MOLECULAR CHARACTERIZATION OF GENES FOR QUALITY TRAITS IN MACEDONIAN WHEAT GENOTYPES (Triticum aestivum L.)

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ABSTRACT
The main goal of this study was to characterize glutenin subunits with high molecular weight (HMW-GS) in Macedonian wheat genotypes by using DNA markers and to analyse the polymorphism of the Glu-A1 and Glu-D1 loci, influencing wheat bread-making quality. Polymorphysm and allelic variations in the Glu-A1 loci was determined through characterization of Ax-null, Ax1 and Ax2* alleles, and in Glu-D1 loci through characterization of Dx2+Dy12 and Dx5+Dy10 alleles. Ax null, that has negative influence on bread-making quality, was detected in 77.66% of the genotypes. The allelic pair Dx5+Dy10 was present in 68.09% of the genotypes. Only 8.51% of the analysed genotypes had the allele Ax1. According to the identified alleles in the Glu A1 and Glu D1 loci, the genotypes were grouped in two main clusters, 64 in the first and 30 genotypes in the second cluster. Both clusters consisted of three subclusters, comprising different number of genotypes. The most of the genotypes belonged to the subgroups 1a (presence of Dx5+Dy10) and 2a (presence of Dx2+Dy12). Genotypes in the 2a subgroup had Ax-null in Glu-A1 locus and Dx2+Dy12 in Glu-D1 locus, negatively influencing the wheat bread-making quality. These genotypes are not recommended to be used in a breeding program for improving wheat bread-making quality. Genotypes from the subgroups 1b and 1c possessed the alleles Ax2* and Ax1 in Glu-A1 locus and Dx5+Dy10 in Glu-D1 locus, indicating good bread-making quality. The superior breeding lines with improved quality, good agronomic characteristics and high yield have to be evaluated for their adaptability and stability. The lines with a complex of positive characteristics may be submitted for registration of new varieties. Further investigations of the material are needed for the other loci influencing the wheat bread-making quality.

Keywords: wheat, glutenins, HMW-GS, DNA markers, bread-making quality.

INTRODUCTION
Wheat (Triticum aestivum L.) is one of the most significant cereal crops in the world, grown on more than 200 million hectares, with a production of over 700 million tons per year (FAO, 2018). Today, wheat is the most widespread cereal crop worldwide, with over 25,000 varieties grouped by chromosome number. Total wheat production is almost entirely based on two modern species: *Triticum aestivum* or bread wheat (2n = 6x = 42, with AABBDD genome), and *Triticum durum* or durum wheat (2n = 4x = 28, with AABB genome). Bread wheat is represented with the largest production volume, or approximately 95% of the total production (Dvorak et al., 2011). Wheat flour is the most unique among the cereals and it can form dough that exhibits the rheological properties required for wide diversity of foods (Goutam et al. 2013). Today, the biggest challenge for wheat breeders is not only to improve the yield, but also to improve the grain quality in order to reach the requirements of end users (Duveiller et al., 2007). Improvement of this significant crop is limited by the size and complexity of its genome (Dubcovsky and Dvorak, 2007) which is approximately 17 Gbp (Ramirez-Gonzales et al., 2018).
Most of the proteins in the dough are converted into gluten complex, and unlike the other cereals, wheat contains gluten, one of the most important storage proteins in the endosperm. Gliadin and glutenin are the main components of gluten, which is the major contributor to the rheological and bread-making properties of wheat flour (Goutam et al., 2013; Lindsay and Skerritt, 1999; Shewry et al., 2002). According to their specific weight, the glutenins are divided into high molecular weight glutenin

subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Payne et al., 1980; Jackson et al., 1983; Singh et al., 1991).

HMW-GS, as components of gluten polymers, have a major role in determining the high elastic properties of the dough (Payne et al., 1987; Nieto-Taladriz et al., 1994). They are encoded by the Glu-1 loci, located on the long arms of the chromosome 1A, labelled Glu-A1, chromosome 1B- Glu-B1 and chromosome 1D-Glu-D1 (Payne et al., 1980, Butow et al., 2003). Allelic variations in the Glu-D1 locus are thought to have a higher impact on bakery quality than the variations in alleles at other Glu-1 loci. Alleles Ax1 and Ax2* in the Glu-A1 locus and Dx5 + Dy10 in Glu-D1 locus have the highest impact on good bakery features, but unlike them, Ax-null and Dx2 + Dy12 are negatively correlated with the baking quality (Kocourková et al., 2008). According to Ahmad (2000), specific PCR primers can be successfully used to identify wheat genotypes with certain glutenin allelic combinations associated with good or poor baking quality. De Bustos et al. (2000, 2001) developed and applied 6 dominant markers based on amplification of whole coding regions of 7 HMW-GS genes (Ax2*, Ax1, Ax-null, Dx5, Dy10, Dx2 and Dy12). HMW-GS are the major determinants of wheat bread making quality (Payne et al., 1987), confirmed more than 30 years ago. As indicators of quality, they are the most explored components of dough composition (Shewry et al., 1992; He et al., 2005; Shitre et al., 2016; Hristov et al., 2009).

In our country, the genetic characterization of high molecular glutenins in wheat using DNA markers was not performed by now. Therefore, the main purpose of this study was to make the first molecular characterization of HMW-GS in Macedonian wheat genotypes, i.e. to analyse the DNA polymorphisms of the Glu-A1 and Glu-D1 loci, by using DNA markers. The genotypes that possess the desired alleles can be used to improve the quality of existing wheat varieties and for creating new varieties.

MATERIAL AND METHODS

Characterization of high-molecular gluten subunits of the Glu-A1 and Glu-D1 loci was performed in order to ascertain which alleles are most common in 94 Macedonian wheat genotypes (Table 1). Ten of them are registered Macedonian varieties, and the rest are improved breeding lines.

Table 1. Wheat genotypes included in the study

Variety	No	**Genotype**	No	**Genotype**	No	**Genotype**	No
Milenka	1	**D31**	16	**D97**	42	**D167**	67
Radika	8	**D33**	17	**D99**	43	**D149**	68
Babuna	23	**2/2-9**	18	**D101**	44	**D151**	69
Treska	33	**D35**	19	**D103**	45	**7/1-125**	70
Orovcanka	51	**2/2-20/2**	20	**SK-19/82**	46	**7/1-143**	71
Bistra	78	**2/2-21**	21	**5/1-200**	47	**D157**	72
Sterna	82	**2/2-161**	22	**D109**	48	**D159**	73
Ohrigjanka	89	**D63**	24	**5/2-21**	49	**7/2-13**	74
Pelagonija	90	**D47**	25	**D117**	50	**7/2-69**	75
Vardarka	92	**D49**	26	**6/1-15**	52	**D163**	76
Genotype	No	**D51**	27	**D125**	53	**D165**	77
D1	2	**D53**	28	**6/1-35**	54	**D191**	79
1/1-10	3	**D57**	29	**D127**	55	**8/1-1/2**	80
1/1-14	4	**3/2-1**	30	**6/1-165**	56	**8/1-10/2**	81
D7	5	**D59**	31	**D129**	57	**D177**	83
1/2-16	6	**D61**	32	**D131**	58	**D179**	84
1/2-47/2	7	**D68**	34	**6/2-2**	59	**D181**	85
2/1-1	9	**D69**	35	**D133**	60	**D182**	86
D21	10	**4/1-147**	36	**D135**	61	**8/2-137/1**	87
2/1-19/5	11	**4/1-194**	37	**D137**	62	**8/2-176**	88
D23	12	**D75**	38	**D139**	63	**D67**	91
2/1-51	13	**4/2-56**	39	**6/2-186/1**	64	**5/1-199**	93
D27	14	**D79**	40	**D143**	65	**D85**	94
D29	15	**4/2-120**	41	**SK-15/90**	66		

DNA from five plants from all genotypes was isolated from the leaves (in the 2-3 leaf faze), using the CTAB (cetyl trimethyl ammonium bromide) method of Gale et al. (2001). Characterization of the Glu-A1 and Glu-D1 loci was made with two DNA markers for each locus (Table 2). The concentration of the primers and PCR amplification (the number of cycles, temperature and duration of denaturation, annealing and extension) depended on the primer combination and were made according to the protocols by D'Ovidio et al. (1994), Smith et al. (1994), Lafiandra et al. (1997), Ma et al. (2003), Schwarz et al. (2004), Lei et al. (2006), Ishikawa и Nakamura (2007) и Liu et al. (2008). PCR amplification was performed with Eppendorf Mastercycler ProS. The products of the PCR were separated by 1.5-2% gel electrophoresis, visualized on UV transilluminator, photographed with digital camera (Carestream Gel Logic 112 Imaging System), and analysed with Carestream Molecular Imaging Software. All analyses were conducted in the laboratory at the Department for Genetics and Plant Breeding at the Faculty of Agricultural Sciences and Food in Skopje (Sandeva, 2016).

Table 2. Markers used for the analyses and their sequences

Locus	Marker	Sequence of the primer (5'-3')	Allele	Expected fragment length	Chromosome	Reference
Glu-A1	UMN19	UMN19F: CGAGACAATATGAGCAGCAAG UMN19R: CTGCCATGGAGAAGTTGGA	*Ax2**	344	1AL	Liu et al. (2008)
			Ax1	362		
			Ax-null	362		
	Null	BFC: CGTAGTAAGGTGCAAAAAAGTGCCACG BRC2: ACAGCCTTATTGTACCAAGACCCATGTGTG	*Null Wx-B1*	668		
Glu-D1	UMN25	UMN25F: GGGACAATACGAGCAGCAAA UMN25R: CTTGTTCCGGTTGTTGCCA	*Dx2*	299	1DL	Liu et al. (2008)
			Dx5	281		
	UMN26	UMN26F: CGCAAGACAATATGAGCAAACT UMN26R: TTGCCTTTGTCCTGTGTGC	*Dy10*	397		
			Dy12	415		

For every combination of DNA markers, the presence or absence of amplified polymorphic product is marked with 1 or 0, accordingly. Only the polymorphic markers were included in the analysis of genetic diversity. The obtained binary matrix was used to calculate the genetic similarity, according to Dice's coefficient (Dice, 1945; Nei and Li, 1979). The genetic distance between analysed genotypes was determined by subtracting the outcomes from the matrix of genetic similarity from 1, according to Safner (2005):

$$d_{NL} = 1 - S_{NL}$$

The S_{NL} value arises from the formula:

$$S_{NL} = \frac{2a}{2a + b + c},$$

where,

a is total number of cases in which the two genotypes (*i* and *j*) contain the same marker, *b* represents the total number of cases in which the genotype *i* contains marker which the genotype *j* does not have, and *c* represents the total number of cases in which the genotype *j* contains markers which the genotype *i* does not.

The genotypes were grouped according to the UPGMA method, based on the genetic distance. Cluster analysis was done by using the SAHN module in NTSTS pc 2.2 program (Rohlf, 2005).

RESULTS AND DISCUSSION

The most frequent allele was the Ax-null allele, which adversely affects the wheat baking quality, present in 77.66% of the genotypes. Dx5+Dy10 alleles were detected in 68.09% of the genotypes. The least present allele was Ax1, determined only in 8.51% of the analysed genotypes (Table 3).

Table 3. Presence of different alleles in analysed genotypes

Alleles	Ax-null	Ax1	Ax2	Dx2 + Dy12	Dx5 + Dy10
Genotypes	Milenka, Radika, Babuna, Orovchanka, Bistra, Sterna, Ohrigjanka, Pelagonija, Vardarka, D21, D1, 1/2-47/2, 2/1-19/5, 2/1-51, D27, D29, D33, 2/2-9, D35, 2/2-20/2, 2/2-21, 2/2-161, D63, D47, D49, D53, D57, 3/2-1, D59, D61, 4/1-147, 4/1-194, D97, D99, D101, D103, 5/1-200, D109, 5/2-21, D117, 6/1-15, D125, 6/1-35, D127, 6/1-165, D129, D131, D133, D135, D137, D139, 6/2-186/1, D143, SK-15/90, D167, D149, D151, 7/1-125, 7/1-143, D157, D159, 7/2-13, 7/2-69, D163, D165, D191, D177, D179, D181, D182, 8/2-137/1, 8/2-176, 5/1-199	Treska, D51, D68, D69, 6/2-2, 8/1-10/2, D67, D85	2/1-1, 1/1-10, 1/1-14, D7, 1/2-16, D31, D75, 4/2-56, D79, 4/2-120, SK-19/82, D23, 8/1-1/2	Radika, Bistra, Sterna, Ohrigjanka, Pelagonija, Vardarka, 2/1-19/5, D23, 2/1-51, 2/2-9, D35, 2/2-20/2, 2/2-21, 2/2-161, D53, 3/2-1, 4/1-147, 4/1-194, 6/2-2, D135, 6/2-186/1, D143, 7/2-69, D191, 8/1-1/2, 8/1-10/2, D179, D181, D182, 8/2-137/1	Milenka, Babuna, Treska, Orovchanka, 2/1-1, D21, D1, 1/1-10, 1/1-14, D7, 1/2-16, 1/2-47/2, D27, D29, D31, D33, D63, D47, D49, D51, D57, D59, D61, D68, D69, D75, 4/2-56, D79, 4/2-120, D97, D99, D101, D103, SK-19/82, 5/1-200, D109, 5/2-21, D117, 6/1-15, D125, 6/1-35, D127, 6/1-165, D129, D131, D133, D137, D139, SK-15/90, D167, D149, D151, 7/1-125, 7/1-143, D157, D159, 7/2-13, D163, D165, D177, 8/2-176, D67, 5/1-199, D85
Presence of alleles (%)	77,66%	8,51%	13,83%	31,91%	68,09%

The classification of the analysed genotypes based on the presence or absence of alleles in the Glu-A1 and Glu-D1 loci is shown in Figure 1. It can be observed that the genotypes are grouped into two main clusters, each divided into three subclusters with different number of genotypes. In the cluster 1 were classified 64 genotypes, while the remaining 30 belonged to cluster 2. Most of the analysed genotypes according to the identified alleles in the Glu A1 and Glu D1 loci were placed into subclusters 1a and 2a. All genotypes belonging to the first cluster were characterized by the presence of Dx5 + Dy10 alleles and the absence of Dx2 + Dy12 alleles at the Glu-D1 locus. Opposite to this cluster, the presence of Dx2 + Dy12 alleles and the absence of Dx5 + Dy10 alleles were observed in cluster 2. Glu-A1 locus alleles were present or absent in genotypes from specific subclusters of each cluster. Genotypes of subcluster 2a were characterized by the presence of the Ax-null allele at the Glu-A1 locus and Dx2 + Dy12 at the Glu-D1 locus, which, according to Kocourková et al. (2008), negatively affect wheat baking quality. Based on the wheat quality rating assigned to each Glu-1 subunit, these genotypes would receive grade 3 based solely on the alleles in the specific loci (Table 4). They cannot be used in breeding programs to improve wheat quality.

Most of the Macedonian wheat varieties (Radika, Bistra, Sterna, Ohrid, Pelagonia and Vardarka) that were included in the study do not have good baking quality. The best baking quality features have the genotypes that were classified into subclusters 1b and 1c. These genotypes possess the Ax2 and Ax1 alleles, respectively, at the Glu-A1 and Dx5 + Dy10 alleles at the Glu-D1 locus. Subunit 5 + 10 has a significant effect on dough mixing time, SDS-sedimentation value, and dough strength, compared to subunit 2 + 12 (Luo et al., 2001; Liang et al., 2010), contributing to better baking quality (Payne et al., 1981; Kolster et al., 1991), bread volume (Rogers et al. 1991), higher gluten strength, dough resistance (Branlard and Dardevet, 1985) and higher effect on mixographic parameters (Radovanovic et al., 2002). The superior effect of subunit 5 + 10 over 2 + 12 is mainly due to the presence of an additional cysteine residue in subunit Dx-5 which contributes to the frequent formation of larger polymers (Buonocore et al., 1996). Branlard and Dardevet (1985) state that subunit 1 (the Ax1 allele) is correlated with gluten strength. These findings were confirmed by Obukhova et al. (1997), which concluded that the high

quality of the dough is primarily determined by the Glu-A1a allele (subunit 1), whereas the low quality is associated with Glu-A1c allele (null allele).

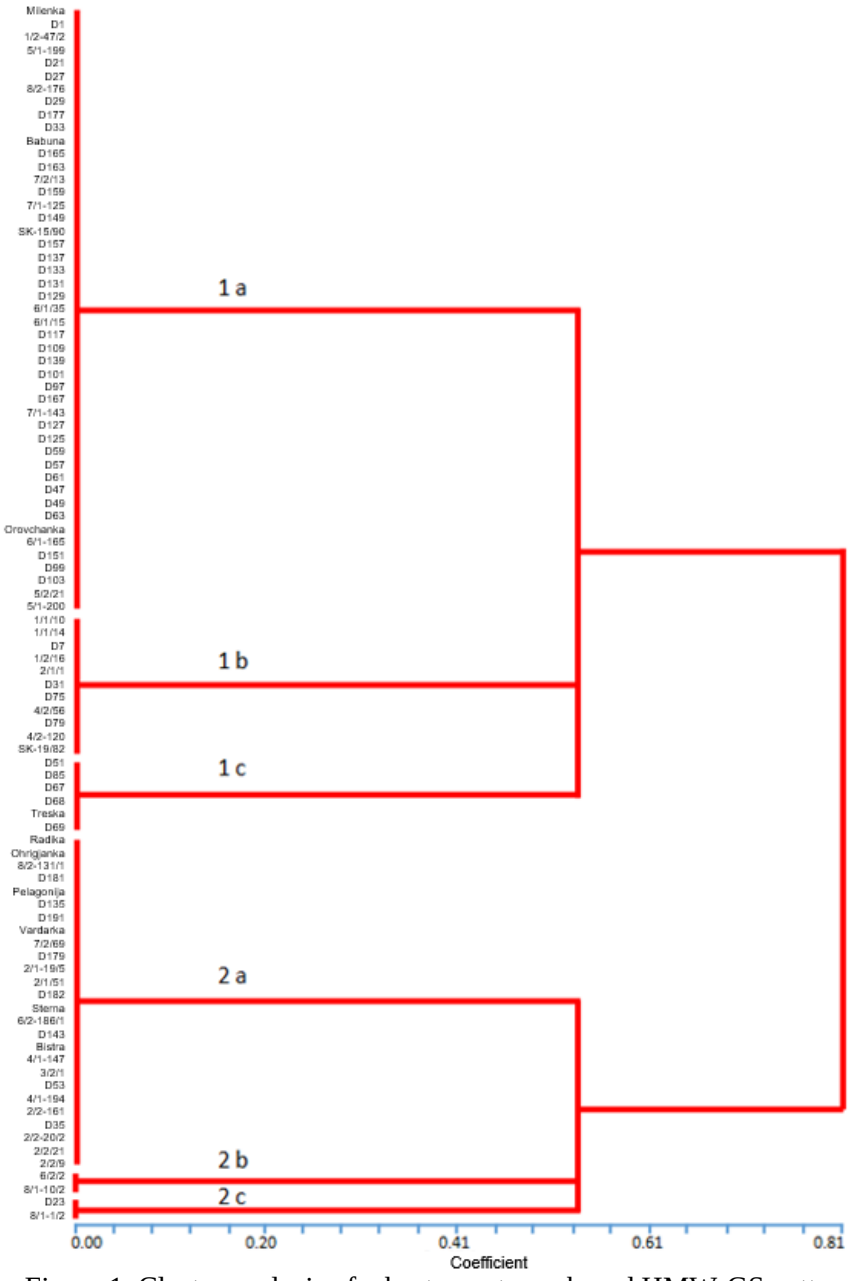

Figure 1. Cluster analysis of wheat genotypes based HMW-GS pattern

Considering the rate of the quality of the high-molecular gluten units (Payne et al., 1987), (Table 4), the genotypes belonging to the subclusters 1b and 1c, according to the presence of the alleles in the two of the characterized loci would be rated with 7 (3 for the alleles in the Glu-A1 locus and 4 for the alleles in the Glu-D1 locus).

In the 1b subcluster were classified only the breeding lines. The presence of the Ax2 and Dx5+Dy10 allele combination indicates good quality of the dough. Treska is the only variety included in this research, characterized with excellent quality features. This variety is part of the 1c subcluster, together with the D51, D67, D68, D69 and D85 breeding lines. The genotypes of these two subclusters should be part of the breeding programs for improving the wheat bread baking characteristics in North Macedonia. In each of the subclusters 2b and 2c belonged two breeding lines, which are distinguished by the presence of a different combination of alleles, compared to the rest of the analysed genotypes. The lines D23 and 8/1-1/2 are part of the 2b subcluster, have good quality, and they contain the Ax1

allele. The lines 6/2-2 and 8/1-10/2 belong to the 2c subcluster. Many studies (Singh and Balyan, 2009; Lai et al., 2014; Singh et al., 2014; Zhang et al., 2016) point at the positive effect of using different techniques for increasing the divergence of the breeding material and for improving agronomical and quality characteristics of wheat.

Table 4. Rate of the quality of the HMW-GS

Rate of the quality	HMW-GS coded by Glu-1		
	Glu-A1	Glu-B1	Glu-D1
1	null	7	4+12
1	-	6+8	-
2	-	7+9	2+12
2	-	-	3+12
3	1	17+18	-
3	2*	7+8	-
4	-	-	5+10

Adapted from Payne et al., 1987

The success of using different breeding methods and techniques for increasing the variability of the initial material for breeding, and the improvement of wheat quality features are confirmed with the results from this research. The variability of the bakery quality among different wheat varieties cannot be explained only based on the composition of the HMW-GS. Gluten with low-molecular weight and some of the gliadins, and their interaction with HMW-GS, also play an important role in determining gluten strength and baking quality. Therefore, additional characterization of the rest of the loci related to the wheat quality is needed for performing complete characterization of the analysed genotypes. Besides the high quality, the breeding lines also need to have good agronomical characteristics, such as high yield and should be graded according to their adaptability and stability when cultivated on different locations. Those with superior features compared to the others could become candidates for registration of new varieties.

CONCLUSIONS

In conclusion, according to the performed classification of the analysed genotypes, most of the Macedonian wheat varieties (Radika, Bistra, Sterna, Ohrigjanka, Pelagonija and Vardarka) don't have good baking quality. The best baking quality showed the genotypes 1/1-10, 1/1-14, D7, 1/2-16, 2/1-1, D31, D75, 4/2-56, D79, 4/2-120 and SK-19/82 classified in the subcluster 1b, the variety Treska and the lines D67, D68, D69, D85, D51 classified in the subcluster 1c. These genotypes are recommended for breeding for improved wheat baking quality. The lines D23 and 8/1-1/2 were classified in the subcluster 2b, and they are characterized with good quality as a result of the presence of the Ax1 allele. The lines 6/2-2 and 8/1-10/2 were classified in the 2c subcluster. The divergence of the studied germplasm can be exploited for further improvement of wheat quality.

REFERENCES

A. S. Shitre, S. Bakshi, D. A. Gadekar, A. P. Padhye, B. K. Das. 2016. Characterization of high molecular weight glutenin subunits of Wheat genotypes. Electronic Journal of Plant Breeding, ISSN 0975-928X. 2016. 282-290.

Ahmad M. 2000. Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. Theor App Genet. 101:892– 896.

Branlard G., Dardevet M. 1985. Diversity of grain proteins and bread wheat quality. I Correlation between gliadin bands and flour quality characteristics. J Cereal Sci. 3: 29-343

Buonocore, Francesco & Hickman, D. & Caporale, Carlo & Porceddu, Enrico & Lafiandra, Domenico & Tatham, Arthur & Shewry, P.. 1996. Characterisation of a Novel High M rGlutenin Subunit Encoded by Chromosome 1D of Bread Wheat. Journal of Cereal Science - J CEREAL SCI. 23. 55-60. 10.1006/jcrs.1996.0005.

Butow B.J., Ma W., Gale K.R., Cornish G.B., Rampling L., Larroque O., Morell M.K., Bekes F. 2003. Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high molecular weight glutenin allele has a major impact on wheat flour dough strength. Theor Appl Genet 107:1524-1532
Day A. 2012. Package "heatmap.plus": Heatmap with more sensible behavior.
De Bustos A., Rubio P., Jouve N. 2000. Molecular Characterization of the inactive allele of the gene Glu-A1 and the development of a set of ASPCR markers for HMW glutenins of wheat. Theor Appl Genet. 100:1085–1094
De Bustos A., Rubio P., Soler C., Garcia P., Jouve N. 2001. Marker assisted selection to improve HMW-glutenins in wheat. Euphytica. 119:69-73.
Dice L.R. 1945. Measurement of the amount of ecological association between species. *Ecology*, 26:297-302
Dubcovsky J., Dvorak J. 2007. Genome plasticity a key factor in the success of polyploidy wheat under domestication. Science, 316 (5833):1862-1866.
D'Ovidio R., Anderson O.D. 1994. PCR analysis to distinguish between alleles of a member of a multigene family correlated with bread making quality. Theor Appl Genet. 88:759- 763.
Duveiller E., Singh R.P., Nicol J.M. 2007. The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica. 157:417–430.
Dvorak J., Luo M.C., Akhunov E.D. 2011. N.I. Vavilov's theory of centers of diversity in light of current understanding of wheat diversity, domestication and evolution. Czech J Genet Plant Breed 47, 2011 (Special Issue): S20–S27
FAO, Statistical Yearbook 2018.
Gale KR, Ma W, Zhang W, Rampling L, Hill AS, Appels R, Morris P, Morell M, In: Proc 10th Australian Wheat Breeding Assembly, Wheat Breeding Soc of Australia. Eastwood R, editor. 2001. Simple high-throughput DNA markers for genotyping in wheat; pp. 26–31.
Goutam U., Kukreja S., Tiwari R., Chaudhury A., Gupta R. K., Dholakia B.B., Yadav R. 2013. Biotechnological approaches for grain quality improvement in wheat: Present status and future possibilities. AJCS 7(4):469-483.
He, Z.H., Liu, L., Xia, X.C., Liu, J.J. and Peña, R.J. 2005, Composition of HMW and LMW Glutenin Subunits and Their Effects on Dough Properties, Pan Bread, and Noodle Quality of Chinese Bread Wheats. Cereal Chemistry, 82: 345-350. doi:10.1094/CC-82-0345
Hristov, Nikola & Mladenov, Novica & DJURIC, V. & Kondic-Spika, Ankica & Jeromela, Ana. (2010). Improvement of Wheat Quality in Cultivars Released in Serbia during the 20th Century. Cereal Research Communications. 38. 10.1556/CRC.37.2009.4.9.
Ishikawa G., Nakamura T. 2007. A new co-dominant PCR-based marker to identify the high-molecular-weight glutenin subunit combination ''5+10'' of common wheat. Wheat Inf Serv 103:1–4.
Jackson E.A., Holt L.M., Payne P.I. 1983. Characterization of high molecular weight gliadin and low-molecular-weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal location of their controlling genes. Theo Appl Genet. 66:29-37.
Jorge Dubcovsky and Jan Dvorak. 2017. Genome Plasticity a Key Factor in the Success of Polyploid Wheat Under Domestication. 316(5833): 1862-1866
Kocourková Z., Bradová J., Kohutová Z., SlámováL., Vejl P., Horcicka P.2008. Wheatbreeding for the iImproved bread-making quality using PCR based markers of glutenins.CzechJ. Genet. Plant Breed. 44(3):105-113.
Kolster P., Van Eeuwijk F.A., Van Gelder W.M.J. 1991. Additive and epistatic effects of allelic variations at the high molecular weight glutenin subunit loci determining the bread making quality of breeding lines of wheat. Euphytica. 55:277-285.
Lafiandra D., Tucci G.F., Pavoni A., Turchetta T., Margiotta B. 1997. PCR analysis of x- and y-type genes present at the complex Glu-A1 locus in durum and bread wheat. Theor Appl Genet. 94:235–240.Lai et al., 2014
Lei Z.S., Gale K.R., He Z.H., Gianibelli C., Larroque O., Xia X.C., Butow B.J., Ma W. 2006. Y-type gene specific markers for enhanced discrimination of high-molecular weight glutenin alleles at the Glu-B1 locus in hexaploid wheat. J Cereal Sci. 43:94-101.
Liang D., Tang J.W., Pena R.J., Singh R., He X.Y., Shen X.Y., Yao D.N., Xia X.C., He Z.H. 2010. Characterization of CIMMYT bread wheats for high- and low-molecular weight glutenin subunits and

other quality-related genes with SDS-PAGE, RP-HPLC and molecular markers. Euphytica. 172:235–250.
Lindsay M.P., Skerritt J.H. 1999. Immunocytochemical Localization of Gluten Proteins Uncovers Structural Organization of Glutenin Macropolymer. Cereal Chemistry, 77(3):360-369.
Liu S., Chao S., Anderson J.A. 2008. New DNA markers for high molecular weight glutenin subunits in wheat. Theor Appl Genet. DOI 10.1007/s00122-008-0886-0.
Luo C., Griffin W.B., Branlard G., McNeil D.L. 2001. Comparison of low- and high molecular-weight wheat glutenin allele effects on flour quality. Theor Appl Genet. 2:1088-1098.
Ma W., Zhang W., Gale K.R. 2003. Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica. 134:51–60.
Nei M., Li W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269-5273.
Nieto-Taladriz M.T., Perretant M.R., Rousset M. 1994. Effect of gliadins and HMW and LMW subunits of glutenin on dough properties in the F6 recombinant inbred lines from a bread wheat cross. Theo Appl Genet. 88:81–88.
Obukhova L.V., Maystrenko O.I., Generalova G.V., Ermakova M.F., Popova R.K. 1997. Composition of HMW Glutenin subunits in common wheat substitution lines from cultivars with contrasting bread making qualities. Russian J Plant Genet. 33:1005-1009.
Payne P.I., Holt L.M., Law C.N. 1981. Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Theor Appl Genet. 60:229–236.
Payne P.I., Law C.N., Mudd E.E. 1980. Control by homoeologous group 1 chromosomes of the highmolecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor Appl Genet 58:113-120.
Payne P.I., Nightingale M.A., Krattiger A.F., Holt L.M. 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. J Sci Food Agric. 40:51–65.
Ramírez-González R. H., P. Borrill, D. Lang, S. A. Harrington, J. Brinton, L. Venturini, M. Davey, J. Jacobs, F. Van Ex, A. Pasha, Y. Khedikar, S. J. Robinson, A. T. Cory, T. Florio, L. Concia, C. Juery, H. Schoonbeek, B. Steuernagel, D. Xiang, C. J. Ridout, B. Chalhoub, K. F. X. Mayer, M. Benhamed, D. Latrasse, A. Bendahmane, International Wheat Genome Sequencing Consortium, B. B. H. Wulff, R. Appels, V. Tiwari, R. Datla, F. Choulet, C. J. Pozniak, N. J. Provart, A. G. Sharpe, E. Paux, M. Spannagl, A. Bräutigam, C. Uauy. 2018. The transcriptional landscape of polyploid wheat. *Science.* 361(6403)
Radovanovic N., Cloutier S., Brown D., Humphreys D.G., Lukow O.M. 2002. Genetic variance for gluten strength contributed by high molecular weight glutenin proteins. Cereal Chem. *Science*. 79:843–849.
Rogers W.J., Payne P.I., Seekings J.A., Sayers E.J. 1991. Effect on bread-making quality of x-type and y-type high molecular weight subunits of glutenin. J Cereal Sci. 14:209- 221.
Rohlf F.J. 2005. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Applied biostatistics inc; Setautec, NewYork.
Safner T. 2005. Upotreba doiminantnih biljega u analizi bioraznolikosti. Magistarski rad. Sveuciliste u Zagrebu, Agronomski fakultet.
Sandeva Nadica. 2016. Molecular characterization of genes for quality traits in Macedonian wheat genotypes (Triticum aestivum L.). Master thesis. Ss Cyril and Methodius University of Skopje. Faculty of Agricultural sciences and food. (in Macedonian).
Schwarz G., Felsenstein F.G., Wenzel G. 2004. Development and validation of a PCR-based marker assay for negative selection of the HMW glutenin allele Glu-B1-1d (Bx-6) in wheat. Theor Appl Genet. 109:1064–1069.
Shewry P.R., Halford N.G., Belton P.S., Tatham A.S. 2002. The structure and properties of gluten: an elastic protein from wheat grain. Philos. Trans. R. Soc. B, 357, pp. 133–142.
Shewry P.R., Halford N.G., Tatham A.S. 1992. High molecular-weight subunits of wheat glutenin. J Cereal Sci. 15:105–120.
Singh N.K., Balyan H.S. 2009. Induced Mutations in Bread Wheat (Triticum aestivum L.) CV. 'Kharchia 65' for Reduced Plant Height and Improved Grain Quality Traits.Advances in Biological Research, 3 (5-6): 215-221.

Singh N.K., Shepherd K.W., Cornish G.B. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J Cereal Sci. 14:203-208.
Singh R., TiwariR., SharmaD., Tiwari V., SharmaI. 2014. Mutagenesis for wheat improvement in the genomics era.Journal of Wheat Research 6(2):120-125.
Smith R.L., Schweder M.E., Barnett R.D. 1994. Identification of glutenin alleles in wheat and triticale using PCR generated DNA markers. Crop Sci. 34:1373–1378.
Zhang L., Chen Q., Su M., Yan B., Zhang X., Jiao Z. 2016. High-molecular-weight glutenin subunit-deficient mutants induced by ion beam and the effects of Glu-1 loci deletion on wheat quality properties. J Sci Food Agric. 96(4):1289-96. doi: 10.1002/jsfa.7221.