Selection of Wheat Quality Genotypes Using Molecular Markers for High Molecular Weight Glutenin Alleles at The *Glu-B1* Locus

M.I. Motawei

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

ABSTRACT

Yeocra Rojo wheat cultivar and the ten selected genotypes, originating from immature embryos culture of such cultivar were evaluated for its productivity in two field experiments during 2005/2006 and 2006/2007 winter seasons. The results indicated that YR-9 and YR-10 wheat genotypes exhibited the tallest plants whereas, the shortest plants were for cultivar Yeocra Rojo. In general, (YR-2, YR-7, and YR-10) genotypes produced the highest grain yield. While, wheat YR-8 genotype produced the lowest grain yield. The latter genotype gave the lowest harvest index. Wheat YR-7 genotype had the largest number of grains per spike, spike length and number of spikelets per spike, compared to its parent, Yeocra Rojo cultivar and the 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 existed in the allele combination *Glu-B1b* (Bx7 + By8), was detected only in one genotype (YR-7). ZSBy9aF1/R3 primer pair gave characteristic banding patterns for Glu-B1c (Bx7+By9) and could therefore, be used to discriminate *By9*- containing alleles from *non-By9* alleles. ZSBy9F2/R2 primer pair produced applicants with a diagnostic banding pattern for *Glu-B1f* allele (Bx13+By16) in YR-10 genotype. While, YR-8 and YR-9 genotypes did not produce any PCR product that was found to be specific for *By-null* or *20* gene. It was concluded that YR-7 and YR-10 wheat genotypes produced the highest grain yield and had the *By8* and *By16* genes, respectively, which were associated with a superior bread-making quality. Whereas, YR-9 genotype produced a high grain yield and had *By-null* or *20* gene, which was associated with a poor bread-making quality. Hence, 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.

INTRODUCTION

Wheat flour is an organic complex in which starch interacts with both gluten and non-gluten proteins (largely albumin and globulins), lipids and

nonstarch carbohydrates. A large portion of variation, observed in flour quality, may be attributed to variation in 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 may improve the volume and the texture of baked loaves considerably. Unfortunately, increasing the protein concentration is hampered by a negative correlation between grain 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, among 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 a 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; namely, Glu-A1, Glu-B1 and Glu-D1, located on the long arms of homologous group-1 chromosomes (Payne and Lawrence, 1983). Molecular studies have shown that each locus contained two tightly linked genes, which encoded two types of HMW-GS, one of a higher molecular weight, designated as the x-type, and the other of a lower molecular weight, designated as the y-type (Harberd *et al*., 1986). A considerable allelic variation exists at each of the HMW glutenin loci (Anderson *et al*., 1998; Payne and Lawrence, 1983).

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 of 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 of 7, 20, and 6+8) (Singh *et al*., 1990).

HMW-GS analysis, using SDS-PAGE, is restricted to be used 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 Eagles *et al*., 2001; Gale, 2005; Gupta *et al*., 1999). 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 previously reported were 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, had 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 the present study were: (1) To evaluate ten wheat genotypes and their parent, Yeocara Rojo cultivar, for their productivity, (2) To identify wheat genotypes carrying *By* genes of HMW glutenin alleles at the *Glu-B1* locus by PCR-generated DNA markers and (3) To select wheat genotypes for superior bread-making quality, based on DNA markers of *By* genes.

MATERIALS AND METHODS

Field trials:

Two field experiments were 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. Wheat Yeocra Rojo cultivar and the ten selected genotypes, originating from immature embryos culture of such cultivar under salt stress (Barakat

and Abdel-Latif, 1996), were sown on 5th and 25th December, 2005 and 2006, respectively, with a seeding rate of 140 kg/ha. The plot size was 4x3 m with row to row spacing of 25 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.

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 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 -20C 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 previously reported (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 *By* genes amplified, 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 was detected by ethidium bromide staining.

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 & 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

Statistical analysis:

Data were statistically analyzed by using a randomized complete block design, with three replicates, according to Snedecor and Cochran (1980). The two growing seasons were analyzed separately. (Costat) computer program was used to perform the analysis of ANOVA (Table 2).

The least significant difference (LSD) test was used to compare means at the 5% level. Only significant differences at P≤0.05 were considered in the text.

RESULTS AND DISCUSSION

Grain yield and its components:

There were significant differences for plant height among wheat genotypes (Table 2). Wheat YR-9 and YR-10 genotypes exhibited the tallest plants (81.0 and 85.3 cm) and (85.3 and 81.3 cm), whereas, the shortest plants were obtained from Yeocra Rojo cultivar (64.6 and 61.3 cm) in both seasons, respectively. Moreover, the differences were not significant among the remaining genotypes in the just season, and the not significantly differences from Yeocara Rojo cultivar. Grain yield is the end result of many complex morphological and physiological processes occurring during the growth and development of crop. Data in Table 2 clearly indicated that grain yield differed significantly among wheat genotypes. In general, genotypes, YR-2, YR-7 and YR-10 produced the highest grain yield (250.0, 259.0 and 259.3 g/m^2) and (219.5, 197.0, and 203.0 g/m^2) in both seasons, respectively. These genotypes produced higher grain yield than its parent, Yeocra Rojo cultivar (214.3 and 203.0g/m^2). While, wheat YR-8 genotype produced the lowest significant grain yield (105.0 and 101.7 g/m^2) in both seasons, respectively. Also, the latter genotype gave the lowest harvest index (39.0 and 36.1 %) in both seasons, respectively. The magnitude of genetic variation among tissue culture derived genotypes has been reported in wheat by Barakat,(1995). Also, Barakat (1994) reported that dominance or dominant types of epistasis might play a role in the immature embryo culture response to *in vitro* culture traits. Besides, YR-3 and YR-2 gave the highest values (46.2 and 47.8 (%) in the two successive seasons.

Furthermore, the number of grains per spike were recorded and wheat YR-7 genotype had more grains per spike (53.3 and 49.4), compared to its parent, Yeocra Rojo cultivar (37.2 and 27.2) and the other genotypes in both seasons, respectively (Table 3). On the other hand, YR-8 genotype had the lowest number of grains per spike (32.6 and 27.1) in both seasons, respectively. Moreover, spike length was recorded and YR-7 genotype, also, had the longest spikes (13.7 and 14.0 cm) in both seasons, respectively. Also, wheat YR-7 genotype had more spikelets per spike (17.2 and 18.5), compared to the other genotypes in both seasons, respectively (Table 3). Lupotto *et al.* (1989) reported that *in vitro* selection methods had been employed to select cell cultures capable of growing in the presence of salt and to regenerate from their plants under selective stress conditions. In cereals, tissue culture approach revealed to be

effective in wheat (Barakat and Abdel-Latif, 1996), in barley (Ye *et al.,* 1987) and in maize (Lupotto *et al*., 1989).

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

1- *By8* gene – specific marker :

Primer pair of ZSBy8F5/R5 was found to be specific for the alleles containing the *By8* gene, which existed in *Glu-B1b* (Bx7 + By8) allele (Lei *et al.,* 2006). Wheat YR-7 genotype produced a 530 bp fragment, while, the other genotypes were negative with this primer pair and did not have *By8* gene (Fig.1). This marker, the first dominant marker available for selection at the *Glu-B1* locus, allowed discrimination of alleles in a cross containing *By8* and *By8**, that were usually difficult to distinguish by SDS-PAGE procedure due to their identical mobility on gel (Lei *et al.,* 2006). It, also, represented an alternative marker to distinguish the 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 existed in the allele combination *Glu-B1b* (Bx7 + By8), was only detected in two spring cultivars (Kargo and Mieszko). Southan and MacRitchie (1999) reported that wheat quality scores assigned to the HMW-GS range from zero (null allele) to four. The HMW-GS pair (7 + 8), coded by *Glu-B1*, had been assigned a score of three as this pair had been associated with a high dough strength. Therefore, the amplification of DNA fragment for *By8* gene in YR-7 genotype was associated with a high bread-making quality.

2- *By9* gene – specific marker:

Primer pair of ZSBy9aF1/R3 was used to amplify *By* gene segments from various genotypes. Wheat YR-1, YR-2, YR-3, YR-4, YR-5, and YR-6 genotypes 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 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).

3- - *By16* gene – specific marker:

Primer pair of ZSBy9F2/R2 was used to amplify the *By16* gene (Fig. 3). Wheat YR-10 genotype produced three PCR fragments for the *By16* gene, which existed in *Glu-B1f* (Bx13 + By16). Yecora Rojo cultivar and YR-1, YR-2, YR-3, YR-4, YR-5, YR-6, and YR-7 genotypes 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 genotypes did not produce any PCR product that was found to be specific for *By-null* or By *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 had opposite genetic effects on wheat quality, but were 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) were associated with a superior bread-making quality.

In the present study, it was shown that wheat YR-7 and YR-10 genotypes produced the highest grain yield and had the *By8* and *By16* genes, respectively, which were associated with a superior bread-making quality. Whereas, YR-9 genotype produced a high grain yield and had By-null or By *20* gene, which was associated with a poor bread-making quality. Hence, molecular 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.

Table2: Plant height, grain yield and harvest index of wheat genotypes during 2005/2006 and 2006/2007 winter seasons.

Wheat genotypes	**Plant height (cm)**		**Grain yield (g/m^2)**		**Harvest index (%)**	
	2005/2006	**2006/2007**	**2005/2006**	**2006/2007**	**2005/2006**	**2006/2007**
YR-1	67.0 b[1]	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
LS.D.(0.05)						

-Data are expressed as means.

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

Table 3: Number of grains per spike, spike winter, 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[1]	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 Rojo (YR)	37.2 bcd	27.2 c	9.3 cde	10.0 bc	15.1 bc	15.7 b
LS.D.(0.05)						

-Data are expressed as mean

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

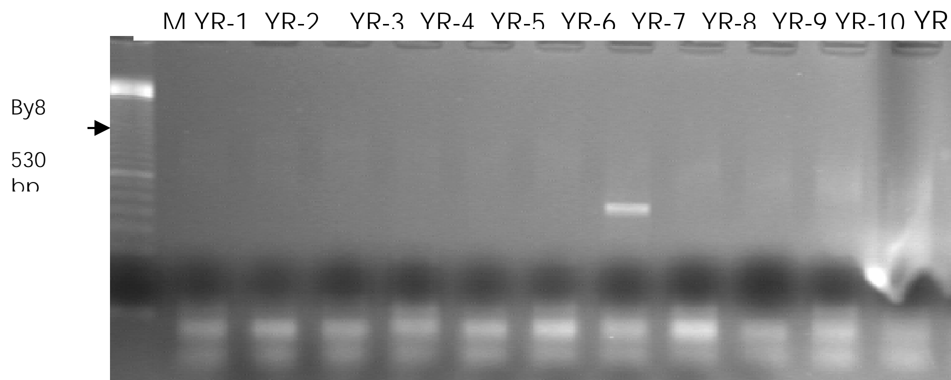

Fig. 1: Detection of alleles encoding HMW gelutenin *By8* in wheat genotypes, using specific primer (ZSBy8F5/R5). M line is kbp DNA marker. The arrow points to a unique fragment of approximately 530 bp present in *By8* gene.

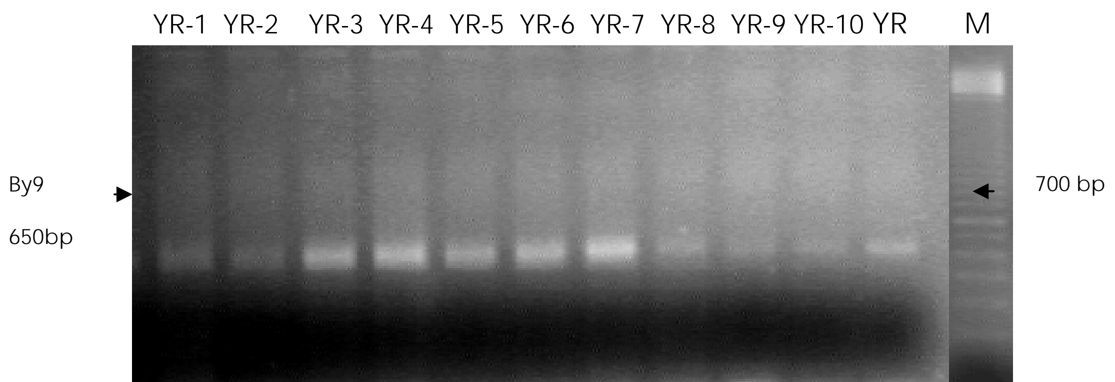

Fig. 2: PCR products obtained by using primer pair ZSBy9aF1/R3 indicate a size difference of 50 bp between By9 genotypes and non-By9 genotypes. M line is kbp DNA marker.

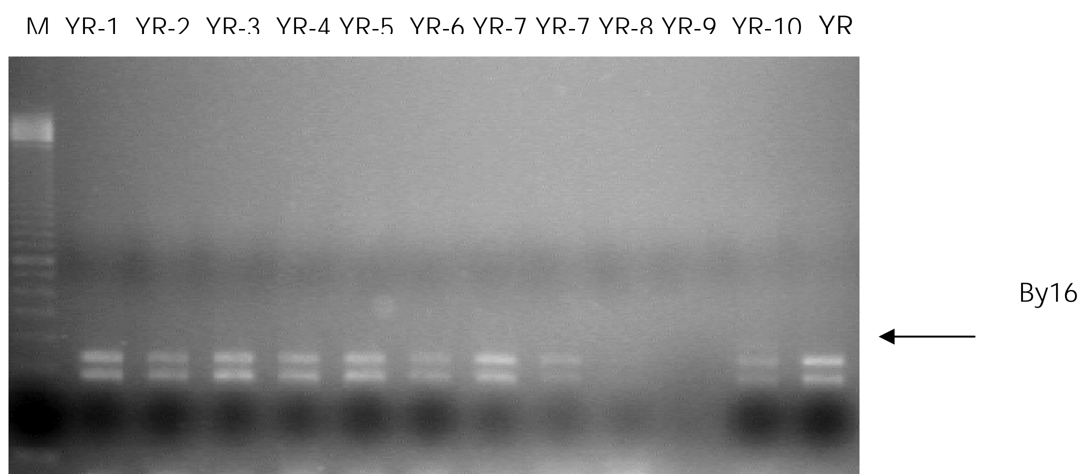

Fig. 3: Primer pair ZSBy9F2/R2 amplified the By16, By-null or 20 genes, producing three fragments for the By16 gene and two fragments for By8, By9, By18, By15 and no amplification for By-null or twenty genes. M line is kbp DNA marker. The arrow points three fragments for the By16 gene.

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الملخص العربي

الانتخاب لجودة التراكيب الراثية في القمح باستخدام الدلائل الجزيئية لأليلات الجلوتين ذو الوزن الجزيئي العالي على الموقع الوراثي *Glu-B1*

محمد إبراهيم مطاوع
معمل التقنية الحيوية – قسم المحاصيل – كلية الزراعة – جامعة الإسكندرية

تم تقويم صنف القمح" يوكورا روجو" و عشرة تراكيب وراثية ناتجة من زراعة الأجنة غير الناضجة للصنف" يوكورا روجو"، من حيث إنتاجيتها في تجربتين حقليتين خلال موسمين شتويين (2006/2005م و 2007/2006م) و أوضحت النتائج ان التراكيب الوراثية "YR-9 و YR-10" كانت أطول النباتات بينما كانت اقصر النباتات هو للصنف" يوكورا روجو"، و أنتجت التراكيب الوراثية "YR-2 و TR-7 و YR-10" اعلى محصول حبوب بينما كان التركيب الوراثي" YR-8" أقل محصولا للحبوب و معامل حصاد. و كان التركيب الوراثي "YR-7" أعلى من الصنف" يوكورا روجو" و التراكيب الوراثية الأخرى من حيث عدد الحبوب بالسنبلة و طول السنبلة و عدد السنيبلات بالسنبلة. استخدمت مجموعة من الدلائل الجزيئية لجينات (*By*) والتي تحتوي على جزيئات الجلوتين ذو الوزن الجزيئي العالي للتعرف على التراكيب الوراثية التي تحمل جينات *By* على الموقع الوراثي *Glu-B1* المسئولة عن جودة صناعة الخبز. وقد وجد الجين" *By8*" الخاص بالاليل (Bx7+ By8) *Blu-B1b* في التركيب الوراثي "YR-7". و أعطى زوجين البادئات ZSBy9aF1/R3 حزم خاصة بتميز أليل (Bx7+By9) *By9* عن الأليلات الأخرى. و أعطى زوجي البادئات ZSBy9F2/R2 حزم لتميز أليل *Glu-B1f* (Bx13+By16) في التركيب الوراثي "YR-10" . بينما لم يعطيا التركيبان الوراثيان أي نواتج PCR مما يدل على احتواهما على الجين" *By-null or By20*" . وخلاصة الدراسة أن التركيبين الوراثيين "YR-7 و YR-10" قد أعطيا أعلى محصول للحبوب و يحتويان على الجينين" By8 و By16" على الترتيب و المسئولان عن جودة عالية لصناعة الخبز، بينما التركيب الوراثي "YR-9" اعطى محصولا عاليا للحبوب و يحتوي على الجين" *By-null or By20* " و المسئول عن جودة منخفضة لصناعة الخبز. و لذلك فأن استخدام الدلائل الجزيئية السريعة و الدقيقة في التعرف على جينات "*By*"في الموقع الوراثي "*Glu*-B1" يمكن أن تكون ذات كفاءة في الانتخاب للتركيب الوراثية في القمح ذات جودة جيدة لصناعة الخبز.