Evaluation of Iranian bread wheats by storage proteins "gliadins"

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INTRODUCTION

Seed storage proteins (about 85% of endosperm proteins) are traditionally classified into monomeric (gliadins) and polymeric (glutenins) proteins, based on their solubility. Gliadins (alcohol soluble) are classified into α-,β-, γ- and ω-gliadins on the basis of charge during A-PAGE and their mobility. Gliadins (γ-, and ω-) are encoded by blocks of genes at the *Gli-1* loci (*Gli-A1*, *Gli-B1* and *Gli-D1*) located on the short arms of homoeologous group 1 chromosomes whereas α-, β-, and some γ-gliadins are encoded by *Gli-2* loci (*Gli-A2*, *Gli-B2* and *Gli-D2*) located on the short arms of homoeologous group 6 chromosomes (Payne et al., 1982). There are also a few gliadin gene loci located on the short arms of homoeologous group 1 chromosomes about 2-20 cM from the *Gli-1* loci: *Gli-A3*, *Gli-A5* (Pogna et al., 1995), *Gli-A6* (Metakovsky et al., 1996), *Gli-B3* (Galili and Feldman, 1984; Metakovsky et al., 1986), *Gli-B5* (Pogna et al., 1993). New gliadin allels have also been reported on the short arm of chromosome 1D. There is considerable variation in gliadin patterns between varieties and combinations of the patterns make it possible to often distinguish wheat genotypes. There are various reports regarding gliadin pattern variations (Metakovsky, 1991; Branlard et al., 1993). Though high molecular weight subunit (HMW-GS) composition of Iranian bread wheat has been studied (Shahnejat-Bushehri et al., 2006), the information on gliadin composition of Iranian bread wheat are not reported so far. This paper is focused to study gliadin patterns of 95 obsolete and commonly bread wheat varieties grown in Iran.

MATERIALS AND METHODS

Plant materials

Ninty five Iranian bread wheats cvs collected from the Field Crops Research and Genetic Resources Unit of the Faculty of Agriculture, University of Tehran, Karaj, Iran were evaluated in this study. Gliadins were extracted from six seeds of each cultivar. Additional seeds (up to 10) were used for those varieties that showed inconsistent gliadin patterns. Chinese Spring and Marquis was used as a control in each gel to identify of gliadin zones. Pedigree information was available for only 64 of the cultivars (Saidi et al., 2005). Gliadin patterns were characterized according to the A-PAGE method of Metakovsky and Novoselskaya (1991) with minor modifications. Gliadins were extracted with 70% (v/v) ethanol from single crushed seed. A-PAGE (aluminium lactate, pH 3.1) was carried out with a Bio-Rad vertical apparatus. Electrophoresis was performed at 40 mA until the end of the electrophoresis. Gliadin patterns of Iranian wheats cvs were categorized into 3 groups (ω-, β+γ- and α-gliadins), based on their mobility on A-PAGE (Tanaka et al., 2003). It was not possible to score gliadin pattern of Iranian wheat in accordance with the Metakovsky et al., (1991) because there was no variety having gliadin pattern similar to Marquis, therefore a different strategy was used to score gliadin patterns (Ram et al., 2005; Tanaka et. al., 2003). Experiment was repeated for six individual seeds from each sample to confirm the pattern of genotypes within each group. The genetic diversity for each gliadin pattern was calculated according to Nei (1973) as follows: $H = 1 - \sum p_i^2$, where H is the Nei's genetic variation index and p_i is the proportion pattern in each group of ω-, β+γ- and α- gliadins separately. Mean value of H was calculated for all the three groups of gliadins. Genetic distance among different regions was calculated according to Nei, 1972. Pair-wise comparisons of gliadin pattern frequencies in different zones were performed using a standard Student's test by calculating a t value that was tested against the desired level of significance.

RESULTS

Ideograms of the observed patterns presented in Fig. 1. Gliadins were classified as ω-, β+γ-, and α- groups according to their mobility based on A-PAGE (Fig. 1). Of 95 varieties, 7 (Chamran, Pitic, Kaveh, Karaj2, Karaj3, Quds, and Bayat) were heterogeneous for ω-gliadin patterns. Gliadins patterns from the 95 bread wheat cultivars classified them into 26 different patterns of which 13 correspond to ω-gliadins, 8 to γ+β-, and 5 to α- gliadins. Three patterns of the α-gliadins (B, C, and E), 5 of the β + γ- gliadins (A, C, D, E and F) and 8 of as Tanaka et. al., 2003 report. Patterns of G and H (α-gliadins), I, J and K (β + γ- gliadins) and N, O, P, Q and R (ω-gliadins) only appeared in Iranian cultivars. The most frequent patterns were E and B in α-gliadins; A, C, and J in β + γ- gliadins; and H, C, E, and Q in the zone of ω-gliadins and some were common among all the zones. There was variation in the gliadin patterns of cultivars grown in different regions in Iran (Saidi et al., 2005). The genetic diversity index was highest in tropical region (H = 0.811) followed by cold region, (H = 0.750), Caspian region, (H = 0.706), and tropical (H=0.669), (Table 1). Genetic distance of different region indicated in table 2. Based on calculated genetic distances, regions divided into 2 groups, tropical-

temperate and Caspian-cold. The α-gliadin pattern E, and B; β+γ-gliadin patterns A, C, and J and ω- gliadins pattern H, C, and Q were present in all the regions.

Student's test analysis (*t*-test) showed that α-gliadin C, β+γ-gliadins A, and ω- gliadins H, C, and E patterns were significantly higher in temperate and tropical zones. β+γ-gliadin C and ω-gliadin Q were significantly higher in Caspian-cold regions (Table 2). The ideogram showed larger variation in β+γ- and ω- gliadins than in α-gliadins.

DISCUSSION

Gliadins with large numbers of patterns were used to study genetic diversity in wheat, and large variation in the gliadin patterns encoded by six main loci was observed. The genetic diversity (Nei's index, 1973) based on gliadin patterns in Iranian wheats cvs was higher (H = 0.734) than those in countries such as France (H = 0.714; Metakovsky and Branlard, 1998), England, and the former Yugoslavia (H = 0.676, and H = 0.728, respectively, Metakovsky et al., 1994), and was lesser than in Spain (H = 0.844; Metakovsky et al., 2000), India (H=0.875; Ram et al., 2005) or Italy (H = 0.754; Metakovsky et al., 1994). The ideogram showed larger variation in ω- and γ+β- gliadins than that in α-gliadins. Similar results were reported for Japanese cultivars (Tanaka et al., 2003). Cultivars from the tropical region exhibited the highest genetic diversity from cultivars grown in three other regions (Table 1). This may be due to different types of parents used for diverse climatic conditions prevailing in different regions.

Table 1. Zone-wise and period-wise genetic diversity indices (H) of wheat cultivars using ω-, β+γ- and α- gliadin patterns.

	Genetic diversity index (H)			
Zone/Period	H (ω)	H (β, γ)	H (α)	Mean
Caspian sea shore region	0.778	0.777	0.562	0.706
Temperate region	0.828	0.645	0.534	0.669
Tropical region	0.810	0.873	0.751	0.811
Cold region	0.789	0.813	0.648	0.750
1951-1960	0.8678	0.843	0.645	0.785
1961-1970	0.780	0.840	0.480	0.700
1971-1980	0.889	0.778	0.4860	0.718
1981-1990	0.750	0.778	0.500	0.676
After 1990	0.852	0.841	0.755	0.816

Table 2. Frequency of some gliadin patterns in cultivars grown in temperate-tropical and Caspian-cold regions and the level of significance between the zones. Values in brackets are total number of cultivars in the zone.

	Number of Cultivars			
Gliadin pattern	Caspian + Cold (27)	Temperate + Tropical (37)	P	t Test
ω-H	3	8	0.038	*
ω-C	2	9	0.020	*
ω-E	1	12	0.039	*
ω-Q	11	1	0.019	*
ω-N	1	1	0.423	NS
ω-L	1	2	0.592	NS
β-γ-A	2	11	0.012	*
β-γ-C	9	2	0.020	*
β-γ-J	3	9	0.486	NS
α-C	1	9	0.030	*
α-H	0	1	0.423	NS
α-E	14	18	0.465	NS

* Indicates t test significant at $P < 0.05$.

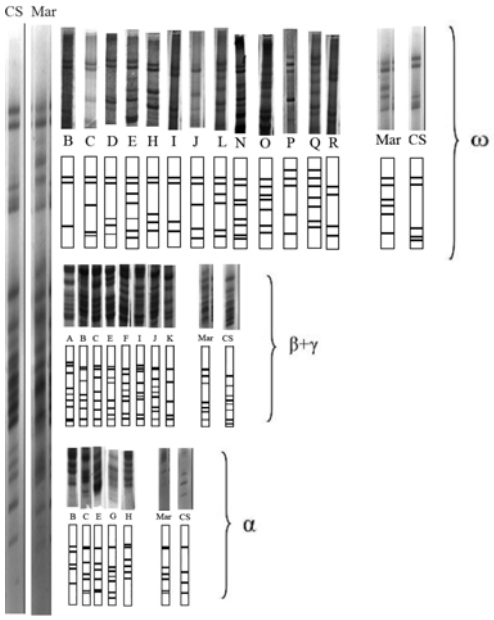

Figure 1. Enlarged picture on the left side is the pattern of gliadin in Marquis (Mar) and Chinese spring (CS). The positions of ω-, β+γ- and α-gliadin are indicated. On the right side the patterns of these gliadins in Iranian bread cultivars were shown with the ideograms.

There were some patterns specific to one region, for example ω-gliadin patterns H, C, and Q were predominantly present in cultivars developed for temperate-tropical conditions. The presence of some patterns may correlate with a higher adaptive value of germplasm to the particular environment (Metakovsky et al., 1991; Ram et al., 2005). The association between genotypes and their environment has also been reported by Nevo et al., (1988, 1995).

However, there is also a report indicated no significant relationship between genotypes and their environments (Dreisigacker et al., 2004). Different combinations of gliadin patterns were prevalent in different regions suggesting the adaptive properties of individual alleles or the chromosome segments in which these alleles reside (Nevo et al., 1995; Metakovsky and Branlard, 1998). Moreover, gliadin patterns may be associate with specific traits such as disease resistance, quality, or adaptation to abiotic stress. Multinational collaborations (especially CIMMYT) also contributed in expanding the genetic base of Iranian germplasm by providing materials with diverse genetic sources suited for different environments. During various years some new gliadin patterns appeared (ω-R and α-H), some lost (ω-P) and some were retained (ω-Q). These alterations mainly associated with changes in goals of breeding programs. It implies that all cultivars should be preserved because these may contain unique gene combinations absent in recent cultivars. To preserve the common wheat germplasm and fight erosion it would be well worth developing and maintaining wheat collection. Analysis of genetic diversity and gliadin pattern of cultivars released in different years indicated some fluctuations. Period-wise highest mean genetic diversity (H = 0.816) was observed in cultivars released after 1990 and lowest (H = 0.676) in cultivars released from 1981 through to 1990. There was a fluctuation of genetic diversity during 3 decades (1961-1990). Higher genetic diversity identified in cultivars released after 1990 might be due to the use of more diverse germplasm than the previous decades. Analyses of genetic distance among groups of cultivars from different zones indicated highest between cold and tropical and the lowest between cold and Caspian regions. Different gliadins might have some advantage over other gliadins in adaptation to the conditions prevailing in these zones or these are closely linked with genes having adaptive values to the specific environment, though that needs to be confirmed by genetic analysis (Ram et al. 2005). Each Iranian bread wheat cultivar showed unique fingerprinting for its gliadin pattern. The data showed that gliadins can be used in the identification of cultivars as a tool for the evaluation of wheat genetic resources. Seven cultivars showed the presence of biotypes. Storage proteins are expected to be a cultivar constant element, being the direct expression of its genotype. Impurity of grain is one cause of varying electrophoretic fingerprints. Occurrence of mutations can give rise to the appearance of biotypes, as was previously reported for some gliadin or glutenin compositions. In addition breeders use the traditional pedigree method for selection from the F_2 to F_7 generation and variety uniformity is an important criterion for variety release and for farmers choosing varieties. Hence a low frequency of heterogeneous genotypes is expected.

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