

Screening Selected Spring Wheat Cultivars for Productivity, Protein Content and the *Glu-B1* Allele Using PCR

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Abstract. Spring wheat cultivars (Sama, Yocra Rojeo, Sids 1, Sakha 8, Sakha 61, Giza 164, Giza 168, Bani Swaf 1, Bani Swaf 3, and Shandawel) were evaluated for their productivity in two field experiments during 2005/2006 and 2006/2007 winter seasons. The results indicated that cultivars (Sids 1 and Giza 164) produced the highest grain yield. While, cultivars (Bani Swaf 3, and Bani Swaf 1) produced the lowest grain yield. Also, the last cultivars gave the lowest harvest index. Wheat cultivars Sama, Giza-164 and Sids 1 were the tallest plants. Moreover, there were significant differences in yield components among wheat cultivars. The highest protein cultivar Sakha 61 had a mean protein percentage of 15.65%. Cultivar Sakha 61 had genetic potential to produce grain with higher protein content than the other cultivars. Bani Swaf 3 *cv.* had the highest protein content and the lowest grain yield.

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 primer pair ZSBy9aF1/R3 in the reaction mixture, which is an allele-specific marker for the wheat HMW glutenin *By9* allele, showed one band of 650 bp in wheat cultivars Sama, Yocra Rojeo, Sids 1, Sakha 8, Sakha 61, and Giza 168. Also, primer set ZSBy9F2/R2, specific for *By16* and *By-null* subunits in wheat cultivars, amplified three different banding patterns for wheat cultivars. Wheat cultivars Sama, Sids 1, and Sakha 61 produced three PCR fragments for the *By16* gene, which exists in *Glu-B1f* (Bx13 + *By16*). Cultivars Yecora Rojo, Sakha 8, Giza 164, Giza 168, and Bani Swaf 3 produced two PCR fragments. These two PCR fragments were found to be specific for *By8*, *By9*, *By18* and *By15* genes. Cultivars Bani Swaf 1, and Shandawel did not produce any PCR product that was found to be specific for *By-null* or *20* gene. It was concluded that

wheat genotypes Sakha 61 produced the highest grain yield and had the highest protein content *By16* gene, which are associated with a superior bread-making quality.

Introduction

Proteins are the most important components of wheat (*Triticum aestivum* L.) grains governing end-use quality (Weegels, *et al.*, 1996). Variations in both protein content and composition significantly modify flour quality for bread-making (Weegels, *et al.*, 1996; Lafiandra, *et al.*, 1999 and Branlard, *et al.*, 2001). Although grain protein composition depends primarily on the genotype, it is significantly affected by the environmental factors and their interactions (Graybosch, *et al.*, 1996; Triboï, *et al.*, 2000 and Zhu and Khan, 2001).

The bread-making quality of wheat is mainly determined by the composition and quantity of gluten-forming storage proteins, in particular the high-molecular-weight glutenin subunits (HMW-GS) (Butow, *et al.*, 2003, 2004). Various alleles of HMW glutenin genes are particularly important for determining wheat gluten and dough elasticity (Shewry, *et al.*, 1992). 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 homologous group-1 chromosomes (Payne and Lawrence, 1983). Each locus is made of two tightly linked genes, *e.g.*, Glu-D1-1 and Glu-D1-2, which encode the x and y subunits, respectively. 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). Based on the different mobility, revealed by SDS-PAGE, a large number of HMW-GS allelic variations of Glu-1 loci are identified and listed in wheat gene catalogs (McIntosh, *et al.*, 2003).

Molecular 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 available. 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: (1) To evaluate ten spring wheat cultivars for their productivity and protein content, and (2) to identify wheat cultivars carrying *By* genes of HMW glutenin alleles at the *Glu-B1* locus by PCR-generated DNA markers.

Materials and Methods

Field Trials

The field experiment was conducted at the Agricultural Research Station, College of Agriculture and Veterinary Medicine, Al-Qassim University, Saudi Arabia during 2005/2006 and 2006/2007 winter seasons. Ten cultivars of spring wheat (Sama "local cultivar", Yocra Rojeo, and Egyptian cultivars: Sids 1, Sakha 8, Sakha 61, Giza 164, Giza 168, Bani Swaf 1, Bani Swaf 3, and Shandawel) were sown on December 1st and 15th, 2005 and 2006, respectively, with a seeding rate of 140 kg/ha. The plot size was 4 × 3 m² with row to row spacing of 15 cm. The recommended fertilizer requirements of wheat in 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 (Steel and Torrie, 2000).

At harvesting time, ten plants were randomly chosen to measure 1000-grain weight and the number of grains per spike. Also, harvest index and grain yield per square meter were recorded.

The grain samples were oven dried at 70°C, then ground in a blender and stored in glass vials for protein determination. Grain protein content was determined, by means of the standard Kjeldahl method, then the percentage of protein was calculated by multiplying Kjeldahl nitrogen (%) by 5.75 (A.O.A.C., 2000).

DNA Extraction

Frozen young leaves (500 mg) were ground to 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-Marooof, *et al.*, 1984). The tubes were incubated at 65°C for 60-90 min. and 4.5 ml chloroform/ octanol (24:1) were added. The 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 were 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 DNAs 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 (Halford, *et al.*, 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 MgCl₂) 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.

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

<i>Glu-B1</i> gene	Primer pair	Forward and reverse PCR primer sequence 5'-3'	PCR cycling
<i>By9</i>	ZSBy9aF1/R3	F:TTCTCTGCATCAGTCAGGA R:AGAGAAGCTGTGTAATGCC	1×95°C 30" 38×(94°C 30"; 59°C 30"; 72°C 1'30") 1×72°C 10' 1×10°C hold
<i>By16 and By-null or (20)</i>	ZSBy9F2/R2	F:GCAGTACCCAGCTTCTCAA R:CCTTGTCTTGTGTTGTGCC	1×95°C 30" 38×(94°C 30"; 62°C 30"; 72°C 1'30") 1×72°C 10' 1×10°C hold

Statistical Analysis

Data were statistically analyzed by using a randomized complete block design with three replicates according to El-Nakhlawy (2008). The two growing seasons were analyzed separately. The Costat computer program (CoHort Software, Monterey, CA) was used to perform the analysis of ANOVA. The least significant differences (LSD) test was used to compare means at the 5% level. Only differences significant at $P \leq 0.05$ are considered in the text.

Results and Discussion

Grain Yield and its Components, and Protein Content

The results presented in Table 2 and 3 indicate that wheat cultivars showed different characteristic response. In general, cultivars (Sids 1 and Giza-164) produced the highest grain yield (355.8 and 386.4 g/m²) and (347.5 and 291.7 g/m²) in both seasons, respectively. While, cultivars (Bani Swaf 3, and Bani Swaf 1) produced the lowest grain yield (41.6 and 83.0 g/m²) and (77.5 and 91.0 g/m²) in both seasons, respectively. Also, the last cultivars gave the lowest harvest index (22.6 and 26.9 %) and (23.6 and 25.9%) in both seasons, respectively. Also, plant height

was recorded and wheat cultivars Sama, Giza-164 and Sids 1 were the tallest plants (85.5, 75.0 and 70.5 cm) and (95.5, 89.5 and 100 cm) in both seasons, respectively. The shortest plants were recorded in cultivar Bani Swaf 3 (46.5 cm) in the first season, and cultivar Yocora Roieo (74.5 cm) in the second season.

Table 2. Means of protein percentage (%), plant height (cm), grain yield (g/m²) and harvest index wheat cultivars during 2005/2006 and 2006/2007 seasons.

Cultivar	Protein (%)	Plant height (cm)		Grain yield (g/m ²)		Harvest index (%)	
	2005/06	2005/06	2006/07	2005/06	2006/07	2005/06	2006/07
Sama (Sa)	13.93 c	85.5 a	99.5 a	289.1 abcd	145.2 cb	31.6 abc	24.5 d
Yocra Rojeo (YR)	13.69 d	53.0 d	74.5 b	310.4 abc	204.0 abc	38.0 a	38.1 abc
Sids 1 (S-1)	13.84 c	70.5 abc	100 a	355.8 ab	347.5 a	31.8 abc	40.1 ab
Sakha 8 (Sk-8)	13.57 d	58.5 cd	95.5 a	165.8 bcde	293.0 ab	33.6 ab	46.3 a
Sakha 61 (Sk-61)	15.65 a	57.5 cd	95.5 a	386.4 a	250.0 ab	24.9 bc	42.5ab
Giza 164 (G-164)	12.78 f	75.0 ab	89.0 ab	289.9 abcd	291.7 ab	30.2 abc	33.8 bc
Giza 168 (G-168)	12.75 f	63.0 bcd	75.0 b	289.9 abcd	280.1 ab	29.0 bc	38.1 abc
Bani Swaf 1 (BS-1)	12.93 e	47.0 d	91.0 ab	83.0 ed	91.0 c	26.9 bc	25.9 d
Bani Swaf 3 (BS-3)	14.15 b	46.5 d	84.0 ab	41.6 e	77.5 c	22.6 c	23.6 d
Shandawel (Sh)	12.17 g	48.5 d	80.0 ab	115.6 cde	199.0 abc	27.5 b	36.9 bc

*Means within the same column and followed by the same letter(s) are not significantly different from each other according to LSD ($p \leq 0.05$).

Table 3. Means of number of grains/spike, spike length (cm), 1000-grain weight (gm) of wheat cultivars during 2005/2006 and 2006/2007 seasons.

Cultivars	No. of gain/spike		Spike length (cm)		1000-grain weight (gm)	
	2005/06	2006/07	2005/06	2006/07	2005/06	2006/07
Sama (Sa)	36.6 bc	35.5 ed	7.5 abc	6.5 e	26.8 c	31.6 abc
Yocra Rojeo (YR)	31.5 dc	31.4 e	6.5 bc	9.0 c	31.3 ab	38.0 a
Sids 1 (S-1)	43.7 b	53.0 a	9.5 a	13.0 a	32.6 ab	31.8 abc
Sakha 8 (Sk-8)	36.1 bc	41.7 bcd	7.0 abc	10.0 c	34.6 a	33.6 ab
Sakha 61 (Sk-61)	39.9 bc	38.5 cde	9.0 ab	11.5 b	32.6 ab	24.9 bc
Giza 164 (G-164)	54.6 a	53.5 a	9.5 a	9.7 c	32.2 ab	30.2 abc
Giza 168 (G-168)	54.8 a	49.0 ab	7.5 abc	7.8 d	31.4 ab	29.0 bc
Bani Swaf 1 (BS-1)	22.5 d	47.1 abc	4.0 d	8.0 d	25.9 cd	26.9 bc
Bani Swaf 3 (BS-3)	23.6 d	43.9 bcd	4.0 d	7.5 d	23.3 d	22.6 c
Shandawel (Sh)	29.7 cd	43.1 bcd	6.0 cd	9.5 c	29.0 bc	27.5 bc

*Means within the same column and followed by the same letter(s) are not significantly different from each other according to LSD ($p \leq 0.05$).

Moreover, the number of grains per spike were recorded and wheat cultivars Giza-164, Giza-168 and Sids 1 had more grains per spike (54.6, 54.8 and 43.7) and (53.5, 49.0 and 53.0) in both seasons, respectively (Table 3). Also, cultivar Sids 1 had the longest spike (9.5 and 3.0 cm),

whereas cultivar Bani Swaf 3 had the shortest spike (4.0 and 7.5 cm) in both seasons, respectively. At the last 1000-grain weight (gm) were recorded and Yocra Rojeo, Sakha 8 and Sids 1 wheat cultivars had the heaviest grains compared to the other cultivars, while the lowest grain weight was found in cultivar Bani Swaf 3.

Wheat cultivars differ slightly in their ability to convert soil nitrogen to grain protein. Tables illustrates the variation in grain protein levels of wheat cultivars. The highest protein cultivar Sakha 61 had a mean protein percentage of 15.65%. Cultivar Sakha 61 had genetic potential to produce grain with higher protein content than the other cultivars. While, cultivar Bani Swaf 3 had a high protein content and the lowest grain yield. Higher protein varieties tend to be lower yielding, and higher yielding varieties tend to have lower protein. Care should be taken when selecting varieties to consider both yield and protein potential, as well as, overall agronomic characteristics (Rharrabti, *et al.*, 2001). Wide phenotypic variability was found for grain yield and protein content, suggesting the potential for selecting the best cultivars in terms of grain quality. Genotypic variation in protein content can be attributed to differences in nitrogen uptake from the soil before anthesis, activity of the root system during grain filling, efficiency of translocation of nitrogenous substances from vegetative tissues to the grains and harvest index (Kramer, 1979; Jenner, *et al.*, 1991).

PCR Analysis of Wheat HMW Glutenin Genes

Table 1 shows sets of gene- or allele-specific PCR markers, which were used in this study for identification of genes encoding HMW glutenin subunits *By9*, *By16*, and *By-null* or *20* at the *Glu-B1* locus in wheat cultivars. In the initial experiments, PCR reactions were performed in modified thermal cycling conditions for each primer pair reported previously for wheat genes by Lei, *et al.* (2006).

The presence of primer pair ZSBy9aF1/R3 in the reaction mixture, which is an allele-specific marker for the wheat HMW glutenin *By9* allele, showed one band of 650 bp (Fig. 1) in wheat cultivars Sama, Yocra Rojeo, Sids 1, Sakha 8, Sakha 61, and Giza 168. This primer pair did not produce any PCR product in wheat cultivars Giza 164, Bani Swaf 1, Bani Swaf 3, and Shandawel. Moreover, primer set ZSBy9F2/R2, specific for *By16* and *By-null* subunits in wheat cultivars (Lei, *et al.*,

2006), amplified three different banding patterns for wheat cultivars. Wheat cultivars containing the subunit *By16* showed three fragments with the use of primer set ZSBy9F2/R2, 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).

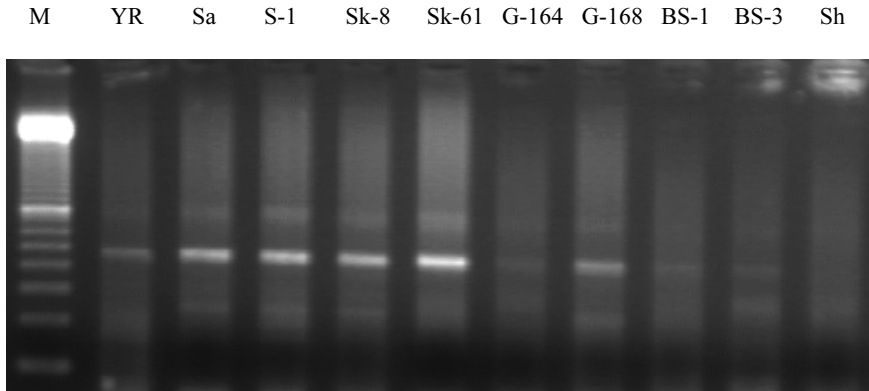


Fig. 1. Detection of alleles encoding HMW glutenin *By9* in wheat cultivars using specific primer ZSBy9aF1/R3. M line is kbp DNA marker.

Wheat cultivars Sama, Sids 1, and Sakha 61 produced three PCR fragments for the *By16* gene (Fig. 2), which exists in *Glu-B1f* (Bx13 + *By16*). Cultivars Yecora Rojo, Sakha 8, Giza 164, Giza 168, and Bani Swaf 3 produced two PCR fragments. These two PCR fragments were found to be specific for *By8*, *By9*, *By18* and *By15* genes (Lei, *et al.*, 2006). Cultivars Bani Swaf 1, and Shandawel did not produce any PCR product that was found to be specific for *By-null* or *20* gene. Lei, *et al.* (2006) demonstrated an enhanced discrimination of alleles at *Glu-B1* locus, including the distinction between the *Glu-B1e* (*By20*) allele from *Glu-B1h* (*By15*) allele, that 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 Sakha 61 produced the highest grain yield and had the highest protein content *By16* gene, which are associated with a superior bread-making quality. Therefore,

fast and accurate identification of molecular markers of *By* genes at the *Glu-B1* locus could be efficient for early selection of useful wheat genotypes with good bread-making quality.

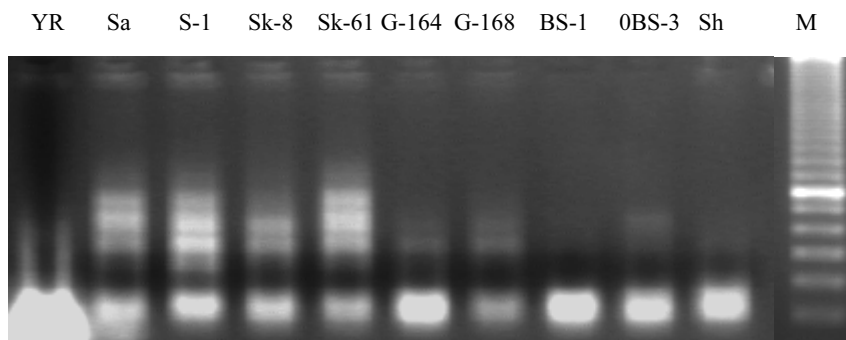


Fig. 2. Primer pair ZSBy9F2/R2 amplified the *By16*, *By-null* or *20* genes, producing 3 fragments for the *By16* gene and 2 fragments for *By8*, *By9*, *By18*, *By15* and no amplification for *By-null* or *20* genes. M line is kbp DNA marker.

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حصر أصناف القمح الربيعي المنتخبة من حيث إنتاجيتها، ومحتوى البروتين، وأليل *Glu-B1* باستخدام PCR

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المستخلص. تم تقييم عشرة أصناف من القمح الربيعي وهي الصامة، و يوكورا روجو، وسدس ١، وسخا ٨، وسخا ٦١، وجيزة ١٦٤، وجيزة ١٦٨، وبني سويف ١، وبني سويف ٣، وشندويل، من حيث الإنتاجية، ومحتوى البروتين في تجربتين حقليتين خلال موسمي ٢٠٠٥/٢٠٠٦م و ٢٠٠٦/٢٠٠٧م. وأوضحت النتائج أن الأصناف سدس ١، وجيزة ١٦٤ من أكثر الأصناف التي أعطت محصولاً للحبوب ومكوناته، بينما الأصناف بني سويف ٣، وبني سويف ١ أقل محصولاً، وأعطت أصناف بني سويف ٣، وبني سويف ١ أقل معامل حصاد. ووجد كذلك أن هناك اختلافات معنوية في مكونات المحصول بين أصناف القمح. و كان صنف سخا ٦١ يحتوي على أعلى نسبة بروتين ١٥,٦٥ ٪ و أيضا أعطى محصولاً عاليًا مقارنة بالأصناف الأخرى. بينما أعطى صنف بني سويف ٣ نسبة بروتين عالية وأقل محصولاً.

استخدمت مجموعة من الدلائل الجزيئية لجينات (*By*) جزيئات الجلوتين ذو الوزن الجزيئي العالي، للتعرف على التركيب الوراثية التي تحمل جينات *By* على الموقع الوراثي *Glu-B1*، المسؤول عن جودة الخبز. وأعطى زوجي البادئات ZSBy9aF1/R3 حزمة خاصة بتميز أليل *By9* (*Bx7+By9*) عند ٦٥٠ Bp في أصناف

القمح الصامة، ويوكورا روجو، وسدس ١، وسخا ٨، وسخا ٦١، وجيزة ١٦٨. وكذلك أعطى زوجي البادئات ZSBY9F2/R2 حزمًا لتمييز أليل (*By16 & By-null*) في أصناف القمح. وأعطت أصناف القمح صامة، وسدس ١، وسخا ٦١، ثلاث حزم للجين *By16*. وأعطت الأصناف يوكورا روجو، وسخا ٨، وجيزة ١٦٤، وجيزة ١٦٨، وبني سويف ٣ حزمتي PCR المتخصصتين للجينات *By8, By9, By18 and By15*. بينما الصنفان بني سويف ١، وشنديول لم يعطيا أي نواتج PCR والتي وجدت متخصصة للجين *By-nul or 20*. وتخلص الدراسة على أن الصنف سخا ٦١ أعطى أعلى محصول، ومحتوى بروتين، واحتوى على الجين *By16* المسؤول عن جودة الخبز.