Theor Appl Genet (2008) 118:177–183
DOI 10.1007/s00122-008-0886-0

ORIGINAL PAPER

New DNA markers for high molecular weight glutenin subunits in wheat

Sixin Liu · Shiaoman Chao · James A. Anderson

Received: 29 April 2008 / Accepted: 30 August 2008 / Published online: 17 September 2008

Abstract End-use quality is one of the priorities of modern wheat (*Triticum aestivum* L.) breeding. Even though quality is a complex trait, high molecular weight (HMW) glutenins play a major role in determining the bread making quality of wheat. DNA markers developed from the sequences of HMW glutenin genes were reported in several previous studies to facilitate marker-assisted selection (MAS). However, most of the previously available markers are dominant and amplify large DNA fragments, and thus are not ideal for high throughput genotyping using modern equipment. The objective of this study was to develop and validate co-dominant markers suitable for high throughput MAS for HMW glutenin subunits encoded at the *Glu-A1* and *Glu-D1* loci. Indels were identified by sequence alignment of allelic HMW glutenin genes, and were targeted to develop locus-specific co-dominant markers. Marker UMN19 was developed by targeting an 18-bp deletion in the coding sequence of subunit Ax2* of *Glu-A1*. A single DNA fragment was amplified by marker UMN19, and was placed onto chromosome 1AL. Sixteen wheat cultivars with known HMW glutenin subunits were used to validate marker UMN19. The cultivars with subunit Ax2* amplified the 362-bp fragment as expected, and a 344-bp fragment was observed for cultivars with subunit Ax1 or the Ax-null allele. Two co-dominant markers, UMN25 and UMN26, were developed for *Glu-D1* by targeting the fragment size polymorphic sites between subunits Dx2 and Dx5, and between Dy10 and Dy12, respectively. The 16 wheat cultivars with known HMW glutenin subunit composition were genotyped with markers UMN25 and UMN26, and the genotypes perfectly matched their subunit types. Using an Applied Biosystems 3130xl Genetic Analyzer, four F_2 populations segregating for the *Glu-A1* or *Glu-D1* locus were successfully genotyped with primers UMN19, UMN25 and UMN26 labeled with fluorescent dyes.

Communicated by B. Friebe.

S. Liu · J. A. Anderson (✉)
Department of Agronomy and Plant Genetics,
University of Minnesota, St Paul, MN 55108, USA
e-mail: ander319@umn.edu

S. Chao
Biosciences Research Lab, USDA-ARS, Fargo, ND 58105, USA

Introduction

Wheat is the most important grain source for human food, and is consumed in many different forms. The end-use quality of wheat is mainly determined by the high molecular weight (HMW) glutenins, which are the major determinants of the unique visco-elastic properties of wheat dough (Payne 1987; Shewry and Halford 2002). HMW glutenins are encoded by the *Glu-1* loci on the long arms of chromosomes 1A, 1B and 1D, and these loci are designated *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively. Each locus consists of two tightly linked HMW glutenin genes, one x-type and one *y*-type. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is used for the separation and identification of HMW glutenin subunits in wheat, which are named numerically based on their mobility. The HMW glutenin subunits are referred to in this study by both the genome from which the subunit is derived and the x-type or y-type of the subunit, such as Ax1 and Dy10. The y-type gene on chromosome 1A is not expressed in most common wheat cultivars.

The HMW glutenin subunit composition alone may account for 47–60% of the variation in bread making quality

of wheat (Payne et al. 1987; Lukow et al. 1989). After comparing the bread making quality and HMW glutenin subunit composition for a large collection of wheat cultivars, Payne et al. (1987) assigned quality scores to each of the common alleles of the *Glu-1* loci. The *Glu-D1* locus has the largest effect on bread making quality. The combination of Dx5 and Dy10 is associated with strong dough and good bread making quality (Payne et al. 1981; Popineau et al. 1994), and the allelic pair Dx2 + Dy12 has negative effects on bread making quality. For the *Glu-A1* locus, both the Ax1 and Ax2* subunits have positive effects on bread making quality and the null allele has a low quality score. Both the alleles *Glu-B1d* (Bx6 + By8) and *Glu-B1e* (Bx20 + By20) (Lukow et al. 1989; Shewry et al. 2003) have negative effects on dough characteristics and bread making quality. However, the over-expressed Bx7 subunit encoded by *Glu-B1al* is associated with improved dough strength of wheat flour (Marchylo et al. 1992; Radovanovic et al. 2002; Butow et al. 2004). The HMW glutenin subunits are subjected to intense selection in most bread wheat breeding programs due to these differences in end-use quality.

Separation of HWM glutenin subunits by SDS-PAGE is not amenable to high throughput analysis. Fortunately, extensive and intensive genomic studies have been documented for the *Glu-1* loci. All six HMW glutenin genes of hexaploid wheat have been cloned and sequenced and BAC clones spanning the *Glu-1* loci have been sequenced from hexaploid, tetraploid and diploid wheat (Gu et al. 2004, 2006; Kong et al. 2004; Ragupathy et al. 2008). All these available genomic sequences are useful for development of DNA markers to facilitate marker-assisted selection (MAS) for the HMW glutenin subunits of wheat.

Several research groups reported DNA markers for HMW glutenin subunits in wheat. Gale (2005) reviewed these markers for HMW glutenins in addition to markers for other quality traits in wheat. Only DNA markers not covered in that review are briefly summarized here. Schwarz et al. (2003) developed and validated a SNP (single nucleotide polymorphism) marker located in the promoter region of the x-type genes at the *Glu-D1* locus. This SNP marker can be used to discriminate subunits Dx2 and Dx5. Schwarz et al. (2004) reported a co-dominant DNA marker to select against the Bx6 subunit, which has a negative effect on the bread making quality of wheat. Based on the sequences of y-type genes at the *Glu-B1* locus, Lei et al. (2006) developed several dominant markers for the *Glu-B1* locus. Recently, Ragupathy et al. (2008) reported DNA markers specific to the *Glu-B1al* allele encoding the over-expressed Bx7 subunit.

Most of these previously available markers for HMW glutenins were developed for genotyping with agarose gel assays, and these markers have one or more of the following drawbacks for high throughput MAS. First, most of them are dominant markers, meaning that homozygous and heterozygous plants cannot be distinguished in positive PCR reactions, and it is difficult to discriminate negative reactions from PCR failure. Second, non-genome specific amplification leads to multiple PCR products and complicates MAS. Finally, some of the previous markers amplify the entire coding region of HMW glutenin genes. Large PCR products are difficult to amplify, especially with low quality DNA templates, and the fragment sizes may exceed the limit of the commercially available molecular weight size standards for DNA sequencers. The objective of this study was to develop and validate co-dominant markers suitable for high throughput MAS of HMW glutenin subunits encoded at the *Glu-A1* and *Glu-D1* loci.

Materials and methods

Three nulli-tetrasomic lines (N1AT1B, N1BT1A, and N1DT1A) (Sears 1966) and three short arm ditelosomic lines (Dt1AS, Dt1BS and Dt1DS) (Sears and Sears 1978) of Chinese Spring were used to confirm the location of new DNA markers for HMW glutenin subunits on the long arms of wheat chromosome 1. Sixteen wheat cultivars (Table 1) were chosen from the database of HMW glutenin composition (http://www.aaccnet.org/GRAINBIN/) to validate these new DNA markers. Four F_2 populations (Table 2) segregating for the HMW glutenin subunits were chosen from the wheat breeding program at the University of Minnesota, and were genotyped to test whether these new markers are suitable for high throughput MAS.

The DNA and protein sequences of HMW glutenin subunits (Table 3) encoded by the *Glu-A1* and *Glu-D1* loci were retrieved from GenBank. The sequences of each locus were aligned using software ClustalW2 (Larkin et al. 2007) to identify fragment size polymorphisms. The polymorphic sites were targeted for primer design using program Primer 3 (Rozen and Skaletsky 2000) with expected product sizes of 200-500 bp. To amplify a single PCR product, all primers have only one prime site on the targeted gene, and are specific to the targeted HMW glutenin locus.

DNA was extracted as described by Liu and Anderson (2003) from the Chinese Spring aneuploid lines and the 16 wheat cultivars used for marker validation. PCR was performed with annealing temperature at 60°C. The PCR protocol and gel analysis were conducted as described by Liu and Anderson (2003). The high throughput genotyping was performed at the USDA-ARS small grains genotyping lab in Fargo, North Dakota. The methods for leaf sample collection and DNA extraction from the four breeding populations were conducted as described by Bodo Slotta et al. (2008). PCR products amplified with primers labeled with fluorescent dyes were separated and analyzed on an Applied Biosystems

Table 1 Wheat cultivars of known HMW glutenin subunits used to validate DNA markers reported in this study

Cultivar	Origin	HMW glutenin alleles[a]	
		Glu-A1	Glu-D1
Oslo	Canada	1	2 + 12
Marquis	Canada	1	5 + 10
Rescue	Canada	1	5 + 10
Maringa	Brazil	2*	2 + 12
Cranbrook	Australia	2*	2 + 12
Wangshuibai	China	2*[b]	2 + 12
Recital	France	2*	5 + 10
Wheaton	USA	2*	5 + 10
Bobwhite	Mexico	2*	5 + 10
Stoa	USA	2*	5 + 10
Roblin	Canada	2*	5 + 10
Glenlea	Canada	2*	5 + 10
Frontana	Brazil	null	2 + 12
Fielder	USA	null	2 + 12
Chinese Spring	China	null	2 + 12
Sumai 3	China	null	2 + 12

[a] HWM Glutenin alleles as reported at http://www.aaccnet.org/GRAINBIN/

[b] The cultivar Wangshiubai used in this study contains a null allele instead of the subunit Ax2* at the *Glu-A1* locus based on our study and using markers reported by Lafiandra et al (1997)

3130xl Genetic Analyzer (Foster City, CA, USA). Chi-square tests were performed for each F_2 population to check if the genotypes fit the expected 1:2:1 ratio.

Results

Development and validation of co-dominant markers for HMW glutenin subunits

Except for one 15-bp deletion identified only in the null allele (AY494981) of durum wheat, there are no other indels among the sequences of Ax1 and Ax-null. However, one 18-bp deletion and one 27-bp deletion in Ax2* were identified. The 18-bp deletion (Fig. 1a) was targeted for primer design and marker UMN19 was developed (Table 4). A single DNA fragment was amplified with marker UMN19. As expected, marker UMN19 was mapped onto chromosome 1AL by aneuploid analysis (Fig. 2).

Table 3 GenBank accessions of wheat HMW glutenin subunits used to develop DNA markers

Subunit	Accession no.	Reference
Ax1	X61009	Halford et al. 1992
Ax2*	M22208	Anderson and Greene 1989
Ax-null	AF145590	De Bustos et al. 2000
Ax-null	U19774	Xin et al. 1992
Ax-null	AY494981	Gu et al. 2004
Dx2	X03346	Sugiyama et al. 1985
Dx5	X12928	Anderson et al. 1989
Dy10	X12929	Anderson et al. 1989
Dy12	X03041	Thompson et al. 1985

Among the 16 wheat cultivars (Table 1) used to validate marker UMN19, the cultivars with subunit Ax1 or the null allele were expected to amplify the 362-bp DNA fragment, and the 344-bp fragment was expected for cultivars with subunit Ax2* (Table 4). Except for cultivar Wangshuibai, the other 15 cultivars had the expected product sizes for marker UMN19 (Fig. 3). To investigate the discrepancy, cultivar Wangshuibai was genotyped with the DNA markers reported by Lafiandra et al. (1997). Wangshuibai was positive for primer combination a and b, and was negative for primer pair c and d (data not shown). Thus, cultivar Wangshuibai used in this study possesses a null allele at the *Glu-A1* locus instead of the Ax2* subunit reported in the database. Therefore, the genotypes with marker UMN19 were consistent with the HMW glutenin composition for all 16 wheat cultivars.

Three indels were revealed by comparison of subunits Dx2 and Dx5. A hexamer amino acid deletion was found near each end of the Dx5 repetitive domain, and a 12 amino acid insertion in Dx5 was located between those two

Table 2 Marker-assisted selection results of four F_2 populations segregating for the HMW glutenin subunits at the *Glu-A1* or *Glu-D1* locus

Pop.	Pedigree	Subunit	Marker	No. of plants				*P* value[a]
				Total	*F*	*H*	*M*	
1.	MN01311-A-1/MN03119	Dx5 + Dy10/Dx2 + Dy12	UMN25, UMN26	321	75	160	86	0.6849
2.	MN97803-3BS + 6BS/MN01311-A-1	Dx2 + Dy12/Dx5 + Dy10	UMN25, UMN26	351	126	146	79	<0.001
3.	MN99126-1-3-7/MN02072-7	Dx2 + Dy12/Dx5 + Dy10	UMN26	317	78	157	82	0.9374
4.	MN99220-4-1/MN01333-A-2	Ax-null/Ax2*	UMN19	296	78	146	72	0.8619

F homozygous for allele from the female, *H* heterozygous, *M* homozygous for allele from the male

[a] *P* value is based on the chi-square goodness-of-fit to a 1:2:1 ratio of *F*:*H*:*M* alleles

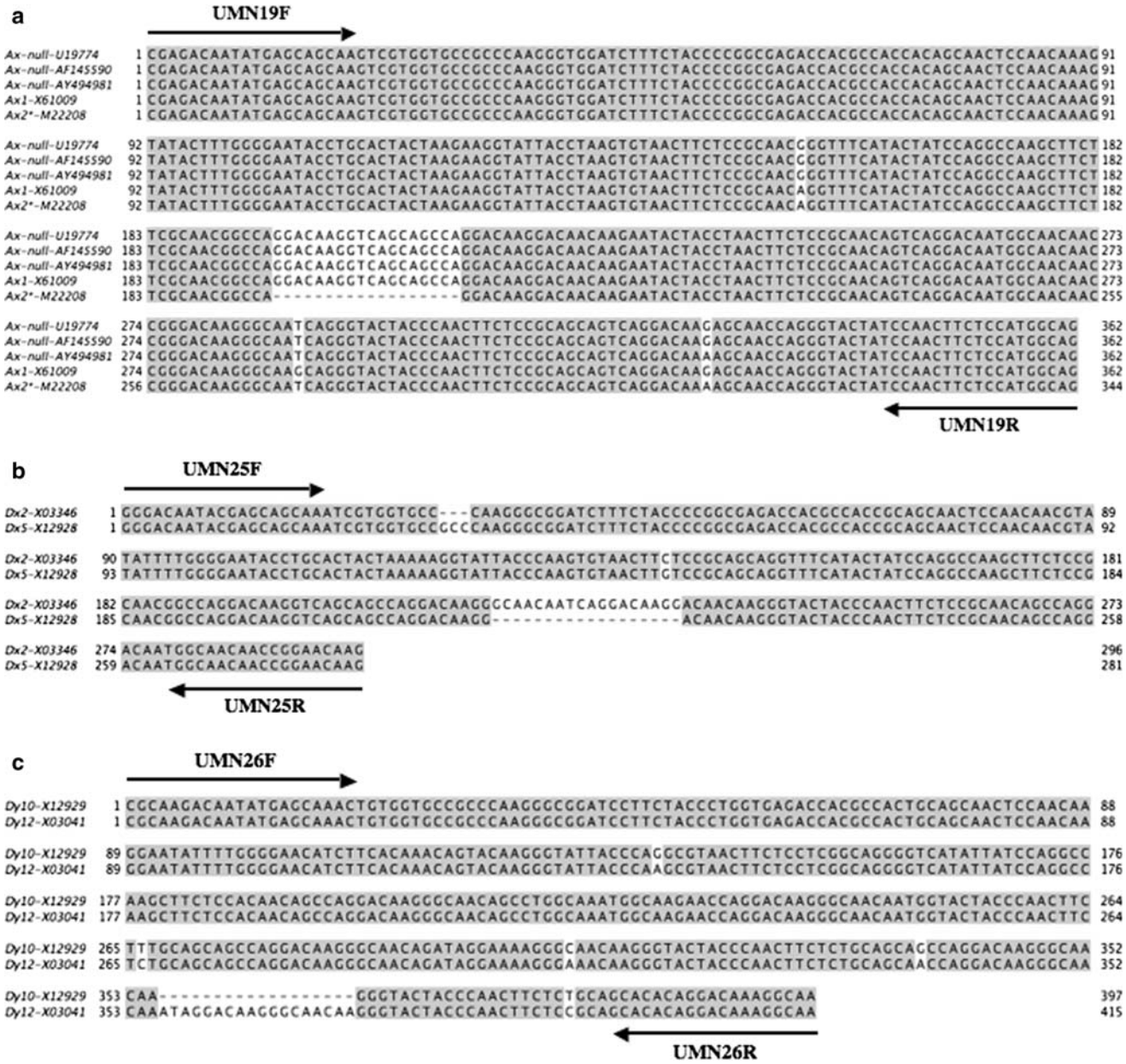

Fig. 1 Nucleotide sequence alignment of the region amplified by each new DNA marker for HMW glutenin genes encoded by the *Glu-A1* and *Glu-D1* loci. Alleles are labeled on the *left side* of the sequences, followed by their GenBank accession number. The deleted nucleotides are shown as *dashes*. The nucleotide polymorphism is highlighted by *lighter* background. The primer binding sites are at the ends of sequence alignment. **a** Marker UMN19; **b** marker UMN25 and **c** marker UMN26. Our unpublished results indicated the tri-nucleotide deletion GCC in subunit Dx2 shown in **b** is due to a sequencing error

hexamer amino acid deletions. Marker UMN25 (Fig. 1b; Table 4) was designed to target the hexamer amino acid deletion near the start site of the repetitive domain. Marker UMN25 produced a single PCR fragment and it was placed onto chromosome 1DL by aneuploid analysis (Fig. 2). The expected product sizes are 281-bp and 299-bp for subunit Dx5 and Dx2, respectively (Table 4).

Sequence alignment of subunit Dy10 and Dy12 revealed four indels. The first hexamer amino acid deletion in Dy10 was targeted for primer design and a co-dominant marker UMN26 (Fig. 1c; Table 4) was developed. As expected, marker UMN26 was also placed on chromosome 1DL based on aneuploid analysis (Fig. 2). The expected sizes for subunits Dy10 and Dy12 are 397 and 415 bp, respectively. The marker genotypes revealed by UMN25 and UMN26 completely match their HMW glutenin subunits for the *Glu-D1* locus for all the 16 cultivars (Table 1; Fig. 3).

Table 4 New DNA markers for wheat HMW glutenin subunits at the *Glu-A1* and *Glu-D1* locus

Locus	Marker	Primer: Sequence (5′–3′)	Expected size (bp)
Glu-A1	UMN19	UMN19F: CGAGACAATATGAGCAGCAAG UMN19R: CTGCCATGGAGAAGTTGGA	Ax2* 344; Ax1, Ax-null 362
Glu-D1	UMN25	UMN25F: GGGACAATACGAGCAGCAAA	Dx2 299
		UMN25R: CTTGTTCCGGTTGTTGCCA	Dx5 281
Glu-D1	UMN26	UMN26F: CGCAAGACAATATGAGCAAACT	Dy10 397
		UMN26R: TTGCCTTTGTCCTGTGTGC	Dy12 415

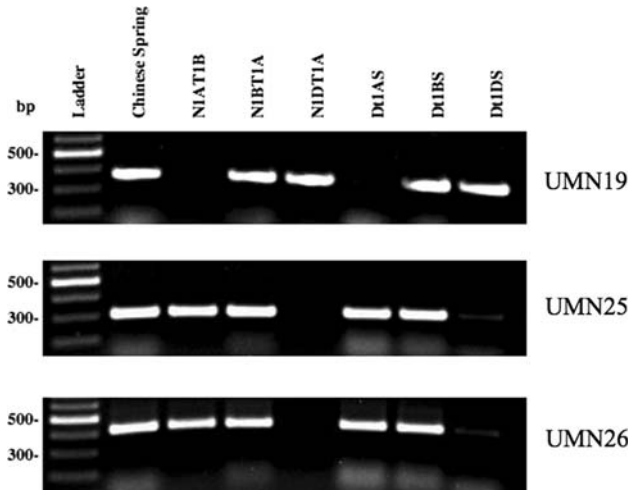

Fig. 2 Validation of the chromosome and chromosome arm locations of the new genome-specific markers for HMW glutenin subunits using chromosome 1 aneuploid lines of Chinese Spring. Faint PCR products were amplified from the Chinese Spring ditelosomic line Dt1DS for both marker UMN25 and UMN26 in repeated experiments. This may be caused by minor DNA contamination of the stock DNA

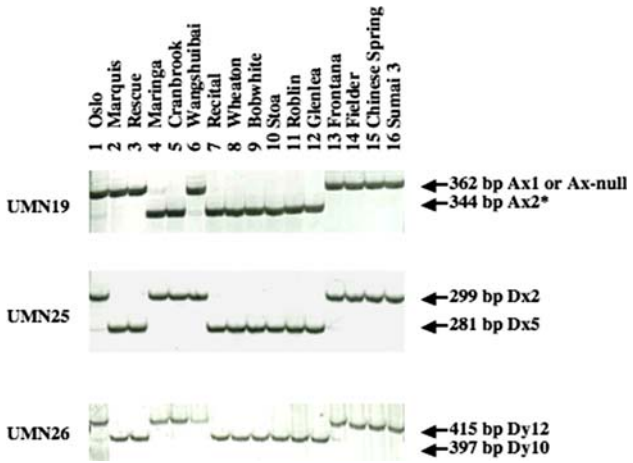

Fig. 3 Validation of the new markers for HMW glutenin subunits using the 16 wheat cultivars with known subunits listed in Table 1. The expected product sizes of each allele are indicated at the *right* of the gel images

MAS for HMW glutenin subunits using the new DNA markers

The expected fragment sizes were observed for all parents of the four F_2 populations, and the MAS results were summarized in Table 2. Populations 1 and 2 were segregating for the *Glu-D1* locus, and were genotyped with markers UMN25 and UMN26. Among 672 F_2 plants, the genotypes for these two markers agreed with each other and no recombination event was observed. The expected 1:2:1 ratio was observed for population 1, but population 2 had more plants with the homozygous alleles derived from the female parent, MN97803-3BS + 6BS. We also genotyped population 2 with other DNA markers, and none of them fit the expected 1:2:1 ratio (data not shown). It is possible that some unknown genetic factors caused the observed segregation distortion in population 2; however, the observed distortion was most likely caused by an error during crossing of the two parents. We suspect this F_2 population was mixed with self-pollinated seeds of the female parent. Population 3 was genotyped with marker UMN26 alone, and the expected 1:2:1 ratio was observed. Population 4 was segregating for the *Glu-A1* locus and was genotyped with marker UMN19. The female parent, MN99220-4-1, possesses the Ax-null allele and the male parent MN01333-A-2 contains the subunit Ax2*. The expected 1:2:1 ratio was observed for population 4.

Discussion

Three co-dominant markers, UMN19, UMN25 and UMN26, for HMW glutenin genes encoded at the *Glu-A1* and *Glu-D1* loci were developed and validated in this study. These three new DNA markers worked well with the DNA extracted using a high throughput protocol, and four breeding populations were successfully genotyped with the Applied Biosystems 3130xl Genetic Analyzer by the USDA-ARS genotyping lab in Fargo, North Dakota. Therefore, these markers can be applied to large-scale MAS for HMW glutenin subunits in wheat.

Because marker UMN19 is monomorphic between the Ax1 subunit and the Ax-null allele (Fig. 3), it is challenging to select against the Ax-null allele whenever the Ax1 subunit is present in segregating populations. Fortunately, the

nucleotide substitution A for G (Fig. 1a) in the region amplified with marker UMN19 can be used as a SNP marker to differentiate the Ax-null allele from subunits Ax1 and Ax2*. We are planning to develop and validate SNP markers for this region in the near future.

Both UMN25 and UMN26 target the *Glu-D1* locus, and either one of them should be adequate to select for the favorable allele *Glu-D1d* (Dx5 + Dy10) as demonstrated above. Recombinants between the x-type and y-type genes can be identified when both markers are used for genotyping. Alleles *Glu-D1b* (Dx3 + Dy12) and *Glu-D1c* (Dx4 + Dy12) are associated with poor bread making quality (Payne et al. 1987). For populations involving these two alleles, marker UMN26 can be used to select for the favorable subunit combination Dx5 + Dy10. The sequences of allele Dx3 and Dx4 are currently not available, and we have not tested whether marker UMN25 is polymorphic between Dx5 and Dx3, or between Dx5 and Dx4.

We did not report DNA markers for the *Glu-B1* locus for two reasons. First, the subunit pairs with negative effects on bread making quality such as Bx6 + By8 or Bx20 + By20 are rare among the parental lines in the breeding program at the University of Minnesota. Thus, the demand for selection of the *Glu-B1* locus is not urgent in our breeding program. Second, currently available markers for *Glu-B1* locus are sufficient. Schwarz et al. (2004) reported a co-dominant marker to select against the *Glu-B1d* allele (Bx6 + By8). The over-expressed Bx7 subunit encoded by the *Glu-B1al* allele is desirable for high dough strength. We have tested the recently reported DNA markers specific for the *Glu-B1al* allele (Ragupathy et al. 2008) and found them to work well with the DNA analyzers used in the USDA-ARS genotyping lab in Fargo, North Dakota (data not shown).

These three markers were not developed as a replacement for other techniques such as SDS-PAGE to identify the HMW glutenin subunit composition of wheat cultivars due to redundancy in PCR product size among different subunits. For example, two additional subunits encoded by the *Glu-A1* locus, Ax2*^{B} (Juhasz et al. 2001) and Ax2.. (Gobaa et al. 2007), also have been cloned and sequenced. Allele Ax2*^{B} (GenBank accession EF055262) contains the same 18-bp deletion as that observed in Ax2*, and thus alleles Ax2*^{B} and Ax2* should have the 344-bp PCR products for marker UMN19. There is no size polymorphism among alleles Ax2.. (GenBank accession DQ533690), Ax1 and Ax-null in the region amplified by marker UMN19, and the 362-bp PCR products are expected for all three types of subunits. Therefore, neither the 344-bp allele nor the 362-bp allele is unique to one type of subunit at the *Glu-A1* locus. At the *Glu-D1* locus, Wan et al. (2005) reported four other x-type genes, in addition to Dx2 and Dx5. On the basis of their nucleotide sequences, the expected product size for marker UMN25 should be 299-bp for all four novel x-type genes. Therefore, marker UMN25 cannot be used alone to postulate the types of *Glu-D1* HMW glutenin subunit of wheat because other Dx subunits may produce the same size PCR fragment as Dx2 or Dx5. In addition to subunits Dy10 and Dy12, dozens of y-type gene sequences of bread wheat and its alien species perfectly match the primer sequences of marker UMN26 based on the results of BLAST searches of the nucleotide sequence collection of GenBank. Even though most of these sequences have not been characterized in detail, it is clear that the product size of 397-bp or 415-bp for marker UMN26 is not limited to wheat lines possessing the Dy10 or Dy12 HMW glutenin subunits.

Acknowledgments We thank Dr. Michael Pumphrey at USDA-ARS, Manhattan, Kansas, for providing DNA of several cultivars used for marker validation, Jennifer Flor and Susan Reynolds for collecting leaf samples for DNA extraction. We also appreciate the technical assistance of Mary Osenga and Richard Sonju at USDA-ARS, Fargo, North Dakota.

References

Anderson OD, Greene FC (1989) The characterization and comparative analysis of high molecular weight glutenin genes from genome A and Genome B of a hexaploid bread wheat. Theor Appl Genet 77:689–700

Anderson OD, Greene FC, Yip RE, Halford NG, Shewry PR, Malpicaromero JM (1989) Nucleotide sequences of the 2 high molecular weight glutenin genes from the D-genome of a hexaploid bread wheat, *Triticum aestivum* L cv Cheyenne. Nucleic Acids Res 17:461–462

Bodo Slotta TA, Brady L, Chao S (2008) High throughput tissue preparation for large-scale genotyping experiments. Mol Ecol Notes 8:83–87

Butow BJ, Gale KR, Ikea J, Juhasz A, Bedo Z, Tamas L, Gianibelli MC (2004) Dissemination of the highly expressed Bx7 glutenin subunit (*Glu-B1al* allele) in wheat as revealed by novel PCR markers and RP-HPLC. Theor Appl Genet 109:1525–1535

De Bustos A, Rubio P, Jouve N (2000) Molecular characterization of the inactive allele of the gene Glu-A1 and the development of a set of AS-PCR markers for HMW glutenins of wheat. Theor Appl Genet 100:1085–1094

Gale KR (2005) Diagnostic DNA markers for quality traits in wheat. J Cereal Sci 41:181–192

Gobaa S, Kleijer G, Stamp P (2007) 2.., a new high molecular weight glutenin subunit coded by Glu-A1: its predicted structure and its impact on bread-making quality. Plant Breeding 126:1–4

Gu YQ, Salse J, Coleman-Derr D, Dupin A, Crossman C, Lazo GR, Huo N, Belcram H, Ravel C, Charmet G, Charles M, Anderson OD, Chalhoub B (2006) Types and rates of sequence evolution at the high molecular weight glutenin locus in hexaploid wheat and its ancestral genomes. Genetics 174:1493–1504

Gu YQ, Coleman-Derr D, Kong X, Anderson OD (2004) Rapid genome evolution revealed by comparative sequence analysis of orthologous regions from four triticeae genomes. Plant Physiol 135:459–470

Halford NG, Field JM, Blair H, Urwin P, Moore K, Robert L, Thompson R, Flavell RB, Tatham AS, Shewry PR (1992) Analysis of HMW glutenin subunits encoded by Chromosome 1A of bread

wheat (*Triticum aestivum* L) indicates quantitative effects on grain quality. Theor Appl Genet 83:373–378

Juhasz A, Tamas L, Karsai I, Vida G, Lang L, Bedo Z (2001) Identification, cloning and characterisation of a HMW glutenin gene from an old Hungarian wheat variety, Bankuti 1201. Euphytica 119:75–79

Kong XY, Gu YQ, You FM, Dubcovsky J, Anderson OD (2004) Dynamics of the evolution of orthologous and paralogous portions of a complex locus region in two genomes of allopolyploid wheat. Plant Mol Biol 54:55–69

Lafiandra D, Tucci GF, Pavoni A, Turchetta T, Margiotta B (1997) PCR analysis of x- and y-type genes present at the complex *Glu-A1* locus in durum and bread wheat. Theor Appl Genet 94:235–240

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and clustal X version 2.0. Bioinformatics 23:2947–2948

Lei ZS, Gale KR, He ZH, Gianibelli C, Larroque O, Xia XC, Butow BJ, Ma W (2006) Y-type gene specific markers for enhanced discrimination of high-molecular weight glutenin alleles at the Glu-B1 locus in hexaploid wheat. J Cereal Sci 43:94–101

Liu S, Anderson JA (2003) Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. Crop Sci 43:760–766

Lukow OM, Payne PI, Tkachuk R (1989) The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. J Sci Food Agric 46:451–460

Marchylo BA, Lukow OM, Kruger JE (1992) Quantitative variation in high molecular weight glutenin subunit 7 in some Canadian wheats. J Cereal Sci 15:29–37

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annu Rev Plant Physiol Plant Mol Biol 38:141–153

Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. J Sci Food Agric 40:51–65

Payne PI, Corfield KG, Holt LM, Blackman JA (1981) Correlations between the inheritance of certain high molecular weight subunits of glutenin and bread-making quality in progenies of 6 crosses of bread wheat. J Sci Food Agric 32:51–60

Popineau Y, Cornec M, Lefebvre J, Marchylo B (1994) Influence of high M(r) glutenin subunits on glutenin polymers and rheological properties of glutens and gluten subfractions of near-isogenic lines of wheat Sicco. J Cereal Sci 19:231–241

Radovanovic N, Cloutier S, Brown D, Humphreys DG, Lukow OM (2002) Genetic variance for gluten strength contributed by high molecular weight glutenin proteins. Cereal Chem 79:843–849

Ragupathy R, Naeem HA, Reimer E, Lukow OM, Sapirstein HD, Cloutier S (2008) Evolutionary origin of the segmental duplication encompassing the wheat GLU-B1 locus encoding the overexpressed Bx7 ($Bx7^{OE}$) high molecular weight glutenin subunit. Theor Appl Genet 116:283–296

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Schwarz G, Felsenstein FG, Wenzel G (2004) Development and validation of a PCR-based marker assay for negative selection of the HMW glutenin allele Glu-B1-1d (Bx-6) in wheat. Theor Appl Genet 109:1064–1069

Schwarz G, Sift A, Wenzel G, Mohler V (2003) DHPLC scoring of a SNP between promoter sequences of HMW glutenin x-type alleles at the *Glu-D1* locus in wheat. J Agric Food Chem 51:4263–4267

Sears ER (1966) Nullisomic-tetrasomic combination in hexaploid wheat. In: Riley R, Lewis KR (eds) Chromosome manipulation and plant genetics. Oliver and Boyd, Edinburgh, pp 29–45

Sears ER, Sears LMS (1978) The telocentric chromosomes of common wheat. In: Ramanujan S (ed) Proceedings of 5th international wheat genetics symposium. Indian society of genetics and plant breeding. Indian Agric Res Inst, New Delhi, pp 389–407

Shewry PR, Gilbert SM, Savage AWJ, Tatham AS, Wan YF, Belton PS, Wellner N, D'Ovidio R, Bekes F, Halford NG (2003) Sequence and properties of HMW subunit 1Bx20 from pasta wheat (*Triticum durum*) which is associated with poor end use properties. Theor Appl Genet 106:744–750

Shewry PR, Halford NG (2002) Cereal seed storage proteins: structures, properties and role in grain utilization. J Exp Bot 53:947–958

Sugiyama T, Rafalski A, Peterson D, Soll D (1985) A wheat HMW glutenin subunit gene reveals a highly repeated structure. Nucleic Acids Res 13:8729–8737

Thompson RD, Bartels D, Harberd NP (1985) Nucleotide sequence of a gene from chromosome 1D of wheat encoding a HMW glutenin subunit. Nucleic Acids Res 13:6833–6848

Wan YF, Yan ZH, Liu KF, Zheng YL, D'Ovidio R, Shewry PR, Halford NG, Wang DW (2005) Comparative analysis of the D genome-encoded high molecular weight subunits of glutenin. Theor Appl Genet 111:1183–1190

Xin H, Jiang Y, Huang W, Gu Q, Sun C (1992) Nucleotide sequence of a high molecular weight glutenin gene in wheat. Acta Bot Sin 34:729–735