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Original article

Identification and characterization of high-molecular-weight glutenin genes in Polish triticale cultivars by PCR-based DNA markers

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Abstract. Molecular markers were used to identify the allele/gene composition of complex loci *Glu-A1* and *Glu-B1* of high-molecular-weight (HMW) glutenin subunits in triticale cultivars. Forty-six Polish cultivars of both winter and spring triticale were analysed with 7 PCR-based markers. Amplified DNA fragments of HMW glutenin *Glu-1* genes were separated by agarose slab-gel electrophoresis. Differences between all 3 alleles at the locus *Glu-A1* [*Glu-A1a* (encoding Ax1), *1b* (Ax2*), and *1c* (AxNull)], 4 alleles at *Glu-B1-1* [*Glu-B1-1a* (Bx7), *1b* (Bx7*), *1d* (Bx6), *1ac* (Bx6.8)], and 5 alleles at *Glu-B1-2* [*Glu-B1-2a* (By8), *2b* (By9), *2o* (By8*), *2s* (By18*), and *2z* (By20*)] were revealed. In total, 16 allele combinations were observed. Molecular markers are particularly helpful in distinguishing the wheat *Glu-A1a* and *Glu-A1b* alleles from the rye *Glu-R1a* and *Glu-R1b* alleles in triticale genotypes, respectively, as well as subunits Bx7 from Bx7* and By8 from By8*, which could not be distinguished by SDS-PAGE. Novel glutenin subunits By18* and By20* (unique to triticale) were identified. HMW glutenin subunit combinations of Polish triticale cultivars, earlier identified by SDS-PAGE analyses, were verified by PCR-based DNA markers. Rapid identification of wheat *Glu-1* alleles by molecular markers can be an efficient alternative to the standard separation procedure for early selection of useful triticale genotypes with good bread-making quality.

Keywords: allelic variation, allele-specific markers, *Glu-1* loci, HMW glutenin subunits, triticale.

Introduction

Hexaploid triticale (× *Triticosecale* Wittmack) is a man-made cereal hybrid created by crossing wheat (*Triticum* spp.) with rye (*Secale cereale* L.) Significant progress in breeding of the species was made in the last two decades. The future of triticale is bright because it has a high yield potential, is environmentally more flexible than other cereals, and shows better tolerance to pathogens than its parental species (Oettler 2005; Barnett et al. 2006; Lelley 2006). Triticale contains high concentrations of the essential amino acid lysine, which makes it more nutritionally valuable than wheat. In comparison with common wheat, most triticale flour usually has a lower protein content and milling yield, higher ash content and inferior loaf volume, which limit its bread-making quality (Serna-Saldivar et al. 2004; Lelley 2006).

In hexaploid triticale (2n=6x=42, AABBRR) the rye (R) genome replaces the D genome of bread wheat. This difference in genomic constitution has a noticeable influence on the bread-making quality of triticale. The secalins, inherited from rye, are distinct from wheat storage proteins and not capable of forming gluten. The bread-making quality of bread wheat is mainly determined by the composition and quantity of gluten-forming storage proteins, in particular the high-molecular-weight glutenin subunits (HMW-GS) (Wieser et al.1998; Butow et al. 2003, 2004). Various alleles of HMW glutenin genes are

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particularly important for determining wheat gluten and dough elasticity (Shewry et al. 1992). In triticale there are only 4 HMW glutenin genes, even though 1–3 subunits are synthesized in any particular cultivar (Igrejas et al. 1999; Amiour et al. 2002a). The HMW glutenin genes are encoded at the complex loci *Glu-A1* and *Glu-B1* on the long arms of chromosomes 1A and 1B (Shewry et al. 1992). Each locus includes 2 tightly linked genes encoding 2 types of HMW glutenin subunits, designated as x-type and y-type. The corresponding rye HMW secalin genes are encoded at the *Glu-R1* locus, also designated as *Sec-3* (Shewry et al. 1984), which is located on the long arm of chromosome 1R. The elimination of the wheat *Glu-D1* locus, with its known positive effects on wheat bread-making quality, causes the total amount of gluten in triticale to be lower and thus its bread-making quality is poorer. Although genetic variability in gluten content exists, the highest gluten content of triticale is still 10–15% lower than that of wheat (Pena 1996).

Traditionally, polyacrylamide gel electrophoresis (SDS-PAGE and/or A-PAGE), reverse-phase high performance liquid chromatography (RP-HPLC) and capillary electrophoresis were the most widely used techniques for identification of wheat HMW glutenin composition and for investigation of their effects on bread quality (Payne and Lawrence 1983; Shewry et al. 1992; Wieser et al. 1998; Bean and Lookhardt 2000). In triticale, the storage proteins (HMW and LMW glutenin, and secalin subunits) identified by SDS-PAGE show a significantly lower range of storage proteins than in wheat, as reported in literature (Rozinek et al. 1998; Igrejas et al. 1999; Amiour et al. 2002a,b). The presence of both wheat and rye HMW proteins in triticale, which appear in different amounts and additionally are often characterized by similar electrophoretic mobilities, interferes to a large extent with the identification of individual HMW subunits by using SDS-PAGE gels. Particularly, some functionally different HMW glutenin subunits, such as Bx7 vs. Bx7* or By8 vs. By8*, have similar relative electrophoretic mobilities and cannot be unambiguously identified by SDS-PAGE (Butow et al. 2003, 2004).

Polymerase chain reaction (PCR) techniques have been used as alternative forms of analysis of HMW glutenin subunits from wheat. Several specific primer sets amplifying fragments of wheat DNA for single genes or alleles present at the complex *Glu-1* loci have been developed (Lafiandra et al. 1997; Ahmad 2000; De Bustos et al. 2000; Radovanovic and Cloutier 2003; Butow et al. 2003, 2004; Lei et al. 2006). DNA fragments of wheat HMW glutenin genes were amplified by single and multiplex PCR methods (Ma et al. 2003; Moczulski and Salmanowicz 2003; Salmanowicz and Moczulski 2004; Gale 2005).

The present paper describes the exploitation of a molecular method to identify hexaploid triticale genotypes carrying different wheat HMW glutenin allelic combinations at *Glu-A1* and *Glu-B1* loci. Seven known wheat primer sets of *Glu-1* loci were examined to detect genes encoding Ax1, Ax2* and AxNull at the *Glu-A1* locus; and Bx6, Bx7, Bx7*, By8, By8*, By18* and By20* at the *Glu-B1* locus in triticale cultivars. The combinations of HMW glutenin subunits of Polish triticale, determined on the basis of the PCR and SDS-PAGE analyses, were compared with published SDS-PAGE data.

Materials and methods

Plant material

Grain samples of 46 Polish cultivars of both winter (36) and spring (10) hexaploid triticale were analysed. The triticale grain samples were obtained from DANKO Plant Breeding at Choryń, and Plant Breeding Companies at Strzelce (Branch at Małyszyn) and Szelejewo (western Poland).

Isolation of HMW storage proteins and separation by SDS-PAGE

Total seed proteins were extracted from single crushed half-seeds (non-embryo half, ca. 20 mg). Extractions were performed with 200 μL SDS reducing sample buffer [62.5 mM Tris, pH 6.8, 4% (w/v) SDS, 10% (v/v) glycerol, 2% (v/v) mercaptoethanol, and 0.001% bromophenol blue] for 1h at room temperature. The samples were centrifuged at 15 400 g for 10 min. Equal volumes of supernatants were then loaded onto the upper 4.5% gel and the proteins were separated on 11.5% (w/v) polyacrylamide in resolving solution at 200 V for 5 h. The gels were stained overnight with Coomassie Brilliant Blue according to Neuhoff et al. (1998). The wheat HMW glutenin alleles/genes in triticale samples were designated according to Payne and Lawrence (1983), Amiour et al. (2002a) and McIntosh et al. (2003).

DNA purification

Genomic DNA was extracted from lyophilized fresh leaves (150 mg) of a single plant (ca. 3 weeks old). Plant material was ground with a pre-cooled plastic pestle after freezing with liquid nitrogen, and an extraction buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) was added. The homogenate was mixed thoroughly and incubated for 15 min at 65°C. The mixture was supplemented with 1/3 volume of 5 M potassium acetate and was left on ice for 10 min. Next the mixture was centrifuged at 4°C for 15 min at 13 000 g (Allegra™ 21R Beckman-Coulter Centrifuge). The clear supernatant was transferred to a fresh tube and precipitated with 100 μg mL^{-1} ribonuclease. Then the solution was incubated for 20 min at 37°C. The DNA was precipitated by adding isopropanol (1 : 1) and was left on ice for 20 min at –20°C. After centrifugation for 10 min at 12 000 g, the obtained DNA pellet was washed with 70% ethanol, air-dried, and re-suspended in 50 μL TE (10 mM Tris-HCl, 1 mM EDTA) pH 8.

PCR analysis

The oligonucleotides used as primers were synthesized according to published data (Table 1), and purchased from Sigma-Genosys (Germany).

PCR analyses were performed in a PTC-200 thermal cycler (MJ Research, USA) with a heated lid in a final volume of 25 μL. To verify the applicability of the PCR-based method for distinguishing wheat HMW glutenin alleles, DNA samples of 5 standard wheat cultivars and 3 triticale cultivars with well-known wheat HMW glutenin combinations were used for testing. The PCR reaction conditions were established by optimization of concentrations of HotStart *Taq* polymerase (Qiagen, Germany, 0.03–1.5 U μL^{-1}), $MgCl_2$ (1.5–3.5 mM), each primer (0.1–0.4 μM), and each deoxyribonucleotide (150–350 μM). The next step was checked at annealing temperature in the range 57°C–64°C for 40 s to 1 min, extension time in the range 1–2.30 min at 72°C, and number of cycles in the range 30–45.

For the detection of individual HMW glutenin subunits in triticale samples, the following PCR reaction mixture was applied: 1 × PCR buffer (Qiagen), 2 mM $MgCl_2$, 300 μM of each dNTP, 0.2 μM of each primer, 50 ng genomic DNA and 0.5 U HotStar *Taq* DNA Polymerase (Qiagen). After initial denaturation at 95°C for 15 min, 35 cycles were performed, depending on the individual markers, in cycling temperatures presented in Table 2.

The final DNA extension temperature was 72°C for 10 min. The PCR products were separated in ethidium bromide-stained 1.5 or 2.0% (w/v) agarose gels run in 1 × TBE buffer and exposed to UV light to visualize DNA fragments.

Table 1. Sets of allele- and gene-specific markers for identification of HMW glutenin genes of triticale

Primer set	HMW glutenin subunits	Expected size of DNA fragment	Forward and reverseprimer sequences (5'-3')	References
PS1	Ax2* Ax1/AxNull	1400 bp 1500 bp	F:CCATCGAAATGGCTAAGCGG R:GTCCAGAAGTTGGGAAGTGC	Lafiandra et al. 1997
PS2	AxNull	920 bp	F:ACGTTCCCCTACAGGTACTA R:TATCACTGGCTAGCCGACAA	Lafiandra et al. 1997
PS3	Ax2*	2650 bp	F:CCGATTTTGTTCTTCTCACAC R:CACCAAGCGAGCTGCAGAT	De Bustos et al. 2000
PS4	Bx6 Bx7 Bx7*	3 groups: (1 band of 850-920 bp; 4-5 bands of 420-640 bp; 2-4 bands of 180-280 bp)	F:CAAGGGCAACCAGGGTAC R:AGAGTTCTATCACTGCCTGGT	Butow et al. 2004
PS5	By18* By20* By8, By8*, By9	1 band 2 bands 3 bands (290-400 bp)	F:GCAGTACCCAGCTTCTCAA R:CCTTGTCTTGTTTGTTGCC	Lei et al. 2006
PS6	By20* By8, By8*, By18* By9	750 bp 710 bp 660 bp	F:TTCTCTGCATCAGTCAGGA R:AGAGAAGCTGTGTAATGCC	Lei et al. 2006
PS7	By8	527 bp	F:TTAGCGCTAAGTGCCGTCT R:TTGTCCTATTTGCTGCCCTT	Lei et al. 2006

Table 2. PCR cycling conditions for detecting DNA fragments encoding glutenin subunits

Ax2*, Ax1/AxNull	AxNull	Ax2*	Bx6, Bx7*, Bx7	By18*, By20*, By8/By8*/By9	By9, By20*, By8/By8*/By18*	By8
94°C, 1'	94°C, 1'	94°C, 1'	94°C, 45"	94°C, 1'	94°C, 1'	94°C, 1'
60°C, 45"	60°C, 1'	57°C, 1'	58°C, 45"	59°C, 1'	59°C, 1'	64°C, 1'
72°C, 2'10"	72°C, 2'30"	72°C, 2'30"	72°C, 1'	72°C, 2'30"	72°C, 2'	72°C, 2'30"

Results and discussion

SDS-PAGE analysis of HMW glutenin subunits

Protein sample extracts from 46 triticale samples were first analysed for HMW glutenin subunits by one-dimensional SDS-PAGE. Five samples from common wheat – Chinese Spring (N/7 + 8/2 + 12), Glenlea (2*/7 + 8*/5 + 10), Hope (1/6 + 8/5 + 10), Olimpia (2*/17 + 18/5 + 10) and Olcha (N/20 + 20/5 + 10) – were used as reference markers. Figure 1 shows electrophoretic patterns of some triticale cultivars differing in their alleles at 2 wheat loci *Glu-A1* and *Glu-B1* and the rye locus *Glu-R1*. Eight triticale cultivars (see Table 3) were heterogeneous (two-three HMW glutenin patterns). Eleven HMW glutenin alleles were identified among Polish triticale cultivars by SDS-PAGE: 3 alleles at the *Glu-A1* locus [*Glu-A1a* (Ax1), *A1b* (Ax2*) and *A1c* (AxNull)], and 9 alleles at the *Glu-B1* locus. Six of the allele combinations at the *Glu-B1* locus [*Glu-B1a* (Bx7), *B1aa* (Bx7*), *B1ak* (Bx7* + By8*), *B1u* (Bx7* + By8), *B1c* (Bx7* + By9) and *B1d* (Bx6 + By8)] were typical of wheat, and 2 allele combinations [*Glu-B1av* (Bx7 + By18) and *B1aw* (Bx6.8 + By20)] were once reported for triticale (Amiour et al. 2002a,b). The analysed triticale accessions were divided into 16 different groups according to their HMW glutenin subunit composition. Two HMW glutenin subunit compositions, 2*/7 + 18 and 2*/6.8 + 20y, were the most frequent (28.3% and 21.8%, respectively) in the analysed material.

PCR analysis of wheat HMW glutenin genes

Table 1 shows sets of 7 gene- or allele-specific PCR markers, which were used in this study for identification of genes encoding HMW glutenin subunits AxNull, Ax1 and Ax2* at the *Glu-A1* locus and Bx6, Bx6.8, Bx7, Bx7*, By8, By8*, By9, By18, By18*, and By20* at the *Glu-B1* locus in triticale genotypes. In the initial experiments, PCR reactions were performed in modified thermal cycling conditions for each primer pair (PS1–PS7) reported previously for wheat genes by Lafiandra et al. (1997), De Bustos et al. (2000), Butow et al. (2004) and Lei et al. (2006). The PCR reactions for genomic DNA from 5 wheat and 3 triticale cultivars with known composition of HMW glutenin subunits gave the expected products (data not shown).

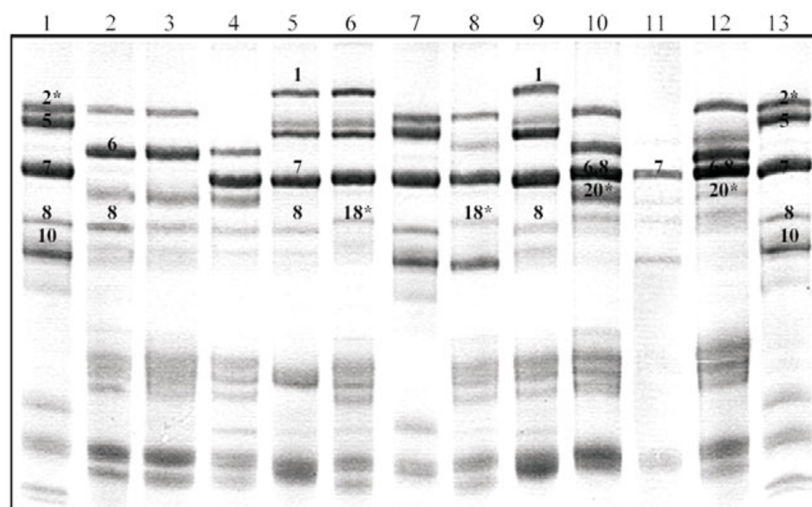

Figure 1. SDS-PAGE pattern of HMW glutenin subunits of selected Polish triticale cultivars with characteristic subunit sets at the *Glu-A1* and Glu-B1 loci. Lanes: 1, 7 and 13 = bread wheat Glenlea as standard (2*/7 + 8*/5 + 10); 2 = Alzo (2*/6 + 8*); 3 = Hewo (2*/6 + 8*); 4 = Prego (N/7* + 8*); 5 = Pawo (1/7* + 8); 6 = Kitaro (1/7 + 18*); 8 = Krakowiak (2*/7 + 18*); 9 = Eldorado (1/7* + 8*); 10 = Sorento (2*/7 + 20*); 11 = Lamberto (N/7 + 20*); 12 = Matejko (2*/7 + 20*).

Table 3. Comparison of wheat HMW glutenin subunit compositions for 46 Polish triticale cultivars determined by PCR to published SDS-PAGE data. Bold letters marked differences between data obtained by PCR and SDS-PAGE.

Cultivars	*Glu-A1*	*Glu-B1*	*Glu-A1/Glu-B1*	*Glu-A1/Glu-B1*
	PCR		SDS-PAGE (Brzeziński 2006)	SDS-PAGE (Amiour et al. 2002b)
Alzo	**2***	6 + **8***	N/6 + 8	2*/6 + 8
Hewo	2*	6 + **8***	2*/6 + 8	
Janko	2*	6 + **8***	2*/6 + 8	
Prado	2*	6 + **8***(7*)	2*/6 + 8 (7)	2*/6 + 8
Ugo	N (2*)	6 + **8***	N (2*)/6 + 8	N/6 + 8
Prego	N	**7*** + **8***	N/7 + 8	N/7 + 8
Bogo	1	**7*** + **8***	1/7 + 8	1/7 + 8
Eldorado	1	**7*** + **8***	1/7 + 8	1/7 + 8
Witon	2*	**7*** + **8***	2*/7 + 8	
Kargo	N	7* + 8 (7 + 8)	N(1)/7 + 8 (/7* + 8)	
Mieszko	N	7* + 8 (7 + 20*)	N/7 + 8 (7* + 25)	
Pronto	N	7* + 8 (7 + 18*)	N/7* + 8 (7* + 26)	
Pawo	1	7* + 8	1/7 + 8	
Mundo	**2***	7* + **9**	N/7 + 26	2*/7 + 18
Woltario	2*	7* + 9 (7 + 18*)	2*/7 + 9 (7* + 26)	
Kazo	N	7 + 18*	N/7 + 26	
Pinokio	N	7 + 18*	N/7* + 26	
Zorro	N	7 + 18*	N/7* + 26	
Disco	1	7 + 18*	1/7* (7* + 26)	1/7 + 18
Kitaro	1	7 + 18*	1/7* + 26	
Aliko	2*	7 + 18*	2*/7 + 26	
Baltiko	2*	7 + 18*	2*/7 + 26	
CHD 503	2*	7 + 18*		
Dinaro	2*	7 + 18*	2*/7 + 26	
Dublet	2*	7 + 18*	2*/7 + 26	
Grenado	2*	7 + 18*	–	
Krakowiak	2*	7 + 18*	2*/7* + 26	
Moderato	2*	7 + 18*	2*/7* + 26	
Moreno	**2***	7 + 18*	N/7* + 26	2*/7 + 18
Nemo	2*	7 + 18*	2*/7 + 26	2*/7 + 18
Piano	2*	7 + **18***	2*/7	1/7 + 9
Presto	2*	7 + 18*	2*/7 + 26	2*/7 + 18
Todan	2*	7 + 18*	2*/7 + 26	
Gabo	N	7 + 20*	N/7* + 25	
Lamberto	N	7 + **20***	N/7*	
Marko	1	7 (7 + 20*)	1/7* (7* + 25)	2*/7 + 18
Fidelio	2*	7 + 20*	N/7* + 8	
Lasko	2* (N)	7 (7 + 20*)	N/7* (7* + 25)	2*/6.8 + 20y
Legalo	**2***	7 + 20*	1/7 + 25	
Matejko	**2***	7 + 20*	1/7 + 25	
Migo	**2***	7 + **20***	N/7*	
Sekundo	2*	7 + 20*	2*/7 + 25	
Sorento	2*	7 + 20*	2*/7 + 25	
Tewo	2*	7 + **20***	2*/7*	2*/6.8 + 20y
Tornado	2*	7 + 20*	2*/7 + 25	2*/6.8 + 20y
Wanad	2*	7 + 20*	2*/7 + 25	

For the identification of *Glu-A1* alleles, 3 primer sets (PS1–PS3) were used. Primer set P1 amplified one-band products for all alleles of the *Glu-A1* locus. The DNA of genotypes with the *Glu-A1b* allele gave in a single PCR reaction a band of 1400 bp (Figure 2, lanes 4, 5, 7, 10 and 11) and with *A1a* and *A1c* alleles gave bands of 1500 bp, similar to previously reported patterns for wheat accessions by Lafiandra et al. (1997). PCR product for the *Glu-A1a* and *A1c* alleles, as in the case of wheat genotypes (De Bustos 2000). In total, for 8 triticale cultivars analysed by PCR assay, results for *Glu-A1* alleles (Table 3) were not in accordance with the data previously presented by Amiour et al. (2002a,b) or Brzeziński (2006). It has already been shown that there is significant difficulty in correct identification of alleles *Glu-A1a* (Ax1) or *Glu-A1b* (Ax2*) in triticale genotypes, which is caused by the simultaneous presence of alleles *Glu-R1a* or *Glu-R1b*, encoding HMW secalin subunits $Rx1^r$ or $Rx2^r$, respectively, with the same electrophoretic mobility (Amiour et al. 2002a). The contradictions in the literature concerning the frequencies of subunit Ax2* in Portuguese cultivars can be explained by this. Amiour and co-workers (2002b) claim that the triticale from Portugal does not have the Ax2* subunit; however, Igrejas et al. (1999) shows its presence in 21.4% of triticale cultivars.

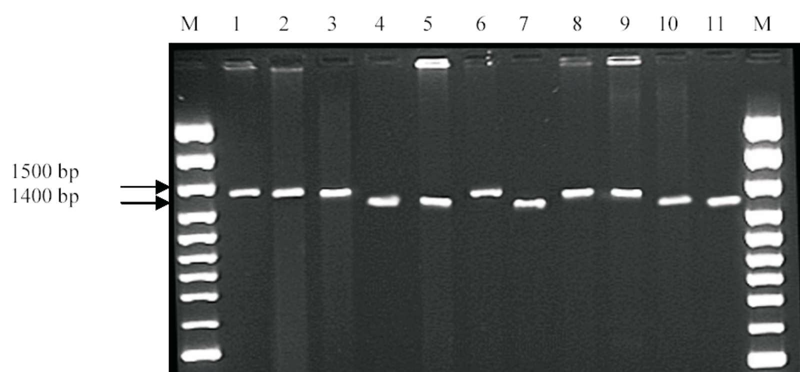

Figure 2. Co-dominant PCR marker for triticale HMW glutenin alleles of the *Glu-A1* locus. Primer set PS1 produces a 1400 bp fragment for the allele *Glu-A1b* (Ax2*) and 1500 bp fragments for *Glu-A1a* (Ax1) and *Glu-A1c* (AxNull). Lanes: M = 100-bp DNA Ladder Plus (Fermentas); 1 = Pawo (Ax1); 2 = Kargo (AxNull); 3 = Eldorado (Ax1); 4 = Hewo (Ax2*); 5 = Krakowiak (Ax2*); 6 = Lamberto (AxNull); 7 = Matejko (Ax2*); 8 = Kitaro (Ax1); 9 = Prego (AxNull); 10 = Sorento (Ax2*); 11 = Tewo (Ax2*).

An amplified product of ca. 920 bp (Figure 3, lanes 2, 4 and 5), which is characteristic of the wheat *Glu-A1c* allele, was observed when PS2 primer set was used for analysis of triticale samples with this allele. As in the case of wheat accessions (Lafiandra et al. 1997), no amplification was obtained with cultivars containing *A1a* and *A1b* alleles.

The presence of primer pair PS3 in the reaction mixture, which is an allele-specific marker for the wheat *Glu-A1b* allele, showed one band of 2650 bp (Figure 4, lanes 3, 4, 5 and 8) in triticale genotypes. This primer pair did not produce any

The application of primer set PS4, which differentiates the alleles of *Glu-B1-1* gene (Butow

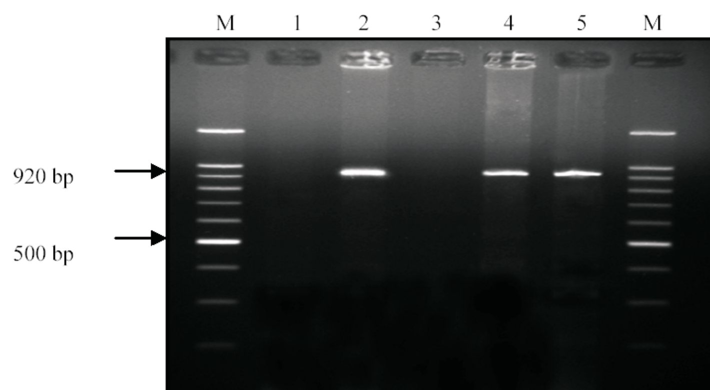

Figure 3. Allele-specific PCR marker (PS2) for the allele *Glu-A1c* (AxNull). The triticale cultivars containing this allele show a DNA fragment of 920 bp. Lanes: M = 100 bp DNA Ladder (Promega); 1 = Eldorado (Ax1); 2 = Mieszko (AxNull); 3 = Pawo (Ax1); 4 = Lamberto (AxNull); 5 = Prego (AxNull)

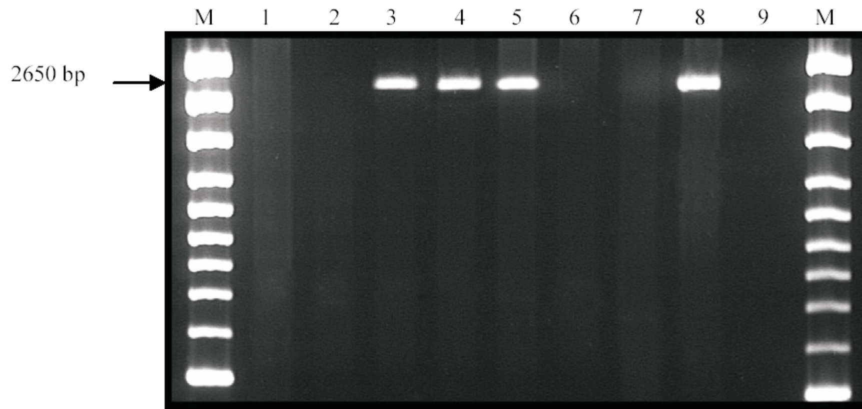

Figure 4. Dominant PCR marker (PS3) specific for the allele *Glu-A1b* encoding HMW glutenin subunit Ax2*. Fragment size 2650 bp. Lanes: M = 100 bp DNA Ladder Plus (Fermentas); 1 = Pawo (Ax1); 2 = Kazo (AxNull); 3 = Krakowiak (Ax2*); 4 = Sorento (Ax2*); 5 = Hewo (Ax2*); 6 = Prego (AxNull); 7 = Kitaro (Ax1); 8 = Todan (Ax2*); 9 = Lamberto (AxNull).

et al. 2004), also showed multi-banding patterns. Similarly to wheat, in triticale cultivars with allele combinations *Glu-B1u* (7* + 8 subunits), *Glu-B1ak* (7* + 8* subunits), *Glu-B1b* (7 + 8 subunits), and *Glu-B1al* (7 + 8* subunits), PCR products in three clear groups (I–III) were amplified (Figure 5, I = a band in the range of 850–920 bp; II = four or five bands of 420–640 bp; and III = two or four bands of 180–280 bp). However, in triticale cultivars containing subunit Bx7 the amplified products (Figure 5, lanes 4 and 8) were different from samples with subunit Bx7* (Figure 5, lanes 1, 2, 3, 5, 7, 10 and 11) in both group II and III bands. The discrimination between By7* and By7 subunits is particularly important, as these subunits have a similar electrophoretic mobility and their respective alleles (*Glu-B1b* and *Glu-B1al*) have contrasting effects on wheat bread-making quality (Butow et al. 2003, 2004). For the allele *Glu-B1-1d*, a new characteristic pattern was observed: a band of ca. 920 bp in group I; five bands (420–640 bp) in group II; and two additional bands in group III (Figure 5, lanes 6 and 9). The *Glu-B1-1d* allele was identified only in five cultivars.

Primer set PS5, specific for By16 and ByNull subunits and the *Glu-B1e* allele in wheat accessions (Lei et al. 2006), amplified three different banding patterns in the range of 180–300 bp for triticale cultivars. Three bands of ca. 280 bp, 330 bp and 380 bp were observed for subunits By8, 8*, 9 (Figure 6). DNA of genotypes with the alleles earlier designated as *By20* (subunit 6.8 + 20y) by Amiour et al. (2002) or *By25* (sub-

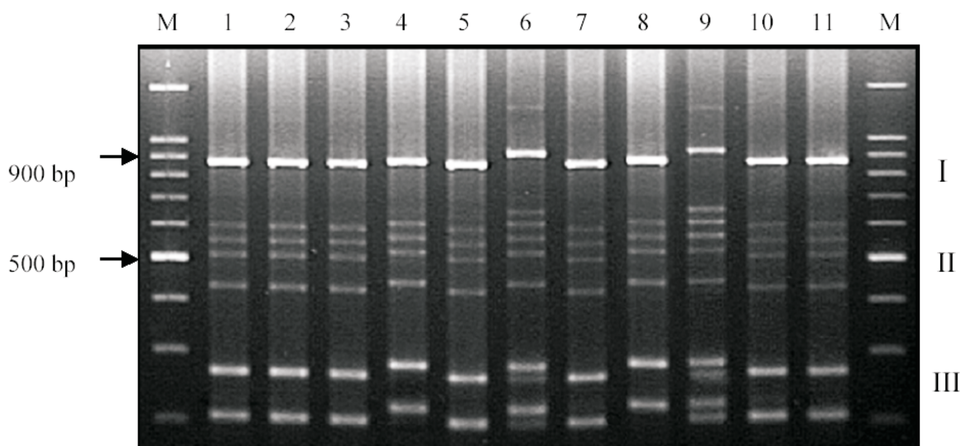

Figure 5. PCR products of primer set PS4 specific for Bx subunits. In the gel, 7–10 PCR products were located in 3 groups (I-III) with different fragment lengths. The genotypes encoding subunits Bx7 and Bx7* produce 1 band of 860 bp in group I; 4 bands of 420-600 bp in group II, and 2 bands of 180-280 bp in group III. PCR products for subunit Bx7, in comparison with subunit Bx7*, have smaller fragments in groups II and III. For the allele *Glu-B1-1d*, the multi-band pattern shows a band of 920 bp in group I; 5 bands (420–640 bp) in group II; and 2 additional bands in group III. Lanes: M = 100 bp DNA Ladder (Promega); 1 = Prego (Bx7* + By8*); 2 = Mundo (Bx7* + By9); 3 = Bogo (Bx7* + By8*); 4 = Kitaro (Bx7 + By18*); 5 = Pronto (Bx7* + By8); 6 = Hewo (Bx6 + By8*); 7 = Kargo (Bx7* + By8); 8 = Piano (Bx7 + By18*); 9 = Alzo (Bx6 + By8*); 10 = Woltario (Bx7* + By9); 11 = Witon (Bx7* + By8*).

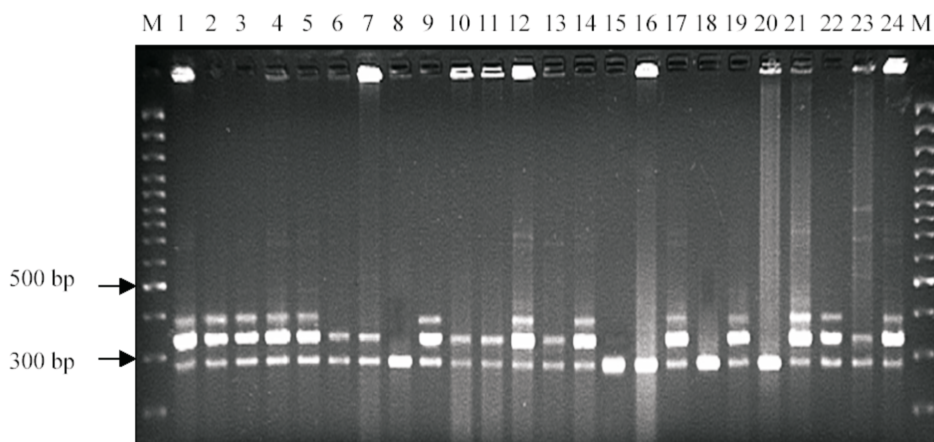

Figure 6. Co-dominant PCR marker for triticale HMW glutenin By subunits. Primer set PS5 produced a band for By18*, 2 bands for By20*, and 3 bands for By8, By8* and By9 each. Lanes: M = 100 bp DNA Ladder (Promega); 1 = Eldorado (Bx7* + By8*); 2 = Mundo (Bx7* + By9); 3 = Pronto (Bx7* + By8); 4 = Prego (Bx7* + By8*); 5 = Alzo (Bx6 + By8*); 6 = Lamberto (Bx7 + By20*); 7 = Sekundo (Bx7 + By20*); 8 = Krakowiak (Bx7 + By18*); 9 = Witon (Bx7* + By8*); 10 = Sorento (Bx7 + By20*); 11 = Wanad (Bx7 + By20*); 12 = Alzo (Bx6 + By8*); 13 = Matejko (Bx7 + By20*); 14 = Mieszko (Bx7* + By8); 15 = Piano (Bx7 + By18*); 16 = Todan (Bx7 + By18*); 17 = Bogo (Bx7* + By8*); 18 = Moderato (Bx7 + By18*); 19 = Kargo (Bx7* + By8); 20 = Kitaro (Bx7 + By18*); 21 = Janko (Bx6 + By8*); 22 = Ugo (Bx6 + By8*); 23 = Fidelio (Bx7 + By20*); 24 = Hewo (Bx6 + By8*).

units 7 + 25) by Brzeziński (2006), gave two bands (Figure 6, lanes 6, 7, 10, 11, 13 and 23). One PCR fragment of ca. 280 bp was observed for genotypes containing the alleles designated earlier as *By18* or *By26* (subunits 7 + 18 or 7 + 26) (Figure 6, lanes 8, 15, 16, 18 and 20). Wheat genotypes containing the subunit By16 showed three fragments with the use of primer set PS5, two fragments were detected for By8, 8*, 9, 15 or 18, and no PCR products were found for ByNull and By20 (Lei et al. 2006). This indicates that the triticale alleles supposed to encode subunits By20 and By18 do not correspond to the wheat alleles with those symbols. For those new HMW glutenin subunits, the following names were attributed: By20* instead of By20 and By18* instead of By18. The discrimination between alleles encoding By20 and By20* by PCR is particularly important, as these two subunits are not resolved by SDS-PAGE.

The statements presented above confirm data obtained by primer set PS6, which is a gene- specific marker for wheat subunit By9 (Lei et al. 2006). For triticale accessions, this primer pair produces single-fragment PCR products; a band of ca. 750 bp for By20* (Figure 7, lanes 4, 5 and 11), a fragment of 660 bp for By9 (Figure 7, lanes 7, 8), and one band of 710 bp for other subunits, i.e. By8, 8*, 18*. This indicates that the triticale By20* subunit differs from the wheat By20 subunit. This subunit was additionally identified in three cultivars (Lamberto, Migo and Tewo), where it was not detected earlier by SDS-PAGE (Table 3).

As in wheat (Lei et al. 2006), primer set PS7 was specific for the By8 gene, which exists in allele combinations *Glu-B1b* and *Glu-B1u* (Figure 8, lanes 3, 7, 8 and 11). This primer pair amplified a 527 bp fragment for the By8 subunit, while no PCR fragment was produced for By8* and the other By subunits. This marker allows discrimination of alleles encoding subunits By8 and By8* in segregating populations, which are difficult to score by SDS-PAGE analysis due to the identical mobility of subunits By8 and By8*. The presence of the gene encoding By8, which exists in the allele combination *Glu-B1u* (Bx7* + By8), was detected only in two spring cultivars (Kargo, Mieszko) and two winter cultivars (Pawo and Pronto).

The capability to distinguish HMW glutenin subunits is of great importance to breeders during early selection of breeding lines. We analysed 145 breeding lines of the cross Kazo × Matejko (F_3 population) by using two primer sets (PS5 and PS6). The lines were segregating at the loci *Glu-A1* (AxNull vs. Ax2*), *Glu-B1* (Bx7 + By18* vs. Bx7 + By20*) and *Glu-R1* (common $Rx6^r$ + $Ry13^r$). The PCR results were compared to those obtained by SDS-PAGE (data presented in Figure 9A, B and C). On the basis of the SDS- PAGE patterns it was especially difficult to identify the presence of subunits By18* and $Ry13^r$ (due to a small difference in electrophoretic mobility) and subunit By20* (due to the presence of other storage protein subunits with the same mobility). The 98.2% similarity of the PCR and SDS-PAGE data for

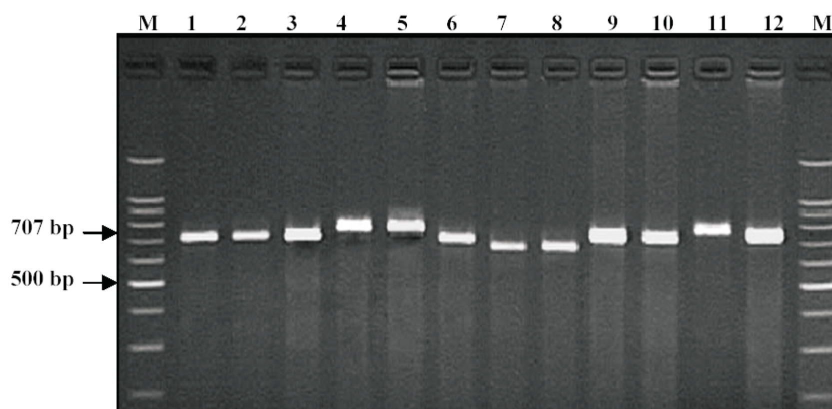

Figure 7. PCR assay of HMW glutenin gene encoding By subunits, with primer set PS6. The PCR product for By20* was ca. 750 bp, for By8, By8* and By18* ca. 710 bp, and for By9 ca. 660 bp. Lanes: M =100 bp DNA Ladder (Promega); 1 = Kitaro (Bx7 + By18*); 2 = Prado (Bx6 + By8*); 3 = Krakowiak (Bx7 + By18*); 4 = Lamberto (Bx7 + By20*); 5 = Sekundo (Bx7 + By20*); 6 = Kazo (Bx7 + By18*); 7 = Mundo (Bx7* + By9); 8 = Woltario (Bx7* + By9); 9 = Alzo (Bx6 + By8*); 10 = Todan (Bx7 + By18*); 11 = Migo (Bx7 + By20*); 12 = Eldorado (Bx7* + By8*).

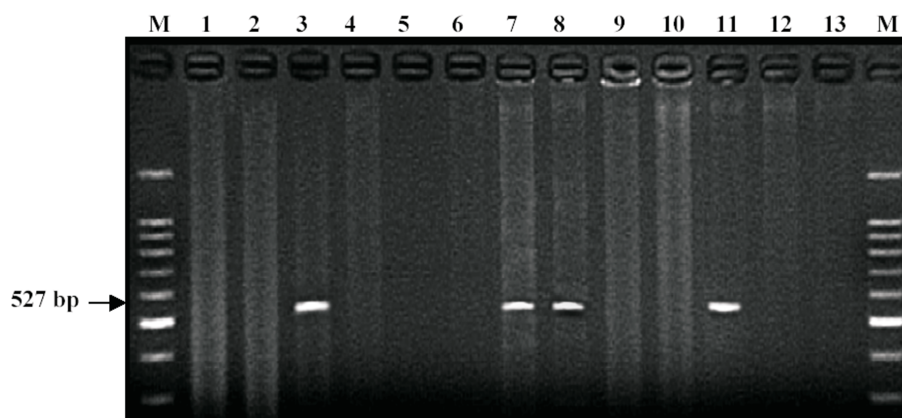

Figure 8. Dominant PCR marker (PS7) specific for By8 subunit. This primer pair produces a 527 bp fragment for genotype containing By8 subunit, and no amplification for other By subunits. Lanes: M =100 bp DNA Ladder (Promega); 1 = Hewo (Bx6 + By8*); 2 = Krakowiak (Bx7 + By18*); 3 = Pawo (Bx7* + By8); 4 = Mundo (Bx7* + By9); 5 = Alzo (Bx6 + By8*); 6 = Lamberto (Bx7 + By20*); 7 = Mieszko (Bx7* + By8); 8 = Pronto (Bx7* + By8); 9 = Kitaro (Bx7 + By18*); 10 = Eldorado (Bx7* + By8*); 11 = Kargo (Bx7* + By8); 12 = Kazo (Bx7 + By18*); 13 = Witon (Bx7* + By8*).

breeding lines shows the usefulness of molecular markers in triticale breeding programs for early selection of desirable HMW glutenin subunits, without the need of carrying out an analysis of storage proteins.

Characterization of wheat *Glu-1* loci in Polish triticale cultivars

Forty-six Polish cultivars of both winter and spring triticale were screened for *Glu-1* alleles encoding the HMW glutenin subunits. In Table 3, results of identification of HMW-GS composition by PCR and SDS-PAGE analyses are compared with earlier data from SDS-PAGE separation presented by Amiour et al. (2002b) and Brzeziński (2006).

At the *Glu-A1* locus, subunit Ax2* was predominant (65.2%). The less frequent alleles were *Glu-A1c* (AxNull), found in 10 cultivars (21.7%), and *Glu-A1a* (Ax1) was only observed in 6 cultivars (13.1%). Unlike Mexican cultivars, which do not have the Ax2* subunit (Amiour 2002b), the Polish cultivars often have this subunit, with a crucial influence on bread-making quality in wheat cultivars.

At the *Glu-B1* locus, high allelic variation was observed, as 8 alleles were found. The most frequently observed allele combinations were *Glu-B1av* (Bx7 + By18*) and *Glu-B1aw* (Bx7 + By20), so far only found in triticale with frequencies of 37.8% and 28.2%, respectively. Frequencies were equal for three allele combinations, i.e. *Glu-B1d* (Bx6 + By8), *Glu-B1ak* (Bx7* + By8*) and *Glu-B1u* (Bx7* + By8), with 8.7% each, and lower for allele combinations *Glu-B1a* (Bx7) and *Glu-B1c* (Bx7 + By9), with 2.2% and 4.4%, respectively. Similar allelic distribution at this locus was observed among 134 European

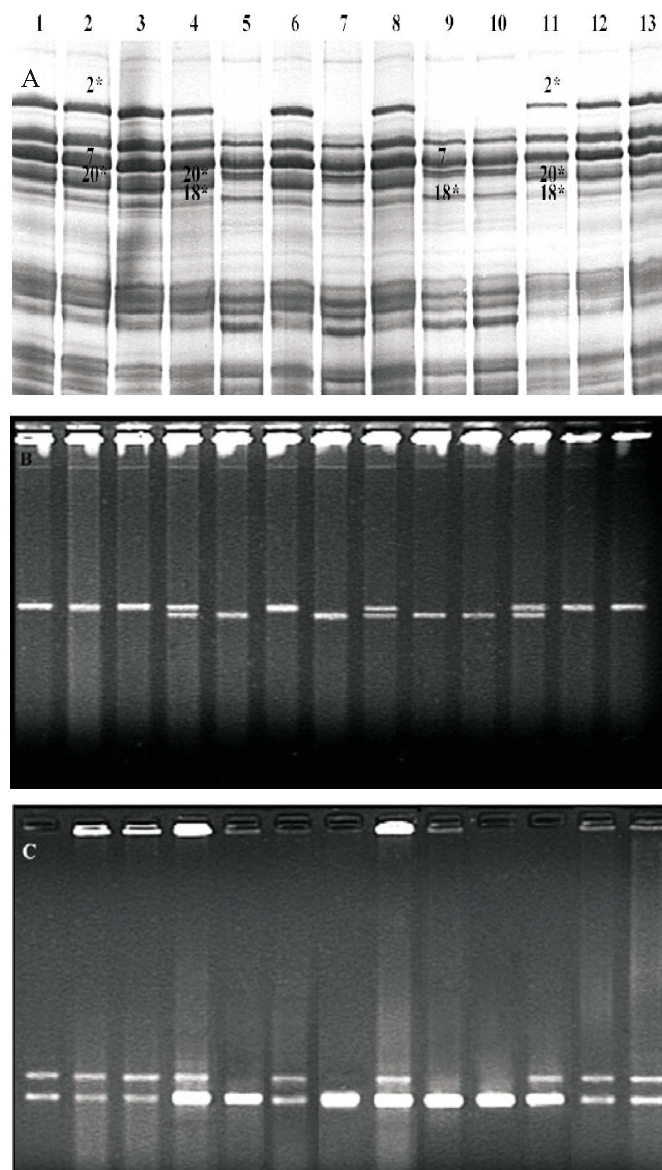

Figure 9. Comparison of patterns between SDS-PAGE (A) and PCR for primer set PS6 (B) and PS5 (C). Equivalent electrophoregrams of 13 (from 145) lines of the cross Kazo Matejko population F3 segregating at the *Glu-A1* (AxNull vs. Ax2*), *Glu-B1* (Bx7 + By18* vs. Bx7 + By20*) and *Glu-R1* loci (common Rx6r + Ry13r) are presented. Primer pair PS6 (Figure 9B) produces a characteristic 750 bp fragment PCR for the allele encoding By20* or a 707 bp fragment for By18*, while PS5 (Figure 9C) gave 1 band for By18* or 2 bands for By20*. Some breeding lines (lanes 4, 8 and 11) are heterozygous.

triticale cultivars (Amiour 2002b). So far, triticale breeding in Poland has been mainly aimed at searching for genotypes with a relatively high grain yield. Breeding selection in early generations for subunits Ax1, Ax2* and Bx7, and in particular detection of over-expression of the gene encoding Bx7 by molecular markers and HPLC and HPCE analyses, should exert a positive impact on triticale bread-making quality improvement. Rapid identification of molecular markers of wheat *Glu-1* genes by PCR can be an efficient alternative to the standard SDS-PAGE procedure separation for early selection of useful and pure lines. A deeper knowledge of quality-quantity relationships of HMW glutenin and secalin composition and dough quality will facilitate identification of breeding lines useful in bread baking with triticale flour.

REFERENCES

Ahmad M, 2000. Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. Theor Appl Genet 101: 892–896.

Amiour N, Bouguennec A, Marcoz C, Sourdille P, Bourgoin M, Khelifi D, Branlard G, 2002a. Diversity of seven glutenin and secalin loci within triticale cultivars grown in Europe. Euphytica 123: 295–305.

Amiour N, Dardevet M, Khelifi D, Bouguennec A, Branlard G, 2002b. Allelic variation of HMW and LMW glutenin subunits, HMW secalin subunits and 75k gamma-secalins of hexaploid triticale. Euphytica 123: 179–186.

Barnett RD, Blount AR, Pfahler PL, Brucker PL, Wesenberg DM, Johnson JW, 2006. Environmental stability and heritability estimates for grain yield and test weight in triticale. J Appl Genet 47: 207–213.

Bean SR, Lookhart GL, 2000. Ultrafast capillary electrophoretic analysis of cereal storage proteins and its application to protein characterization and cultivar differentiation. J Agric Food Chem 48: 344–353.

Brzeziński W, 2006. Electrophoretic patterns of wheat, triticale, barley and oat cultivars registered in Poland. Wiadomości Odmianoznawcze. Band 84, Słupia Wielka, Poland.

Butow BJ, Ma W, Cornish GB, Rampling L, Larroque O, Morell MK, Bekes F, 2003. Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high-molecular-weight glutenin allele has a major impact on wheat flour dough strength. Theor Appl Genet 107: 1524–1532.

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 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.

Igrejas G, Guides-Pinto H, Carnide V, Branlard G, 1999. Seed storage protein diversity in triticale varieties commonly grown in Portugal. Plant Breeding 118: 303–306.

Lafianrda 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.

Lei ZS, Gale KR, He ZH, Gianibelli C, Larroque O, Xia XC, et al. 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.

Lelley T, 2006. Triticale: A low-input cereal with untapped potential. In: Singh RJ, Jauhar PP, eds. Genetic Resources, Chromosome Engineering, and Crop Improvement, Cereals, Vol. 2. CRS Press, Taylor & Francis Group, Boca Raton, USA: 395–430.

Ma W, Zhang W, Gale KR, 2003. Multiplex-PCR typing of high molecular weight glutenin subunit alleles in wheat. Euphytica 134: 51–60.

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R, 2003. Catalogue of gene symbols for wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G, eds. Proc. 10th International Wheat Genetics Symposium, Vol. 4. Istituto Sperimentale per la Cerealicoltura, Rome.

Moczulski M, Salmanowicz BP, 2003. Multiplex PCR identification of wheat HMW glutenin subunit genes by alleles-specific markers. J Appl Genet 44: 459–471.

Neuhoff V, Arnold N, Taube D, Ehrhardt W, 1998. Improved staining of protein in polyacrylamide gels including isoelectric focusing with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. Electrophoresis 9: 221–233.

Oettler G, 2005. The fortune of a botanical curiosity – triticale: past, present and future. J Agricultural Sci 143: 329–346.

Payne PI, Lawrence GJ, 1983. Catalogue of alleles for the complex loci, *Glu-A1*, *Glu-B1*, and *Glu-D*1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11: 29–35.

Pena RJ, 1996. Factors affecting triticale as a food crop. In: Guedes-Pinto A, ed. Triticale Today and Tomorrow. Kluwer Academic Publishers, Dordrecht, The Netherlands: 753–761.

Radovanovic N, Cloutier S, 2003. Gene-assisted selection for high molecular weight glutenin subunits in wheat doubled haploid breeding programs. Mol Breed 12: 51–59.

Rozinek B, Gunther T, Hesemann CU, 1998. Gel electrophoretic investigations of prolamins in eu- and alloplasmatic octoploid primary triticale forms. Theor Appl Genet 96: 46–51.

Salmanowicz BP, Moczulski M, 2004. Multiplex polymerase chain reaction analysis of *Glu-1* high-molecular-mass glutenin genes from wheat by capillary electrophoresis with laser-induced fluorescence detection. J Chromatogr A 1032: 313–318.

Serna-Saldivar S, Guajardo-Flores S, Viesca-Rios R, 2004. Potential of triticale as a substitute for wheat in flour tortilla production. Cereal Chem 81: 220–225.

Shewry PR, Bradberry D, Franklin J, White RP, 1984. The chromosomal locations and linkage relationships of the structural genes for the prolamin storage proteins (secalins) of rye. Theor Appl Genet 69: 63–69.

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

Wieser H, Antes S, Seilmeier W, 1998. Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography. Cereal Chem 75: 644–650.