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# The protein fingerprinting and genetic variations among different genotypes of *Aegilops* belonging to four sections

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## Abstract

The significance of this research is the study of genetic relationships between genotypes which is one of the first steps taken to preserve genetic diversity from loss and one of the key processes for supporting and developing breeding programs. Wheat-associated wild *Aegilops* species are considered to be an important source of genetic resources and to reveal the relationships and genetic variations between genotypes by using storage proteins to obtain a protein fingerprint. The research aims to obtain a protein fingerprint of genotypes of the genus *Aegilops* using the methods of Acid Polyacrylamide Gel Electrophoresis (A-PAGE) and Sodium Dodecyl Sulphate Polyacrylamide Electrophoresis (SDS-PAGE) and to study genetic diversity using storage proteins as biochemical markers. Laboratory work was carried out in 2019, at the Atomic Energy Commission in Damascus. Ten genotypes belong to four sections (*Sitopsis*, *Aegilops, Cylindropyrum, and Vertebrata*) were compared (*Ae.var speltoides, Ae.speltoides, Ae.tauschii, Ae.umbellulata, Ae.vavilovii, Ae.biuncialis, Ae.cylindrica, Ae.geniculata, Ae.kotschyi, Ae.peregrina)*. Using Unweighted Pair Group Mean Arithmetic Average (UPGMA) to illustrate the genetic relationships between the studied genotypes, the phylogenetic tree was constructed based on data on gliadin and glutenin proteins. The total band count was 110 lines. The smallest number of bands was *Ae.cylindrica* (30), whereas the highest number of bands was 44 (*Ae.peregrina*). The least genetic distance was observed among the genotypes (*Ae.speltoides* and *Ae.tauschii*) (0.24), while the most genetic distance was observed among the genotypes (*Ae.biuncialis* and *Ae.tauschii*) (0.48). The lowest average of Percent Disagreement Values (PDVs) was for *Ae.kotschyi* and *Ae.speltoides* (0.32), meaning they are the closest to the rest of the genotypes studied. While the highest average of (PDVs) was for *Ae.biuncialis* (0.37), meaning it is the farthest to the rest of the genotypes studied.

**Keywords**: **Genetic Relationships, *Aegilops*, Protein Fingerprint, A-PAGE, SDS-PAGE.**

## Introduction

Wild relatives of wheat played an important role in the evolution of varieties with desirable characteristics and have been able to persevere and adapt under different environmental conditions (Jiang *et al*.,1993; Nevo *et al*., 2002) making them a rich source of many valuable genes, especially after wheat crops have been exposed to many biotic and abiotic stresses (Medouri *et al*., 2015), and the erosion of the plant genetic base for this crop under climate and environmental fluctuations and human activities.

Like genus *Aegilops*, which was known as Goatgrass, which is an annual autogamous weed, belonging to *Poaceae*, grows at different heights around the Mediterranean basin and in West and Central Asia (Van Slageren, 1994; Kilian *et al*., 2011; Al-ahmar *et al*., 2010). It is the genus most closely related to *Triticum* L. which includes the cultivated wheat *Triticum aestivum* (Raskina *et al*.,2004) and the species of *Aegilops* are considered a suitable genetic resource for the genetic improvement of wheat, resistant to diseases and pests (Gill *et al*., 1985) and have desirable agricultural traits such as tolerance to drought and salinity (Colmer *et al*., 2006; Molnár *et al*., 2004), which explains their ability to persevere and adapt under stressful environmental conditions (Nevo *et al*., 2002). They are also excellent sources of resistance to rust and rot (Damania & Pecetti, 1990; Dimov *et al*., 1993).

Depending on the phenotype and analysis of the genome, it was divided into five sections: (*Aegilops, Comopyrum, Cylindropyrum, Sitopsis,* and *Vertebrata*) contains different Ploidy Levels:

1. Diploid: (Diploid 2n = 14).

2. Polyploid: It was divided into two groups of tetraploid (Tetraploid 2n = 28) and hexaploid (Hexaplpid 2n = 42).

*Aegilops* L. has various genome groups (D, B = S, U, C, T, N, and M) (Van Slageren, 1994). Research has shown that *Ae.speltoides* was the main donor of the B genome to polyploid wheat and the donor for the D genome is *Ae.tauschii* (Huang et al., 2002).

Therefore, it is important to study the genetic variation between the *Aegilops* species, for their conservation and use in improving the cultivated wheat (Monneveux *et al*., 2000; Hatami *et al*., 2010).

Storage proteins in grains are important biochemical markers that can be used in the detection of genetic variances, as they reflect part of the genetic information of the genotype, and have been used to assess different germplasm and identify varieties in crops (Alnaddaf, 2013; Payne *et al*., 1984; Belhais, 2014).

Storage proteins have been the focus of many genetic studies, due to their economic importance and being one of the taxonomic tools in plants, and the wild relatives showed great genetic diversity in storage proteins, which had a major role in the technological properties of wheat (Ciaffi *et al*., 1993; Nevo & Payne, 1987). Gluten is the main storage protein in wheat grains and its wild relatives, consisting of the proteins gliadin (alcohol-soluble) and glutenin (insoluble) (Carrillo *et al*., 1990), representing about 80% of the total proteins in the grain (Bietz & Wall, 1973) and has been known for its role in determining the properties of the dough and the quality of bread for a long time (MacRitchie, 1992). Gliadin is a monomeric polypeptide protein that contributes to the physical properties of the dough. It is classified into four groups of (α, β, γ and ω) based on molecular mobility at low pH in Acid Polyacrylamide Gel Electrophoresis (pH=3.1) (Wrigley *et al*., 2006; Woychik *et al*., 1961; Bushuk & Zillman, 1978). The glutenin is a polymeric protein stabilized by disulfide bonds, and it separated into two groups, high molecular weight (HMW) and low molecular weight (LMW) subunits glutenin after being treated with reducing agents such as mercaptoethanol (Payne & Lawrence, 1983) and it is responsible for the elasticity characteristic of the dough (Payne et al., 1984; Mir Ali, 2000).

Gliadins are encoded by six Gli loci mapped to the short arms of homoeologous group 1(Gli-A1, Gli-B1, and Gli-D1) and group 6 (Gli-A2, Gli-B2, and Gli-D2) chromosomes (Payne, 1987) and The HMW-GS are encoded by genes at three loci, Glu-A1, Glu-B1 and Glu-D1, located on the long arms of homoeologous group1 chromosomes, while The LMW-GS are encoded by genes at three loci, (Glu-A3, Glu-B3, Glu-D3), located on the short arms of the same chromosome (Singh & Shepherd, 1988).

Storage proteins are good markers in assessing taxonomic relationships and phylogenetic evolution at different levels of species (Kharazian, 2008), and were used to study the genetic variation in wild relatives of wheat (*Ae.tauschii, Ae.speltoides, T.monococcum* L*., T.uartu*) (Xynias *et al*., 2007).

The *Aegilops* L. species showed great genetic diversity in the gliadin and glutenin proteins (Nevo, & Payne, 1987; Ciaffi *et al*., 1993). Electrophoresis techniques made it possible to identify storage proteins in grains (Belhais, 2014).

Most research has focused on studying genetic diversity using A-PAGE to separate gliadin and SDS-PAGE to separate glutenin into their smaller parts (Murray, 1997) depending on their molecular weight in an electric field, where different protein patterns appear as bands which become visible after staining (Godfrey, 2008), A-PAGE and SDS-PAGE provide an easy and convenient method to obtain protein fingerprinting for varieties. It can also be used to give a comprehensive knowledge of storage proteins in wild wheat grains. (Sofalian & Valizadeh, 2009) confirmed in their results that there was genetic variation between storage proteins in the seeds of wild relatives, and it could be used in genetic improvement programs.

This research aims to obtain a protein fingerprint of genotypes of the genus *Aegilops* using the methods of Acid Polyacrylamide Gel Electrophoresis (A-PAGE) and Sodium Dodecyl Sulphate Polyacrylamide Electrophoresis (SDS-PAGE) and to study genetic diversity using storage proteins as biochemical markers.

## Materials and Methods

**Plant material:** grains of Ten genotypes belong to Genus *Aegilops* were obtained from The International Center for Agricultural Research in the Dry Areas (ICARDA), which belong to four sections (*Sitopsis*, *Aegilops*, *Cylindropyrum* and *Vertebrata*) (Table 1).

**Table (1): The names of the studied genotypes**

| The sequence | Name of genotype | Genome | Ploidy level | Section |
|---|---|---|---|---|
| 1 | *Ae.var speltoides* | SS | 2x=14 | *Sitopsis* |
| 2 | *Ae.speltoides* | SS | 2x=14 | |
| 3 | *Ae.tauschii* | DD | 2x=14 | *Vertebrata* |
| 4 | *Ae.umbellulata* | UU | 2x=14 | *Aegilops* |
| 5 | *Ae.vavilovii* | DDSSMM | 6x=42 | *Vertebrata* |
| 6 | *Ae.biuncialis* | UUMM | 4x=28 | *Aegilops* |
| 7 | *Ae.cylindrica* | DDCC | 4x=28 | *Cylindropyrum* |
| 8 | *Ae.geniculata* | UUMM | 4x=28 | |
| 9 | *Ae.kotschyi* | UUSS | 4x=28 | *Aegilops* |
| 10 | *Ae.peregrina* | UUSS | 4x=28 | |

**Analytical methods:** Two electrophoretic systems were utilized A-PAGE (Bushuk and Zillman, 1978) for gliadin separation and the SDS-PAGE (laemmli, 1970) for glutenin separation as modified by (Mir Ali, 2000).

About 20 mg of crushed grains were used from each genotype. The analysis was performed in the laboratory of biotechnology department at the atomic energy commission in Damascus-Syria in 2019.

**A-PAGE:** The gliadin was extracted by adding 66µl of 70% ethanol, vortexed and mixed for 2.30 h then centrifuged for 15 min at 14000 rpm in an Eppendorf microcentrifuge. 100µl from the supernatant were added to 85µl of 60% (v/v) glycerin and a 25µl of the mixture was run on 6% Acrylamide gels (160x180x1.5mm) using an electric current of 50 mA for 3.30 h. The gel contained 10 genotypes in addition to the Canadian variety Marquis as a control.

**SDS-PAGE:** The glutenin was extracted from the residue of the same samples and fractionated in 10%(w/v) polyacrylamide gels. Each sample was suspended in a medium containing 2%(w/v) SDS, 5%(w/v) 2-mercaptoethanol, 0.001%(w/v) pyronin, 10%(v/v) glyceroland 1M Tris-HCl (pH 6.8). The samples were left for 90 minutes at room temperature and shaken every 15 minutes. Later, they were placed in a boiling water bath for 3 min and allowed to cool and were put in an Eppendorf microcentrifuge for 15 min at 14000 rpm. 25µl from each sample were placed into each slot of a vertical slab gel electrophoresis unit. A constant current of 25 mA was used to run two gels for 17 h.

Gels were stained overnight with 0.01% (w/v) Coomassie Brilliant Blue, Ethanol and acetic acid (10%) and then distained overnight in water for at least 24 h.

**Data analysis:** The data obtained from A-PAGE and SDS-PAGE were scored for the presence (1) or absence (0) of the bands and entered in a data matrix**.** Followed by setting up the cluster analysis by Unweighted Pair Group Mean Arithmetic Average method (UPGMA) and Percent Disagreement Values (PDVs) of the STATISTICA program were used to construct the matrixes and the dendrograms (STATSOFT Inc. 2003) to illustrate the genetic relationships among the studied genotypes, and the phylogenetic tree was constructed based on data on gliadin and glutenin proteins.

## Results and Discussion

The number of protein bands obtained from the data of A-PAGE and SDS-PAGE for the studied genotypes ranged from 30 to 44 bands. The total number of bands was 351, with an average of 35.1. The total bands count were 110 lines, which were analysed to get cluster analysis resulting from the protein bands obtained from the two previous methods. The lowest number of bands was 30 (*Ae.cylindrica*), whereas the highest number of bands was 44 (*Ae.peregrina*) shown in (Table 2).

The obtained data were combined, analyzed and presented in one dendrogram showing that species were grouped in two main clusters (Fig. 1). The first included (*Ae.var speltoides*, *Ae.kotschyi*, *Ae.speltoides*, *Ae.tauschii*, *Ae.cylindric*, *Ae.vavilovii*) and the second included all remaining species (*Ae.geniculata*, *Ae.biuncialis*, *Ae.umbellulata, Ae.peregrina*). The least genetic distance was observed among the genotypes (*Ae.speltoides* and *Ae.tauschii*) PDV=0.24, while the most genetic distance was observed among the genotypes (*Ae.biuncialis* and *Ae.tauschii*) PDV=0.48. The lowest average of PDVs was for *Ae.kotschyi* and *Ae.speltoides* (0.32), meaning they are the closest to the rest of the studied genotypes, while the highest average of PDVs was for *Ae.biuncialis* (0.37), meaning it is the farthest to the rest of the studied genotypes.

**Table (2): Number of total bands obtained from A-PAGE and SDS-PAGE**

| The sequence | Name of genotype | Number of bands |
|---|---|---|
| 1 | *Ae.varspeltoides* | 34 |
| 2 | *Ae.speltoides* | 31 |
| 3 | *Ae.tauschii* | 35 |
| 4 | *Ae.umbellulata* | 40 |
| 5 | *Ae.vavilovii* | 35 |
| 6 | *Ae.biuncialis* | 33 |
| 7 | *Ae.cylindrica* | 30 |
| 8 | *Ae.geniculata* | 34 |
| 9 | *Ae.kotschyi* | 35 |
| 10 | *Ae.peregrina* | 44 |

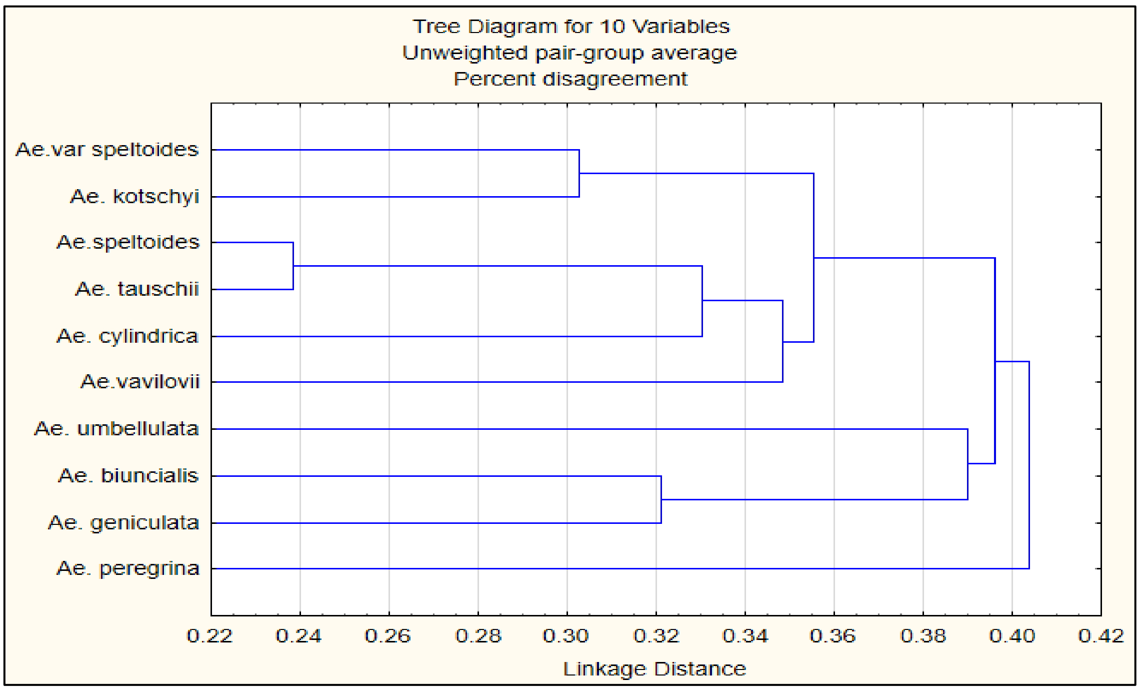


**Fig (1): Cluster analysis of *Aegilops* species based on A–PAGE and SDS–PAGE**

Our results were in agreement with many previous studies that proved a close genetic relationship between the D-genome and the S-genome (Haider *et al*., 2010; Huang *et al*., 2002), in which diploid *Ae.tauschii* (DD) grouped close to the diploid *Ae.speltoides* (SS) and they were the most closely related species to each other based on protein analysis (PDV=0.24), and this agrees with previous studies, (Haider *et al*., 2015) and (Monneveux *et al*., 2000) revealed that *Ae.tauschii* was the closest genetically to *Ae.speltoides*, and (Alnaddaf *et al*.,2013) also showed that *Ae.speltoides* separated from the remaining four genotypes from the *Sitopsis* section and grouped with *Ae.tauschii*. In addition, both of these genotypes contributed to the origin of wheat *T. aestivum* (McFadden & Sears, 1946) and proved to be important in the cultivation of cultivated wheat. The storage protein analysis showed *Ae.umbellulata* clustering with *Ae.biuncialis* and *Ae.geniculata* in the second cluster of the cluster analysis (Fig. 1) where *Ae.umbellulata* was a parent for these two genotypes, and it reflected the genetic relationships between *Ae.biuncialis* and *Ae.geniculata*, which appeared as sister species and they belong to the same section because they had the same genome formula (UUMM) as suggested by (Resta *et al*., 1996), and this was in agreement with (Al-ahmar *et al*., 2010) and (Alnaddaf, 2013), where these two genotypes were closely related genetically.

**Table (2): The PDVs matrix based on the combination of A-PAGE and SDS-PAGE**

| Variable | *Ae.var speltoides* | *Ae.speltoides* | *Ae. tauschii* | *Ae.umbellulata* | *Ae.vavilovii* | *Ae. biuncialis* | *Ae. cylindrica* | *Ae.geniculata* | *Ae.kotschyi* | *Ae.peregrina* |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Ae.var speltoides*** | 0.00 | | | | | | | | | |
| ***Ae.speltoides*** | 0.36 | 0.00 | | | | | | | | |
| ***Ae. tauschii*** | 0.36 | **0.24** | 0.00 | | | | | | | |
| ***Ae.umbellulata*** | 0.39 | 0.34 | 0.47 | 0.00 | | | | | | |
| ***Ae.vavilovii*** | 0.36 | 0.33 | 0.37 | 0.41 | 0.00 | | | | | |
| ***Ae. biuncialis*** | 0.38 | 0.46 | **0.48** | 0.38 | 0.42 | 0.00 | | | | |
| ***Ae. cylindrica*** | 0.41 | 0.31 | 0.35 | 0.36 | 0.35 | 0.42 | 0.00 | | | |
| ***Ae. geniculata*** | 0.37 | 0.36 | 0.36 | 0.40 | 0.41 | 0.32 | 0.32 | 0.00 | | |
| ***Ae. kotschyi*** | 0.30 | 0.35 | 0.33 | 0.41 | 0.35 | 0.42 | 0.33 | 0.36 | 0.00 | |
| ***Ae. peregrina*** | 0.46 | 0.43 | 0.45 | 0.40 | 0.38 | 0.39 | 0.38 | 0.42 | 0.32 | 0.00 |
| **Average** | 0.34 | 0.32 | 0.34 | 0.36 | 0.34 | 0.37 | 0.33 | 0.33 | 0.32 | 0.36 |

In addition, *Ae.biunacialis* and *Ae.geniculata* were sister species in the phylogenetic trees of (Haider *et al*.,2010) based on DNA analysis data using RAPDs and ISSRs, (Badaeva *et al*.,2004; Bandou *et al*., 2009) confirmed that the genome (UUMM) of *Ae.biuncialis* and *Ae.geneculata* originated from the diploid ancestors *Ae.umbellulata* (UU) and *Ae.comosa* (MM) and this agreed with our current study.

The results of this study were in agreement with (Alnaddaf *et al*., 2012) and (Haider *et al*., 2012) of the presence of genetic relations between the genotypes *Ae.umbellulata*, *Ae.biuncialis* and *Ae.geniculata*, which were classified in U-genome group, they were clustered in the same cluster in the tree except for *Ae.peregrina* and *Ae.kotschyi*, which separated into different clusters although they were classified within the same section *Aegilops* (Van Slageren, 1994) and had very similar genome (UUSS) (Przewieslik-Allen *et al*., 2018).

The tetraploid *Ae.kotcshyii* (SSUU) was clustered close to *Ae.varspeltoides* (SS) in this studied phylogenetic tree, and the reason for this genetic affinity may be attributed to *Ae. var speltoides* being one of the parents of *Ae.kotschyii* because any genotype of *Sitopsis* may be the donor of the S-genome (Yamane & Kawahara, 2005), and morphological, geographic, and cellular studies, as well as molecular evidences, assumed that *Ae.speltoides* or other genetically close relatives were nominated as donors to the S-genome (Alnaddaf, 2013; Dovrak & Zhang, 1992).

Both (Alnaddaf *et al*., 2012) and (Konstantinos & Bebeli, 2010) indicated the presence of a genetic relationship between *Ae.kotcshyi* and *Ae.umbellulata*, and this was in disagreement with the results of our studied phylogenetic tree, where *Ae.kotcshyi* separated from *Ae.umbellulata* (PDV=0.41) although that *Ae.umbellulata* was the donor parent of U-genome, and *Ae.kotcshyi* was closer to the second donor parent of S-genome (PDV=0.30).

The diploid *Ae.tauschii* (DD) was a parent of tetraploid *Ae.cylindrica* (DDCC) (Wan *et al*., 2002) and they had similar genomes according to (Jaaska, 1981; Kharazian, 2008), the results of the studied phylogenetic tree analysis reflected these facts. It showed the existence of a genetic relationship between these genotypes *Ae.tauschii* and *Ae.cylindrica*, where they clustered in same cluster, (Alnaddaf *et al*., 2013) confirmed that based on ITS data.

In addition, a genetic relationship has been shown between *Ae.cylindrica* (DDCC) and the hexaploid *Ae.vavilovii* (DDSSMM) and this relationship can be explained by the similarity in the D genome of the common ancestor *Ae.tauschii* (Przewieslik-Allen *et al*., 2018) that clustered closely in the same cluster with these two genotypes in the studied tree.

The most genetic distance was observed among the genotypes *Ae.biuncialis* and *Ae.tauschii* (PDV=0.48), (Yen *et al*. 2005) indicated that the results of molecular analyses have shown that genomes S, B, D and A were much more closely related to each other than to other genomes (Dvorak and Zhang, 1990).

The results of our study of gliadin and glutenin proteins were consistent with the classification by

(Van Slageren, 1994) of the genus *Aegilops*, as A-PAGE and SDS-PAGE technology reflect the genetic similarities between *Aegilops* species belonging to each of the four sections. The results showed that the genotypes with similar genomes were clustered close together in the phylogenetic tree that showed relationships between them may have the same parents and this corresponds to (Baranduzi *et al*., 2013).

It was believed that the genetic variation present within each genetic loci was responsible for the variances between species with regard to protein quality expressed by gene silencing or genes that did not express themselves (Mir Ali *et al*., 1999). The genetic variation that can be detected at the level of proteins is based on allelic variation for a limited number of protein loci responsible for gliadins and glutenins (Haider *et al*., 2010).

## Conclusions

This study showed the efficiency of the A-PAGE and SDS-PAGE electrophoresis methods applied to storage proteins in detecting the genetic differences between the studied genotypes of the genus *Aegilops*, indicating the possibility of using these proteins (gliadin and glutenin) as useful biochemical markers for studying relationships and genetic diversity. Our study confirmed the genetic relationships between the genotypes of the genus *Aegilops* according to their genetic origin. The PDVs matrix of the genetic distance between the *Ae.speltoides* and *Ae.tauschii* reflected a strong relationship between the S and D genomes.

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