Molecular evaluation of genetic diversity using gliadin alleles in Iranian landrace wheat *Triticum aesativum* L.

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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. 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) (Metakovki *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, make 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*^{t}*7 and Gli-D*^{t}*1*) which each controls a few minor gliadin bands (Gianibelli *et al.* 2001, and Hassani *et al.* 2004). 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. Therefore, analysis of gliadin alleles in Iranian landrace wheats may adequately describe process of change in wheat germplasm including genetic erosion caused 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 (Bahraee *et al.* 2004 and Saidi *et al.* 2005).

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.

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.

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.

The genetic diversity at each locus was calculated according to Nei (1973) as $H = 1 - \sum p_i^2$. 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.

RESULTS

Landrace wheats grown in different parts of the country, ranging from North-west region to the western region and South-west region, were analyzed. Gliadins were

separated into α, β, γ, and ω groups according to their mobility in followed Acid-PAGE. The gliadin allelic compositions in 73 landraces are shown in Table 1. 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.

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

No. Cultivars	Province	*H* (province)		*H*
5	Khuzestan	0.520	tropical	0.71
6	Boushehr	0.583		
5	Hormozg-an	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				

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. 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. Generally, genetic diversity in the set of Iranian landrace wheats studied was rather high (*H*=0.726). 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). Analyses of genetic distances among groups of landraces release-ed in different zones in studied parts of Iran showed that landraces re-presenting province of Boushehr and province of Kerman were closer to each other than to cultivars from other zones (Fig. 1). 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. In the past few decades, introgression of landrace germplasm in wheat improvement has been increasing (Campbell, 1997). 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).

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

Loci	number 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

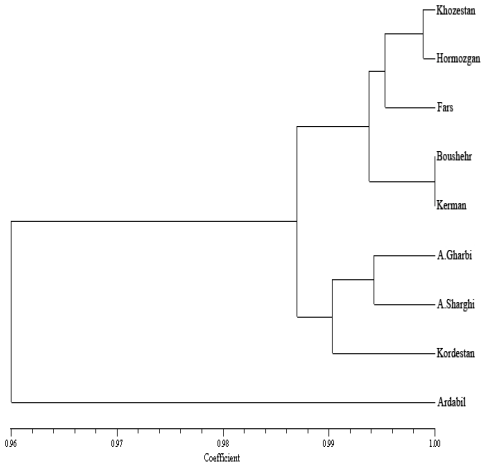

Figure 1. Dandrogram of Genetic diversity according to gliadin alleles based on growing region.

CONCLUSION

We think that the study of any species must be performed in its natural conditions, where the evolutionary study mechanisms work. The cause of the heterogeneity within the population could be seed dispersal, because the plants appear distributed in cattle zones where 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. 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 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, in the group of 46 landraces with the highest cold resistance, which mean that grow in colder area, as compared with the group of 27 landraces with the lowest resistance grown in warmer habitat. 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 environments. Natural selection may recognize the adaptive properties of individual alleles of any locus, or the chromosome segments in which this locus reside.

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