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Genetic Variation of Durum Wheat Landraces and Cultivars Using Morphological and Protein Markers

M. R. Naghavi, S. Rashidi Monfared, A. H. Ahkami, and M. A. Ombidbakhsh

Abstract**—**Knowledge of patterns of genetic diversity enhances the efficiency of germplasm conservation and improvement. In this study 96 Iranian landraces of *Triticum turgidum* originating from different geographical areas of Iran, along with 18 durum cultivars from ten countries were evaluated for variation in morphological and high molecular weight glutenin subunit (HMW-GS) composition. The first two principal components clearly separated the Iranian landraces from cultivars. Three alleles were present at the Glu-A1 locus and 11 alleles at Glu-B1. In both cultivars and landraces of durum wheat, the null allele (Glu-A1c) was observed more frequently than the Glu-A1a and Glu-A1b alleles. Two alleles, namely Glu-B1a (subunit 7) and Glu-B1e (subunit 20) represented the more frequent alleles at Glu-B1 locus. The results showed that the evaluated Iranian landraces formed an interesting source of favourable glutenin subunits that might be very desirable in breeding activities for improving pasta-making quality.

Keywords**—*Triticum turgidum*** var. durum, glutenin subunits, morphological characters.

I. Introduction

POSSIBLE segregations in morphological and molecular investigations are essential for breeding programs such as germplasm propagation. Genetic diversity of wheat genotypes has been well evaluated using morphological [1] and protein [2] variation. Phenotypic identification based a description of the morphological and has been successfully used for genetic diversity analyses and cultivar development. However, morphological traits have a number of limitations, including low polymorphism, low heritability, late expression, and may be controlled by epistatic and pleiotropic gene effects [3]. While molecular markers such as isozymes and seed storage proteins reflect the genotype more directly, independent of environmental influences [4].

The HMW subunits account for about 25-35% of the total glutenins [5] and have been studied extensively. High molecular weight glutenin subunits are mainly responsible for dough strength in wheat. Dough strength determines the quality of bread and pasta made from bread and durum wheat, respectively [6].

Authors are with Agronomy and Plant Breeding Department, Agricultural College, University of Tehran, Karaj, Iran (phone: 0098-261-2246074; fax: 0098-261-2227605; e-mail: mnaghavi@ut.ac.ir).

Genetic studies have revealed that glutenins are encoded at several, complex and highly polymorphic loci [7]. In tetraploid wheat Glu-A1 loci code for one (1Ax) or none, Glu-B1 usually code for one (1Bx) or both (1Bx and 1By), subunits. Thus a tetraploid wheat genotype produces one to three subunits [8]. Little information is available regarding genetic variation in Iranian landraces of durum wheat.

The main objective of this study was to study genetic diversity in landraces and cultivars of wheat durum using morphological data and seed storage proteins. This information will be useful to improve techniques for sampling wheat genetic variation which might increase efficiency of conservation of germplasme.

II. Materials and Methods

The materials for this study comprised 96 Iranian landraces of durum wheat (*Triticum turgidum* var. durum), collected from different geographical regions, along with 18 durum cultivars from ten countries provided by the Gene Bank of the Agricultural College at the University of Tehran, Iran.

These accessions were evaluated in a RCBD design with three replications at experimental station of Agriculture College of the University of Tehran, Iran during two years (2004 and 2005). The accessions were sown by hand. Each accession was planted in a 1 m long row with 0.5 m row spacing. Morphological data on spiklet per spike, seed per spike, 100 grain weight (g), plant height (cm), peduncle length (cm) and spike length (cm) were recorded from five plants which had been randomly chosen in each row and mean of quantitative data sets were used for analysis. The data recorded were analyzed for simple statistics, i.e., mean, range and coefficient of variation (CV) for both landraces and cultivars. As the characters were recorded on different scales, the data were standardized to a mean of zero and a variance of unity prior to principal components analyses to eliminate scale differences. The computer software SPSS version 10.0 was used for these computations.

The seeds were crushed finely after removal from the embryo. The flour was mixed in an extraction buffer of 0.125M Tris-Hcl (PH 6.8), buffer 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.03% bromophenol blue and 5%2-mercaptoethanol. Samples were boiled for 2 minute at 90 and then centrifuge for 10 minutes at 6500 rpm fractionated by SDS-PAGE according to methods Payne and Lawrence (1983) using stacking and separating gels containing 4%

acrylamide, 0.3% bis acrylamide, 10% SDS and 0.125 M Tris-Hcl (PH=6.8), and 14% acrylamide, 0.03% bis acrylamide, 10% SDS, and 0.125 M Tris-Hcl (PH 8.8), respectively.

Gels were stained overnight with 0.13% comassie Brilliant Blue and then destained overnight in water. The HMW subunits of glutenin were designated according to the numbering system of Payne and Lawrence (1983) and the alleles found at the two HMW loci have been identified for both landraces and cultivars. Subunit mobility was compared with that of hexaploid wheat (chinese spring) determined previously [8].

III. Results

The first two principal component scores, which are the most important components, explained 40.74% and 39% of total variation, respectively. The plot of these two components mainly separated the Iranian landraces from cultivars (Fig. 1).

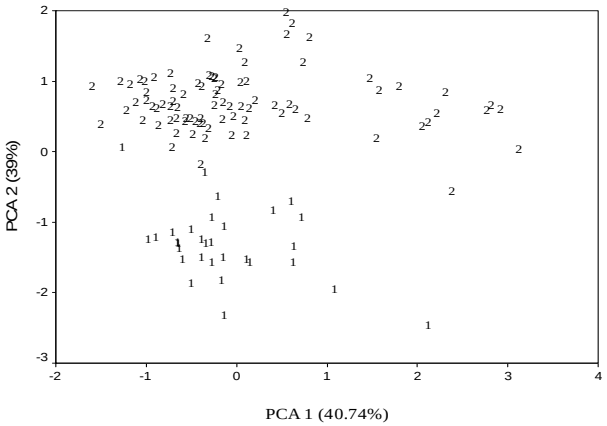

Fig. 1 Bi-plot of principal component analysis (PCA) for 6 characters in durum wheat landraces and cultivars. All cultivars along with a few landraces symbolize as 1, while the remaining landraces represent as 2

One hundred fourteen durum wheat landraces and cultivars seeds were evaluated for high molecular weight glutenin subunits (HMWGS) by SDS-PAGE. It was apparent that HMWGS bands had resolved into one to three bands each with a different electrophoretic mobility. The HMWGS were numbered according to Payne and Lawrence (1983). Subunit mobility was compared with that of hexaploid wheats determined by Payne *et al*. (1984) and 14 different alleles were identified (Table I). Three out of 14 alleles belonged to the Glu-A1 locus (chromosome A1) and 11 belonged to Glu-B1(chromosome B1). The most frequent allele in Glu-A1 for both landraces and cultivars was null allele. Out of the 11 alleles observed at Glu-B1, two alleles, namely Glu-B1a (subunit 7) and Glu-B1e (subunit 20) were present more frequently in landraces and cultivars. Five alleles (Glu-B1b, Glu-B1f, Glu-B1h, Glu-B1i and Glu-B1l) were found also in Iranian landraces whereas 'Glu-B1k' allele was observed only in one cultivar. The composition of high-molecular weight glutenin subunits (HMWGS) of landraces and cultivars (Glu-A1+Glu-B1) are presented in Table 2. Each genotype included one to three HMWGS subunits bands.

TABLE I
Comparison of Alleles and Subunits in Landraces and Cultivars of Durum Wheat for 'Glu-A1' and 'Glu-B1' Loci

Locus	Allele	Subunit	Number of accessions	
			Landraces	Cultivars
Glu-A1	a	1	1	1
	b	2*	43	2
	c	Null	52	15
Glu-B1	a	7	30	6
	b	7+8	8	-
	d	6+8	2	1
	e	20	24	6
	f	13+16	3	-
	g	13+19	7	1
	h	14+15	6	-
	i	17+18	10	-
	j	21	2	3
	k	22	-	1
	L	Unknown	4	-

The frequency of most subunit compositions was quite low. There are eleven codominant alleles, Glu-B1a to Glu-B1k (chromosome B1) along with unknown allele. Most frequent combinations for landraces were Glu-ba (16 samples), Glu-be (12 samples), Glu-ca (13 samples) and Glu-ce (12 samples), while the combinations Glu-ca (5 samples) and Glu-ce (6 samples) were more frequent in cultivars. Only subunits 7+9, controlled by alleles on Glu-B1c, were not found in any of the landraces or cultivars tested. Subunits named 7+8, 14+15, 17+18, 13+16 controlled by the Glu-B1b, Glu-B1h, Glu-B1i and Glu-B1f alleles were not detected in any cultivars. Both 7+8 and 17+18 subunits, were found only in Iranian landraces. These differences may be due to the dissimilar materials and show that the cultivars studied are not originated from Iranian landraces.

IV. Discussion

In this study the genetic diversity for agronomical traits and HMWG subunits in the 96 Iranian landraces and 18 cultivars of durum wheat were investigated. The first two principal component scores separated Iranian landraces from cultivars showing that the studied cultivars are quite different from Iranian landraces. A high genetic variability was also observed for HMWGS compositions in the studied genotypes. We found that null allele is the most frequent allele in Glu-A1 for both landraces and cultivars, while prevalence of Glu-B1a

allele for landraces and Glu-B1e allele for cultivars were obtained. Previous studies showed also that the HMWG subunit genes on chromosome 1A appear to have a negligible relationship to durum quality parameters when compared to genes on chromosome 1B [9, 10].

TABLE II
COMBINATIONS OF HMW GLUTENIN SUBUNITS (GLU-A1 + GLU-B1) ALLELES IN THE LANDRACES AND CULTIVARS OF DURUM WHEAT

Allele		Subunit	Number of accessions
		Landraces	Cultivars
aa	1,7	1	1
ba	2*,7	16	-
bb	2*,7+8	2	-
be	2*,20	12	-
bf	2*,13+16	3	-
bg	2*,13+19	2	1
bi	2*,17+18	6	-
bk	2*,22	-	1
ca	Null,7	13	5
cb	Null,7+8	6	-
cd	Null,6+8	2	1
ce	Null,20	12	6
cg	Null,13+19	5	-
ch	Null,14+15	6	-
ci	Null,17+18	4	-
cj	Null,21	2	3
bl	2*,unknown	2	-
cl	Null, unknown	2	-

Other authors reported that certain HMWG subunits were correlated with the quality of durum wheat [11, 12]. The most significant result was that the HMWGS 20, coded at Glu-B1, presents a differential and negative effect on gluten strength and mixing properties [6, 13]. The same authors have also reported the positive correlation of 13+16 and 7+8 subunits and negative correlation of subunit 20 with the dough strength.

We observed that the subunits 7+9, controlled by alleles on Glu-B1c, were not found in any of the landraces or cultivars tested. In a previous study reported by Ram (2003), these subunits were found only in *T. turgidum* var. dicoccum. Subunits named 7+8, 14+15, 17+18, 13+16 controlled by the Glu-B1b, Glu-B1h, Glu-B1i and Glu-B1f alleles were not detected in any cultivars in our study. Both 7+8 and 17+18 subunits were found only in Iranian landraces, which have significant effects on dough extensibility [14].

Overall, most of landraces showed different HMWGS compositions compared with cultivars. Moreover, cultivars were also separated from landraces by principle component analysis (PCA). These differences may be due to the dissimilarity of materials and/or the fact that these cultivars are not originated from Iranian landraces.

In conclusion these two methods could be used to study genetic diversity in different wheat genotypes. The choice of the method for genetic diversity estimation depends largely upon the tools available and how it fit in breeding scheme. Both HMWGS and agronomical traits will be useful to breeders to formulate crosses by choosing genotypes with appropriate characters.

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