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QUALITY PROPERTIES AND EXPRESSION PROFILING OF PROTEIN DISULFIDE ISOMERASE GENES DURING GRAIN DEVELOPMENT OF THREE SPRING WHEAT NEAR ISOGENIC LINES

Liwei DONG[1,4], Ning LI[1,4], Xiaobing LU[1,4], Slaven PRODANOVIC[2*], Yanhao XU[3], Wenying ZHANG[3], Yueming YAN[1,3*]

[1]College of Life Science, Capital Normal University, 100048 Beijing, China
[2]Hubei Collaborative Innovation Center for Grain Industry, 434025 Jingzhou, China
[3]Faculty of Agriculture, University of Belgrade, Belgrade, Serbia
[4]Contributed equally to this work

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Three wheat glutenin near isogenic lines (NILs) CB037A, CB037B and CB037C were used to investigate their quality properties and the transcriptional expression profiles of PDI gene family during grain development. Our purpose is to understand the relationships between the dynamic expression of different PDI genes and glutenin allelic compositions related to gluten quality. The results showed that glutenin allelic variations had no significant effects on main agronomic traits and yield performance, but resulted in clear gluten quality changes. CB037B with 5+10 subunits had higher glutenin macropolymer (GMP) content and better breadmaking quality than CB037A with 2+12 while the lack of *Glu-B3h* encoding one abundant B-subunit in CB037C significantly reduced GMP content, dough strength and breadmaking quality. The dynamic expression patterns of eight protein disulfide isomerase (PDI) genes during grain development detected by quantitative real-time polymerase chain reaction (qRT-PCR) showed the close correlations between higher expression levels of *PDI3-1*, *PDI5-1* and *PDI8-1* and the presence of 5+10 subunits. Meanwhile, *Glu-B3h* silence resulted in significant decrease of expression levels of five PDI genes (*PDI3-1, PDI5-1, PDI6-1, PDI7-2* and *PDI8-1*), suggesting the vital roles of certain PDI genes in glutenin and GMP synthesis and gluten quality formation.

Key words: Wheat, NILs, glutenins, *PDI*s, qRT-PCR

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*Corresponding author***:** Prof. Dr. Slaven Prodanovic, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade, Serbia, phone: +381638687246, fax: +38-11-13161987, E-mail: slavenp@agrif.bg.ac.rs
Prof. Dr. Yueming Yan, College of Life Science, Capital Normal University, Beijing, XisanhuanBeilu 105, 100048 Beijing, China, phone: +86-10-68902777, fax: +861068902777, E-mail: yanym@cnu.edu.cn

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important grain crops in the world because of its huge need. Wheat seeds contain abundant starch and proteins, and serve as vital food and vegetable protein source for human. Not only its flour and dough have unique physical properties for producing bread, cakes, biscuits, pasta and noodles, but also people pursue a high-level living standard with the rapid society progress. It is known that wheat processing quality is greatly affected by seed proteins. According to different solubilities, wheat seed proteins are consist of four classes: the water-soluble albumins, the salt-soluble globulins, the alcohol-soluble gliadins and the acid- or base-soluble glutenins. Glutenins are further divided into high and low molecular weight glutenin subunits (HMW-GS, LMW-GS) based on the differences of their molecular weight, which impact the dough resistance predominantly (WANG *et al*., 2013). Gliadins and glutenins are the major seed storage proteins and they are main determinant for dough extensibility and viscoelasticy, respectively. Particularly, glutenins form glutenin macropolymer (GMP) via disulfide bonds and its content affects the rheological properties of dough directly (WEEGELS *et al*., 1996).

Protein disulfide isomerase (PDI) gene family would be classified into eight subfamilies according to phylogenetic analyses results from several plants (D'ALOISIO *et al*., 2010; ZHU *et al*., 2014). The typical or classical PDIs have five discrete domains: a, b, b', a', and c, and consist of two subunits with approximately 57 kDa (HOUSTON *et al*., 2005; ZHU *et al*., 2014). PDI gene family encodes PDI and PDI-like (PDIL) which contain thioredoxin (TRX) domains. They assist in the proteins to participate in forming both intra- and inter-chain disulfide bonds and isomerisation during protein folding, correctly folding and assembling protein bodies as well as resistance to various biotic and abiotic stresses, and therefore they function as chaperone (D'ALOISIO *et al*., 2010; ZHU *et al*., 2014).

The folding and assembly of the glutenin proteins are assisted by ER lumenal proteins such as PDI and the molecular chaperone binding protein (BiP). Studies showed that the synthesis and accumulation of glutenins during wheat grain development were closely related with the expression of PDI and BiP genes (GRIMWADE *et al*., 1996; DUPONT *et al*., 1998). The recent reports in our laboratory have showed that PDI and PDI-like genes generally displayed high expression levels in the early stages of wheat grain development, consistent with the rapid accumulation of glutenin proteins (WANG *et al*., 2013). In the model species *Brachypodium distachyon* L., *BdPDIL1-1* and *BdPDIL5-1* were found to express abundantly in developing grains, suggesting their important roles in synthesis and accumulation of seed storage proteins (ZHU *et al*., 2014).

In the present study, we used three wheat glutenin near isogenic lines (NILs) developed in our laboratory as materials and performed the first investigation on the transcriptional expression profiles of PDI gene family during grain development. Our purpose is to investigate the relationships between the dynamic expression of different PDI genes and glutenin compositions. The results obtained provide new information for further understanding the roles of PDI gene family involved in glutenin synthesis and wheat quality formation.

MATERIALS AND METHODS

Plant materials

The materials used in this study included three spring wheat glutenin NILs recently developed in our laboratory, named as CB037-A, CB037-B and CB037-C. The NILs with

different HMW-GS allelic compositions at *Glu-D1* locus were developed through crossing between CB037-A with 1Dx2+1Dy12 and powdery mildew resistance and Jing 771 with 1Dx5+1Dy10, and consecutive backcross, self cross and selection while the NILs with different LMW-GS alleles at *Glu-B3* locus were obtained through variant screening during tissue culture. HMW-GS and LMW-GS compositions of three NILs were: CB037-A: HMW-GS (1, 17+18, 2+12), LMW-GS (*Glu-A3c, Glu-B3h, Glu-D3a*); CB037-B: HMW-GS (1, 17+18, 5+10), LMW-GS (*Glu-A3c, Glu-B3h, Glu-D3a*), and CB037-C: HMW-GS (1, 17+18, 5+10), LMW-GS (*Glu-A3c, Glu-B3Null, Glu-D3a*). Two Aroona NILs Ari124-3 (*Glu-A3c, Glu-B3d, Glu-D3c*) and Ari127-6 (*Glu-A3c, Glu-B3g, Glu-D3c*) as well as Chinese Spring (*Glu-A3a, Glu-B3a, Glu-D3a*) were used as controls for LMW-GS allele identification.

Field planting and sampling

Three NILs were planted in the experimental fields of the China Agricultural University, Beijing during the 2014–2015 wheat growing season. Field experiments were performed in randomized block design with three biological replicates (each plot with 12 m^2). The cultivation and management were same as local field cultivation conditions. The developing grains were collected from middle ears at 5, 8, 11, 14, 17, 21, 23, 26, 29 days post anthesis (DPA).The collected samples were immediately put in liquid nitrogen and then stored in -80°C prior to use.

Agronomic traits, grain yield and quality testing

The mature grains from each plot were harvested and the main agronomic traits and grain yield were measured, including plant height, effective spikelet number, grain number per spike, thousand grain weight (TKW) and grain yield (GY, kg/ha.). The main dough quality parameters were tested according to SUN *et al*. (2010) with minor modification. Flour protein content was determined according to American Association of Cereal Chemists Approved Methods (AACC) 39-10A with a near-infrared (NIR) analyzer (Instalab610, Dickey-john Co. Ltd, USA). Falling number (Perten, Sweden) were determined with the methods of AACC 56-81B. Mixograph indexes (Nationalmfg, USA) including water absorption rate, peak time, and time×width were determined according to the method of AACC 54-40A. American Association of Cereal Chemists Approved Method (2000) 54-21 was followed to obtain Farinograph parameters (10gBrabenderFarinograh-E). Bread-making was followed with the method of AACC 10-10B. The internal structure of bread was tested and evaluated by the means of C-CELL (UK CCFRA Company). Data analysis was performed using independent Student’s T-test with SPSS statistical software (version 17.0).

Glutenin macropolymer (GMP) extraction and size-exclusion high-performance liquid chromatography (SE-HPLC)

Grain flours (20 mg) from three biological replicates were oscillated for about 20 min in 1800µL extraction buffer (0.05M PBS with 2% sodium dodecyl sulphate polyacrylamide). After centrifugation for 15 min at 12000g, the supernatant was thrown away and 1800 µL extraction buffer was added again, following by oscillating for about 20 min in ultrasonic instrument and keeping on oscillating for about 1h. After centrifugation for 15 min at 12000g, the supernatant was filtrated into another centrifugal tube, and then centrifuged for 10 min at 13000g and used for following analysis.

Separation and characterization of GMP by SE-HPLC were based on the modified

method of RAKSZEGIA *et al.* (2008). Agllent bio sec-5 column with 5 μm diameter was used, which can produce a column pressure at around 80 bar. 0.05M PBS with 0.1% SDS was used as the mobile phase.

Glutenin extraction and SDS-PAGE

Glutenin proteins were extracted from a half kernel (about 20 mg) and analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) based on YAN *et al.* (2003).

mRNA extraction and qRT-PCR

Total mRNA from 9 grain developmental stages of three NILs was extracted by using Trizol extraction kit (Invitrogen) according to the modified manufacturer's instructions. mRNA with 20~50μg was purified to synthesize cDNA using the PrimeScript™ RT reagent Kit with gDNA Eraser provided by TaKaRa based on the manufacturer's instructions. Specific primers for PDI genes were designed using Primer Premier 5.0. ADP-ribosylation factor was selected as the internal reference gene based on PAOLACCI *et al.* (2009). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed in CFX96 Real Time system (Bio-Rad) with the following conditions: an initial denaturation of 95°C for 4 min followed by 40 cycles of 95°C for 20s, 58°C for 15s and 72°C for 10s. The transcription levels of PDI genes in three biological replicates were quantified using qRT-PCR with SYBR-green as the intercalating dye, and the 2^{-CT} method (LIVAK *et al.*, 2001). Real-time melting temperature curves for each of the PDI genes were tested to obtain a single peak, which was confirmed by agarose gel electrophoresis. The qRT-PCR efficiency was determined by serial five-fold dilutions of cDNA, and high RT-PCR efficiency rates of the standard curve were obtained.

RESULTS

Agronomic traits, yield performance and glutenin compositions of three wheat glutenin NILs

The field performance of three wheat glutenin NILs was showed in Figure S1 and the results of main agronomic traits and yield testing were listed in Table S1. In general, three NILs had similar growing properties, agronomic traits and yield performance. They had strong ability for tilling and disease resistance, especially for powdery mildew (Figure S1a). The dynamic developmental patterns of grains in three NILs were similar (Figure S1b). In addition, ear morphology, grain sizes and weight after maturity also had no significant differences (Figure S1c).

The glutenin compositions at *Glu-1* and *Glu-3* loci were determined by SDS-PAGE (Figure 1). CB037-A and CB037-B showed same compositions at *Glu-A1* (1Ax1) and *Glu-B1* (1Bx17+1By18), but had different allelic compositions at *Glu-D1* locus with 1Dx2+1Dy12 and 1Dx5+1Dy10, respectively. Similarly, CB037-C only had one allele difference at *Glu-B3* locus with CB037-B: *Glu-B3h* silence. These results demonstrated that the differences at glutenin compositions have no significant effects on plant development, agronomic trait and yield performance.

Comparative analysis of quality properties of three wheat glutenin NILs

The main quality parameters of three wheat glutenin NILs were showed in Table 1. The results indicated that flour quality in CB037-B with 1Dx5+1Dy10 was significantly improved compared to CB037-A with 1Dx2+1Dy12, including significantly increasing GMP content,

MixographandFarinograph parameters, loaf sizes and scores as well as much improved bread interior structures. Particularly, the GMP content during main grain developmental stages of CB037-B determined by SE-HPLC was significant higher than those of CB037-A (Figure 2). Our results further confirmed previous reports that 1Dx5+1Dy10 can improve gluten strength and lead to superior breadmaking quality compared to 1Dx2+1Dy12 (LIU *et al*., 2012; WANG *et al*., 2013).

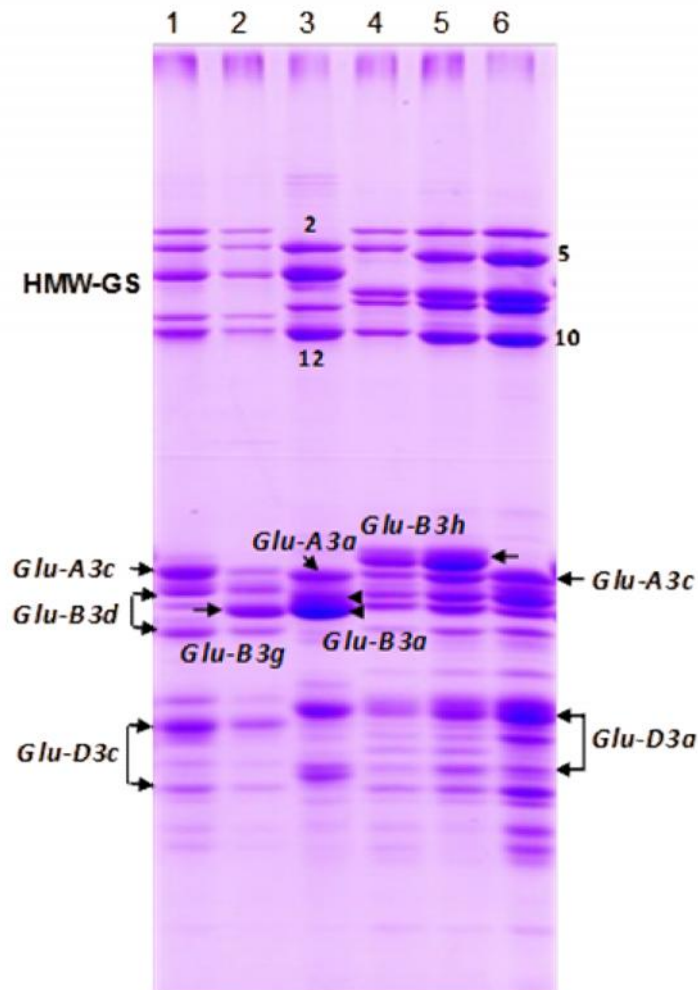

Figure 1. SDS-PAGE of glutenins in three CB037 NILs. 1. Ari124-3 (*Glu-A3c, Glu-B3d, Glu-D3c*); 2. Ari127-6 (*Glu-A3c, Glu-B3g, Glu-D3c*); 3. Chinese Spring (*Glu-A3a, Glu-B3a, Glu-D3a*); 4. CB037-A (*Glu-A3c, Glu-B3h, Glu-D3a*); 5. CB037-B (*Glu-A3c,Glu-B3h, Glu-D3a*); 6. CB037-C (*Glu-A3c, Glu-B3 null, Glu-D3a*).

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Table 1. Main quality parameters of three wheat glutenin near isogenic lines (NILs).

Quality parameters	CB037-A	CB037-B	CB037-C
Protein(%)	13.3±0.8	13.3±0.9	14.5±0.9
GMP (%)	6.38±0.2	7.66±0.3**	6.51±0.2**
Falling number	332.6±9.8	308.3±6.1**	325.7±6.8**
Peak time (min)	2.42±0.04	3.15±0.05**	2.67±0.04**
Time×width(%)	5.57±0.8	6.26±0.9**	5.46±0.8**
Development time (min)	3.9±0.2	4.6±0.2**	3.5±0.2**
Stability(min)	4.5±0.1	5.8±0.1**	4.2±0.1**
Loaf volume (ml^3)	750±15	820±18*	720±12**
Loafscore (100)	69±2	78±3**	65±1**
Slice brightness	128.4±0.5	156.8±0.7**	132.5±0.6**
Slice area/px	236721±1406	249855±1524**	240817±1485*
Attenuation ratio	58.2±0.82	66.5±0.75*	56.7±0.65**
Cell contrast	0.6±0.001	0.7±0.001*	0.6±0.001*
Cell extension	1.6±0.03	1.7±0.02*	1.6±0.02*
Cell diameter/px	17.5±0.21	18.4±0.23*	17.7±0.25*
Cell quantity	2147±25	2286±26*	2196±22*

* and **: significance at 5% and 1% levels, respectively.

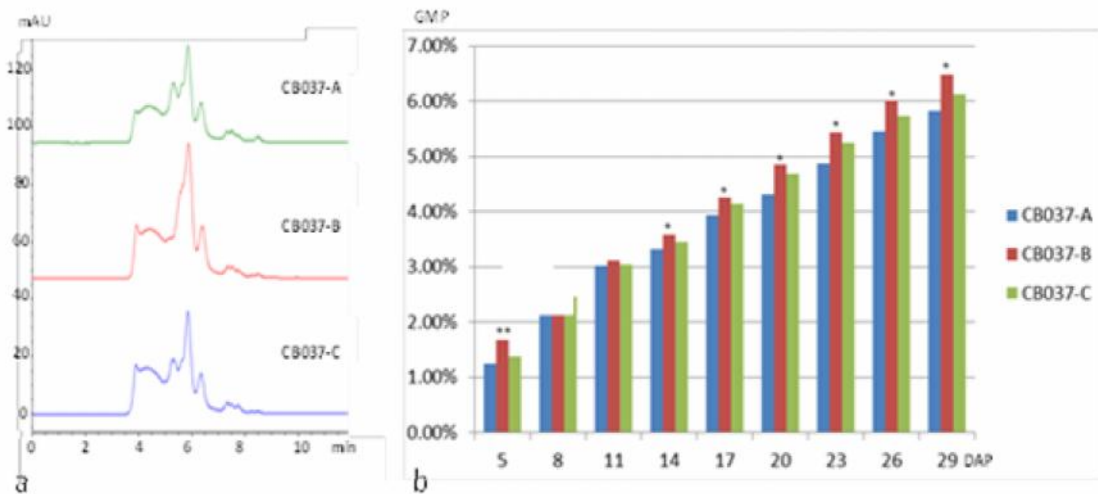

Figure 2. GMP content changes during nine grain developmental stages of three spring wheat NILs detected by SE-HPLC. a. GMP detection of mature grains from CB037-A, CB037-B and CB037-C by SE-HPLC; b. Changes of GMP content during nine grain developmental stages of three NILs.

When *Glu-B3h* is silent in CB037-C, dough strength and breadmaking quality were significantly decreased (Table 1). For example, GMP content from main grain developmental stages of CB037-C (Figure 2), MixographandFarinograph parameters, loaf sizes and scores, and C-Cell parameters in CB037-C (Table 1) were significantly lower than those of CB037-B. Since *Glu-B3h* encodes a larger B-subunit with higher expression amount (Figure 1), its silence could result in significant decrease of gluten strength and breadmaking quality.

Dynamic expression profiles of different PDI genes during grain development of three wheat glutenin

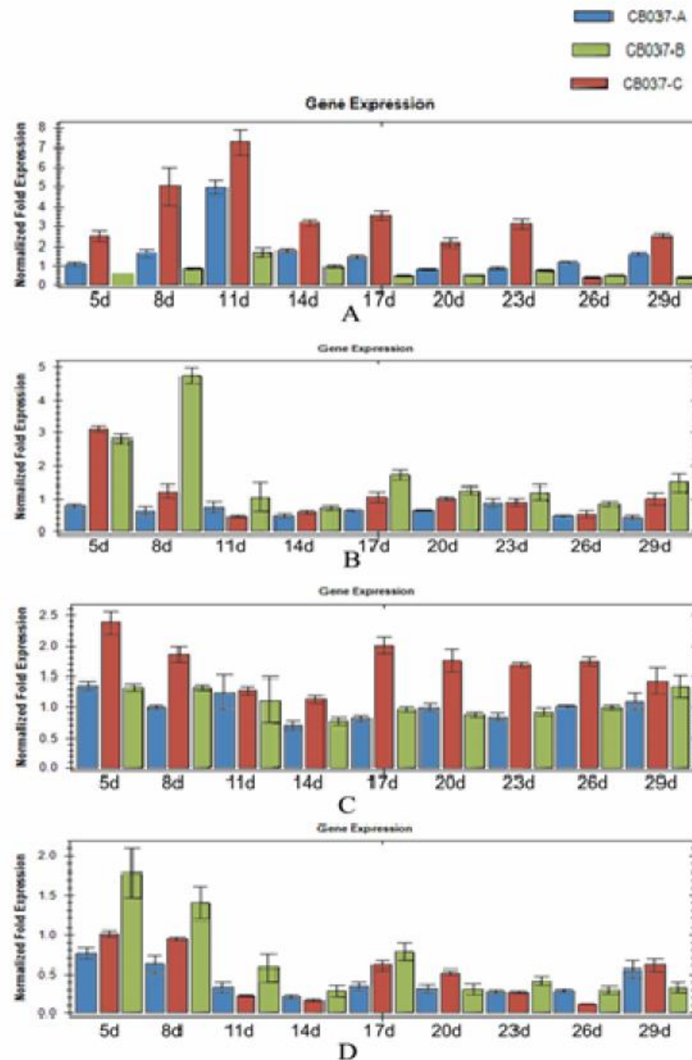

Figure 3. Transcriptional expression profiles of 4 PDI genes during nine grain developmental stages of three spring wheat NILs. A. *PDI1-1*; B. *PDI3-1*; C. *PDI4-1*; D. *PDI5-1*.

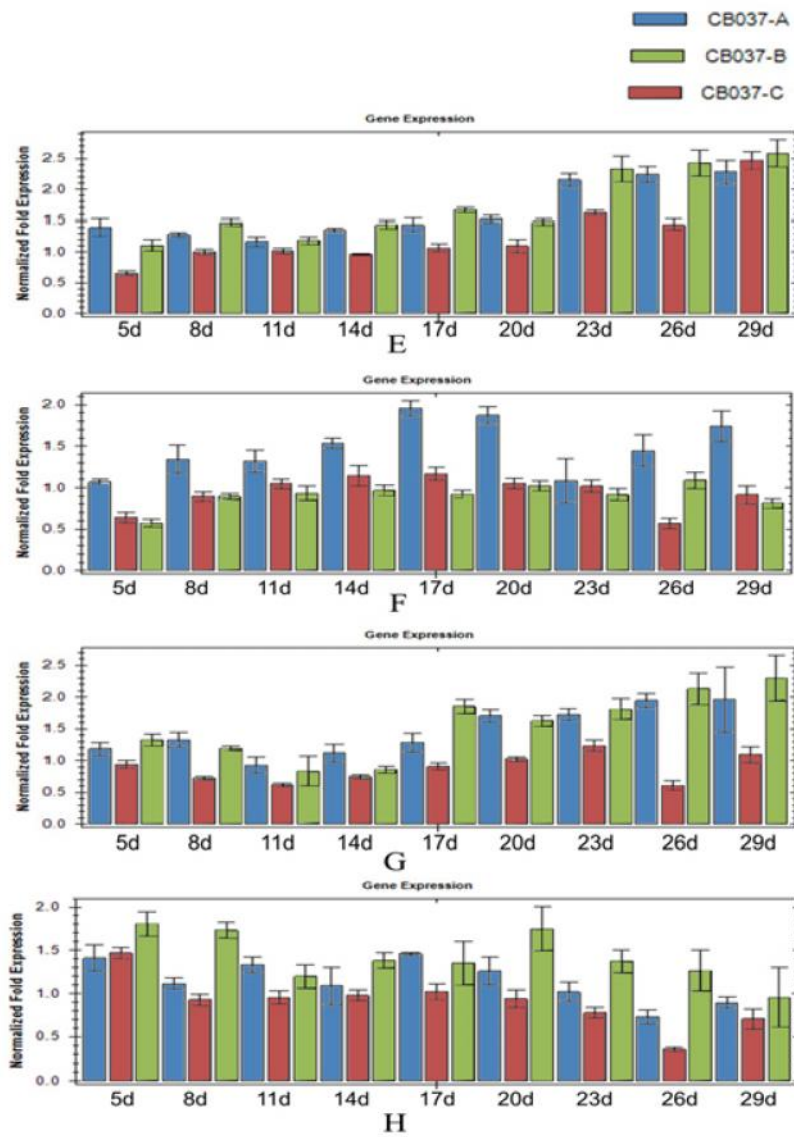

Figure 4. Transcriptional expression profiles of 4 PDI genes during nine grain developmental stages of three spring wheat NILs. E. *PDI6-1*; F. *PDI7-1*; G. *PDI7-2*; H. *PDI8-1*.

Dynamic expression profiles of eight PDI genes (*PDI1-1*, *PDI3-1*, *PDI4-1*, *PDI5-1*, *PDI6-1*, *PDI7-1*, *PDI7-2* and *PDI8-1*) during eight grain developmental stages in three wheat glutenin NILs were detected by qRT-PCR (Figures 3 and 4). Specific primers for eight PDI genes from eight PDI subfamilies respectively were designed (Table 2). RT-PCR amplification specificity and efficiency for each gene were optimized, and high specificity and efficiency rates were obtained as shown in Figure S2-7.

Table 2. qRT-PCR primers for PDI genes

PDI genes	Forward	Reverse
PDI1-1	5'-GGATGCCCAGATGGTTCCT-3'	5'-CCGATTTGCTGAATGCTGACA-3'
PDI3-1	5'-GATATGCACTTGCCTTGTTG-3'	5'-GACTTCTTCCGTTTTCAG-3'
PDI4-1	5'-CTGGACCTTCTGTGAATCAT-3'	5'-ACAGCGCCCCTCTGTAATTTC-3'
PDI5-1	5'-AAACACGTATTGGTGGCGA-3'	5'-TTATGCTAGTCACTCTACGA-3'
PDI6-1	5'-AGCGCCATTCAACCTTATC-3'	5'-TACTCTGGCCCTCATCGGG-3'
PDI7-1	5'-ATGGGCAATTGCTTCTAGG-3'	5'-AATCAGTGCTGAGTACTCC-3'
PDI7-2	5'-ATTGGACCTGATGTTCACC-3'	5'-GAAATGCAGTGAGACCTCT-3'
PDI8-1	5'-GACTTATGCAAGTTTCA-3'	5'-AACTTTGCCAAGGCGACC-T3'
ADP	5'- GACATATCTAAGCTAGC-3'	5'- GCATCTGTAACCCGTGACC -3'

In general, eight PDI genes showed multiple expression patterns during grain development among three glutenin NILs (Figure 3 and 4). The expression level of *PDI3-1* in CB037-A as well as *PDI4-1* in both CB037-A and CB037-B were relatively stable and had no clear changes during grain development. *PDI6-1* showed an upregulated expression in three NILs while *PDI3-1, PDI5-1*and *PDI8-1* were downregulated in CB037-A or CB037-C. *PDI1-1* in three NILs, *PDI3-1* and *PDI7-1* in CB037-B exhibited an up-down expression while *PDI4-1* in CB037C, *PDI7-2* in both CB037-A and CB037-C, and *PDI8-1* in CB037-C displayed an opposite down-up expression. In addition, *PDI7-1* in both CB037-A and CB037-C showed an up-down-up pattern while *PDI5-1*, *PDI7-2* and *PDI8-1* in CB037-B displayed an opposite down-up-down model. *PDI5-1* in CB037-C showed a down-up-down-up expression pattern.

Comparison of PDI gene expression during grain development between CB037-A and CB037-B showed that the expression levels of *PDI3-1* and *PDI5-1* in CB037-B were significantly higher than CB037-A, especially at the early developmental stages 5-8 DPA (Figure 3B and 3D). *PDI8-1* in CB037-B showed clearly higher expression level than CB037-A during all grain developmental stages (Figure 4H). On the contrary, the expression of *PDI7-1* in CB037-A was remarkably higher than CB037-B (Figure 4F). The remaining PDI genes had no clear expression differences between them during grain development.

Glu-B3h silence could result in significant expression changes of PDI genes. Five PDI genes (*PDI3-1, PDI5-1, PDI6-1, PDI7-2* and *PDI8-1*) in CB037-B showed significantly reduced expression levels in CB037-C. Particularly, both *PDI3-1* and *PDI5-1* in CB037-B had a remarkable upregulation from 5 to 8 DPA (Figure 3B and 3D). Contrarily, the expression levels of both *PDI1-1* and *PDI4-1* in CB037-C were significantly higher than those in CB037-B during all grain developmental stages (Figure 3A and 3C).

DISCUSSION

It is generally accepted that HMW-GS 5+10 can produce superior dough quality compared to 2+12 subunits (SARKAR *et al.*, 2014). Possible reasons include the number and position of cysteine residues (WEEGELS *et al.*, 1996; PIROZI *et al.*, 2008), the accumulation rate differences during grain development (XU *et al.*, 2006; GAO *et al.*, 2012; LIU *et al.*, 2012; WANG *et al.*, 2013) and the formation of inter- or intra-molecular disulfide bonds which can stabilize the structure and increase the number of glutenin macropolymer (ALTENBACH *et al.*, 2007; LAUDENCIA-CHINGCUANCO *et al.*, 2007; SHEWRY *et al.*, 2009; LI *et al.*, 2010). Our results from this work further confirmed that CB037-B with HMW-GS 5+10 subunits has higher GMP content and better mixing property and breadmaking quality than CB037-A with 2+12 subunits (Table 1 and Figure 2). When *Glu-B3h* gene encoding one abundant B-subunit lacked, dough strength and breadmaking quality were significantly reduced (Table 1), demonstrating its important roles in gluten formation and quality performance. Previous studies confirmed that HMW-GS overexpression and LMW-GS with higher expression amount such as B-subunits have significant effects on dough properties and gluten quality (D'OVIDIO *et al.*, 2004; LI *et al.*, 2014).

PDI family proteins can catalyze the formation of disulfide bonds, and likely repair the cleaved disulfide bonds or re-form new disulfide bonds between glutenin subunits that are important for GMP formation (KOH *et al.*, 2010). Wheat cultivars with high GMP content have strong dough strength, good dough elasticity, superior breadmaking quality and fresh noodle extension characters (LIU *et al.*, 2014). Our results demonstrated that the expression patterns of certain PDI genes at early developmental stages are closely associated with glutenin allelic compositions, suggesting their important roles for glutenin subunits assembling, folding, and GMP formation. Among 8 PDI genes investigated, we found the close correlations between higher expression levels of *PDI3-1*, *PDI5-1* and *PDI8-1* during grain development, particularly at early stages and the presence of 5+10 subunits conferring superior gluten quality (Figure 3 and 4). High expression of PDI gene at early stages from 5 to 14 DPA would facilitate right disulfide bond formation or wrong disulfide bond cleaving, and then enhance the synthesis and accumulation rate of glutenin subunits. When the abundant B-subunit encoded by *Glu-B3h* lacked, the GMP content was significantly reduced and the expression levels of five PDI genes (*PDI3-1, PDI5-1, PDI6-1, PDI7-2* and *PDI8-1*) were significantly decreased, indicating that these PDI genes are involved in glutenin synthesis and GMP formation.

Previous studies showed that PDI proteins TaPDIL1-1, TaPDIL3-1, TaPDIL4-1, TaPDIL5-1, TaPDIL6-1, TaPDIL7-1 and TaPDIL7-2 contain -CXXC- active site, except TaPDIL8-1, catalyzing the thiol–disulfide exchange reactions essential for the maintenance of the correct thiol redox state of proteins, which boost disulfide bonds formation between substrate. Thus, PDIs may play important roles in forming GMP (D'ALOISIO *et al.*, 2010; SHEPHERD *et al.*, 2013). Every PDI subfamily has conserved arginine modulating the pKa of the active-site cysteine residues by moving into and out of the active-site locale. To act as an oxidant, the dithiol state of the enzyme must be stabilised relative to the disulphide state. To some extent, through the stabilisation of the thiolate state of the N-terminal cysteine residue and the destabilisation of the thiolate state of the C-terminal cysteine residue, the pKa value of the N-terminal cysteine residue of PDI is very low and that of the C-terminal cysteine residue is very high (LAPPI *et al.*, 2004).This not only helps make the protein more oxidising, but also increases the nucleophilicity of the N-terminal cysteine and decreases that of the C-terminal cysteine residue, promoting the formation of mixed disulphides with substrate and inhibiting the reverse reaction (LAPPI *et al.*,

2004). The structural features of PDIs suggested that they would be involved in disulfide bonds formation and storage protein folding (ELLGAARD *et al.*, 2005). There are two conserved charged pairs near the active site in TaPDIL1-1, TaPDIL4-1 and TaPDIL5-1, which are important for the catalytic activity of the thioredoxin (D'ALOISIO *et al.*, 2010) and for the oxidative activity of the human PDI (ELLGAARD *et al.*, 2005).

Our results indicated that *PDI3-1, PDI5-1, PDI6-1, PDI7-2* and *PDI8-1* are closely related to glutenin synthesis and GMP formation (Figure 3 and 4). Particularly, PDI5-1 includes three determinants benefiting disulfide bonds formation. *PDI6-1* has both -CXXC- active site and conserved arginine, while *PDI7-2* contains a -CXXC- active site in combination with three other determinants: the conserved arginine residue, the charged pair near the active site and a high-affinity substrate-binding site in a non-catalytic domain. These structural features may be helpful for the function of PDI family.

CONCLUSION

Analysis from three wheat glutenin NILs showed that glutenin allelic variations significantly affect dough strength and breadmaking quality, but main agronomic traits and yield performance had no clear changes. HMW-GS 5+10 led to higher GMP content and better breadmaking quality than the allelic pair 2+12. The slience of *Glu-B3h* encoding one abundant B-subunit significantly reduced GMP content, dough strength and breadmaking quality. The genotypes with 5+10 subunits had higher expression levels of *PDI3-1*, *PDI5-1* and *PDI8-1* compared to those with the allelic pair 2+12. Five PDI genes (*PDI3-1, PDI5-1, PDI6-1, PDI7-2* and *PDI8-1*) were significantly downregulated when *Glu-B3h* was silent, suggesting their important roles in glutenin and GMP synthesis and gluten quality formation.

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KVALITATIVNA SVOJSTVA I PROFILISANJE EKSPRESIJE GENA PROTEIN DISULFID IZOMERAZE TOKOM RAZVOJA ZRNA TRI JARE BLISKO IZOGENE LINIJE PŠENICE

Liwei DONG[1,4], Ning LI[1,4], Xiaobing LU[1,4], Slaven PRODANOVIC[2*], Yanhao XU[3], Wenying ZHANG[3], Yueming YAN[1,3*]

[1]Biološki fakultet, Capital Normal Univerzitet, 100048 Peking, Kina
[2]Poljoprivredni fakultet, Univerzitet u Beogradu, 11080 Beograd, Srbija
[3]Hubei saradni ki inovacioni centar za industriju zrna - 434025 Jingzhou, Kina
[4] podjednak doprinos autora

Izvod

U ovom istraživanju koriš ene su tri gluteninske blisko izogene linije (BIL) pšenice CB037A, CB037B i CB037C radi ispitivanja njihovih kvalitativnih svojstava i transkripcionih profila ekspresije PDI familije gena tokom razvoja zrna. Cilj našeg rada je razumevanje odnosa izme u dinami ke ekspresije razli itih PDI gena i sastava gluteninskih alela povezanih sa kvalitetom glutena. Rezultati su pokazali da alelne varijacije glutenina nemaju zna ajan uticaj na glavne agronomske osobine i prinos, ali su uticale na jasne promene kvaliteta glutena. CB037B sa 5+10 subjedinicama je imao ve i sadržaj gluteninskog makropolimera (GMP) i bolji pekarski kvalitet nego CB037A sa 2+12, dok je nedostatak *Glu-B3h* koji kodira za jednu obilnu B-subjedinicu kod CB037C zna ajno smanjio sadržaj GMP, rastegljivost testa i pekarski kvalitet. Dinami ki obrasci ekspresije osam protein disulfid izomeraznih (PDI) gena tokom razvoja zrna, koji su detektovani pomo u reakcije kvantitativne PCR u stvarnom vremenu (qRT-PCR), pokazali su blisku povezanost izme u ve ih nivoa ekspresije *PDI3-1*, *PDI5-1* i *PDI8-1* i prisustva 5+10 subjedinica. Me utim, utišavanje *Glu-B3h* rezultirao je u zna ajnom smanjenju nivoa ekspresije pet PDI gena (*PDI3-1, PDI5-1, PDI6-1, PDI7-2* i *PDI8-1*), ukazuju i na vitalnu ulogu odre enih PDI gena u sintezi glutenina i GMP i obrazovanju kvaliteta gluten.

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Supplementary Table:

Table S1. Agronomic traits and yield performance of three wheat glutenin near isogenic lines (NILs)

Traits	CB037-A	CB037-B	CB037-C
Tilling number	6.2±0.07	6.1±0.05	5.9±0.06
Plant height (cm)	60.3±0.41	61.9±0.43	61.6±0.42
Growing period (day)	112.3±0.58	111.2±0.41	111.4±0.65
Main ear length (cm)	9.3±0.08	9.7±0.07	10.6±0.06
Effective ears	17.1±0.05	16.6±0.08	16.7±0.09
Ear grain number	63.3±0.31	64.2±0.42	61.8±0.45
Ear grain weight (g)	2.9±0.03	2.7±0.04	2.8±0.03
1000-grain weight (g)	36.1±0.12	35.6±0.23	36.3±0.22
Grain yield (kg/ha.)	4312±55	4363±62	4378±58

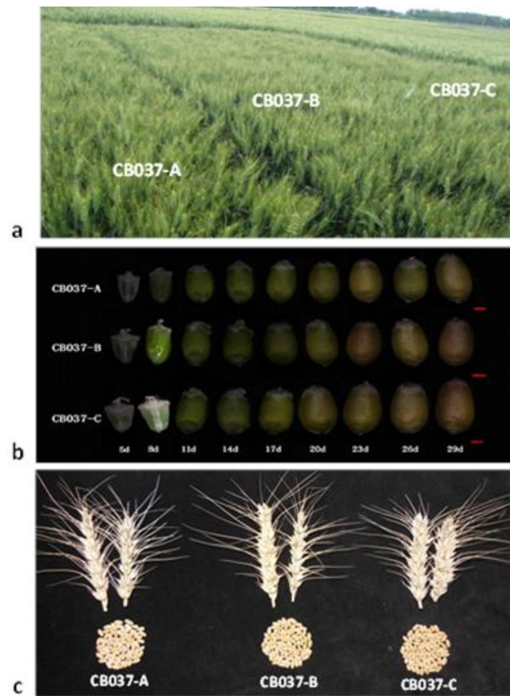

Figure S1. Field performance (a), grain development (b) and morphological features of mature spikes and seeds (c) of three spring wheat NILs.

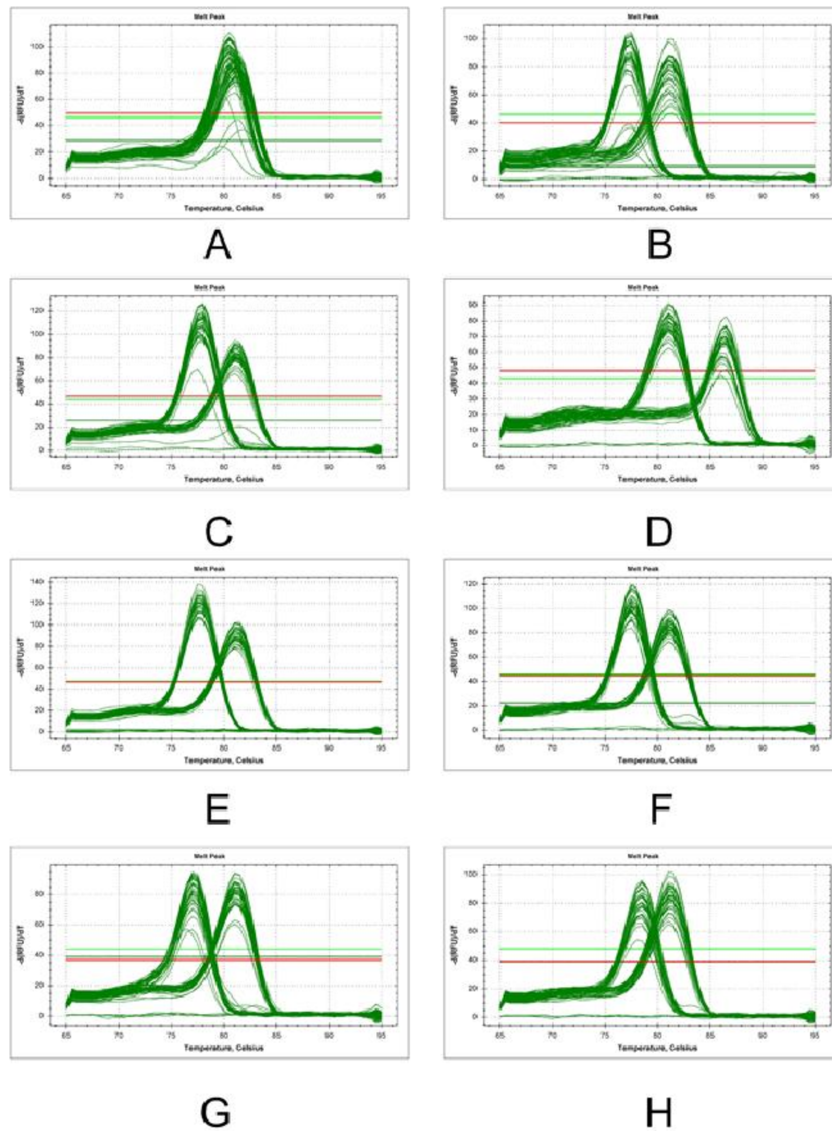

Figure S2. Dissolution curves of 8 PDI genes in CB037A. A. *PDI1-1*; B. *PDI3-1*; C. *PDI4-1*; D. *PDI5-1*; E. *PDI6-1*; F. *PDI7-1*; G. *PDI7-2*; H. *PDI8-1*. The red standard curves represent PDI genes and the green standard curves represent the reference gene. The dissolution curves of different genes are indicated.

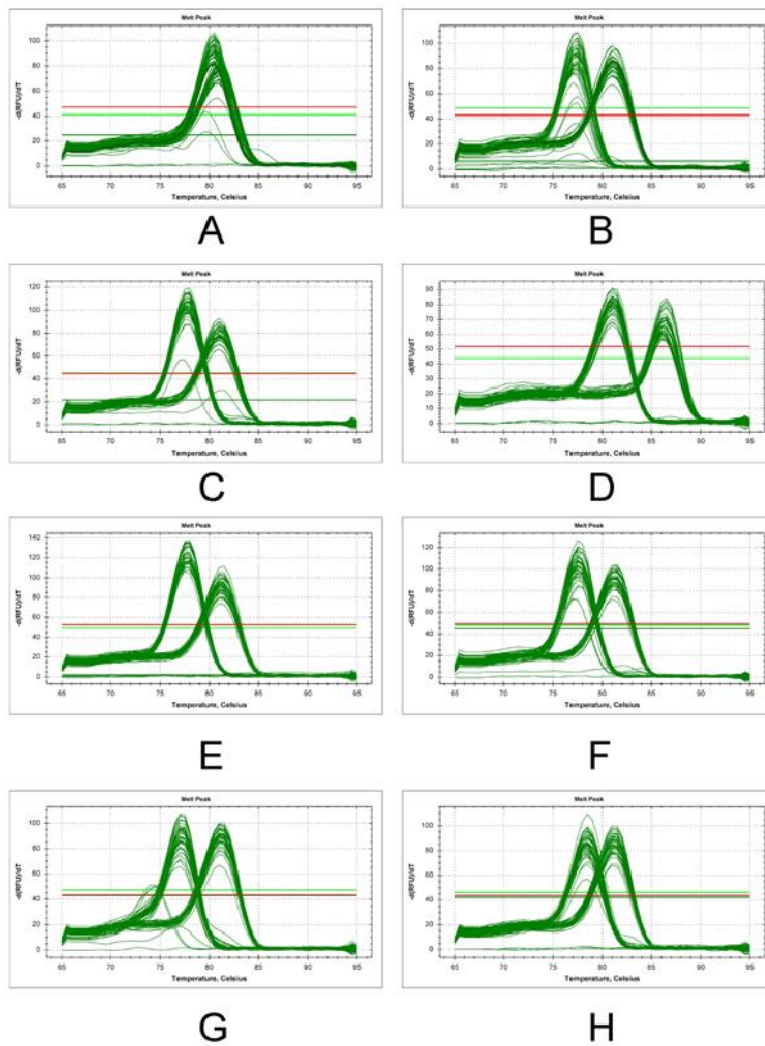

Figure S3. Dissolution curves of 8 PDI genes in CB037B. A. *PDI1-1*; B. *PDI3-1*; C. *PDI4-1*; D. *PDI5-1*; E. *PDI6-1*; F. *PDI7-1*; G. *PDI7-2*; H. *PDI8-1*. The red standard curves represent PDI genes and the green standard curves represent the reference gene. The dissolution curves of different genes are indicated.

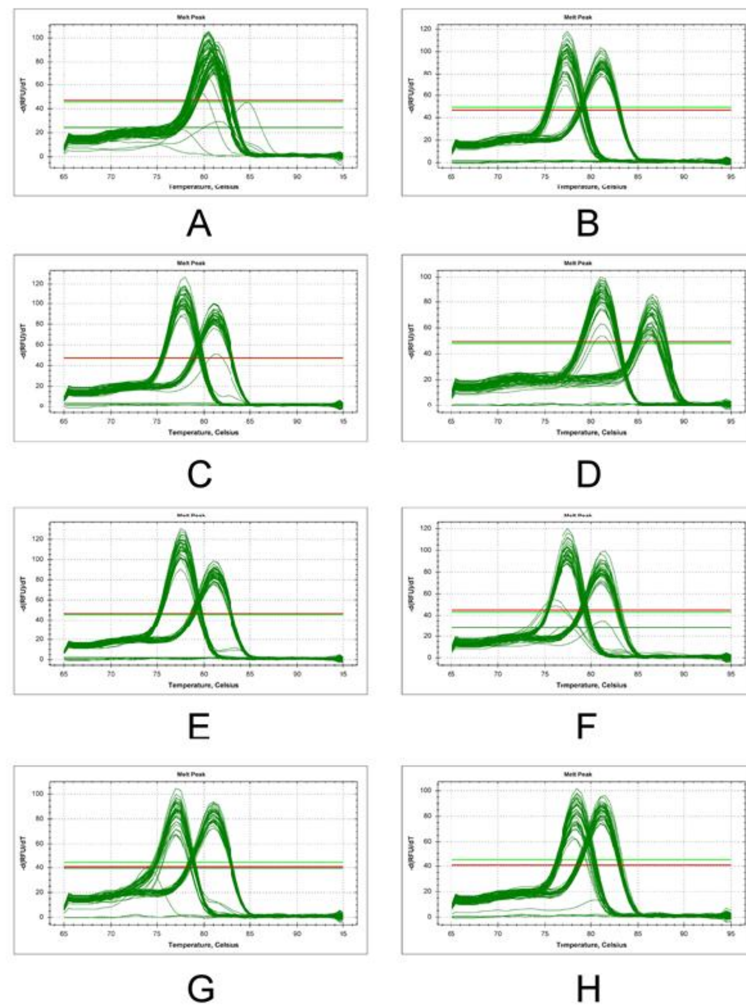

Figure S4. Dissolution curves of 8 PDI genes in CB037C. A. *PDI1-1*; B. *PDI3-1*; C. *PDI4-1*; D. *PDI5-1*; E. *PDI6-1*; F. *PDI7-1*; G. *PDI7-2*; H. *PDI8-1*. The red standard curves represent PDI genes and the green standard curves represent the reference gene. The dissolution curves of different genes are indicated.

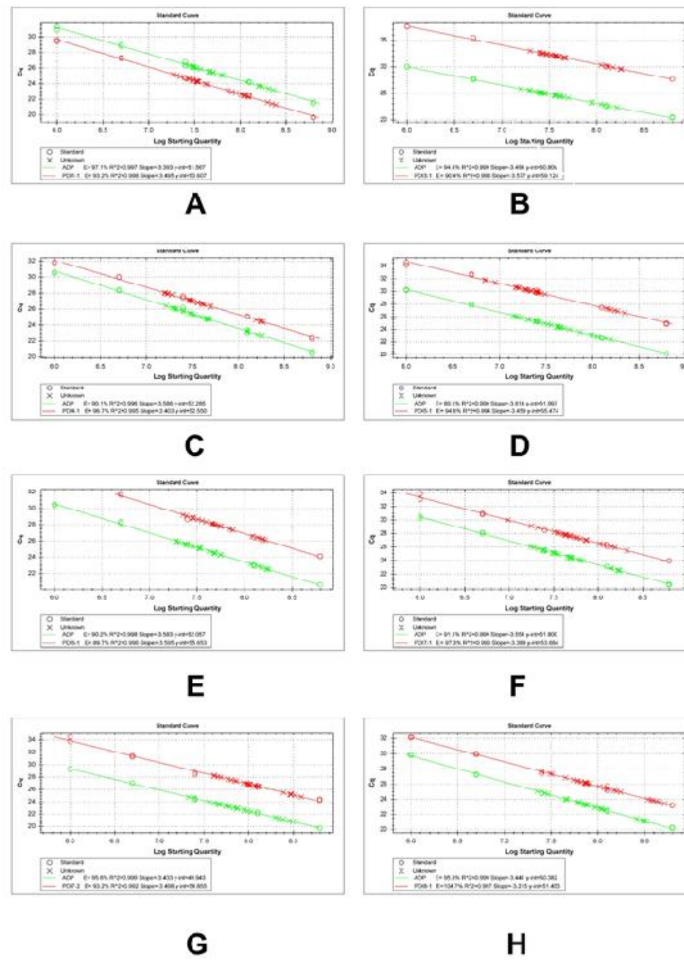

Figure S5. Double standard curves of 8 PDI genes in CB037A. A. *PDI1-1*; B. *PDI3-1*; C. *PDI4-1*; D. *PDI5-1*; E. *PDI6-1*; F. *PDI7-1*; G. *PDI7-2*; H. *PDI8-1*.

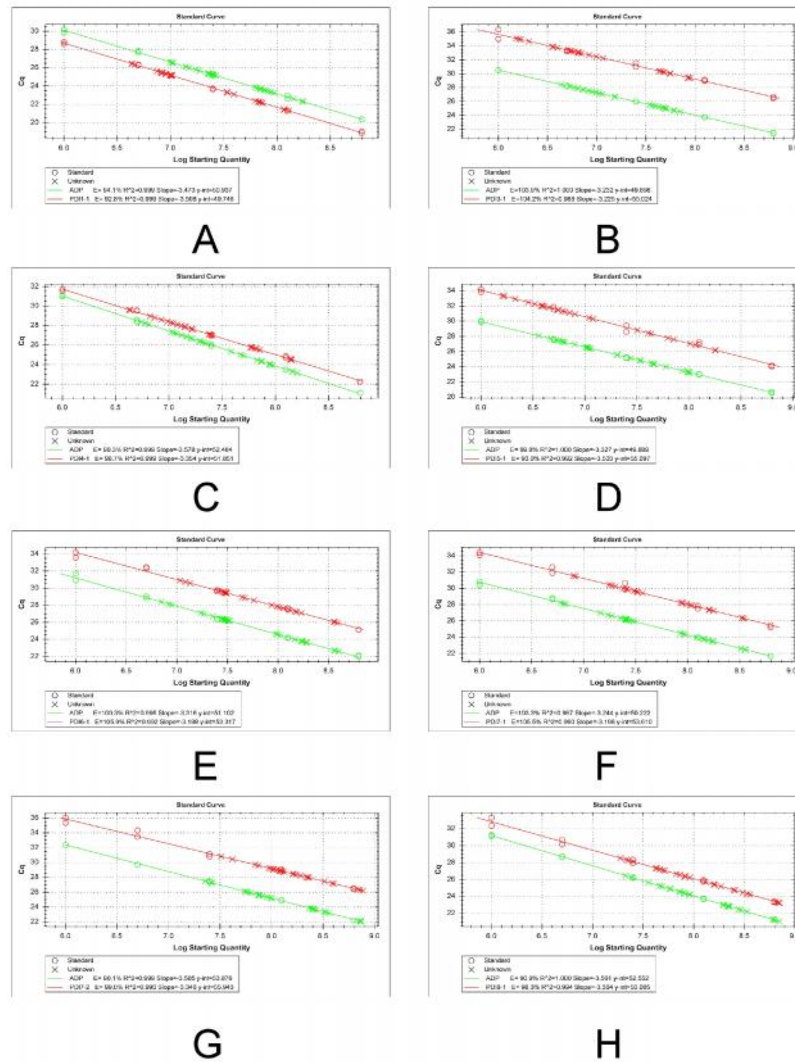

Figure S6. Double standard curves of 8 PDI genes in CB037B. A. *PDI1-1*; B. *PDI3-1*; C. *PDI4-1*; D. *PDI5-1*; E. *PDI6-1*; F. *PDI7-1*; G. *PDI7-2*; H. *PDI8-1*.

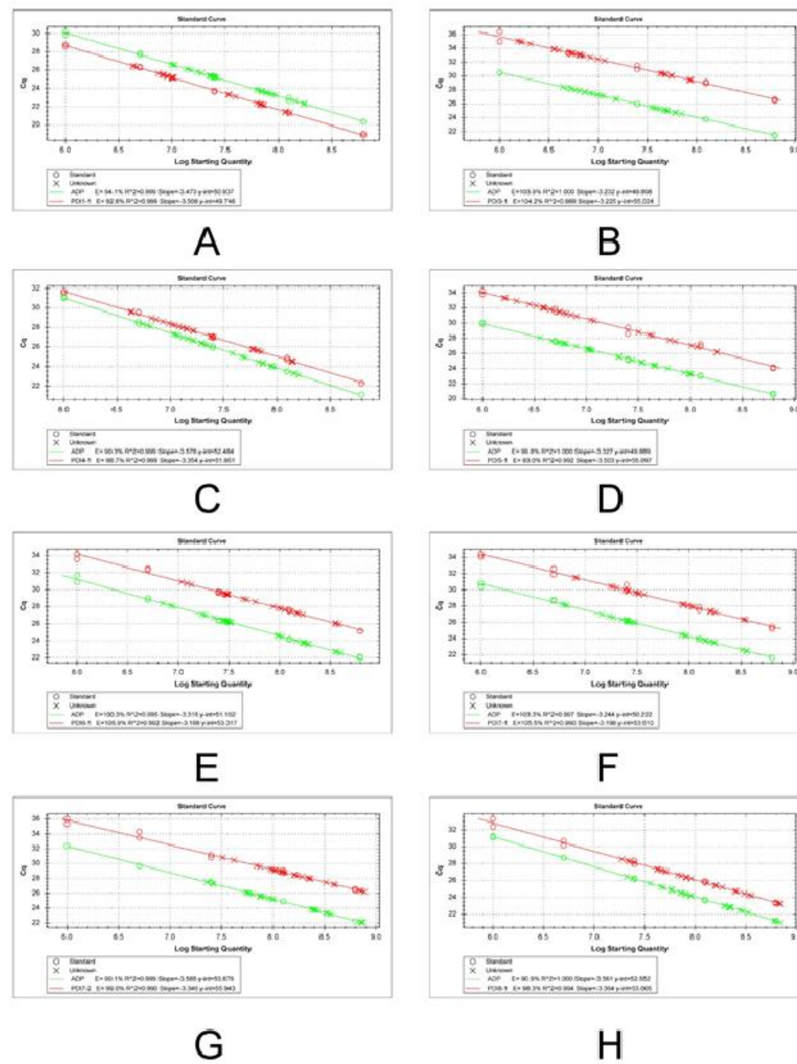

Figure S7. Double standard curves of 8 PDI genes in CB037C. A. *PDI1-1*; B. *PDI3-1*; C. *PDI4-1*; D. *PDI5-1*; E. *PDI6-1*; F. *PDI7-1*; G. *PDI7-2*; H. *PDI8-1*.