with 0.01% TFA twice, and completely dried. The peptide ions generated by autolysis of trypsin (with m/z 2163.333 and 2273.434) were used as internal standards for calibration. The list of peptide masses from each PMF was saved for database analysis.

Data analysis

Monoisotopic peptide masses generated from the PMFs were analyzed with Auto Flexanalysis (Bruker Daltonics, Germany) and Mascot distiller (Matrix Science) and used to search for NCBInr database using MASCOT (http://www.matrixscience.com/home.html). Matches to protein sequences from the Viridiplantae taxon in NCBInr database were considered acceptable if: (1) A MOWSE score was obtained from MASCOT, which rates scores as significant if they are above the 95% significance threshold ($P < 0.05$); (2) At least four different predicted peptide masses need to match the observed masses for an identification to be considered valid; (3) The coverage of protein sequences by the matching peptides should be higher than 10%; (4) The mass deviation between the experimental and theoretical peptide masses should be less than 0.2 Da (Donnelly et al. 2005; Porubleva et al. 2001). In addition, some proteins successfully identified have substantial discrepancies between the experimental and calculated p*I* and *M*r, which could be caused by numerous factors such as post translational modification (PTM), polymeric forms of proteins, proteolytic degradation of proteins, matches to proteins from different organisms, or genomic sequence, which could contain segments that are spliced out of the functional protein. Such protein identifications were deemed acceptable as long as the other statistical criteria were met (Donnelly et al. 2005). Parameters for searches are as follows: Viridiplantae (green plant) for taxon consideration; tryptic peptides with up to one missed cleavage site; 0.2 Da mass tolerances for peptide or MS/MS; oxidation of methionine and carbamidomethylation of cysteine were specified as variable and fixed modifications.

Quantitative real-time PCR analysis

Specific PCR primers (Table 1) were designed according to corresponding cDNA sequences. Reaction was carried out in 20 µl reactions system containing 10 mmol/l Tris–HCl (pH8.5), 50 mmol/l KCl, 2 mmol/l $MgCl_2$, 0.4 µl DMSO, 200 mmol/l dNTPs, Specific PCR primers 10 pmol, Taq DNA polymerase 1U, SYBR GREEN I fluorescence dye 0.5 µl. Three biological replications for each sample were conducted. A 350 bp β-actin gene fragment was amplified as a positive control using the primer pair 5′-CAGCA ACTGGGATGATATGG-3′ and 5′-ATTTCGCTTTCAG CAGTGGT-3′. Thermocycling was conducted using an Opticon2 DNA Engine (MJ Research Inc.) initiated by a 5 min incubation at 94°C, followed by 40 cycles (94°C, 30 s; anneal temperature 58°C, 30 s; 72°C,30 s.) with a single fluorescent reading taken at the end of each cycle. Each run was completed with a melting curve analysis to conform the specificity of amplification. Ct values were determined by the Opticon2 software using a fluorescence threshold manually. *C* value of hybrid and parents were calculated according to Ct value. $C = 2 - \Delta CT$, $\Delta Ct = Ct$ target gene − Ct internal gene.

Results

Two-dimensional electrophoresis analysis of total proteins in seedling leaves of wheat hybrid and its parental lines

For wheat hybrid 3338/2463 and its parental lines (3338 and 2463), four characters of aerial parts, including leaf fresh weight (LFW), leaf dry weight (LDW), total leaf number (TLN), and total tiller number (TTN), were measured, and the middle- and best-parent heterosis was calculated. Analysis indicated that middle-parent heterosis (MPH) was significant for leaf fresh weight (LFW) ($P < 0.01$), leaf dry weight (LDW) ($P < 0.01$) and total leaf number (TLN) ($P < 0.05$), and best-parent heterosis (BPH) was also significant for LFW ($P < 0.05$) and LDW

Table 1 Specific PCR primers for quantitative real-time PCR

Spot no.	Primer	Sequence
222	Sense	5′-AGATTAAGGCTGCTATCAAGGAG-3′
	Antisense	5′-TTGACAAAGTTGTCGTTCAGAG-3′
991	Sense	5′-AGGAAAATCATTCCAGTGTGAG-3′
	Antisense	5′-CTTACCCTTCTTGATCATGTCTG-3′
865	Sense	5′-CTTGCTCTTGCTACAGTTAAACG-3′
	Antisense	5′-TACTTTCCAGAGGATTAGCTTCC-3′
1050	Sense	5′-GCTACTCTCCTCAAATCGTCTTT-3′
	Antisense	5′-GTTAGCCTCAGTGTTCTCAAGG-3′

($P < 0.01$) (Table 2), suggesting that the wheat hybrid at the seedling stage produced higher biomass than its parental lines. Therefore, the first four leaves of main stems from three individuals were harvested and pooled as one biological replicate, and three biological replicates were set for subsequent 2DE analysis. In order to make a more comprehensive comparison between wheat hybrid and its parental lines, two partially overlapped pH ranges, that is 4–7 and 6–11, were used for isoelectric focusing at the first dimension. Moreover, with each biological replicate, at least triplicate gels were performed, with the purpose to ensure reproducibility of 2D-gel. At acid (pH 4–7) and basic (pH 6–11) pH ranges, the number of protein spots that were reproducibly detected by image 5.0 software on silver-stained gels was about 630 and 260, respectively (Fig. 1a, b). Further quantitative image analysis revealed that a total of 49 protein spots were differentially accumulated between wheat hybrid and its parental lines with the fold changes of more than 1.5 and significant at $P < 0.05$ (Supplementary Table 1).

When comparing the patterns of differentially expressed protein spots between hybrid and its parents, both quantitative and qualitative differences were observed (Fig. 2). 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.

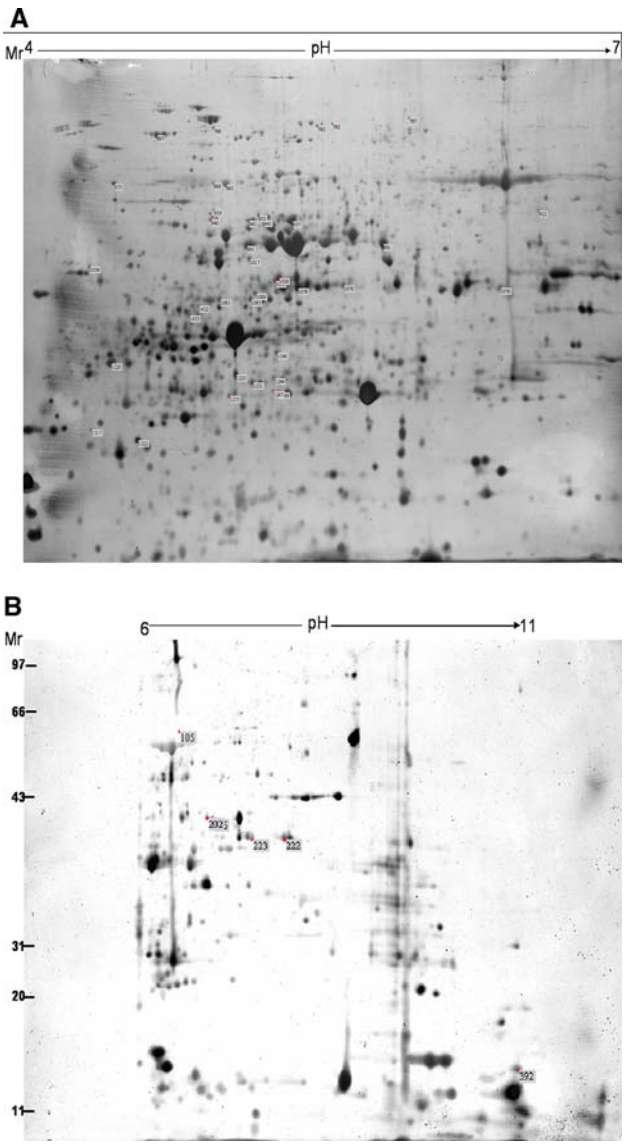

Fig. 1 Wheat leaf 2D gel maps of two pH ranges **a** 4–7 and **b** 6–11

Table 2 Heterosis of hybrid 3338/2463 at seedling stage

	Aerial part traits			
	LFW (g)	LDW (g)	TLN	TTN
Parent 3338	1.779 ± 0.287[a]	0.275 ± 0.055	4.6 ± 0.53	2.7 ± 0.76
Parent 2463	1.732 ± 0.649	0.266 ± 0.109	4.6 ± 0.53	2.6 ± 0.53
Hybrid 3338/2463	2.213 ± 0.344	0.321 ± 0.054	5.0 ± 0.00	3.1 ± 0.38
MPH (%)	26.06**	18.70**	8.70*	16.98
BPH (%)	24.40*	16.73**	8.70	14.81

LFW leaf fresh weight, *LDW* leaf dry weight, *TLN* total leaf number, *TTN* total tiller number, *MPH* mid-parent heterosis, calculated using formula: MPH = (mean F1 − mean *P*)/mean *P* in %, *BPH* best-parent heterosis, calculated using formula: BPH = (mean F1 − mean best *P*)/mean best *P* in %

** Significant at $P < 0.01$; * Significant at $P < 0.05$

[a] Standard deviations ($n = 7$)

Identification of differentially expressed protein spots

The 49 differentially accumulated protein spots in seedling leaves between wheat hybrid and its parental lines were eluted from representative 2D gels for identification, and 30 spots were successfully identified, which correspond to 34 proteins or protein isoforms. According to the criteria adopted in a previous study (Bevan et al. 1998), these 34

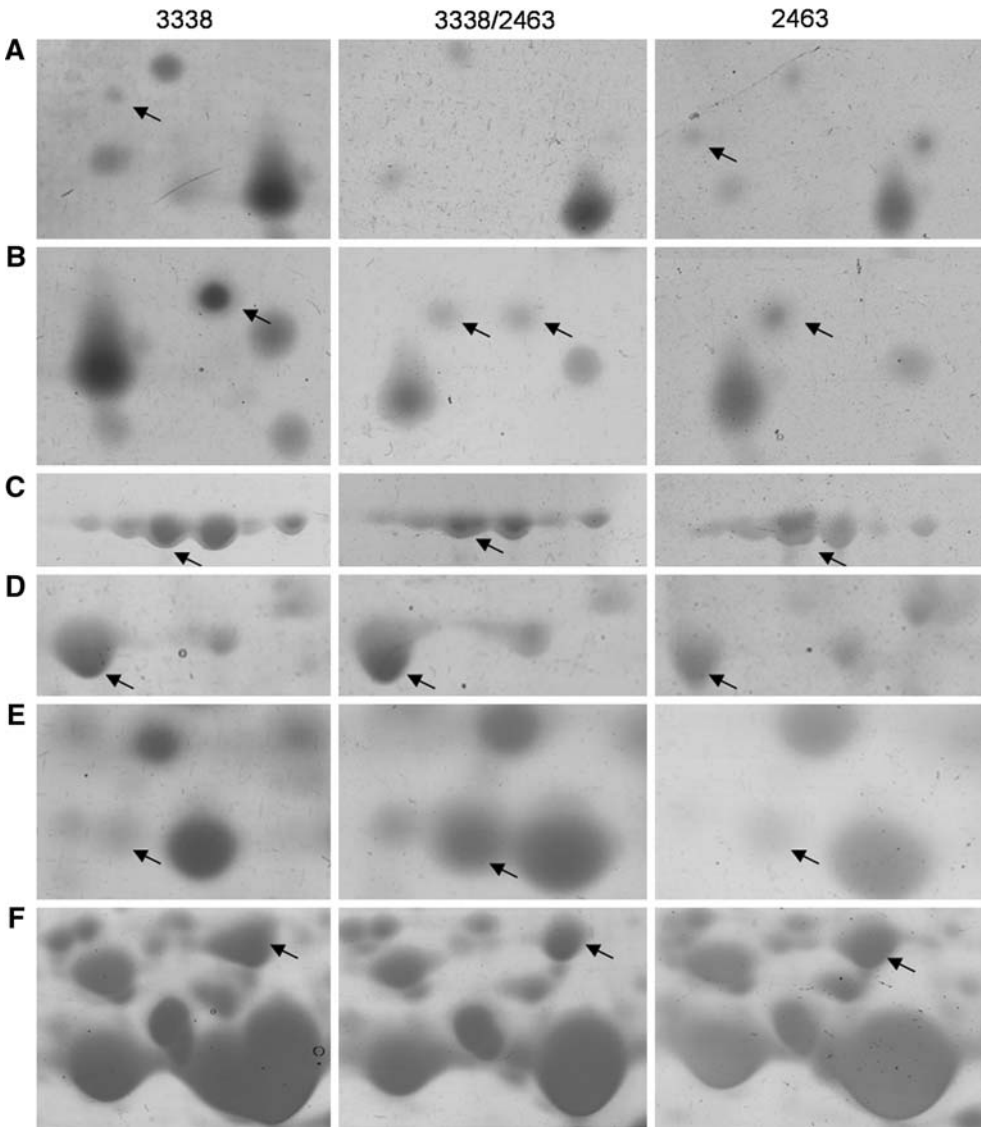

Fig. 2 Differential protein expression patterns between hybrids and its parents in wheat leaves. **a** (spot 1317-1, 1317-2) $UPnF_1$: dominant expression of uniparental proteins but not in hybrids, expression in either paternal or maternal parents of protein not expressed in hybrid; **b** (spots 1322-1, 1322-2) UPF_1: dominant expression of uniparental proteins in hybrids, expression in hybrid of protein only expressed either paternal or maternal parents; **c** (spot 807) LDH: low-dominant in hybrid, expression in hybrid is equal to the lowly expressed parent; **d** (spot 865) HDH: high-dominant in hybrid, expression in hybrid is equal to the highly expressed parent; **e** (spot 1269) URH: up-regulated in hybrid, expression in hybrid is higher than in both female and male parents; **f** (spot 937) DRH: down-regulated in hybrid, expression in hybrid is lower than in its parents

identified proteins or isoforms were classified into eight functional classes, including energy (nine spots, fructose 1,6-bisphosphate aldolase, ATP synthase CF1 beta subunit, glyceraldehyde-3-phosphate dehydrogenase, ribulose-1,5-bisphosphate carboxylase, ribulose 1,5-bisphosphate carboxylase activase), metabolism (seven spots, sucrose synthase, cysteine synthase, glucose-6-phosphate 1-dehydrogenase, proline dehydrogenase, cytochrome P450), signal transduction (three spots, toll/interleukin-1 receptor, leucine-rich repeat (LRR) protein, serine/threonine protein kinase), transposable elements (four spots, copia retroelement, polyprotein-like, reverse transcriptase, hypothetical proteins), disease and defense (two spots, ascorbate peroxidase, heat shock protein 70), cell structure (two spots, actin, kinesin), transcription and translation (one spot, pentatricopeptide repeat) and six unclassified proteins (Fig. 3; Table 3).

Expression analysis of selected transcripts of differentially accumulated proteins

To further investigate whether changes in the profiles of protein expression between hybrid and parents were correlated to changes in transcript expression, we conducted quantitative real-time RCR to examine the mRNA expression in parallel with our proteomics assay. Four proteins differentially expressed were selected for further transcritps analysis. The total mRNA was isolated from the same materials as the ones for total protein extraction using the protocol provided by TRIZOL® Reagent (Invitrogen). In this analysis, we found only one consistency instance of the two expression levels. Fructose 1,6-bisphosphate aldolase precursor (spot 1050) showed high-dominant expression in hybrid both on protein level and mRNA level (Figs. 2, 4). The expression patterns of three genes between hybrid and

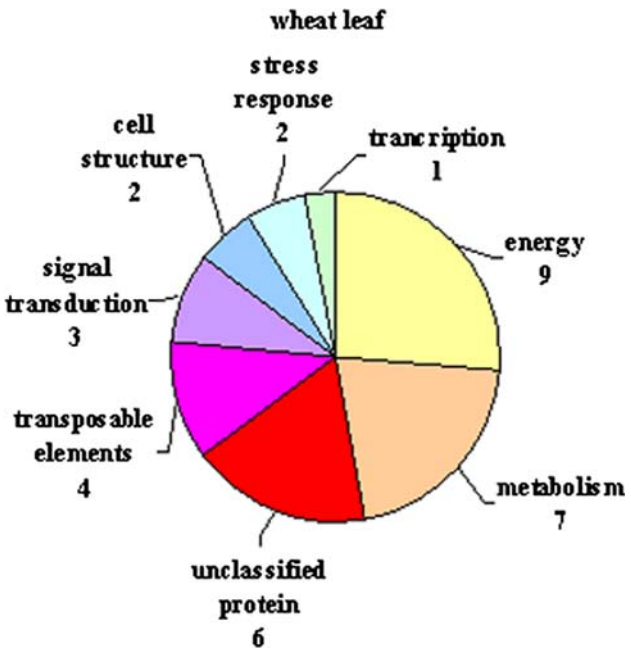

Fig. 3 Functional classification of the 34 identified proteins and protein isoforms differentially expressed in leaves between hybrid and its parents

its parents on mRNA level were inconsistent to the patterns of protein abundance. For example, the expression of cytosolic glyceraldehyde-3-phosphate dehydrogenase (spot 222) was detected to be preferentially expressed in Line 3338 on protein level. And Ribulose 1,5-bisphosphate carboxylase activase isoform 1 (spot 991) showed high-dominant expression in hybrid on protein level. However, these two genes shown no expression differences between hybrid and parents on mRNA level ($P < 0.05$) (Fig. 4). ATP synthase CF1 beta subunit with two protein isoforms identified in leaves (spots 865 and 859) were expressed as the patterns of HDH and UPF1 on protein level, respectively, but were detected to be up-regulated in hybrid on mRNA level (Fig. 4).

Discussions

Differential protein expression profiles in leaves of wheat hybrid and its parental lines

In modern breeding programs, the discovery and exploitation of hybrid vigor is one of the most important advances in plant improvement. However, molecular basis of heterosis is poorly understood. Recently, with novel molecular tools at hand, it was reported that some patterns of differential expression on transcriptional level detected in leaves are correlated with heterosis in some traits, and a number of genes that probably contribute to wheat heterosis were identified (Sun et al. 2004; Yao et al. 2005; Wu et al. 2003). However, whether protein accumulation is also changed between hybrids and their parents is still unclear since changes on the level of mRNA do not necessarily indicate changes on the protein level. In the present study, by using 2DE, difference in protein accumulation in leaves of wheat hybrid and its parental lines were investigated, and a total of 49 differentially accumulated protein spots were detected, which provided evidence that some proteins were also differentially accumulated between wheat hybrid and its parents.

Further analysis revealed that these 49 differentially expressed protein spots represented six differential expression patterns, and the number of protein spots exhibited high-parent dominance (HDH), low-parent dominance (LDH), uniparent silencing (UPF1) and uniparent dominance ($UPnF_1$) is 11, 11, 15 and 5, respectively. In addition, seven protein spots exhibited underdominance (DRH) or overdominance (URH). In a study on the inheritance of specific protein amount in maize inbreds and their hybrids, dominant gene expression was proposed to be important for heterosis (Leonardi et al. 1987; Romagnoli et al. 1990). Based on our present data, that is, 42 of 49 (85.7%) differentially expressed protein spots between wheat hybrid and its parents exhibited dominance, we suggest that dominance at protein accumulation level could be important for the leaf heterosis, which is similar to our previous report on wheat root (Song et al. 2007).

Comparison of protein abundance and mRNA expression

Understanding how protein abundance is related to mRNA transcript levels is essential for interpreting gene expression, protein interactions, structures and functions in a cellular system. There have been many studies attempting to elucidate this kind of correlation. However the correlation coefficient varied among different studies or tissues. Although there appears to be a good correlation between transcript and protein abundance in *Escherichia coli* (Corbin et al. 2003), global comparisons of yeast, mammalian cells and *Methanococcus maripaludis* yielded correlations coefficients of 0.36, 0.48 and 0.24 between transcript and protein abundance, respectively (Anderson and Seilhamer 1997; Gygi et al. 1999; Xia et al. 2006). A relatively low correlation has also been reported for *A. thaliana* chloroplasts (Baginsky et al. 2005), pollen (Holmes-Davis et al. 2005; Noir et al. 2005), root and leaf (Mooney et al. 2006), suggesting a widespread lack of correlation between transcript and protein abundance. A parallel genomic and proteomic analysis of gene expression was conducted in the reproductive tract of 3-day-old unmated and mated female *Drosophila melanogaster* (Mack et al. 2006). Using proteomics methods, 84 differentially expressed proteins were

Table 3 Differentially expressed proteins between hybrid and parents

Spot number	Differential expression pattern	Functions	Functional categories	Taxonomy	Accession number	Experimental MW(kDa)/pI	Calculated MW(kDa)/pI	Database	Top score	Peptide matched	Sequence coverage (%)
105	LDH	expressed protein	Unclassified	*Oryza sativa* (japonica cultivar-group)	gil108708001	57/6.41	37.93/5.96	NCBInr	70	8	12
205	LDH	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	Energy		gil120680	36/6.87	36.49/6.67	NCBInr	95	17	43
222	LDH	Cytosolic glyceraldehyde-3-phosphate dehydrogenase	Energy	*Triticum aestivum*	gil32478662	33/7.84	18.17/6.34	NCBInr	105	16	84
223	LDH	Putative cytochrome P450	Metabolism	*Vitis vinifera*	gil147835240	33/7.42	55.71/8.61	NCBInr	70	14	26
807	LDH	heat shock protein 70	Stress response	*Cucumis sativus*	gil1143427	63/4.65	75.37/5.15	NCBInr	87	14	20
865	HDH	ATP synthase CF1 beta subunit	Energy	*Triticum aestivum*	gil14017579	51/5.00	53.82/5.06	NCBInr	205	28	63
869	UPF1	ATP synthase CF1 beta subunit	Energy	*Triticum aestivum*	gil14017579	52/4.94	53.82/5.06	NCBInr	130	20	45
930	UPF1	Putative leucine-rich repeat (LRR) protein	Signal Transduction	*Vitis vinifera*	gil147778302	46/4.92	160.71/8072	NCBInr	80	39	22
931	LDH	Ppass2 (PHRAGMOPLAST ORIENTING KINESIN 2)	Cell structure	*Arabidopsis thaliana*	gil145338697	45/5.17	314.87/5.13	NCBInr	79	28	13
937	DRH	actin	Cell structure	*Setaria italica*	gil9965319	44/5.34	41.66/5.31	NCBInr	72	14	41
981	LDH	Glyceraldehyde-3-phosphate dehydrogenase B	Energy		gil120663	42/5.80	48.07/7.57	NCBInr	79	12	24
991	HDH	ribulose 1,5-bisphosphate carboxylase activase isoform 1	Energy	*Hordeum vulgare* subsp. *vulgare*	gil167096	41/5.11	47.11/8.62	NCBInr	164	25	42
1017	HDH	ATRAD51D/RAD51D (*Arabidopsis* homolog of RAD51 D)	Unclassified	*Arabidopsis thaliana*	gil145323780	40/5.12	33.39/8.87	NCBInr	73	11	51
1050	HDH	fructose 1,6-bisphosphate aldolase precursor	Energy	*Avena sativa*	gil8272480	38/5.27	41.90/9.01	NCBInr	114	10	21
1060	HDH	Fructose-1,6-bisphosphate aldolase	Energy	*Vitis vinifera*	gil147835267	38/5.25	42.92/8.13	NCBInr	68	11	31
1070	LDH	Cysteine synthase	Metabolism		gil585032	38/5.60	34.09/5.48	NCBInr	103	16	48
1076	URH	S-locus-related I	Unclassified	*Erucastrum abyssinicum*	gil21321236	38/6.40	47.89/6.44	NCBInr	70	9	29
1078	LDH	Hypothetical protein OsI_014952	Transposable elements	*Oryza sativa* (japonica cultivar-group)	gil125547897	38/5.37	154.26/9.27	NCBInr	74	42	31
1089	UPnF1	Putative cysteine synthase	Metabolism	*Oryza sativa* (indica cultivar-group)	gil125529334	37/5.15	29.72/5.44	NCBInr	70	9	46

Table 3 continued

Spot number	Differential expression pattern	Functions	Functional categories	Taxonomy	Accession number	Experimental MW(kDa)/pI	Calculated MW(kDa)/pI	Database	Top score	Peptide matched	Sequence coverage (%)
1093	DRH	Putative cysteine synthase	Metabolism	*Oryza sativa* (japonica cultivar-group)	gil125529334	37/5.13	29.72/5.44	NCBInr	70	9	42
1235	HDH	Cytosolic ascorbate peroxidase	Stress response	*Zea mays*	gil600116	30/5.05	27.30/5.28	NCBInr	77	13	54
1244	UPF1	TIR (Toll/interleukin-1 receptor)	Signal Transduction	*Medicago truncatula*	gil124361170	29/5.25	119.43/6.69	NCBInr	79	19	22
1251	HDH	Pentatricopeptide repeat	Transcription	*Medicago truncatula*	gil92877013	28/5.14	43.53/7.14	NCBInr	84	15	43
1267	UPF1	Copia retroelement pol polyprotein-like	Transposable elements	*Arabidopsis thaliana*	gil9294244	26/5.24	59.40/9.20	NCBInr	70	15	21
1269	URH	Unnamed protein product	Unclassified	*Ostreococcus tauri*	gil116058247	26/5.27	78.84/4.62	NCBInr	74	15	22
1275	HDH	Sucrose synthase	Metabolism		gil26454671	25/5.02	92.74/5.84	NCBInr	83	18	20
1095 (mixture[a])	DRH	Ribulose-1,5-bisphosphate carboxylase, large subunit	Energy	*Impatiens gordonii*	gil4034729	37/4.97	51.42/6.34	NCBInr	56	9	21
	DRH	Unknown protein	Unclassified	*Arabidopsis thaliana*	gil18397646	37/4.97	69.35/9.10	NCBInr	48	10	16
1220 (mixture)	HDH	Putative serine/threonine protein kinase	Signal Transduction	*Oryza sativa* (japonica cultivar-group)	gil125602510	32/4.42	35.72/7.63	NCBInr	131	13	43
	HDH	Putative proline dehydrogenase	metabolism	*Oryza sativa* (japonica cultivar-group)	gil125575622	32/4.42	42.99/8.05	NCBInr		15	25
1322-1 (mixture)	UPF1	Unknown protein	Unclassified	A*rabidopsis thaliana*	gil42561854	18/4.49	38.47/6.35	NCBInr	59	12	38
	UPF1	Hypothetical protein OsJ_002231	Transposable elements	*Oryza sativa* (japonica cultivar-group)]	gil125570891	18/4.49	155.61/8.95	NCBInr	58	22	16
202 (mixture)	LDH	Cytoplasmic glucose-6-phosphate 1-dehydrogenase	Metabolism	*Mesembryanthemum crystallinum*	gil4206114	36/6.82	59.02/6.07	NCBInr	51	13	23
	LDH	Hypothetical protein	Transposable elements	*Vitis vinifera*	gil147773537	36/6.82	152.54/8.39	NCBInr	43	20	14

The spot number from the 2D gel, the accession number, putative protein identification, functional classification, experimental and calculated MW/pI, peptide match information and differential expression pattern between hybrid and its parents for each protein identity are presented

URH up-regulated in hybrid, *DRH* down-regulated in hybrid, *HDH* high-dominant in hybrid, *LDH* low-dominant in hybrid, *UPF1* dominant expression of uniparental genes in hybrids, *UPnF1* dominant expression of uniparental genes but not in hybrids

[a] Refered to "protein mixture" which indicated that this single spot on 2D gel contains peptides from more than one protein

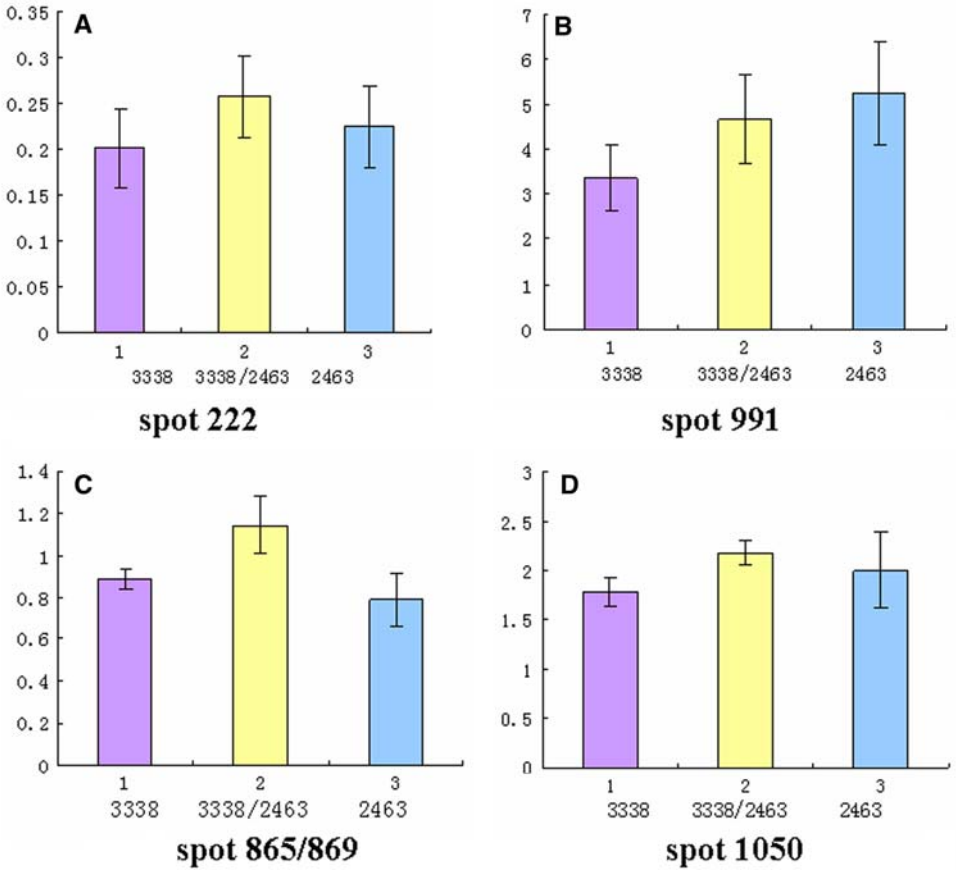

Fig. 4 Quantitative real-time PCR of mRNA expression patterns for selected protein spots. **a** *(spot 222)* No difference between hybrid and its parents after *t*-test ($P < 0.05$); **b** *(spot 991)* no difference between hybrid and its parents after *t*-test ($P < 0.05$); **c** *(spots 865 and 869)* URH: up-regulated in hybrid, expression in hybrid is significantly higher than in both female and male parents after *t*-test ($P < 0.05$); **d** *(spot 1050)* HDH: high-dominant in hybrid, expression in hybrid is not significantly different from the highly expressed parent Line 2463 but is significantly higher than lowly expressed parent Line 3338 after *t*-test ($P < 0.05$)

detected. However, none of the differentially expressed proteins exhibited corresponding up-or down-regulation at transcript levels (Mack et al. 2006).

In this study, first, a comparison of protein abundance with transcript abundance was conducted, using previously reported SHH data of our lab with similar material set. As a result, eight identified proteins in our present work were also detected in previous subtracted cDNA libraries with homology identities. These genes included heat shock protein 70, leucine-rich repeat protein, ribulose 1,5-bisphosphate carboxylase activase, fructose-bisphosphate aldolase, cysteine synthase (two genes), ribulose-bisphosphate carboxylase, serine/threonine protein, some of which exhibited similar differential expression profiles at the two expression level. For example, heat shock protein 70, cysteine synthase (two genes) were detected to be expressed at lower level in hybrid at both expression level; fructose-bisphosphate aldolase, serine/threonine protein kinase there detected highly expressed at the two levels, while the other three genes exhibited opposite differential expression tendency between mRNA and protein levels. Secondly, several proteins differentially expressed were selected for further transcritps analysis with quantitative real-time RCR in parallel with our proteomics assay. However, only one of the selected differentially accumulated proteins exhibited same differential expression patterns at transcript level.

The possible reasons for the inconsistency at protein and mRNA expression levels could be due to: (1) The differences in half-lifes of individual mRNAs and proteins within a given eukaryotic cell (Day and Tuite 1998); (2) differences in translational efficiency which is controlled by post-transcriptional regulations and posttranslational modifications. Although we cannot exclude experimental error and the limitations of different detection methods, our observations suggest that some differential expression patterns at protein abundance might be mediated by post-translational modification mechanisms.

Possible roles of the differentially expressed proteins in wheat heterosis

Experimental data presented in this paper demonstrated that at least 49 protein spots in the hybrid F_1 seedling leaves were differentially accumulated as compared to the parents. To get an insight into the possible role of each individual differentially accumulated protein spots in heterosis, 30 spots were successfully identified, which correspond to 34 different proteins or protein isoforms. As the major source of organic carbon, leaves play a vital role in photosynthesis. Therefore, it is not surprising that, among the 34 differentially expressed proteins identified, 10 of which (29.4%) are involved in the photosynthesis and carbon metabolisms.

First, three important photosynthetic proteins, including ATP synthase CF1 beta subunit, Rubisco large subunit (RcbL) and Rubisco activase (RcbA), were identified to be differentially accumulated between hybrid and its parents. The chloroplast ATP synthase utilizes the energy of the proton gradient formed during light-driven electron transport to catalyze the formation of ATP from ADP and inorganic phosphate (Mills and Richter 1991). Rubisco is the major photosynthetic enzyme in plants, and its activity in vivo is regulated by Rubisco activase. In our present study, ATP synthase CF1 beta subunit was detected in isoforms of two protein spots, and their differential expression patterns were HDH and UPF_1, respectively. In addition, the abundance of one protein, which represented Rubisco activase, is equal to that of the highly expressed parent Line 2463. Second, sucrose and starch are the main products of photosynthesis, and genes involved in carbon metabolism are active in leaves. In our present study, sucrose synthase and fructose-1, 6-bisphosphate aldolase were found to be highly expressed in wheat hybrid. Sucrose synthase, an important enzyme in sucrose metabolism, catalyzes the reversible conversion of sucrose and UDP to UDP-glucose and fructose in vitro (Martin et al. 1993). Fructose-1, 6-bisphosphate aldolase (FBA) is a glycolytic and Calvin cycle enzyme. Thus the high expression of these carbon metabolism related proteins may contribute to the observed vigorous growth in hybrid leaves. It should also be noted that one Rubisco Large subunit and three glyceraldehyde-3-phosphate dehydrogenases were detected to be down-regulated in wheat hybrid, the causal reason need further investigation.

Interestingly, some candidate components involved in signal transduction were identified to be differentially expressed between hybrid and parents, i.e., putative leucine-rich repeat (LRR) protein (spot 930), putative serine/threonine protein kinase (spot 1220mix-1) and TIR (Toll/interleukin-1 receptor) (spot 1244). Among the three proteins, the functions of LRR protein and serine/threonine protein kinase have been well characterized. For Leucine-rich repeat proteins (LRRs), besides the well known roles in numerous developmental, environmental and defense-related pathways, they are also involved in such diverse processes such as pollen tube growth, root development, Ran GTPase activation, transcription regulation and meristem cell organization (Forsthoefel et al. 2005). In addition, activation of serine/threonine protein kinase, and the subsequent phosphorylation of their target proteins are thought to be common strategies in the transduction of environmental and developmental signals among yeasts, animals, and plants (Tsuyoshi et al. 1994). As a signal receptor, TIR has ectodomains with characteristic blocks of leucine-rich repeats and a cytoplasmic signaling domain of ~200 residues named as Toll-interleukin 1 receptor domain (Dunne et al. 2003). The Toll-interleukin-1 receptor (TIR) domain is found in innate immune molecules of insects and animals. In addition, Toll protein is involved in establishment of dorso-ventral polarity in the embryo of *Drosophila melanogaster* (Nature Signaling Gateway 2007). In plants, pathways involving TIR domain-containing proteins are not as well understood, but data show that plants have used their TIR domains to perform a multitude of duties and have expanded the functions of the TIR domain beyond those seen in animals (Frost et al. 2004; Seo et al. 2007). Interestingly, all of the three proteins were expressed in hybrid at the level equal to the highly expressed parent. However, at present it would be premature to determine how expression changes in genes involved in signaling transduction in hybrid might affect heterosis, these expression changes might be important to regulate down-stream gene expression in hybrids that affect heterosis in their turn.

Retrotransposons are ubiquitous in plants and play a major role in plant gene and genome evolution (Kumar and Bennetzen 1999). Recently, some retrotransposons, which differentially expressed between hybrids and their parents, has been identified by using DDRT, SSH or cDNA microarray (Wu et al. 2003; Yao et al. 2005; Zhang et al. 2006). In the present study, four proteins were identified as retroelement coded proteins. Retrotransposons can generate mutations by inserting near or within genes, and these elements may provide regulatory sequences for gene expression and alter the expression of adjacent genes (Fedoroff 2000; Kashkush et al. 2003). Taken together, the changes in the expression profiles of retrotransposon at transcriptional and translational levels might contribute to alterations in the expression of other genes in hybrid.

Comparison of differential expression profiles between hybrid and parents in wheat leaf and root

Our previous and present studies indicated that leaf and root at seedling stage exhibited significant heterosis (Yao et al. 2005). Recently, by using 2D gel, we analyzed the differential protein expression between wheat hybrid and its parental inbreds in seedling roots and found that about 10% of total displayed protein spots was polymorphic between hybrids and its parental inbreds (Song et al. 2007). In the present study, we found that less differentially expressed proteins could be detected in the seedling leaves and approximately 5.5% displayed protein spots were found to be polymorphic, which is approximately half of those displayed in seedling roots. Further comparison revealed that both quantitative and qualitative differences can be detected in both seedling leaves and roots. Interestingly, differential expression pattern $UPnF_1$, that is expression in either of the parents but not in F_1, was observed in leaves (10.2%), but not in seedling roots (Song et al. 2007). In addition, expres-

sion patterns of HDH (22.4% in leaves, 6.7% in root) and LDH (22.4% in leaves, 8.9% in root) were over-represented in leaves, while UPF_1 (84.4% in root, 30.6% in leaf) was under-represented in seedling leaves. Moreover, comparison of differentially expressed proteins identified from roots and leaves revealed no protein spots in common. Gene Ontology (GO) analysis indicated that, in leaves, a large proportion (25%) of the identified differentially expressed proteins between hybrid and its parental inbreds was involved in energy, whereas only 8% of differentially expressed proteins in roots can be classified in this category. On the other hand, the largest category in roots is signaling transduction (32%), whereas only three proteins (9%) in this category were identified in leaves. The possible reason may be the functional specialization of leaves and roots, since leaves are major organ for energy harvesting and carbon metabolism, and roots mainly function in water and nutrients absorption, stresses and phytohormones response.

In our previous study using seedling roots (Song et al. 2007) and present study using seedling leaves, a total of 450 and 890 protein spots were displayed, respectively. Theoretically, these protein spots could be considered as a representation of part of the expressed proteins in the hybrids and their parents. Moreover, both leaves and roots were collected from the same developmental stage, and the same hybrid and parents were used for this and previous studies. Therefore, the differences in the protein expression obtained from seedling leaves and roots suggested that the level and pattern of differential protein expression between wheat hybrids and their parents are tissue specific.

Acknowledgments The authors wish to thank Mr. Jidong Feng for his assistance in mass spectrometry analysis. 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

Anderson L, Seilhamer J (1997) A comparison of selectedmRNA and protein abundances in human liver. Electrophoresis 18:533–537

Baginsky S, Kleffmann T, von Zychlinski A, Gruissem W (2005) Analysis of shotgun proteomics and RNA profiling data from *Arabidopsis thaliana* chloroplasts. J Proteome Res 4:637–640

Bevan M, Bancroft I, Bent E, Love K, Goodman H, Dean C, Bergkamp R, Dirkse W (1998) Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of the *Arabidopsis thaliana*. Nature 391:485–488

Broughton W-J, Dilworth M-J (1971) Control of leghaemoglobin synthesis in snake beans. Biochem J 125:1075–1080

Corbin R-W, Paliy O, Yang F, Shabanowitz J, Platt M, Lyons C-E, Root K, McAuliffe J, Jordan M-I, Kustu S, Soupene E, Hunt D-F (2003) Toward a protein profile of *Escherichia coli*: comparison to its transcription profile. Proc Natl Acad Sci USA 100:9232–9237

Damerval C, Hebert Y, De Vienne D (1987) Is the polymorphism of protein amounts related to phenotypic variability? A comparison of two-dimensional electrophoresis data with morphological traits in maize. Theor Appl Genet 74:194–202

Day D-A, Tuite M-F (1998) Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview. J Endocrinol 157:361–371

Donnelly B-E, Madden R-D, Ayoubi P, Porter D-R, Dillwith J-W (2005) The wheat(*Triticum aestivum* L.) leaf proteome. Proteomics 5:1624–1633

Dunne A, Ejdebäck M, Ludidi P-L, O'Neill L-A, Gay N-J (2003) Structural complementarity of Toll/Interleukin-1 Receptor domains in Toll-like Receptors and the adaptors Mal and MyD88. J Biol Chem 278:41443–41451

Fedoroff N (2000) Transposons and genome evolution in plants. Proc Natl Acad Sci 97:7002–7007

Forsthoefel N-R, Cutler K, Port M-D, Yamamoto T, Vernon D-M (2005) PIRLs: a novel class of plant intracellular leucine-rich repeat proteins. Plant Cell Physiol 46:913–922

Frost D, Way H, Howles P, Luck J, Manners J, Hardham A, Finnegan J, Ellis J (2004) Tobacco Transgenic for the flax rust resistance gene L expresses allele-specific activation of defense responses. MPMI 17:224–232

Gonella J-A, Peterson P-A (1978) Isozyme relatedness of inbred lines of maize and performance of their hybrids. Maydica 23:55–61

Guo M, Rupe M-A, Zinselmeier C, Habben J, Bowen B-A, Smith O-S (2004) Allelic variation of gene expression in maize hybrids. Plant Cell 16:1707–1716

Gygi S-P, Rochon Y, Franza B-R, Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19:1720–1730

Hadjinov M-I, Scherbok V-S, Benko N-I, Gusev V-P, Sukhorzhevokaya T-B, Voronova L-P (1982) Interrelationships between isozymic diversity and combining ability in maize lines. Maydica 27:135–149

Heidrich-Sobrinho E, Cordeiro A-R (1975) Codominant isoenzymic allela as markers of genetic diversity correlated with heterosis in maize (*Zea mays* L.). Theor Appl Genet 46:197–199

Holmes-Davis R, Tanaka C-K, Vensel W-H, HurkmanW-J McCormick S (2005) Proteome mapping of mature pollen of *Arabidopsis thaliana*. Proteomics 5:4864–4884

Huang Y, Li L-H, Chen Y, Li X-H, Xu C-G, Wang S-P, Zhang Q-F (2006) Comparative analysis of gene expression at early seedling stage between a rice hybrid and its parents using a cDNA microarray of 9198 uni-sequences. Sci China 49:519–529

Hunter R-B, Kannenberg L-W (1971) Isozyme characterization of corn (*Zea mays*) inbreds and its relationship to single cross hybrid performance. Can J Genet Cytol 13:197–199

Kashkush K, Feldman M, Levy A-A (2003) Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nature Genet 33:102–106

Kumar A, Bennetzen J-L (1999) Plant retrotransposon. Annu Rev Genet 33:479–532

Leonardi A, Damerval C, De Vienne D (1987) Inheritance of protein amounts: comparison of two-dimensional electrophoresis patterns of leaf sheath of two maize lines (*Zeamays* L.) and their hybrids. Genet Res 50:1–5

Mack P-D, Kapelnikov A, Heifetz Y, Bender M (2006) Mating-responsive genes in reproductive tissues of female Drosophila melanogaster. Proc. Natl. Acad. Sci. 103:10358–10363

Martin T, Frommer W-B, Salanoubat M, Willmitzer L (1993) Expression of an *Arabidopsis* sucrose synthase gene indicates a role in metabolization of sucrose both during phloem loading and in sink organs. Plant J. 4:367–377

Matuschke I, Mishra RR, Qaim M (2007) Adoption and impact of hybrid wheat in India. World Dev 35:1422–1435

Mills D-A, Richter M-L (1991) Nucleotide binding to the isolated β subunit of the chloroplast ATP synthase. J Biol Chem 266:7440–7444

Mooney B-P, Miernyk J-A, Greenlief C-M, Thelen J-J (2006) Using quantitative proteomics of *Arabidopsis* roots and leaves to predict metabolic activity. Physiol Plant 128:237–250

Nature Signaling Gateway (2007) Nature publishing group. http://www.signaling-gateway.org. Cited 29 Oct 2007

Ni Z-F, Sun Q-X, Wu L-M, Xie C-J (2002) Differential gene expression between wheat hybrids and their parental inbreds in primary roots. Acta Bot Sin 44:457–462

Ni Z-F, Sun Q-X, Liu Z-Y (2000) Identification of a hybrid-specific expressed gene encoding novel RNA-binding protein in wheat seedling leaves using differential display of mRNA. Mol Gen Genet 263:934–938

Noir S, Brautigam A, Colby T, Schmidt J, Panstruga R (2005) A reference map of the *Arabidopsis thaliana* mature pollen proteome. Biochem Biophys Res Commun 337:1257–1266

Porubleva L, Velden K-V, Kothari S, Oliver D-J, Chitnis P-R (2001) The proteome of maize leaves: use of gene sequences and expressed sequence tag data for identification of proteins with peptide mass fingerprints. Electrophoresis 22:1724–1738

Ramagli L-S (1999) Quantifying protein in 2-D PAGE solubilization buffers. Methods Mol Biol 112:99–103

Romagnoli S, Maddaloni M, Livini C, Motto M (1990) Relationship between gene expression and hybrid vigor in primary root tips of young maize. Theor Appl Genet 80:769–775

Seo Y-S, Jeon J-SE, Rojas M-R, Gilbertson R-L (2007) Characterization of a novel Toll/interleukin-1 receptor (TIR)-TIR gene differentially expressed in common bean (*Phaseolus vulgaris* cv. Othello) undergoing a defence response to the geminivirus Bean dwarf mosaic virus. Mol Plant Pathol 8:151–162

Song X, Ni Z-F, Yao Y-Y, Xie C-J, Li Z-X, Wu H-Y, Zhang Y-H, Sun Q-X (2007) Wheat (*Triticum aestivum* L.) root proteome and differentially expressed root proteins between hybrid and parents. Proteomics 7:3538–3557

Sun Q-X, Ni Z-F, Liu Z-Y (1999) Differential gene expression between wheat hybrids and their parental inbreds in seedling leaves. Euphytica 106:117–123

Sun Q-X, Wu L-M, Ni Z-F, Meng F-R, Wang Z-K, Lin Z (2004) Differential gene expression patterns in leaves between hybrids and their parental inbreds are correlated with heterosis in a wheat diallel cross. Plant Sci 166:651–657

Swanson-Wagner R-A, Jia Y, DeCook R, Borsuk L-A, Nettleton D, Schnable P-S (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F 1 hybrid and its inbred parents. Proc Natl Acad Sci 103:6805–6810

Tsaftaris A-S (1987) Isozymes in plant breeding. In: Rattazzi MC, Scandalios GJ, Whitt GS (eds) Isozymes: current topics in biological and medical research. Alan R. Liss, New York, pp 103–119

Tsuyoshi M, Nobuaki H, Kazuko Y-S, Hiroshi K, Kazuo S (1994) Cloning and sequencing of a nove1 serine/threonine protein kinase in *Arabidopsis thaliana*. Plant Physiol 106:1229–1230

Wang Z-K, Ni Z-F, Wu H-L, Nie X-L, Sun Q-X (2006) Heterosis in root development and differential gene expression between hybrids and their parental inbreds in wheat (*Triticum aestivum* L.). Theor Appl Genet 113:1283–1294

Wu L-M, Ni Z-F, Meng F-R, Lin Z, Sun Q-X (2003) Cloning and characterization of leaf cDNAs that are differentially expressed between wheat hybrids and their parents. Mol Gen Genet 270:281–286

Xia Q, Hendrickson E-L, Zhang Y, Wang T, Taub F, Moore B-C, Porat I, Whitman W-B, Hackett M, Leigh J-A (2006) Quantitative proteomics of the archaeon Methanococcus maripaludis validated by microarray analysis and real time PCR. Mol Cell Proteomics Feb 24:868–881

Xiong L-Z, Yang G-P, Xu C-G, Zhang Q-F, Maroof M-A-S (1998) Relationships of differential gene expression in leaves with heterosis and heterozygosity in a rice diallel cross. Mol Breed 4:129–136

Yao Y-Y, Ni Z-F, Zhang Y-H, Chen Y, Ding Y-H, Han Z-F, Liu Z-Y, Sun Q-X (2005) Identification of differentially expressed genes in leaf and root between wheat hybrid and its parental inbreds using PCR-based cDNA subtraction. Plant Mol Biol 58:367–384

Zhang Y-H, Ni Z-F, Yao Y-Y, Zhao J, Sun Q-X (2006) Analysis of genome-wide gene expression in root of wheat hybrid and its parents inbreds using Barley1 GeneChip. Prog Nat Sci 16:712–720