Effect of gluten formation on wheat quality

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Abstract: Wheat gluten contains two type of protein molecules namely gliadins and glutenins. Gliadin and glutenins play main role in determining viscoelastic properties of wheat dough and other technological quality parameters. Gluten proteins can cause intestinal disorders or celiac disease in some gluten intolerant individuals. This require the selection of cultivars having low gluten content. In this paper gluten content of 10 old wheat cultivars created in former Yugoslavia were analyzed during two year (2011-2013) with different climatic conditions (temperature and precipitation). In first year the dry gluten content varied between 24.21% (Lasta) and 32.16% (Macvanka 2), while in the second year all cultivars had higher gluten content and changed from 26.95% (Loznicanka) to 36.36% (Crvenkapa). The protein content and loaf volume were also higher in all cultivars in the second year in comparison to the values of the first experimental year. The presence of gliadin alleles at *Gli-A1* and *Gli-A2* loci, controlling α-, β-, γ- and ω-gliadins were also analyzed and probability of their origin were estimated. Five alleles (***a, b, f, h, k***) were present in *Gli-A1* locus in Yugoslav cultivars while 7 alleles (***b, e, g, j, k, o, p***) were present at *Gli-A2* locus in cultivars originating from other countries (Italy, Hungary, Romania, France, Great Britain, Mexico and the late Soviet Union), *Gli-A1h* allele was not present in any studied foreign cultivars. Lasta variety was identified with the lowest gluten content and being the most suitable for breeding and bread making for celiac patient.

Key words: gliadin alleles, gluten, quality, wheat.

Introduction

Gluten is a complex protein which formed from proteins of flour, such as gliadins and glutenins, by mechanical mixing with water. Hydrated gliadins and glutenins begin to stick to each other and begin to interact through the formation of chemical bonds. Properties of dough, -viscoelasticity, -strength and -resistance depends on the structure and interaction of these proteins (Menkovska et al., 2002; Torbica et al., 2007). Gliadins and glutenins composed of many different molecular types, which differed in their molecular weight. Gliadin contain single polypeptide chains, globular confirmation, with intra disulfide bonds (Bietz, 1997; Shewry and Halford, 2002) and most of them have low molecular weight (16kDa to 50kDa), glutenins have intermolecular disulfide bonds between polypeptides. One group of glutenins consists of subunits with low molecular weight 20kDa to 50kDa (LMW GS) while the other group of glutenins have high molecular weight 50kDa to 200kDa (HMW GS). Gliadins are encoded by six *Gli*-loci, positioned at short arm of chromosomes 1. and 6. on A, B and D genomes of wheat. Each locus characterized multiple alleles (Metakovsky et al., 1991). Gliadin proteins are similar, -considering their amino acid sequence and molecular weight and these proteins are rich in proline (~14%) and glutamine (~40%) Wrigley and Bietz (1988). Large number of gliadin and glutenin subunits were identified by electrophoretic separation (Bietz, 2002). In standard gel electrophoresis, the mobility of the proteins depend on the size and the charge (Waga and Zientarski, 2007).

Gliadins and glutenins are important for human nutrition. World Health Organization (WHO) recommends bread eating several times per day (WHO, 2003). However, wheat products which consist of gliadins and glutenins can cause, celiac toxicity and allergenicity as well (Matsuo et. al. 2008). Three illnesses are associated with gluten intake: food allergy is present in 0.2-0.5% of the population; celiac disease (CD) appear in both children and adults in various frequencies (Kagnoff,, 2007) and gluten sensitivity with frequency of 6% in USA but the tendency is increasing all over the world (Kasarda, 2013).

The aim of this paper was to identify the alleles at the *Gli-A1* and *Gli-A2* loci, encoding gliadins and to study the variability of quality traits, such as: gluten content, protein content and loaf volume obtained from flour of wheat cultivars and grown under different environmental condition.

Materials and methods

Grain samples of 10 wheat cultivars (Bacvanka 1, Balkan, Baranjka, Crvenkapa, Dukat, Gruza, Krajinka, Lasta, Loznicanka and Macvanka 2) were analyzed for gliadin composition, especially focusing on proteins encoded by alleles at the *Gli-A1* and *Gli-A2* loci. Electrophoresis was carried out by Metakovsky et al. (1991). Gliadins were extracted with 70% ethanol from 30 single kernels. Gliadin extract (20*µl*) were loaded on the gel by micropipette. Polyacryl-amide gel was polymerized by 10*µl* 3% hydrogen peroxid. Gel electrophoresis was performed in 8.33% polyacrylamide for 2.5-3 hours, under constant voltage of 550 V and in 5*m*M aluminum lactate buffer at pH=3.1 (Novoselskaya *et al.,* 1983). After running electrophoresis, gels were removed and immersed in 300*ml* of fixative for 15 minutes, and stained in 0.05% ethanol solution of Coomassie Briliant Blue R-250 by adding 250*ml* 10% trichloroacetic acid (TCA). Staining was carried out during night. Next day. Gels were washed in water and photographed. Photographs were used for determination of gliadin alleles (Metakovsky, 1991).

Weather condition during growing seasons

In the years of experiment temperature and the quantity of precipitation were different. The values in comparison with 10 year's average were also different (tab. 1). The average temperatures (8.13^{o}C) of the first year was slightly lower than the 10 year's average (9.08^{o}C), while the average temperature (9.73^{o}C) in the second year of the experiment (2012/13) was higher than in first year and the 10 yearr's average. In the first year (2011/12) the average amount of precipitation (474.7mm) was significantly lower than in the second year of experiment (611.5mm), and lower than the 10 year's average (543.8mm).

The amounts of precipitation was high and had suitable distribution from sowing to ripening time in the second year of the experiment. In the first year, precipitation was extremely low at the period of germination, while from December to ripening time the

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distribution of precipitation was enough at each stage of plant development. Precipitation in June of the first year (17.8mm) was enough for seed maturity at the end of grain filling stage, however this quantity of precipitation was much lower than the 67.6mm in second year, the 74.4mm average precipitation in the last ten year.

Table 1. Monthly and mean temperatures and monthly and cumulative precipitation

Month	Temperature °C			Precipitation (mm)		
	2011/12	2012/13	2001-2010	2011/12	2012/13	2001-2010
October	10.4	13.7	12.2	30.4	56.7	64.3
November	3.2	9.1	7.0	1.7	11.1	57.4
December	3.3	0.4	2.0	63.7	97.6	48.5
January	-0.1	2.9	0.9	107.1	62.4	42.8
February	-4.2	4.0	2.4	54.9	84.3	44.7
March	8.8	6.4	7.6	24.5	102.0	52.5
April	12.7	13.3	12.0	69.1	41.2	66.6
May	16.0	18.0	17.2	105.5	70.8	74.9
June	23.1	19.8	20.4	17.8	85.4	92.2
Average	**8.13**	**9.73**	**9.08**	52.7	67.94	60.4
Total				**474.7**	**611.5**	**543.8**

Results and discussion

***Variation in gliadin alleles*.**

The gliadin allele composition at the *Gli-A1* and *Gli-A2* loci and the quality traits of wheat showed differences among the analyzed wheat cultivars. Five different alleles were identified (***a, b, f, h, k***) at the *Gli-A1* locus and 7 alleles (***b, e, g, j, k, o, p***) at the *Gli-A2* locus. The frequency of the identified alleles was different. The ***b*** allele (40.0%) was the most frequent at the *Gli-A1* locus, while frequency of alleles ***a, f*** were 20% and the lowest frequency had alleles ***h, k*** with 10%. At the ***Gli-A2*** locus the most frequent allele was the ***g*** allele (40.0%), while frequency of allele ***e*** was 20.0% and frequency for alleles ***b, k, o, p*** was 10%. Krajinka cultivar was heterozygous at the *Gli-A2* locus, where two different alleles *Gli-A2g, Gli-A2j* (table 2). The established polymorphisms of ***Gli-A1*** and ***Gli-A2*** was in agreement with previous finding (Menkovska et al., 2002; Djukić et al., 2011; Knezevic et al., 2016a). The variation of gliadin alleles identified in this analysis indicated that there is a lagre gene-pool which could be useed in breeding programmes to transfer superior genes to other varieties in order to improve the protein composition. The high frequency of the similar alleles is coming from the frequent use of parental genotypes carrying the sam protein alleles. Other reason could be the use of selection method based on phenotypic characters, that might also lead to high allele frequency of certain protein alleles (Knezevic et al.2016a).

The alleles identified at the *Gli-A1* locus were mainly γ-gliadins, but there were smaller number of β- and ω-gliadins as well. Alleles at the *Gli-2* locus encodes mainly α-gliadins components and smaller number of β-gliadins.

***Grain protein content*.**

The protein is the main compositional quality trait of the grain. In this study, the grain protein content varied from 10.40% (Lasta) to 13.50% (Crvenkapa) in the first year of investigation. In the second year of investigation grain protein content varied from 12.10% (Lasta) to 14.50% (Crvenkapa). Generally the protein content of all varieties were higher in the second year than in the first year of research (table 2).

The gentic control of the protein content is very complex, and it, also, depends on the environmental factors. The protein content is genetically determined. Althogh the environment/year can influence it, but the order of the variaties will be very similar in each year (Jolánkai et al, 2008; Johansson et al., 2008; Knezevic et al., 2016b). The pre- and post-anthesis N application, the temperature and the timings, the post-anthesis temperature, the regime of watering all have strong influence on the accumulation of the protein content (Martre et al., 2006; Godfrey et al., 2010).

Protein content and protein quality are highly associated with the baking quality and positively correlated with wet gluten content, farinogrph dough stability and bread loaf volume (Menkovska et al. 2002).

Table 2. Gliadin allele composition and technological quality of winter wheat cultivars

	Gli alleles		Dry gluten %		Grain protein content %		Loaf volume (*ml*)	
	A1	*A2*	2011/12	2012/13	2011/12	2012/13	2011/12	2012/13
Bacvanka 1	a	b	28.22	32.44	11.60	13.20	440	480
Balkan	f	g	30.46	34.42	13.40	14.60	450	500
Baranjka	b	e	26.89	32.28	12.80	13.40	420	450
Crvenkapa	k	g	31.12	36.36	13.50	14.50	520	540
Dukat	f	o	24.52	29.10	11.20	12.30	340	390
Gruza	b	k	29.23	32.21	12.30	12.80	400	420
Krajinka	h	g+j	25.34	31.89	12.00	13.25	360	400
Lasta	b	p	24.21	27.04	10.40	12.10	340	380
Loznicanka	b	g	29.57	26.95	11.60	12.85	420	420
Macvanka 2	a	e	32.16	30.63	12.10	12.65	430	430

Gluten content.

Gluten is like a rubbery mass, which remains after washing of starch granule and water-soluble components from wheat dough. The dry gluten content varied much depending on the wheat cultivars, wheater conditions and the year. In the first year of investigation, the dry gluten content varied between 24.21% (Lasta) and 32.16% (Macvanka 2). The gluten content in second year of experiment varied from 26.95% (Loznicanka) to 36.36% (Crvenkapa). Generally, each wheat cultivar had the higher gluten content in the second year than in the first year (table 2).

The variation of the quality characters determined by the genotype and its interaction with the environmental factors (Naeem et al., 2012; Horváth et al., 2014). Temperature sums is the main factor in the polymersation of gluten proteins (Triboi et al., 2003). The high temperature contribute to shortening stage of development (grain filling, ripening). The high temperature at the end of the grain-filling period caused the greatest reduction in the mean gluten index (Vida et al., 2014). It will takes a long time to improve wheat quality characters by breeding (Zečević, et al., 2013). Amount of gluten protein fraction will be higher when using fertilizer than without using. The high temperature increasing of gluten protein content per grain which is resulted by the inhibited synthesis of starch under high temperature (Hurkman et al., 2013). Gluten matrix have main role in determining baking quality of wheat flour by affecting capacity of water absorption, viscosity and elasticity of the dough, what is important for food industry and quality products in human nutrition. Gliadins determines the extensibility of the dough while glutenins determines the strength of the (Shewry et al., 2003). Their quantitative ratio is important determinant of gluten quality (Menkovska et al., 2002; Wrigley et al., 2006).

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Bread quality

Loaf volume is a parameter characterizing bread qualiy. In this study, 'Crvenkapa' variety had the highest loaf volume (520*ml*) in the first and second (540*ml*) year, while the lowest loaf volume was found for 'Dukat' and 'Lasta' (340*ml*) in the first year and 'Lasta' (380*ml*) in the second year of investigation (table 2). The quality characteristics are important for processing industries and bakeries. Uniform quality could be reached by blending of the flours originating form different trial locations, seasons and varieties. This can help for the industries to produce uniform quality wheat products. Further research would be needed to evaluate allergenic factors, and to use it for selection in breeding programs.

Conclusions

On the basis of the identified gliadin alleles, polymorphisms were established at *Gli-A1* and *Gli-A2* loci i.e. 5 at *Gli-A1* and 7 at *Gli-A2* locus. The study of the ten wheat cultivars showed differences in their quality characteristics such as gluten content, protein content and loaf volume. The quality characteristics of wheat cultivars differed and varied among cultivars and years. The wheat cultivar 'Crvenkapa', had the highest dry gluten content (36.36%) in second year, grain protein content (13.50% and 14.50%) and loaf volume (520*ml* and 540*ml*) in both years of the investigation. 'Lasta' cultivar had low gluten content (24.21%) in the first year of investigation, and low protein content in both years. Flour from 'Lasta' and flour from other low protein varieties can be used to blend with flour with high protein content to getting uniforme quality. The decreasing of gluten content is important for prevention of "toxicity" and decreasing cause of intestinal disorders. Also, this cultivar is interesting for breeding wheat cultivars with low protein content, which flour and its products are more suitable for consumers untolerant to gluten.

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References

Bietz, J. A. (1997): Recent Advances in the Isolation and Characterization of Cereal Proteins. Cereal Foods World. 24:199-202.

Bietz, J. A. (2002): HPLC of Cereal Endosperm Storage Proteins. In: K. M. Gooding and F. E. Regnier, eds., HPLC of Biological Macromolecules, 2nd edition, Marcel Dekker, Inc., New York, pp. 547-587.

Djukić, N., Knežević, D., Horvat, D., Živančev, D., Torbica, A. (2011): Similarity of cultivars of wheat (*Triticum durum*) on the basis of composition of gliadin alleles. Genetika. 43: 3. 527-536.

Godfrey, D., Hawkesford, M. J., Powers, S. J., Millar, S., Shewry, P. (2010): Effects of crop nutrition on wheat grain composition and end-use quality. J. Agric. Food Chem. 58: 3012-3021.

Hurkman, W. J., Tanaka, C. K., Vensel, W. H., Thilomony, R., Altenbach, S. B. (2013): Comparative proteomic analysis of the effect of temperature and fertilizer on gliadin and glutenin accumulation in developing endosperm and flour *Triticum aestivum* L. cv. Butte 86. Proteome Science. 11:8 http://www.proteomesci.com/content/11/1/8

Horváth, Cs., Kis, J., Tarnawa, Á., Kassai, K., Nyárai, H.F., Jolánkai, M. (2014): The effect of nitrogen fertilization and crop year precipitation on the protein and wet gluten content of wheat (*Triticum aestivum* L.) grain. Agrokémia és Talajtan 63: 159–164.

Johansson, E., Prieto-Linde, M.L., Gissén, C. (2008): Influences of weather, cultivar and fertiliser rate on grain protein polymer accumulation in field-grown winter wheat, and relations to grain water content and falling number. J. of the Science of Food and Agric. 88: 11. 2011-2018.

Jolánkai, M., Nyárai, H.F., Tarnawa, Á., Klupács, H., and Farkas I. (2008): Plant and soil interrelations. Cereal Res. Commun. 36, Suppl. 7–10.

Kagnoff, M.F. (2007): Celiac disease: pathogenesis of a model immunogenetic disease. J. Clin. Investig. 117: 41–49.

Kasarda, D. (2013) Can an increase in celiac disease be attributed to an increase in the gluten content of wheat as a consequence of wheat breeding? J. Agric. Food Chem. 61: 1155–1159.

Knezevic, D., Rosandic, A., Kondic, D., Radosavac, A., Rajkovic, D. (2016a): Impact of quality of grain wheat on food value. Növénytermelés, 65:99-102.

Knežević, D., Maklenović, V., Kolarić, Lj., Mićanović, D., Šekularac, A., Knežević, J. (2016b): Variation and inheritance of nitrogen content in seed of wheat genotypes (*Tr. aestivum* L.). Genetika. 48: 2. 579-586.

Lookhart, G., Zečević Veselinka, Bean, S.R., Knežević, D. (2001): Breeding of Small Grains for Quality Improvement. In: Monograph Genetic and Breeding of Small Grains. (eds. S.Quarrie et all) pp. 349-375**.**

Matsuo, H., Dahlström, J., Tanala, A., Kohno, K., Takahashi, H., Furumura, M., Morita, E. (2008): Sensitivity and Specificity of Recombinant ω-5 Gliadin-Specific IgE Measurement for the Diagnosis of Wheat-Dependent Exercise-Induced Anaphylaxis. Allergy. 63:233-236.

Martre, P., Jamieson, P.D., Semenov, M.A., Zyskowski, R.F., Porter, J.R., Triboi, E. (2006): Modelling protein content and composition in relation to crop nitrogen dynamics for wheat. Europ. J. Agron.. 25: 2. 138-154.

Menkovska, M., Knežević, D., Ivanoski, M. (2002): Protein allelic composition, dough rheology, and baking characteristics of flour mill streams from wheat cultivars with known and varied baking qualities. Cereal Chemestry. 79: 5. 720-725.

Metakovsky, E. V., Knežević, D., Javornik Branka (1991): Gliadin allele composition of Yugoslav winter wheat cultivars. Euphytica. 54:285-295.

Metakovsky E.V. 1991. Gliadin allele identification in common wheat. II. Catalogue of gliadin alleles in common wheat. Journal of Genetics and Breeding, 45**:**325-344.

Naeem, H.A., Paulon, D., Irmak, S., MacRitchie, F. (2012): Developmental and environmental effects on the assembly of glutenin polymers and the impact on grain quality of wheat. J. Cer. Sci. 56: 1. 51-57.

Novoselskaya, A.YU., Metakovsky, E.V., Sozinov A.A. (1983): Study of polymorphisms of gliadin in some wheat by using one- and two-dimensional electrophoresis. Citologija i Genetika, 17: 5. 45-49. (in Russian)

Shewry, P.R., Halford, N.G. (2002): Cereal seed storage proteins: structures, properties and role in grain utilization. J. Exp. Bot**.** 53: 947–958.

Shewry, P.R., Halford, N.G., Lafiandra, D. (2003): Genetics of wheat gluten proteins. Adv. Genet. 49:111-184.

Triboi, E., Martre, P., Triboi-Blondel, A.M. (2003): Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content. Journal of Experimental Botany. 54: 388. 1731-1742.

Torbica, A., Antov, M., Mastilović, J., Knežević, D. (2007): The influence of changes in gluten complex structure on technological quality of wheat (*Triticum aestivum* L.). Food Res.Int. 40: 1038-1045.

Vida, G., Szunics, L., Veisz, O., Bedo, Z., Láng, L., Árendás, T., Bónis, P., Rakszegi, M. (2014). Effect of genotypic, meteorological and agronomic factors on the gluten index of winter durum wheat. *Euphytica, 197:*1. 61-71.

Zečević, V., Bosković, J., Knežević, D., Mićanović, D., Milenković, S. (2013): Influence of cultivar and growing season on quality properties of winter wheat (*Triticum aestivum* L.). Afric. J. Agr. Res. 8: 21. 2545-2550

Waga, J., Zientarski, J. (2007): Isolation and Purification of Individual Gliadin Proteins by Preparative Acid Polyacrylamide Gel Electrophoresis (A-PAGE) for Allergenic Research," Polish Journal of Food and Nutrition Sciences. 57:91-96.

World Health Organization (2003): Food based dietary guidelines in the WHO European Region. Copenhagen, Denmark: WHO.

Wrigley, C.W., Bietz, J.A. (1988): Proteins and Amino Acids. In: Y. Pomeranz, Ed., Wheat Chemistry and Technology, American Association of Cereal Chemists, St. Paul, Inc., St. Paul. pp. 159-275

Wrigley, C.W., Bekes, F., Bushuk, W. (2006): Gluten: a balance of gliadin and glutenin. In: Wrigley C, Bekes F, Bushuk W (eds) Gliadin and glutenin. The unique balance of wheat quality. AACC Int Press, St Paul, pp. 3–32.

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