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Identification of HMW Subunits in Iranian landrace wheat by using STS-PCR Method

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INTRODUCTION

Wheat flour bread-making quality is mainly determined by the composition and quantity of endosperm storage proteins, in particular the high-molecular-mass (HMW) glutenin subunits (Aahmad M, 2000). Little was known about the structure of these proteins until 20 years ago. Then, boosted by new technology developments such as molecular cloning, it became possible to isolate cloned cDNA and genes for all the major groups of gluten proteins (D'Ovidio *et al.* 1999; Hsia and Anderson 2001). This has allowed the complete amino acid sequences of the proteins encoded by these DNA to be deduced, providing a basis for modeling and biophysical studies. Such molecular and biophysical studies have been combined to give a detailed picture of HMW-GS structure (De Bustos. and Jouve, 2003). (HMW) HMW glutenin genes are encoded by homologous loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, each locus includes two tightly linked genes encoding two types of HMW glutenin subunits, designated as *x*- and *y*-types. Therefore, there are six HMW glutenin genes even though only three to five subunits are synthesized in any particular cultivar. The allelic variation of these subunits is associated with flour quality. In particular, the HMW glutenin genes *1Ax1* and *1Ax2** encoded by *Glu-1A* locus, and the *1Dx5* and *1Dy10* subunit pair at *Glu-1D* locus have been suggested to be associated with stronger dough and better baking properties. Traditionally, polyacrylamide gel electrophoresis sodium dodecylsulfate (SDS-PAGE) and/or A-PAGE and RP-HPLC are the most widely used techniques for identification of HMW glutenin composition in wheat. Glutenin subunit screening is accomplished using electrophoresis (SDS-PAGE). Recently, steady and cost-effective systems based on the identification of genes rather than gene products are required. Once such systems are developed and verified they can be easily upscaled and expanded, taking advantage of Polymerase Chain Reaction (PCR) procedures. Sequence-tagged site (STS) is a short fragment of DNA whose exact sequence is found nowhere else in the genome; typically about 200 to 300 bp. Polymerase chain reactions can be used to amplify the known sequences, which can serve as physical landmarks for mapping. This technique is using for evaluate of exceeded plants such as barely, soybean, peach, wheat. In this research, using different regions between *1Dx2* and *1Dx5* genes and *1Dy10* and *1Dy12* genes to design site-specific primers for PCR analysis.

One of nucleotide's differences between *1Dx5* and *1Dx2* is Cystein residues codon in start of repetitive domain. In comparing 5+10 and 2+12, an extra cysteine residue in *Dx5* was suggested as a possible explanation (Kasarda 1999).

Here we report the utility of specific PCR primers to identify wheat genotypes carrying glutenin allelic combinations which are related with good or poor bread-making quality.

MATERIALS AND METHODS

This study was conducted in the biotechnology laboratory of Department of Agronomy in University of Tehran, in 2007.

Genomic DNA was extracted from 2 mg of fresh leaves from single plants of five wheat cultivars with different glutenin genotypes covering a range of bread quality allelic combinations. Leaves were crushed to a fine powder in a mortar after freezing with liquid nitrogen and 800 µl of extraction buffer (2% cTAB, 100 m*M* TRIS-HCl, 25 m*M* NaCl, and 50 m*M* EDTA, pH 8) was added. The homogenised mixtures was transferred into a 1.5 ml plastic tube, mix and incubate at 60°C for 30 min with occasional gentle swiveling then add 600 µl chloroform/isoamylalcohol (24:1) was added and the mixture was incubated at room temperature for 45 min with mixing. After centrifugation for 10 min at 6500 rpm, the supernatant was carefully poured into a silica matrix tube.

Where DNA was precipitated by adding 600 µl of isopropanol, After centrifugation for 10 min at 13000 rpm, the DNA pellet was washed with 70% ethanol, air-dried and resuspended in 350 µl of R 40 (40 µl RNase A added to 1 ml of 10 m*M* TRIS-HCl, 1 m*M* EDTA, pH 8.0, boiled for 10 min to destroy DNase, stored at –20°C) overnight at 4°C. Absorbance at 260 nm and 280 nm was used to evaluate DNA quality and to standardize DNA concentrations.

The 25-µl amplification reaction contained 10 m*M* TRIS-HCl, pH 8.3, 50 m*M* KCl, 2 m*M* $MgCl_2$, 0.001% gelatine, 300 µ*M* of each dNTP (deoxyribonucleotide), 250 ng of each primer

(table 1), 50 ng genomic DNA and 1 U *Taq* DNA polymerase (Cinnagen Company, Iran).

Table 1. Primer sequences used to amplify HMW glutenin subunits

Primer	Sequence (5´−3´)	Allele
P12	GCCTAGCAACCTTCACAATC	Dx2,
P13	GAAACCTGCTGCGGACAAG	Dx5
P14	GTTGGCCGGTCGGCTGCCATG	Dy10,
P15	TGGAGAAGTTGGATAGTACC	Dy12
P16	ATGGCTAAGCGCCTGGTC	Bx7
P17	TGCCTGGTCGACAATGCGTCG CTG	

In this study, we use specific PCR primers to recognize wheat genotypes carrying glutenin allelic combinations which are related with good or poor bread-making quality (*Dx5+Dy10*, *Dx2+Dy12*) according to Ahmad 2000 with minor modifications. Amplifications were performed in a Bio-Rad Mastercycler Gradient programmed at 94°C for 5 min, followed by 45 cycles at 94°C for 1 min, 63°C for 1 min and 72°C for 1 min, except for primers P5 and P6 where the annealing temperature was 60°C for 1 min and extension was 72°C for 3 min. After 45 cycles, the extension temperature was kept at 72°C for 10 min. half of each total PCR product was analyzed on 2% agarose gels and then stained in 0.5 µg/ml ethidium bromide for 1 h before visualization under UV light.
The seed were crushed after removal of the embryo. The flour was mixed in an extraction buffer of 62 m*M* Tris-HCL (PH=6.8) buffer containing 10% (W/V) glycerol, 4% (W/V) sodium dodecyl sulfate(SDS), 0.01%(W/V) bromophenol blue and 4% 2-mercaptoethanol.

Table 2 Allelic amplification with specific primers and their expected fragments Allele Primer Expected DNA fragment

Name of Allele	Name of Primer	Expected fragment
1Dx5	P1+P2	450 bp
1Dy10	P3+P4	576 bp
1Dy12	P3+P4	612 bp

Samples were allowed to stand at room temperature for at least 12h with occasional vortexing. They were then placed in a boiling water-bath for 2 minutes and then centrifuged for 5 minutes at 6500 rpm and 15 µl of each sample was loaded on the gel. Proteins were fractionated by SDS-PAGE according of Laemmli procedure, using stacking gel Proteins were fractionated using stacking gel containing 4% acrylamide, 0.05% bis acrylamide, 0.1% SDS and 0.00009 *M* Tris-HCL; and separating gel containing 10% acrylamide, 0.13% bis acrylamide, 0.1% SDS and 6.78 *M* Tris-HCL. Gels were stained overnight with 0.01% (W/V) Coomassie Brilliant Blue R 250 in water and acetic acid (10%) and then destained overnight in water for at least 24h (Laemmli, 1970).

RESULTS

The efficiency of paired, specific primers for *1Dx5* allele was validated using some commercial wheat for which the existence of *1Dx5* allele was confirmed previously by Bushehri *et al.* (2006) and Bahraeie *et al.* (2004). In all cultivars such as Marmareh, Ghafghaz, Falat, Naz and Inia the segment of 450 paired base was absorbed indicating the presence of *1Dx5* allele PCR analysis was performed on Azadi, Qouds, Azar, Bezotaia, Karaj 1 and Zagros wheat cultivars using paired specific primers for *1Dy10* and *1Dy12* that cause the multiplication of 567 and 612 paired base segments for *1Dy10* and *1Dy12*, respectively.
After the validation of the efficiency of paired primers by commercial wheat cultivars, a SDS-PAGE was performed on bread wheat native lines proteins. In the next step using above primers and PCR analysis on wheat native lines of genetic resource unit of the Directorate of Wheat Research Karaj, the given alleles were probed.
The utilization of paired specific primers for *1Dy10* and *1Dy12* also resulted in the multiplication of 567 paired base segments specified for *1Dy10* allele, indicating the correlation between these two alleles. PCR analysis showed no bared with *1Dx6* primer. Results of comparing two methodologies of SDS-PAGE and STS-PCR are represented in table 1. Protein subunits in migration SDS-PAGE is not always correlated with their molecular weights, making the selection of parental lines a difficult task in breeding programs (Shewry *et al.* 1992). The results of this study showed that the misleading results of a glutenin heavy subunits SDS-PAGE analysis could be avoided by PCR. The precision of this method was confirmed for all cultivars both in the absence or presence of *1Dx5*, *1Dy10* and *1Dy12*.
The dissimilarity of SDS-PAGE methods with that of PCR could be resulted from the existence of within line biotypes where in conventional methods of wheat breeding is unavoidable. Biotypes even were present among some common cultivars such as Chamran, Bistoon, Kavir, Shole, Sabalan, Karaj 1, Karaj 2, Karaj 3, Qouds, Pitic, Aljazaier 4820, Punjab, Kaveh and Darab (Bushehri *et al.* 2006). DNA based recombination in the flunking region acids codon of glutenin protein (Payne *et al.* 1987).

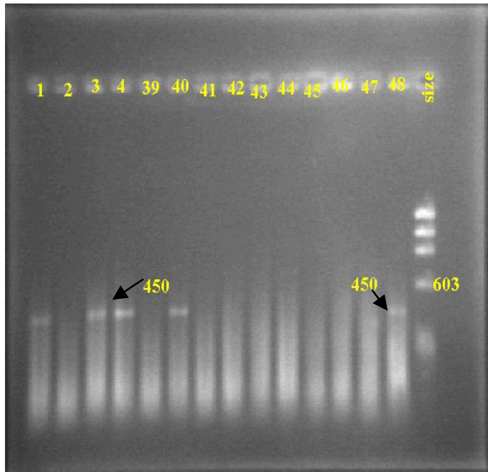

Fig. 1. Primers (P1 and P2) specific for the 1Dx5 allele were used for PCR reaction on 50 ng of genomic DNA from 48 bread wheat lines for the 450-bp (barrow) DNA fragment amplification.

The relatedness of alleles may further be ideal by usage of primers. The increased information about sequences and PCR results could lead into more precisely identification of novel alleles. *1Dx5**, *1Dx2.2*, 1Dx2.2*, *1Dx4*, *1Dx3*, for example are not the transformations of *1Dx5* as *1Dx5* specific primers did not multiply the 450 paired based segment of interest. More alleles are needed to understand whether these novel alleles are closer to *1Dx2* gene or not. Precision, rapidity, simplicity as well as avoiding toxic chemicals such as acryl amid made PCR a reliable substitution for standardized methods of

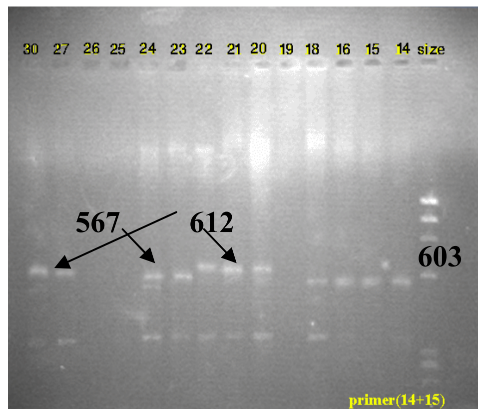

Fig. 2 Primers (P3 and P4) specific for the 1Dy10 and 1Dy12 alleles were used for PCR reaction on 50 ng of genomic DNA from five

selecting genotypes with *1Dx5* and other genes. With PCR, furthermore, it is possible to screen hundreds of plant in a day for breeding programs by using only a small amount of (about 10 mg) leaf, root or endosperm tissues, facility the rapid evaluations of primary results. In next stage the primary plants or embryonic section may be cultivated for subsequent characterization of next generation. In breeding programs for quality improvement, if selection is based on a marker probing only a single allele of *Dx2* or *Dx5* (assuming *Dx2* is completely linked with *Dy12* and *Dx5* with *Dy10*) would lead to the selection of inappropriate allele combination. Thus, simultaneously selection for both alleles *Dx2+Dx12* or *Dx5+Dy10* is a critical factor in breeding for quality. In addition to the selection for both alleles, PCR based discrimination system, hampers mistakes result from in appropriate allele combinations such as *Dx2+Dy10* and *Dx5+Dy12* that are corresponded with low quality. When the allele amount of heavy glutenin subunits of commercial cultivars were compared with these of gene bank lines by SDS-PAGE system it was absorbed that the *Glu-A1* genome of gene bank lines has a high frequency of novel allele, however, considered as an unsatisfactory attribute. Alternatively, the *Glu-B1* genome of lines revealed a high amount of 7+8 allele which is potentially a valuable resource for transferring their genes to commercial cultivars, which is considered a pronounced advantage for gene bank lines. Finally, it was illustrated that in *Glu-D1* genome the level of the worthy 10+5 allele is less than that of commercial cultivars.

CONCLUSION

Accuracy, simplicity and speed make the proposed PCR generated DNA markers a valid alternative to standard techniques for selecting genotypes containing the high molecular- weight glutenin subunits related to bread quality.
Moreover, MAS could allow the screening of hundreds of plants in 1 day for a quick, early-generation evaluation, thereby saving time and resources otherwise required for seed increase and physical quality testing in a wheat breeding programmed.

REFERENCES

1. Ahmad. M. 2000. Molecular Marker-assisted selection of HMW Glutenin Alleles Related to Wheat Bread Quality by PCR-generated DNA Markers. Teor Appl Genet. 101: 892-896.
2. Bahraeie. S, A. Saidi, D. Alizadeh. 2004. High Molecular Weight Glutenin Subunits of Current Bread Wheats Grown In Iran. Euphytica. 137:173-179
3. Bushehri,A.A , M. Gomarian, B. Yazdi-Samadi. 2006. The high molecular weight Glutenin subunit composition in old and modern bread wheats cultivated in Iran. Australian Journal of Agricultutal Research. 57:1109-1114