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Effect of Climate Change on Wheat Quality and HMW Glutenin Subunit Composition in the Pannonian Plain

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ABSTRACT

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The primary goal of this study is to improve our understanding of the extent of influence of climatic factors in Serbia and high-molecular-weight glutenin subunit (HMW-GS) composition upon wheat end-use quality. In-depth analyses were performed on four bread wheat cultivars that are the most common in agricultural practice in Serbia. Total glutenin content showed significant difference between the production years, in opposition to gliadins. Cluster analysis of different percentages of glutenin and gliadin subunit molecular weight ranges (<40,000, 40,000–80,000, 81,000–120,000, and >120,000) indicated that the year of production and the cultivar did not have a significant effect on the percentage ranges for glutenins. However, they had a considerable impact on the percentage ranges for gliadins. Production year and the interaction of year and cultivar had the strongest influences on the percentage of SDS-unextractable polymeric proteins. A synergistic effect of the HMW-GS composition and climatic conditions revealed that all eight samples with HMW-GS composition 2*, 5 + 10, 7 + 9 along with the highest *Glu 1* score of 9 (out of a maximum of 10) produced in the year 2011 belonged to two clusters with the best wheat end-use quality. Furthermore, the climate conditions in 2011 made it possible for the wheat cultivars with HMW-GS composition –, 2 + 12, 7 + 9 to possess similar qualities as cultivars with HMW-GS composition 2*, 5 + 10, 7 + 9 produced in 2012.

When wheat flour is mixed with water, it possesses ability to form dough that has unique rheological properties that are always connected with wheat end-use quality. Different factors affect wheat end-use quality; most researchers claim that gluten proteins, gliadins and glutenins, are the key elements regarding viscoelastic properties of dough and baking quality (Panozzo et al. 2001). Therefore, numerous studies have been carried out to examine the influence of gliadins (Payne et al. 1984; Van Lonkhuijsen et al. 1992; Huebner et al. 1997; Gil–Humanes et al. 2012) and glutenins (Payne et al. 1984; Branlard and Dardevet 1985; Ng and Bushuk 1988; MacRitchie et al. 1991; Blumenthal et al. 1995; Huebner et al. 1999; Pirozi et al. 2008) on the technological quality of wheat. Since Payne et al. (1984) determined positive correlation between high-molecular-weight glutenin subunit (HMW-GS) composition and the technological quality of wheat, the studies of end-use wheat quality have been mainly focused on determining the presence or absence of certain glutenin subunits. Also, they assigned quality scores to each subunit or pair of subunits and calculated the *Glu 1* score as a sum of the individual values obtained for subunit or pair of subunits (the maximum *Glu 1* score for a wheat genotype is 10). However, evaluation of *Glu 1* score was only based on the sedimentation test. Because this test belongs to the group of small-scale tests, Kang et al. (2007) and Oury et al. (2010) suggest that grading wheat by values of glutenin subunits and *Glu 1* score could be quite different from end-use quality. It is well known that the 5 + 10 pair of HMW-GS from *Glu-D1* loci forms stronger dough than the 2 +12 pair of HMW-GS from the same loci because of an extra cysteine residue on the repeating amino acid domain of glutenin subunit 5 (Lafiandra et al. 1993). Contrary to this, findings in a study by Kaur et al. (2013) indicated that wheat cultivars with the 5 + 10 or 2 +12 pair of HMW-GS in combination with the 7 + 9 pair of HMW-GS formed dough with weak dough stability (2.8–4.4 min), whereas those with the 17 + 18 pair of HMW-GS in combination with the 2 + 12 or 5 + 10 pair of HMW-GS had strong dough stability (8.8–13.8 min). Dumur et al. (2010) came to the conclusion that duplication of the 2 +12 pair of HMW-GS and deletion of 2* HMW-GS had a positive influence on Zeleny sedimentation values and significantly increased dough strength (W in the alveograph parameters) and elasticity index. Also, in a recent study, Barak et al. (2013) confirmed that subunit 2* and the HMW-GS pair 5 + 10 contributed substantially to the improved end-use quality of wheat, which confirmed the hypothesis of Salmanowicz et al. (2008) that breadmaking quality should be considered only within particular glutenin subunit compositions. Jin et al. (2013) and Hernández-Estrada et al. (2012) obtained similar findings about the effect that specific HMW-GS compositions have on dough rheology. Besides glutenin and gliadin subunits, another parameter of wheat protein composition, the proportion of larger glutenin polymers classified as SDS-unextractable polymeric protein (UPP), could influence the viscoelastic properties of dough, and it is strongly correlated with dough strength properties (Gupta et al. 1993; Edwards et al. 2007; Moldestad et al. 2014).

In the past, from 1990 to 2008 the recorded climatic fluctuation on the Balkan Peninsula was moderate. Cold winters and relatively warm, cloudy springs resulted in a reduction of wheat yields owing to droughts during a grain filling period (Supit et al. 2012). Also, the study by Olesen et al. (2011) confirmed that the regions that came out as the most severely affected in terms of changing conditions for crop production (especially winter wheat production owing to increased incidents of heat waves and droughts) belonged to the Pannonian Plain, where agriculture still plays an important role in the national economies of countries in the immediate vicinity such as Romania, Bulgaria, Hungary, and Serbia. What is more, climatic conditions have a tendency to influence gluten protein composition and end-use quality of wheat. Borghi et al. (1995) discovered that temperature can have an impact on gluten quantity and composition. Additionally, the percentage of gliadins and glutenins depends on the wheat genotype and climatic conditions, but gliadins are more susceptible to the influence of climatic conditions than glutenins (Graybosch et al. 1995). However, the effect of temperature on the wheat storage protein composition is not fully understood, because differences depend on wheat genotype. For example, wheat cultivars that possess the 5 + 10 pair of HMW-GS from *Glu-D1* loci accumulate HMW-GS faster during the stage of grain filling than do wheat cultivars that possess the 2 + 12 pair of HMW-GS from *Glu-D1* loci (Gupta et al. 1996). This distinction becomes increasingly apparent 28 days after anthesis. Nevertheless, the effects of HMW and LMW glutenin subunits on polymer size are relatively less distinct, which is contrary to the study by Stone and Nicolas (1996). Climate conditions can instigate various

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effects on the technological properties of wheat. According to Daniel and Triboi (2000), rising temperatures and decreasing rainfall increase protein content in wheat grain. However, the studies of Garrido-Lestache et al. (2004) and López-Bellido et al. (2001) showed that large amounts of rainfall in the spring (April and May, or May exclusively) increased protein content. The protein content in grain is a result of complex interactions between nitrogen and water availability. The optimum range of rainfall during the period from September to May is 500–550 mm (Garrido-Lestache et al. 2004), and it is 80 mm in May when protein accumulation takes place in the grain (López-Bellido et al. 2001). In combination with applied nitrogen fertilization, optimal rainfall results in protein content increase. The relatively warm and dry weather heat stress during 2008 in Israel had a major impact on a significant decline in gluten index value (Gil et al. 2011). Yet Garrido-Lestache et al. (2004) stated that the influence of climatic factors on the rheological properties of wheat was not so clearly defined as was the case with protein content.

Because various factors such as HMW-GS composition and climatic conditions influence dough properties and cause remarkably diverse effects, it is important to better understand the relationship that exists among them. In recent decades, climate changes have intensified, and the prediction of climate conditions according to Ruml et al. (2012) indicates that average daily temperature in Serbia will increase by 4.0–4.4°C, and the average annual rainfall might increase by up to 50 mm by the end of 2100, which could result in entirely different effects on gliadin-to-glutenin ratio and wheat end-use quality. Also, breeders have developed new wheat cultivars with different technological properties, which makes it imperative to reexamine the well-established principles obtained in earlier studies. Thus, the first objective of this study was to investigate how production year influences the rheological properties of wheat cultivar and different parameters of wheat protein composition. The second objective was to examine the impact of HMW-GS composition on wheat end-use quality.

MATERIALS AND METHODS

Materials. Examinations were carried out on four bread wheat (*Triticum aestivum* L.) cultivars (Pobeda, Gordana, Zvezdana, and Apache), grown in Serbia at four localities in the Pannonian Plain (Sremska Mitrovica, Vršac, Sombor, and Bačka Topola, with the following geographic coordinates: N45°20′ E19°20′, N45°06′ E18°13′, N45°46′ E19°09′, and N45°49′ E19°39′, respectively) in two production years (2010–2011 and 2011–2012). The chosen wheat cultivars are among the most common ones used in agricultural practice in Serbia. The first three cultivars were bred at the Institute of Field and Vegetable Crops (Novi Sad, Serbia), and Apache was produced by Limagrain (Novi Sad, Serbia). All cultivars used in this research were medium-early and medium-late winter wheat cultivars characterized by excellent resistance to lodging (Hristov et al. 2012). Pobeda represents the standard of breadmaking quality of wheat in Serbia and can be used as an improver (Denčić et al. 2011). Zvezdana also has an excellent technological quality (Živančev et al. 2009). Apache has been one of the leading wheat cultivars in France since 2001. In the last decade, it has been successfully expanded in the Danube Region (Romania, Serbia, Bulgaria, Hungary, and Croatia) and Italy (Limagrain France 2013). Apache also has good protein content and high falling number values (Limagrain France 2013; Limagrain Central Europe 2014).

Weather data from May to July (periods from wheat anthesis to full kernel maturity and harvest) were collected from neighboring meteorological stations from annual reports of the Republic Hydrometeorological Service of Serbia.

Lab-on-a-Chip Electrophoresis. Wheat samples were milled with an MLU-202 laboratory mill (Bühler, Uzwil, Switzerland), and the obtained flour was used for further analysis. The percentages of gliadin and glutenin subunits were determined from 30 mg flour samples after removing albumins and globulins. Gliadin proteins were extracted with 300 μL of 70% ethanol solution and, after a 24 h extraction period at room temperature, were centrifuged for 20 min at 14,500 rpm. Supernatant (200 μL) was dried under air flow at room temperature. The full range of glutenin subunits was subsequently extracted with a treatment solution (2% SDS solution containing 5% β-mercaptoethanol), and the same treatment solution was added to the dried residue of gliadins. A volume of 350 μL of the extract solution was added and afterward heated for 5 min to 100°C. Final solutions of gliadins and glutenins were prepared by mixing a volume of 4 μL of the clarified sample extract with 2 μL of Agilent sample buffer and 84 μL of deionized water. Separation of proteins was performed with the chip electrophoresis technique on an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, U.S.A.) with a Protein 230 Plus lab-on-a-chip kit. After analysis, every subunit was manually integrated, and their percentage was calculated from the time-corrected area.

UPP and SDS-Extractable Polymeric Proteins (EPP). Extraction of UPP and EPP was carried out according to the method of Vensel et al. (2014) with slight modifications. After addition of 0.5% SDS phosphate buffer to 10 mg of flour, the samples were vortexed and then incubated 1 h at 40°C and 800 rpm (TS 100 thermo shaker, Labconsult, Brussels, Belgium). After centrifugation of samples and collection of EPP supernatant, 1 mL of 0.5% SDS phosphate buffer was added to samples. The samples were then stirred for 30 s with a biovortex and then sonicated for 10 min at 40°C (ATM40- 3LCD, Aplicationes Técnicas de Ultrasonidos, Valencia, Spain). Concentrations of UPP and EPP were measured with the Lowry method by using a Specord spectrophotometer (M40, Carl Zeiss, Jena, Germany). All measurements were performed in triplicate, and the results were expressed as UPP% (the percentage of UPP protein content in the total UPP + EPP protein content) and as UPP/EPP ratio.

Dough Rheology. The first set of dough rheology parameters was measured with Brabender devices (C. W. Brabender, Duisburg, Germany) using the following equipment: farinograph, extensigraph, and amylograph (method numbers 115/1, 114/1, and 126/1, respectively; ICC 2006). The second set of parameters was measured with Chopin devices (Chopin, Paris, France) using the alveograph and mixolab (method numbers 121 [ICC 2006] and 173 [ICC 2011]). Gluten index for flour samples was determined according to method number 155 (ICC 2006) and also by the improved method (Torbica et al. 2007).

Statistical Analysis. The data were statistically analyzed by Statistica 12.0 software (StatSoft, Tulsa, OK, U.S.A.). Descriptive statistics were used to explore the dependence of technological quality of the examined wheat cultivars on the climatic conditions, and for that purpose mean and standard deviation as well as coefficient of variation (CV) were calculated. Analysis of variance (ANOVA) was performed to study the influence of the main effect (year) and interaction between the effects (year, locality, and cultivar) on total glutenin and gliadin content as well as on UPP% and UPP/EPP ratio. Hierarchical cluster analysis was used to investigate associations among wheat samples according to the percentage of gliadins and glutenins as well as the rheological properties. Clustering was performed following Ward's method, and the Euclidean distance metric was used.

RESULTS AND DISCUSSION

Climate Conditions. Grain filling and ripening (i.e., from flowering to ripeness) are the final stages of growth in cereals, in which fertilized ovaries develop into caryopses. The climate conditions during this period of wheat development were followed from May to July over two production years, because high temperature and rainfall during this period could cause various adverse effects on wheat kernels. Absolute daily maximal temperatures in June 2011 were not above 34°C (Table I). This indicates that heat stress in 2011 could not have an adverse impact on wheat quality. During examination of durum wheat, Borghi et al. (1995) found that the threshold temperature for heat stress is 35°C, and Ciaffi et al. (1996) suggested that a short period of high temperatures (35–40 °C) during grain development has a negative impact on the technological quality of wheat. In 2012, critical daily maximal temperatures were recorded in Sombor and Bačka Topola at the end of June and the beginning of July (Table I), during full kernel maturity, which may have an adverse effect on wheat quality. According to Jajić et al. (2008), average rainfalls in Vojvodina for May and June in the period from 2004 to 2007 were ~60 and ~80 mm, respectively. Except for the Vršac locality in May 2012, rainfalls in May and June were average or below the determined average values (Table I). Insolation in June 2012 for all four localities was over 45 h higher than in June 2011 (Table I). Even sharper distinctions were recorded between insolation in July 2012 and July 2011. According to Atkinson et al. (2008), a long insolation period during grain filling can increase some of the individual wheat technological properties. Furthermore, the number of rainy days in June and July of 2011 for all four localities was higher than the number in June and July of 2012. According to Atkinson et al. (2008), an unpredictable wet day has a completely different impact on the individual wheat technological properties during grain ripening than insolation. The number of rainy days has an adverse effect on the individual wheat technological properties; thus, rain in early grain growth

TABLE I
Main Meteorological Characteristics of 2011 and 2012 Production Seasons at Localities in Serbia[a]

	2011						2012					
Locality, Month	**T_{max} (°C)**	**T_{max} Days**	**T ≥ 30°C**	**RF (mm)**	**Ins. (h)**	**RD**	**T_{max} (°C)**	**T_{max} Days**	**T ≥ 30°C**	**RF (mm)**	**Ins. (h)**	**RD**
Sremska Mitrovica												
May	28.6	31	0	63.3	265.1	17	30.6	2	3	71.1	239.1	14
June	34.0	22	7	70.1	258.1	13	36.2	30	16	26.8	319.8	9
July	38.0	9	12	93.5	267.6	12	37.0	9	21	39.6	345.4	6
Vršac												
May	29.9	15	0	68	253.9	15	31.6	2	4	93.1	240.7	11
June	33.5	23	5	65.1	297.0	11	34.0	21	16	12.8	350.8	6
July	36.1	19	13	98.1	294.7	13	37.2	8	11	115.7	363.1	6
Sombor												
May	30.3	31	1	42.0	270.6	16	31.1	1	3	40.2	264.7	12
June	33.3	22	5	42.0	288.6	15	36.8	30	16	56.5	339.9	9
July	36.1	9	11	84.4	277.4	13	37.1	1	20	24.3	358.3	8
Bačka Topola												
May	28.6	31	0	63.3	294.7	15	30.8	2	2	64.7	262.1	13
June	31.9	22	4	60.0	290.5	10	35.6	30	12	18.2	336.0	8
July	35.3	10	12	59.8	265.0	13	37.5	5	20	40.4	354.9	12

[a] T_{max} = maximum temperature; T_{max} Days = days with maximum temperature in that month; T ≥ 30°C = number of days with temperature ≥ 30°C; RF = rainfall; Ins. = insolation; and RD = rainy days.

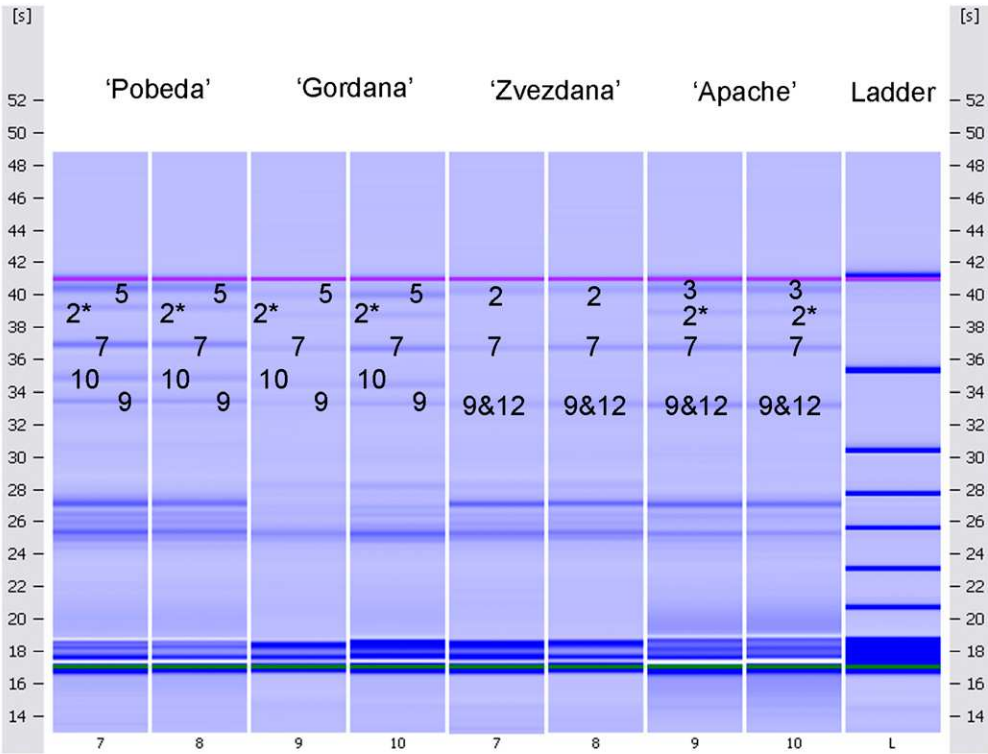

Fig. 1. Gel-like image of high molecular weight glutenin subunits (2*, 3, 5, 7, 9, 10, and 12) of the examined wheat cultivars obtained by lab-on-a-chip electrophoresis.

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should indirectly reduce the specific weight of wheat through low insolation (Kettlewell et al. 2003).

HMW-GS and Protein Similarity of Wheat Cultivars. First, the influence of three main factors (year, locality, and cultivar) on total glutenin and gliadin content was determined. Significant difference between 2011 and 2012 was obtained for total glutenin content, whereas total gliadin content did not differ significantly between the years (data not shown). Total glutenin content was influenced mainly by year, to a lesser extent by cultivar, and locality had the least impact. In contrast, total gliadin content was most influenced by the cultivar, then locality, and year had the least impact.

The HMW-GS compositions of the examined cultivars and their position on a gel-like image are shown in Figure 1. A number of studies have shown that one of the key genetic factors influencing the rheology of wheat dough is the composition of HMW-GS (Payne et al. 1987). To predict the quality of wheat, it is not enough

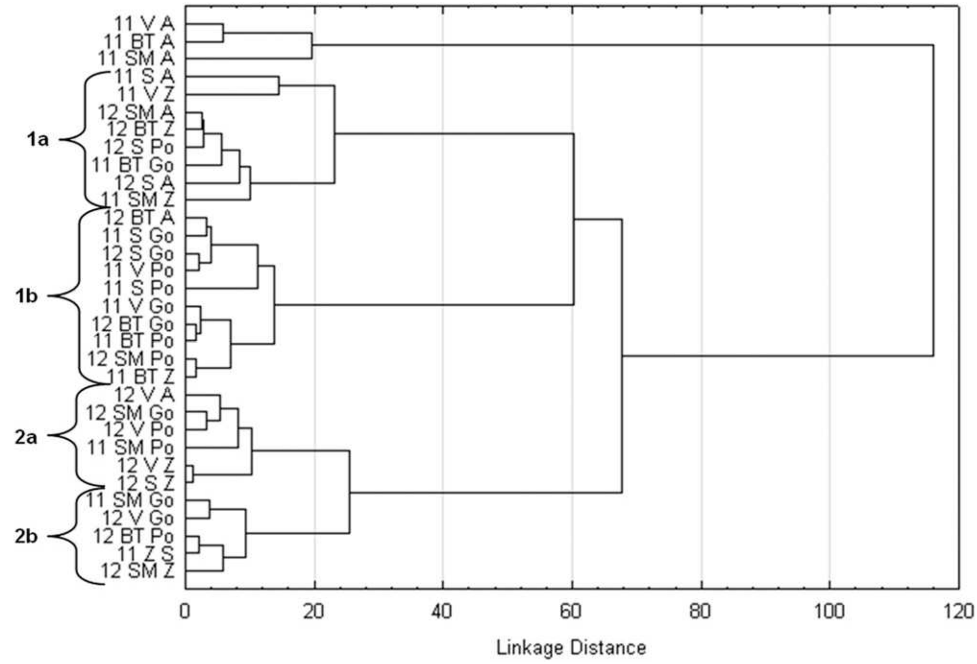

Fig. 2. Dendrogram based on the glutenin percentage molecular weight ranges of four cultivars produced at four localities in two production years. Numbers 11 and 12 indicate production years (2011 and 2012, respectively); abbreviations SM, V, S, and BT indicate localities Sremska Mitrovica, Vršac, Sombor, and Bačka Topola, respectively; and abbreviations Po, Go, Z, and A indicate cultivars Pobeda, Gordana, Zvezdana, and Apache, respectively.

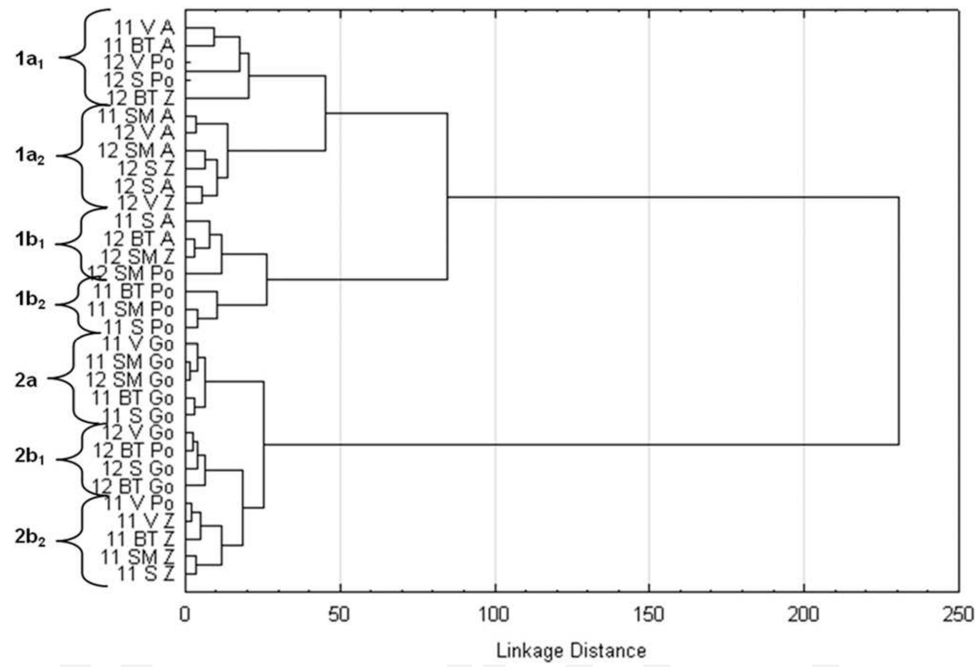

Fig. 3. Dendrogram based on gliadin percentage molecular weight ranges of four cultivars produced at four localities in two production years. Numbers 11 and 12 indicate production years (2011 and 2012, respectively); abbreviations SM, V, S, and BT indicate localities Sremska Mitrovica, Vršac, Sombor, and Bačka Topola, respectively; and abbreviations Po, Go, Z, and A indicate cultivars Pobeda, Gordana, Zvezdana, and Apache, respectively.

to determine the presence or absence of certain HMW-GS: it is necessary to determine the relative share of HMW-GS (MacRitchie et al. 1991). It is evident that Pobeda and Gordana had the same HMW-GS composition (i.e., 2*, 5 + 10, 7 + 9, and a high *Glu 1* score of 9). Apache had the composition 2*, 3 + 12, 7 + 9 and a medium *Glu 1* score of 7, and the HMW-GS composition of Zvezdana was –, 2 + 12, 7 + 9, and its *Glu 1* score was the lowest (5).

To determine similarities among the examined wheat samples according to the different percentages of glutenin and gliadin subunit molecular weight ranges (<40,000, 40,000–80,000, 81,000–120,000, and >120,000), cluster analysis was applied.

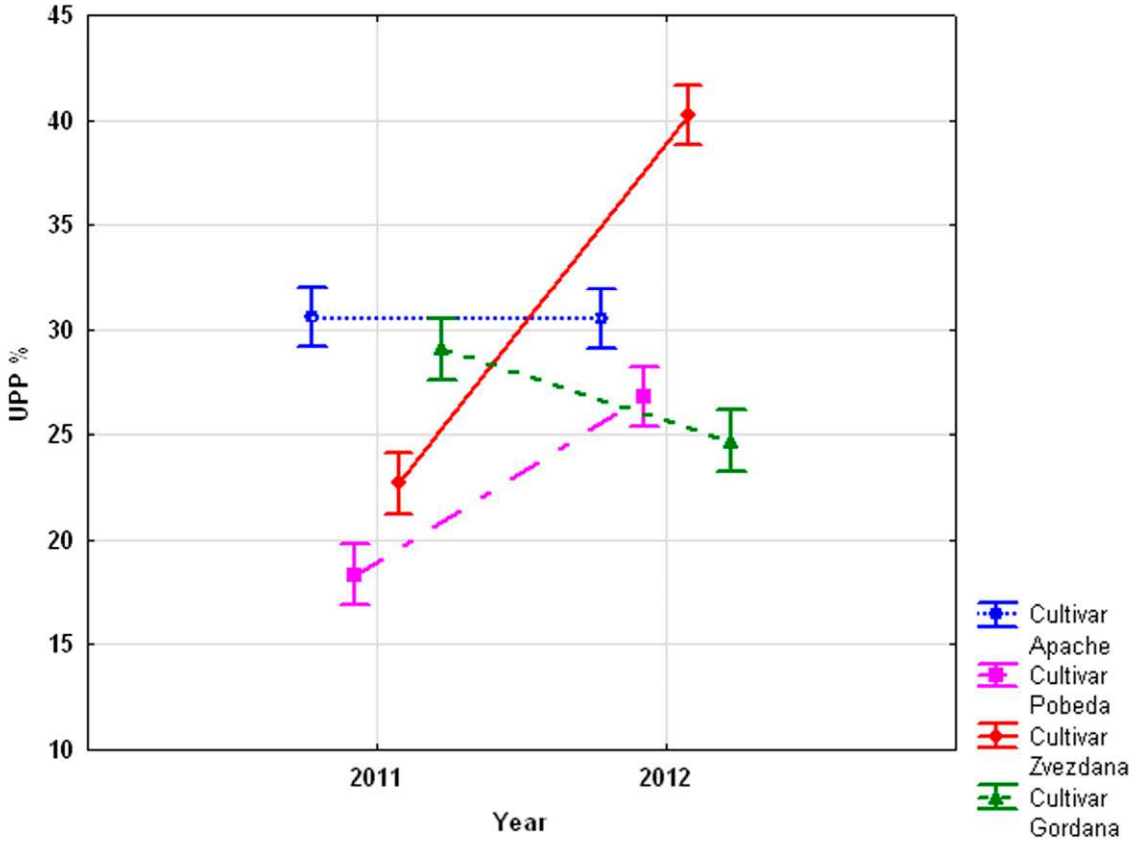

Fig. 4. LS means of interaction year × cultivar for SDS-unextractable polymeric proteins (UPP%); vertical bars denote 0.95 confidence intervals.

TABLE II
Descriptive Statistics and Univariate ANOVA of the Technological Properties Calculated According to Wheat Cultivar Produced in 2011[a]

	Farinograph					Extensigraph					Alveograph				
Cultivar	WA* (%)	DDT* (min)	Stab* (min)	SD* (BU)	QN*	E (cm^2)	R (BU)	Ex* (mm)	R/Ex	PV* (BU)	P* (mm$H_2$0)	L* (mm)	G*	W* (×10^{-4} J)	P/L*
Pobeda															
Mean	61.5	3.0	5.3	30	80	84	245	166	1.5	573	91	99	22	251	0.9
Min	60.7	2.5	4.0	20	73	58	170	153	1.0	370	78	90	21	217	0.8
Max	62.5	4.0	6.5	50	83	95	300	176	2.0	730	101	105	23	274	1.1
SD	0.8	0.7	1.2	14	4	17	58	10	0.4	181	10	7	1	24	0.1
CV	1.2	23.6	22.7	47.1	5.4	20.6	23.7	6.3	28.9	31.6	10.5	6.7	3.4	9.7	14.9
Zvezdana															
Mean	62.4	3.0	1.4	58	65	81	288	154	1.9	1,238	97	78	20	235	1.3
Min	61.2	2.0	0.5	45	59	58	220	144	1.3	1,000	85	72	19	197	1.1
Max	63.2	4.0	2.0	75	70	94	350	176	2.4	1,700	109	85	21	253	1.5
SD	0.9	0.8	0.8	13	5	16	72	15	0.6	314	10	6	1	26	0.2
CV	1.5	27.2	54.5	23.0	8.1	20.2	25.1	9.5	30.9	25.4	10.1	8.1	4.0	10.9	14.1
Gordana															
Mean	61.2	4.6	6.5	14	93	92	325	145	2.2	558	81	99	22	253	0.8
Min	59.9	3.0	3.0	5	87	76	260	139	1.7	390	75	94	22	223	0.8
Max	63.2	6.0	12.0	20	100	104	370	150	2.5	690	87	112	24	291	0.9
SD	1.4	1.6	3.9	8	5	12	47	5	0.3	125	6	9	1	31	0.1
CV	2.3	34.6	60.6	54.5	5.9	12.9	14.3	3.3	15.4	22.4	7.9	9.0	4.4	12.2	7.0
Apache															
Mean	55.5	2.4	2.1	58	65	86	235	172	1.4	1,521	54	108	23	174	0.5
Min	54.8	1.5	0.5	40	49	32	130	154	0.8	1,430	39	76	19	86	0.4
Max	56.5	3.0	3.5	100	74	136	350	179	2.0	1,585	66	131	26	227	0.6
SD	0.8	0.6	1.4	28	12	44	91	12	0.5	71	11	23	3	64	0.1
CV	1.4	26.5	64.8	49.4	17.8	50.9	38.9	6.9	36.0	4.7	21.2	21.4	11.2	36.7	12.1

[a] Asterisk (*) indicates quality parameters that differed significantly between the cultivars in 2011. WA = water absorption; DDT = dough development time; Stab = stability; SD = softening degree; QN = quality number; E = energy; R = resistance; Ex = extension; PV = amylograph peak viscosity; P = tenacity; L = extensibility; G = index of swelling; and W = deformation energy.

The dendrogram of glutenin percentage ranges (Fig. 2) showed that four cultivars investigated in two years (2011 and 2012) at four localities grouped into several clusters at different levels only when outliers formed by the three samples of Apache produced in 2011 were eliminated. Only two clusters were recorded at the distance between 60 and 68. Cutting points were determined at the distance 24 (four clusters obtained) and at the distance 62 (two clusters obtained), in which the members fully belonged to the same group. The results showed that the year of production, locality, and cultivar did not have a considerable impact on glutenin percentage ranges, because there were no similarities within the clusters regarding these factors.

The dendrogram of gliadin percentage ranges (Fig. 3) showed that the examined wheat samples could be distinguished into several clusters at different levels. Namely, two clusters formed for the distance between 85 and 250. When the cutting point was set at the distance 50, three clusters were obtained, whereas at the distance 22 there were six clusters. The findings indicated that the year of production and cultivar had a powerful effect on gliadin percentage ranges. Namely, cluster $1b_2$ was composed of Pobeda produced in

TABLE III

Descriptive Statistics and Univariate ANOVA of the Technological Properties Calculated According to Wheat Cultivar Produced in 2012[a]

	Farinograph					Extensigraph				PV	Alveograph				
Cultivar	WA* (%)	DDT (min)	Stab (min)	SD* (BU)	QN*	E (cm^2)	R (BU)	Ex (mm)	R/Ex	* (BU)	P* (mmH$_2$0)	L* (mm)	G	W (×10^{-4} J)	P/L*
Pobeda															
Mean	64.3	5.1	2.3	44	77	58	225	149	1.6	1,098	93	74	19	208	1.3
Min	61.5	5.0	2.0	35	72	32	120	121	0.8	930	82	68	18	186	1.0
Max	66.8	5.5	2.5	50	82	82	310	174	2.6	1,350	109	85	21	249	1.6
SD	2.6	0.3	0.3	8	5	21	79	23	0.8	194	12	8	1	29	0.2
CV	4.1	4.9	12.8	17.1	6.7	35.7	34.9	15.2	48.1	17.7	12.4	10.6	5.1	13.7	19.3
Zvezdana															
Mean	67.3	3.1	1.0	66	61	60	275	137	2.0	1,605	114	62	17	217	2.2
Min	64.7	2.0	0.5	45	52	51	210	121	1.4	1,550	92	33	13	154	1.0
Max	71.5	4.5	1.5	85	68	80	310	152	2.6	1,680	147	94	22	259	3.4
SD	2.9	1.1	0.4	17	7	13	44	16	0.5	56	24	27	4	45	1.2
CV	4.3	35.5	40.8	24.9	10.9	22.4	16.1	11.7	24.7	3.5	21.1	44.0	22.7	20.6	54.7
Gordana															
Mean	66.4	5.9	4.1	21	84	68	334	126	2.7	723	110	58	17	230	2.0
Min	63.2	2.0	0.5	0	59	59	260	117	1.8	490	94	39	14	149	1.5
Max	68.2	11.0	8.0	55	100	80	380	141	3.3	1,150	127	71	19	264	2.4
SD	2.3	4.4	3.1	24	18	9	58	11	0.6	293	14	14	2	54	0.4
CV	3.4	74.3	74.3	112.6	21.0	13.0	17.4	8.8	22.9	40.6	12.9	24.3	12.8	23.6	22.8
Apache															
Mean	57.3	2.4	1.5	64	65	66	273	141	1.9	1,718	57	97	22	157	0.6
Min	55.8	1.5	0.5	55	60	25	125	133	0.9	1,490	49	76	19	134	0.4
Max	58.8	4.0	2.0	75	70	91	360	150	2.7	2,000	66	121	25	173	0.8
SD	1.3	1.1	0.7	10	4	29	109	7	0.8	211	9	19	2	17	0.2
CV	2.3	46.7	47.1	16.2	6.5	43.4	40.0	5.3	41.7	12.3	16.3	20.0	10.0	10.6	34.1

[a] Asterisk (*) indicates quality parameters that differed significantly between the cultivars in 2012. WA = water absorption; DDT = dough development time; Stab = stability; SD = softening degree; QN = quality number; E = energy; R = resistance; Ex = extension; PV = amylograph peak viscosity; P = tenacity; L = extensibility; G = index of swelling; and W = deformation energy.

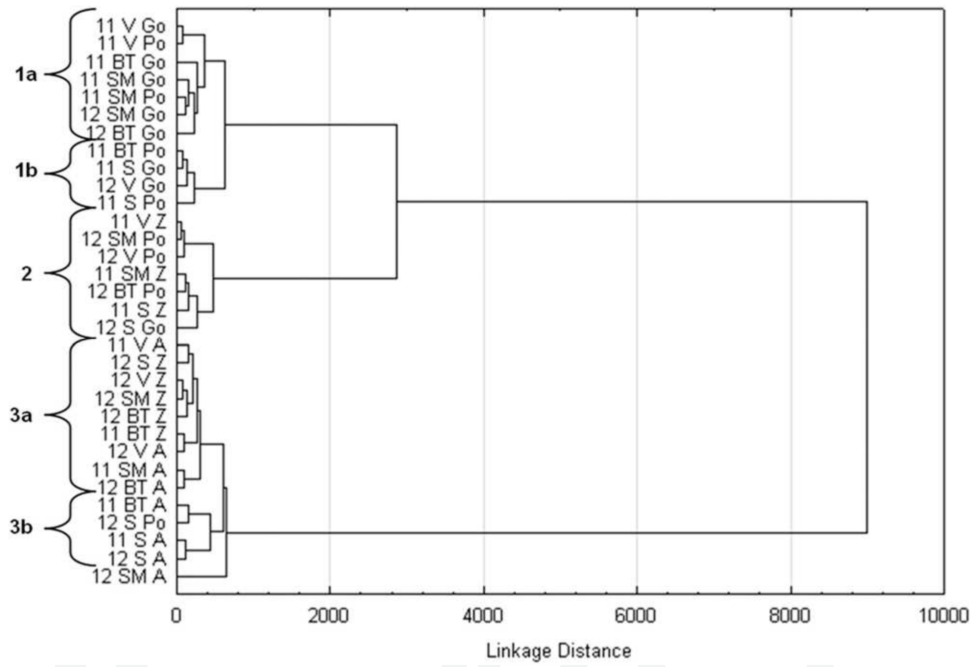

Fig. 5. Dendrogram based on the rheological properties of four wheat cultivars produced at four localities in two production years. Numbers 11 and 12 indicate production years (2011 and 2012, respectively); abbreviations SM, V, S, and BT indicate localities Sremska Mitrovica, Vršac, Sombor, and Bačka Topola, respectively; and abbreviations Po, Go, Z, and A indicate cultivars Pobeda, Gordana, Zvezdana, and Apache, respectively.

2011 at three different localities, whereas cluster 2a was formed from Gordana produced at all four localities in 2011, with Sremska Mitrovica as the only one produced in 2012. However, clusters $2b_1$ and $2b_2$ were not composed of only one cultivar. Cluster $2b_1$ was composed of four wheat samples produced in 2012, 75% of them being Gordana, whereas cluster $2b_2$ was formed from five wheat samples produced in 2011, with 80% being Zvezdana produced at all four localities. These results confirmed that the year of production and genetic factors achieved greater impact on gliadin percentage ranges than on glutenin percentage ranges, because gliadins are particularly sensitive to climatic conditions (Graybosch et al. 1995).

Three-way ANOVA showed that the factor that mostly influenced UPP% was year (F = 116.61), followed by year × cultivar interaction (F = 94.32). In the case of UPP/EPP ratio, these two factors possessed almost the same influence (F = 128.36 and 128.175, respectively). UPP% of two cultivars (Zvezdana and Pobeda) was statistically higher ($P < 0.05$) in 2012, characterized by higher temperature during May and June (Table I), than in 2011 (Fig. 4). These results are in accordance with the results of Malik et al. (2011, 2013), who showed that temperature increase led to increase of UPP%. An opposite trend of UPP% between these two years was depicted by cultivar Gordana, and levels of UPP% of cultivar Apache did not change. These observations confirm that there are no consistent differences in UPP% levels influenced by temperature, as shown in study of Johansson et al. (2005).

Similarity of Technological Properties of Examined Wheat Cultivars. For a detailed investigation, descriptive statistics on the dependence of cultivar technological quality on climatic changes were performed in regard to wheat cultivars produced in 2011 and 2012 (Tables II and III, respectively). The results showed that the examined cultivars produced in these two years differed in the values for the following quality traits: water absorption (WA), energy (E), peak viscosity (PV), and alveograph extensibility (L). Values of E and L for three cultivars (Pobeda, Gordana, and Zvezdana) produced in 2011 were higher than in 2012, whereas WA and PV showed an opposite trend, indicating that climate conditions undoubtedly have a real impact on technological quality.

Furthermore, technological quality parameters that differed significantly between the cultivars in 2011 and 2012 are presented in Tables II and III, respectively. In 2011, the following technological properties differed significantly between the cultivars: WA, dough development time (DDT), stability (Stab), softening degree (SD), quality number (QN), extension (Ex), PV, tenacity (P), L, index of swelling (G), deformation energy (W), and P/L. In 2012, a much smaller number of quality parameters differed between the cultivars: WA, SD, QN, PV, P, L, and P/L. On the other hand, locality influenced only E and resistance (R) of all quality parameters in 2011 and none in 2012 (data not shown).

Values of these parameters caused grouping in clusters, which became evident when cluster analysis was applied. Also, CV of these parameters in 2011 indicated that differences in technological quality among examined localities were less than in 2012, because only CV of Pobeda and Zvezdana PV produced in 2011 were higher, whereas CV of WA, Pobeda E, Gordana PV, and Zvezdana L showed opposite trends. As for Apache cultivars, these parameters distinguished only E in these two years with high CV (higher than 40 in both years), whereas WA, PV, and L were on the same level. This indicates that different climate conditions in the examined two years had the least impact on cultivar Apache.

Cluster analysis was applied to find similarities in quality traits among the examined wheat cultivars (Fig. 5). A dendrogram was

TABLE IV
Mean Values of the Rheological Parameters of Wheat Samples That Belong to the Same Ward Cluster[a]

Samples	Ward 1a	Ward 1b	Ward 2	Ward 3a	Ward 3b
Farinograph					
Water absorption (%)	61.5	62.6	62.7	60.8	57.8
Development time (min)	7.8	9.6	5.7	3.8	3.9
Stability (min)	13.4	13.5	9.9	5.7	7.0
Softening degree (BU)	41	43	53	65	71
Quality number (mm)	164	189	121	79	90
Extensigraph					
Energy (cm^2)	83	79	73	82	41
Resistance (BU)	314	260	280	307	146
Extension (mm)	144	153	147	148	157
Resistance/extension (R/E)	2.25	1.74	1.97	2.12	0.92
Amylograph peak viscosity (BU)	494	700	1063	1599	1509
Mixolab					
Water absorption, mixolab (%)	59.1	59.6	59.6	56.9	54.2
Maximum torque during mixing (C1, Nm)	1.091	1.091	1.090	1.092	1.103
Development time, mixolab (min)	5.681	5.611	4.939	3.017	3.143
Stability, mixolab (min)	8.916	8.868	9.193	8.697	7.628
Elasticity (Nm)	0.074	0.065	0.079	0.087	0.090
Dough protein weakening (C2, Nm)	0.456	0.466	0.502	0.506	0.476
Maximum viscosity (C3, Nm)	1.806	1.829	1.865	1.995	2.109
Stability of the formed starch gel (C4, Nm)	1.471	1.579	1.695	1.691	1.829
Starch retrogradation (C5, Nm)	2.119	2.250	2.461	2.908	3.074
Cooking stability range (C4 – C3, Nm)	0.335	0.250	0.170	0.304	0.280
Gelling range (C5 – C4, Nm)	0.648	0.671	0.766	1.217	1.245
α (Nm/mm)	–0.075	–0.056	–0.059	–0.068	–0.077
β (Nm/mm)	0.458	0.477	0.434	0.462	0.470
γ (Nm/mm)	–0.051	–0.029	–0.026	–0.052	–0.106
Alveograph					
Tenacity (mmH_2O)	91.7	92.3	97.7	89.4	57.8
Extensibility (mm)	86.0	88.3	74.1	81.8	94.5
Index of swelling (mm)	20.4	20.9	19.1	19.8	21.5
Deformation energy ($\times 10^{-4}$ J)	240	247	229	210	144
Tenacity/extensibility (P/L)	1.26	1.07	1.34	1.42	0.67
Gluten index (%)	90	90	89	92	93
Gluten index (37°C) (%)	81	72	69	73	37

[a] See Figure 5 for a dendrogram showing the clusters.

drawn for the technological properties of the winter wheat grain samples. The dendrogram shows that four cultivars investigated in two production years (2011 and 2012) at four localities grouped into clusters at different levels only when an outlier formed by one sample of Apache (produced at Sremska Mitrovica in 2012 at the distance 600) was eliminated. The cutting point was set at the distance 600 (five clusters were obtained), because the average distance among samples was 600, and at the distance 3,000 there were only two clusters. The findings indicated that HMW-GS composition had a noticeable effect on the grain quality under favorable climatic conditions. Namely, clusters 1a and 1b were formed from Pobeda and Gordana with the same HMW-GS composition and a high *Glu 1* score of 9 (out of a maximum 10).

Also, the mean values of the technological parameters (Table IV) of the wheat samples that belong to the same cluster indicate that clusters 1a and 1b possess several superior quality traits (Stab, SD, QN, gluten index [37°C], W, PV, and maximum viscosity [C3]) compared with the other three clusters, which were formed mainly from the other two cultivars with lower *Glu 1* than Pobeda and Gordana. The detected positive influence of HMW-GS composition (*Glu 1* score) on quality traits is in accordance with the results that other authors have obtained (Horvat et al. 2006; Oury et al. 2010). Additionally, the mean values of the technological parameters show that similarity between these two clusters (1a and 1b) is predominantly characterized by farinograph, extensigraph, amylograph, and alveograph properties (Table IV) and that difference is caused by some mixolab properties (Table IV) (elasticity, starch retrogradation [C5], gelling range [C5 – C4], and α). Furthermore, all eight samples of wheat cultivars with the highest *Glu 1* score produced in 2011 belong to these two clusters.

On the other hand, cluster 2 was composed of three cultivars with different *Glu 1* scores. Although HMW-GS composition and *Glu 1* score of Zvezdana strongly differ from Pobeda and Gordana, the mean values of the technological parameters (Table IV) indicate that technological quality of this cluster is slightly inferior to clusters 1a and 1b, because of the values of the following quality traits: Stab, SD, QN, E, P, L, W, P/L, PV, and C3. Also, all three samples of Zvezdana that belong to this cluster were produced in 2011, whereas the rest of the samples from this cluster were produced in 2012. This could be the reason why cultivars from this cluster possess similar qualities, because in numerous studies it has already been proved that climate factors such as insolation and number of rainy days during grain filling and ripening periods affect some of the individual wheat technological properties (Kettlewell et al. 2003; Atkinson et al. 2008). These factors present the difference between climate conditions in June and July of production years 2011 and 2012. Also, according to Carceller and Aussenac (1999) and Carceller (2000), the accumulation of the UPP fractions starts during the late stages of wheat grain development, more specifically, from 30 to 45 days after anthesis. Because in Serbia these stages of wheat grain development begin in June, insolation and number of rainy days could also affect the second level of aggregation of UPP fractions. According to Rhazi et al. (2003), stabilization by hydrogen bonding and forming of disulfide bridges thus has an indirect influence on the rheological properties. Additionally, cluster 2 was distinguished from other clusters by the values of mixolab properties development time (Dev_{MIX}) and stability ($Stab_{MIX}$) (Table IV). These parameters are related to the protein network and represent the properties of dough strength (Rosell et al. 2013), and their high values indicate good wheat end-use quality of cluster 2.

Furthermore, clusters 3a and 3b were mainly formed from Zvezdana and Apache, which possess three identical HMW-GS: 12 and 7 + 9. These two clusters differ from the other three clusters in regard to mean values of the following farinograph and mixolab parameters: WA, SD, QN, mixolab WA (WA_{MIX}), Dev_{MIX}, $Stab_{MIX}$, mixolab elasticity ($Elast_{MIX}$), PV, C3, stability of the starch gel (C4), and cooking stability range (C4 – C3) (Table IV). The mean values of these technological parameters of clusters 3a and 3b measured by farinograph, amylograph, and mixolab (WA_{MIX}, Dev_{MIX}, and $Stab_{MIX}$) were lower than values of the other three clusters, and they indicated a low technological quality of dough. However, the mean values of $Elast_{MIX}$ of these two clusters (0.087 and 0.090 for 3a and 3b, respectively) show completely opposite trends. Živančev (2014) reported that during three years' examination of numerous wheat cultivars showed $Elast_{MIX}$ values ranging from 0.05 to 0.012, indicating a good quality of wheat from clusters 3a and 3b. Regarding the starch properties of the mixolab curve, the wheat flour of these two clusters showed the highest C3, C4, and C4 – C3. This is in accordance with the findings by Rosell et al. (2013) that the wheat flour with the lowest protein content, which indicated low end-use quality, showed the highest C4. The difference between end-use quality of clusters 3a and 3b is mainly caused by parameters of dough strength measured by alveograph and extensigraph (Table IV). Namely, cluster 3a possesses higher mean values of E, R, R/Ex, GI (37°C), P, W, and P/L than cluster 3b, whereas Ex and L behave differently. Also, the values of these parameters indicate that samples of wheat flour from cluster 3a possess stronger, firmer, and less elastic gluten than samples of cluster 3b.

CONCLUSIONS

This study allowed us to better understand how year of production (climate conditions), genetic factors, and locality influence glutenin and gliadin percentage ranges and wheat end-use quality. Total glutenin content showed significant difference between the production years, in opposition to gliadins. However, in the case of glutenin percentage ranges, the year of production, locality, and cultivar did not have a significant impact, whereas in the case of gliadin percentage ranges, the year of production, and cultivar had a considerable effect, because gliadins are sensitive to climatic conditions. Also, production year and interaction of year and cultivar had the strongest influence on UPP% and UPP/EPP ratio. The HMW-GS composition had a substantial effect on the dough rheological properties under the favorable climatic conditions such as in the production year 2011. Results of descriptive statistics showed that examined cultivars produced in these two years differed in values of the following quality traits: WA, E, PV, and L. Values of E and L of Pobeda, Gordana, and Zvezdana produced in 2011 were higher than those produced in 2012, whereas for WA and PV the trend was opposite, which indicated that climate conditions undoubtedly had an impact on technological quality. In addition, all eight samples of Pobeda and Gordana (with HMW-GS composition 2*, 5 + 10, 7 + 9 and the highest *Glu 1* score of 9) produced in 2011 belonged to two clusters with the best technological properties. Also, in cluster 3, three samples of Zvezdana (with HMW-GS composition –, 2 + 12, 7 + 9 and the lowest *Glu 1* score of 5) produced in 2011 possessed similar quality as Pobeda and Gordana produced in 2012. Naturally, all of these statements are valid within the scope of the study, and for obtaining general conclusions it is necessary to examine numerous wheat samples from a larger number of localities during several consecutive production years.

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