Turk J Bot
27 (2003) 433-440

Research Article

Isozyme Variations in Some *Aegilops* L. and *Triticum* L. Species Collected from Central Anatolia

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Received: 01.11.2002
Accepted: 02.05.2003

Abstract: In this study the aspartate aminotransferase (AAT, E.C. 2.6.1.1), phosphoglucomutase (PGM, E.C. 5.4.2.2) and phosphoglucose isomerase (PGI, E.C. 5.3.1.9) isozyme patterns of nine different diploid and tetraploid wild wheat species belonging to the *Aegilops* L. and *Triticum* L. genera were analysed electrophoretically, using horizontal starch gel and non-denaturating polyacrylamide gel (only for AAT). All species were found to have three AAT isozyme zones (except for the AAT of *Aegilops speltoides* Tausch on starch gel) on both gels. While the migration distances of AAT-1 isozyme were similar, the AAT-2 and AAT-3 band patterns were different on both gels.

A single PGI enzyme zone was detected for diploid and tetraploid wild wheats, except for *Aegilops triuncialis* L. and *Aegilops biuncialis* Vis. These two species had two PGI enzyme zones on starch gel.

All the test species except for *Ae. biuncialis* showed only one PGM enzyme band, this species having two PGM zones.

The utilization of PGM and PGI isozymes as genetic markers to distinguish interspecific variation among different wild wheat species seems encouraging, particularly PGM for *Ae. biuncialis* and PGI for both *Ae. triuncialis* and *Ae. biuncialis*. However, the usefulness of the AAT-2 and AAT-3 zones as genetic markers needs further study.

Key Words: *Aegilops*, *Triticum*, isozyme polymorphism, genetic markers, genetic variation, electrophoresis.

Orta Anadolu'dan Toplanan Bazı *Aegilops* L. ve *Triticum* L. Türlerinde İzozim Varyasyonları

Özet: Bu çalışmada, *Aegilops* L. ve *Triticum* L. cinslerine ait dokuz adet diploid ve tetraploid yabani buğday türünün aspartat aminotransferaz (AAT, E.C. 2.6.1.1), fosfoglukomutaz (PGM, E.C. 5.4.2.2) ve fosfoglukoz izomeraz (PGI, E.C. 5.3.1.9) izozim modelleri yatay nişasta jel ve denatüre-etmeyen poliakrilamid jel (yalnız AAT için) kullanılarak, elektroforetik olarak analiz edilmiştir. Bütün türlerde (*Aegilops speltoides* Tausch nişasta jel sonucu hariç), her iki jelde farklı elektroforetik hıza sahip üç AAT izozim bölgesi bulunmuştur. Test edilen türler arasında AAT-1 izozimi için göç uzaklıkları çok az değişken olduğu halde, AAT-2 ve AAT-3 izozimleri her iki jelde farklı hızlarda göç etmişlerdir.

Aegilops triuncialis L. ve *Aegilops biuncialis* Vis. hariç, diğer diploid ve tetraploid yabani buğday türlerinde sadece bir PGI enzim bandı gözlendiği halde, bu iki türde nişasta jelde iki PGI bandı bulunmuştur.

Ae. biuncialis örnekleri hariç, bütün diploid ve tetraploid türler tek bir PGM enzim bandı vermiştir. Buna karşılık *Ae. biuncialis*'in iki PGM bölgesine sahip olduğu saptanmıştır.

Yabani buğday türleri arasındaki türler-arası değişkenlikleri ayırt etmede PGM ve PGI izozimlerinin genetik markır olarak kullanılabilecekleri görülmektedir. Özellikle *Ae. biuncialis* için PGM, *Ae. biuncialis* ve *Ae. triuncialis* için ise PGI enzimi umut verici sonuçlar vermiştir. Bununla birlikte, AAT-2 ve AAT-3 izozimlerinin genetik markır olarak kullanılabilmesi için ek çalışmalara gereksinim duyulmaktadır.

Anahtar Sözcükler: *Aegilops*, *Triticum*, izozim polimorfizmi, genetik markır, genetik çeşitlilik, elektroforez.

Introduction

Landraces and their wild relatives are an essential raw material source for genetic diversity maintenance and improvement programmes. Whether for pragmatic agricultural purposes or theoretical problem solving, particular plant genetic resources must be conserved, made available and characterised (Goodman, 1990). The characterization and conservation of plant genetic variation is difficult. In the first instance, characterization usually involves the description of variation for morphological traits. The genetic information provided by morphological characters is often limited. These limitations have resulted in the deployment of biochemical techniques such as isozyme and protein electrophoresis (Gottlieb, 1977; Crawford, 1989). Electrophoretic surveys of proteins play an important role in the quantitative evaluation and management of genetic resources. This is because information concerning the geographical and taxonomic distribution of genetic variation provides guidelines for sampling strategies and germplasm preservation (Machon et al., 1997). Gene-controlled enzyme variations constitute a rich source of taxonomic characters (Ayala, 1983). Isozyme analysis is an economical and effective method for the determination of mutation and recombination in genes and chromosomes (Brown, 1978; Jaaska, 1993). Isozymes are used as genetic markers to observe the recombination and segregation of linked qualitative and quantitative characters (Fleischmann, 1990). In addition, high or low genetic diversity among and within natural populations can be deduced by using different isozyme patterns (Stuber et al., 1980; Price et al., 1984; Michaud et al., 1995). Isozyme patterns obtained electrophoretically are frequently used as biochemical markers

i) in linkage studies (Golenberg, 1986; McMillin et al., 1986; Vahl et al., 1987; Melz & Thiele, 1990),

ii) in establishing whether or not chromosomes or chromosome segments among different individuals are transferred and in identifying different chromosomes (Salinas & Benito, 1985),

iii) in detecting the localization of genes on chromosomes (Drefahl & Buschbeck, 1991),

iv) in detecting gene expression changes seen in different developmental and differentiation stages (Vences et al., 1986; Chawla, 1988; Drefahl & Buschbeck, 1991),

v) in providing qualitative and quantative estimates of gene flow and divergence in switching on and off genes (Loxdale, 1994),

vi) in determining spreading limits and species separation of natural plant populations (Murphy et al., 1990; Jaaska, 1993).

Aspartate aminotransferase (AAT, E.C. 2.6.1.1.), phosphoglucomutase (PGM, E.C. 5.4.2.2.) and phosphoglucose isomerase (PGI, E.C. 5.3.1.9.) are three important enzymes which occur in glycolysis and amino acid metabolic pathways. It appears that culture varieties of wheat, barley and rye were employed as test materials in most of the studies dealing with the AAT, PGM and PGI isozymes.

The aim of this study was to characterize electrophoretic isozyme phenotypes of AAT, PGM, PGI and their variation patterns among some wild wheat species which grow in Central Anatolia.

Materials and Methods

Wild wheat species were collected from the provinces of Kayseri and Ankara (in squares B_5 and B_4, respectively) in Turkey. The names, ploidy levels and collection localities of the wild wheat samples are illustrated in Table 1. One gram of leaf tissue obtained from at least 15 individuals which were 14 days old were crushed into dust in a mortar with the help of a pestle by adding liquid nitrogen. A cold extraction buffer was added to this homogenate (modified from Marburger & Jauhar, 1989); 10% (w/v) hydrolysed potato starch (Sigma) was used for starch gel electrophoresis. Lithium–borate (pH

Table 1. Wild wheat species and collection localities.

Species name	Collection locality		Ploidy level
	Kayseri	Ankara	
Aegilops mutica Boiss.	+	+	Diploid (2n)
Aegilops umbellulata Zhuk.	+	+	Diploid (2n)
Aegilops speltoides Tausch	-	+	Diploid (2n)
Triticum monococcum L.	+	+	Diploid (2n)
Aegilops triuncialis L.	+	+	Tetraploid (4n)
Aegilops biuncialis Vis.	+	+	Tetraploid (4n)
Aegilops cylindrica Host	+	+	Tetraploid (4n)
Aegilops columnaris Zhuk.	+	-	Tetraploid (4n)
Aegilops ovata L.	-	+	Tetraploid (4n)

8.1), Tris–citrate I (pH 8.4) and Tris–citrate II (pH 7.0) were used as gel and running buffers. A 7.5% polyacrylamide vertical slab gel was used for PAGE (Bollag & Edelstein, 1991). The gels were stained using the methods of Pasteur et al. (1988) for AAT and of Harris & Hopkinson (1976) for PGM and PGI.

Results

All the materials showed three AAT regions with different electrophoretic mobilities (Figures 1-8). These three AAT zones were consistent with the earlier data obtained by Hart (1975) and Jaaska (1976) and named AAT-1, AAT-2 and AAT-3, respectively, from fast to slow migration rates.

The mobilities of the AAT-1 isozyme varied slightly among the diploid species studied, and the patterns in general were similar on both gels (Figures 1-4). The AAT-1 bands of all tetraploid group members except for *Aegilops cylindrica* Host and *Ae. triuncialis*, displayed the same mobilities on starch gel. On the other hand, the migration distances of the AAT-1 bands of these two species were at the same level, although shorter than the others (Figures 5 & 6). Nevertheless, few migration differences were observed among the AAT-1 bands of tetraploid species on PAGE (Figures 7 & 8).

The AAT-2 of all diploid species migrated to similar distances and formed bands from the origin on starch gel (Figures 1 & 2). This similarity applied to also PAGE, except for the Ankara and Kayseri samples of *Aegilops*

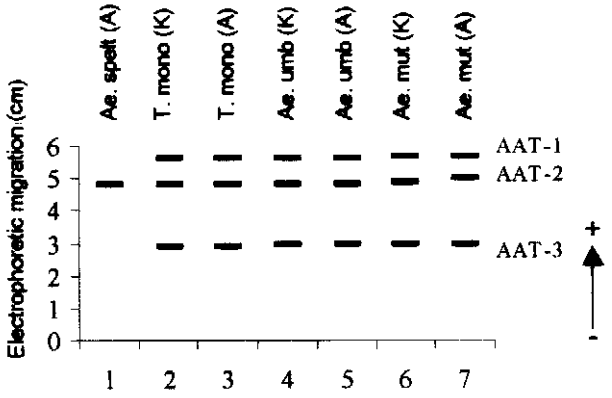

Figure 1. Schematic representation of AAT zymograms on starch gel. 1: *Ae. speltoides*; 2,3: *T. monococcum*; 4,5: *Ae. umbellulata*; 6,7: *Ae. mutica* A = Ankara, K = Kayseri

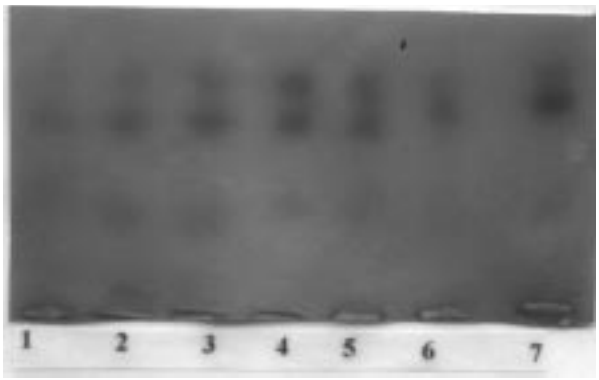

Figure 2. Zymograms of AAT on starch gel. 1: *Ae. speltoides*; 2,3: *T. monococcum*; 4,5: *Ae. umbellulata*; 6,7: *Ae. mutica* A = Ankara, K = Kayseri

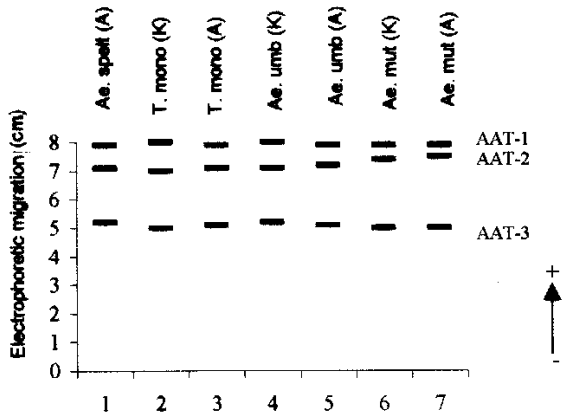

Figure 3. Schematic representation of AAT zymograms on polyacrylamide gel. 1: *Ae. speltoides*; 2,3: *T. monococcum*; 4,5: *Ae. umbellulata*; 6,7: *Ae. mutica* A = Ankara, K = Kayseri

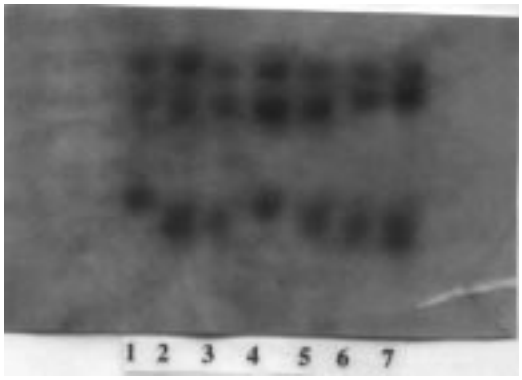

Figure 4. Zymograms of AAT on polyacrylamide gel. 1: *Ae. speltoides*; 2,3: *T. monococcum*; 4,5: *Ae. umbellulata*; 6,7: *Ae. mutica* A = Ankara, K = Kayseri

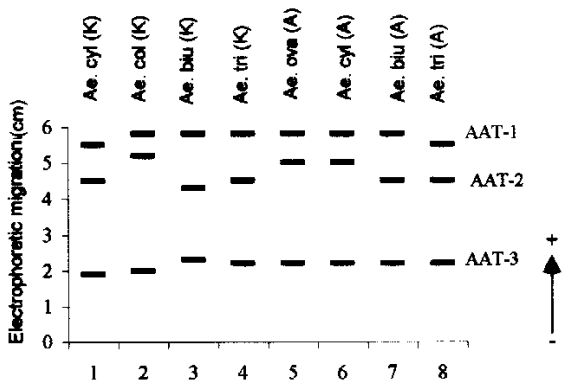

Figure 5. Schematic representation of AAT zymograms on starch gel. 1,6: *Ae. cylindrica*; 2: *Ae. columnaris*; 3,7: *Ae. biuncialis*; 4,8: *Ae. triuncialis*; 5: *Ae. ovata* A = Ankara, K = Kayseri

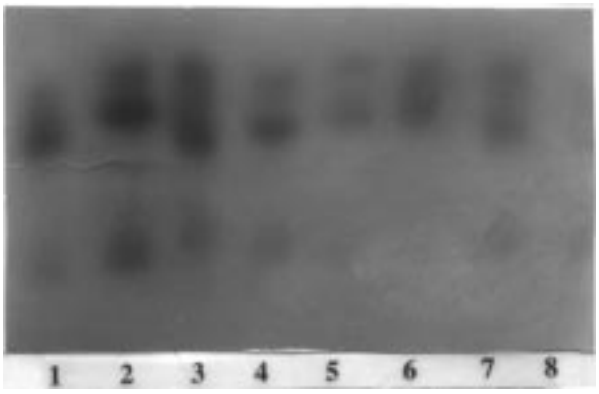

Figure 6. Zymograms of AAT on starch gel. 1,6: *Ae. cylindrica*; 2: *Ae. columnaris*; 3,7: *Ae. biuncialis*; 4,8: *Ae. triuncialis*; 5: *Ae. ovata* A = Ankara, K = Kayseri

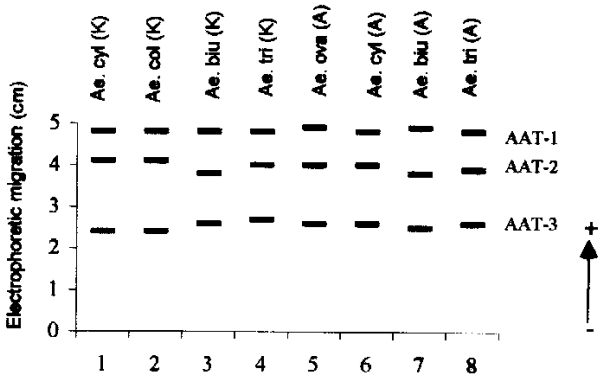

Figure 7. Schematic representation of AAT zymograms on polyacrylamide gel. 1,6: *Ae. cylindrica*; 2: *Ae. columnaris*; 3,7: *Ae. biuncialis*; 4,8: *Ae. triuncialis*; 5: *Ae. ovata* A = Ankara, K = Kayseri

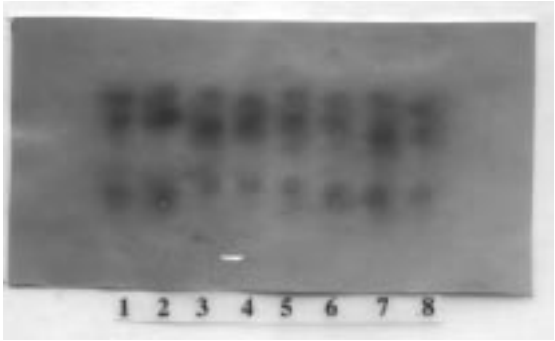

Figure 8. Zymograms of AAT on polyacrylamide gel. 1,6: *Ae. cylindrica*; 2: *Ae. columnaris*; 3,7: *Ae. biuncialis*; 4,8: *Ae. triuncialis*; 5: *Ae. ovata* A = Ankara, K = Kayseri

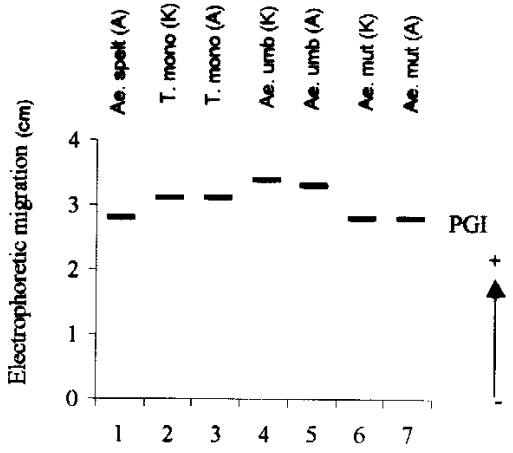

Figure 9. Schematic representation of PGI zymograms on starch gel. 1: *Ae. speltoides*; 2,3: *T. monococcum*; 4,5: *Ae. umbellulata*; 6,7: *Ae. mutica* A = Ankara, K = Kayseri

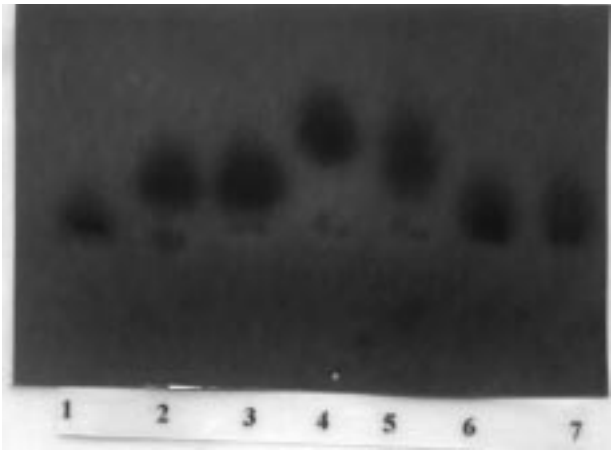

Figure 10. Zymograms of PGI on starch gel. 1: *Ae. speltoides*; 2,3: *T. monococcum*; 4,5: *Ae. umbellulata*; 6,7: *Ae. mutica* A = Ankara, K = Kayseri

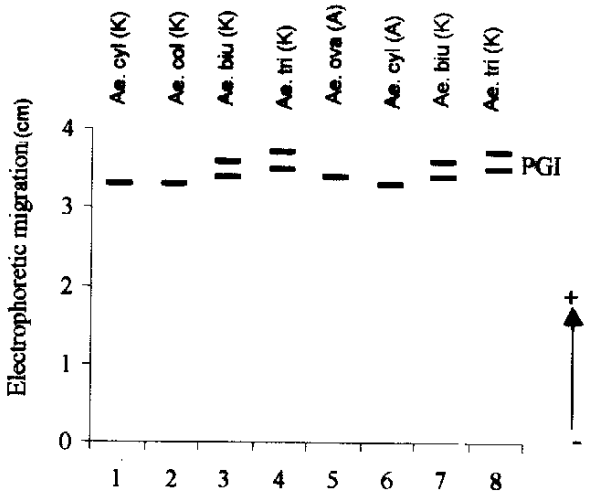

Figure 11. Schematic representation of PGI zymograms on starch gel. 1,6: *Ae. cylindrica*; 2: *Ae. columnaris*; 3,7: *Ae. biuncialis*; 4,8: *Ae. triuncialis*; 5: *Ae. ovata* A = Ankara, K = Kayseri

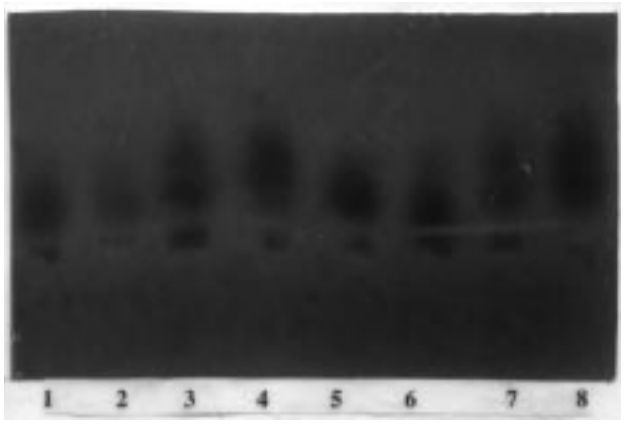

Figure 12. Zymograms of PGI on starch gel. 1,6: *Ae. cylindrica*; 2: *Ae. columnaris*; 3,7: *Ae. biuncialis*; 4,8: *Ae. triuncialis*; 5: *Ae. ovata* A = Ankara, K = Kayseri

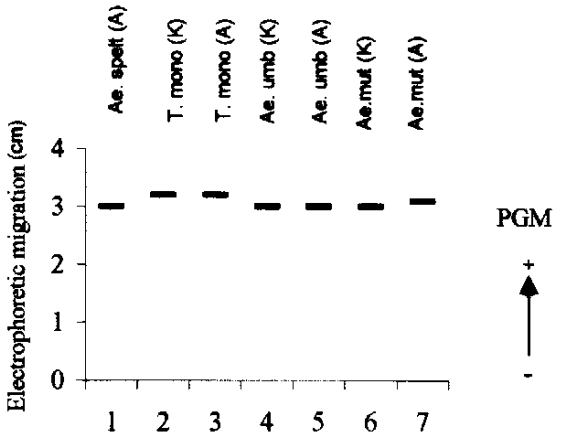

Figure 13. Schematic representation of PGM zymograms on starch gel. 1: *Ae. speltoides*; 2,3: *T. monococcum*; 4,5: *Ae. umbellulata*; 6,7: *Ae. mutica* A = Ankara, K = Kayseri

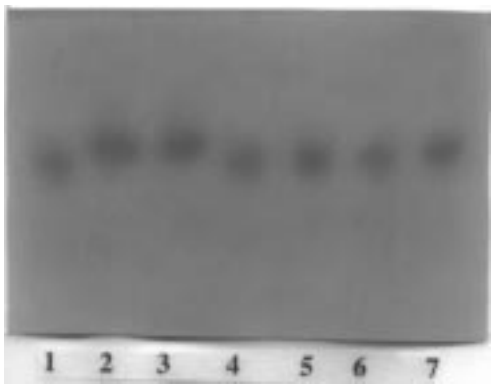

Figure 14. Zymograms of PGM on starch gel. 1: *Ae. speltoides*; 2,3: *T. monococcum*; 4,5: *Ae. umbellulata*; 6,7: *Ae. mutica* A = Ankara, K = Kayseri

mutica Boiss., which had different migration rates from the others (Figures 3 & 4). *Ae. speltoides*, one of the diploid group members, showed a single band for AAT (namely AAT-2) on starch gel, while all three isozyme bands were present on PAGE. This is a typical difference between the two gels (Figures 1-4). The AAT-2 bands of the tetraploid group had both inter-and intra-specific mobility differences on starch gel (Figures 5 & 6). The Ankara and Kayseri samples of *Ae. cylindrica* and *Ae. biuncialis* are good examples of these intra-and inter-specific variations.

The AAT-3 zones of all diploid wheats were almost on one level on starch gel (Figures 1 & 2). On the other hand, they had dissimilar migration levels on PAGE (Figures 3 & 4). A minor difference was observed among the AAT-3 zones of tetraploid wheats on starch gel (Figures 5 & 6). A similar finding also applied to the PAGE results (Figures 7 & 8).

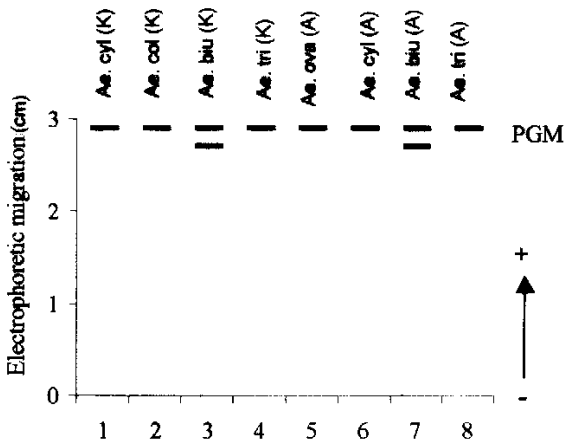

Figure 15. Schematic representation of PGM zymograms on starch gel. 1,6: *Ae. cylindrica*; 2: *Ae. columnaris*; 3,7: *Ae. biuncialis*; 4,8: *Ae. triuncialis*; 5: *Ae. ovata* A = Ankara, K = Kayseri

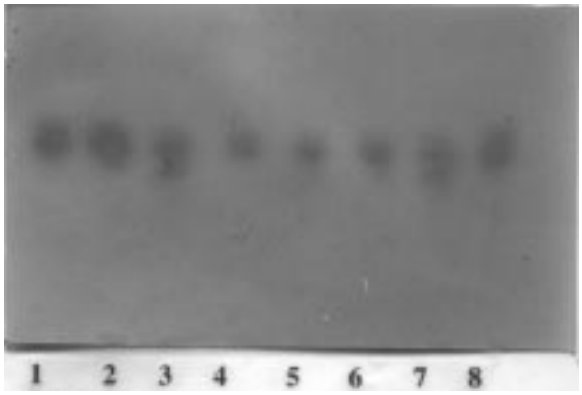

Figure 16. Zymograms of PGM on starch gel. 1,6: *Ae. cylindrica*; 2: *Ae. columnaris*; 3,7: *Ae. biuncialis*; 4,8: *Ae. triuncialis*; 5: *Ae. ovata* A = Ankara, K = Kayseri

In diploid wild wheat species we found only one enzyme zone belonging to PGI. However, the PGI band of *Aegilops umbellulata* Zhuk. migrated faster than the other PGIs belonging to other diploids (Figures 9 & 10). Tetraploid wild wheat species, except for *Ae. triuncialis* and *Ae. biuncialis*, had a single PGI enzyme zone. On the other hand, these two species had two PGI enzyme zones on starch gel (Figures 11 & 12).

All diploid and tetraploid species, except for *Ae. biuncialis*, showed only one PGM band (Figures 13-16), while the *Ae. biuncialis* samples had two zones (Figures 15 & 16). Having an extra band for PGM makes *Ae. biuncialis* easily distinguished from the other tetraploids. Diploid *Triticum monococcum* L. had a faster PGM band, differing from the other diploid species (Figures 13 & 14).

Discussion

Unfortunately, little data covering the wild wheat species which we selected to analyse were available in the literature. Benito et al. (1987) reported three AAT zones in *Ae. umbellulata* which were compatible with our results for this species. On the other hand, Fleischmann (1990) reported the presence of two AAT zymotypes in *Ae. umbellulata* strains. These zymotypes were defined as ZT1u and ZT2u. In spite of his experimental results, which had a visually undetectable AAT-1 band (zone I), Fleischmann (1990) predicted the occurrence of a hypothetical zymotype corresponding to the AAT-1 band for the species *Ae. umbellulata* and *Ae. triuncialis*.

The conclusions related to the differing mobilities of AAT-2 and AAT-3 zones suggest that although the genes which code isozymes in different species are conservative, some degree of differentiation may occur in certain zones. Changes in the DNA of an enzyme locus may change which amino acids are in a sequence and thus may change the amount of static charge on an enzyme molecule.

Due to the very low amounts of isozymes, these cannot be stained or seen very well. This may be a reason for *Ae. speltoides* showing only one band for AAT (namely AAT-2) on starch gel, while all three AAT isozymes were observed on polyacrylamide gel. Therefore, the use of both PAGE and starch gel electrophoresis at the same time is useful and makes it possible to obtain results which could not be obtained using only one of them.

Benito et al. (1987) have reported five bands belonging to the PGI enzyme in *Ae. umbellulata*, in contrast to our results, which showed only one PGI zone in this species.

We found only one band for PGM in *Ae. umbellulata*. This conclusion is in accordance with the findings of Benito et al. (1987).

The existence of an extra band for PGI in *Ae. biuncialis* and *Ae. triuncialis* and for PGM in *Ae. biuncialis* probably indicates a different allele for these two enzymes. This second allele may have occurred from the first one by duplication and have separated by point mutations. Gene duplication in diploid species and the addition of genomes

in polyploid species may increase the number of isozymes (Gottlieb, 1982).

Our experiments showed different migration rates for at least two enzymes, PGI and PGM, and these isozymes belonging to different species or different populations of the same species may well represent some degree of genetic variation among them.

Interspecific enzyme variation may also be explained by the effect of environmental conditions (Cleland et al., 1996). There are some findings that environmental heterogeneity, especially climatic factors and changes in geographical continuity, may play an important role in genetic variability (Nevo et al., 1982; Michaud et al., 1995). In addition to these, soil factors were proposed as an important factor in genetic diversity (Nevo et al., 1988). Enviromental factors may operate on the marker loci proper and/or on any block of genes with which they are associated (Nevo et al., 1982).

In conclusion, the differences in isozyme phenotypes, although not reflected in the phenotypic appearance of the species, may be a sign of genetic diversity of some isozyme loci. Therefore, it appears that electrophoresis can detect the most important variability in the structural genome via its expressed protein products.

It seems probable that this method will continue to be the cheapest and easiest for studying genetic variation for years to come.

Acknowledgements

We are grateful to Dr. Alptekin Karagöz and Dr. Meral Peşkircioğlu for their help in classifying the collected samples used in this study. This work was supported by a grant from the Hacettepe University, Research Foundation and by The Scientific and Technical Research Council of Turkey.

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