Genes, Genomes and Genomics ©2008 Global Science Books

Molecular Weight Glutenin Alleles at the *Glu-B1* Locus

Mohamed Ibrahim Motawei*

Biotechnology Lab, Agronomy Department, College of Agriculture, Alexandria University, Egypt

Correspondence: * **rumotawei@hotmail.com**

ABSTRACT

Wheat cultivar 'Yeocra Rojo' and ten selected genotypes originating from immature embryo culture of this cultivar were evaluated for their productivity in two field experiments during 2005/2006 and 2006/2007 winter seasons. Wheat genotypes YR-9 and YR-10 exhibited the tallest plants whereas the shortest plants were those of cv. 'Yeocra Rojo'. In general, genotypes YR-2, YR-7, and YR-10 produced the highest grain yield while YR-8 produced the lowest. YR-8 also gave the lowest harvest index. YR-7 had the most grains per spike, spike length and number of spikelets per spike compared to its parent cultivar and all other genotypes. A set of PCR-based markers for specific HMW glutenin genes encoding By-subunits were used to identify wheat genotypes carrying *By* genes at the *Glu-B1* locus for its bread-making quality. The presence of the gene encoding *By8*, which exists in the allele combination *Glu-B1b* (Bx7 + By8), was detected only in one genotype: YR-7. Primer pair ZSBy9aF1/R3 gave characteristic banding patterns for Glu-B1c (Bx7+By9) and can therefore be used to discriminate *By9*-containing alleles from non-*By9* alleles. Primer pair ZSBy9F2/R2 produced individuals with a diagnostic banding pattern for allele *Glu-B1f* (Bx13+By16) in genotype YR-10 while YR-8 and YR-9 did not produce any PCR product that was found to be specific for *By-null* or the *20* gene. YR-7 and YR-10 produced the highest grain yield and had the *By8* and *By16* genes, respectively, which are associated with superior bread-making quality. YR-9, however, produced high grain yield but had the *By-null* or *20* gene, which is associated with poor bread-making quality. Therefore, fast and accurate identification of *By* genes by molecular markers at the *Glu-B1* locus could be an efficient way for early selection of useful wheat genotypes with good bread-making quality.

Keywords: embryo culture, wheat

INTRODUCTION

Wheat flour is an organic complex in which starch interacts with both gluten and non-gluten proteins (largely albumins and globulins), lipids, and non-starch carbohydrates. A large portion of variation observed in flour quality may be attributed to variation in the gluten protein content and composition (Bietz 1988). Thus, breeding for improved bread-making quality can be done either by increasing the protein concentration or altering the protein composition of cultivars. Increased grain protein concentration will improve the volume and the texture of baked loaves considerably. Unfortunately, increasing the protein concentration is hampered by a negative correlation between the yield and the protein concentration of the grains (Johnson *et al.* 1985). Breeding for increased protein concentration is therefore a difficult task, although not impossible.

Flour protein composition, or the distribution of flour protein in classes based on molecular size and solubility, has been reported to be a major variable influencing wheat processing quality (Graybosch *et al*. 1996). A large part of the variation in bread-making quality between cultivars has been ascribed to the high molecular weight glutenin subunit (HMW-GS) genotype (Payne *et al*. 1987). The contribution of the HMW glutenin subunit alleles to the quality has been determined by relating the presence or absence of an allele in cultivars or in segregating progenies to the bread-making quality. The identified HMW glutenin subunit alleles, influencing the dough gluten strength in a positive or negative way, can be used by plant breeders for improving bread-making quality. The HMW-GS are encoded by genes at three Glu-1 loci; Glu-A1, Glu-B1 and Glu-D1, located on the long arms of homoeologous group-1 chromosomes (Payne and Lawrence 1983). Molecular studies have shown that each locus contains two tightly linked genes which encode two types of HMW-GS, one of higher molecular weight, designated the x-type, and the other of lower molecular weight, designated the y-type (Harberd *et al*. 1986). Considerable allelic variation exists at each of the HMW glutenin loci (Payne and Lawrence 1983; Anderson *et al*. 1998).

Among allelic HMW subunits controlled by the Glu-A1 locus on chromosome 1A, bands 1 and 2 have an equal positive effect over the null allele, suggesting a quantitative effect. Similarly, among several alleles at the Glu-B1 locus on chromosome 1B, those producing double bands or intensely staining bands (for example subunits 7+8, 13+16, and 17+18) are associated with superior bread-making quality compared with those with single or faint bands (for example subunits 7, 20, and 6+8) (Singh *et al*. 1990).

HMW-GS analysis using SDS-PAGE is restricted to use on grain material and therefore the selection of breeding lines cannot be made in the field before harvest. The polymerase chain reaction (PCR) has been used as an efficient and reliable approach for the determination of HMW-GS allelic composition (Ahmed 2000; Radovanovic and Cloutier 2003) and is applicable to screening leaf material from the field prior to harvest of lines containing the desired genotypes (reviewed by Gupta *et al*. 1999; Eagles *et al*. 2001; Galle 2005). PCR markers may be used to discriminate alleles based on very small differences in sequence identity between alleles, with as little as 1 bp polymorphism being sufficient for the development of allele-specific PCR primers (Zhang *et al.* 2003). The effectiveness of this approach in plant breeding is limited by the number of available markers, which is in turn influenced by the degree of characterization of the gene families being selected.

A number of markers targeting different glutenin alleles have been reported, including markers for *Glu-B1* alleles that are based on sequence variations of Bx type genes (Ma *et al*. 2003). However, all markers reported previously are co-dominant and no markers based on *By* genes were avail-

Received: 7 June, 2008. Accepted: 12 Augsut, 2008.

Original Research Paper

able. Recently, Lei *et al*. (2006) concluded that the discovery and application of *Glu-B1* allelic variation in breeding programs have made possible the development of specific molecular markers for a range of *By*-type genes, to facilitate the further differentiation of various *Glu-B1* alleles.

The objectives of this study were to (1) evaluate ten wheat genotypes and their parent cv. 'Yeocara Rojo' for their productivity, (2) identify wheat genotypes carrying *By* genes of HMW glutenin alleles at the *Glu-B1* locus by PCR-generated DNA markers, and (3) select wheat genotypes for superior bread-making quality based on DNA markers of *By* genes.

MATERIALS AND METHODS

Field trials

Field experiments were conducted at the Agricultural Research Station, College of Agriculture and Veterinary Medicine, Al-Qassim University, Saudi Arabia, during the 2005/2006 and 2006/2007 winter seasons. Wheat cv. 'Yeocra Rojo' and 10 selected genotypes originating from immature embryo culture of cv. 'Yeocra Rojo' under salt stress (to produce salt stress tolerant lines) (Barakat and Abdel-Latif 1996) were sown on the 5th and 25th December 2005 and 2006, respectively, with a seeding rate of 140 kg/ha. The plot size was 4 × 3 m with a row-to-row spacing of 25 cm. The recommended fertilizer requirements of wheat in the Al-Qassim region, Saudi Arabia, as NPK, were 200, 200 and 100 kg/ha, respectively for a growing season of 120 days on wheat, according to Bashour and Al-Jaloud (1984). A randomized complete block design with three replicates was used.

At harvesting time, ten plants were randomly chosen to measure plant height, spike length, the number of spikelets per spike, and the number of grains per spike. Also, harvest index and grain yield per square meter were recorded.

DNA extraction

Frozen young leaves (500 mg) were ground to a powder in a mortar with liquid nitrogen. The powder was poured into tubes containing 9.0 ml of warm (65°C) CTAB extraction buffer (Sagahi-Maroof *et al.* 1984). The tubes were incubated at 65°C for 60-90 min. 4.5 ml chloroform/octanol (24: 1) was added and tubes were rocked to mix for 10 min, and centrifuged for 10 min at 3200 rpm. The supernatants were pipetted off into new tubes and 6 ml isopropanol was added. After 60 min., the tubes were centrifuged for 10 min. and the pellets obtained were put in sterile Eppendorf tubes, containing 400 µl of TE buffer of a pH 8.0 (10 mM Tris-HCl, pH 8.0 + 1.0 mM EDTA, pH 8.0). The DNA's from genotypes were, then, extracted and stored at -20°C until use.

Specific PCR amplification for *By* genes of high-molecular weight glutenin alleles at the *Glu-B1* locus

The DNA sequence of the *By* genes from the *Glu-B1* locus has been reported previously (Genbank accession: X61026; Halford 1992). Based on this sequence, a number of primers were used to amplify segments of various *By* genes from wheat genotypes (Lei *et al.* 2006). The amplified *By* genes, primer sequences and PCR cycling required for these primers are shown in **Table 1**. PCR amplifications were performed using a thermal cycler (Thermolyne Amplitron). Amplifications were carried out in 25 µL reaction volumes, containing 1X *Taq* polymerase buffer (50 mM KCl, 10 mM Tris, pH 7.5, 1.5 mM $MgCl_2$) and 1 unit of *Taq* polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 25 pmol primer, and 50 ng of total genomic DNA. The PCR products were separated by electrophoresis in 1.5% agarose using TBE buffer and detected by ethidium bromide staining.

Statistical analysis

The two growing seasons were analyzed separately. Costat computer program (CoHort Software, Monterey, CA) was used to perform the analysis of variance (Snedecor and Cochran 1980). The protected least significant differences (LSD) test was used to compare means at the 5% level.

RESULTS AND DISCUSSION

Grain yield and yield-contributing traits

There were differences in plant height among wheat genotypes (**Table 2**). Wheat genotypes YR-9 and YR-10 were the tallest plants while the shortest plants were those of cv.

Table 1 PCR primers information and PCR cycling conditions for the amplification of specific *Glu-B1* genes.

Glu-B1 gene	Primer pair	Forward and reverse PCR primer sequence 5′-3′	Marker type	PCR cycling
By8	ZSBy8F5/R5	F: TTAGCGCTAAGTGCCGTCT R: TTGTCCTATTTGCTGCCCTT	Dominant	1×95°C 30″ 38×(94°C 30″; 64°C 30″; 72°C 1′30″) 1×72°C 10′ 1×10°C hold
By9	ZSBy9aF1/R3	F: TTCTCTGCATCAGTCAGGA R: AGAGAAGCTGTGTAATGCC	Co-dominant	1×95°C 30″ 38×(94°C 30″; 59°C 30″; 72°C 1′30″) 1×72°C 10′ 1×10°C hold
By16 and By-null or (20)	ZSBy9F2/R2	F: GCAGTACCCAGCTTCTCAA R: CCTTGTCTTGTTTGTTGCC	Co-dominant	1×95°C 30″ 38×(94°C 30″; 62°C 30″; 72°C 1′30″) 1×72°C 10′ 1×10°C hold

Table 2 Plant height, grain yield and harvest index of wheat genotypes during 2005/2006 and 2006/2007 seasons.

Wheat genotypes	Plant height (cm)		Grain yield g/m²		Harvest index (%)	
	2005/2006	2006/2007	2005/2006	2006/2007	2005/2006	2006/2007
YR-1	67.0 b	70.3 de	250.0 a	148.0 df	45.5 ab	38.6 cd
YR-2	69.0 b	70.0 de	250.0 a	219.5 a	46.2 ab	47.8 a
YR-3	65.0 b	67.3 e	234.0 ab	169.7 bcde	52.3 a	38.9 cd
YR-4	67.6 b	72.6 cd	249.3 ab	189.4 abcd	50.4 ab	39.8 bcd
YR-5	71.0 b	69.6 de	218.3 ab	187.7 abcd	47.9 ab	44.5 abc
Yr-6	62.6 b	67.0 e	171.0 b	152.0 cde	50.3 ab	42.4 abcd
YR-7	71.3 b	74.0 c	259.0 a	197.0 bcd	47.9 ab	39.8 bcd
YR-8	64.0 b	63.0 f	105.0 c	101.7 f	39.0 b	36.1 d
YR-9	81.0 a	85.3 a	220.0 ab	193.0 abcd	46.2 ab	44.8 abc
YR-10	85.0 a	81.3 b	259.3 a	203.0 ab	39.9 b	42.2 abcd
Yeocra Rojo (YR)	64.6 b	61.3 f	214.3 ab	140.5 e	47.7 ab	46.8 ab

Data are expressed as means; Means within the same column and followed by the same letters are not significantly different from each other according to LSD test ($p \leq 0.05$).

Table 3 Number of grain per spike, spike length, 1000-grain weight of wheat genotypes during 2005/2006 and 2006/2007 seasons.

Wheat genotypes	No. of grains/spike		Spike length (cm)		No. of spikelets/spike	
	2005/2006	2006/2007	2005/2006	2006/2007	2005/2006	2006/2007
YR-1	40.0 bcd	38.1 b	10.0 bc	10.0 bc	16.4 ab	15.9 ab
YR-2	35.4 d	35.6 b	9.7 bcd	9.7 bcd	15.3 bc	16.1 bcd
YR-3	43.5 cd	34.2 b	9.0 cde	10.7 b	14.8 bc	13.8 d
YR-4	35.9 cd	34.3 b	9.0 cde	9.3 bcd	16.3 ab	15.8 b
YR-5	37.3 bcd	38.4 b	10.7 b	10.7 b	13.7 c	14.5 b
Yr-6	40.0 bcd	39.0 b	9.3 cde	8.3 d	15.4 bc	15.0 b
YR-7	53.3 a	49.4 a	13.7 a	14.0 a	17.2 a	18.5 a
YR-8	32.7 d	27.1 c	9.0 cde	8.7 cd	14.3 c	15.1 b
YR-9	45.0 b	37.4 b	8.3 de	9.0 cd	16.6 ab	16.5 ab
YR-10	40.4 bcd	32.3 b	8.0 e	9.7 bcd	14.2 c	16.0 ab
Yocra Rojeo (YR)	37.2 bcd	27.2 c	9.3 cde	10.0 bc	15.1 bc	15.7 b

Data are expressed as means; Means within the same column and followed by the same letters are not significantly different from each other according to LSD test ($p \leq 0.05$).

'Yeocra Rojo', YR-6, and YR-8 in both seasons. **Table 2** clearly indicates that grain yield differed among wheat genotypes. In general, genotypes YR-2, YR-7, and YR-10 produced the highest grain yield in both seasons. YR-8 produced the lowest grain yield and had the lowest harvest index in both seasons.

YR-7 had the highest while YR-8 the lowest number of grains per spike in both seasons (**Table 3**). YR-7 also had the longest spikes and the most spikelets per spike in both seasons (**Table 3**). The magnitude of genetic variation among tissue culture-derived wheat genotypes has been reported in wheat by Barakat (1995). In cereals, tissue culture was effective in wheat for producing tolerant lines to salt stress (Barakat and Abdel-Latif 1996), barley (Ye *et al.* 1987) and maize (Lupotto *et al*. 1989).

Specific PCR markers for *By* genes of high-molecular weight glutenin alleles at the *Glu-B1* locus

***By8* gene-specific marker:** Primer pair ZSBy8F5/R5 was found to be specific for the alleles containing the *By8* gene, which exists in the *Glu-B1b* (Bx7 + By8) allele (Lei *et al.* 2006). YR-7 produced a 530 bp fragment while other genotypes were negative for this gene banding (**Fig. 1**). This marker, the first dominant marker available for selection at the *Glu-B1* locus, allows discrimination of alleles in a cross containing *By8* and *By8** that are usually difficult to distinguish by SDS-PAGE due to their identical mobility on gel (Lei *et al.* 2006). It also represents an alternative marker to distinguish two *Glu-B1i* alleles: *Glu-B1i* (Bx17 + By18) and *Glu-B1b* (Bx7 + By8) (Ma *et al*. 2003). Salmanowicz and Dylewicz (2007) found that the presence of the gene encoding *By8*, which exists in the allele combination *Glu-B1b* (Bx7 + By8), was detected only in two spring cvs., 'Kargo' and 'Mieszko'. Southan and MacRitchie (1999) reported that wheat quality scores assigned to the HMW-GS ranged from 0 (null allele) to 4. The HMW-GS pair (7 + 8)

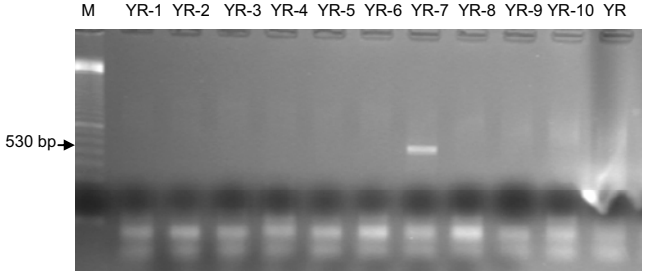

Fig. 1 Detection of alleles encoding HMW gglutenin *By8* in wheat genotypes using a specific primer (ZSBy8F5/R5). M: kbp DNA marker. Arrow points to a unique fragment of approximately 530 bp present in the *By8* gene.

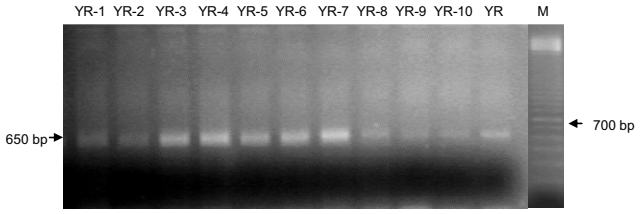

Fig. 2 PCR products obtained by using primer pair ZSBy9aF1/R3 indicate a size difference of 50 bp between *By9* genotypes and non-*By9* genotypes. M: kbp DNA marker.

coded by *Glu-B1* has been assigned a score of 3 as this pair has been associated with a high dough strength. Therefore, the amplification of a DNA fragment for the *By8* gene in YR-7 was associated with high bread-making quality.

***By9* gene-specific marker:** Primer pair ZSBy9aF1/R3 was used to amplify *By* gene segments from various genotypes. YR-1, YR-2, YR-3, YR-4, YR-5, and YR-6 produced a 650 bp fragment that was found to be specific for the *By9* gene (**Fig. 2**) while the other genotypes produced a 700 bp fragment. The PCR products of this primer pair (ZSBy9aF1/R3) resulted in a co-dominant marker that discriminated the *By9* gene from other *By* genes, via a 50 bp size difference (Lei *et al.* 2006). The PCR products of this primer pair resulted in a co-dominant marker that discriminated the *By9* gene, a 650 bp fragment, from other *By* genes, via a 50 bp size difference (Lei *et al.* 2006).

***By16* gene-specific marker:** Primer pair ZSBy9F2/R2 was used to amplify the *By16* gene (**Fig. 3**). YR-10 produced three PCR fragments for the *By16* gene, which exists in *Glu-B1f* (Bx13 + By16). Cv. 'Yecora Rojo' and genotypes YR-1, YR-2, YR-3, YR-4, YR-5, YR-6, and YR-7 produced two PCR fragments. These two PCR fragments were found to be specific for *By8*, *By9*, *By18* and *By15* genes (Lei *et al.* 2006). YR-8 and YR-9 did not produce any PCR product that was found to be specific for the *By-null* or *20* genes. Lei *et al.* (2006) demonstrated an enhanced discrimination of alleles at the *Glu-B1* locus, including the distinction between the *Glu-B1e* (*By20*) allele from the *Glu-B1h* (*By15*) allele which have opposite genetic effects on wheat quality, but are difficult to identify using SDS-PAGE gel. Also, Singh *et al.* (1990) concluded that among several alleles at the *Glu-B1* locus on chromosome 1B, those producing double bands or intensely staining bands (for example subunits 7+8 and 13+16) are associated with a superior bread-making quality.

In this study, it was shown that wheat genotypes YR-7 and YR-10 produced the highest grain yield and had the *By8* and *By16* genes, respectively, which are associated with superior bread-making quality. YR-9, on the other hand, produced a high grain yield and had the *By-null* or the *20* gene, which is associated with poor bread-making quality (Singh *et al*. 1990; Shewry *et al.* 2003). Therefore, molecu-

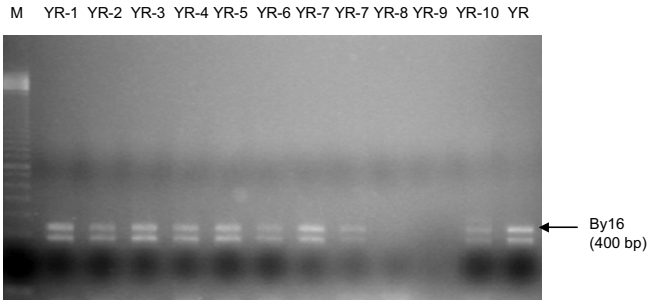

Fig. 3 Primer pair ZSBy9F2/R2 amplified the *By16*, *By-null* or *20* genes, producing 3 fragments for the *By16* gene and 2 fragments for *By8*, *By9*, *By18*, *By15* and no amplification for *By-null* or *20* genes. M: kbp DNA marker. The arrow points to one extra faint fragment for the *By16* gene. (Note: YR-7 was repeated twice)

lar markers for amplifying *By* genes of high-molecular weight glutenin alleles could be valuable for wheat breeding programs for selection of desirable *Glu-B1* alleles for new, high-quality wheat genotypes.

REFERENCES

Ahmad M (2000) Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. *Theoretical and Applied Genetics* **101**, 892-896

Anderson OD, Abraham-Pierce FA, Tam A (1998) Conservation in wheat high-molecular-weight glutenin gene promoter sequences: comparisons among loci and among alleles of the Glu-B1-1 locus. *Theoretical and Applied Genetics* **96**, 568-576

Bashour II, Al-Jaloud AA (1984) Fertilization of wheat in central region of Saudi Arabia. Extension Bulletin, Regional Agriculture and Water Research Center, Ministry of Agriculture and Water, Riyadh, Saudi Arabia

Barakat MN (1995) Estimation of genetic parameters for *in vitro* traits in wheat immature embryo cultures involving high X low regeneration capacity genotypes *Euphytica* **87**, 119-125

Barakat MN, Abdel-Latif TH (1996) *In vitro* selection of wheat callus tolerant to high levels of salt and plant regeneration. *Euphytica* **91**, 127-140

Bietz JA (1988) Genetic and biochemical studies of non-enzymatic endosperm proteins. In: Heyne EG (Ed) *Wheat and Wheat Improvement* (2nd Edn) Agronomy Monograph 13, ASA, CSSA & SSSA, Madison, WI, pp 215-242

Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ, Hollamby GJ, Henry RJ, Henschke PH, Carter M (2001) Implementation of markers in Australian wheat breeding. *Australian Journal of Agricultural Research* **52**, 1349-1356

Gale KR (2005) Diagnostic DNA markers for quality traits in wheat. *Journal of Cereal Science* **41**, 181-192

Grayboasch RA, Peterson CJ, Shelton DR, Baenziger PS (1996) Genotypic and environmental modification of wheat flour protein composition in relation to end-quality. *Crop Science* **36**, 296-300

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. *Plant Breeding* **118**, 369-390

Harberd NP, Bartels D, Thompson RD (1986) DNA restriction fragment variation in the gene family encoding high-molecular-weight (HMW) glutenin subunits of wheat. *Biochemical Genetics* **24**, 579-592

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 1 A of bread wheat (*Triticum aestivum* L.) indicates quantitative effects on grain quality, *Theoretical and Applied Genetics* **83**, 373-378

Johnson VA, Mattern PJ, Peterson CJ, Kuhr SL (1985) Improvement of wheat protein by traditional breeding and genetic techniques. *Cereal Chemistry* **62**, 350-355

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. *Journal of Cereal Science* **43**, 94-101

Lupotto E, Lusardi MC, Mongodi M (1989) *In vitro* selection of maize (*Zea mays* L.) salt tolerant somaclones and plant regeneration. *Journal of Genetics and Breeding* **43**, 215-222

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

Payne PI, Nightingale MA, A.F. Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *Journal of the Science of Food and Agriculture* **40**, 51-65

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci GluA1, GluB1, GluD1, which code for the highmolecular-weight subunits of glutenin in hexaploid wheat. *Cereal Research Communication* **11**, 29-35

Radovanovic N, Cloutier S (2003) Gene-assisted selection for high molecular weight glutenin subunits in wheat doubled haploid breeding programs. *Molecular Breeding* **12**, 51-59

Sagahi-Maroof M, Soliman K, Jorgensen R, Allard R (1984) Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of the National Academy of Sciences USA* **81**, 8014-8018

Salmanowicz BP, Dylewicz M (2007) Identification and characterization of high-molecular-weight glutenin genes in Polish triticale cultivars by PCR-based DNA markers. *Journal of Applied Genetics* **48**, 347-357

Singh NK, Donovan GR, Macritchie F (1990) Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of gluten in as a measure of bread-making quality. *Cereal Chemistry* **67**, 161-170

Shewry PR, Gilbert SM, Savage AW, Tatham AS, Wan YF, Belton PS, Wellner N, D'Ovidio R, Bekes F, Halford NG (2003) Seqence and properties of HMW subunit 1Bx20 from pasta wheat (*Triticum durum*) which is associated with poor end use properties. *Journal of Applied Genetics* **106**, 744-750

Snedecor GW, Cochran WG (1980) *Statistical Methods* (6th Edn) Iowa State University Press, Ames, Iowa, 593 pp

Southan M, Macritchie F (1999) Molecular weight distribution of wheat proteins. *Cereal Chemistry* **76**, 827-836

Zhang W, Gianibelli MC, Ma W, Rampling L, Gale KR (2003) Identification of SNPs and development of allele-specific PCR markers for γ-gliadin alleles in *Triticum aestivum*. *Theoretical and Applied Genetics* **107**, 130-138

Ye JM, Kao KN, Harvey BL, Rossnagel BG (1987) Screening salt-tolerant barley genotypes via F1 anther culture in salt stress media. *Theoretical and Applied Genetics* **74**, 426-429