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Intra- and Inter-population Diversity of Iranian *Aegilops tauschii* Based on Seed Storage Protein Electrophoresis

R. GHASEMZADE[1], M.R. BEHAMTA, M.J. AGHAII, M. OMIDI, V. MOHAMMADI AND M.I. HASANI
Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
[1]Corresponding author's e-mail: rahele_ghasemzade@yahoo.com

ABSTRACT

The variation of high molecular weight glutenin subunits were studied in 115 populations of *Aegilops tauschii* (DD, 2n = 2x =14) collected from different part of Iran using SDS-PAGE. The results showed a wide variation in HMW-glutenin subunits among populations, whereas no diversity was found intra-populations. Twenty one Glu-D_t1 subunit combinations were found among different accessions. The endosperm of *Ae. tauschii* contains many allelic variants of glutenin storage proteins, which are not present in wheat and it could be utilized in breeding varieties with improved bread-making qualities.

Key Words: *Aegilops tauschii*; Diversity; Iran; HMW-glutenin subunits

INTRODUCTION

Common wheat (*Triticum aestivum* L. em. Tell) is a natural amphiploid (2n=6x=42) that is originated from interspecific hybridization between the tetraploid *T. turgidum ssp. Dicoccum* (2n=4x=28, AABB) and the diploid *Aegilops tauschii* (2n=2x=14, DD) (Dovorak *et al*., 1998). It is known that southwestern Asia is the origin center of the *Triticum* species. The diploid Asia goat grass, *Ae. tauschii* is mainly distributed in the area of northeastern Syria, Iran, Iraq, Transcaucasia and the western slopes of the Himalayas (Dudnikov & Kavahara, 2006).

Common wheat is a unique cereal crop, which is grown extensively across a wide range of environments around the world and contains proteins (mainly giuten), whose physico-chemical properties play an important role in determining the end use of flour by conferring unique biomechanical properties, such as dough elasticity and extensibility (Shewry *et al.*, 1992).

The gluten protein comprises about 80-90% of the storage proteins in endosperm, gliadin and glutenin proteins, which are the main contributors to the reological and bread making properties of wheat flour. Glutenin proteins are polymeric, wheat disulphide bonds linking the individual glutenin polyprptides. Subunits from SDS polyacrylamid gel electrophoresis (SDS-PAGE) and size exclusion high performance liquid chromatography (SE-HPLC) has identified two distinct group; high and low molecular weight glutenin subunit (HMW-GS & LMW-GS, respectively) (Kassard, 1999). The gliadins are monomeric proteins, soluble in aqueous alcohol solutions with a molecular mass of ~30-60 kda. The α-, β- and γ- gliadins have intra molecular disulphide bonds but some γ- gliadins may be linked with glutenin subunits (Kohler *et al*., 1993). Most of the ω gliadins, also called sulphurpoor prolamins (Shewry *et al*., 1986), do not contain disulphide bonds.

Although high molecular weight (HMW) glutenins (90-150 kDa) contain a small proportion of total seed proteins, they play an important part in grain quality (Payne *et al*., 1983). The HMW- GS of wheat are controlled by genes at three loci; Glu-A_1, Glu-B_1 and Glu-D_1, located on the long arms of chromosomes 1A, 1B and 1D, respectively. Each Glu-1 locus contains two genes, one encoding a X-type subunit with slower mobility and a Y- type subunit with faster mobility (Payne *et al*., 1981; Lafiandra *et al*., 1993). Genetic variability among storage proteins in bread wheat is limited. Of three genomes of bread wheat, D genome has the lowest variability suggesting that only a small number of *Ae. tauschii* genotypes of restricted geographic origin were involved in the origin of hexaploid bread wheat (Lagudah & Halloran., 1988a). Recent studies of various accessions of *Ae. tTauschii* have revealed greater genetic variability in comparison to the D genome of bread wheat. Many genes are now identified from different accessions of *Ae. tauschii*, conferring leaf rust resistance (Roupp *et al*.,1983), resistance to green bug (Harvey *et al*., 1980) and a novel HMW- Gs gene Glu-$D_{1\text{-}2}$($Dy12^t$) subunits (Mackie *et al*., 1996a).

Genetic diversity in bread wheat is limited and closely related species serve as a source of new gene (Zohary *et al*., 1969; Feldman & Sears, 1981). Iran is located in an area, which is the center of origin for many crop plants. Furthermore, Iran is very rich in habitat variation due to the diversity in its geomorphology, topography and climate.

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This has helped the survival of diverse plant species in the wild Iranian flora. There are some important food crops such as wheat and its wild relatives (Arzani, 2005).

Although many landraces of wheat were collected in Iran, wild wheat and *Aegilops* from Iran, still need to be explored (Skovemand *et al*., 2002). The objectives of present study were to evaluate the allelic variation in Glu-D_1 of *Ae. tauschii* and compare to bread wheat, identify accessions that contain HMW-GS, which relative to higher bread making quality and study the association between geographical and genitival variability.

MATERIALS AND METHODS

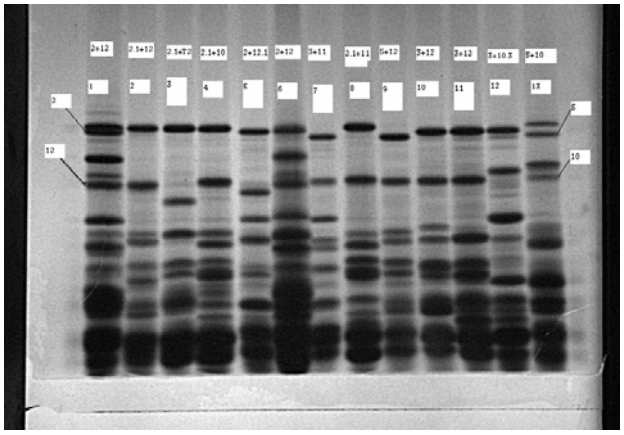
Plant material. One hundred and fifteen populations of Iranian *Aegilops tauschii* held by National Plant Gene Bank of Iran (NPGBI) were used for the study of intera and inter population HMW-Gs variation. The origin of all population was known.

Storage protein analysis. In order to evaluate genetic diversity of high molecular weight subunit of glutenin within and between populations, four samples were taken and analyzed from each of them 115 populations of *Ae. tauschii*. Chines spring, Sunbrook and Sunvale cultivars were used as standard to compare subunit composition. The seed were crushed after removal from the embryo. The flour was mixed in an extraction buffer of 62 mm Tris-HCL (PH=6.8) buffer containing 10% (w/v) glycerol, 4% (w/v) sodium dodecyl sulfate (SDS), 0.01% (w/v) bromophenol blue and 4% ß-mercaptoethanol. Samples left stand at room temperature for at least 12 h with occasional vortexing. They were then placed in a boiling waterbath for 2 minutes and then centrifuged for 5 minutes at 6500 rpm and 15 µL of each sample was loaded on the gel. Proteins were fractionated by SDS-PAGE according modified procedure of Laemmli (1970) by using stacking gel Proteins were fractionated using stacking gel containing 4% acrylamide, 0.05% bis acrylamide, 0.1% SDS and 0.00009 M Tris-HCL; and separating gel containing 10% acrylamide, 0.13% bis acrylamide, 0.1% SDS and 6.78 M Tris-HCL. Gels were stained overnight with 0.01% (w/v) Coomassie Brilliant Blue R 250 in water and acetic acid (10%) and then distained overnight in water for at least 24 h.

Table I. Relative frequency of high molecular subunit composition

Glu-D^t1 variants	Number of accessions	Relative frequency
2+11	2	1.73
2+10	24	20.86
2.1+10.1	2	1.73
1.5+11	1	0.87
2+t2	6	5.21
2+12	27	23.47
5+10	8	6.95
2.1+10	11	9.59
5+12	1	0.87
5+10.3	1	0.87
2+12.1	1	0.87
3+10.3	2	1.73
1.5+10.3	2	1.73
3+12	2	1.73
2.1+10.3	3	2.6
1.5+12	2	1.73
3+10	3	2.6
2.1+12	5	4.35
5+12	5	4.35
11.5+10	2	1.73
2+10.3	2	1.73
2+10.1	1	0.87
1.5+10.1	1	0.87
2.1+t2	11	0.87

Fig. 1. High molecular subunit composition of 11 *Aegilops* and 3 hexaploid wheat by SDS-PAGE with 10% acrylamid gels. Lanes and accessions: (1)Sunvale, (2) TN02007, (3) TN00698, (4) TN00839, (5)TN01984, (6) Chinese Spring, (7)TN02013, (8) TN02017, (9) TN002011, (10) TN01366, (11) 050136, (12) TN00621, (13) Sunvale



RESULTS AND DISCUSSION

Results revealed 7a wide variation in HMW–Gs subunits between different accessions, but no diversity was found within accession. Among 115 accessions, showing no diversity, within populations, 24 types of HMW–GS subunits combinations were identified at the Glu-D^t1 locus (Fig. 1). All of allelic variants were generally characterized by a pair of subunits, namely one X-type subunit with slower mobility and one Y-type subunit with faster mobility, similar to the HMW–GS documented in Common wheat *Triticum aestivum* by Payne *et al*. (1981). None of these alleles showed null-forms. These subunit combinations were indicative of a larger polymorphism in *Ae. tauschii* than one reported at the Glu-D^t1 locus of bread wheat (Payne *et al*., 1983). The allelic forms of 2+10 and 2+12 had the highest relative frequency among populations with 21 and 23.4%, respectively. Different allelic forms appeared in 115 accessions of *Ae. tauschii* (Table I). Five X-type subunits were identified: 2.1, 1.5, 2, 3, 5 in order of ascending mobility. Seven diverse Y-type subunits were detected, 10, 10.1, 10.3,11, 12, 12.1, T^2 according to the

nomenclature of William *et al*. (1993) and Yan *et al.* (2003). The accessions collected from Gorgan and Mazandran provinces in west-east of Caspian showed highest variation with different allelic forms. This is in good agreement with the findings of Lagudah and Halloran (1988b).

In bread wheat, a tight linkage between genes encoding subunits 5+10 and 2+12 has been observed, but in *Aegilops tauschii*, subunit 5 was found along with subunit 10 or 12 (Pfluger *et al*., 2001). Rare recombination between subunits may have given rise to the subunit combinations 5+10 and 2+10. Furthermore, workers employing different methodologies have determined that 2+12 and 5+10 subunitt pairs of *Ae. tauschii* are not the same as those present in *T. aestivum* (Lagudah & Halloran, 1988a; Makie *et al*., 1996a; Gianibelli *et al*., 2000).

In summary, wide variability in HMW glntenin subunits, are not present in hexaploid wheat, suggests that *Ae. taschii* is a valuable source for breeders to improve quality and other characteristics of bread wheat. The results can help breeders to select the *Ae. tauschii* accessions with subunits for used in the development of synthetic wheat.

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