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Evaluation of Genetic Diversity in Iranian Landrace Wheat *Triticum aesativum* L. by Using Gliadin Alleles

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Abstract: Seventy three Iranian landrace wheat were characterized by acid-PAGE (A-PAGE) analysis of gliadins. Extensive polymorphism (H = 0.821) in gliadin pattern was observed in the Iranian landrace wheat analysis of gliadin electrophoretic (A-PAGE) patterns made it possible to identify 52 alleles at six *Gli-1* and *Gli-2* loci (from 6 to 11 per locus) and 73 gliadin genotypes in Iranian landrace bread wheat which from 9 province of century (Tropical and subtropical) were collected. Considered four new alleles were observed among the landrace varieties. The genetic diversity of Iranian landrace wheats was found to be high (H = 0.726). Genetic distances between Iranian landrace wheat and common Iranian wheat were analyzed. The considerable differentiation of landrace wheat genotypes from different countries and Iranian common wheat might be caused by breeders' personal preferences and by hidden natural selection specific to each local environment. In Iranian landrace wheats, genetic variation in the tropic/cold habit of the landraces studied. In order to investigation the gliadin band pattern of landrace varieties and population, banding patterns each of population and varieties were determinate. At zone-wise genetic diversity index was highest in cold (H = 0.71) followed by tropical region (H = 0.708). Landraces from cold region exhibited the largest genetic distance from landraces grown in other zone, this landrace placed in one main group excepted one province (Ardabil) that made a separate group. Some gliadin alleles were probably associated with cold resistance. The frequency of alleles, *Gli-A2r* and *Gli-D2g* was significantly higher and alleles *Gli-A1a*, *Gli-B2c* and *Gli-D2m* significantly lower landraces with the highest cold resistance.

Key words: Landrace wheat, gliadin alleles, genetic diversity, zone-wise genetic diversity, genetic distances, gliadin band pattern

INTRODUCTION

Genetic erosion, or the reduction of the genetic base of the common wheat germplasm caused by frequent use of the same parental genotypes for breeding activities, is becoming a serious problem (Porceddu *et al*., 1988). It restricts the genetic potential of wheat, complicates wheat improvement and could lead to problems. Few plant characteristics, however, serve as effective genetic markers to monitor and evaluate the changes occurring in wheat germplasm over the course of time. Gliadins, which are alcohol-soluble seed storage proteins, show the highest level of inter-varietals polymorphism when studied by a standard method of acid electrophoresis (A-PAGE) (Metakovski *et al*., 1991). The gliadin pattern of a landraces is not affected by the area of plant growth. Most gliadins are controlled in common wheat by six main *Gli* loci located on the chromosomes of the first (*Gli-1*) and sixth (*Gli-2*) homoeological groups. A vast multiple-allelism has been described at each of these loci; an allele encodes several gliadin A-PAGE bands inherited as a Mendelian unit (block). Alleles of a locus differ in the number and electrophoretic mobility of the encoded gliadins (Metakovsky, 1991). Combinations of different alleles at the six main loci ensure a great diversity of A-PAGE patterns and, therefore, makes it possible to distinguish a number of common wheat genotypes and to describe them in terms of gliadin allele composition. There are also several minor, *Gli* loci (*Gli-3*, *Gli-5*, *Gli-6*, *Gli-D*^{1}*7* and *Gli-D*^{1}*1*) which each controls a few minor gliadin bands (Sobko *et al*., 1986; Pogna *et al*., 1993; Metakovsky *et al*., 1997; Gianibelli *et al*., 2001; Hassani *et al*., 2006).

Two genotypes identical at *Gli-1* and *Gli-2* may be distinguished by alleles at minor *Gli* loci using the same gliadin pattern (Metakovsky *et al*., 1994). Common and landrace wheat bred in Iran are widely implemented in different scientific and breeding programmes, but their genotypes are still not well described or classified using efficient genetic markers. Also, gliadin alleles have never been subjected to direct selection by breeders.

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Therefore, analysis of gliadin alleles in Iranian landrace wheats may adequately describe process of change in wheat germplasm including genetic erosion cause by breeding activities and natural selection. It is known that most Iranian common wheat cultivars registered in the period 1940-90 originated from a rather restricted group of parental genotypes (Bahraei *et al*., 2004; Saidi *et al*., 2005). Therefore, the risk of narrowing the genetic variation of new cultivars and advanced lines has been discussed (Branlard and Dardevet, 1994).

In the present study, gliadin alleles in more than 80 samples of landrace wheat cultivars registered in Iran were identified. Genetic variation in different groups of Iranian landrace wheats was compared, genetic distances between these groups and between Iranian wheats and those from other countries were studied and correlations between gliadin alleles and some agronomically characteristics were revealed. In this research also, distribution of allelic variation among Iranian landrace wheats and relationship between gliadin alleles and some agronomically characters was studied.

MATERIALS AND METHODS

Seeds of 73 Iranian landrace bread wheats were used for this study. Seed were obtained from the Field Crops Research and Genetic Resources Unit of the faculty of Agriculture, University of Tehran. This research was carried out in department of agronomy and plant breeding, faculty of Agriculture, University of Tehran, Karaj/Iran, in 2006. Gliadins were extracted from four seeds of each landrace. Additional seeds (up to eight) were used for those varieties that inconsistent gliadin patterns.

Gliadin analysis: Acid polyacrylamide-gel electrophoresis (pH = 3.1) was performed as described by Metakovsky and Novoselskaya (1991). Gliadin was extracted from flour from single grains by 70% ethanol and analysed by A-PAGE (aluminium lactate, pH = 3.1). The *Gli-1* and *Gli-2* alleles in the protein spectra were identified by using a standard catalogue of Gliadin alleles (Metakovsky, 1991). At the least four grains characterization. At the next step, for zone-wise analysis, gliadin alleles were arranged in groups of cold and tropical regions. Since Marquis were used as check in each gel, comparison of band pattern among different varieties was easy.

Analysis of data: The genetic diversity at each locus was calculated according to Nei (1973) as $H = 1-\Sigma p^2$, where H is Nei's genetic variation index and p_i the frequency of a particular allele and pattern at that locus. Mean value of H was calculated for all groups of gliadins. Pair-wise comparisons of frequencies of gliadin patterns in different zones were performed with standard Fisher's test by calculating a z-value that was tested against the desired level of significance. Dendrogram representing genetic relationships among landraces of different zones were constructed on the basis of distances by the Neighbor-joining algorithm. The genetic distance was computed with the POPGENE software (version 1.32) and Dandrogram was draw by ANTSYS software (version 2.02).

RESULTS

Landrace wheats grown in different parts of the country, ranging from North-west region to the western region and South-west region (Fig. 1), were analyzed for gliadin band pattern in this study. Gliadins were separated into α, β, γ and ω groups according to their mobility in followed Acid-PAGE (Fig. 2). Authenticity of the grain samples studied and gliadin allelic compositions of Iranian wheat cultivars in total, 100 Landraces grown in west and North-west from Iran, which from these population 73 varieties were studied.

However, gliadin genotypes of three landraces were either not identical in different grain samples analyzed, or were very heterogeneous and carried heterozygous grains. These three landraces were excluded from the study. The gliadin allelic compositions in 73 landraces are shown in Table 1.

Fig. 1: The map of Iran and selected provinces. Yellow zone including Tropical provinces and Gray ones incouding Cold province

Table 1: Gliadin allelic composition in Iranian landrace wheat that grown in some parts of Iran

	Gliadin allelic composition					
Name of cultivars	A1	B1	D1	A2	B2	D2
Tropical						
Nigro graecum	k	f	b	p	e	a
Sub erernum	k	f	b	l	l	m
Kosestanicum	a	f	j	p	l	a
Sub turticicum	f	b	b	l	e	a
Sub Miltum	b	f	b	e	g	m
FarsiCum	a	b	b	p	e	m
Ocasium	o	b	b	p	e	n
Honobicum	o	b	a	g	e	a
Ahwazicum	a	f	f	l	o	b
Bamie	o	f	b	l	l	h
Kermanicu	a	f	b	p	o	a
Dezfoulium	o	g	b	g	e	n
Sub shiraz	a	f	b	g	e	a
Sub pseudo shiraz	o	f	a	g	h	a
Abasicum	f	e	g	l	o	h
Sub hostianum	f	f	b	f	e	m
Hormozicum	f	g	b	g	m	n
Enbaleusum	k	m	b	t	o	n
Sub pseudo Aerbarosum	f	g	b	p	o	n
Pseudo Karoun	f	b	l	j	r	n
Garmium	b	b	b	g	l	a
Lutes	f	f	b	g	o	h
Bushehricum	b	b	b	g	h	a
Orubriftum	c	m	b	l	o	v
Sub Dez	f	f	j	p	e	n
Pseudo Sommericum	f	m	l	g	g	n
Sommericum	b	e	b	g	g	h
Cold						
Fergugineum	a	f	b	c	v	a
Erithrospermum	f	b	b	g	l	n
Delfi	f	f	b	l	g	h
Graecum	m	q	j	l	g	g
Sub meridionala	o	f	b	l	b	g
Sub graecum	o	e	a	r	o	n
Pseudo meridinale	e	f	b	r	o	v
Pseudo turcicum	o	f	b	t	b	a
Turcicum	f	f	b	p	l	h
Erythroleucom	f	f	b	t	l	h
Alborubroiflatum	o	b	b	l	l	g
Milturum	k	f	b	g	o	a
Caesium	b	f	b	g	o	g
Luteseeus	k	m	l	r	o	q
Pseudo barbarossa	b	f	b	r	o	n
Leucospermum	m	f	a	l	o	v
Sub ferrugineum	b	b	g	g	g	a
Barbarossa	c	f	b	r	l	h
Hastianum	f	f	b	h	g	e
Alborubrum	a	h	b	l	l	a
Sub hastianum	f	f	l	o	h	h
Larostanicum	o	b	b	g	m	g
Fulginosum	c	e	f	j	o	b
Transeaspicum	k	h	b	l	e	h
Compactum	o	f	b	p	g	e
Pseudo hostianum	o	f	b	l	h	g
Kmrassaniam	f	f	b	j	o	a
Lutinflatum	o	f	b	l	g	n
Bengaleus	f	m	b	t	g	g
Sub pseudo turcicum	o	b	b	l	b	h
Kermanchanicum	f	f	b	r	o	a
Kurdistanium	b	m	b	j	g	h
Sub mesopotanium	f	b	b	j	g	q
Pyrothria	f	b	b	t	l	g
Albinflatum	o	f	b	r	g	g

Table 1: Continued

	Gliadin allelic composition					
Name of cultivars	A1	B1	D1	A2	B2	D2
Nigrecens	o	f	b	p	g	a
Sub meridionala	m	e	b	g	g	h
Albidum	o	f	a	g	o	h
Meridionala	c	e	b	j	c	n
Sub mesopotanium	k	k	b	g	o	n
S. pseudo meridionale	f	b	a	g	o	g
Sub graecum	f	f	a	h	p	a
Hamadanicum	o	f	b	p	b	n
Hostianum	b	f	g	g	l	h
Sub erythroleucon	f	f	b	l	b	g
Sub nigricens	o	b	b	e	o	g

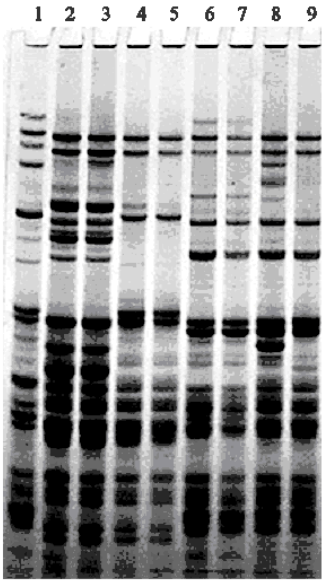

Fig. 2: A-PAGE patterns of the international standard cultivars Marquis (lane 8) and Cheyenne (lane 1), some Iranin landrace wheats: (2) Leucospwrmum, (3) Barbarossa, (4) Pesudo hostinum, (5) Albinflatum, (6) Alborubroiflatum, (7) Ocasium, (9) Sommericum

The results showed large variation in gliadin pattern encoded by six main coding loci. In total, considering *Gli-1* and *Gli-2* loci, 73 gliadin allelic compositions were found.

Although the 73 landraces had not been selectively chosen they represented an appropriate and representative set of genotypes for the evaluation of Iranian landrace wheat germplasm.

In order to comparison the present varieties in differing climate area and comparison their banding pattern, the patterns within each gliadin loci of *Gli-A1*, *Gli-B2*, *Gli-D1*, *Gli-A2*, *Gli-B2* and *Gli-D2* were identified by comparing banding pattern of each variety.

Table 2: Genetic diversity (H) in Iranian landrace wheats that classified based on province of and region of grown

No. of cultivars	Province	H (province)	Region	H
5	Khuzestan	0.520	Tropical	0.708
6	Boushehr	0.583		
5	Hormozgan	0.600		
4	Kerman	0.458		
7	Fars	0.626		
		27	Cold	0.71
12	Ardabil	0.648		
13	A. Gharbi	0.750		
14	A. Sharghi	0.621		
7	Kordestan	0.660		
		46		

Table 3: Number of alleles in six main loci in landraces grown in Iran

Loci	No. of alleles	H	
A1	8	0.794	
B1	8	0.663	
D1	6	0.436	
Mean H for *Gli-1*			0.631
A2	11	0.825	
B2	10	0.816	
D2	9	0.820	
Mean H for *Gli-2*			0.820
Mean H for all of six loci			0.726

Genetic diversity analysis: Nei's genetic variation index (H) was calculated at each gliadin loci (Table 3). The *Gli-2* loci displayed a much higher genetic diversity (0.820) than the *Gli-1* loci, showing H values of 0.631. The mean genetic diversity index was 0.726. At the *Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A2*, *Gli-B2* and *Gli-D2* loci, eight (a, b, c, e, f, k, m and o), eight (b, e, f, g, h, k, m and q), six (a, b, f, g, j and l), eleven (c, e, f, g, h, j, l, o, p, r and t), ten (b, c, g, h, l, m, o, p, r and v) and nine (a, b, e, g, h, m, n, q and *v*) alleles were found and genetic diversity indices were 0.794, 0.663, 0.436, 0.825, 0.816 and 0.820, respectively.

Distribution of allelic variation: The distribution of the 73 gliadin alleles found in of Iranian landrace wheat was clearly uneven: upwards of six alleles were found at *Gli-D1* and up to 11 alleles at *Gli-A2* (Table 3). The frequency of the most common allele at a *Gli* locus ranged from 2% to 74%. The uneven distribution of gliadin alleles has also been found in groups of cultivars from other countries (Metakovsky *et al*., 1991, 1993, 1994; Chernakov and Metakovsky, 1994; Metakovsky and Branlard, 1998). Generally, genetic diversity in the set of Iranian landrace wheats studied was rather high (H = 0.726), (Table 2, 3). About 2 catalogued gliadin alleles only were present in cultivars that grown in cold region, on other hand, one allele was rare in cold than tropical wheats. *Gli-D1g* and *Gli-A2r* did not absorbent in tropical landraces. Genetic variation in group of cold landraces was higher than tropical (Table 2).

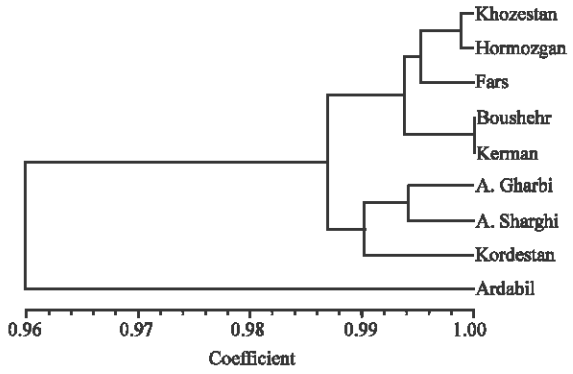

Fig. 3: Dandrogram of Genetic diversity according to fliadan alleles based of growing region

Gliadins have been used by several workers around the world for genetic diversity studies in wheat and large numbers of patterns have been reported. The data this investigation showed higher genetic variability.

In Iranian landrace wheats as compared with reports from different parts in the world. The divergence may because of selection pressure exerted because of diverse climatic conditions prevailing in different zones. For example, cold conditions in Northern region during winter and comparatively warm and dry condition in tropical region spectrum also varies from one zone to another. Analyses of genetic distances among groups of landraces released in different zones in studied parts of Iran showed that landraces representing province of Boushehr and province of Kerman were closer to each other than to cultivars from other zones (Fig. 3). landraces from cold region exhibited the largest genetic distance from landraces grown in other zone, all of this landraces placed in one main group excepted province of Ardabil that made a separate group. 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 value to the specific environment, though that needs to be confirmed by genetic analysis.

Cluster analysis: In order to determine the genetic relationship between Iranian landrace wheats were used for cluster analysis.

In the past few decades, introgression of landrace germplasm in wheat improvement has been increasing (Campbell, 1997). Thus, it is important to understand the genetic diversity of the available landrace wheat collections as well as the relationship to common wheat. Storage proteins of wheat endosperm have been shown to be reliable markers not only in the genetic improvement of bread-making quality (Payne, 1987), but also in the

studies of crop origin and evolution (Fernandez-Calvin and Orellana, 1990; Yan *et al*., 2003). As shown in this study, although limited variation at the *Gli-1* loci was detected in Iranian landrace wheats, genetic diversity of gliadins was higher than previously reported among France common wheats (H = 0.714; Metakovsky and Branlard, 1998), England and Yugoslavia (H = 0.676 and H = 0.728, respectively, Metakovsky *et al*., 1994)

Cluster analysis showed that landrace wheats were separated into three groups suggesting that significant differences in gliadin compositions exist in these three groups. Some investigations indicated high polymorphisms between landrace wheat in gliadin patterns (Harsch *et al*., 1997).

CONCLUSIONS

The use of gliadin protein markers allowed us to reveal a high level of genetic diversity in Iranian landrace wheat germplasm as well as its differentiation between different cereal companies and regions of Iran.

Previous studies have showen that gliadin markers are an powerful tool for the evaluation of wheat genetic resources. Using DNA sequences coding alone for wheat storage-proteins are known to give an appropriate level of intervarietal polymorphism for wheat genotype identification. Common wheat, being an inbreeding species and having a large genome size, is characterized by PCR molecular markers (Talbert *et al*., 1994) or by a low level of inter-varietals polymorphism of DNA detectable by a conventional RFLP approach (Sharp *et al*., 1989).

The present study is a thorough evaluation of 73 primitive landraces of *T. aestivum* which were was collected from Iran in their natural habitats, using seed storage protein, gliadin.

We believe that the study of any species must be performed in its natural conditions, in which the evolutionary mechanisms work. Consequently, in the present work, the original spikes were and stored at +4°C in own germplasm Bank.

Heterogeneity present within population probability was due to seed dispersal in the cattle zoon in which pasturing could be an efficient mechanism of dispersion. On the contrary, the presence of heterogeneity within the seeds implicates the possibility of the sporadic cross pollination, which appear with more or less intensity in all the self-pollinated species.

For wheats, especially winter wheats, time of flowering and subsequent maturity is determined by a complex assemblage of genes which strongly influence yield potential, therefore is one of the most important selection characters. In addition, all landraces with *Gli-D2g* were grown only in the cold region where the frequency of this allele was therefore significantly higher than in the tropical region. Some gliadin alleles which were probably associated with cold resistance are: *Gli-A2r* and *Gli-D2g* (significantly higher) and *Gli-A1a*, *Gli-B2c* and *Gli-D2m* (significantly lower) in the group of 46 landraces with the highest cold resistance, comparing with the group of 27 landraces with the lowest resistance grown in warmer habitat.

Iranian landrace wheats with this allele is grown in colder area (mainly in general the North-west of Iran). Wherease, landraces with the allele *Gli-D2m* were are cold sensitive and grown in the South-west of Iran (which has a warmer climate). It is reasonable to suggest that chromosomal segments marked by these alleles may be involved in multilocus combinations affecting the degree of plant adaptation to local. Natural selection may recognize the adaptive properties of individual alleles of any locus, or the chromosome segments in which this locus.

Knowledge of the structure of genetic diversity within the distribution zone of *T. aestivum* may be important in deciding on breeding strategies. Previous works with this species could not establish any clear relation between variability of gliadin and geographic origin. This work indicated that there was a continuous distribution of the variability for storage proteins within the entire distribution zone. However, this work mainly focused on the characterization of the variability for gliadin composition and, therefore, the distribution of the variability within geographic regions was studied in a descriptive way. We found some degree of association between endosperm storage protein and geographic localization (Table 3, Fig. 3).

These results showed that a low part of the variation is related to geographic distance. Since gliadin is neutral towards the environment do not provide any selection advantage, this was expected. These results reveal a high degree of polymorphism for endosperm storage proteins. These proteins are very useful as molecular markers for the study of variability in *T. aestivum*. In spite of the neutral behavior of these proteins towards the environment, we have detected a low degree of differentiation among regions, which could not be detected using the descriptive analyses in previous. This variation may be caused by the probable association between endosperm storage protein and genes for adaptation. Therefore, it can be suggested that collection of new entries from as many different regions as possible would be an adequate strategy to increase the for endosperm storage proteins variability of this species for breeding programmes of wheat.

It is known that inbreeding plant species, including wheat, show very intense geographic and microgeographic differentiation (Brown, 1979; Nevo *et al.*, 1988, 1995). A decrease in the genetic base of common wheat germplasm in a country is conditioned both by breeders' activities and natural selection. Obviously, only the first of these may be controlled and reduced by breeding genotypes from other countries as well as land races and old cultivars from the same country. Local genotypes would be an especially valuable source of alleles and multi-locus combinations already suitable for specific environments of the country concerned (Allard, 1996). Genetic diversity in breeding material may be monitored by means of an analysis of polymorphic markers.

These advices emphasize the importance of the correct maintenance, evaluation and use in breeding of the world wheat collections (Porceddu *et al.*, 1988). To preserve the common wheat germplasm of a country and fight erosion it would be well worth developing and maintaining local wheat collections which would both include both old cultivars and land races.

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