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# Genetic variation of gliadins and some quality characteristics in spelt wheat

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## Abstract

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The aim of the study was to asses the genetic variation in 22 accessions of *Triticum spelta* L. on the basis of quality characteristics and *A-PAGE* markers. Eleven quality characteristics of grain and whole spelt wheat flour were included in the study. The results of analysis of variance showed high significant differences between spelt wheat accessions. Phenotypic coefficient of variation (PCV) was greater than genotypic coefficient of variation (GCV) for all the traits. High PCV and GCV were observed for sedimentation and fermentation values. High heritability coupled with high genetic advance as percentage of mean was recorded for vitreousness, sedimentation value and fermentation value that indicate selection of such traits may be effective. The cluster analys base on studied characteristics using the Between-groups linkage method group the genotypes into four clusters. Genotypes in the fourth cluster BGR 19018 and BGR 28710 were in the highest rate with respect to thousand kernel weight, vitreousness, crude protein content, wet gluten content, dry gluten content, sedimentation value and fermentation value. In total, 51 polymorphic and 2 monomorphic bands and 20 gliadin patterns were identified by A-PAGE. Fourteen different mobility bands and 13 gliadin patterns were identified in the ω-gliadin zone, 17 bands and 18 patterns were noted in the γ-gliadins, 14 patterns and 10 mobility bands were found for β-gliadins and 12 bands with 16 different α -gliadin patterns were determined. The genetic diversity index (H) was the highest for γ-gliadins (0.908), followed by α -gliadins (0.844), respectively and the lowest value was detected in ω-gliadin patterns (0.804). The genetic similarities (GS) among 22 spelt genotypes estimated by Jaccard's coefficient ranged from 0.103 to 1. The lowest GS was found between genotypes: BGR 26757 and B2000280, respectively, which indicates that the genetic diversity within these pairs of accessions was very high. Three pairs of genotypes had GS equal to 1 (BGR 10978 and BGR 23639; B2000277 and B2000279; BGR 28710 and BGR 10975), which indicated that they are genetically identical and do not differ in their gliadin spectra. Cluster analysis base on gliadin bands data classified genotypes into 11 main groups.

*Keywords:* A-PAGE; gliadin; genetic variability; *Triticum spelta* L.; quality characteristics; cluster analysis

## Introduction

Spelt wheat (*Triticum spelta* L.) was one of the major feed and food grains in ancient Europe. In the second half of the 20th century, the interest to spelt was revived on account of its health benefits, high nutritional value, and suitability for breeding programs aiming to develop varieties characterized by high grain quality and high resistance to pathogens (Paka et al., 2019) as well as because of the increasing demand for unconventional foods and low-input agriculture (Xueli et al., 2005). Spelt wheat (*Triticum spelta* L.) as a representative of hexaploid series of the Triticum genome constitution, is characterized by a great adaptation to a wider range of environments (Dinu et al., 2018). Therefore knowledge of genetic variation in spelt wheat is a useful tool to selecting of desirable genotypes to be used in breeding programs

(Radwan et al., 2013). Variability, in particular determines the effectiveness of selection. It is fact that the higher the variability among the genotypes betters the chances for further improvement in the crop (Subhashchandra et al., 2009). There are different analyses and techniques available to compute the genetic parameters and the index of transmissibility of characters (Eivazi et al., 2007; Khodadadi et al., 2011; Waqar- Ul-Haq et al., 2012). The knowledge of genetic variability present in a given crop species for the character under improvement is of paramount importance for the success of any plant breeding program (Bisne, 2009).

Characterization of germplasm using biochemical and molecular markers has received a great attention in the last decades. They are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar's identification at early stages of plant development. Biochemical characterization of cultivars is also useful to evaluate potential genetic erosion, identification of a variety and genetic diversity (Shuaib et al., 2010). Protein markers, like seed storage proteins (gliadins and glutenins) remain highly effective for wheat breeding purposes as genetic markers (Zheleva et al., 2007). Genetic identification of plant genotypes using protein markers is easier, time and cost effective in comparison with the DNA markers (Dvořáček & Čurn, 2003; Sadigov, 2015). Radwan et al. (2013) noted that seed storage proteins have been used as genetic markers in: (1) identifying variation among the taxa of each species; (2) screening the purity of the ever expanding number of cultivars; (3) establishing genome relationships; (4) exploiting the important traits of landraces and wild relatives to provide increasing crop production and stabilizing yield, and (5) using information on genetic diversity to make decisions regarding selection of superior genotypes for improvement yield of plants through breeding.

The present study was initiated to study genetic variation in accessions of *Triticum spelta* L. on the basis of quality characteristics and A-PAGE markers.

## Material and Methods

### Plant material

Twenty two spelt wheat accessions maintained in the National genebank of Bulgaria were examined (Table 1).

### Experimental conditions

The present study was conducted in the experimental field of the Konstantin Malkov Institute of Plant Genetic Resources at Sadovo (IPGR-Sadovo), Bulgaria during the 2014-2015, 2015-2016 and 2016-2017 growing seasons. The experiment was carried out in a randomized block design in three replications on a 1 $m^2$ plot size. Each plot consisted of five rows of 1.0 m length with 20 cm and 5 cm spacing between and within rows, respectively. Traditional agronomic and cultural practices were applied to the experiment throughout the growing seasons.

**Table 1. List of accessions included in the study**

| № | Accession number | Genus | Species | Spaut. | Origin |
|---|---|---|---|---|---|
| 1 | BGR 32889 | *Triticum* | *spelta* | L. | DEU |
| 2 | BGR 17293 | *Triticum* | *spelta* | L. | SRB |
| 3 | BGR 10978 | *Triticum* | *spelta* | L. | DEU |
| 4 | BGR 23639 | *Triticum* | *spelta* | L. | DEU |
| 5 | BGR 19018 | *Triticum* | *spelta* | L. | RUS |
| 6 | BGR 31344 | *Triticum* | *spelta* | L. | DEU |
| 7 | BGR 10973 | *Triticum* | *spelta* | L. | DEU |
| 8 | BGR 17294 | *Triticum* | *spelta* | L. | DEU |
| 9 | BGR 31240 | *Triticum* | *spelta* | L. | DEU |
| 10 | BGR 31892 | *Triticum* | *spelta* | L. | DEU |
| 11 | BGR 28710 | *Triticum* | *spelta* | L. | ESP |
| 12 | BGR 33817 | *Triticum* | *spelta* | L. | ESP |
| 13 | BGR 10974 | *Triticum* | *spelta* | L. | ESP |
| 14 | BGR 10975 | *Triticum* | *spelta* | L. | ESP |
| 15 | BGR 26757 | *Triticum* | *spelta* | L. | IRN |
| 16 | B2000277 | *Triticum* | *spelta* | L. | BEL |
| 17 | B2000278 | *Triticum* | *spelta* | L. | BEL |
| 18 | B2000279 | *Triticum* | *spelta* | L. | BEL |
| 19 | B2000280 | *Triticum* | *spelta* | L. | BEL |
| 20 | B2000281 | *Triticum* | *spelta* | L. | BEL |
| 21 | B2000282 | *Triticum* | *spelta* | L. | BEL |
| 22 | B2000283 | *Triticum* | *spelta* | L. | BEL |

### Quality characteristics of grain and whole spelt wheat flour

The main characteristics of grain were analyzed in the biochemical and technological laboratories in the IPGR-Sadovo. The grain physical characteristics such as thousand kernel weight and general vitreousness were determined according to the methods described in BDS ISO 520 (2003) and BDS 13378 (1976). The following chemical characteristics of grain were studied: crude protein content (%) by Kejeldahl's method, and grain lysine (%) by the method of Ermakov et al. (1972) and lysine content (% of protein). Wet and dry gluten content was estimated after grinding the grain into flour and hand washing according to BDS 13375 (1988). The sedimentation value of the whole wheat flour was determined using the method of Pumpyanskiy (1971). Bread-making strength index and gluten weakness were

detected according to BDS 13375 (1988). The whole spelt wheat meal flour of each accession was also tested for fermentation value (Pelshenke et al., 1953).

**Statistical analysis**

The mean data from all eleven characteristics were used to analysis of variance according to Lidansky (1988). LSD test was carried out to explore the significance of differences between the mean standard and the respective accession in the data set. Genotypic, phenotypic and environmental variances, genotypic and phenotypic coefficient of variability, broad sense heritability were calculated according to Singh and Chaudhary (1985). Genetic advance in terms of percentage of means was assessed by Brim et al. (1959). Hierarchical cluster analysis (Between groups-linkage method) using Euclidean distance was performed by using the SPSS 13.0 software.

***Acid Polyacrilamyde Gel Electrophoresis (Acid-PAGE)***

Acid-PAGE is carried out according to the standard reference method of ISTA (Draper, 1987; Anonymous, 2003). Proteins 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 and 14ºC. Then, 10 µl of the extracts were loaded into wells. Gliadin electrophoresis was performed on a vertical polyacrylamide gel with a thickness of 1.5 mm and an electrode buffer with a pH of 3.2 using a Consort E835 vertical unit (with gel cassette 200 × 200 mm). Electrophoresis was carried out at 20 mA for 5 hours and 15 min. 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 GelPro32 was used to create databases for the gliadin spectra of the studied genotypes. The presence and absence of each band polyacrylamide gel electrophoresis was coded as "1" and "0" respectively. Jaccard's similarity index (JSI) was calculated by the formula of Sneath and Sokal (1973). The data were subjected to tree clustering using SPSS 13.0 software. Cluster analysis was done based on the Between groups-linkage method and Euclidean distance.

The genetic diversity for each gliadin pattern was calculated as per Nei (1973), as $H = 1 - \Sigma pi^2$, where H is the genetic variation index and pi is the proportion of a particular pattern in each group of α, β, γ and ω gliadins separately. The mean value of H was calculated for all the four groups of gliadins.

## Results and Discussion

***Genetic variations in studied quality characteristics of grain and whole spelt wheat flour***

Knowledge of genetic variation is a useful tool in genebank management, helping in the establishment of core collections, facilitating efficient sampling and utilization of germplasm, and selecting of desirable genotypes to be used in breeding programs (Radwan et al., 2013). Some studies have suggested that the wheat breeding programs centered on high-yield cultivars could have eroded the genetic variability from the quality traits among and within cultivars (Esquinas-Alcazar, 2005). It is therefore of the utmost to search for species that could be useful in contributing genes for wheat quality improvement (Alvarez & Guzmán, 2019). The spelt wheat is an important source of genetic diversity for endosperm proteins that are associated with bread-making quality in wheat (Pu et al., 2007). Spelt wheat has been described as a valuable nutrient source of protein with good amino acid composition, lipids and crude fiber, vitamins and minerals (Bonafaccia et al., 2000; Wieser, 2001; Capouchova, 2001; Abdel-Aal & Hucl, 2002; Marconi et al., 2002; Bojňanská & Francáková, 2002; Wiwart et al., 2004; Pruska-Kedzior et al., 2008). The yield of wet gluten from spelt wheat is usually higher, but with a weaker structure (Schober et al., 2002; 2006; Pruska-Kedzior et al., 2008). It varied in dependent of varieties and nitrogen fertilization (Schober et al., 2002; Zielinski et al., 2008; Bojnanská & Francáková, 2002). In the present study the genetic variation in some quality characteristics of grain and whole flour in 22 spelt genotypes maintained in the National genebank of Bulgaria was evaluated (Table 2). The results of the analysis of variance showed high significant differences between spelt wheat accessions, which was confirmed by the mean square values (MS). The value of mean square varied in wide ranges, respectively from 0.07 for lysine to 8853.7 for fermentation value (Table 3).

The physical characteristics of grain – thousand kernel weight and vitreousness showed significant variation among different accessions (Table 2). The thousand kernel weight ranged from 26.29 to 48.57 g. The highest thousand kernel weight was observed in B2000281 (48.57 g), followed by BGR 19018 (45.96 g), B2000278 (44.39 g) and BGR 28710 (42.45 g). The grain vitreousness is important trait for kernel grading and bread-making properties (Wang et al., 2002). The highest values for vitreousness above 65% were found for BGR 23639, BGR 19018, BGR 31892 and BGR 32889 (67.25, 66.50, 65.25 and 65.25%, respectively), while the lowest values were confirmed statistically for five accessions (Table 2).

**Table 2. Mean values of quality characteristics of grain and whole spelt wheat flour**

| Accessions | TKW, g | V, % | CP, % | L, % | LC, % of protein | WGC, % | DGC, % | SV, $cm^3$ | FV, min | GW, mm | BSI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| St | 36.81 | 56.20 | 14.42 | 0.36 | 2.51 | 36.51 | 12.26 | 25.61 | 78.95 | 8.80 | 64.65 |
| BGR 32889 | 29.62- | 65.25++ | 13.44- | 0.43+++ | 3.16+++ | 35.66 | 11.82 | 24.5 | 50 | 11.25++ | 54.00- |
| BGR 17293 | 33.89 | 54.5 | 14.85 | 0.41+++ | 2.72 | 38.32 | 12.44 | 27.5 | 94 | 9.63 | 63.25 |
| BGR 10978 | 33.46 | 56.25 | 14.49 | 0.38+ | 2.64 | 35.04 | 11.58 | 17.00--- | 33.50- | 7.13 | 73 |
| BGR 23639 | 35.32 | 67.25+++ | 15.36+ | 0.35 | 2.27- | 41.10++ | 13.66++ | 22.00- | 53.5 | 11.75++ | 53.25-- |
| BGR 19018 | 45.96+++ | 66.50+++ | 14.17 | 0.34- | 2.37 | 35.26 | 11.68 | 37.25+++ | 181.25+++ | 9.5 | 63.5 |
| BGR 31344 | 35.5 | 62.50+ | 13.94 | 0.34- | 2.41 | 37.17 | 11.82 | 26 | 88 | 10.5 | 57 |
| BGR 10973 | 35.24 | 56 | 15.19 | 0.36 | 2.37 | 36.96 | 12.79 | 25 | 78 | 9.88 | 59.75 |
| BGR 17294 | 34.84 | 55.5 | 15.37+ | 0.40++ | 2.6 | 39.62+ | 13.57++ | 24.75 | 77 | 9.75 | 59.25 |
| BGR 31240 | 26.29--- | 65.00++ | 14.32 | 0.42+++ | 2.96+++ | 35.6 | 11.89 | 23 | 52.5 | 7 | 71.5 |
| BGR 31892 | 33.48 | 65.25++ | 13.97 | 0.40++ | 2.89++ | 36.36 | 12 | 22.00- | 63 | 8.75 | 63.5 |
| BGR 28710 | 42.56+ | 52.75 | 15.27+ | 0.38+ | 2.48 | 39.53+ | 13.39+ | 30.50+++ | 159.50+++ | 7.5 | 70 |
| BGR 33817 | 35.18 | 58.75 | 15.65++ | 0.40++ | 2.52 | 40.45++ | 13.32+ | 38.25+++ | 114.5 | 8.75 | 69.5 |
| BGR 10974 | 35.9 | 55 | 14.33 | 0.36 | 2.51 | 35.82 | 11.98 | 26 | 70.5 | 9.75 | 59.5 |
| BGR 10975 | 34.94 | 60.25 | 14.69 | 0.37 | 2.36 | 38.21 | 12.84 | 25 | 88.5 | 7.75 | 68.75 |
| BGR 26757 | 39.92 | 57 | 14.09 | 0.34- | 2.43 | 36.12 | 12.12 | 21.00-- | 67 | 9.25 | 61 |
| B2000277 | 35.65 | 46.75--- | 13.95 | 0.34- | 2.46 | 33.21- | 11.62 | 22.25- | 64 | 7 | 74.25+ |
| B2000278 | 44.39++ | 40.50--- | 13.57- | 0.33- | 2.49 | 33.12- | 11.27- | 18.50--- | 39.00- | 6.50- | 72.5 |
| B2000279 | 37.43 | 62.75+ | 14.37 | 0.34- | 2.42 | 36.27 | 12.11 | 31.50+++ | 74 | 10.75+ | 58.25 |
| B2000280 | 36.37 | 43.75--- | 14.07 | 0.32-- | 2.28- | 35.33 | 12.22 | 29.75++ | 88.75 | 6.50- | 74.50+ |
| B2000281 | 48.57+++ | 52.5 | 13.59 | 0.30--- | 2.23- | 32.64-- | 11.74 | 29.25+ | 126.00+ | 6.88- | 73.75+ |
| B2000282 | 29.30-- | 43.25--- | 14.1 | 0.32-- | 2.30- | 36.84 | 12.17 | 22.50- | 40 | 9.25 | 61.25 |
| B2000283 | 38.2 | 49.00- | 14.42 | 0.36 | 2.55 | 34.67 | 11.64 | 20.00--- | 34.50- | 8.5 | 61 |
| LSD5% | 5.28 | 5.48 | 0.84 | 0.02 | 0.22 | 2.71 | 0.89 | 2.79 | 39.6 | 1.81 | 8.47 |
| LSD1% | 6.95 | 7.25 | 1.11 | 0.03 | 0.29 | 3.59 | 1.18 | 3.69 | 52.39 | 2.4 | 11.21 |
| LSD0.1% | 8.89 | 9.32 | 1.43 | 0.04 | 0.38 | 4.62 | 1.52 | 4.75 | 67.39 | 3.08 | 14.42 |
| Std. Deviation | 8.20 | 7.92 | 0.63 | 0.04 | 0.24 | 2.29 | 0.70 | 5.44 | 38.41 | 1.58 | 6.85 |
| CV, % | 22.27 | 14.09 | 4.37 | 9.73 | 9.34 | 6.28 | 5.71 | 21.25 | 48.65 | 17.95 | 10.59 |
| MS | 199.7*** | 375.1*** | 2.7*** | 0.07*** | 0.33*** | 30.5*** | 2.9*** | 177.7*** | 8853.7*** | 14.9*** | 278.84** |

TKW-Thousand kernel weight, g; V- Vitreousness, %; CP-Crude protein, %; L- Lysine, %; LC- Lysine content (% of protein); WGC- wet gluten content, %; DGC- dry gluten content, %; SV- Sedimentation value, $cm^3$; FV-Fermentation value, min; GW- Gluten weakness, mm; BSI- Bread-making strength index; MS-Mean square; CV-coefficient of variation, %

Most studies indicate higher protein content in spelt than in wheat (Bonafaccia et al., 2000; Matuz et al., 2000; Oliveira, 2001, Marconi et al., 2002; Pruska-Kedzior et al., 2008; Escarnot et al., 2016). Protein content is significantly influenced by the genotype, environment, field and cultivation technique (Marconi et al., 2002; Loje et al., 2003; Abdel -Aal, 2007; Zielinski et al., 2008; Bojňanská & Francáková, 2002; Lacko-Bartošová et al., 2019). It is generally accepted that the higher the protein, the better is the quality (Vázquez et al., 2012). In our study crude protein value varied between 13.44 and 15.65%. Four accessions (BGR 33817, BGR 17294, BGR 23639 and BGR 28710) were proven to have the highest crude protein content ranging from 15.27 to 15.65% (Table 2). However the crude protein content is not the crucial factor of grain quality. It is also important for describing the structure of protein fractions, baking quality and the composition of amino acids (Desheva, 2016). Lysine is the most limiting essential amino acid in cereal grains (Shewry, 2007). The lysine in the studied accessions varied from 0.30% to 0.43%. The lysine content above 0.40% was observed in BGR 32889 (0.43%), BGR 31240 (0.42%) and BGR 17293 (0.41%). These accessions were characterized also with high lysine content in comparison to protein content in the kernel, respectively 3.16%, 2.96% and 2.70% (Table 2).

The quantity and quality of gluten are considered the most important quality parameters of wheat flour. Gluten

quality is characterized by the degree of extensibility and the elasticity (Curic et al., 2001). The gluten content is directly correlated to the grain protein, which is strongly influenced by the pedoclimatic conditions (Ionescu & Stoenescu, 2010). A wide variation in gluten quality was found among the spelts (Schober et al., 2006). Bojňanská & Francáková (2002) noted that in general all the cultivars of *Triticum spelta* L. showed high wet gluten contents – 37.2%, but their disadvantages are lower swelling values (9.3 ml) and lower sedimentation values (37.4 ml) which have a negative influence on the bread volume and the specific volume (under 310 ml.100 $g^{-1}$). The results of our study also confirm that in general the tested genotypes are characterized with high wet gluten content (36.51%) but with low sedimentation value (25.61). The wet gluten varied from 32.64 for B2000281 to 41.10% for BGR 23639. The value of gluten weakness varied between 6.50 and 11.75 mm. BGR 23639, BGR 32889 and B2000279 showed high values of gluten weakness relating to low gluten quality. The sedimentation valus was found to range from 17 to 38.25 $cm^3$. Proven higher values than the average standard in the experiment were noted in 6 samples. The most relative variable character during the period of study was the fermentation value (CV = 48.65%). Four accessions showed above 100 mm, respectively BGR 19018-181.25 mm, BGR 28710-159.50 mm, B2000281-126 mm and BGR 33817 -114.5 mm. The best combination of grain gluten properties were observed in BGR 33817 and BGR 19018. The bread-making strength index of above samples was also high as an indication for good gluten quality (respectively 69.5 and 63.5) (Table 2).

In Table 3 are presented genetic parameters of analyzed quality characteristics. The estimates of phenotypic, genotypic and environmental variances were the highest for fermentation value and the lowest for lysine. As expected, phenotypic coefficient of variation (PhCV) was greater than genotypic coefficient of variation (GCV) for all of the investigated characteristics indicating the environmental influence on the expression of these traits (Gyawali et al., 2018). The extent of the influence of growing environment on observed traits is explained by the magnitude of the differences between GCV and PhCV. Large difference between PhCV and GCV indicate high environmental influence on the expression of particular traits (Adhikari et al., 2018). High PCV and GCV were observed in the following characteristics: sedimentation value (PhCV = 23.77%, GCV = 20.71) and fermentation value (PhCV = 69.48%, GCV = 43.3%). High PhCV and moderate GCV was recorded for thousand kernel weight (PhCV = 23.41%, GCV = 13.6%) and gluten weakness (PhCV = 27.33%, GCV = 15.41%). Moderate PCV and GCV were found for vitreousness (PhCV = 17.17%, GCV = 13.36%), while low for crude protein content (PhCV = 8.63%, GCV = 3.23%), wet gluten content (PhCV = 9.77%, GCV = 5.16%), dry gluten content (PhCV = 9.32%, GCV = 4.67%). Moderate PCV and low GCV were noted for lysine (PhCV = 12.41%, GCV = 9.07%), lysine content in % of protein (PhCV = 14.61%, GCV = 7.82%) and bread-making strength index (PhCV = 18.36%, GCV = 8.35%). The largest differences between PCV and GCV values were found for fermentation value, gluten weakness, bread-making strength index and thousand kernel weight indicating the high contribution of environmental variance to phenotypic variance (Table 3).

Plant breeders use heritability estimates to determine the influences of the environmental and genetic factors on the trait of interest and choose the selection procedure that should be implemented to make improvements (Kaya & Akcura,

**Table 3. Genetic parameters of various quality characteristics in 22 spelt wheat genotypes**

| Characters | EV | GV | PhV | GCV, % | PhCV, % | HBS, % | GA,% of means |
|---|---|---|---|---|---|---|---|
| Thousand kernel weight, g | 49.23 | 25.08 | 74.30 | 13.6 | 23.41 | 33.74 | 16.28 |
| Vitreousness, % | 36.71 | 56.40 | 93.11 | 13.36 | 17.17 | 60.57 | 21.42 |
| Crude protein content, % | 1.34 | 0.22 | 1.56 | 3.23 | 8.63 | 14 | 2.49 |
| Lysine, % | 0.001 | 0.001 | 0.002 | 9.07 | 12.41 | 53.45 | 13.67 |
| Lysine content in % of protein | 0.096 | 0.039 | 0.135 | 7.82 | 14.61 | 28.62 | 8.62 |
| Wet gluten content, % | 9.17 | 3.56 | 12.73 | 5.16 | 9.77 | 27.94 | 5.62 |
| Dry gluten content, % | 0.98 | 0.33 | 1.30 | 4.67 | 9.32 | 25.09 | 4.82 |
| Sedimentation value, $cm^3$ | 8.92 | 28.14 | 37.06 | 20.71 | 23.77 | 75.93 | 37.17 |
| Fermentation value, min | 1840 | 1168.9 | 3008.96 | 43.3 | 69.48 | 38.85 | 55.6 |
| Gluten weakness, mm | 3.94 | 1.84 | 5.78 | 15.41 | 27.33 | 31.8 | 17.91 |
| Bread-making strength index | 108.6 | 28.37 | 136.99 | 8.35 | 18.36 | 20.71 | 7.83 |

EV – Environmental variance; GV – Genotypic variance; PhV – Phenotypic variance; GCV – Genotypic coefficient of variability, %; PhCV – Phenotypic coefficient of variability, %; HBS – Heritability in broad sense, %; GA – Genetic advance, % of means

2014). Heritability estimates are classified as low (5-10%), medium (10-30%) and high (30-60%) (Robinson, 1966). In our investigation the high estimates of heritability in broad sense were recorded for sedimentation value (75.93%), vitreousness (60.57%), lysine (53.45), fermentation value (38.85), thousand kernel weight (33.74%) and gluten weakness (31.80%). The remaining characters had medium heritability. The expected genetic advance expressed as a percentage of the mean varied between 2.49 to 55.6%. Deshmukh et al. (1986) classified genetic advance as percent of mean as low (<10%), moderate (10-20%) and high (>20%). The high genetic advance as percentage of mean was recorded for fermentation value (55.6%), followed by sedimentation value (37.17%), and vitreousness (21.42). Moderate value was observed for thousand kernel weight (16.28%), lysine (13.67%) and gluten weakness (17.91%), while the low value for remaining characters. High heritability accompanied with high genetic advance indicates that most likely the heritability is due to additive gene effects and selection may be effective (Devesh et al., 2018). In our study high heritability coupled with high genetic advance as percentage of mean was recorded for the following characters: vitreousness, sedimentation value and fermentation value, that indicates selection of such traits may be effective (Table 3).

***Genetic variations of gliadins through A-PAGE***

Protein electrophoresis is considered a reliable, practical and reproducible method because seed storage proteins are the third hand copy of genomic DNA and largely independent of environmental fluctuations (Javaid et al., 2004; Iqbal et al., 2005). Gliadins are generally considered to contribute to the viscosity and extensibility of gluten (Zarghani & Imamjomeh, 2011). Although some authors have associated specific gliadin alleles with bread-making quality, Gianibelli et al. (2001) noted that these proteins may not have a direct effect on wheat. Gliadins are monomeric prolamins, controlled by the Gli-1 and Gli-2 loci, located on the short arm of chromosome of the homoeologous group 1 and 6, respectively (Payne, 1987; Payne et al., 1982) and when are separated by acid-polyacrylamide gel electrophoresis (A PAGE) they form a pattern composed of a certain number of subunits, depending on the genotype (Waga, 2002). Gliadins are usually divided into four fractions ω, γ, β and α-based on decreasing electrophoretic mobility in A-PAGE (Piergiovanni & Volpe, 2014). Escarnot et al. (2012) noted that Abdel-Aal et al. (1996) and Harsch et al. (1997) have found that spelt gliadins do not have slow-moving ω-gliadin or strong-staining fast-moving ω-gliadin, but these are present in common wheat. Spring and winter spelt are characterized by a large number of slow-moving α-gliadins. A γ-gliadin band was also observed in spring spelt, but not in winter spelt or wheat, and this could be a useful point of distinction.

In Table 4 are presented the number of gliadin bands, patterns, and the genetic variation index in gliadins for 22 investigated genotypes of *Triticum spelta* L. Among the 22 spelt wheat accessions analysed, 53 different bands (51 polymorphic and 2 monomorphic bands) were detected assuming that the bands with the same relative mobility represent the same protein. Each zone (α, β, γ and ω) was considered as a single locus and the different patterns as allelic variants. The patterns within each gliadin group of α, β, γ and ω were identified by comparing banding patterns of each spelt accession with all the other spelt accessions (Aliyeva et al., 2012).

A total of 14 different mobility bands and 13 gliadin patterns were identified in the ω-gliadin zone. Bands varied between four and nine in each ω-gliadin pattern, as patterns with six bands being the most frequent (36.36%). Nine accessions presented its unique ω-gliadin pattern, while genotypes -BGR 17293, BGR 17294 and BGR 10974 had the ω-gliadin pattern 2, genotypes – BGR 10978, BGR 23639 and BGR 19018 had the pattern 3 and genotypes – BGR 31892, BGR 28710 and BGR 10975 had the pattern 7. The ω-gliadin pattern 10 is noted respectively in four accessions (B2000277, B2000278, B2000279 and B2000281) (Figure 1).

In the γ-gliadin zone, 17 bands and 18 different patterns were noted. The γ -gliadin pattern 3, were observed in accessions BGR 10978 and BGR 23639. The pattern 10 was detected in BGR 28710 and BGR 10975. The γ -gliadin patterns 11 and 13 were marked respectively in two groups of accessions – BGR 33817 and BGR 10974; B2000277 and B2000279, respectively (Figure 1).

Fourteen β-gliadin patterns and totally 10 different mobility bands were found. The bands in the gliadin patterns varied from 2 to 4 bands, as patterns with three bands being the most frequent (54.54%). Ten accessions had specific patterns in the β- gliadin zone, while BGR 10978 and BGR 23639 with pattern 3, BGR 19018 and BGR 31240 with pattern 4, BGR 28710, BGR 10975, B2000277, B2000279 and B2000281 with pattern 9, BGR 33817, BGR 10974 and BGR 26757 with pattern 10 had the similar patterns (Figure 1).

Twelve bands were encountered in α gliadin region and sixteen different α -gliadin patterns were determined. Twelve accessions had specific patterns, while α -gliadin pattern with number 3 included 2 genotypes (BGR 10978 and BGR 23639), pattern 10 – 2 genotypes (BGR 28710 and BGR 10975), pattern 14 – 4 genotypes (B2000277, B2000279, B2000281 and B2000283) and pattern 16- 2 genotypes (B2000280 and B2000282). The accessions with four bands in the α-gliadin zone predominate (45.45%) (Table 4, Figure 1).

**Table 4. Number of gliadin bands, patterns, and the genetic diversity in gliadins for 22 genotypes of *Triticum spelta* L.**

| № | Accession number | Number of gliadin bands | | | | Total bands |
|---|---|---|---|---|---|---|
| | | α | β | γ | ω | |
| 1 | BGR 32889 | 2 | 2 | 4 | 7 | 15 |
| 2 | BGR 17293 | 4 | 3 | 5 | 5 | 17 |
| 3 | BGR 10978 | 4 | 4 | 4 | 6 | 18 |
| 4 | BGR 23639 | 4 | 4 | 4 | 6 | 18 |
| 5 | BGR 19018 | 3 | 2 | 4 | 6 | 15 |
| 6 | BGR 31344 | 5 | 3 | 3 | 5 | 16 |
| 7 | BGR 10973 | 4 | 4 | 5 | 9 | 22 |
| 8 | BGR 17294 | 4 | 3 | 4 | 5 | 16 |
| 9 | BGR 31240 | 4 | 2 | 4 | 7 | 17 |
| 10 | BGR 31892 | 5 | 3 | 4 | 8 | 20 |
| 11 | BGR 28710 | 4 | 3 | 5 | 8 | 20 |
| 12 | BGR 33817 | 2 | 2 | 5 | 7 | 16 |
| 13 | BGR 10974 | 3 | 2 | 5 | 5 | 15 |
| 14 | BGR 10975 | 4 | 3 | 5 | 8 | 20 |
| 15 | BGR 26757 | 4 | 2 | 6 | 4 | 16 |
| 16 | B2000277 | 3 | 3 | 6 | 6 | 18 |
| 17 | B2000278 | 4 | 3 | 5 | 6 | 18 |
| 18 | B2000279 | 3 | 3 | 6 | 6 | 18 |
| 19 | B2000280 | 3 | 2 | 3 | 8 | 16 |
| 20 | B2000281 | 3 | 3 | 5 | 6 | 17 |
| 21 | B2000282 | 3 | 3 | 6 | 7 | 19 |
| 22 | B2000283 | 3 | 3 | 5 | 6 | 17 |
| Range of gliadin bands | 2-5 | 2-4 | 3-6 | 4-9 | 15-22 | |
| Number of gliadin patterns | 16 | 14 | 18 | 13 | 20 | |
| H, % | 0.844 | 0.735 | 0.908 | 0.734 | 0.805 | |

H, % – genetic variation index

**Fig. 1. Ideograms of different gliadins patterns in the α** (1 – BGR 32889; 2 – BGR 17293; 3 – BGR 10978 and BGR 23639; 4 – BGR 19018; 5 – BGR 31344; 6 – BGR 10973; 7 – BGR 17294; 8 – BGR 31240; 9 – BGR 31892; 10 – BGR 28710 and BGR 10975; 11 – BGR 33817; 12 – BGR 10974; 13 – BGR 26757; 14 – B2000277, B2000279, B2000281 and B2000283; 15 – B2000278; 16 – B2000280 and B2000282), β (1 – BGR 32889; 2 – BGR 17293; 3 – BGR 10978 and BGR 23639; 4 – BGR 19018 and BGR 31240, 5 – BGR 31344; 6 – BGR 10973; 7 – BGR 17294; 8 – BGR 31892; 9 – BGR 28710, BGR 10975, B2000277, B2000279 and B2000281; 10 – BGR 33817, BGR 10974 and BGR 26757; 11 – B2000278; 12 – B2000280; 13 – B2000282; 14 – B2000283), γ (1 – BGR 32889; 2 – BGR 17293; 3 – BGR 10978 and BGR 23639; 4 – BGR 19018; 5 – BGR 31344; 6 – BGR 10973; 7 – BGR 17294; 8 – BGR 31240; 9 – BGR 31892; 10 – BGR 28710 and BGR 10975; 11 – BGR 33817 and BGR 10974; 12 – BGR 26757; 13 – B2000277 and B2000279; 14 – B2000278; 15 – B2000280; 16 – B2000281; 17 – B2000282; 18 – B2000283) and ω (1 – BGR 32889; 2 – BGR 17293, BGR 17294 and BGR 10974; 3 – BGR 10978, BGR 23639 and BGR 19018; 4 – BGR 31344; 5 – BGR 10973; 6 – BGR 31240; 7 – BGR 31892, BGR 28710 and BGR 10975; 8 – BGR 33817; 9 – BGR 26757; 10 – B2000277, B2000278, B2000279 and B2000281; 11 – B2000280; 12 – B2000282; 13 – B2000283) regions

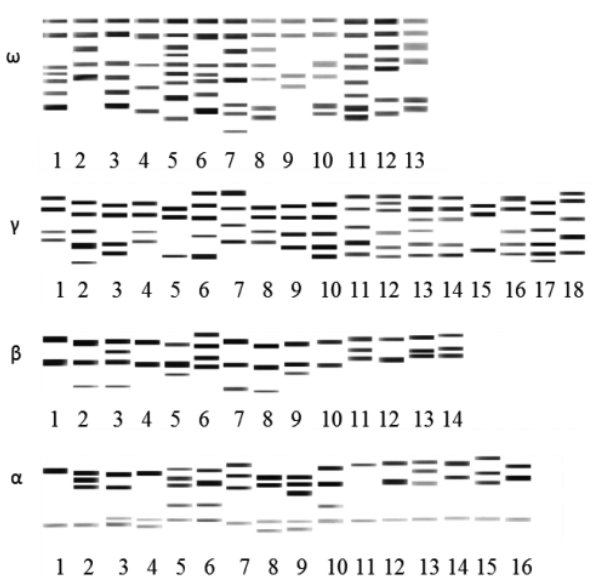

**Table 5. Average genetic similarity matrix of Jaccard coefficient based on A-PAGE gliadin patterns for the 22 spelt accessions**

| Case | Jaccard Measure | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| 1:BGR 32889 | 1.000 | | | | | | | | | | | | | | | | | | | | | |
| 2:BGR 17293 | 0.231 | 1.000 | | | | | | | | | | | | | | | | | | | | |
| 3:BGR 10978 | 0.269 | 0.346 | 1.000 | | | | | | | | | | | | | | | | | | | |
| 4:BGR 23639 | 0.269 | 0.346 | 1.000 | 1.000 | | | | | | | | | | | | | | | | | | |
| 5:BGR 19018 | 0.500 | 0.391 | 0.435 | 0.435 | 1.000 | | | | | | | | | | | | | | | | | |
| 6:BGR 31344 | 0.292 | 0.269 | 0.259 | 0.259 | 0.292 | 1.000 | | | | | | | | | | | | | | | | |
| 7:BGR 10973 | 0.276 | 0.219 | 0.212 | 0.212 | 0.194 | 0.407 | 1.000 | | | | | | | | | | | | | | | |
| 8:BGR 17294 | 0.240 | 0.435 | 0.308 | 0.308 | 0.348 | 0.280 | 0.267 | 1.000 | | | | | | | | | | | | | | |
| 9:BGR 31240 | 0.391 | 0.417 | 0.296 | 0.296 | 0.455 | 0.435 | 0.219 | 0.320 | 1.000 | | | | | | | | | | | | | |
| 10:BGR 31892 | 0.207 | 0.276 | 0.357 | 0.357 | 0.250 | 0.286 | 0.313 | 0.241 | 0.321 | 1.000 | | | | | | | | | | | | |
| 11:BGR 28710 | 0.296 | 0.321 | 0.267 | 0.267 | 0.346 | 0.565 | 0.400 | 0.241 | 0.321 | 0.429 | 1.000 | | | | | | | | | | | |
| 12:BGR 33817 | 0.292 | 0.222 | 0.308 | 0.308 | 0.409 | 0.333 | 0.357 | 0.524 | 0.320 | 0.333 | 0.385 | 1.000 | | | | | | | | | | |
| 13:BGR 10974 | 0.250 | 0.333 | 0.222 | 0.222 | 0.304 | 0.348 | 0.370 | 0.632 | 0.333 | 0.296 | 0.296 | 0.722 | 1.000 | | | | | | | | | |
| 14:BGR 10975 | 0.296 | 0.321 | 0.267 | 0.267 | 0.346 | 0.565 | 0.400 | 0.241 | 0.321 | 0.429 | 1.000 | 0.385 | 0.296 | 1.000 | | | | | | | | |
| 15:BGR 26757 | 0.348 | 0.375 | 0.214 | 0.214 | 0.292 | 0.333 | 0.267 | 0.391 | 0.222 | 0.200 | 0.333 | 0.231 | 0.292 | 0.333 | 1.000 | | | | | | | |
| 16:B2000277 | 0.269 | 0.296 | 0.385 | 0.385 | 0.375 | 0.478 | 0.250 | 0.259 | 0.400 | 0.407 | 0.462 | 0.478 | 0.435 | 0.462 | 0.259 | 1.000 | | | | | | |
| 17:B2000278 | 0.222 | 0.207 | 0.440 | 0.440 | 0.320 | 0.360 | 0.333 | 0.214 | 0.296 | 0.357 | 0.407 | 0.360 | 0.269 | 0.407 | 0.308 | 0.565 | 1.000 | | | | | |
| 18:B2000279 | 0.269 | 0.296 | 0.385 | 0.385 | 0.375 | 0.478 | 0.250 | 0.259 | 0.400 | 0.407 | 0.462 | 0.478 | 0.435 | 0.462 | 0.259 | 1.000 | 0.565 | 1.000 | | | | |
| 19:B2000280 | 0.192 | 0.138 | 0.360 | 0.360 | 0.292 | 0.231 | 0.226 | 0.185 | 0.269 | 0.385 | 0.241 | 0.333 | 0.292 | 0.241 | 0.103 | 0.417 | 0.360 | 0.417 | 1.000 | | | |
| 20:B2000281 | 0.280 | 0.259 | 0.346 | 0.346 | 0.391 | 0.435 | 0.219 | 0.269 | 0.360 | 0.370 | 0.423 | 0.500 | 0.455 | 0.423 | 0.269 | 0.944 | 0.522 | 0.944 | 0.435 | 1.000 | | |
| 21:B2000282 | 0.172 | 0.241 | 0.370 | 0.370 | 0.308 | 0.207 | 0.242 | 0.250 | 0.161 | 0.258 | 0.219 | 0.296 | 0.308 | 0.219 | 0.129 | 0.276 | 0.276 | 0.276 | 0.522 | 0.286 | 1.000 | |
| 22:B2000283 | 0.222 | 0.207 | 0.286 | 0.286 | 0.222 | 0.259 | 0.379 | 0.259 | 0.250 | 0.520 | 0.357 | 0.360 | 0.320 | 0.357 | 0.172 | 0.440 | 0.385 | 0.440 | 0.478 | 0.400 | 0.370 | 1.000 |

Considering the four zones together, 20 gliadin patterns were identified. The number of bands varied between 15 and 22. The highest number of bands had BGR 10973, while the lowest was found in BGR 32889, BGR 19018 and BGR 10974 genotypes (Table 2). Three groups of genotypes (BGR 10978 and BGR 23639), (BGR 28710 and BGR 10975) and (B2000277 and B2000279) showed identical gliadin patterns in their gliadin spectra.

The genetic diversity based on the patterns was calculated for each of the four zones. The genetic variation indexes varied between 0.734 and 0.908. Gama (γ) zone was found to have the most diversity (H = 0.908), followed by α (H = 0.844) and the least diversity being that of ω (H = 0.734). The mean value of H calculated for all the four groups of gliadins was also high (H = 0.805) (Table 4). It indicated that the high genetic variation existed in the investigated spelt wheat (Zhao-cai et al., 2006).

Caballero et al. (2004) reported that in spelt wheat, the gliadins encoding by 1A, 1B and 1D (i.e. ω- and γ-gliadins) had higher genetic variability than that of encoding by 6A, 6B and 6D (i.e. α and β-gliadins). Pu et al. (2007) also confirm that the relatively lower variations were detected in α and β-gliadin zones. They noted also that the polymorphism of spelt wheat on *Gli-1* loci was higher than that on *Glu-1* loci. In this study, it was observed that α, β, γ and ω zones had high allelic variants, but the least allelic variants were observed in ω area. The highest number of allelic variants was observed for γ gliadins (Figure 1). Our results are inconsistent with those noted above and this may be due to the relatively small number of analyzed genotypes. Keskin San et al. (2015) also noted that differences might be due to different species, and the number of samples used as representatives of accessions or populations in other studies. Although care was taken to separate all the bands, more than one protein may be present in a band (Medouri et al., 2015).

The genetic similarities (GS) among 22 spelt genotypes estimated by Jaccard's coefficient are presented in Table 5. It ranged from 0.103 to 1. The lowest GS was found between genotypes: BGR 26757 and B2000280, respectively, which indicates that the genetic diversity within these pairs of accessions was very high. Three pairs of genotypes had GS equal to 1 (BGR 10978 and BGR 23639; B2000277 and B2000279; BGR 28710 and BGR 10975), which indicated that they are genetically identical and do not differ in their gliadin spectra. These pairs are obtained from Germany, Spain and Belgium, respectively and probably they are duplicates in the gene bank's collection (Table 1). However, to be sure of our conclusion other electrophoretic tests should be applied for discrimination (Wrigley et al., 1982).

***Cluster analyzes***

The analysis of genetic diversity through the cluster analyzes are shown in Figure 2 and Figure 3. The cluster diagram for 11 studied variables (quality characteristics of grain and whole spelt wheat flour) based on Euclidean dissimilarity us-

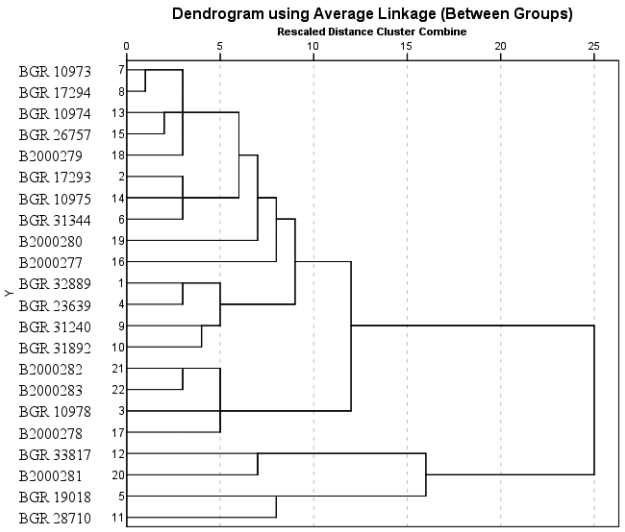


**Fig. 2. Tree dendogram of 22 spelt genotypes for 11 studied variables using hierarchical cluster analysis (Between-group linkage method and Euclidean distance)**

**Table 6. The average value of characters for each cluster and difference between each cluster and the total mean (Diff).**

| Clusters | | TKW, g | V, % | CP, % | L, % | LC, % | WGC, % | DGC, % | SV, cm$^3$ | FV, min | GW, mm | BSI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | Mean | 34.60 | 58.34 | 14.42 | 0.37 | 2.56 | 36.84 | 12.35 | 25.02 | 72.05 | 9.25 | 62,70 |
| | Diff | -4.67 | 3.13 | -0.05 | 0.01 | 0.10 | 0.42 | 0.08 | -3.02 | -27.80 | 0.90 | -4.31 |
| II | Mean | 36.34 | 47.25 | 14.15 | 0.35 | 2.50 | 34.92 | 11.67 | 19.50 | 36.75 | 7.85 | 66.94 |
| | Diff | -2.93 | -7.96 | -0.33 | -0.01 | 0.03 | -1.51 | -0.60 | -8.54 | -63.11 | -0.51 | -0.06 |
| III | Mean | 41.88 | 55.63 | 14.62 | 0.35 | 2.38 | 36.55 | 12.53 | 33.75 | 120.25 | 7.82 | 71.63 |
| | Diff | 2.61 | 0.42 | 0.14 | -0.01 | -0.09 | 0.12 | 0.26 | 5.71 | 20.39 | -0.54 | 4.62 |
| IV | Mean | 44.26 | 59.63 | 14.72 | 0.36 | 2.43 | 37.40 | 12.54 | 33.88 | 170.38 | 8.50 | 66.75 |
| | Diff | 4.99 | 4.42 | 0.24 | 0.00 | -0.04 | 0.97 | 0.27 | 5.84 | 70.52 | 0.15 | -0.25 |
| Total mean | 39.27 | 55.21 | 14.48 | 0.36 | 2.46 | 36.42 | 12.27 | 28.04 | 99.86 | 8.35 | 67.00 | |

**Table 7. Proximity Matrix for 22 spelt wheat on the base of studied quality characters**

| Case | Euclidean Distance | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| 1:BGR 32889 | 0.00 | | | | | | | | | | | | | | | | | | | | | |
| 2:BGR 17293 | 46.65 | 0.00 | | | | | | | | | | | | | | | | | | | | |
| 3:BGR 10978 | 28.36 | 62.34 | 0.00 | | | | | | | | | | | | | | | | | | | |
| 4:BGR 23639 | 9.66 | 44.15 | 31.67 | 0.00 | | | | | | | | | | | | | | | | | | |
| 5:BGR 19018 | 133.24 | 89.49 | 150.32 | 129.68 | 0.00 | | | | | | | | | | | | | | | | | |
| 6:BGR 31344 | 38.74 | 12.14 | 58.02 | 35.57 | 94.84 | 0.00 | | | | | | | | | | | | | | | | |
| 7:BGR10973 | 30.70 | 16.76 | 47.29 | 28.28 | 105.14 | 12.40 | 0.00 | | | | | | | | | | | | | | | |
| 8:BGR 17294 | 30.07 | 17.83 | 46.65 | 27.21 | 106.35 | 13.74 | 3.08 | 0.00 | | | | | | | | | | | | | | |
| 9:BGR 31240 | 18.57 | 44.65 | 22.97 | 21.89 | 131.30 | 39.82 | 31.07 | 30.73 | 0.00 | | | | | | | | | | | | | |
| 10:BGR31892 | 16.96 | 33.36 | 32.73 | 15.47 | 119.90 | 26.44 | 18.46 | 18.29 | 15.19 | 0.00 | | | | | | | | | | | | |
| 11:BGR28710 | 112.43 | 66.56 | 127.23 | 108.96 | 28.07 | 73.93 | 82.79 | 83.83 | 109.28 | 98.39 | 0.00 | | | | | | | | | | | |
| 12:BGR33817 | 68.55 | 24.52 | 84.09 | 65.81 | 68.57 | 32.28 | 40.30 | 41.30 | 65.03 | 54.94 | 46.67 | 0.00 | | | | | | | | | | |
| 13:BGR10974 | 24.51 | 24.08 | 40.59 | 23.03 | 112.44 | 19.27 | 7.84 | 7.96 | 26.02 | 14.17 | 90.13 | 47.19 | 0.00 | | | | | | | | | |
| 14:BGR10975 | 42.14 | 10.24 | 56.01 | 39.36 | 94.63 | 12.45 | 14.69 | 15.88 | 37.60 | 26.83 | 72.04 | 29.35 | 21.20 | 0.00 | | | | | | | | |
| 15:BGR26757 | 23.01 | 28.72 | 36.48 | 20.11 | 115.98 | 23.12 | 12.82 | 12.71 | 24.08 | 11.55 | 93.65 | 51.72 | 7.75 | 24.11 | 0.00 | | | | | | | |
| 16:B2000277 | 31.85 | 33.86 | 32.53 | 32.60 | 120.81 | 34.11 | 22.91 | 23.06 | 23.82 | 21.84 | 96.62 | 55.08 | 18.89 | 29.13 | 17.98 | 0.00 | | | | | | |
| 17:B2000278 | 36.87 | 59.48 | 20.13 | 38.61 | 146.15 | 57.39 | 45.62 | 45.12 | 33.75 | 37.65 | 121.96 | 81.15 | 38.97 | 54.93 | 35.10 | 27.53 | 0.00 | | | | | |
| 18:B2000279 | 26.69 | 22.97 | 46.27 | 24.25 | 107.95 | 15.26 | 10.64 | 11.41 | 29.22 | 16.28 | 87.17 | 43.10 | 10.38 | 19.71 | 14.44 | 26.92 | 46.58 | 0.00 | | | | |
| 19:B2000280 | 49.81 | 17.33 | 58.17 | 48.70 | 96.71 | 26.32 | 22.86 | 23.85 | 43.85 | 36.35 | 71.88 | 31.97 | 26.64 | 18.46 | 30.47 | 26.14 | 51.83 | 29.43 | 0.00 | | | |
| 20:B2000281 | 82.09 | 37.40 | 94.64 | 78.89 | 58.64 | 45.16 | 52.34 | 53.78 | 78.16 | 67.34 | 35.04 | 22.83 | 58.99 | 41.59 | 61.85 | 63.98 | 88.59 | 56.63 | 40.27 | 0.00 | | |
| 21:B2000282 | 25.44 | 55.64 | 20.13 | 29.78 | 144.90 | 52.39 | 40.65 | 39.63 | 27.40 | 32.20 | 121.24 | 78.47 | 33.59 | 52.35 | 32.15 | 28.57 | 20.03 | 41.17 | 51.62 | 89.88 | 0.00 | |
| 22:B2000283 | 25.63 | 60.55 | 15.20 | 28.69 | 149.02 | 55.81 | 44.56 | 43.77 | 29.06 | 33.35 | 126.01 | 83.35 | 37.13 | 56.15 | 33.57 | 32.67 | 16.51 | 43.56 | 57.06 | 93.53 | 12.43 | 0.00 |

ing the Between-groups linkage method categorized the genotypes into four clusters at a 20% linkage distance. The average of characters for each cluster and the difference between each cluster with the total mean (Diff) are presented in Table 6. The first cluster comprises 14 accessions including 63.64% of total genotypes. The genotypes in this group were in the highest rate with respect to lysine (0.37%), respectively lysine content in % of protein) (2.56), and gluten weakness (9.25 mm).

The genotypes in this cluster had the lowest values for bread-making strength index (62.70) (Table 6). In the second group, 4 genotypes (BGR 10978 from DEU and B2000278, B2000282, B2000283 from Belgium) were classified including 43.75% of total accessions. The values for the most of studied charaters in this cluster were the lowest compared with the total means of all genotypes (Table 6).

The third group included 2 accessions – BGR 33817 from ESP and B2000281 from Belgium. The genotypes in this group were in the highest rate with respect to bread-making strength index (71.63). The fourth cluster group BGR 19018 from Rusia and BGR 28710 from Spain. The genotypes in this group were in the highest rate with respect to thousand kernel weight (44.26 g), vitreousness (59,63%), crude protein content (14.72%), wet gluten content (37.40%), dry gluten content (12.54%), sedimentation value (33.88 $cm^3$) and fermentation value (170.38 mm) (Table 6). These genotypes are suitable for breeding programs aimed to improve quality characteristic of grain in wheat varieties. The most phenotypically distant genotypes were BGR 10978 (from Germany) and BGR 19018 (from Rusia), following from BGR 19018 and B2000277 (from Belgium) and the closest were BGR 10973 (from Germany) and BGR 17294 (from Germany), following from BGR 10974 (from Spain) with BGR 26757 (from Iran) and BGR 10973 (from Germany) with BGR 10974 (from Spain) (Table 7, Figure 2).

Cluster analysis base on gliadin bands data using Between groups-linkage method and Euclidean distance classified genotypes into 11 main groups at 20 linkage distance (Figure 3). Cluster 1 included 4 genotypes (B2000277, B2000279, B2000281 and B2000278) which had pattern 10 in the β zone and origin from Belgium. Cluster 2 combined 3 genotypes divided to two subgroups. The first subgroup grouped BGR 28710 and BGR 10975, which are similar by gliadin spetra and origin from Spain and the second subgroup included BGR 31344 from Germany. Cluster 3 included genotypes- BGR 31892 from Germany and B2000283 from Belgum. Genotypes BGR 17293 and BGR 26757 are separated in individual groups, respectively cluster 4 and cluster 5. In Cluster 6 are included 3 genotypes. The cluster is divided to two subgroups. The first subgroup grouped BGR 33817 and BGR 10974 from Spain, which had pattern 11 in the γ zone and pattern 10 in β

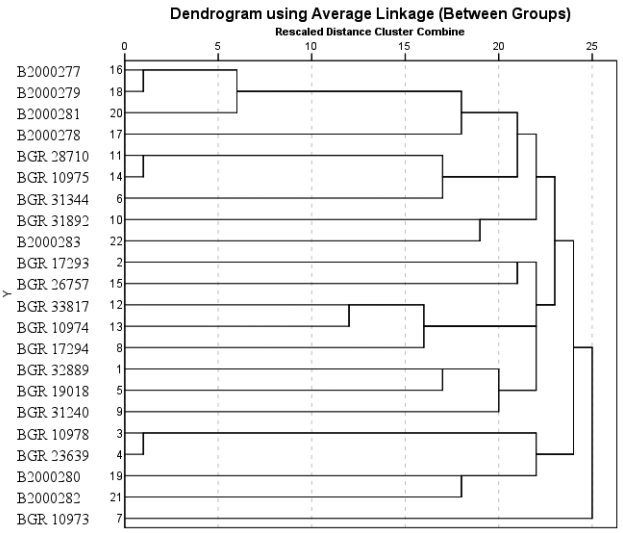


**Fig. 3. Tree dendogram of 22 spelt genotypes base on *A-PAGE* using hierarchical cluster analysis (Between-group linkage method and Euclidean distance)**

zone. The second subgroup included BGR 17294 from Germany. Cluster 7 combined BGR 32889 from Germany and BGR 19018 from Russia. Genotype BGR 31240 from Germany is separated in cluster 8. In cluster 9 are included BGR 10978 and BGR 23639, which are identical by gliadin spectra and origin from Germany. Cluster 10 combined B2000280 and B2000282 from Belgium, while BGR 10973 from Germany is separated in the cluster 11 (Figure 3).

Comparing the two cluster analyzes, no relationships were established regarding to the distribution of genotypes in the individual clusters. The genotypes which are genetically similar on the base of gliadin bands in their gliadin spectra fall into different groups when are clustering on the base of the studied qualitative parameters. Genetic diversity differentiated primarily by ecological factors such as soil mineral content, moisture stress, and microclimatic condition (Zarghani & Imamjomeh, 2011). Therefore the gliadin electrophoresis of of spelt wheat genotypes as qualitatively independent of growth locations and years (Anjum et al., 2000) is a strong system for identifying similar genotypes (Ojaghi & Akhundova, 2010). The results of our investigation also show that electrophoretic spectra of gliadins are highly polymorphic for genotype identification in spelt wheat.

## Conclusions

The present study showed the existence of wide ranges of variations for all of the investigated quality characteristics among spelt wheat genotypes. High heritability accompa-

nied with high expected genetic advances in the case of vitreousness, sedimentation value and fermentation value, indicates that selection may be effective in early generations for these traits. BGR 19018 from Russia and BGR 28710 from Spain were characterized with high thousand kernel weight, vitreousness, crude protein content, wet and dry gluten contents, sedimentation value and fermentation value and are suitable for breeding programs aimed to improve quality characteristics in wheat varieties. The investigated 22 spelt wheat genotypes were also characterized with high genetic diversity on the basis of their alcohol soluble proteins-gliadins by the acid-PAGE method. Gamma (γ) gliadin zone was found to have the higest genetic variation, followed by α and the least variation being that of ω. The highest genetic similarity (GS) was found between accessions: BGR 10978 and BGR 23639; B2000277 and B2000279; BGR 28710 and BGR 10975, respectively while the lowest GS was observed between BGR 26757 and B2000280.

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