RESEARCH ARTICLE

Quality of synthetic hexaploid wheat containing null alleles at *Glu-A1* and *Glu-B1* loci

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Abstract

Triticum turgidum ssp. *dicoccon* PI94668 and PI349045 were identified as containing null alleles at *Glu-A1* and *Glu-B1* loci in previous investigation. Sequencing of the respective HMW-GS genes *Ax*, *Bx*, *Ay* and *By* in both accessions indicated equal DNA lengths with gene silencing caused by 1 to 4 in-frame stop codon(s) in the open reading frames. Six synthetic hexaploid wheat lines were produced by crossing PI94668 or PI349045 with six *Aegilops tauschii* by spontaneous chromosome doubling of unreduced gametes. As expected, these amphiploids had three different HMW-GS: Dx $3.1^t + Dy11^{*t}$, Dx$2.1^t + 10^t$ and Dx$2^t + Dy12^t$ in *Glu-D1* but double nulls in *Glu-A1* and *Glu-B1*. Quality tests showed that most quality parameters in two *T. turgidum* ssp. *dicoccon* parents were very low due to the lack of HMW-GSs. However, incorporation of HMW-GS from *Ae. tauschii* in six synthetic hexaploid wheat lines significantly increased most quality related parameters. The potential values of these wheat lines in improving the quality of wheat are discussed.

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Introduction

High-molecular-weight (HMW) glutenins are important quality determinants of wheat flours. In bread wheat, *Triticum aestivum* L. ($2n = 6x = 42$), HMW glutenins are encoded by three sets of gene loci *Glu-A1, Glu-B1* and *Glu-D1* located on the long arm of homologous group one chromosomes (Shewry *et al.* 1992). Each locus contains two tightly linked genes that encode a larger 'x' subunit and a smaller 'y' subunit (Harberd *et al.* 1986). These HMW glutenin genes are silenced at varying degrees in hexaploid wheat with *Glu-Ay* gene always silenced, *Glu-Ax*, *Glu-Bx* and *Glu-By* genes occasionally silenced while the x and y genes in *Glu-D1* always expressed. Consequently, the numbers of HMW glutenin subunits (HMW-GS) among bread wheat cultivars ranges from three to five (Payne *et al.* 1981).

The association of end-use quality of wheat flours with the quantity and quality of functional HMW-GS has long been recognized (Shewry *et al.* 2003). Integration of additional HMW-GS using transgenic approaches have indicated that overexpression of a single HMW-GS gene is sufficient to alter the gluten complex structure and processing properties of wheat (Barro *et al.* 1997; Rooke *et al.* 1999). In bread wheat, deficiencies of a single or a pair of HMW-GS on dough and baking quality of wheat flours were studied extensively using near isogenic lines (NILs) deficient in various subunit combinations at each locus (Payne *et al.* 1987; Lawrence *et al.* 1988; Rogers *et al.* 1991; Payne and Seekings 1996; Margiotta *et al.* 2000). In lines lacking the x or y subunit encoded by *Glu-D1*, the end-use quality of wheat flours was greatly reduced suggesting the negative influence of decreased HMW-GS numbers on dough and baking quality of wheat flour (Lawrence *et al.* 1988; Rogers *et al.* 1991).

The effect of silencing the HMW-GS genes in *Glu-A1* and *Glu-B1* loci in *Triticum turgidum* ssp. *dicoccon* accessions PI94668 and PI349045 and their quality potentials has not been studied thus far. Therefore, the present study was conducted with the following objectives: (i) to elucidate the reasons behind HMW-GS gene silencing in PI94668 and

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PI349045, and (ii) to evaluate the quality of HMW-GS double null alleles in hexaploid wheat background. In this study, we identified six synthetic hexaploid wheat lines PI94668/As 60 (As 2387, As 2388) and PI349045/As 66 (As 88, As 85) containing null alleles at *Glu-A1* and *Glu-B1* loci in two *T. turgidum* ssp. *dicoccon* accessions, PI94668 and PI 349045 (Vallega and Waines 1987), and *Glu-D1* alleles in six *Aegilops tauschii* including As 60, As 88, As 85, As 2387, As 2386 and As 66 ($2n = 2x = 14$, DD).

Material and methods

Plant material

T. turgidum ssp. *dicoccon* accessions, PI94668 and PI349045 ($2n = 4x = 28$, AABB) were obtained from the USDA-ARS germplasm bank (http://www.ars-grin.gov). Six *Ae. tauschii* accessions ($2n = 2x = 14$, DD) belonging to two subspecies *tauschii* (As 60, As 66, As 85 and As 88) and *strangulata* (As 2386 and As 2387) originated from Iran. Six synthetic hexaploid wheat lines were produced in our laboratory by spontaneous chromosome doubling of unreduced gametes of F_1 obtained from a cross between *T. turgidum* ssp. *dicoccon* and *Ae. tauschii* ($2n = 3x = 21$, ABD). These were selfpollinated for at least four generations. A weak gluten wheat cv. Chuannong 16 and six synthetic wheat lines were planted in Chengdu Wenjiang farm in the year 2012 and *T. turgidum* ssp. *dicoccon* accessions were grown in the same farm in years 2011 and 2012 with three replications. The seeds from the synthetic wheat lines were manually harvested.

Sodium-dodecyl sulphate (SDS)-PAGE

HMW-GS in the seeds of newly produced wheat lines were analysed using 10% nondenaturing and 10% denaturing SDS-PAGE gels containing 4% urea (Yan *et al.* 2002). Briefly, proteins from four μg of powdered endosperms were extracted in 100 μL extraction buffer containing 0.0625 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 1.5% DTT and 0.002% bromophenol blue at room temperature for 3 h with occasional mixing. After denaturing for 5 min in boiling water homogenates were centrifuged at 10,000 rpm for 5 min. Five μL of supernatant was then separated on vertical SDS-PAGE gels.

PCR amplification and cloning

To amplify the four ORFs of HMW-GS in PI94668 and PI349045 by PCR, primers P1: 5′-ATCACCCACAACACCGAGCA-3′ and P2: 5′-AGCTGCAGAGAGTTCTATCA-3′ were used. All amplifications were carried out in an ABI 9700 DNA cycler Q1 87 (PE company, USA) in 50 μL reaction volumes containing 200–300 ng template DNA, 1.25 U high fidelity *ExTaq* polymerase (Takara, Dalian, China), 1× *ExTaq* PCR buffer, 0.2 mM of each dNTP and 1 μM of each primer. PCR conditions were: denaturation of the template DNA at 94°C for 5 min, followed by 24 cycles at 94°C for 40 s and 68°C for 8 min, and a final extension at 68°C for 15 min.

All PCR products were purified using BioTeke Gel Recovery kit (DP1403, Beijing, China) after separation on 0.8% agarose gels followed by ligation into *pMD18-T* vectors (Takara, Dalian, China) and transformation into chemically competent *Escherichia coli* DH 10B. Full length sequences were obtained by sequencing a set of overlapping subclones, which were made by nested deletion methods (Yan *et al.* 2002).

Testing quality parameters

Flour samples were produced by milling wheat on a Buhler laboratory mill. Wheat flour gluten index, dry gluten and wet gluten were evaluated by Glutomatic 2100/2102 (Perten Instruments, Hägersten, Sweden) based on 14% moisture content. SDS sedimentation values were determined using the CAU-B sedimentation value analyser (Chinese Agricultural University, Beijing, China). Farinograph parameters were tested using Farinograph E (Brabender, Duisburg, Germany) and protein content was evaluated by Foss Tecator Infratec 1241 Grain Analyzer ver. 3.40 (Foss NIRSystems, Laurel, USA) using WH982126 Wheat STM program. All quality parameters were from three replications.

Results

HMW-GS composition in six synthetic wheat lines and their parents

HMW-GS composition in six *Ae. tauschii* involved in the crosses that resulted in six synthetic wheat lines were analysed by 10% nondenaturing (figure 1a) and denaturing SDS-PAGE gels (figure 1b). Three different HMW-GS combinations were observed among them. The migration rates of HMW-GS in six *Ae. tauschii* based on this and previous studies (Wan *et al.* 2005; Chen *et al.* 2012) were Dx 3.1^t + Dy 11^{*t} (As 60 and As 85), Dx 2.1^t + Dy 10^t (As 2388) and Dx 2^t + Dy12^t (As 66, As 88 and As 2387).

As expected, all six synthetic wheat lines showed the presence of HMW-GS in *Glu-D1* from their *Ae. tauschii* parents and double nulls in *Glu-A1* and *Glu-B1* from *T. turgidum* ssp. *dicoccon* parents PI94668 or PI349045 (figure 2). Additional protein bands, similar to *T. turgidum* ssp. *dicoccon* or *Ae. tauschii* parents, were also identified (shown in figure 2a–c, e–f with arrows).

Sequencing of HMW-GSs in PI94668 and PI349045

All four HMW-GS open reading frames (ORFs) were amplified (figure 3) and sequenced from both accessions PI94668 and PI349045 (table 1). GenBank accession numbers for the nucleotide sequences are from JQ007586 to JQ007593.

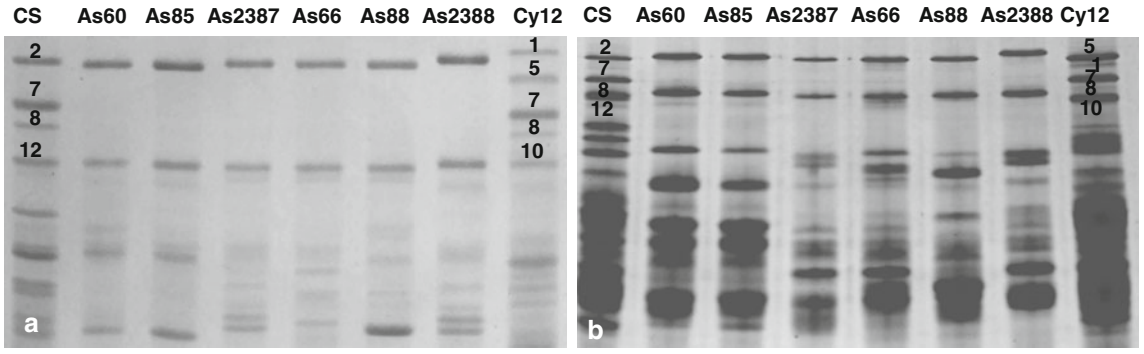

Figure 1. SDS-PAGE patterns of HMW-GS in six *Aegilops tauschii* revealed by 10% nondenaturing gel (a) and 10% denaturing gel with 4 M urea (b).

For further comparisons, four DNA sequences corresponding to the genes *Ax* (AF145590), *Ay* (AY260548), *Bx* (AY621068), and *By* (Eu137874) were used. The ORFs of all these genes in *Glu-A1* and *Glu-B1* loci in both accessions were interrupted by in-frame stop codons. Compared to *Ax*-AF145590, the stop codons of *Ax* genes in PI94668 and PI349045 were located at the amino acid (aa) residue 139 rather than 406. In contrast, the four stop codons in *Ay* in both PI94668 and PI349045 were similar to *Ay*-AY 260548 located at aa positions 142, 151, 286 and 355. Similarly, *Bx* in PI94668 and PI349045 had the stop codon in the same location aa 213. Besides, an additional in-frame stop codon was found at aa 186 in *Bx* in PI349045. An in-frame stop codon at aa position 237 led to the silencing of *By* genes in PI349045 and PI94668.

Quality parameters of the newly developed synthetic wheat lines

Quality parameters of *T. turgidum* ssp. *dicoccon* parents and their amphiploids with six *Ae. tauschii* accessions were investigated (table 2). Compared with the weak gluten wheat control cv. Chuannong 16, the *T. turgidum* ssp. *dicoccon* parents had the lowest SDS sedimentation (<10 mL), development time (<1 min), stability time (<0.6 min) and Farinograph quality number (<11). However, these parameters in the D genome incorporated synthetic hexaploid wheat lines were 9.2–17.6 mL (SDS sedimentation), 0.9–1.4 min (development time), 1.1–1.4 min (stability time) and 14–17 (Farinograph quality number) that were between the values in control and *T. turgidum* ssp. *dicoccon* parents. The gluten index, wet gluten and dry gluten parameters

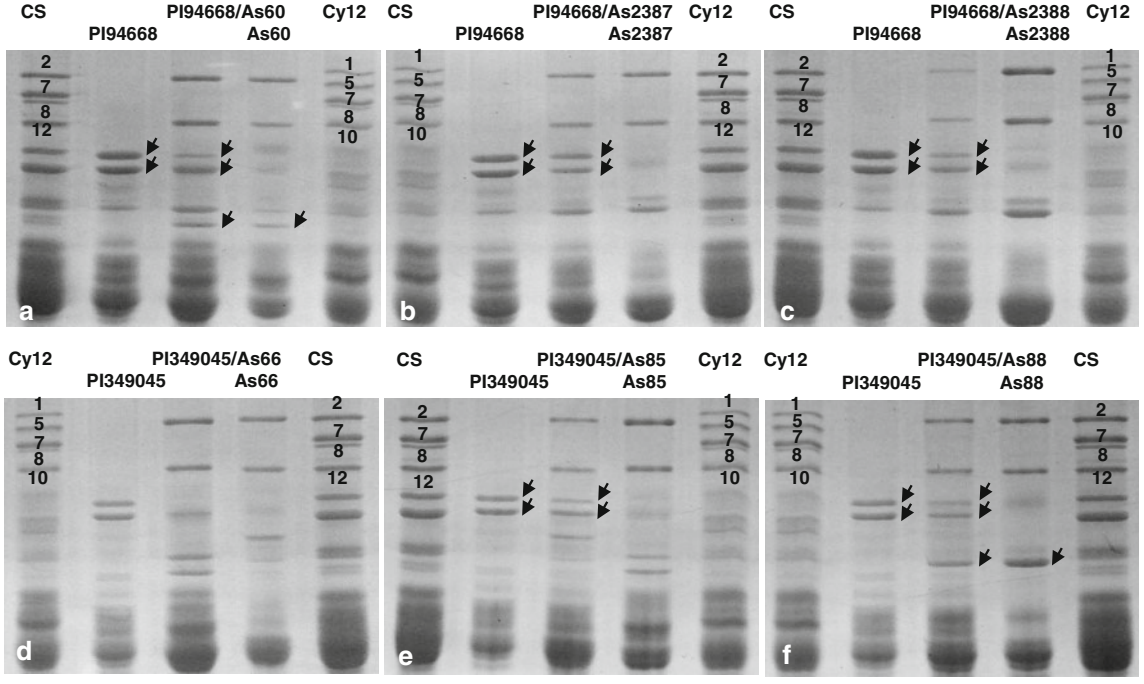

Figure 2. SDS-PAGE patterns of HMW-GS in six newly developed synthetic wheat lines. Arrowheads indicate protein bands similar to *Triticum turgidum* ssp. *dicoccon* or *Aegilops tauschii* parents.

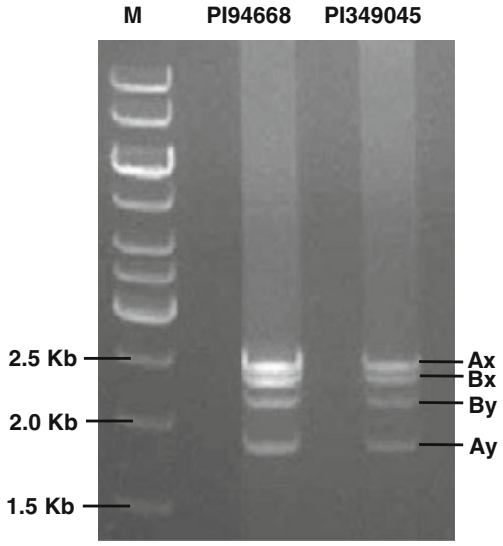

Figure 3. PCR amplification of the ORFs of HMW-GS genes in *Triticum turgidum* ssp. *dicoccon* PI94668 and PI349045.

in *T. turgidum* ssp. *dicoccon* parents were too low to be detected. As expected, a significant increase in these gluten parameters were observed in the D genome incorporated synthetic hexaploid wheat lines. Amount of wet gluten (13.43–35.82) in the six synthetic wheat lines were much higher but dry gluten (6.83–22.75) and gluten index (47.41–62.20) were lower compared to Chuannong 16. However, the protein content in synthetic wheat lines and *T. turgidum* ssp. *dicoccon* parents were significantly higher than Chuannong 16.

Discussion

Dough elasticity and viscosity are critical properties of wheat flours for the food industry and the balance between these two properties determines the end-use quality of wheat flours. Relatively strong (i.e. highly elastic) dough are suitable for pan breads and weaker dough are used for flat breads, noodles, cakes and biscuits. Changes in HMW-GS by overexpression or deficiency of HMW-GS in wheat background could alter the structure of gluten complex and therefore change the processing properties of wheat flours (Payne *et al.* 1987; Lawrence *et al.* 1988; Rogers *et al.* 1991; Payne

Table 1. HMW-GS in PI94668 and PI349045.

Gene	Source	DNA length (bp)	Accession number
Ax	PI 94668	2,496	JQ007589
Ax	PI 349045	2,496	JQ007593
Bx	PI 94668	2,373	JQ007588
Bx	PI 349045	2,373	JQ007592
Ay	PI 94668	1,830	JQ007586
Ay	PI 349045	1,830	JQ007590
By	PI 94668	2,157	JQ007587
By	PI 349045	2,157	JQ007591

Table 2. Quality parameters of *Triticum turgidum* ssp. *dicoccon* parents and their amphiploids with *Ae. tauschii*.

Material	SDS sedimentations (at 14% water content, mL)	Protein content	Gluten index	Dry gluten (g)	Wet gluten (based on 14% moisture)	Development time (min)	Stability time (min)	Farinograph quality number
PI94668[a] (2011)	9.5 ± 0.5D	21.03 ± 0.12B	–	–	–	0.6 ± 0.1C	0.3 ± 0.2C	9.0 ± 0.2D
PI94668[b] (2012)	8.4 ± 1.1D	19.37 ± 0.06D	–	–	–	0.5 ± 0.1C	0.2 ± 0.1C	6.0 ± 1.4D
PI349045[a] (2011)	8.5 ± 0.3D	20.00 ± 0.01C	–	–	–	1.0 ± 0.1BC	0.6 ± 0.1C	11.0 ± 0.1C
PI349045[b] (2012)	8.1 ± 0.8D	17.07 ± 0.06E	–	–	–	0.6 ± 0.1C	0 ± 0.2C	7.0 ± 1.4D
PI94668/As2387	11.6 ± 0.3C	21.00 ± 0.10B	25.78 ± 4.21E	13.61 ± 2.42BC	49.09 ± 0.06B	1.4 ± 0.4AB	1.4 ± 0.5AB	16.0 ± 2.0AB
PI94668/As2388	17.6 ± 0.9B	22.87 ± 0.12A	32.14 ± 0.87D	20.12 ± 0.98A	58.49 ± 1.29A	1.3 ± 0.2AB	1.1 ± 0.2B	16.0 ± 2.4AB
PI94668/As60	10.5 ± 0.8CD	20.70 ± 0.11B	13.43 ± 2.21G	6.83 ± 0.24C	47.41 ± 1.01B	1.4 ± 0.3AB	1.1 ± 0.1B	14.0 ± 1.2B
PI349045/As66	17.1 ± 0.2BC	22.40 ± 0.20A	35.82 ± 0.98B	22.75 ± 1.18A	59.29 ± 1.44A	1.3 ± 0.1AB	1.2 ± 0.1B	15.0 ± 0.1B
PI349045/As85	16.1 ± 0.9BC	19.10 ± 0.10D	34.51 ± 4.04C	17.76 ± 2.40AB	48.00 ± 0.88B	0.9 ± 0.4BC	1.1 ± 0.1B	15.7 ± 3.3AB
PI349045/As88	9.2 ± 0.5D	22.77 ± 0.07A	23.68 ± 1.25F	15.72 ± 0.52B	62.20 ± 0.97A	1.2 ± 0.1BC	1.2 ± 0.1B	17.0 ± 1.5AB
Chuannong 16	28.0 ± 0.8A	13.33 ± 0.06F	69.67 ± 2.50A	22.19 ± 0.13A	29.70 ± 1.22C	1.9 ± 0.2A	1.9 ± 0.1A	26.5 ± 3.5A

[a] and [b] represent flour samples from wheat collected in 2011 and 2012, respectively. –, Indicate parameters that were not detectable.

and Seekings 1996; Barro *et al.* 1997; Rooke *et al.* 1999; Margiotta *et al.* 2000). In most cases, overexpression of HMW-GS increased quality related parameters and thereby improved the dough quality of wheat flours (Barro *et al.* 1997; Rooke *et al.* 1999). In contrast, deficiency in the number of HMW-GS could reduce quality parameters of wheat dough (Lawrence *et al.* 1988; Rogers *et al.* 1991). Absence of one, two or three subunits was reported to have significant effects on SDS sedimentation values and bread making performance (Lawrence *et al.* 1988; Payne *et al.* 1988).

T. turgidum ssp. *dicoccon* accessions PI94668 and PI349045 were found to contain double null alleles in *Glu-A1* and *Glu-B1* loci (Vallega and Waines 1987). Sequencing of four HMW-GS genes in these accessions indicated that they were silenced by in-frame stop codons (1 to 4) at different positions within their ORFs. Double null alleles of *Glu-A1* and *Glu-B1* in hexaploid wheat allow determination of the effects of *Glu-D1* subunits on the rheological and processing properties of wheat flours. Further, we produced six hexaploid wheat lines that express only the subunits encoded by *Glu-D1* by incorporating the double null alleles of HMW-GS from *T. turgidum* ssp. *dicoccon* accessions PI94668 and PI349045.

Although differences in the defined quality parameters were observed among the six wheat lines derived from two *turgidum* ssp. *dicoccon* accessions PI94668 and PI349045 with three different *Ae. tauschii* accessions. Quality test consistently indicated that most parameters were very low in the double null alleles of both parents and the newly developed wheat lines when compared with weak gluten wheat control cv. Chuannong 16, thus indicating that lack of x and y subunits in *Glu-A1* and *Glu-B1* resulted in weaker doughs with faster development and stability times but lower Farinograph quality number in *turgidum* ssp. *dicoccon* accessions PI94668 and PI349045 also reflected in their derivatives with *Ae. tauschii* in synthetic hexaploid wheat. Thus, introduction of null alleles of HMW-GS into wheat may be a possibly and feasibility strategies for developing new germplasm with weak gluten.

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