Characterization of low-molecular-weight glutenin subunit genes at *Glu-B3* and *Glu-D3* loci and development of functional markers in common wheat

Wang L[1], Zhao X[1,2], He Z[1,3], Xia X[1,*]

[1]Institute of Crop Science, National Wheat Improvement Centre/The National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences (CAAS), Zhongguancun South Street 12, Beijing 100081, China; [2]Wheat Research Institute, Henan Academy of Agricultural Sciences, Nongye Road 1, Zhengzhou, Henan, 450002, China; [3]International Maize and Wheat Improvement Centre (CIMMYT) China Office, c/o CAAS, Zhongguancun South Street 12, Beijing 100081, China

****Author for correspondence: Xianchun Xia, Email address: xiaxianchun@caas.net.cn***

SUMMARY

Four genes with 17 allelic variants at *Glu-B3* locus and 6 genes with 12 variants at *Glu-D3* locus were isolated in common wheat using the technique of in-silico cloning in combination with PCR amplification. The relationship between the *Glu-B3* and *Glu-D3* alleles defined by protein mobility at SDS-PAGE and the allelic variants were determined, and 10 STS markers for different *Glu-B3* alleles and 7 markers for different *Glu-D3* genes were developed based on the allelic variations among different gene haplotypes. The functional STS markers were validated by wheat varieties and advanced lines with different *Glu-B3* and *Glu-D3* alleles.

INTRODUCTION

Low-molecular-weight glutenin subunits (LMW-GS) showed large effects on dough extensibility, and thus influence the quality of end-use products of wheat, particularly for Asian noodles[1, 2, 3]. Compared with high-molecular-weight glutenin subunits (HMW-GS), the extensive allelic variations of LMW-GS and their overlapping mobility with the abundant gliadin proteins[4] make it difficult to discriminate the role of individual alleles. Characterization of LMW-GS genes at DNA level and development of functional markers is therefore very important for the discrimination of different LMW-GS alleles in wheat breeding.

MATERIALS AND METHODS

Aroona (*Glu-B3b*) and its seven near-isogenic lines (NIL), Aroona-B3a (*Glu-B3a*), Aroona-B3c (*Glu-B3c*), Aroona-B3d (*Glu-B3d*), Aroona-B3f (*Glu-B3f*), Aroona-B3g (*Glu-B3g*), Aroona-B3h (*Glu-B3h*), Aroona-B3i (*Glu-B3i*) and Cheyenne (*Glu-B3e*) with different *Glu-B3* alleles defined by protein mobility were used to isolate *Glu-B3* genes. The Aroona and its NILs were kindly provided by Dr Marie Appelbee at SARDI Grain Quality Research Laboratory, Australia. Cheyenne was provided by Dr. Baoyun Li at China Agricultural University. Eight common wheat cultivars (Tasman, Chinese Spring, Silverstar, Sunco, Aroona, Norin61, Hartog, and BT2288A) carrying five common *Glu-D3* alleles (a, b, c, d, and e)[5] were used to amplify *Glu-D3* genes. Chinese Spring and its nulli-tetrasomic lines N1A-T1D, N1B-T1A, N1B-T1D and N1D-T1B, kindly provided by Prof. R. A. McIntosh at the Plant Breeding Institute Cobbitty, University of Sydney. Besides the eight NILs and Cheyenne, 20 varieties from our laboratory collection and 141 wheat varieties and advanced lines from CIMMYT with different *Glu-B3* alleles detected by SDS-PAGE were employed to validate the allele-specific markers.

Gene-specific primers for cloning *Glu-B3* and *Glu-D3* genes were developed according to the description of Zhang et al.[6, 7]. Eight reference genes with complete coding region, including seven LMW-GS genes (GenBank accessions AB119006, AB164415, AB164416, AB262661, Y14104, AB062852 and AJ007746) located on the short arm of chromosome 1B and one (AY542898) with high similarity to *Glu-B3* available in GenBank were used for cloning *Glu-B3* genes (http://www.ncbi.nlm.nih.gov). Seven reference genes *X13306*, *AB062851*, *AB062865*, *AB062872*, *AB062873*, *AB062875* and *AY223396* available in GenBank (www.ncbi.nlm.nih.gov) were employed for the isolation of *Glu-D3* genes. The PCR fragments with expected sizes were recovered from agarose gel, followed by cloning into the pGEM-T Easy Vector, and the recombinant clones with expected size were sequenced after PCR test. All the sequencings were performed by the Sangon Biotechnology Co. Ltd (Shanghai, China). Each PCR and sequence procedure was repeated 3-6 times to avoid any technical errors. Sequence analysis and characterization were performed using software DNAMAN (http://www.lynnon.com). Allele-specific PCR markers were designed based on the allelic variants of *Glu-B3* and *Glu-D3* genes following the method of Zhang et al. [6,]. These markers were validated with wheat varieties and advanced lines from CIMMYT, Australia and France with different protein mobility alleles.

RESULTS

Four *Glu-B3* genes with complete coding sequence were obtained, designated as *GluB3-1*, *GluB3-2*, *GluB3-3* and *GluB3-4*, respectively. *GluB3-1* had five allelic variants, *GluB3-11*, *GluB3-12*, *GluB3-13*, *GluB3-14* and *GluB3-15* that were present in the NILs and varieties with *Glu-B3* alleles *a*, *b*, *e*, *f* and *g*, respectively. Compared with *GluB3-12*, four, one and two triplet nucleotides (CAA) deletion at the position 319-330 were found in the coding region of *GluB3-11*, *GluB3-13* and *GluB3-14*, leading to four, one and two glutamines absence in the glutamine-rich repetitive domain, respectively. *GluB3-15* had a 63-bp deletion at the position 292-354, resulting in twenty-one amino acids deletion in the deduced peptide. *GluB3-2* contained three allelic variants *GluB3-21*, *GluB3-22* and *GluB3-23*. *GluB3-21* was present in the NIL with the allele *Glu-B3a*, *GluB3-22* in the genotypes with *Glu-B3* alleles *b*, *e*, and *f*, and *GluB3-23* in those with *Glu-B3g*. Compared with *GluB3-21*, both *GluB3-22* and *GluB3-23* had a 3-bp deletion (CAA) at the position 378-380 in the coding region, leading to a glutamine deletion in the repetitive domain of the deduced peptide. *GluB3-22* had two SNPs at the positions 671 and 1246 with the latter leading to an amino acid mutation from serine to asparagine in the C-terminal conserved region. *GluB3-3* was amplified from the NILs containing *Glu-B3c*, *d*, *h* and *i,* with four allelic variants, designated as *GluB3-31*, *GluB3-32*, *GluB3-33* and *GluB3-34*, respectively. Compared with *GluB3-31*, 4-5 SNPs were found in the coding region of *GluB3-32*, *GluB3-33* and *GluB3-34*, and a double base substitution in *GluB3-33*. In addition, *GluB3-34* had a 3-bp (CAA) deletion at the position 603-605 and a 3-bp (CAA) insertion at 651-653, leading to a glutamine deletion and insertion, respectively. *GluB3-4* contained five allelic variants designated as *GluB3-41* (in the genotypes with *Glu-B3a*, *c* and *d*), *GluB3-42* (*Glu-B3b*), *GluB3-43* (*Glu-B3e* and *h*), *GluB3-44* (*Glu-B3f* and *g*) and *GluB3-45* (*Glu-B3i*). Compared with *GluB3-41*, three SNPs were detected in the coding region of *GluB3-42*, *GluB3-43* and *GluB3-44*, and six SNPs and a 21-bp deletion in *GluB3-45*. Ten allele-specific PCR markers were designed based on the SNPs presented in the identified allelic variants for the discrimination of *Glu-B3* protein mobility alleles *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h* and *i*. These markers were validated with the 8 NILs and Cheyenne, and 161 wheat varieties and advanced lines with different *Glu-B3* alleles, indicating they may be useful in marker-assisted breeding to improve wheat quality. The detailed information of the *Glu-B3* genes and functional markers associated with different *Glu-B3* alleles are available at our lab upon request.

Six *Glu-D3* genes, designated as *GluD3-1*, *GluD3-2*, *GluD3-3*, *GluD3-4*, *GluD3-5* and *GluD3-6*, respectively, were identified in the eight cultivars[8, 9]. Computer program DNAMAN was used to predict the protein outputs from these genes. *GluD3-1* showed two allelic variations, one amplified from cultivars containing *b* and *c* alleles (designated *GluD3-1bc*) and the other from *a*, *d*, and *e* alleles (designated *GluD3-1ade*). Compared with *GluD3-1bc*, a 3-bp deletion was found in the coding region of N-terminal repetitive domain of *GluD3-1ade* , leading to a glutamine deletion at the 116th position. *GluD3-2* had three variants in the eight cultivars, which were designated as $GluD3\text{-}2ae_2$, $GluD3\text{-}2be_1$ and *GluD3-2cd*, respectively. In comparison to $GluD3\text{-}2ae_2$, a single nucleotide polymorphism (SNP) was detected for $GluD3\text{-}2be_1$ in the signal peptide region, resulting in an amino acid change from alanine to threonine at the 11th position; and 11 mutations were found at *GluD3-2cd*, with five in upstream region, four in coding region and two in downstream region, respectively. *GluD3-3* had two allelic variations, designated as *GluD3-3abe* and *GluD3-3cd*, encoding LMW-m and LMW-i types of glutenin subunits, respectively. Compared with the GenBank *GluD3* genes, nucleotide sequences of $GluD3\text{-}2ae_2$ and *GluD3-2cd* were the same as *X13306* and *AB062875*, respectively. $GluD3\text{-}2be_1$ and *GluD3-1ade* had only one-base difference from *U86027* and *AB062865*, respectively. *GluD3-1bc* gene was not found in the GenBank database, indicating a newly identified *GluD3* gene variation. *GluD3-3* was a new gene different from any other known *Glu-D3* genes. *GluD3-4* showed three allelic variants or haplotypes at the DNA level in the eight cultivars, which were designated as *GluD3-41*, *GluD3-42* and *GluD3-43*. Compared with *GluD3-42*, a single nucleotide polymorphism (SNP) was detected for *GluD3-43* in the coding region, resulting in a pseudo-gene with a nonsense mutation at the 119th position of deduced peptide, and a 3-bp insertion was found in the coding region of *GluD3-41*, leading to a glutamine insertion at the 249th position of its deduced protein. The coding regions for *GluD3-5* and *GluD3-6* showed no allelic variation in the eight cultivars tested, indicating that they were relatively conservative in common wheat. Based on the 12 allelic variants of three *Glu-D3* genes identified in this study and three detected previously, seven STS markers were established to amplify the corresponding gene sequences in wheat cultivars containing five *Glu-D3* alleles (*a*, *b*, *c*, *d* and *e*). The seven primer sets M2F12/M2R12, M2F2/M2R2, M2F3/M2R3, M3F1/M3R1, M3F2/M3R2, M4F1/M4R1 and M4F3/M4R3 were specific to the allelic variants *GluD3-21/22*, *GluD3-22*, *GluD3-23*, *GluD3-31*, *GluD3-32*, *GluD3-41* and *GluD3-43*, respectively, which were validated by amplifying 20 Chinese wheat cultivars containing alleles *a*, *b*, *c* and *f* based on protein electrophoretic mobility. These markers will be useful to identify the *Glu-D3* gene haplotypes in wheat breeding programs.

REFERENCES

1. Cornish GB, Burridge PM, Palmer GA, Wrigley CW (1993) Mapping the origins of some HMW and LMW glutenin subunit alleles in Australian germplasm. Proceedings of the 42nd Australian Cereal Chemistry Conference, Sydney, pp 255–260
2. Gupta RB, Shepherd KW (1990) Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutenin. I. Variation and genetic

control of the subunits in hexaploid wheats. Theor Appl Genet 80: 65–74
3. He ZH, Liu L, Liu JJ, Xia XC, Peña RJ (2005) Composition of HMW and LMW glutenin subunits and their effects on dough properties, pan bread, and noodle quality of Chinese bread wheats. Cereal chem 82:345–350
4. Singh NK, Shepherd KW (1988) Linkage mapping of genes controlling endosperm storage proteins in wheat.
5. McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols for wheat. Proceedings of the 9th International Wheat Genetics Symposium, Saskatoon, Canada, pp. 1–235.
6. Zhang W, Gianibelli MC, Ma W, Rampling L, Gale KR (2003) Identification of SNPs and development of AS-PCR markers for γ-gliadin alleles in *Triticum aestivum*. Theor Appl Genet 107:130–138
7. Zhang W, Gianibelli MC, Rampling L, Gale KR (2004) Characterisation and marker development for low molecular weight glutenin genes from *Glu-A3* alleles of bread wheat (*Triticum aestivum* L.). Theor Appl Genet 108:1409–1419
8. Zhao XL, Xia XC, He ZH, Gale KR, Lei ZS, Appels R, Ma WJ (2006) Characterization of three low-molecular-weight *Glu-D3* subunit genes in common wheat. Theor Appl Genet.113:1247–1259
9. Zhao XL, Xia XC, He ZH, Lei ZS, Appels R, Yang Y, Sun QX, Ma W (2007) Novel DNA variations to characterize low molecular weight glutenin *Glu-D3* genes and develop STS markers in common wheat. Theor Appl Genet 114:451–460