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Identification and Classification of Gliadin Genes in Iranian Diploid Wheat

Jafar Ahmadi, Alireza Pour-Aboughadareh

***Abstract*—**Wheat is the first and the most important grain of the world and its bakery property is due to glutenin and gliadin qualities. Wheat seed proteins were divided into four groups according to solubility including albumin, globulin, glutenin and prolamin or gliadin. Gliadins are major components of the storage proteins in wheat endosperm. It seems that little information is available about gliadin genes in Iranian wild relatives of wheat. Thus, the aim of this study was the evaluation of the wheat wild relatives collected from different origins of Zagros Mountains in Iran, in terms of coding gliadin genes using specific primers. For this, forty accessions of *Triticum boeoticum* and *Triticum urartu* were selected for this study. For each accession, genomic DNA was extracted and PCRs were performed in total volumes of 15 µl. The amplification products were separated on 1.5% agarose gels. In results, for Gli-2A locus three allelic variants were detected by Gli-2As primer pairs. The sizes of PCR products for these alleles were 210, 490 and 700 bp. Only five (13%) and two accessions (5%) produced 700 and 490 bp fragments when their DNA was amplified with the Gli.As.2 primer pairs. However, 93% of the accessions carried allele 210 bp, and only 8% did not any product for this marker. Therefore, these germplasm could be used as rich gene pool to broaden the genetic base of bread wheat.

***Keywords*—**Diploied wheat, gliadin, *Triticum boeoticum, Triticum urartu*.

I. Introduction

THE wild relatives of crop plants constitute an increasingly important genetic resource for improving agricultural production and maintaining sustainable agro-ecosystems. Wild species of *Aegilops* and *Triticum* provide a useful source of new genetic variation for wheat improvement. Einkorn wheat is a diploid and self- pollinated species. Three species *T. monococcum*, *T. urartu* and *T. boeoticum* are belonging to the einkorn wheat. *T. boeoticum* and *T. urartu* are separated by crossing barriers [1], differ slightly in plant morphology [2], biochemical and molecular marker loci [3], [4]. It has been widely recognized that *T. monococcum* was among the first crops domesticated in the Fertile Crescent starting from the wild progenitor *T. boeoticum* [5]. *T. urartu* has played an important role in wheat evolution and this species donated the A genome to all tetraploid and hexaploid wheats [4]. Wheat seed proteins were divided into four groups according to solubility. Two groups are albumin and globulin dissolved in water and salt solutions possess metabolic activities. Two other groups are inactive and non-dissolvable and contain glutelins or glutenins and prolamins or gliadins [6]. Wheat glutenins and gliadins are located in glutelin and prolamin groups, respectively [6]. Wheat glutenin is divided into two groups according to molecular weight, glutenin subunits with high molecular weight (HMW-GS) and glutenin subunits with low molecular weight (LMW-GS). HMW-GS are coded by Glu1 loci located on the long arm of chromosomes group 1, and each locus includes two coding genes for subunits type X and Y [7]. Genes X and Y control subunits with slower and faster movement, respectively. LMW-GS are coded by *Glu-3* genes including *Glu-A3*, *Glu-B3* and *Glu-D3* that are like a genetic block and located on the short arm of 1A, 1B and 1D chromosome [8]. Six patterns were identified for loci *Glu-A3*, five for *Glu-D3* and nine for *Glu-B3*. For the first time, glutenin subunits were divided into three groups A, B and C according to their movement in SDS-PAGE gel. It was shown that the group A belonged to HMW-GS and groups B and C belonged to LMW-GS. Gliadins with high molecular weight show similar movements with B and C and LMW subunits. The gliadin is a major component of the storage proteins in wheat endosperm. Gliadin proteins were separated into three groups based on electrophoretic mobility: α/β-gliadin, γ-gliadin, and ω-gliadin. The α/β-gliadin are located on the Gli-2 loci in the short arm of the homoelogous group 6 chromosomes and the γ-gliadin, and ω-gliadin are tightly linked and are located on the Gli-1/Gli-3 loci in the short arm of the homoelogous group 1 chromosomes [9]. The origins of wild wheat in Iran are potentially ideal areas for discover suitable genes to further transfer into cultivated wheat [10]. However, it seems that little information is available about these wild relatives of wheat in Iran. Thus, the aim of this study was the evaluation on the wild relatives in terms of coding gliadin genes.

Jafar Ahmadi is Associate Professor with the Imam Khomeini International University, Qazvin, Iran (corresponding author, phone: +982833901139; fax: +982833780073; Qazvin, Iran, e-mail: njahmadi910 @yahoo.com).

Alireza Pour-Aboughadareh is Ph.D Student with the Imam Khomeini International University, Qazvin, Iran.

II. Material and Methods

Thirty two accessions of *T. boeticum* along with eight accessions of *T. urartu* which located in different parts of Zagros Mountains in northwestern to southwestern Iran were selected for this study. A summary of geographical distributions of these accessions are shown in Table I. For each accession, genomic DNA was extracted from the leaves according to the method of [11] with minor modification. DNA quantity and quality was determined using NanoDrop-2000c spectrophotometer as well as 0.8% agarose gel. PCR were performed in total volumes of 15 µl, including 150 mM Tris-HCl pH 8.5, 40 mM (NH4)2S04, 4.0 mM of MgCl2, 0.4 mM of each dNTPs, 0.05 units/µl of ampliqon Taq DNA

polymerase and 2 µl of DNA. Sequences of primers synthesized according to [12] and [13].

TABLE I
THE ORIGIN AND SITE DESCRIPTION OF THE 40 ACCESSIONS OF *TRITICUM BOEOTICUM* AND *TRITICUM URARTU*

Accession code	Geographical position in Zagros area	Province	Altitude (m)	Longitude (E)	Latitude (N)
IUGB-0003	Northwest	West Azerbaijan	1649.2	45° 45′	36° 39′
IUGB-0004	Central	Kermanshah	2267.6	46° 35′	34° 51′
IUGB-0010	Central	Kurdistan	1588.2	46° 28′	36° 19′
IUGB-0012	Central	Kermanshah	1936.0	47° 32′	34° 19′
IUGB-0015	Northwest	Kurdistan	1509.0	46° 16′	34° 27′
IUGB-0016	Central	Kermanshah	1760.4	46° 34′	35° 41′
IUGB-0018	Central	Lorestan	2431.8	48° 26′	34° 53′
IUGB-0052	Central	Lorestan	1679.4	48° 48′	34° 50′
IUGB-0077	Central	Kermanshah	1971.4	46° 19′	33° 17′
IUGB-0102	Northwest	Kurdistan	1291.8	46° 44′	33° 12′
IUGB-0113	Northwest	Qazvin	1291.8	*	*
IUGB-0114	Northwest	Qazvin	1709.2	50° 04′	34° 23′
IUGB-0118	Central	Lorestan	1573.6	47° 54′	35° 25′
IUGB-0120	Central	Lorestan	1367.2	48° 51′	36° 17′
IUGB-0125	Central	Kermanshah	1153.3	47° 37′	33° 19′
IUGB-0126	Northwest	Hamadan	1270.2	48° 08′	33° 11′
IUGB-0127	Central	Kermanshah	1851.2	46° 46′	34° 18′
IUGB-0154	Central	Kohgiluyeh & Boyer-Ahmad	1796.2	51° 11′	34° 47′
IUGB-0155	Central	Lorestan	1613.6	48° 41′	34° 45′
IUGB-0162	Central	Chaharmahal and Bakhtiari	1673.2	50° 51′	33° 53′
IUGB-0165	Central	Kermanshah	1961.8	47° 22′	33° 31′
IUGB-0171	Central	Lorestan	2249.4	48° 13′	30° 44′
IUGB-0176	Central	Lorestan	1652.0	48° 41′	33° 23′
IUGB-0177	Central	Lorestan	2105.0	48° 29′	32° 12′
IUGB-0179	Central	Kermanshah	2047.0	47° 50′	34° 44′
IUGB-0181	Central	Kermanshah	2152.8	47° 37′	32° 13′
IUGB-0206	Central	Kermanshah	1637.2	47° 52′	34° 16′
IUGB-0216	Northwest	East Azerbaijan	1706.8	47° 33′	38° 40′
IUGB-0230	Central	Lorestan	2096.0	48° 10′	33° 17′
IUGB-0257	Central	Kermanshah	1522.2	47° 33′	33° 24′
IUGB-0372	Central	Lorestan	1757.4	48° 45′	33° 55′
IUGB-0277	Central	Kermanshah	2093.8	47° 32′	34° 52′
IUGB-0285	Central	Kermanshah	1551.0	47° 25′	36° 25′
IUGB-0316	Central	Lorestan	1967.8	48° 40′	34° 48′
IUGB-0320	Central	Kermanshah	2044.6	47° 33′	34° 12′
IUGB-0368	Central	Ilam	1072.2	46° 31′	33° 47′
IUGB-0407	Central	Kermanshah	1895.0	46° 23′	34° 47′
IUGB-0484	Unknown	Unknown	*	*	*
IUGB-0200	Northwest	Kurdistan	1518.4	42° 19′	35° 37′
IUGB-0300	Northwest	Kurdistan	1692.2	46° 28′	34° 39′

* data not available

PCR conditions were first denaturation at 95°C for 5 min, followed by 35 cycles consisted of denaturation at 95°C for 40 s, annealing at 58.3ºC for 40 s, extension at 72ºC for 60 s, and final extension at 72ºC for 7 min. The amplification products were separated on 1.5% agarose gels. Finally, PCR products were stained with ethidium bromide and visualized using UV light.

III. Results and Discussion

The allelic state at *Gli-2A* for each of the accessions is given in Table II. For *Gli-2A* locus three allelic variants were detected by Gli-2A-s primer pairs (Table III). The sizes of PCR products for these alleles were 210, 490 and 700 bp. With respect to the allelic status at *Gli-2A* locus, the only five (13%) and two accessions (5%) produced 700 and 490 bp fragments when their DNA was amplified with the Gli.As.2 primer pair diagnostic for *Gli-2A*. However, 37 of the 40 accessions (93%) carried 210 bp allele, and three accessions (8%) did not any product for this marker. Only two and three accessions of *T. boeoticum* and *T. urartu* carried 700 bp allele diagnostic for *Gli-2A*. The presence of gliadin genes can be monitored directly at every stage of wheat growth. In this regard, the gliadin genes were detected with respect to their effect on quality and other aspects of bread making [13]-[16].

In this study, at *Gli-A2* loci, 2 new alleles were detected using *Gli-A2-s* primer pairs. As shown in Fig. 1, at the *Gli-A2* locus, we also identified two new alleles with size of 700 and 490 bp. In accordance with our results, [17] and [9] reported a high allelic variation for gliadin genes in wheat germplasm. Zhao-cai et al. [14] indicated that, among the Einkorn wheat, *T. urartu* and *T. boeoticum* species have a high level of allelic variation in *Gli-A1* and *Gli-A2* loci. Furthermore, there still existed several gliadin genes couldn't be detected in A-genome ancestors wheat. Thus it seems that, there might be some new gliadin alleles in these accessions and this need to be further study [13], [14]. Allelic status in breeding materials might be monitored by means of DNA markers. Therefore, the gliadin patterns of Einkorn wheat could be used as helpful markers in selecting breeding parents from Einkorn wheat germplasm collection. In the present study, we characterized two new alleles for *Gli-A2*, which it indicated that the higher genetic variations exist in the species of Einkorn wheat from Iran. Moreover, the results revealed a remarkable potential in accessions collected from west and central of Zagros Mountains especially, parts of Kurdistan and Kermanshah provinces as well as capability accessions of *T. urartu* collected from regions of Chaharmahal & Bakhtiari and Kohgiluyeh & Buyer Ahmad provinces, which carried different alleles types.

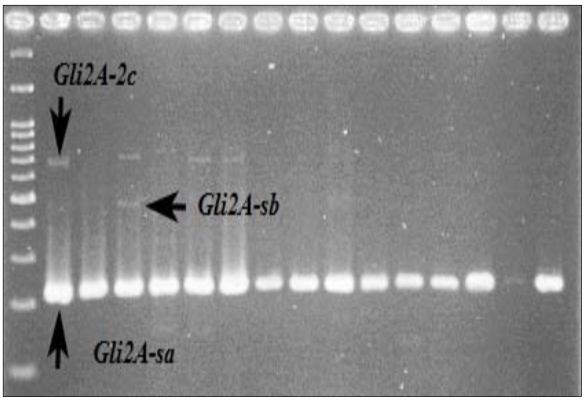

Fig. 1 The amplified segments (alleles) from *T. boeoticum* and *urartu* amplified by *GliAs-2* specific primers

TABLE II
THE ALLELIC STATE AT *GLI-2A* FOR EACH OF THE ACCESSIONS STUDIED IN THIS EXAMINES

Accession code	*Gli-As.2* alleles		
	700	490	210
IUGB-0003	-	-	+
IUGB-0004	-	-	+
IUGB-0010	-	-	+
IUGB-0012	-	-	+
IUGB-0015	-	-	+
IUGB-0016	-	-	+
IUGB-0018	-	-	+
IUGB-0052	-	-	+
IUGB-0077	-	-	-
IUGB-0102	-	-	+
IUGB-0113	-	-	+
IUGB-0114	-	-	+
IUGB-0118	-	-	+
IUGB-0120	-	-	+
IUGB-0125	-	-	+
IUGB-0126	+	-	+
IUGB-0127	-	-	+
IUGB-0154	+	+	+
IUGB-0155	+	+	+
IUGB-0162	+	-	+
IUGB-0165	+	-	+
IUGB-0171	-	-	+
IUGB-0176	-	-	+
IUGB-0177	-	-	+
IUGB-0179	-	-	+
IUGB-0181	-	-	+
IUGB-0206	-	-	+
IUGB-0216	-	-	+
IUGB-0230	-	-	-
IUGB-0257	-	-	+
IUGB-0372	-	-	+
IUGB-0277	-	-	+
IUGB-0285	-	-	+
IUGB-0316	-	-	+
IUGB-0320	-	-	+
IUGB-0368	-	-	-
IUGB-0407	-	-	+
IUGB-0484	-	-	+
IUGB-0200	-	-	+
IUGB-0300	-	-	+

TABLE III
ALLELIC FREQUENCIES FOR GLIADIN SUBUNITS IN *T. BOETICUM* AND *T. URARTU* ACCESSIONS FROM CENTRAL AND NORTHWEST OF ZAGROS MOUNTAINS

Locus	Allele	Frequency	%	Northwest	Central	*T. boeticum*	*T. urartu*	Fragment size
Gli-As.2	*a*	5	12.5	1	4	2	3	700
	b	2	5.0	0	2	1	1	500
	c	37	92.5	9	28	29	7	210

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