Theor Appl Genet (2000) 101:892–896

M. Ahmad

Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers

Received: 14 August 1999 / Accepted: 21 March 2000

Abstract While quality in hexaploid wheat (*Triticum aestivum* L. em Thell.) is a very complex trait, it is known that the water-insoluble gluten proteins are responsible for the elasticity and chohesiveness (strength) of dough and are therefore important determinants of breadmaking quality. High-molecular-weight (HMW) glutenin subunits encoded by genes on the long arm of group 1 chromosomes have been associated with gluten strength, and a portion of the variability between cultivars can be attributed to glutenin subunit composition. Good or poor wheat breadmaking quality is associated with two allelic pairs at the *Glu-D1* complex locus, designated 1Dx5–1Dy10 and 1Dx2–1Dy12, respectively. Among the HMW glutenin subunits encoded at *Glu-B1*, Bx7 is quite common, being associated with either of two subunits, By8 or By9. Both allelic pairs contribute moderately well to good breadmaking quality by increasing dough elasticity. Glutenin subunit screening is accomplished using electrophoresis (SDS-PAGE). In this paper, I report the development of an alternative screening method based on glutenin genes themselves using the polymerase chain reaction (PCR). This easy, quick and non-destructive PCR-based approach is an efficient alternative to standard procedures for selecting bread-wheat genotypes with good breadmaking characteristics.

Key words Marker-assisted selection · PCR · Glutenin subunits · Bread quality · Hexaploid wheat

Introduction

Bread quality of hexaploid wheat is a complex feature which depends on many elements. However, the water-insoluble glutenin proteins encoded by the group 1 homoeologous chromosomes of the wheat A, B and D genomes are mainly responsible for the elasticity and extensibility (visco-elasticity) of dough, which contribute to breadmaking quality. Doughs that have high elasticity and reasonable extensibility are ideal for making bread; doughs which are highly extensible are good for cookies; doughs with intermediate properties are used for making the flat breads in the Middle East and the Indian subcontinent, or noodles in the Far East. Glutenins are composed of both high-molecular-weight (HMW) and low-molecular-weight (LMW) subunits based on their mobility on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and are numbered according to their migration in the gel (Payne et al. 1979; Zhen and Mares 1992). The higher molecular-weight glutenin subunits (HMW-GS) are controlled by pairs of tightly linked loci on the long arm of the group 1 homeologous chromosomes. The locus pair contains genes that encode two types of HMW glutenin subunits, one of greater molecular weight, designated the x-type, and the other of lower molecular weight, designated the y-type (Harberd et al. 1986). The A genome y-type gene(s) are silent in hexaploid bread wheats but are active in many diploid and tetraploid wheats. Allelic variation exists at each of the HMW glutenin loci (Payne and Lawrence 1983), and numerous studies have demonstrated that allelic composition significantly affects dough properties. Most significantly, the HMW glutenin subunits 1Dx5–1Dy10 have been associated with high dough strength and good breadmaking quality, whereas allelic subunits 1Dx2–1Dy12 are associated with poor breadmaking properties (Payne et 1987; Hamer et al. 1992). Among the HMW glutenin subunits encoded at *Glu-B1*, Bx7 is quite common, being present in many different bread and durum wheat cultivars (Shewry et al. 1992; Lookhart et al. 1993). Bx7 is usually associated with either of two By subunits, By8 or By9. Although it is not possible to separate the quality contributions of Bx7 from its corresponding y-type subunits, both allelic pairs apparently contribute moderately well to good bread quality.

Communicated by F. Salamini

M. Ahmad (✉)
New Zealand Institute for Crop & Food Research Limited,
P.O. Box 4704, Christchurch, New Zealand
e-mail: Ahmadm@crop.cri.nz

The identification of allelic pairs present in a genotype is currently carried out at the protein level by comparing the relative migration of these HMW glutenin subunits on SDS-PAGE. However, I wanted to develop an alternative efficient, accurate and reliable diagnostic technique based on the gene sequence of a genotype using the polymerase chain reaction (PCR) (Saiki et al. 1988, 1985) instead of the polypeptide gene product. In fact, PCR has already been used to identify the gliadin gene and LMW genes of durum wheat (D'Ovidio et al. 1992; D'Ovidio 1993) and HMW glutenin genes (D'Ovidio and Anderson 1994). Here I report the utility of specific PCR primers to identify wheat genotypes carrying glutenin allelic combinations which are related with good or poor breadmaking quality.

Materials and methods

DNA extraction

Genomic DNA was extracted from 2 g of fresh leaves from single plants of five wheat cultivars with different glutenin genotypes covering a range of bread quality allelic combinations (Table 1). Leaves were crushed to a fine powder in a mortar after freezing with liquid nitrogen and 4 ml of extraction buffer (1% sarkosyl, 100 m*M* TRIS-HCl, 100 m*M* NaCl, 10 m*M* EDTA, pH 8.5) was added. The homogenised mixture was transferred into a 15-ml plastic tube, 4 ml of phenol/chloroform/isoamylalcohol (25:24:1) was added and the mixture was incubated at room temperature for 15 min with mixing. After centrifugation for 10 min at 5000 rpm, the supernatant was carefully poured into a silica matrix tube and re-extracted with 4 ml of phenol/chloroform/isoamylalcohol (25:24:1) for 10 min. After further centrifugation at 5000 rpm for 10 min, the supernatant was transferred to a fresh plastic tube where DNA was precipitated by adding 400 µl of 3 *M* NaOAc (pH 4.8) and 4 ml of isopropanol. The DNA pellet was washed with 70% ethanol, air-dried and resuspended in 350 µl of R 40 (40 µl RNase A added to 1 ml of 10 m*M* TRIS-HCl, 1 m*M* EDTA, pH 8.0, boiled for 10 min to destroy DNase, stored at –20°C) overnight at 4°C. Absorbance at 260 nm and 280 nm was used to evaluate DNA quality and to standardise DNA concentrations.

Table 1 Bread-wheat cultivars subjected to this study and the composition of their relative allelic HMW glutenin subunits

Number	Cultivar	HMW-glutenin subunits				
		1Ax	1Bx	1By	1Dx	1Dy
1	Klasic	1	17	18	5	10
2	Hartog	1	17	18	5	10
3	Neepawa	2*	7	9	5	10
4	Rata	N	7	9	3	12
5	Chinese Spring	N	7	8	2	12

PCR analysis

The 50-µl amplification reaction contained 10 m*M* TRIS-HCl, pH 8.3, 50 m*M* KCl, 2 m*M* $MgCl_2$, 0.001% gelatine, 300 µ*M* of each dNTP (deoxyribonucleotide), 250 ng of each primer (Table 2), 50 ng genomic DNA and 1 U *Taq* DNA polymerase (Boehringer Mannheim, Germany).

Amplifications were performed in a Eppendorf Mastercycler® Gradient programmed at 94°C for 5 min, followed by 45 cycles at 94°C for 1 min, 63°C for 1 min and 72°C for 1 min, except for primers P5 and P6 where the annealing temperature was 60°C for 1 min and extension was 72°C for 3 min. After 45 cycles, the extension temperature was kept at 72°C for 10 min. Half of each total PCR product was analysed on 3% agarose gels and then stained in 0.5 µg/ml ethidium bromide for 1 h before visualisation under UV light.

Results

The availability of the nucleotide sequences of the HMW glutenin subunit genes designated 1Dx2 (Sugiyama et al. 1985), 1Dx5 (Anderson et al. 1989), 1Bx7 (Anderson and Greene 1989), 1Dy10 and 1Dy12 (Smith et al. 1994) enabled the construction of six oligonucleotides specific for the HMW glutenin 1Dx5, 1Bx7, 1Dy10 and 1Dy12 alleles (Table 3). The 1Dx2 and 1Dx5 genes have a high degree of homology and a large repetitive region, which are characteristics of HMW glutenin genes. 1Dy10 and 1Dy12 genes also have a high degree of similarity among their sequences, but is was possible to find regions differing between 1Dx2 and 1Dx5 genes and 1Dy10 and 1Dy12 genes to design site-specific primers for PCR analysis.

To verify the applicability of this PCR-based method of distinguishing between wheat cultivars possessing the HMW glutenin 1Dx5 gene, DNA samples of five cultivars were analysed (Table 1) using primers P1 and P2. In all the cases the 450-bp amplification product was present only in cultivars known to possess the HMW glutenin 1Dx5 gene (lane 2, Klasic; lane 3, Hartog, lane 4, Neepawa) (Fig. 1). Cultivars Rata (lane 5) and Chinese Spring (lane 6), encoding subunits 3 and 2 at the 1Dx gene, respectively, did not show the characteristic 450-bp amplification band (Fig. 1).

With primer combinations P3 and P4, the Dy10 templates amplified a 576-bp fragment, and the Dy12 tem-

Table 2 Primer sequences used to amplify HMW glutenin subunits

Primer	Sequence (5´.....3´)	Allele	Reference
P1	GCCTAGCAACCTTCACAATC	Dx2, Dx5	(Anderson et al. 1989)
P2	GAAACCTGCTGCGGACAAG		
P3	GTTGGCCGGTCGGCTGCCATG	Dy10, Dy12	(Smith et al. 1994)
P4	TGGAGAAGTTGGATAGTACC		
P5	ATGGCTAAGCGCCTGGTCCT	Bx7	(Anderson and Greene 1989)
P6	TGCCTGGTCGACAATGCGTCGCTG		

Table 3 Allelic amplification with specific primers and their expected fragments

Allele	Primer	Expected DNA fragment
1Dx5	P1+P2	450 bp
1Dy10	P3+P4	576 bp
1Dy12	P3+P4	612 bp
1Bx7	P5+P6	2373 bp

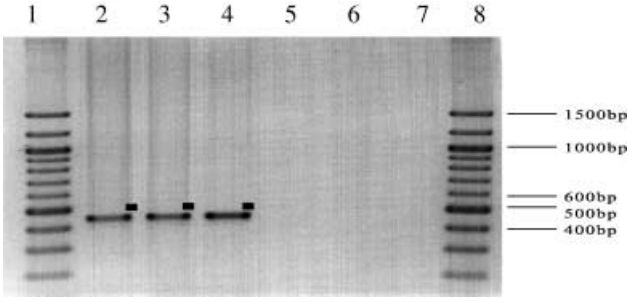

Fig. 1 Primers (P1 and P2) specific for the 1Dx5 allele were used for PCR reaction on 50 ng of genomic DNA from five bread wheat cultivars for the 450-bp (■) DNA fragment amplification. *Lanes: 1* 100-bp DNA ladder (New England Biolabs no. 323-1L); the molecular weight of the fragments are reported in base pairs (bp), *2* Klasic (1Dx5–1Dy10), *3* Hartog (1Dx5–1Dy10), *4* Neepawa (1Dx5–1Dy10), *5* Rata (1Dx3–1Dy12), *6* Chinese Spring (1Dx2–1Dy12), *7* control (no DNA), *8*, same as *lane 1*. The *block* (■) indicates the 450-bp band specific to the 1Dx5 allele

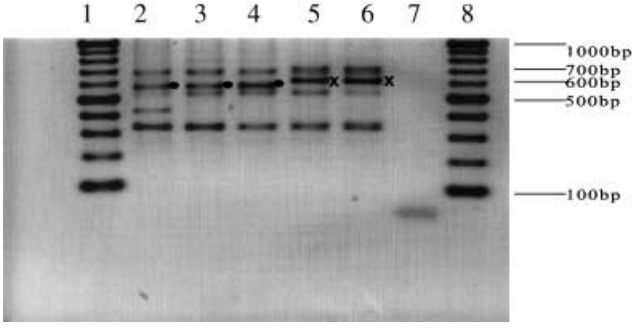

Fig. 2 Primers (P3 and P4) specific for the 1Dy10 and 1Dy12 alleles were used for PCR reaction on 50 ng of genomic DNA from five bread wheat cultivars for the 576-bp and 612-bp DNA fragment amplifications, respectively. *Lanes: 1* 100-bp DNA ladder (New England Biolabs no. 323-1L); the molecular weight of the fragments are reported in base pairs (bp), *2* Klasic (1Dx5-1Dy10), *3* Hartog (1Dx5–Dy10), *4* Neepawa (1Dx5–1Dy10), *5* Rata (1Dx3–1Dy12), *6* Chinese Spring (1Dx2–1Dy12), *7* control (no DNA), *8* same as *lane 1*. The *dot* (●) indicates the 576-bp fragment specific to the 1Dy10 allele, and the cross (*X*) indicates a 612-bp fragment specific to the 1Dy12 allele

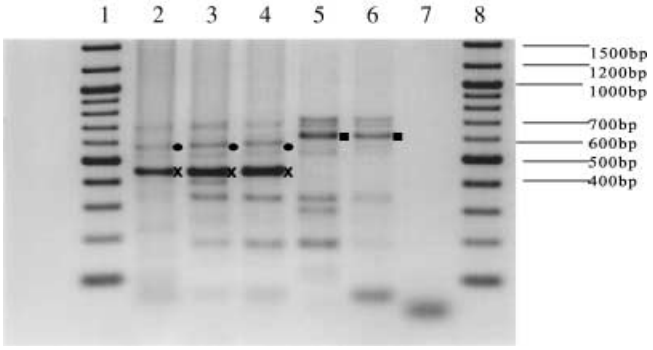

Fig. 3 Primer pairs (P1+P2 and P3+P4) specific for the 1Dx5 and 1Dy10/1Dy12 alleles were used for multiplex PCR reaction on 50 ng of genomic DNA from five bread wheat cultivars for the 450-bp and 576-bp/612-bp DNA fragment amplifications, respectively. *Lanes 1*, 100-bp DNA ladder (New England Biolaps no. 323-1L); the molecular weight of the fragments are reported in base pairs (bp), *2* Klasic (1Dx5–1Dy10), *3* Hartog (1Dx5–1Dy10), *4* Neepawa (1Dx5–1Dy10), *5* Rata (1Dx3–1Dy12), *6* Chinese Spring (1Dx2–1Dy12), *7* control (no DNA), *8* same as *lane 1*. The cross indicates (X) the 450-bp DNA fragment specific to the 1Dx5 allele, the *dot* (●) the 576-bp DNA fragment specific to the 1Dy 10 allele, the *block* (■) the 612-bp DNA fragment specific to the Dy12 allele

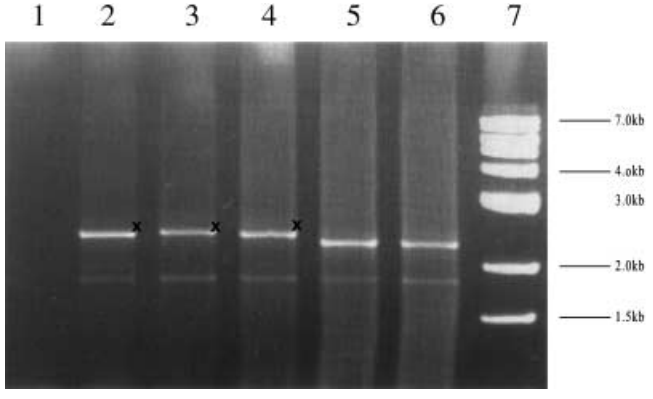

Fig. 4 Primers (P5 and P6) specific for the 1Bx7 allele were used for PCR reaction on 50 ng of genomic DNA from five bread wheat cultivars for the 2373-bp DNA fragment amplification. *Lanes: 1* Control (no DNA), *2* Chinese Spring (1Bx7–1By8), *3* Rata (1Bx7–1By9), *4* Neepawa (1Bx7–1By9), *5* Hartog (1Bx17–1By18), *6* Klasic (1Bx17–1By18), *7* 1-kb DNA ladder (New England Biolabs no. 323-2L); the molecular weight of the fragments are reported in base pairs (bp). The *cross* (X) indicates the 2373-bp DNA fragment specific to the 1Bx7 allele

plates amplified a 612-bp fragment that is easily distinguished from the Dy10 fragment (Fig. 2). These fragment sizes were expected from the gene sequence. DNA from three cultivars, Klasic (lane 2), Hartog (lane 3) and Neepawa (lane 4) directed amplification of the Dy10 marker fragment, whereas Rata (lane 5) and Chinese Spring (lane 6) amplified the Dy12 marker fragment. Four primer combinations, P1, P2, P3 and P4 were used to develop a simple multiplex PCR assay to distinguish both alleles of HMW glutenin at the 1Dx and 1Dy levels related to bread quality. Cultivars Klasic (lane 2), Hartog (lane 3) and Neepawa (lane 4), containing the 1Dx5 and 1Dy10 alleles, showed two fragments, the 450-bp fragment specific to the 1Dx5 allele and the 576-bp fragment specific to the 1Dy10 allele (Fig. 3). Two cultivars, Rata (lane 5) and Chinese Spring (lane 6) did not produce any amplification of the 450-bp 1Dx5 fragment but did pro-

duce a 612-bp fragment specific to the 1Dy12 allele. The combination of four primers without any addition of genomic DNA produced a primer dimer fragment less than 100 bp in size (lane 7).

PCR analysis using primers P5 and P6 specific for the coding region of Bx7 gene, based on the nucleotide sequences of the Bx7 gene of wheat cv. Cheyenne (Anderson and Greene 1989), were carried out to identify wheat cultivars possessing this sequence. Cultivars Klasic (lane 6), Hartog (lane 5) did not show the amplification specific to Bx7, while cvs. Neepawa (lane 4), Rata (lane 3) and Chinese Spring (lane 2) produced a fragment of 2373 bp, as expected from the nucleotide sequence informaiton (Anderson and Greene 1989) specific to the Bx7 gene (Fig. 4).

Discussion

Recent developments in DNA marker technology together with the concept of marker-assisted selection provide new solutions for selecting and maintaining desirable genotypes. Diagnostic PCR analysis with primers specific not only for specific genes but also for nearly identical alleles of a given gene represents a useful new tool for identifying genotypes for specific purposes.

At the *Glu-D1* locus of most bread wheat cultivars two allelic pairs, Dx5+Dy10 (good quality) and Dx2+Dy12 (poor quality) have been detected (Payne et al. 1987). Along with pairs Dx5+Dy10 and Dx2+Dy12, the pairs Dx2+Dy10 and Dx5+Dy12 have also been reported to be present at the *Glu-D1* locus in wheat cultivars (Payne and Lawrence 1983; Margiotta et al. 1993). Marker-assisted selection of single alleles, Dx2 or Dx5 assuming their tight linkage with Dy12 (poor quality) and Dy10 (good quality), respectively, as reported by D'Ovidio and Anderson (1994) may lead plant breeders to select wrong allelic combinations when aiming for bread quality improvement. In this situation, marker-assisted selection of a single allele, either Dx2 or Dx5, is not useful for selecting genotypes for bread quality. Therefore, marker-assisted selection of both alleles (Dx2+Dy12 or Dx5+Dy10) at the same time is a critical factor in wheat breeding for bread quality. The results reported here clearly demonstrate that this diagnostic PCR system can help to select both alleles and avoid the misleading marker-assisted selection of Dx2+Dy10 and Dx5+Dy12, both poor quality allelic combinations.

SDS-PAGE is one of the most widespread techniques used in wheat breeding laboratories for detecting allelic forms related to good or poor quality. Shewry et al. (1992) reported that the mobility of HMW glutenin subunits in SDS-PAGE is not always correlated to their actual molecular weights, which might be a problem for breeders selecting parental lines for breeding programmes. The results of this study show that marker-assisted selection (MAS) can help avoid the misleading interpretation of the results obtained either on SDS-PAGE analysis or single allelic selection through PCR analysis of HMW glutenin subunits at *Glu-D1* locus for bread quality. Bx7 either with By8 or By9 contributes to increase dough elasticity and resistance to some degree and might be a useful incorporation into new wheat cultivars if a strong mixing character is required.

Conclusion

Accuracy, simplicity and speed make the proposed PCR-generated DNA markers a valid alternative to standard techniques for selecting genotypes containing the high-molecular-weight glutenin subunits related to bread quality. Moreover, MAS could allow the screening of hundreds of plants in 1 day for a quick, early-generation evaluation, thereby saving time and resources otherwise required for seed increase and physical quality testing in a wheat breeding programme.

Acknowledgements The author is thankful to the Foundation for Research, Science and Technology, Wellington for funding this project (Contract No. C02625) and the New Zealand Lottery Grants Board for awarding a lottery research science grant (SR 86018).

References

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

Anderson DD, Greene FC, Yip RE, Halford NG, Shewry PR, Malpica-Romero J-M (1989) Nucleotides sequences of the two 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

D'Ovidio R (1993) Single-seed PCR of HMW glutenin genes to distinguish between durum wheat cultivars with good and poor technological properties. Plant Mol Biol 22:1173–1176

D'Ovidio R, Anderson OD (1994) PCR analysis to distinguish between alleles of a member of a multigene family correlated with wheat bread-making quality. Theor Appl Genet 88: 759–763

D'Ovidio R, Tanzarella OT, Masci S, Lafiandra D, Porceddu E (1992) RFLP and PCR analyses at *Gli-1, Gli-2, Glu-1* and *Glu-3* loci in cultivated and wild wheats. Hereditas 116:79–85

Hamer RJ, Weegels WP, Marseille JP (1992) Prediction of bread-making quality of wheat: the use of HMW glutenin A subunit based quality scoring systems. J Cereal Sci 15:91–102

Harberd NP, Bartels D, Thompson RD (1986) DNA restriction fragment variation in the gene family encoding high-molecular-weight (HMW) glutenin subunits of wheat. Biochem Genet 24:579–596

Lookhart GL, Hagman K, Kasarda DD (1993) High-molecular-weight glutenin subunits of the most commonly grown wheat cultivars in the U.S. in 1984. Plant Breed 110:48–62

Margiotta B, Colaprico G, D'Ovidio R, LAfiandra D (1993) Characterization of high-M*r* subunits of glutenin by combined chromatographic and electrophoretic separations and restriction fragment length polymorphism (RFLP) analysis of their encoding genes. J Cereal Sci 17:221–236

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1, Glu-B1,* and *Glu-D1* which code for high molecular weight subunits in glutenin in hexaploid wheat. Cereal Res Commun 11:29–35

Payne PI, Corfield KG, Blackman JA (1979) Identification of a high molecular weight subunit of glutenin whose presence correlated with breadmaking quality in wheats of related pedigree. Theor Appl Genet 55:153–159

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

Saiki RK, Scharf SJ, Fabona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1355

Saiki RK, Gelgand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science 239:487–491

Shewry PR, Halford NG, Tatham AS (1992) High-molecular-weight subunits of wheat glutenin. J Cereal Sci 15:105–120

Smith RL, Schweder ME, Barnett RD (1994) Identification of glutenin alleles in wheat and triticale using PCR-generated DNA markers. Crop Sci 34:1373–1378

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

Zhen Z, Mares D (1992) A simple extraction and one-step SDS-PAGE system for separating HMW and LMW glutenin subunits of wheat and high molecular weight proteins of rye. J Cereal Sci 15:63–78