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Investigation of Seed Storage Proteins in some Wild Wheat Progenitors Using SDS-PAGE and ACID-PAGE

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Abstract

Wheat storage proteins accounted for up to 60% of the total grain proteins. They form gluten proteins, which make a visco-elastic network enables dough to be processed into bread, pasta and other products. In order to study genetic variation of wild wheat relatives, electrophoretic patterns of seed storage proteins, the high-molecular-weight glutenins and gliadins from about 12 wild species and some check improved cultivars were fractionated by SDS-PAGE and Acid-PAGE. The results showed some close relationship between *T. urartu*, *T. dicoccum* and bread wheat in the case of glutenin and gliadin. Therefore It was speculated that progenitor of A genome of cultivated wheat could be *T. urartu* strongly. A high level of polymorphism was detected in the glutenin and gliadin subunits of the wild wheat relatives, showing some similarities with cultivated bread wheat, useful breeding perspectives. The electrophoresis proved to be a suitable method to discriminate wheat variety and species. Also results of this study confirmed that the genetic variation amongst seed storage proteins of wild relatives were considerable. The wild progenitors are important genetic resources and therefore observed genetic variability could be use in any selection strategies.

Keywords: A-PAGE, Gliadin, Glutenin, SDS-PAGE

Introduction

This concern is well known among wheat breeders that remaining genetic diversity in the bread wheat gene pools may be insufficient to access current and future breeder's goals (Rejesus *et al.*, 1996). It is anticipated that germplasm accessions most distinct from modern cultivars would be contain the greatest potentially useful alleles for broadening the genetic basis of cultivars (Vavilov, 1940). Wild relatives of crop plants, rich genetically gene pools, are the best hope for crop improvement at future (Feldman and Sears, 1981; Nevo, 1986; and Plucknett *et al.*, 1987). Wild and progenitors species of *Triticum* L. and *Aegilops* L. provide a useful source of new accessible genetic variation for wheat improvement including tolerance against abiotic (drought, cold, heat, salinity and herbicides) and biotic (pathogens, parasites and competitors) stresses. They also maintain rich genetic resources for various agricultural traits, photosynthetic yield and good quality proteins (Nevo, 1998). Because of progenitors of bread wheats are endogenous of semidry areas of central and west Asia, they have been well adapted to biotic and abiotic stresses and climate fluctuations that are specific these regions. Subsequently, they contain various tolerance and adapting genes against these stresses that are particular value for wheat breeding at the numerous regions of center and west Asia and north of Africa with low-input rain fed farming systems (Valkoun, 2001).

There are numerous examples of successful transfers of genes carrying resistance to various pathogens, environmental stresses or nutritionally and technologically useful characteristics from wild diploid relatives into the genome of polyploid wheats (Gale and Miller, 1987; Appels and Lagudah, 1990).

Wild emmers, *Triticum dicoccoides* (= *T. turgidum ssp. dicoccoides*) has been recognized as donors of A and B genomes of durum and bread wheats (Kimber and Feldman, 1987) and wild diploied, *Aegilops tauschii* Coss. has provided the D genome of bread wheats (Kihara, 1944).

Study of structure and diversity of wheat progenitors is significant 1) theoretically viewpoint for perception of evolutionary process of wheat progenitors and 2) applicably viewpoint for potentially use of these genetic resources in crop improvement at future.

Particular attention must be given to storage proteins, glutenins and gliadins, which have proved to be important for technological properties both in durum and bread wheats. The most important effective proteins in bread making quality of common wheats are glutenins and gliadins. The high molecular weights glutenins (HMW-Gs) are controlled by two closely linked genes at three loci are

located on the long arms of 1A, 1B and 1D chromosomes respectively (Payne, 1987). The Low molecular weight glutenins encoded by Glu-A3, Glu-B3 and Glu-D3 loci are located on short arms of group 1 chromosomes (Singh and Shepherd, 1988). Gliadins are classified to four main α, β, γ and ω groups. The ω gliadins and the most of the γ gliadins are controlled by genes on the short arms of group 1 chromosomes and tightly linked to the genes of LMW glutenins, and the α gliadins and the most of β gliadins encoded by genes on the short arms of group 6 chromosomes (Payne *et al*., 1984).

The allelic variation of HMW-Gs influence on end-use quality in the bread wheats (Shewry *et al*., 1992), spatially Dx5+ Dy10 subunit associated with good bread-making quality and Dx2+ Dy12 related to weak bread making quality (Payne, 1987).

The increasing use of wild relatives in wheat breeding has also led to the need to understand the genome structure and differentiation of these species and its evolutionary relationships with cultivated wheats in more detail.

Commonly, the wild relatives and progenitors have been indicated high level genetic diversity in storage proteins compositions (Nevo and Payne, 1987; and Ciaffi *et al*., 1993) that could be use for quality improvement in the bread wheats.

Materials and methods

Seeds of 12 wild wheat progenitors together with some local and improved varieties of Northwest of Iran obtained from national gene bank of Iran (Tab. 1). Seed storage proteins were extracted according to procedure described by Payne and Lawrence (1983). Based on Lawrence and Shepherd (1981) method with slight modification, extracted protein fractionated by one-dimensional (sodium dodecyl sulfate polyacrylamid gel electrophoresis) SDS-PAGE. Also Acid-PAGE was performed based on procedure described by Bushuk and Zillman (1978). Identification of the subunits in SDS-PAGE was performed based on catalogue described by Payne *et al*. (1981) using standard varieties such as Inia and Enza. However gliadin band identification performed using Marquis cultivar. Subsequently cluster analysis was made with SPSS software after gel scoring.

Tab. 1. List of studding wild wheat relatives

Number	Name of the wild wheat relative
1	*Ae. speltoides*
2	*Ae. cylindrical*
3	*Ae. triuncialis*
4	*Ae. crassa*
5	*Ae. squarrosa*
6	*Ae. biuncialis*
7	*Ae. umbellolata*
8	*Ae. colomnaris*
9	*Tb. araraticum*
10	*T. boeticum*
11	*T. dicoccum*
12	*T. urartu*

[a, b]: *Aegilops* and *Triticum*

Data Analysis

The data obtained from SDS-PAGE and Acid-PAGE was scored for the presence (1) or absence (0) of the bands and entered in a binary data matrix. Based on the results of electrophoretic band spectra, similarity index was calculated for all possible pair of electrophoregrams. The similarity matrix thus generated was converted to a dissimilarity matrix and used to construct the dendrogram by the unweighted pair group average method (UPGMA).

Results and discussions

SDS-PAGE

For an effective breeding program, information regarding the extent and nature of genetic diversity within a crop species is essential. Protein electrophoresis is a useful method for describing the genetic structure of crop germplasm (Ciaffi *et al*., 1993). Northwest of Iran is one of the major center of origin and diversity of wheat and were home of wild relatives and wheat progenitors that were great importance in producing new diversity resource (Harlan and Zohary, 1966).

Cluster analysis of banding pattern of studding species based on simple matching coefficient and UPGMA resulted in 4 groups. First group comprised *T. araraticum* and *T. boeticum*. The similarities of subunits of *T. araraticum* and *T. boeticum* was outlined previously by researchers and it was outlined that the A genome of *T. araraticum* and *T.zhokowsky* was inherited from *T. boeticum* species (Ciaffi *et al*., 1998). Second group composed of *Triticum dicoccum and T. urartu* that was placed together in this group. This was confirmed that the A genome of *T. dicoccum* was originated from *T. urartu* along with the evolutionary process of crossing. Kimber and Feldman (1987) also observed the similarities of A genome of these two species. This result was formed the speculation that A genome of today improved cultivars originated from *T. urartu* species. Moghaddam *et al*., (2000) in the studding the isozies was maked similar conclusion. According to the results it ccould be concluded that The detected differences that were between these species might reflect their reproductive isolation and are consistent with recent nomenclatural and biosystematic treatments that recognise *T. urartu* as separate species from *T. boeoticum*. Third group comprised from another relatives. In this group some closer relation between the *Ae. cylindrica* and *Ae. squarossa* was clear and it could be close relation between the D genome of these two species. The last group comprised of Inia and Anza cultivars that were used as check in discriminant of the glutenin subunits. Also it was concluded that amont of

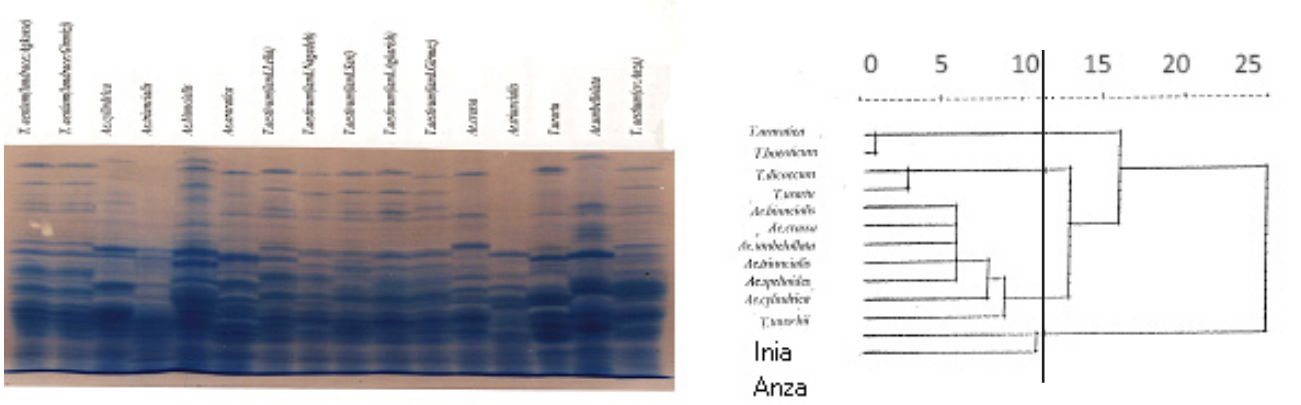

Fig. 1. Banding pattern of glutenin subunits using SDS-PAGE of studding genotypes and cluster analysis using UPGMA.

genetic variability of Glutenin subunits in the wild species was great and can be use in plant preeding (Levy *et al.*, 1988). However not only the glutenin subunit of the relatives was new and could be valuable in the bread making quality but also the A genome encoding subunits of *T. urartu* had close similarities with *T. dicoccum* and it can exploitated in the synthetic hexaploid wheat production. The potential of the glutenin subunits as biochemical marker in discrimination of the species was emphasized another time.

Acid-PAGE

According to the results (Fig. 2) cluster analysis of gliadins bands form 6 groups. First group comprised of *Ae. cylindrica, Ae. biuncialis, Ae. crassa and T. araratica*. Second group composed of Ae.umbelolatta, Ae.biunciallis and T. dicoccum. Third group also was formed from local landraces with code number 11477, 11488 and OMID cultivar and fourth one conformed of Marquis, Zarrin and Mv17; the fifth group formed by landrace with code number 12079 and Ae. taucshii. The last group conformed of *T. paresh* and *T. urartu*. The results showed that gliadin electrophoreisis showed higher level of polymorphism than glutenin and therefore could be better use in variety and species identification purposes. This criterion was based on their monomeric habit and cause more real classification. Some protein band that was so similar to 42 and 45 band was observed in some wild relatives such as *T. paresh, Ae. cylindrica, T. urartu, Ae. taucshii* and *Ae. Cressa* that was confirmed the ability of possible use of relatives through crossing for transferring valuable bands and increase the bread making quality.

Based on the results some close similarities observed between *T. urartu* and *T. aestivum*. This could be the evidence of that A genome of common wheat was derived from *T. urartu.*

Conclusion

The high level of polymorphism was detected in the glutenin and gliadin subunits of the wild wheat relatives. This polymorphism had some similarities with cultivated bread wheat and could be used in any breeding perspectives. It was concluded that Gliadin electrophoreses is a suitable method to discriminate wheat variety and species.

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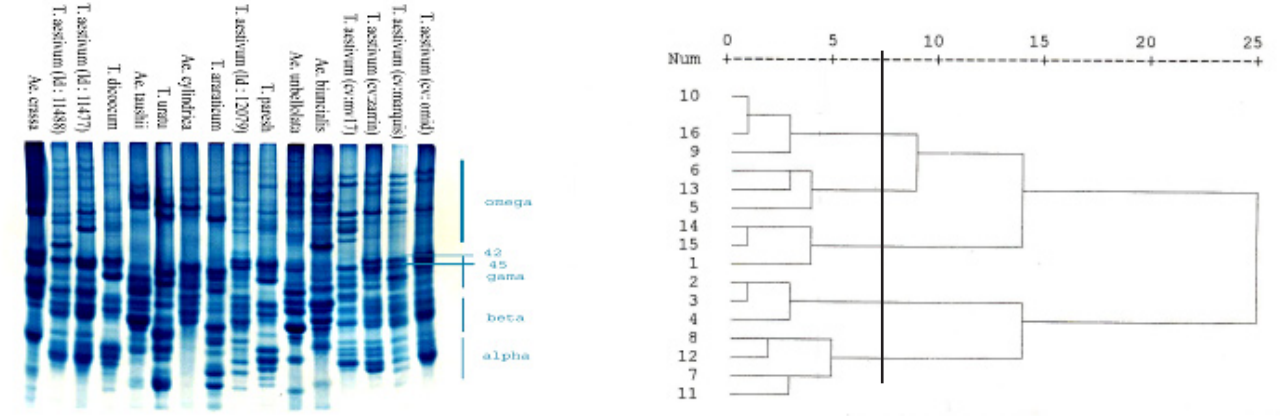

Fig. 2. Banding pattern of gliadin subunits using Acid-Page of studding genotypes and cluster analysis using UPGMA

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