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Evaluation of grain morphometry and gliadin diversity among twenty accessions of the genus *Aegilops* from the National collection of Bulgaria

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Abstract. The objective of the study was to evaluate the genetic diversity in twenty *Aegilops* accessions belonging to five plant species with respect to specific grain morphometric parameters and gliadin proteins, employing digital image analysis and A-PAGE electrophoresis. The considerable variation was observed among accessions for grain morphometric traits - area, perimeter, length, width and colour of grain. A total of 96 gliadin polymorphic bands were identified, with the number of bands varying between 13 and 22 per accession. The overall genetic diversity in the samples based on the patterns observed for each of the four gliadin regions showed that the ω, γ and β regions had high genetic variation index (H = 0.950), while α regions (H = 0.938), respectively. The mean genetic diversity estimate was high (H = 0.947). The average genetic similarity between all 20 genotypes ranges from 0.14 to 0.93 with a mean of 0.56, indicating that the studied accessions exhibited considerable genetic variability. The study found that the clustering of *Aegilops* genotypes was not linked to their geographic origin. Two-dimensional Principal Coordinates Analysis (PCoA) based on the gliadin and morphometric analyses revealed wide genetic dissimilarity between most of the genotypes, explaining 97.16% of the variations, with the model explaining 97.16% of the observed variation. Of this, PCo1 accounted for 93.65% and PCo2 for 3.51%. Genotypes with analogous genomes were grouped in close proximity within the phylogenetic tree, indicating that their evolutionary relationships may have originated from the same parental lineage.

Key words: *Aegilops*, A-PAGE, diversity, gliadins, grain morphometry.

INTRODUCTION

Aegilops is a genus within the grass family, Poaceae. They are commonly referred to as goatgrasses. *Aegilops* comprises 23 species, each with a distinct genome, including D, S, U, C, N, and M (Kishii, 2019). These wild relatives of wheat are valuable genetic resources for crop improvement due to their diverse genetic makeup (Fatemeh et al., 2014;

Aljrf et al., 2021; Adhikari et al., 2023). Despite the difficulties associated with working with wild species, such as inbreeding and incompatibility, they have made a significant contribution to wheat breeding. *Aegilops* species have been identified and incorporated into wheat varieties, resulting in the introgression of a number of genetically-encoded resistance genes. For example, *A. speltoides* possesses the Sr32 gene for stem rust resistance in wheat (Friebe et al., 1996). Stripe rust resistance gene *YrAS2388* is found in *A. tauschii* (Huang et al., 2011). The powdery mildew resistance gene(s) are localized to chromosome 2Mb of *A. biuncialis* (Li et al., 2019), Lr58 resistance gene against leaf rust originates in *A. triuncialis* carrying effective for many years (Arora et al., 2021). It is established that this genus of plants contributes to the enhancement of traits such as productivity, in addition to tolerance to abiotic stressors such as drought and elevated temperatures (Kishii, 2019).

Gliadins are a group of proteins found in wheat and related species like *Aegilops*. They play a crucial role in determining the extensibility properties of gluten dough, which is essential for bread-making (Medouri et al., 2015). The gliadins are alcohol-soluble monomeric proteins. They are encoded by the Gli-1 and Gli-2 loci, on the short arms of group 1 and 6 chromosomes and are divided into α-, β-, γ-, and ω-gliadins based on electrophoretic mobility in Acidic Polyacrylamide Gel Electrophoresis (A-PAGE) (Xiong, 2008). Chromosomal location studies have shown that clusters of genes, Gli-A1, Gli-Bl, and Gli-Dl, take control of γ- and ω-gliadins. These clusters are observed on the short arms of group 1 chromosomes. In addition, Gli-A2, Gli-B2, and Gli-D2, which are located on the short arms of group 2 chromosomes, control α- and β-gliadins (Pourmohammadi et al., 2023). As evidenced in the study undertaken by Žilić (Žilić et al., 2011; Žilić, 2013), a prominent and highly stained polypeptide chain with an approximate molecular weight of 42 to 44 kDa was observed across all durum wheat genotypes. This polypeptide chain is situated within the γ-gliadin region and was notably absent in the examined bread wheat genotypes. Aljrf et al. (2021) observed that A-PAGE is a straightforward and efficient approach for obtaining protein fingerprints from cultivars, which could be employed to investigate their composition and variation. Additionally, it could be utilized to gain a comprehensive understanding of storage proteins in the grains of wheat wild relatives. Furthermore, Utebayev et al. (2019) posit that protein synthesis is genetically determined and is subject to modulation in response to environmental stimuli, which in turn gives rise to variations in the levels of the corresponding proteins.

Gliadin electrophoresis has been shown to offer certain advantages over DNA molecular markers when assessing plant genetic resources. Firstly, it is a less expensive and less labour-intensive method of detecting polymorphism. Secondly, gliadin electrophoresis presents variation independently of genotype/environment. This is the primary reason why it is utilised to compare diversity within and between accessions during the maintenance and propagation/regeneration of *ex situ* collections. The A-PAGE method has been widely applied as standard reference method of ISTA for verifying varieties (ISTA, 2023).

A number of studies have analysed the genetic polymorphism of gliadin in cereal collections from various countries (Anjum et al., 2000; Metakovsky et al., 2000; Novoselskaya-Dragovich et al., 2003; Alvarez et al., 2006; Mohd et al., 2007; Ruiz et al., 2007; Ojaghi & Akhundova, 2010; Zaefizadeh et al., 2010; Aliyeva et al., 2012;

Ma et al., 2012; Khabiri et al., 2013; Dziuba et al., 2014; Ahmadi et al., 2015; Desheva et al., 2021). The evaluation of the gliadin patterns provide additional information on certain parameters related to the quality and stability of cultivars, and it can be used for the selection of new gene sources in wheat breeding (Stoyanova, 2002). Durum wheat with a gliadin fraction of γ-45 is considered to be characterised by strong gluten and good culinary potential. In contrast, wheat with a gliadin fraction of γ-42 is characterised by weaker gluten and diminished culinary potential (Rashed et al., 2007; Khabiri et al., 2013). The chromosome translocation marker of rye and wheat (Gli-B3=1BL/1RS) has been indicated as a marker for varieties with high protein content and increased resistance to pathogens, but lower baking quality (Stoyanova, 2002; Tyrka et al., 2002). According to Doneva (2017) gliadins are a suitable biochemical marker to study the degree of homogeneity of new genotypes as well as to identify them. The application of this marker in crosses involving synthetic wheat and hybrids facilitates the selection and characterization of elite plants with introduced genetic material from wild wheat species.

Genetic erosion in cultivated wheat is the reason to look for new donors for breeding purposes (Diordiieva et al., 2022). Therefore, the study of genetic diversity in *Aegilops* species is important to achieve wheat resistance and yield improvement through the introgression of their genomes (Baranduzi et al., 2013). The study of genetic diversity in conserved genetic resources of *Aegilops* species is also important for better utilization and management of resources.

The aim of the present investigation was to assess the diversity in a collection of *Aegilops* species stored in the Bulgarian gene bank with respect to some grain morphometric parameters and gliadin proteins.

MATERIALS AND METHODS

Plant materials

Twenty *Aegilops* accessions belonging to five plant species were evaluated by gliadin electrophoresis and some morphometric characters. Seventeen of the specimens were collected from an expedition conducted in 2017 and preserved in the National Genbank of Bulgaria. The geographical coordinates of the habitats are presented in Table 1. Three specimens were obtained by introduction from France and Russia. Furthermore, the Marquis wheat variety (*Triticum aestivum* L.) was utilised as the baseline for the documentation of the band patterns (Table 1). All accessions were propagated at the Institute of Plant Genetic Resources (IPGR-Sadovo) during the 2020–2021 growing season. During two consecutive growing seasons (2021/2022 and 2022/2023), the accessions were planted in the experimental field located between 42°07'6.7008"N, 24°56'1.5"E and 42°07'6.8988"N, 24°55'59.4012"E in a completely randomized block design with two replications. All varieties were grown in six-row plots, 1 m long with row spacing of 20 cm. The field was managed according to local agricultural practices. At the full maturity stage, 20 spikes per sample, respectively 10 spikes per replication were randomly collected. After manual dehulling, seeds were used for digital image analysis and A-PAGE electrophoresis.

Table 1. List of accessions included in the study

No.	Number of accession	Plant species	Geographical coordinates	Altitude	Origin
1	BGR44236	*Aegilops triuncialis* L.	41°32′28″N, 26°10′02″E	103	BGR
2	BGR43678	*Aegilops neglecta* Req. ex Bertol.	41°46′36″N, 25°20′33″E	485	BGR
3	BGR43704	*Aegilops geniculata* Roth	41°45′00″N, 25°40′08″E	297	BGR
4	BGR44232	*Aegilops triuncialis* L.	41°27′52″N, 26°07′42″E	270	BGR
5	BGR19081	*Aegilops cylindica* Host	59°55′55″N, 30°18′31″E		RUS
6	BGR43668	*Aegilops neglecta* Req. ex Bertol.	41°48′44″N, 25°57′14″E	225	BGR
7	C3E0144	*Aegilops triuncialis* L.	42°07′27″N, 26°07′27″E	298	BGR
8	BGR43704 A	*Aegilops neglecta* Req. ex Bertol.	41°45′00″N, 25°40′08″E	297	BGR
9	BGR44234	*Aegilops cylindica* Host	41°43′53″N, 24°25′22″E	700	BGR
10	BGR44617	*Aegilops biunialis* Vis.	42°27′04″N, 26°08′15″E	218	BGR
11	BGR43676	*Aegilops cylindica* Host	42°02′06″N, 24°50′40″E	208	BGR
12	BGR43660	*Aegilops biunialis* Vis.	41°37′31″N, 25°40′30″E	166	BGR
13	BGR43684	*Aegilops triuncialis* L.	43°36′51.48″N, 3°52′18.12″E		FRA
14	BGR43687	*Aegilops neglecta* Req. ex Bertol.	43°36′51.48″N, 3°52′18.12″E		FRA
15	BGR43663	*Aegilops geniculata* Roth	42°27′04″N, 26°08′15″E	218	BGR
16	BGR43702	*Aegilops geniculata* Roth	41°45′19″N, 25°59′01″E	282	BGR
17	BGR43677	*Aegilops triuncialis* L.	41°27′52″N, 26°07′42″E	270	BGR
18	BGR43675	*Aegilops cylindica* Host	42°00′04″N, 24°50′41″E	608	BGR
19	BGR43540	*Aegilops triuncialis* L.	41°52′22″N, 26°00′15″E	88	BGR
20	BGR30391	*Aegilops neglecta* Req. ex Bertol.	42°5′58.77″N, 27°56′29.78″E	0	BGR
21	BGR9315 (Marquis)	*Triticum aestivum* L.			CAN

Digital image analysis

GrainScan software was used to measure grain size and colour (Whan et al., 2014). A scanner-Canon MF4010 Series UFRII was used to capture images of the studied *Aegilops* genotypes. The seeds were scattered on the scanner glass at a distance for accurate measurements. Two replicates were scanned for each accessions and each replicate included 20 seeds. To avoid shadows on the seeds and to increase contrast, the equipment was covered with black cardboard. The scanned image resolution was 300 dpi, and the number of pixels was 2,480×3,508. The raw images were imported into the GrainScan software and processed in accordance with the developer's recommended procedure, which included segmentation, processing, and data mining. The data generated by digital image analysis were: area (mm^2), perimeter (mm), length (maximum length of the grain ellipse, marked as majellipse, mm) and width (minor axis of the best fitting ellipse, marked as minellipse, mm). For colour, three values were obtained for red, green, and blue codes (Whan et al., 2014; Petcu et al., 2021).

The data were subjected to Duncan's Multiple Range Test and Two-Way ANOVA using SPSS 13.0 software. The two-factor analysis of variance was applied to estimate the strength of influence of the sources of variation - genotype, year, genotype X year interaction by the method of Plohinskii (1970), using the formula: $\eta^2 = Ci/Cy$,where η^2

is the strength of the influence of the sources of variation-genotype, year and genotype X year interaction, Ci is the variance of the respective factor (genotype, year or genotype X year interaction), and Cy is the total variance.

Cluster analysis was done based on the unweighted pair group average method (UPGAM) and Euclidean distance using Past 4.03 software.

A-PAGE

Acid-PAGE was carried out according to the standard reference method of ISTA (Draper, 1987). Gliadins were extracted from a bulk sample of 50 mg finely ground powdered seeds with 300 µl extracting solution (0.05 g Pyronin G; 25 mL 2-chloroethanol), stained overnight at room temperature, and centrifuged for 30 min at 17,000 g. Then, 5 µl of the extracts were loaded into wells. Gliadin electrophoresis was performed on a vertical polyacrylamide gel with a thickness of 1.0 mm and an electrode buffer with a pH of 3.2 using a Scie Plast TV400 vertical unit, with gel cassette 200×200 mm. Electrophoresis was carried out at 20 mA for 6 hours. Staining of gels was performed in a solution of Coomasie Brilliant Blue G-250: Coomasie Brilliant Blue R-250 (1:3), dissolved in trichloroacetic acid/methanol for 48 hours. Specialized software BIO-1D++, version 11.07 was used to create databases for the gliadin patterns of the studied genotypes. A Nei & Li (1979) genetic similarity coefficient was calculated for all pairs of electrophoregrams, using the results of the electrophoretic band pattern as a basis. The similarity matrix was used to construct the dendrogram by the UPGMA.

The diversity for each gliadin pattern was calculated in accordance with the methodology proposed by Nei (1973).

Two-dimensional Principal Coordinates Analysis (PCoA) based on the gliadin and morphometric analysis was performed using Past 4.03 software.

RESULTS AND DISCUSSION

The morphology of seeds is an invaluable tool in the process of discriminating between different genotypes. Furthermore, the findings derived from this process hold immense significance for the field of systematic classification. The measurement of seed size and shape, as well as the correlation and relationship between these variables, play an indispensable role in the process of breeding for seed yield (Cervantes et al., 2016). It is difficult to measure and describe seed size, colour and morphology using conventional methods. Even for a small amount of samples, this takes a long time and uses many resources (Baek et al., 2020). Image analysis is a modern approach for seed quality testing. This instrument provides a wide use for the evaluation of various morphological characteristics of seeds with a more comprehensive perception. It is based on the extraction of digital data from the captured image for characteristics such as colour, size, shape of seeds and their subsequent processing with using appropriate computer software (Hemender et al., 2018).

In this study, measurement of *Aegilops* seed morphological parameters (length, width, area and perimeter) and colour were determined using a scanner and the GrainScan software developed by Whan et al. (2014). A significant variation was observed among accessions for all grain morphometric traits (Table 2).

Table 2. The mean morphological characteristics of grain recorded for 20 Aegilops accessions and the standard variety Marquis

No.	Accession number	Area, mm^2	Peri-meter, mm	Length, mm	Width, mm	Grain ch1 red	Grain ch2 green	Grain ch3 blue
1	BGR44236	16.8 ± 0.6fg	23.4 ± 0.4e-g	8.7 ± 0.1e	2.4 ± 0.0c-e	158.7 ± 1.9fg	115.7 ± 1.6hi	72.2 ± 1.2d
2	BGR43678	15.8 ± 0.6d-f	22.7 ± 0.4c-f	8.1 ± 0.2d	2.5 ± 0.1c-e	120.0 ± 2.4a	85.8 ± 1.7ab	55.9 ± 1.4ab
3	BGR43704	12.1 ± 0.6a	20.4 ± 0.5a	7.1 ± 0.2b	2.2 ± 0.1a	135.0 ± 2.1d	102.8 ± 1.9e	76.6 ± 1.8d-f
4	BGR44232	17.3 ± 0.7f-h	25.5 ± 0.4h-j	9.0 ± 0.1e	2.4 ± 0.1c-e	155.9 ± 1.2f	117.4 ± 1.1hi	80.3 ± 1.1f-h
5	BGR19081	17.6 ± 0.5g-i	24.8 ± 0.5gh	8.8 ± 0.2e	2.5 ± 0.0d-f	155.4 ± 1.5f	119.7 ± 1.7i	87.9 ± 1.6i
6	BGR43668	12.7 ± 0.5ab	20.8 ± 0.5ab	7.1 ± 0.3b	2.3 ± 0.0a-c	126.1 ± 1.8bc	92.8 ± 1.6cd	66.2 ± 2.1c
7	C3E0144	18.9 ± 0.8ij	23.7 ± 0.5fg	8.9 ± 0.2e	2.7 ± 0.1f	137.3 ± 2.6d	103.3 ± 2.7e	74.7 ± 1.8de
8	BGR43704A	14.7 ± 0.6cd	21.3 ± 0.5a-c	7.3 ± 0.2bc	2.6 ± 0.0ef	120.4 ± 2.7a	84.9 ± 2.0a	54.9 ± 1.4ab
9	BGR44234	12.8 ± 0.4ab	20.1 ± 0.5a	7.3 ± 0.1bc	2.2 ± 0.0ab	159.7 ± 1.2h	117.0 ± 1.6hi	79.0 ± 2.4e-g
10	BGR44617	21.7 ± 0.4k	26.2 ± 0.3ij	8.7 ± 0.1e	3.2 ± 0.0h	137.9 ± 1.5d	100.9 ± 1.3e	65.5 ± 1.0c
11	BGR43676	14.3 ± 0.4b-d	21.4 ± 0.4a-c	7.6 ± 0.1c	2.4 ± 0.1b-d	150.0 ± 2.0e	112.9 ± 2.0gh	82.1 ± 1.8gh
12	BGR43660	13.6 ± 0.3a	20.6 ± 0.2ab	7.3 ± 0.1bc	2.40.0b-d	117.6 ± 1.3a	85.0 ± 1.0a	54.2 ± 0.9a
13	BGR43684	17.9 ± 0.6g-j	24.4 ± 0.6gh	8.9 ± 0.1e	2.6 ± 0.1e-f	138.9 ± 1.7d	105.6 ± 2.0ef	74.4 ± 1.7de
14	BGR43687	13.9 ± 0.2bc	21.0 ± 0.2ab	7.5 ± 0.0bc	2.4 ± 0.0a-c	126.7 ± 1.4c	89.1 ± 0.9a-d	56.9 ± 0.7ab
15	BGR43663	19.3 ± 0.1j	26.7 ± 0.3j	8.7 ± 0.0e	2.9 ± 0.0g	122.0 ± 2.0ab	93.3 ± 1.3d	65.7 ± 0.7c
16	BGR43702	18.4 ± 0.8h-j	25.2 ± 0.6hi	8.8 ± 0.1e	2.7 ± 0.1f	149.1 ± 1.4 e	110.1 ± 1.4fg	76.0 ± 1.3d-f
17	BGR43677	13.9 ± 0.3bc	21.3 ± 0.4ab	7.5 ± 0.1bc	2.4 ± 0.0b-d	119.3 ± 3.1a	87.8 ± 2.5a-c	57.5 ± 1.8ab
18	BGR43675	15.1 ± 0.5c-e	22.8 ± 0.9d-f	8.1 ± 0.2d	2.4 ± 0.1b-d	146.2 ± 2.2e	110.3 ± 2.2fg	80.7 ± 1.9f-h
19	BGR43540	14.7 ± 0.7cd	22.2 ± 0.6a-e	7.5 ± 0.2bc	2.5 ± 0.1c-e	162.0 ± 1.2fg	119.7 ± 1.0i	79.6 ± 1.2f-h
20	BGR30391	15.0 ± 0.4c-e	21.5 ± 0.2b-e	7.6 ± 0.1c	2.5 ± 0.1c-e	126.2 ± 1.8bc	90.5 ± 1.5b-d	59.3 ± 1.8b
21	BGR9315	16.4 ± 0.4e-g	20.5 ± 0.3a	6.6 ± 0.1a	3.2 ± 0.0h	159.9 ± 2.0fg	120.4 ± 2.1i	83.88hi
Mean		15.86	22.69	7.96	2.54	139.24	103.10	70.64
Std. error		0.54	0.45	0.17	0.06	3.44	2.83	2.36
Variance		6.22	4.32	0.59	0.07	247.92	168.61	117.29
Stand. dev		2.49	2.08	0.77	0.26	15.75	12.99	10.83
CV, %		15.73	9.16	9.62	10.37	11.31	12.59	15.33

Means within a column that have different superscript letters are significant different from each other (Duncan's multiple range test, $p \leq 0.05$), CV-Coefficient of variation.

Maximum value of 21.7 m^2 (BGR44617, *Aegilops biunialis* Vis.) and minimum value of 12.1 m^2 (BGR43704, *Aegilops geniculata* Roth) were recorded for area with mean value of 15.86 m^2, respectively.
The perimeter ranged from 20.11 mm (BGR44234, *Aegilops cylindica* Host) to 26.7 mm (BGR43663, *Aegilops geniculata* Roth) with a mean of 22.69 mm.

The largest proven grain length was reported for BGR44232, *Aegilops triuncialis* L. (9.0 mm), and the smallest for BGR9315, *Triticum aestivum* L. (6.6 mm). The grain width ranged from 2.2 mm for BGR43704 (*Aegilops geniculata* Roth) to 3.2 mm for BGR44617 (*Aegilops biunialis* Vis.) (Table 2).

Whan et al. (2014) reported that seed colour is an important character for breeding cereal varieties. A number of factors, including genetic and environmental factors, as well as cultivation practices, influences the colour of the grain. In this study, the values obtained for red, green, and blue codes varied significantly among the accessions. The highest red value was recorded for BGR43540, green for BGR9315 and blue for BGR19081.

Bivariate analysis of variance showed that the genotype, followed by the year of cultivation, had a dominant effect on the variation of the studied grain morphometric parameters. The strength of influence of genotype on trait variability ranged from 36.0% for seed width to 55.2% for grain ch1 red, while year had the greatest influence on seed area and grain ch2 green. The genotype x year interaction exerted no significant effect on trait expression (Table 3).

Table 3. Two-way ANOVA and degree of influence of sources of variation on the grain morphometric characteristics in 21 *Aegilops* accessions

SV	*df*	*MS*	η^2, %	*SV*	*df*	*MS*	η^2, %
Area, mm^2				Grain ch1 red			
G	20	354.44***	45.73	G	20	8,430.20***	55.25
Y	1	5,950.53***	38.39	Y	1	101,399.99***	33.23
GxY	20	50.64	6.53	GxY	20	227.53	1.49
Error	672	2.16		Error	672	45.58	
Perimeter, mm				Grain ch2 green			
G	20	145.87***	40.27	G	20	9,234.74***	52.32
Y	1	2,166.07***	29.90	Y	1	139,944***	39.64
GxY	20	52.33	14.45	GxY	20	300.59	1.70
Error	672	1.66		Error	672	33.30	
Length, mm				Grain ch3 blue			
G	20	19.91***	37.03	G	20	6,488.48***	53.36
Y	1	229.94***	21.38	Y	1	84,438.50***	34.72
GxY	20	3.93	7.31	GxY	20	144.83	1.19
Error	672	0.55		Error	672	38.86	
Width, mm							
G	20	7.38***	36.01				
Y	1	130.38***	31.81				
GxY	20	1.43	6.97				
Error	672	0.15					

SV – sources of variation; df-degree of freedom; MS – mean square; η^2 – degree of influence of sources of variation genotype, year and interaction genotype x year; *** – significant at $p \leq 0.001$.

Cluster analysis based on Euclidean dissimilarity using the unweighted pair group average method (UPGAM) grouped the accessions into six clusters at 20% linkage

distance Fig. 1). The first cluster comprises the genotypes 6-BGR43668 and 15- BGR43663 belong to the species *Aegilops neglecta* Req. ex Bertol. and *Aegilops geniculata* Roth, respectively. The genotypes in this cluster had the low values for seed length and colour values (Table 3). In the second cluster, six genotypes (2- BGR43678, 8- BGR43704 A, 12- BGR43660, 17- BGR43677, 14- BGR43687 and 20- BGR30391) were grouped including 23.57% of total accessions. Four of them belong to the *Aegilops neglecta* Req. ex Bertol, and the rest to *Aegilops biunialis* Vis. (12- BGR43660) and *Aegilops geniculata* Roth (17- BGR43677). The values for the all of studied characters in this cluster were the lowest compared with the total means of all genotypes (Table 4). The third group included six accessions – 4- BGR44232 (*Aegilops triuncialis* L.), 5- BGR19081 (*Aegilops cylindica* Host), 9- BGR44234 (*Aegilops cylindica* Host), 19- BGR43540 (*Aegilops triuncialis* L.), 21- BGR9315(*Triticum aestivum* L.) and 1- BGR44236 (*Aegilops triuncialis* L.). The genotypes in this group were in the highest rate with respect to colour values (158.61 red, 118.35 green and 80.48 blue). The fourth cluster grouped 11- BGRR43676 and 18- BGR43675 from species *Aegilops cylindica* Host in one subcluster and 16- BGR43702 from *Aegilops geniculata* Roth in separate subcluster. The genotypes in this group were with the high values of all characters above mean values, except for grain width (Table 4).

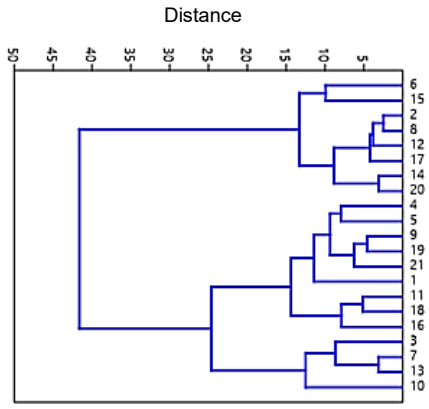

Figure 1. UPGMA dendrogram generated by Euclidian Similarity index based on the studied morphological characteristics of grain. (The numbers of genotypes in the dendograme are the same as given in the Table 1).

Table 4. The average values of the grain morphometric characteristics for each cluster and difference between each cluster and the total mean

Clusters	Area, mm^2	Perimeter, mm	Length, mm	Width, mm	Grain ch1	Grain ch2	Grain ch3
I	16.02	23.72	7.86	2.61	124.05	93.06	65.97
diff.	0.16	1.04	-0.10	0.06	-15.19	-10.04	-4.67
II	14.49	21.40	7.55	2.45	121.69	87.17	56.43
diff.	-1.37	-1.28	-0.42	-0.09	-17.55	-15.93	-14.21
III	15.94	22.75	8.02	2.55	158.61	118.35	80.48
diff.	0.08	0.06	0.05	0.01	19.37	15.25	9.84
IV	15.94	23.12	8.18	2.49	148.41	111.10	79.63
diff.	0.08	0.43	0.22	-0.06	9.17	8.00	8.99
V	16.31	22.83	8.29	2.49	137.05	103.91	75.23
diff.	0.45	0.15	0.33	-0.05	-2.19	0.81	4.60
VI	21.72	26.23	8.72	3.20	137.86	100.87	65.54
diff.	5.86	3.54	0.76	0.66	-1.37	-2.23	-5.10
Mean	15.86	22.69	7.96	2.54	139.24	103.10	70.64

Table 5. Proximity Matrix for 20 *Aegilops* accessions and Standard variety Marquise on the base of the studied grain morphometric parameters

Case	Euclidean Distance																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	0																				
2	51.6	0.0																			
3	28.0	31.1	0.0																		
4	9.0	53.9	26.9	0.0																	
5	16.6	58.7	29.7	8.0	0.0																
6	40.7	14.4	17.0	41.7	45.8	0.0															
7	25.0	31.2	8.3	24.2	27.9	18.9	0.0														
8	52.2	2.5	31.8	54.7	59.6	15.1	32.3	0.0													
9	8.8	55.8	28.6	8.3	12.3	43.4	27.7	56.3	0.0												
10	27.1	26.3	16.2	29.0	34.4	17.9	10.2	27.4	32.3	0.0											
11	14.0	48.3	19.0	9.3	11.5	35.1	18.4	49.0	11.2	25.5	0.0										
12	54.6	4.4	33.5	57.0	61.7	16.7	34.4	3.2	58.5	29.9	51.1	0.0									
13	22.4	33.2	9.0	21.6	25.6	21.0	3.1	34.3	25.1	11.0	16.1	36.4	0.0								
14	44.6	8.0	25.5	47.3	52.5	10.2	25.8	7.9	48.6	20.7	41.8	10.3	27.6	0.0							
15	43.7	13.6	21.7	44.2	48.1	9.9	20.5	15.6	47.4	17.7	38.6	17.1	22.8	13.5	0.0						
16	12.0	43.1	17.8	10.9	16.5	31.3	13.8	44.0	15.1	18.3	8.9	46.3	11.3	36.8	33.5	0.0					
17	50.6	3.7	29.0	52.7	57.2	12.2	29.9	4.2	54.4	25.9	46.7	4.7	31.9	7.6	12.9	42.0	0.0				
18	16.2	43.6	14.6	12.6	15.4	30.5	13.4	44.5	15.7	21.1	5.2	46.5	11.2	37.5	33.6	6.9	42.1	0.0			
19	9.5	59.1	32.2	8.0	11.4	46.9	30.5	59.6	4.5	34.7	14.1	61.9	27.9	52.0	50.3	17.2	57.8	18.5	0.0		
20	43.2	8.7	23.2	45.6	50.5	7.7	23.4	9.2	47.2	18.8	39.9	11.5	25.3	3.1	10.6	34.9	7.7	35.4	50.5	0.0	
21	13.2	59.9	31.7	8.3	7.8	47.3	30.2	60.6	7.0	35.6	12.9	62.7	27.8	53.1	50.5	17.7	58.5	17.7	5.5	51.4	0.0

(The numbers of genotypes in the table are the same as given in the Table 1).

Cluster 5 included 7- C3E0144 and 13- BGR43684 (*Aegilops triuncialis* L.), as well as genotype 3- BGR43704, which was separated into individual subclusters and belonged to *Aegilops geniculata* Roth. The genotype 10- BGR44617 (*Aegilops biunialis* Vis.) was in the last sixth cluster. The most phenotypically closest genotypes were 2- BGR43678 and 8-BGR43704 A (belong to *Aegilops neglecta* Req. ex Bertol.), following from 14- BGR43687 and 20- BGR30391 (belong to *Aegilops neglecta* Req. ex Bertol.), 7- C3E0144 and 13- BGR43684 (belong to *Aegilops triuncialis* L.). The most distant genotypes were 12- BGR43660 (*Aegilops biunialis* Vis.) with 21- BGR9315 (*Triticum aestivum* L.), following from 12- BGR43660 (*Aegilops biunialis* Vis.) with 19- BGR43540 (*Aegilops triuncialis* L.), 8- BGR43704 A (*Aegilops neglecta* Req. ex Bertol.) with 21- BGR9315 (*Triticum aestivum* L.), 2- BGR43678 (*Aegilops neglecta* Req. ex Bertol.) with 21- BGR9315, 8- BGR43704 A (*Aegilops neglecta* Req. ex Bertol.) with 19- BGR43540 (*Aegilops triuncialis* L.), 5- BGR19081 (*Aegilops cylindica* Host) with 8- BGR43704 A (*Aegilops neglecta* Req. ex Bertol.), 2- BGR43678 (*Aegilops neglecta* Req. ex Bertol.) with 19- BGR43540 (*Aegilops triuncialis* L.), and 2- BGR43678 (*Aegilops neglecta* Req. ex Bertol.) with 5- BGR19081 (*Aegilops cylindica* Host) (Table 5).

A-PAGE

The genus *Aegilops* has received minimal attention with regard to gliadins, with the exception of a few species, namely *Aegilops cylindrical* Host, *Aegilops biuncialis* Vis., *Aegilops geniculata* Roth (Kozub et al., 2012; Khabiri et al., 2013; Medouri et al., 2015; Vorosvary et al., 2000). Gregova et al. (2011) investigated variations in seed storage protein patterns in six accessions of jointed goatgrass (*Aegilops cylindrica* Host) populations collected from Slovakia and noted that gliadin electrophoresis showed a higher level of polymorphism than glutenin and therefore should be better for use in population identification. In this study, 20 accessions belonging to six *Aegilops* species (Table 6) were studied to assess the variability of gliadin proteins by *A-PAGE* electrophoresis. A total of 96 gliadin polymorphic bands were identified, with the number of bands varying between 13 and 22 per accession. Thirteen bands were observed in 5- BGR19081 (*Aegilops cylindica* Host) and 17- BGR43677 (*Aegilops geniculata* Roth), while the highest number was detected in 3- BGR43704 (*Aegilops geniculata* Roth) (Fig. 2, Table 6). The considerable variation observed in the gliadin spectra indicates that gliadins are a valuable and accurate diversity marker for *Aegilops* accessions. These results are consistent with those of Aljrt et al. (2021), who also observed a high degree of diversity and identified specific bands in the pattern that are species-specific. Medouri et al. (2015) identified 61 polymorphic bands and 35 gliadin patterns in *Aegilops geniculata* Roth, while Ahmadpoora et al. (2014) separated 36 types of bands for *Aegilops biunialis* Vis. and 35 types of bands for *Aegilops umbellulata* Zhuk. Baranduzi et al. (2013) observed a high degree of polymorphism among 33 accessions belonging to six *Aegilops* species. They reported the presence of 23 bands, with the number of bands per accession ranging from nine to 20.

Twenty gliadin patterns and 28 distinct bands were detected in the ω-gliadin zone. The bands varied between 2 and 10, as the pattern with five bands dominated over all others. The accession with the highest numbers of bands in the ω-gliadin zone was

3- BGR43704 (*Aegilops geniculata* Roth), following from 14- BGR43687 (*Aegilops neglecta* Req. ex Bertol.) and 15- BGR43663 (*Aegilops geniculata* Roth) (Fig. 2, Table 6).

Table 6. Gliadins diversity for 20 *Aegilops* genotypes

No.	Name of accession	Species	Number of gliadin bands				Total bands
			ω	γ	β	α	
1	BGR44236	*Aegilops triuncialis* L.	5	3	5	2	15
2	BGR43678	*Aegilops neglecta* Req. ex Bertol.	6	3	3	2	14
3	BGR43704	*Aegilops geniculata* Roth	10	5	5	2	22
4	BGR44232	*Aegilops triuncialis* L.	8	2	4	2	16
5	BGR19081	*Aegilops cylindica* Host	5	2	5	1	13
6	BGR43668	*Aegilops neglecta* Req. ex Bertol.	8	3	5	3	19
7	C3E0144	*Aegilops triuncialis* L.	5	3	5	2	15
8	BGR43704 A	*Aegilops neglecta* Req. ex Bertol.	5	5	5	2	17
9	BGR44234	*Aegilops cylindica* Host	5	2	6	1	14
10	BGR44617	*Aegilops biunialis* Vis.	7	1	5	1	14
11	BGRR43676	*Aegilops cylindica* Host	5	3	7	1	16
12	BGR43660	*Aegilops biunialis* Vis.	7	4	6	2	19
13	BGR43684	*Aegilops triuncialis* L.	5	2	5	2	14
14	BGR43687	*Aegilops neglecta* Req. ex Bertol.	9	2	6	2	19
15	BGR43663	*Aegilops geniculata* Roth	9	1	4	2	16
16	BGR43702	*Aegilops triuncialis* L.	2	2	9	2	15
17	BGR43677	*Aegilops geniculata* Roth	4	1	7	1	13
18	BGR43675	*Aegilops cylindica* Host	4	4	5	3	16
19	BGR43540	*Aegilops triuncialis* L.	2	2	6	4	14
20	BGR30391	*Aegilops neglecta* Req. ex Bertol.	6	4	5	4	19
Range of gliadin bands			2–10	1–5	4–9	1–4	13–22
Number of gliadin patterns			20	20	20	17	20
Genetic diversity index (H, %)			0.950	0.950	0.950	0.938	0.947

Twenty patterns and 22 different mobility bands were detected in the γ-gliadin region. All accessions had specific patterns. The accessions with the highest numbers of γ-bands were 3- BGR43704 (*A. geniculate* Roth) and 8- BGR43704 A (*Aegilops neglecta* Req. ex Bertol.), while with one band were 10- BGR44617 (*Aegilops biunialis* Vis.) and 15- BGR43663 (*Aegilops geniculata* Roth) (Fig. 2, Table 6).

A total of 28 different mobility bands formed 20 patterns in β gliadin region. The number of bands varied four to nine in each pattern. Two accessions (11- BGRR43676, *Aegilops cylindica* Host; 17- BGR43677, *Aegilops geniculata* Roth) had seven mobility bands in your patterns, while 16- BGR43702 (*Aegilops triuncialis* L.) had nine bands (Fig. 2, Table 6).

In α gliadin region, 18 different mobility bands were detected, whose combination formed 17 patterns. Three accessions (3- BGR43704, *Aegilops geniculata* Roth; 4- BGR44232, *Aegilops triuncialis* L., 14- BGR43687, *Aegilops neglecta* Req. ex Bertol.) had two similar mobility bands in your patterns. Seventeen patterns were specific for the remaining accessions (Fig. 2, Table 6).

Gregova et al. (2011) identified ω, γ and β zones of gliadins in the jointed goatgrass populations, but not α. Baranduzi et al. (2013) reported bands in α area in all 33 *Aegilops*

accessions except Sarab-Bostanabad accession of *A. cylindrica* Host species, while Khabiri et al. (2013) observed in some of the *A. cylindrica* Host populations, just a fair band in the α region. Kozub et al. (2012) reported that only some populations of *A. biuncialis* Vis. have bands in α region. In our study, the presence of bands were found in α zone of all the studied samples. This is in agreement with the suggestion of Khabiri et al. (2013) that the discrepancy in all the results is due to the different germplasm used in these studies or different electrophoresis methods.

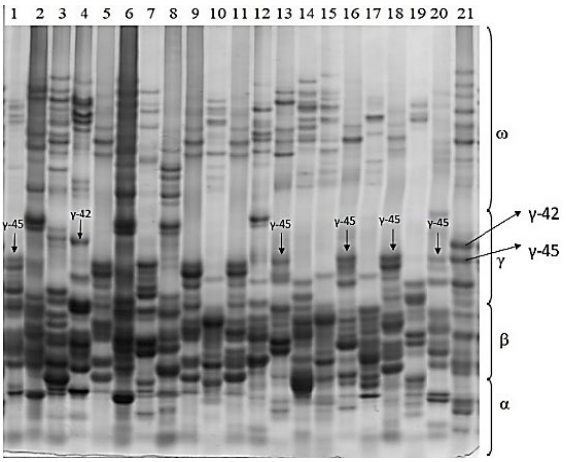

Figure 2. Gliadin patterns of 20 accessions of *Aegilops* species in A-PAGE profile.

The name of the accesions is listen in Table 1. The arrows note the positions of the electrophoregrams equivalent to gliadins- γ-45 and γ-42.

The overall diversity in the samples based on the patterns observed for each of the four gliadin regions showed that the ω, γ and β regions had the highest diversity (H = 0.950). The lowest diversity was observed in α regions (H = 0.938). In all the *Agelops* samples all bands were polymorphic and the mean diversity estimate was high (H = 0.947) (Table 6). Therefore, populations of the studied Aegilops species can be used to increase the genetic diversity of wheat germplasm. Medouri et al. (2015) also found a higher diversity index (H) for ω-gliadins (0.968) and the lowest for α-gliadin patterns (0.944) in 36 accessions of *A. geniculata* Roth collected in northern Algeria.

Ahmadpoora et al. (2014) reported the highest diversity for bands at ω regions, but the lowest at β zone in *A. biuncialis* Vis. and *A. umbellulata* Zhuk. Khabiri et al. (2013) noted that diversity among genotypes of *A. cylindrica* was greater than the diversity exhibited at the intra-population level, with the highest total variability in region β and, the lowest belonged to region ω.

Sherman et al. (2018) reported that allelic variation at the Gli-B1 locus exerts a significant effect on dough properties and bread production quality, irrespective of genetic background or environmental conditions (Pourmohammadi et al., 2023). Qi et al. (2009) found that the γ-gliadins with a short repetitive domain are relatively more nutritional, since they contain a higher proportion of essential amino acids. Moreover, these short γ-gliadins almost contain no toxic epitopes. Therefore, it is possible to breed wheat varieties, the γ-gliadins of which are less, even nontoxic and more nutritional. The presence of gliadin γ-45 in durum wheat results in the production of cooking pasta that is characterised by increased hardness and viscoelasticity. Conversely, the absence of gliadin type γ-42 leads to the opposite effect (Stoyanova & Kolev, 1996). It has been established that components γ-42 and γ-45, which are encoded by alleles at the Gli-B1 locus, are closely related to two different low molecular weight glutenins, which are encoded by alleles at the Glu-B3 locus (Payne et al., 1984; Chacón et al., 2020).

Table 7. Average genetic similarity matrix of Nei & Li (1979) coefficient based on the A-PAGE gliadin patterns

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	1																				
2	0.34	1																			
3	0.38	0.5	1																		
4	0.45	0.4	0.47	1																	
5	0.36	0.22	0.46	0.48	1																
6	0.35	0.48	0.34	0.40	0.38	1															
7	0.53	0.48	0.49	0.32	0.43	0.47	1														
8	0.38	0.45	0.46	0.30	0.27	0.61	0.50	1													
9	0.34	0.36	0.50	0.53	0.89	0.36	0.41	0.32	1												
10	0.34	0.36	0.50	0.47	0.44	0.36	0.28	0.45	0.50	1											
11	0.39	0.48	0.53	0.44	0.83	0.40	0.45	0.48	0.93	0.47	1										
12	0.41	0.30	0.39	0.34	0.50	0.42	0.35	0.33	0.55	0.48	0.51	1									
13	0.21	0.14	0.39	0.40	0.52	0.36	0.41	0.39	0.50	0.50	0.47	0.48	1								
14	0.35	0.48	0.44	0.48	0.44	0.47	0.59	0.28	0.48	0.42	0.51	0.53	0.55	1							
15	0.32	0.33	0.47	0.31	0.34	0.40	0.32	0.24	0.40	0.60	0.44	0.34	0.40	0.51	1						
16	0.47	0.28	0.32	0.32	0.50	0.35	0.60	0.50	0.55	0.34	0.45	0.65	0.62	0.53	0.39	1					
17	0.50	0.37	0.34	0.41	0.54	0.44	0.43	0.40	0.59	0.44	0.48	0.44	0.44	0.44	0.55	0.57	1				
18	0.45	0.33	0.42	0.31	0.48	0.46	0.52	0.30	0.47	0.47	0.50	0.51	0.47	0.40	0.50	0.58	0.41	1			
19	0.55	0.36	0.50	0.40	0.44	0.48	0.69	0.45	0.50	0.43	0.53	0.42	0.50	0.67	0.47	0.69	0.52	0.53	1		
20	0.47	0.42	0.39	0.40	0.50	0.42	0.65	0.50	0.48	0.36	0.51	0.47	0.55	0.37	0.34	0.53	0.38	0.57	0.48	1	
21	0.27	0.39	0.55	0.47	0.40	0.44	0.54	0.46	0.50	0.39	0.58	0.49	0.44	0.44	0.37	0.54	0.46	0.47	0.50	0.59	1

1- BGR44236, 2- BGR43678, 3- BGR43704, 4- BGR44232, 5- BGR19081, 6- BGR43668, 7- C3E0144, 8- BGR43704 A, 9- BGR44234, 10- BGR44617, 11- BGRR43676, 12- BGR43660, 13- BGR43684, 14- BGR43687, 15- BGR43663, 16- BGR43702, 17- BGR43677, 18- BGR43675, 19- BGR43540, 20- BGR30391, 21- BGR9315 (Marquis).

In our study, the international standard variety Marquis (Bushuk & Zillman, 1978; Metakovsky et al., 2018) was used to identify γ-45 and γ-42 in the studied genotypes (Yupsanis & Moustakas, 1988). In the gliadin patterns of five genotypes (1, 13, 16, 18 and 20), a band corresponding to the electrophoretic mobility of γ-45 gliadin encoded by the Gli-B1 locus was observed (Fig. 1). These genotypes would be of interest for breeding programs related with breeding of synthetic and high quality wheat varieties. Ahmadpoora et al. (2014) found also the highest rate for γ-45 band in the populations of *Ae. biuncialis* and *Ae. umbellulata*, while Khabiri et al. (2013) observed a band similar to γ-45 band, in 17 populations of *Ae. cylindrica*.

In order to find the genetic relationship between the *Aegilops* accessions, the genetic similarity was calculated based on the Nei & Li (1979) coefficient. The average genetic similarity between all 20 genotypes range from 0.14 to 0.93 with a mean of 0.56. This indicates that the studied accessions exhibited considerable genetic variability. The highest genetic similarity was observed between 9- BGR44234 and 11- BGRR43676, following from 5- BGR19081 and 9- BGR44234, 5- BGR19081 and 11- BGRR43676. All of them belong to species *Aegilops cylindica* Host. The lowest genetic similarity was recorded between 2- BGR43678 (*Aegilops neglecta* Req. ex Bertol) and 13- BGR43684 (*Aegilops triuncialis* L.) (Table 7).

Fig. 3 shows a UPGMA dendrogram, illustrating the genetic distance among *Aegilops* genotypes based on gliadin analysis. The dendrogram enabled the identification of four main clusters. Cluster I contains one accession (1-BGR44236) of *Aegilops triuncialis* L. Cluster II, included twelve accessions, which were divided into two subgroups. Subgroup 1 comprised three accessions (5- BGR19081, 9- BGR44234, 11- BGRR43676) belonging to *Aegilops cylindica* Host, two accessions (13- BGR43684, 16-BGR43702 from Russia) to *Aegilops triuncialis* L., one accession (12-BGR43660) to *Aegilops biunialis* Vis. and one (17-BGR43677) to *Aegilops geniculata* Roth. Subgroup 2 was composed of two *Aegilops triuncialis* L. accessions (7- C3E0144 from France, 19- BGR43540), two *Aegilops neglecta* Req. ex Bertol accessions (14- BGR43687, 20- BGR30391) and one accession (18- BGR43675) from *Aegilops cylindica* Host. Cluster III contained three accessions from one species- *Aegilops neglecta* Req. ex Bertol. (2- BGR43678, 6- BGR43678 and 8- BGR43704 A from France). Finally, cluster IV included the remaining five accessions, which were divided into three subgroups. Subgroup 1 consisted of 3-BGR43704 (*Aegilops geniculata* Roth) and the standard variety Marqus (21-BGR9315). Subgroup 2 combined specimens 10- BGR44617 and 15- BGR43663 belonging to the species *Aegilops geniculata* Roth. The accession 4-BGR44232,

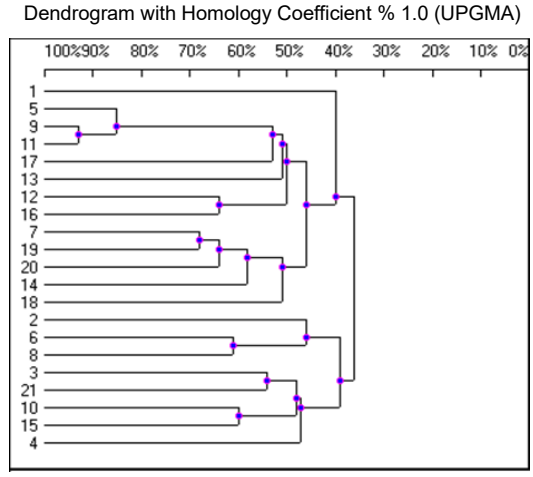

Figure 3. Clustering of 20 *Aegilops* genotypes using UPGMA method.

(The numbers of genotypes in the dendrogram are the same as given in the Table 1).

Aegilops triuncialis L. was separated individually insubgroup 3 (Fig. 3). The study found that the clustering of *Aegilops* genotypes was not linked to their geographic origin. This agrees with a previous study by Khabiri et al. (2013) on 17 populations of *Aegilops cylindrica* Host. Their findings showed that the observed diversity did not align with the geographical distribution. Ma et al. (2012) noted that the geographical origin of accessions might represent different macro-environments, thereby explaining a significant proportion of the genetic variation observed among accessions from disparate regions. Medouri et al. (2015) point out that the observed variation in Algerian genotypes of the species *A. geniculata* Roth is highlighted by a significant correlation between gliadin polymorphism and ecological parameters.

Two-dimensional PCoA was conducted based on the gliadin and morphometric analyses to better understand the interspecies linkages between the genotypes (Fig. 4). It revealed wide genetic dissimilarity between most of the genotypes, explaining 97.16% of the variations of which PCo1 explained 93.65%, while PCo2 3.51% of the variation, respectively. All genotypes were distributed into four quadrants (groups). Quadrants 1 (top left) included the following genotypes-5- BGR19081, 11- BGRR43676, 18- BGR43675 belong to *Aegilops cylindica* Host and 13- BGR43684 and 7- C3E0144 belong to *Aegilops triuncialis* L. The first three genotypes were more distant than the last two in the quadrant. However, the genetic relationship between specimens belonging to these two species could be explained by the similarity in genome C of the common ancestor *Aegilops caldata* L.

Sliai & Amer (2013) observed that the discrepancy in the positions of genotypes with analogous genomes in trees might be attributed to divergence in their respective ecological ecosystems or the soil type in which they are cultivated.

Quadrants 2 (top right) contained three genotypes-3- BGR43704, 15- BGR43663 from species *Aegilops geniculata* Roth and 6- BGR43668 from *Aegilops neglecta* Req. ex Bertol. All genotypes in this group share similar genomes (UUMM).

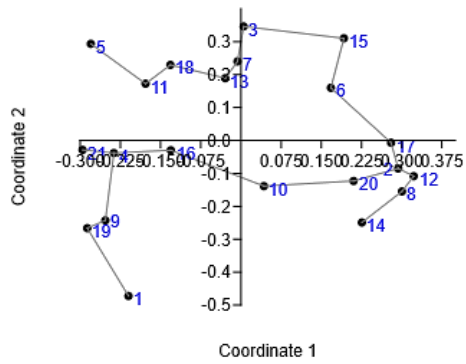

Figure 4. Relation among the genotypes of *Aegilops* species and minimum spanning tree based on the gliadin and morphometric analyses. (The numbers of genotypes in the figure are the same as given in the Table 1).

Quadrants 3 (bottom left) unites the genotypes 21- BGR9315 (the standard variety Marquis), 4- BGR44232 (*Aegilops triuncialis* L.), 16- BGR43702 (*Aegilops geniculata* Roth), 19- BGR43540 (*Aegilops triuncialis* L.), 9- BGR44234 (*Aegilops cylindica* Host) and 1- BGR44236 (*Aegilops triuncialis* L.).Genotypes 9 and 19 were located in close proximity in the minimum spanning tree, which explained by the fact that these annual allotetraploids contain in their genomic formula the C-genome of their common progenitor, as well as the close morphometric indices.

Quadrants 4 (bottom right) was the largest and included seven genotypes - 10- BGR44617 (*Aegilops biunialis* Vis.), 20- BGR30391 (*Aegilops neglecta* Req. ex Bertol.), 2- BGR43678 (*Aegilops neglecta* Req. ex Bertol.), 17- BGR43677 (*Aegilops*

triuncialis L.), 12- BGR43660 (*Aegilops biunialis* Vis.), 8- BGR43704 A (*Aegilops neglecta* Req. ex Bertol.), 14- BGR43687 (*Aegilops neglecta* Req. ex Bertol.). With the exception of 17- BGR43677 (*Aegilops triuncialis* L.), all other specimens had the same genome (UUMM). On the other hand, Mackey (1954, 1966) and Zohary (1966) classified the species *Aegilops biuncialis* Vis., *Aegilops triuncialis* L., and *Aegilops geniculata* Roth in the CU genome group (Haider et al., 2010).

The PCoA results demonstrated that genotypes with analogous genomes were grouped in close proximity within the phylogenetic tree, indicating that their evolutionary relationships may have originated from the same parental lineage. This finding is consistent with the hypothesis that genotypes with similar genomes may have a shared evolutionary history (Baranduzi et al., 2013; Aljrf et al., 2021).

The genetic and systematic relationship between grain morphology (size, shape, color and structure) and protein composition (gliadin) is complex and influenced by multiple genetic loci and environmental factors -soil type, climate, and water availability (Blanco et al., 2012; Alemu et al., 2020; Hacini et al., 2022; Liu et al., 2023). Genetic mapping techniques, such as Quantitative Trait Loci (QTL) mapping and genome-wide association studies (GWAS), have been used to identify specific regions of the genome that affect grain morphology and protein composition. Guo et al. (2023) found that QTL on chromosomes 3B, 4A, 6B, and 7A influence both grain size and protein content. Genes on chromosome 3B have been found to play a significant role in the synthesis of gliadins and amylopectin (Guo et al., 2023). Understanding the genetic relationship between grain morphology and protein composition is crucial for wheat breeding programs for develop wheat varieties with desired grain size and protein content. Therefore, to clarify the complex relationship between grain morphology and protein composition (gliadins) in *Aegilops*, genetic mapping techniques need to be used in future research.

Knowledge of the genetic diversity in *Aegilops* collections as wild relatives of wheat is essential for the development of effective genebank conservation, for understanding the evolutionary process and for its effective use in breeding programs. Interest in the genetic structure of natural populations of *Aegilops* species has increased in recent years, and it is therefore necessary to continue research using other molecular-genetic methods to increase knowledge of genetic variation in Bulgarian *Aegilops* populations. It is imperative to accord paramount importance to the ecogeographic diversification of *Aegilops* germplasm collections, in addition to augmenting sample sizes, with a view to optimising the utilisation and conservation of genetic resources.

CONCLUSIONS

The studied *Aegilops* accessions exhibited notable diversity with respect to morphometric indices of grain and the gliadin pattern to increase the genetic diversity of wheat germplasm. The considerable variation observed among genotypes based on the polymorphism of protein patterns is indicative of the potential of gliadin pattern as a reliable and precise diversity marker for *Aegilops* accessions. Genotypes identified with γ-45 in their gliadin patterns would be of interest for breeding programs related to wheat flour quality. The PCoA results demonstrated that genotypes with analogous genomes were grouped in close proximity within the phylogenetic tree, indicating that their evolutionary relationships may have originated from the same parental lineage. To reveal

the complex genetic and systematic relationship between grain morphometric parameters and gliadins in *Aegilops*, other molecular genetic techniques should be used in future studies.

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