

## Electrophoretic profiles of gliadin subunits to evaluate genetic diversity of *Triticum persicum* Boiss. and *Triticum pyramidale* Percival.

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### Abstract

The genetic diversity of two tetraploid wheat species were analyzed by examining seed storage protein markers at *Gli-A1* and *Gli-B1*; gliadin loci. Samples were collected from Iran, some areas of the Middle East and north of Africa (Egypt) comprising 30 populations of *Triticum persicum* and *Triticum pyramidale*. High rate of electrophoretic polymorphism was detected at studied loci in two species. In *T. persicum* a total of 72 allelic variants on 2 loci were detected, including 41 for *Gli-A1* and 31 for *Gli-B1*. In the *T. pyramidale* samples, 50 allelic variants were observed totally. In *Triticum pyramidale* the number of alleles were 28 and 22 for *Gli-A1* and *Gli-B1* respectively. No null alleles were observed in the *T. persicum* samples and the total number of allelic variants in Iranian and samples from the other countries were distinguished 42 and 30, respectively. In *T.pyramidale* conversely, the total number of allelic variants for Egyptian samples were 19 which was the highest number among samples from the other countries. Iranian population of *T.persicum* (No 1 from Noorabad of Iran) showed the highest level of genetic diversity (A = 3.00, Ne = 2.11, He = 0.63 and GD = 0.51 ). Conversely, population No 7 from Cairo had the highest diversity ( A = 3.00, Ne = 2.50, He = 0.67 and GD = 0.57) in *T.pyramidale*.

**Keywords:** Alleles; A-PAGE; *Gli-A1*; *Gli-B1*; Marker; Variation; Wheat

### Introduction

Although in the recent years the world yield of wheat has increased remarkably but seems that is not enough for population growth and therefore continuing genetic and plant breeding programs is necessary to raise grain yield and quality. Glutenins and gliadins are the most important proteins in the wheat grain which can be used for assessing genetic diversity of species or varieties (Lafiandra et al. 1990; Pfluger et al.2001) and for genotype identification in

different wheat species (Bushuk and Zillman 1978; Nevo and Payne 1987). The gliadins are controlled by the *Gli* loci which have been located on the short arms of chromosomes 1 and 6 of homoeologous groups (Payne et al. 1982; Payne 1987; Caballero et al., 2004). Each *Gli* locus codes for a group of gliadin polypeptides that are inherited as a block. Because of the multiple allelism at these loci, the different blocks generate an extremely complex

**Table 1.** Geographical regions for collecting populations of *Triticum persicum*.

Population Number	Country	Zone(City)	Longitude	Latitude	Altitude
1	Iran	Noorabad	48.30	33.42	1246
2	Iran	Marivan	46.16	34.50	1200
3	Iran	Dehloran	42.6	34.5	1850
4	Turkey	20KmN	27.12	38.22	30
5	Turkey	28KmN	38.20	38.27	650
6	Turkey	9KmSE	35.59	38.37	1200
7	Egypt	Cairo	31.23	30.22	158
8	Egypt	Cairo	31.32	30.26	150
9	Egypt	Aswan	32.54	23.38	212
10	Syria	Deiraljamal	37.00	36.25	580
11	Syria	Rawada	36.00	33.36	1320
12	Syria	Ratieyeh	36.02	33.34	1302
13	Iraq	20KmE	43.25	36.23	470
14	Iraq	Jabelsingar	41.47	36.20	1200
15	Iraq	Imadira	41.51	36.21	1000

gliadin pattern in hexaploid wheats (Sozinov and Poperelya 1980; Metakovski et al., 1984). Genes coding for most  $\gamma$ - and  $\omega$ -gliadins have been located on short arm of chromosomes 1A, 1B and 1D at the *Gli-A1*, *Gli-B1* and *Gli-D1* loci respectively, whereas genes coding for most  $\alpha$ - and  $\beta$ -gliadins exist on short arm of group 6 chromosomes at the *Gli-A2*, *Gli-B2* and *Gli-D2* (Payne, 1987). Study of seed storage protein in various crops, particularly in wheat, has been utilized as powerful tool (Gepts, 1990). The gliadins are heterogeneous monomeric proteins, which are separated into groups on the basis of their mobility on electrophoresis at low pH (A-PAGE gels). Fractionation of the gliadin proteins create three groups, the  $\omega$ ,  $\gamma$ ,  $\beta$  and  $\alpha$ -gliadins, in order of increasing mobility (Bietz & Wall, 1973). Gliadins are encoded by six *Gli* loci (*Gli-1* and *Gli-2*) mapped on the short arms of the group 1 and 6 chromosomes, respectively (Wrigley & Shepherd, 1973; Payne et al., 1982). Many researchers demonstrated the relationship of this polymorphism with genetic diversity in crops (Nevo & Payne, 1987; Felsenburg et al., 1991; Ciaffi et al., 1993; Levy & Feldman 1998). The gliadin markers successfully were used to illustrate genetic diversity of *Triticum urartu* and *Triticum boeoticum*, in the Middle east region (Bahraei S, 1996; Bahraei et al., 1997).

In this study we evaluated *T. persicum* Boiss. (*T.turgidum subsp persicum*) and *T.pyramidale* Percival. (*Triticum durum subsp pyramidale*) on the basis of their seed storage gliadins by the electrophoresis methods in the Middle East region.

## Materials and methods

### Plant Material

Seeds and mature plants of various population of *Triticum persicum* and *Triticum pyramidale* were collected from some parts of Iran, Middle east and the north of Africa (Fig 3) by field trips between years 2000 to 2004 and samples were deposited in the herbarium. Fifteen populations from each species including 270 accessions were selected to estimate the genetic variability (Table 1 and 2), by assessment of gliadin subunits at two loci (*Gli-A1* & *Gli-B1*). Seeds without embryo were ground in the mortar and then the flour immersed in 1.5 M Dimethylformamide (DMF) for about 1 hour at room temperature and then centrifuged for 10 minutes. After all, the gliadin subunits were separated from the upper section of tube and analyzed using polyacrylamide gel electrophoresis which contained Aluminium lactate buffer with pH=3 (Khan et al. 1985).

**Table 2.** Geographical regions for collecting populations of *Triticum pyramidale*.

Population Number	Country	Zone(City)	Longitude	Latitude	Altitude
1	Iran	Kermanshah	47.00	34.42	736
2	Iran	Sanandaj	46.82	35.36	950
3	Iran	Khoramabad	48.30	33.62	780
4	Turkey	Ankara	32.73	39.90	1120
5	Turkey	Orfa	38.10	37.20	720
6	Turkey	Bitlis	43.91	38.33	149
7	Egypt	Cairo	31.25	30.23	172
8	Egypt	Asiot	31.75	27.16	132
9	Egypt	Portsaeid	32.52	31.35	229
10	Syria	Damascus	36.21	33.42	312
11	Syria	Hems	36.44	34.38	920
12	Syria	Halab	37.12	36.42	818
13	Iraq	Basra	47.56	30.92	89
14	Iraq	Baghdad	44.58	33.14	112
15	Iraq	Najaf	44.18	32.41	68

Electrophoresis was performed at 25 mA at 18° C for 45 min after the tracking dye (methyl violet) migrated off the gel. Gels were stained overnight by 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250. Destaining was carried out with tap water (Caballerro et al. 2004). Some standard cultivars (such as Marquis and Chinese Spring) were used to identify new alleles and subunits. Subunits were divided on the basis of their molecular weight into four section  $\omega$ ,  $\gamma$ ,  $\beta$ ,  $\alpha$  from high to low molecular weights, respectively.

#### **Statistical analysis**

The total number of allelic variants were counted and recorded in each population, based on the gliadin loci, and distribution of alleles around the four sections. Final analysis was done after zymogram preparation to detect genetic identity and distance between populations (Nei 1972,1973,1975) and then expected heterozygosity (He), average number of alleles per locus (A) and effective number of alleles per locus (Ne) were calculated to evaluate the genetic diversity within populations.

#### **Results and discussion**

##### ***Gliadin composition and polymorphisms***

High rate of polymorphisms were remarkably observed in *T.persicum* and *T. pyramidale* (Fig 1 and 2). The gliadin subunits were divided into four sections in both species mainly,  $\omega$ ,  $\gamma$ ,  $\beta$ ,  $\alpha$ , where  $\omega$  has the heaviest molecular weight and the other subunits ( $\gamma$ ,  $\beta$ ,  $\alpha$  were lighter, respectively). In *T. persicum* most of the variations were observed at Gli-A1 locus and the area of Gli-A1 was distinctively longer than Gli-B1 in long. The subunits of  $\omega$ ,  $\gamma$  were distinguished in the Gli-A1 locus area and the  $\alpha$ ,  $\beta$  subunits only were found in the Gli-B1 locus. In *T. persicum* some confusions between bands was distinctively detected (Fig 1). These confusions were particularly apparent in the  $\alpha$  subunits at Gli-B1 locus. It shows that subunits in this area are close together in the molecular weight and can be separated apart by 2-Dimensional electrophoresis method (Jackson et al. 1983, Lafiandra & Kasarda 1985). In all *T. pyramidale* samples (originating from Egypt) bands were visibly separated from each other and there was no confusion (Fig 2). In Iranian samples of *T. pyramidale* a null allele at Gli- A1 locus with

**Table 3.** Number of allelic variants in different countries of two species

	Country					Total
	Iraq	Turkey	Syria	Egypt		
<i>Triticum persicum</i>	42	10	9	7	4	72
<i>Triticum pyramidale</i>	3	7	10	11	19	50

**Table 4.** Genetic diversity within 15 population of *Triticum persicum* based on the storage proteins at the Gli-A1 and Gli-B1.

Populations	Country	Sample size	A	Ne	He	Genetic diversity
1	Iran	18	3.00	2.11	0.63	0.51
2	Iran	18	3.67	2.46	0.76	0.49
3	Iran	18	3.33	2.00	0.61	0.42
4	Turkey	18	2.00	1.66	0.48	0.33
5	Turkey	18	2.00	1.94	0.47	0.33
6	Turkey	18	1.67	1.57	0.52	0.31
7	Egypt	18	1.33	1.17	0.54	0.12
8	Egypt	18	2.00	1.12	0.12	0.11
9	Egypt	18	1.33	1.13	0.28	0.09
10	Syria	18	2.33	1.46	0.45	0.30
11	Syria	18	2.33	1.37	0.39	0.25
12	Syria	18	1.67	1.16	0.21	0.13
13	Iraq	18	2.67	1.80	0.55	0.37
14	Iraq	18	2.33	1.50	0.39	0.35
15	Iraq	18	2.33	1.78	0.44	0.35
Total mean		270	2.26	1.61	0.45	0.297

(He) Expected heterozygosity, (A) Average number of alleles per locus, (Ne) Effective number of alleles per locus

$\omega$  subunits was observed in one genotype from Sanandaj, No 2 (Table 2). Also another null allele was found in a Turkish sample, No 4 (Table 2) at the Gli-B1 locus and  $\alpha$  subunits in this species. No null alleles were seen in *T. persicum* samples.

#### Number of allelic variants

The total number of allelic variants in 15 populations of *T. persicum* was 72, of which 41 belonged to Gli-A1 and 31 to Gli-B1. For *T. pyramidale*, a total of 50 allelic variants were seen, of which 28 and 22 were

found at Gli-A1 and Gli-B1, respectively. Iranian populations of *T. persicum* showed the greatest number of allelic variants, which more than half of allelic variants calculated (42 out of 72) in Iran. The number of allelic variants of *T. persicum*, in Iraqi populations was counted 10, Turkish 9, Syrian 7 and Egyptian 4 (Table 3). This suggests that the rate of variation in Iranian populations of *T. persicum* is remarkably higher than populations from the other countries, especially for Gli-A1 locus. Conversely, in *T. pyramidale*, the total number of allelic variants were counted 50.

**Table 5.** Genetic diversity within 15 population of *Triticum pyramidale* based on the storage proteins at the Gli-A1 and Gli-B1.

Populations	Country	Sample size	A	Ne	He	Genetic diversity
1	Iran	18	1.33	1.08	0.19	0.07
2	Iran	18	1.33	1.22	0.21	0.13
3	Iran	18	1.33	1.13	0.30	0.09
4	Turkey	18	2.33	1.81	0.56	0.37
5	Turkey	18	1.67	1.53	0.44	0.29
6	Turkey	18	1.67	1.16	0.21	0.13
7	Egypt	18	3.00	2.50	0.67	0.57
8	Egypt	18	2.67	2.28	0.66	0.44
9	Egypt	18	2.67	1.41	0.29	0.28
10	Syria	18	2.33	1.69	0.51	0.36
11	Syria	18	2.00	1.91	0.58	0.48
12	Syria	18	2.00	1.25	0.22	0.20
13	Iraq	18	2.00	1.44	0.51	0.26
14	Iraq	18	1.67	1.16	0.41	0.13
15	Iraq	18	1.33	1.04	0.10	0.04
Total mean		270	1.95	1.50	0.39	0.256

(He) Expected heterozygosity, (A) Average number of alleles per locus, (Ne) Effective number of alleles per locus

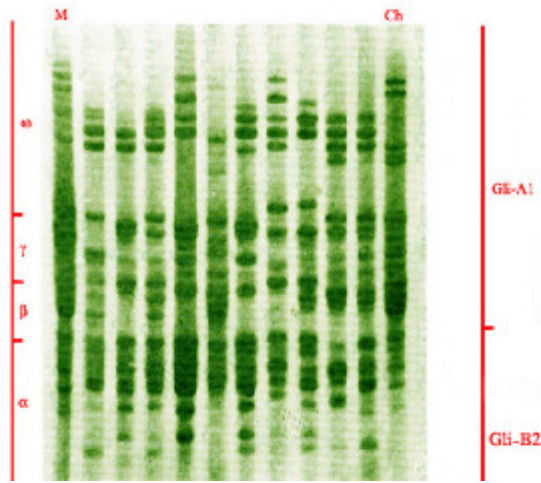
The 19 variants were detected in Egypt solely, 11 in Syria, 10 in Turkey, 7 in Iraq and 3 in Iran out of 50 (Table 3).

#### **Genetic diversity and expected heterozygosity**

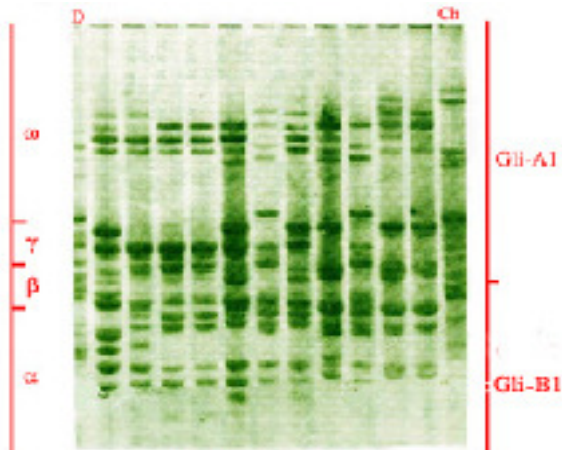
Tables 4 and 5 show genetic diversity and other variability factors such as expected heterozygosity (*He*), average number of alleles per locus (*A*) and effective number of alleles per locus (*Ne*) in of *T.persicum* and *T.pyramidale* accessions, respectively. In *T.persicum*, population No 1 from Noorabad of Iran showed highest level of genetic diversity (*A* = 3.00, *Ne* = 2.11, *He* = 0.63 and *GD* = 0.51). Other accessions from Iran No 2 and 3 (Table 4) also showed high values for genetic diversity of 0.49 and 0.42, respectively. The Egyptian samples had lower values, for example population No 9 had the lowest diversity (*A* = 1.33, *Ne* = 1.13, *He* = 0.28 and *GD* = 0.09) (Table 4). In *T.pyramidale* population No 7 from Cairo had the highest values (*A* = 3.00,

*Ne* = 2.50, *He* = 0.67 and *GD* = 0.57) and conversely population No 15 from Najaf region of Iraq showed the lowest diversity (*A* = 1.33, *Ne* = 1.04, *He* = 0.10 and *GD* = 0.04) (Table 5). Comprehensively, high rate of polymorphism was detected in the electrophoretic pattern of both species as previously recorded in wild wheat accessions (Ciaffi et al. 1992, 1993). Tables 3 and 6 show totally 122 allelic variants, for two loci in both species. This high rate of variation is only found in the wild species of wheat (Bahraei et al., 1996). Distribution of alleles shows the highest variation rate for *T. persicum* in Iran than other countries (42 out of 72).

Conversely, in *T. pyramidale* most of the allelic variants have been found in Egypt (19 out of 50) The remainder of variants (31 out of 50) have been distributed in other regions. The mean value of genetic diversity in the accessions of *T.persicum* is 0.297 (*A* = 2.26, *Ne* = 1.61, *He* = 0.45 and *GD* = 0.297) and for *T.pyramidale*, 0.256 (*A* = 1.95, *Ne* = 1.50, *He* = 0.39 and *GD* = 0.256) which is lower



**Fig 1.** Electrophoretic pattern of gliadin subunits in *Triticum persicum*. M:Marquis and Ch:Chinese spring (Standard cultivars of Bread wheats).



**Fig 2.** Electrophoretic pattern of gliadin subunits in *Triticum pyramidale*. Ch: Chinese spring and D: Drago (Standard cultivars of Bread and durum wheats).

than *persicum* species (Tables 4 and 5). The glutenin subunits of these species were also examined and the similar variation in the grain quality was seen (Kharabian, 2005). This result probably is due to relationship between two species and centre of diversity.



**Fig 3.** Map of sampling situations (approximately) Red stars (\*) show collecting zone of *Triticum persicum* and Black stars (\*) for *Triticum pyramidale*

The centre of origin and primary diversity and for *T. persicum* is probably Iran and other middle eastern countries are secondary centres. For *T. pyramidale* the primary centre of origin and diversity is Egypt and the other middle eastern countries are secondary centres based on previous suggestions (Gepts 1990, Lafiandra et al. 1990,1993)(Table 6).

In the tables 3 and 6 the number of allelic variants have been given briefly, which is conform with our prediction about country of origin. In *T.persicum* most of variability was seen in Iran and it was decreased toward Egypt (The north of Africa) and in *T.pyramidale* the number of variants decreased from Egypt toward Iran, vice versa (Tables 3 and 6). The total number of *Gli-A1* variants was 69 in comparison to 53 for *Gli-B1*. This shows that most of alleles were found in the *Gli-A1* locus and were in the  $\omega$ ,  $\gamma$ ,  $\beta$  subunits area while the other subunits are in the *Gli-B1* locus and  $\alpha$  subunits. This implies that the *Gli-A1* locus produces heavier subunits with higher molecular weight than *Gli-B1* (Bietz & Wall 1973, Ciaffi et al. 1992 ). Some slow bands particularly in  $\omega$  section have been associated with the D genome in hexaploid wheats, but their absence does not indicate that those accessions have not hexaploid genome (Caballero et al., 2001, 2004).

**Table 6.** A summary of allelic variants situation on two species and two loci.

Species	Loci		Various countries	Areas	
	Gli-A1	Gli-B1		Iran	Total
T. persicum	41	31	30	42	72
T. pyramidale	28	22	47	3	50
Total	69	53	77	45	122

Presence of these subunits in some populations of *T.pyramidale* (number 2 and 4) indicates that these accessions may have the D genome which this problem can be investigated by cytogenetic tests but absence of these slow bands confirms tetraploidy of all other studied samples. These polymorphisms are presumably due to high rate of spontaneous mutations in these populations (Porceddu et al. 1998). In *T.persicum* 65.8% of the genetic variation was within and only 34.2 among populations and in *T.pyramidale* 78.2% within and just 21.8% were among populations.

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