

Full Length Research Paper

Molecular markers for predicting end-products quality of wheat (*Triticum aestivum* L.)

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The high molecular weight glutenin subunits (HMW-GS) are key factors in bread making quality since they are major contributors of glutenin elasticity and polymer formation of wheat dough. DNA markers for quality traits are currently used by wheat breeders for direct measurement of these traits without waiting for advancing generations of breeding materials to conduct biochemical tests. The goal of this study was to use DNA markers for screening newly developed Saudi wheat varieties for the presence of HMW-GS genes. Four new Saudi wheat lines (KSU 102, KSU 103, KSU 105 and KSU 106) and two American cultivars Yecora rojo and West Bread (popular in the Kingdom) were utilized in screening for the presence of the HMW-GS using primers covering the three wheat genomes. From the A genome, Ax2* was used. While two sets of primer pairs were used in the B genome. One primer pair for the Bx7 allele and another primer pair for By8 allele. From the D genome, primer pairs for Dx2/Dx5 and another primer pair for Dy10/Dy12 were used. Our results showed that both KSU 102 and 106 were missing Ax2* in the Glu alleles A1 and contain the Dy12 in the Glu-D1 locus which is indication of the poor bread making quality. On the other hand KSU 103 and 105 contained subunit Ax2* as well as Dx5 and Dy10 indicates that these two varieties are of moderate bread making quality. In contrast, both West Bread and Yecora Rojo contained all the five genes indicating that these two cultivars are of good quality.

Key words: DNA markers, wheat varieties, high molecular weight glutenin, quality traits.

INTRODUCTION

Wheat (*Triticum aestivum*) gluten contains both high molecular weight (HMW-GS) and low molecular weight (LMW-GS) glutenin subunits. The effects of (HMW-GS) on dough properties (strength and elasticity) may be additive or synergistic with significant interactions with (LMW-GS) subunits (Beasley et al., 2002). The HMW-GS are encoded by polymorphic genes at the Glu 1 loci that are present on the long arm of group 1 chromosome (Payne and Lawrence, 1983). At each locus (Glu-A1, GluB1, GluD1) there are two tightly linked HMW-GS genes; one of them is x-type -with higher molecular weight- and the other is y-type. For instance, at the Glu-1 there are subunits 1, 2* and null (there is no y allele), at the Glu-B1 locus there are Bx17+By18, Bx7+By8, Bx7+By9, Bx6+By8, and at the Glu-D1 locus, there are Dx5+Dy10, Dx2+Dy12, Dx3+Dy12, Dx4+Dy12. The presence of different allelic composition of the HMW-GS in one specific wheat variety is one of the most important genetic factors in determining the bread making quality

(Payne et al., 1987). For example, wheat varieties containing allelic compositions of (Dx5 paired with Dy10) at the Glu-D1 locus will form stronger dough than those containing (Dx2 paired with Dy12). Due to the large contribution of allelic interaction in bread making quality, Békés et al. (2006) suggested targeting different allelic combinations rather than individual glutenin allele in developing new lines with certain quality attributes.

Baking quality is a major target in wheat breeding programs. However, due to small population size of wheat that can be obtained in early generations of breeding programs, full-scale mill and bakery testing is not routinely feasible. Alternatively, DNA marker screening of these quality traits may be performed on leaf materials at early stage and before grain setting (De Bustos et al., 2000). DNA markers for HMW-GS allelic composition have proven to be superior to electrophoresis of SDS-PAGE in some instances (D'Ovidio and Anderson, 1994). Moreover, this assay can be used to select individual plants

Table 1. PCR primer sequence for the amplification of specific HMW- SG genes.

Gene	Primer	Amplicon size	Reference
Ax2*	F: 5' ATGACTAAGCGGTTGGTTCTT 3' R: 5' ACCTTGCTCCCTTGTCTTT 3'	1200 bp	Ma et al., (2003)
Dx2, Dx5	F: 5' GCCTAGCAACCTTCACAATC 3' R: 5' GAAACCTGCTGCGGACAAG 3'	450 bp for Dx5	Anderson and Green (1989)
Dy10, Dy12	F: 5' GTTGCCGGTTCGGCTGCCATG 3' R: 5' TGGAGAAGTTGGATAGTACC 3'	576 bp for Dy10 612 bp for Dy12	Smith et al. (1994)
Bx7	F: 5' ATG GCTAAGCGCCTGGTCTT 3' R: 5'TGCCTGGTTCGACAATGCGTCGCTG 3'	2373 bp	Anderson and Green (1989)
By8	F: 5' TTAGCGCTAAGTGCCGTCT 3' R: 5':TTGTCCTATTTGCTGCCCTT 3'	527 bp	Lei et al. (2005)

within populations and to overcome the environmental variation originating from both field and laboratory.

Data presented by Uthayakumaran et al. (2006) showed that the markers assisted selection (MAS) for the HMW-GS are especially valuable tool for breeding programs since the information about the bread making quality can be obtained at an earliest stages of breeding program, thus poor-quality lines are not propagated. Moreover, Kuchel et al. (2007) reported that MAS for the HMW and LMW glutenin alleles is widely performed by breeding programs throughout the world to select for improved dough characters. Blechl et al. (2007) suggested that contribution of allele-allele interactions, and different allelic combinations should be targeted rather than the individual glutenin alleles in breeding program to develop new lines with certain quality attributes, especially to improve extensibility. The main reason given was the significant differences found among the β values-, which described the contribution of HMW-GS \times LMW-GS interactions on extensibility.

Similarly, Gale (2005) reported that some diagnostic markers had being used in wheat breeders in identifying the bread making quality of varieties through identifying their different genes and alleles coding for special proteins, without the need for the direct measurement of those traits in early generation screening. DNA markers applied on leaf tissue from individual plants will further increase the efficiency and speed of the development of improved cultivars in the future. More recently, Kuchel et al. (2007) reported that MAS or -selection using molecular markers- could increase the genetic gain and economic efficiency of a specific breeding strategy. In this study PCR was utilized -using published primers of the HMW-GS alleles- as diagnostic DNA markers for newly developed wheat lines in Saudi Arabia.

MATERIALS AND METHODS

The present investigation was carried out at the Biotechnology Laboratory, Department of Plant Production, Collage of Food and Agricultural Sciences, King Saud University.

Plant materials

Six wheat cultivars were used in this study; four newly developed varieties (KSU 102, KSU 103 , KSU 105, KSU 106), obtained from the Department of Plant Production, King Saudi University, and two cultivated varieties in Saudi Arabia Yecora rojo, and West Bred were obtained from the local market.

Genomic DNA extraction

Genomic DNA isolation was done using DNeasy Plant Kit, (Qiagen, USA) as described in the manufacturer manual.

PCR conditions

PCR analyses were performed in MWG-Biotech Thermal-Cycler in the final volume of 25 μ l. The alleles used as MAS, sequence of the primers used and amplicon size with references are listed in Table 1. Primers were purchased from TIB MOLBIOL Syntheses Labor GmbH (Germany). PCR reaction conditions were as described in the references listed in Table 1.

RESULTS AND DISCUSSION

The importance of the HMW-GS on dough properties in wheat have been widely demonstrated (Payne et al., 1987). Summary of the presence of the different HMW-GS alleles in the studied germplasm is presented in Table 2. Our results showed that the Glu-1D locus (Dx5 and Dy10) alleles were found in KSU 103, KSU105, Yecora rojo, and West Bred cultivars. Meanwhile both KSU 102 and KSU 106 were found to have Dy12 which is correlated to the poor quality glutenin. Concerning the Glu1B locus, the (Bx7and By8) alleles were found in Yecora rojo, and West Bred cultivars. However, KSU 103 and KSU 105 were missing the Bx7 allele. Concerning the Glu-1A locus, Ax2* allele was found in KSU 103, 105 and Yecora rojo, and West Bred cultivars.

The strong association between the HMW-GS composition -as protein markers- and dough properties developed by Payne et al. (1987), and known as Payne score has been successfully used in wheat breeding pro-

Table 2. Summary of glutenin composition of cultivars used in this study.

Cultivars	Ax2*	Bx7	By8	Dx5	Dy10	Dy12
102	-	-	-	-	-	+
103	+	-	+	+	+	-
105	+	-	+	+	+	-
106	-	-	-	-	-	+
Yecora rojo	+	+	+	+	+	-
West Bred	+	+	+	+	+	-

grams to select newly developed varieties with good bread making quality (Eagles et al., 2002). This score provides a single number to estimate dough strength from the HMW-GS glutenin allelic composition (giving a score of 3 for the presence of each of Glu-A1 and Glu-B1 and score of 4 for the presence of Glu-D1 allele (Dx5 and Dy10), with a maximum score of 10 for each variety). Applying this score to our results (Table 2), both Yecora rojo and West Bred cultivars gave score of 10. On the other hand both KSU 102 and KSU 106 are of poor quality glutenin (score of 0), and that KSU 103 and KSU 105 are of moderate quality since they have the Glu-1A allele (Ax2*) and Glu-D1 allele (Dx5 and Dy10), and missing only the Bx7 of the Glu-1B allele. However, since By8 alone is extremely rare and there is possible that Bx 6 is linked to it (for which we did not have primer), this combination is given a score of 1 (Payne et al. 1984). This means that both KSU 103 and 106 have a total Payne score of 8.

Given the successful use of DNA based markers in breeding strategies, it is likely that MAS for improved dough properties can be effectively incorporated into breeding programs at an early stage of variety development instead of waiting for six to seven years before evaluating newly developed genotypes for baking quality. Békés et al. (2006) based on statistical investigation of more than 3000 samples covering most of the glutenin alleles and allelic combinations in Australian bread wheat cultivars, found that there was a strong association between glutenin alleles targeted with molecular markers and dough quality. The conclusion of that study was that MAS for both HMW-GS and LMW-GS alleles is very efficient in improving dough parameters and end-product attributes. Therefore, MAS using PCR based markers were used to examine the bread making quality (dough parameters and end-product attributes) of newly developed inbred lines of wheat.

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