The high molecular weight glutenin subunit composition in hexaploid wheat

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INTRODUCTION

Bread wheat endosperm is essentially composed of approximately 70% starch and 10–15% protein (MacRitchie 1992). Seed storage proteins (about 85% of endosperm proteins) are traditionally classified as gliadins and glutenins, based on their solubility (Osborne 1907). The elasticity (strength) and extensibility (viscosity) of the dough properties essential for bread making are closely correlated with glutenins and gliadins, respectively (Payne *et al*. 1984).The glutenins are divided into 2 groups: high-molecular weight (80–120 kDa) and low-molecular-weight glutenin subunits (30–50 kDa) (Payne and Corfield 1979). The high-molecular-weight glutenin subunits (HMW-GS) are encoded at genes *Glu-A1*, *Glu-B1*, and *Glu-D1* with a large of number alleles (Payne and Lawrence 1983) and were updated by McIntosh *et al*. (1994). Although HMW-GS only occupy around 10% of the wheat seed storage proteins, they play a major role in bread-making quality (Payne *et al*. 1984). A quality score was assigned to each HMW-GS by Payne *et al*. (1987). Allelic variation exists at each of the *Glu-1* loci (*Glu-A1*, *Glu-B1*, *Glu-D1*), with 2 tightly linked genes encoding a higher Mr x-type HMW-GS and a lower Mr y-type HMW-GS (Payne 1987). A single hexaploid variety could contain 6HMW-GS, but due to the silencing of some of these genes, most common wheat cultivars contain between 3 and 5 subunits (Payne and Lawrence 1983; Harberd *et al*. 1986; Gianibelli *et al*. 2001). The effect that different HMW-GS alleles have on bread-making quality has been widely studied (Lawrence *et al*. 1987; Payne *et al*. 1987; Gupta *et al*. 1994; Cornish *et al*. 2001). It has been shown that certain alleles, such as 5+10, have a positive influence, whereas others, such as Null, have a negative effect on dough strength and bread-making quality (Payne *et al*. 1987). A correlation has also been drawn between the presence of specific HMW-GS alleles and bread-making quality, and most *Glu-1* alleles have been given a score on the basis of their effect on the sodium dodecyl sulfate (SDS) sedimentation volume (Payne *et al*. 1987), or their gluten strength, as measured by the alveographic parameter W (Pogna *et al*. 1989). High correlations between bread-making quality and HMW-GS have been reported in wheat from different countries (Sontag *et al*. 1986; Payne *et al*. 1987, 1988; Lukow *et al*. 1989; Rogers *et al*. 1989; Uhlen 1990). There are a number of studies on HMW-GS composition of wheats from Australia, Canada, India, Italy, The Soviet Union, Yugoslavia, France, China and Japan, Portugal, Pakistan, and the US (Lawrence 1986; Bhagwat and Bhatia 1988; Vapa and Savic 1988; Ng *et al*. 1989; Pogna *et al*. 1989; Morgunov *et al*. 1990; Lookhart *et al*. 1993; Tahir *et al*. 1995; Redaelli *et al*. 1997; Igrejas *et al*. 1999; Nakamura 2000; Branlard *et al*. 2003). This paper is based on the most extensive study on HMW-GS in Iran. It covers all the cultivars so far cultivated in Iran. The objective of this study was to investigate the HMW-GS composition of 95 old and modern bread wheat varieties grown in Iran to provide information for improving the quality of Iranian wheat. Waste of flat breads is high in Iran; therefore the results help to modernise baking, consumption, and increase production of pan breads in Iran.

MATERIALS AND METHODS

Seeds of 95 Iranian bread wheats were used for this study. Seeds were obtained from the Field Crops Research and Genetic Resources Unit of the Faculty of Agriculture, University of Tehran. Total proteins were extracted from 6 single seeds of each cultivar. Additional seeds (up to 10) were used for those varieties that showed inconsistent HMW-GS patterns. Control varieties Bezostaya-1, Champlein, Chinese- Spring, Danchi, Dunav, Federation, Gabo, Hobbit, Hope, Lancota, Norin 61, Sappo, and Serbian were used as standards (Payne and Lawrence 1983). The seeds were finely crushed after removal of the embryo. The flour was mixed in extraction buffer of 62.5mM Tris-HCl (pH 6.8) containing 12% (w/v) glycerol, 2%(w/v) sodium dodecyl sulfate (SDS), 0.003% (w/v) bromophenol blue, and 5% 2-mercaptoethanol. The samples were boiled for 5 min, then centrifuged for 5 min at 1000 rpm, and 15mL of each sample extract were loaded on the gel. Proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970), using separating gel containing 8.7% acrylamide, 0.3% bis-acrylamide, 0.1% SDS, and 0.38 M Tris-HCl (pH 8.8). Gels were stained overnight with 0.13% Coomassie Brilliant Blue R-250 in water and acetic acid (53:40:7 v/v) and destained overnight in water, butanol, and acetic acid (65:25:10 v/v) (He *et al*. 1992). TheHMW-GS were identified using the numbering system proposed by Payne and Lawrence (1983). A quality score was assigned to each HMWGS of a subunit pair using the scoring described by Payne *et al*. (1987). The genetic diversity at each locus was calculated according to Nei (1973) as follows: $H = 1 - \sum p_i^2$.

RESULTS AND DISCUSSION

Among the 95 varieties, 77 were homogeneous and 18 were heterogeneous for HMW-GS. The Iranian wheat varieties in this study could be divided into 33 groups based on allelic compositions. Twelve groups had only one genotype each and the most frequent HMW-GS compositions were N, 7+8, 2+12 and 2*, 7+8, 2+12, which were observed in 24 and 15 cultivars and biotypes, respectively. In total, 18 *Glu-1* alleles were identified, 3 at *Glu-A1*, 9 at *Glu-B1*, and 6 at *Glu-D1*. At *Glu-A1* the frequencies of occurrence of Null, 2*, and 1 were 46.4, 37.7, and 15.7%, respectively. At *Glu-B1*, subunits 7+8, 17+18, 7+9, 7, 13+16, 13+19, 20, 6+8, and 14+15 were found in 51.7, 18.4, 12.2, 8.7, 4.3, 1.7, 1.7, 0.8, and 0.8 of the cultivars and biotypes, respectively. At *Glu-D1*, subunits 2+12, 5+10, 2***+12_, 3+12, 10, and Null were detected at a frequency of 61.4, 30.7, 5.2, 0.8, 0.8, and 0.8%, respectively. At *Glu-A1* the prevalent allele was Null with a frequency of 46.4%. This is almost certainly due to the Iranian baking requirement for medium-elastic dough rather than strong gluten that results in less extensible dough (Branlard *et al*. 2003). The most frequent HMW-GS at the *Glu-B1* locus were 7+8 and 17+18 as shown in 51.7 and 18.4% of the varieties, respectively. The difference between the report of Bahraei *et al*. (2004) and this study in the frequency of the null allele at the *Glu-A1* locus is due to an inadequate number of varieties included in that study. Subunits 13+19, 20, 14+15, and 6+8 were present in 1.7, 1.7, 0.8, and 0.8% of varieties and biotypes, respectively. The scarcity of these subunits is advantageous because they have all been associated with poor bread-making quality (Payne *et al*. 1987; Dong *et al.* 1991). The high frequency of the 2+12 allele at *Glu-D1* is comparable with that already reported in several collections (He *et al.* 1992; Redaelli *et al*. 1997; Igrejas *et al*. 1999; Nakamura *et al.* 1999; Nakamura 2000; Branlard *et al*. 2003) with the exception of the material grown in Slovakia and Pakistan (Tahir *et al*. 1995; Gregov *et al*. 1997). As shown in Fig. 1, at each locus, one allele (e.g. Null at *Glu-A1*, 7+8 at *Glu-B1*, and 2+12 at *Glu-D1*) has the highest frequency. An already reported rare subunit pair, 2***+12_, at the *Glu-D1* locus, was found in cvs. Boolani, Sorkhtokhm, Afghani, and in one biotype of Karaj2 and Kavir. A characteristic apparently unique to Iranian wheat is the presence of the 2***+12_ subunits similar to Pakistani landraces (Tahir and Lafiandra 1994). Subunits 2***+12_ are observed more frequently in Iranian bread wheat landraces (Bahraei *et al*. 1999). A previous study of Iranian wheat stated that subunits 2***+12 are correlated with good flat bread making quality (Bahraei *et al*. 1999). Cultivar Cooleh showed Null x and y type subunits at *Glu-D1* and *Glu- A1* loci, whereas at the *Glu-B1* locus subunit 20 was present. In cv. Cooleh there are 2 subunits present (*1Bx20*, *1By20*). Cultivar Kalate mahali showed the Null x subunit at the *Glu-D1* locus, and the banding pattern of this cultivar is null, 13+16, and 10. Most wheat breeding programs in Iran have focussed on yield improvement and resistance to biotic and abiotic stresses. An interesting finding of this study was the presence of biotypes in some of the cultivars analysed. Some cultivars were found to be heterogeneous only at *Glu-A1*, *Glu-B1*, or *Glu-D1* loci, e.g. Khalij (N/2*), Darab2(N/2*), Kavir(N,2*), Khazar1(1/2*), and Chamran (N/2*) at *Glu-A1*, cv. Atrak (7+8/7+9), Zagros (7+9/17+18), Karaj3 (7+8/20), Quds (7+8/17+18), Karaj1 (7+9/7+8), and Sholeh (7+8/13+16) at *Glu-B1*, cv. Karaj2 (2***+12_/5+10) and Aljazaier 4820 (2+12/5+10) at the *Glu-D1* locus. Three cultivars were simultaneously heterogeneous at both *Glu-A1* and *Glu-B1* loci, namely Sabalan (2*, 7+8, 5+10/N, 14+15, 5+10), Chenab (1, 17+18, 2+12/2*, 7+8, 2+12), and Pitic (2*, 7+8, 2+12/1, 17+18, 2+12). Two cultivars including Punjamo (2*, 17+18, 2+12/2*, 7+8, 5+10) and Kaveh (2*, 7+8, 2+12/2*, 17+18, 5+10) at *Glu-B1* and *Glu-D1* loci were also a biotype. Storage protein composition is expected to be a cultivar constant element, being the direct expression of its genotype. Impurity of grain samples is one cause of varying electrophoretic fingerprints. In our study the genetic index for the 3 glutenin loci was H=0.66 (Table 1). The genetic index for *Glu-A1*, *Glu-B1*, and *Glu-D1* loci was 0.75, 0.67, and 0.52, respectively. The genetic index for the *Glu-D1* locus was lower compared with other loci. At this locus, subunits 2+12 were fixed in more than 60% of cultivars. In order to maintain diversity, continued cultivation of old varieties and local landraces and introduction of new wheat germplasm from different sources, will be required.

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Table1. Allelic frequencies (%for the Glu1 loci of 95 Iranian wheats

Locus	Allele	Subunit composition	Frequencies	Relative frequencies	Genetic variation	Genetic variation mean
Glu-A1	a	1	18	0.1578		
	b	2*	43	0.3771	0.7556	
	c	N	53	0.4649		
Glu-B1	a	7	10	0.0877		
	b	7	59	0.5175		
	c	7+8	14	0.1228		
	d	6+8		0.0438		
	e	20		0.0175	0.6727	0.66
	f	13+16		0.0087		
	g	13+19		0.1842		
	h	14+15		0.0175		
	i	17+18		0.0087		
Glu-DA1	a	2+12	70	0.6140		
	b	5+10	35	0.3070	0.5257	
	d	3+12		0.0526		
	?	2***+12’		0.008		
	?	N		0.008		
	?	10		0.008		