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Effect of HMM Glutenin Subunits on Wheat Quality Attributes

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Summary

Glutenin is a group of polymeric gluten proteins. Glutenin molecules consist of glutenin subunits linked together with disulphide bonds and having higher (HMM-GS) and lower (LMM-GS) molecular mass. The main objective of this study is the evaluation of the influence of HMM-GS on flour processing properties. Seven bread wheat genotypes with contrasting quality attributes and different HMM-GS composition were analyzed during three years. The composition and quantity of HMM-GS were determined by SDS-PAGE and RP-HPLC, respectively. The quality diversity among genotypes was estimated by the analysis of wheat grain, and flour and bread quality parameters. The presence of HMM glutenin subunits 1 and 2* at *Glu-A1* and the subunits 5+10 at *Glu-D1* loci, as well as a higher proportion of total HMM-GS, had a positive effect on wheat quality. Cluster analysis of the three groups of data (genotype and HMM-GS, flour and bread quality, and dough rheology) yielded the same hierarchical structure for the first top three levels, and similarity of the corresponding dendrograms was proved by the principal eigenvalues of the corresponding Euclidian distance matrices. The obtained similarity in classification based on essentially different types of measurements reflects strong natural association between genetic data, product quality and physical properties. Principal component analysis (PCA) was applied to effectively reduce large data set into lower dimensions of latent variables amenable for the analysis. PCA analysis of the total set of data (15 variables) revealed a very strong interrelationship between the variables. The first three PCA components accounted for 96 % of the total variance, which was significant to the level of 0.05 and was considered as the level of experimental error. These data imply that the quality of wheat cultivars can be contributed to HMM-GS data and should be taken into account in breeding programs assisted by computer models with the aim to improve flour technological quality.

Key words: wheat, technological quality, HMM-GS, principal components, cluster analysis

Introduction

High molecular mass glutenin subunits (HMM-GS) are a group of closely related gluten proteins that play an important role in determining the viscoelastic properties essential for the formation of wheat dough (*1*). HMM-GS are encoded by polymorphic genes at *Glu-1* loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) present on the long

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arms of the group 1 chromosomes (*2*). Although they account for only 10 % of the wheat storage proteins, HMM-GS are one of the most important genetic factors in determining flour technological quality. Different combinations of HMM-GS alleles influence the bread quality of wheat cultivars in different ways. It is accepted that subunits 1 and 2* at *Glu-A1*, 7+9 and 17+18 at *Glu-B1* and 5+10 at *Glu-D1* loci are related to higher dough strength and loaf volume, whereas their allelic variants such as null (N) at *Glu-A1*, 6+8 at *Glu-B1* and 2+12 at *Glu-D1* loci have negative effects on bread quality (*3–5*). Dough properties and baking performance depend on both genotype and environment. The environmental conditions (growing seasons, locations, agrotechnical treatments) largely contribute to the quantitative variation of HMM-GS, while HMM-GS compositions (fingerprint) generally appear constant for genotypes across growing seasons and locations (*6–9*). The aim of the present investigation is to study the value of HMM-GS in evaluation of wheat quality attributes assisted by multivariate statistical models.

Materials and Methods

Material

The sample set comprised 7 Croatian winter wheat (*Triticum aestivum* L.) genotypes grown at the Agricultural Institute Osijek in 2000, 2001 and 2002. The genotypes represented a relatively wide variation in technological quality. Cultivars Žitarka, Kata, Monika, Ana and Demetra originated from the wheat breeding program at the Agricultural Institute Osijek, Croatia. Cultivars Sana (Bc Institute for Breeding and Production of Field Crops, Zagreb, Croatia) and Divana (Jošt Seed Research, Križevci, Croatia) are a Croatian bread improver and a wheat yield standard, respectively.

Grain and flour analysis

The protein content of sample grains was determined by near infrared transmission (NIT) spectroscopy using an Infratec 1241 (Foss Tecator, Höganäs, Sweden). Wet gluten content and gluten index were determined according to the International Association for Cereal Science and Technology (ICC) Standard No. 155 (*10*) on a Glutomatic 2200 (Perten Instruments, Huddinge, Sweden). Farinograph and extensograph (Brabender, Duisburg, Germany) were used to assess the rheological properties of wheat flour in accordance with ICC No. 115/1 (*11*) and ICC No. 114/1, respectively (*12*). Quality parameter values were the average of two replicates for each sample. Three loaves of bread were made from each genotype according to the following recipe (based on flour mass): water (farinographic absorption), 1.5 % salt, 1.86 % sucrose, 1.8 % dry yeast and 0.005 % ascorbic acid. The components were mixed in San Cassiano spiral mixer (Trecate, Italy) with slow (3 min) and high speed (6 min). Dough was divided, rounded and prooved for 50 min (28 °C, 87 % RH) and baked in Roto oven (MIWE roll-in, Arnstein, Germany) for 32 min at a temperature gradient from 250 to 230 °C. Loaf specific volumes were measured using rapeseed displacement. Loaf shape was defined as the ratio of loaf height and diameter measured at the midpoint of the loaf length. An analysis of variance was performed using the GLM procedure (SAS Institute Inc., Cary, USA, v. 9.1.2) and the means were compared using the LSD test ($p<0.05$) (*13*).

Bread crumb structure

In preparing for quality assessment by image analysis, bread loaves weighing 700 g were sliced in the middle, providing two cross sections. Slices were illuminated by halogen indirect illumination ((760±5) lux) and images were captured by digital camera (Panasonic Lumix FZ-30) set at manual focus mode. Images were captured and presented with the surface size of 10×8 cm (960 pixels per cm^2). Three loaves were produced from each of seven wheat cultivars and images of each loaf cross-section were recorded as 8-bit bitmap file. The same image processing operations were applied to all records (removing a 10-pixel wide outer ring of each slice and conversion to a grayscale image). In comparison with the work of Gonzales-Barron and Butler (*14*), threshold value was not determined manually on histograms but directly on images of full bread slices with the crust excluded (Fig. 1). The threshold value was determined by the trail and error method with the aim of enhancing the best cell resolution. The final threshold value was defined as a value when cells with 5 connected pixels (in any direction) were visible as a black area and smaller cells became invisible. Under these sample illumination conditions, the threshold value of 128 was found to be the optimal one. The bread crumb attribute was evaluated by Global Lab Image/2 program (Data Translation Inc., Marlborough, MA, USA), v. 2.6 (*15*).

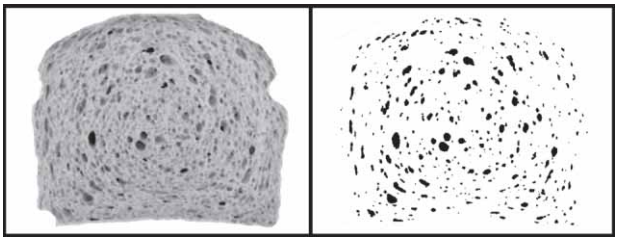

Fig. 1. Original image before and after applying the threshold operation

Electrophoresis and RP-HPLC analysis of HMM glutenin subunits

HMM-GS composition of the analyzed genotypes was identified by SDS-PAGE (Phast System, Pharmacia LKB, Uppsala, Sweden). The nomenclature system used for HMM-GS bands was according to Payne and Lawrence (*16*). The *Glu-1* quality score was calculated according to Payne *et al.* (*17*) by summing up the scores for the individual subunits. Gluten proteins were extracted from the samples according to the stepwise quantitative extraction procedure of Wieser *et al.* (*18*). A Supelcosil LC--318 column (25×0.46 cm) connected to a HPLC Integral 4000 (Perkin Elmer, Waltham, USA) was used for analysis. The separation of gluten proteins was carried out at 50 °C. A linear elution gradient (0 min 28 % ACN/0.1 % trifluoroacetic acid, 30 min 56 % ACN/0.1 % trifluoro-

acetic acid) was applied to separate gluten components. Eluted proteins were monitored at 210 nm. The areas under the RP-HPLC chromatograms, expressed as arbitrary units (AU), were used as a direct measure of the quantity of HMM-GS. Values were the average of two replicates for each sample. The fraction (%) of HMM-GS was calculated from the total protein chromatographic area obtained by summing up the areas under the chromatographic curves for the albumins and globulins, gliadins and glutenins.

Chemometric analysis

Chemometric analysis extracts functional information from variables. Collinearity among multivariate data enables reconstruction of a few latent variables (principal components) that can be very useful for inference of cause-effect relationships in a complex biological system. The principal component analysis was performed on X data matrix with the dimension 21×15. Rows of the matrix X correspond to 7 wheat genotypes analyzed during 3 crop years, while columns correspond to 15 analyzed quality parameters divided into three groups (genotype and HMM-GS, rheological and bread quality data). For numerical evaluation and graphical plotting, Statistica (StatSoft, Inc., Tulsa, USA, 2006) v. 7.1 was applied (*19*).

Results and Discussion

The genotypes differed in HMM-GS composition and quantity as well as in the quality properties (Tables 1, 2 and 3). According to the catalogue of alleles for the

Table 1. Composition and relative quantity of HMM-GS in wheat cultivars

Genotype	HMM-GS locus and allele						*Glu-1* score	HMM-GS[1]/AU	*w*(HMM-GS)[2]/%
	Glu-A1		*Glu-B1*		*Glu-D1*			Mean±SD	
Žitarka	a	1	b	7+8	a	2+12	8	943.9±98.9	10.1±0.3
Kata	c	N	c	7+9	a	2+12	5	796.3±84.3	8.8±0.7
Monika	c	N	c	7+9	a	2+12	5	842.4±123.1	10.0±0.4
Ana	a	1	c	7+9	d	5+10	9	887.3±134.5	10.6±0.1
Demetra	a	1	c	7+9	d	5+10	9	971.1±179.3	11.3±0.6
Divana	b	2*	c	7+9	d	5+10	9	1442.8±214.0	13.1±0.9
Sana	c	N	d	6+8	a	2+12	4	654.7±70.6	8.0±0.3
			Mean±SD					933.9±264.4	10.3±1.6
			$LSD^3_{0.05}$					36.53	0.66
			Source of variation					Mean square (s^2)	
			Genotype (G)					366892*	17.95*
			Year (Y)					260060*	1.25*
			G×Y					10463*	0.19^{ns}
			Error					932	0.16

[1]absorbance unit (AU) under HMM-GS chromatogram area expressed as mean values of three years
[2]proportion of HMM-GS in total protein chromatogram area (albumins, globulins+gliadins+glutenins) expressed as mean values of three years
[3]least significant difference test at p=0.05
*significant at p=0.05 level
[ns]not significant

Table 2. Flour and bread quality parameters of wheat cultivars

Genotype	P[a]	WG	GI	V	H/D	TCA
	Mean[b]±SD					
Žitarka	13.0±1.1	37.9±6.1	75.0±7.6	466.0±35.1	0.69±0.07	13.5±0.2
Kata	12.9±0.7	36.8±5.4	61.0±1.0	483.0±56.3	0.71±0.06	12.7±1.5
Monika	12.3±1.8	29.7±7.2	68.7±11.1	448.3±44.7	0.73±0.02	18.0±0.8
Ana	12.3±1.3	27.1±6.3	98.0±1.0	449.3±13.8	0.78±0.03	20.2±2.3
Demetra	12.6±1.5	28.0±7.9	97.7±1.5	463.0±18.0	0.74±0.05	15.3±1.8
Divana	15.6±2.1	38.2±7.2	94.7±4.2	572.0±19.5	0.87±0.02	17.2±3.7
Sana	12.2±1.0	31.5±4.9	68.3±9.9	451.0±82.9	0.69±0.01	12.4±1.8
Mean±SD	13.0±1.6	32.8±7.1	80.5±16.0	476.1±56.2	0.74±0.07	15.6±3.3
$LSD_{0.05}$	1.03	4.18	11.8	74.50	0.08	3.84

[a]P=protein content (%), WG=wet gluten (%), GI=gluten index, V=loaf volume (cm^3/100 g flour), H/D=loaf height/diameter ratio, TCA=total gas cell area (%)
[b]mean values of three years

Table 3. Dough rheological properties of wheat cultivars

Genotype	DDT[a]	DS	FQN	E	R_{max}	R/Ext
	Mean[b]±SD					
Žitarka	3.0±0.5	102.7±26.3	56.3±2.3	46.5±26.9	180.7±77.6	0.7±0.2
Kata	2.7±0.7	129.0±46.8	44.7±12.4	34.3±13.6	131.0±44.3	0.6±0.1
Monika	3.1±1.2	114.0±17.6	62.7±9.0	35.6±9.9	187.3±29.0	1.1±0.3
Ana	2.8±1.4	75.7±5.0	103.3±89.6	101.5±21.5	417.7±6.8	1.5±0.4
Demetra	2.7±1.6	69.7±2.1	59.3±34.2	111.3±26.1	459.0±15.6	1.7±0.7
Divana	8.9±6.1	20.7±24.9	168.7±54.3	134.0±9.5	441.3±38.5	1.1±0.4
Sana	2.4±0.7	119.0±18.5	47.0±9.2	43.2±3.9	179.0±16.5	0.8±0.2
Mean±SD	3.7±3.1	90.1±41.2	77.4±55.2	72.3±42.4	285.1±142.3	1.1±0.5
$LSD_{0.05}$	3.90	29.45	62.13	17.69	64.93	0.50

[a]DDT=dough development time (min), DS=degree of softening (in Farinograph Units, FU), FQN=farinograph quality number, E=dough energy (cm^2), R_{max}=maximum resistance (in Extensograph Units, EU), R/Ext=resistance to extensibility ratio
[b]mean values of three years

complex gene loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, which encode for HMM-GS in hexaploid wheat (*16*), the alleles *a*, *b* and *c*, which encode for HMM-GS 1, 2* and N (null), respectively, were detected at the *Glu-A1* locus (Fig. 2). At the *Glu-B1* locus, alleles *b*, *c* and *d*, which encode for HMM-GS 7+8, 7+9 and 6+8, respectively, were identified. At the *Glu-D1* locus, alleles *a* and *d*, responsible for encoding HMM-GS 2+12 and 5+10, respectively, were identified. In this study, the most frequent HMM-GS at the *Glu-A1* locus was N, at the *Glu-B1* locus 7+9 and at the *Glu-D1* locus 2+12, which is in accordance with Jošt *et al.* (*20*).

Cultivar Divana (2*, 7+9, 5+10) obtained the best quality attributes regarding gluten extensibility and elasticity properties, as well as the highest amount (1442.8 AU) and fraction (13.1 %) of HMM-GS, which confirms its position as the bread quality improver. From the cluster diagrams (Figs. 3 and 4) of the analyzed wheat quality parameters, it is clear that Divana has a distinct position compared to other cultivars, which is in accordance with our previous findings (*21*). Based on favourable HMM-GS composition and high *Glu-1* quality score, cultivars Ana (1, 7+9, 5+10) and Demetra (1, 7+9, 5+10) belong to the same cluster group as Divana. Like cultivar Divana, Ana and Demetra had a high fraction of HMM-GS (10.6 and 11.3 %, respectively) and these cultivars had statistically significantly higher values of the most important gluten strength indicators (GI=98 and 97.7, E=101.5 and 111.3 cm^2, and R_{max}=417.7 and 459.0 EU, respectively) as well as statistically significantly lower values of degree of softening (DS=75.7 and 69.7 FU, respectively). In comparison with the excellent baking performance of cultivar Divana (V=572 cm^3, H/D= =0.87 and TCA=17.2 %), the lower protein (12.3 and 12.6 %) and wet gluten content (27.1 and 28.0 %) of cultivars Ana and Demetra, respectively, had negative impacts on their loaf volumes (449.3 and 463.0 cm^3). However, they retained a spherical loaf shape (H/D=0.78 and 0.74) and satisfactory crumb porosity (TCA=20.2 and 15.3 %) and they are described as a group with higher gluten strength (Tables 1, 2 and 3). Regarding cultivars Žitarka (1, 7+8, 2+12), Kata (N, 7+9, 2+12), Monika (N, 7+9, 2+12) and Sana (N, 6+8, 2+12), the presence of HMM-GS 2+12 at Glu-D1 locus in combination with lower proportion of HMM-GS (8.0–10.1 %) allocated these cultivars into the quality group with medium to weak gluten strength. These cultivars had statistically significantly lower values of gluten strength indicators (GI=61.0–75.0, E=34.3–46.5 cm^2 and R_{max}=131.0–187.3 EU) as well as unsatisfactory higher values of the degree of softening (DS=102.7–129.0 FU) than cultivars Divana, Ana and Demetra (Tables 1, 2 and 3). The classification of analyzed cultivars in relation to gluten strength characteristics contributed by HMM glutenin subunits is in accordance with the findings of other authors (*5–8*). Considering the small structural differences of HMM glutenin subunits in different wheat genotypes, the observed quality differences among them cannot be explained only by HMM-GS composition. Therefore, HMM-GS quantity must be taken into consideration. Although the quantity of HMM-GS per protein unit of flour is strongly affected by the environment, the different HMM-GS respond so similarly to ex-

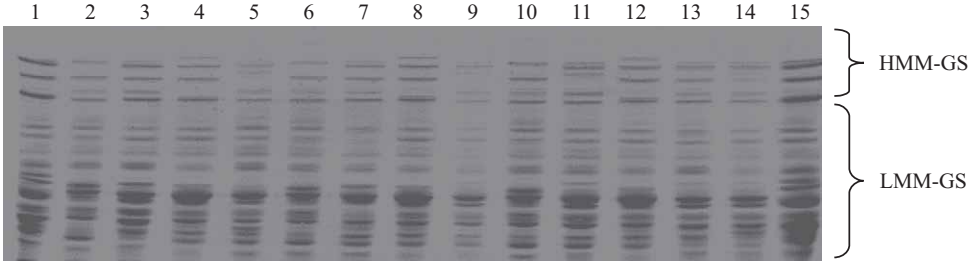

Fig. 2. SDS-PAGE separations of HMM-GS and LMM-GS from the wheat cultivars (from the left: lines 2 and 6 correspond to cultivar Kata, line 4 corresponds to cultivar Ana, lines 5 and 11 correspond to cultivar Sana, lines 8 and 12 correspond to cultivar Demetra)

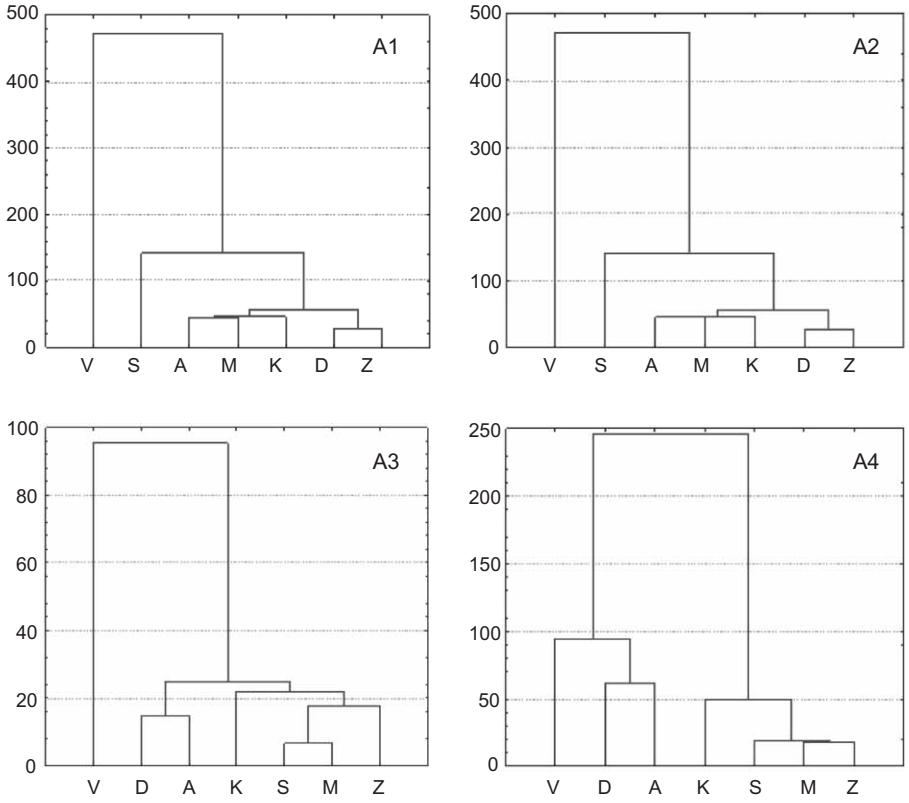

Fig. 3. Cluster dendrograms based on: A1) genotype and HMM-GS data, A2) flour and bread quality data, A3) dough rheological data, A4) all data, HMM-GS+flour and bread quality+rheology. The wheat cultivars are marked with letters (Z – Žitarka, K – Kata, M – Monika, A – Ana, D – Demetra, V – Divana, S – Sana)

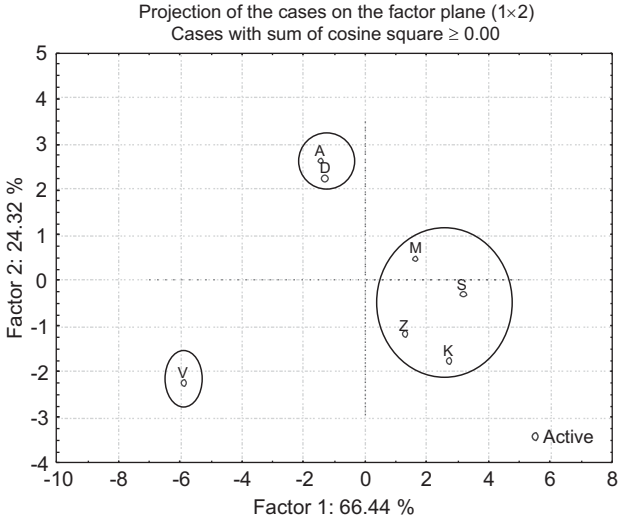

Fig. 4. Cultivar clusters on the plane of the first two principal components. HMM-GS, flour and bread quality and rheological data determine the clusters. Cultivars are marked with letters (Z – Žitarka, K – Kata, M – Monika, A – Ana, D – Demetra, V – Divana, S – Sana)

ternal conditions that their final proportions appear to be determined mainly by genetic factors (*22,23*). In this study, the analysis of variance has been used to partition sources of variation of HMM-GS quantity and proportion due to genotype (G), year (Y) and G×Y interaction. Information about the greater impact of genotype on HMM-GS proportions, compared to their quantity, should lead to more effective selection in breeding programs, because this parameter was more stable under different environmental conditions (Y variation accounted for 6.37 % of the total variation) and was practically not affected by G×Y interaction (Table 1). These conclusions were confirmed by cluster and principal component analyses (PCA). PCA of the total set of data revealed very strong interrelationship between the variables (Fig. 5). The first three PCA components accounted for 96 % of the total variance, which was up to the level of experimental error. The dendrograms (Fig. 3) were based on individual groups of parameters (A1: genotype and HMM-GS, A2: flour and bread quality, A3: rheology), and the complete set of the parameters, A4. Individual cultivars were denoted by the letters (Z – Žitarka, K – Kata, M – Monika, A – Ana, D – Demetra, V – Divana, S – Sana). The following partition sets for the first three top layers were obtained: A1={V,{S,{{A,M,K},{D,Z}}}}; A2={V,{S,{{A,M,K},{D,Z}}}}; A3={V,{{D,A},{K,{S,M,Z}}}} and A4={{D,A},{K,{S,M,Z}}}}. The cluster sets A1 for genotype and A2 for quality data were identical, and were similar to the sets A3 and A4 for rheological properties and the complete data sets. The degree of similarities was indicated by the evaluation of the first three eigenvalues of the corresponding Euclidian matrices (A1 (17.50, 6.91, 3.71), A2 (19.91, 7.75, 6.02), A3 (19.14, 10.5, 4.81), A4 (13.79, 7.98, 3.23)). This conclusion was further supported by the PCA. The same clusters were observed when the cultivars were projected on the plane of the first two principal components (Fig. 4). The strong congruence of the parameters was

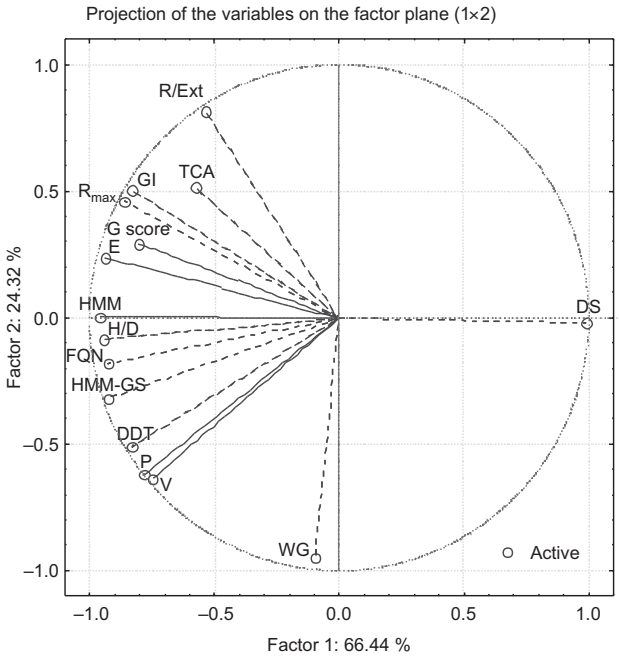

Fig. 5. Projections of the variables onto the first two principal components. Cumulative contributions of the first three projections are: 66.44, 90.76 and 95.96 %

further observed when each of the parameters was projected on the plane of the first two principal factors, shown in Fig. 5. HMM-GS parameters were very closely projected and mainly contributed to the construction of the first principal component. Projections of the two quality parameters, loaf height/diameter ratio (H/D) and farinograph quality number (FQN) almost coincided with HMM-GS data. On the opposite side of these projections was the parameter degree of softening (DS), indicating very high negative correlation with the HMM-GS data. Based on the projections on the plane of the first two principal components, the cause and the effect among the cultivars and the parameters can be inferred. The joint projections are presented in a form of a bi-plot in Fig. 6. Cultivar Divana was projected as a singular (isolated) point associated with high values of the first principal component (HMM-GS data) and the wet gluten (WG) as the second principal component. The cluster with Ana and Demetra was closely related to the parameter resistance to extensibility ratio (R/Ext). The importance of the R/Ext ratio in defining the differences in the quality of cultivars was emphasized in our previous work (*24*). The cluster of cultivars Sana, Monika, Žitarka and Kata is associated with the parameter degree of softening (DS).

Conclusions

The results obtained by multivariate chemometric analysis indicate considerable stability of the quality traits contributed by HMM-GS and this fact should be taken into consideration in breeding programs assisted by computer models that aim to improve wheat technological quality. The wheat technological parameters analyzed in this paper can be efficiently estimated by the first and second principal component projections. Unlike standard quality methods, analysis of HMM-GS requires only a small grain sample, making the estimation of quality more efficient in earlier stages of breeding.

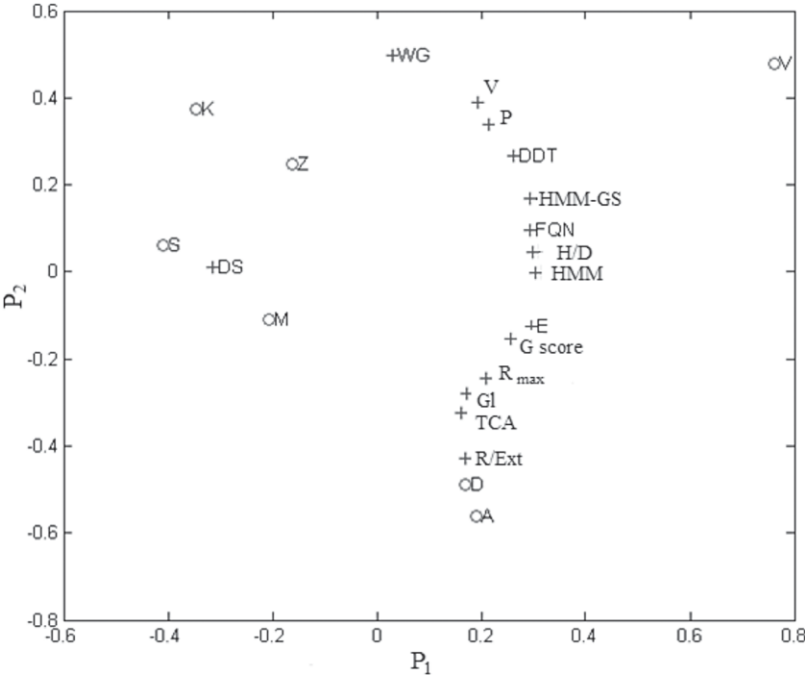

Fig. 6. Bi-plot of the cultivars and variables projected on the plane of the first two principal components P_1 and P_2. The cultivars are marked with open circles o and letters (Z – Žitarka, K – Kata, M – Monika, A – Ana, D – Demetra, V – Divana, S – Sana), while the variables are marked with plus signs and letters: P=protein content, WG=wet gluten, GI=gluten index, V=loaf volume, H/D=loaf height/diameter ratio, TCA= total gas cell area, DDT=dough development time, DS=degree of softening, FQN=farinograph quality number, E=dough energy, R_{max}=maximum resistance, R/Ext=resistance to extensibility ratio

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