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Characterization of Bread Wheat Cultivars (*Triticum aestivum* L.) by Glutenin Proteins

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Glutenin polymers composed of HMW and LMW subunits are important contributors to the wheat end-use properties. Twenty-six winter wheat cultivars differing in bread processing quality were collected at the experimental fields of the Agricultural Institute Osijek, Croatia and Institute of the Field and Vegetable Crops Novi Sad, Serbia, in 2008/2009 season. The HMW glutenins composition and glutenin proteins content were determined by SDS-PAGE and RP-HPLC, respectively, with aim to determine the relationship between glutenin protein fractions and wheat quality properties. Significant differences were found between Croatian and Serbian cultivars in several quality attributes (GI, WA, DDT, DS and R/EXT) as well as in the content of total glutenins and LMW glutenins and GLI/GLU ratio. The dominant HMW subunits in analyzed cultivars were 2*, 7 + 9/7 + 8 and 5 + 10. Principal component analysis (PCA) confirmed the presence of association between HMW glutenins composition and GI, dough E, R and R/EXT, while the glutenins quantitative data showed pronounced relation with P, DDT, DS, E, R and R/EXT. GLI/GLU ratio had the opposite effect on these parameters.

Keywords: wheat, end-use quality, glutenins, SDS-PAGE, RP-HPLC

Introduction

In the last decades cereal scientists have made considerable efforts in better understanding of the molecular basis for the viscoelastic properties of wheat gluten with aim to improve the wheat end-use properties. High-molecular weight (HMW) and low-molecular weight (LMW) glutenin polymers play an important role in determination of processing quality of common wheat flour (Shewry et al. 2001; Pena et al. 2005; Goesaert et al. 2005; Wieser 2007). Compared to HMW and LMW subunits, allelic variations of HMW-GS have more significant effects on wheat bread quality. For instance, the subunits 1 and 2* at the Glu-A1 locus, 5 + 10 at the Glu-D1 locus as well as subunits 17+18 and 7 + 8 at the Glu-B1 locus are associated with better physical dough properties (Payne et al. 1987). Wheat

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end-use quality also highly depends on different amounts and proportions of gluten proteins. Within each group of gluten, the amount of the glutenin components explains the variation in all quality parameters better than the gliadin components (Wieser and Kieffer 2001; Lasztity 2003; Johansson et al. 2004). A number of authors confirmed that environmental factors (soil, fertilization, climate fluctuations) affect the structure and functionality of gluten complex (Triboi et al. 2003; Dupont and Altenbach 2003; Torbica et al. 2007).

The present study was focused on investigating the end-use quality characteristics of some Croatian and Serbian bread cultivars in relation to HMW glutenins composition and glutenin proteins content.

Materials and Methods

Wheat cultivars

Experiments have been performed with 13 bread wheat cultivars (Srpanjka, Žitarka, Divana, Aida, Felix, Zlata, Ilirija, Ružica, Sana, Seka, Golubica, Soissons and Olimpija) grown in Croatia at the Agricultural Institute Osijek and with 13 bread cultivars grown in Serbia (Vojvodina, Angelina, Dragana, Ljiljana, Pobeda, Bastijana, Evropa 90, NS 40S, Simonida, Etida, Renesansa, Zvezdana and NS3-5299/2) at the Institute of Field and Vegetable Crops Novi Sad in 2009. French cultivar Soissons was included in set because this cultivar is well distributed in Croatian wheat market.

Wheat quality characteristics

The wheat quality parameters were defined by grains crude protein content (Infratec 1241, Foss Tecator), wet gluten and gluten index (ICC No. 155). The dough rheological properties were examined by the Brabender farinograph and extensograph (ICC Standard 115/1 and 114/1, respectively).

Proteins characterization

Composition of HMW-GS was previously analyzed by SDS-PAGE (Hoefer SE 600) and identified according to the Payne and Lawrence (1983). The wheat proteins extraction as well as the RP-HPLC method was based on Wieser et al. (1998). Perkin Elmer LC 200 chromatograph was used with a Supelco Discovery Bio Wide Pore C18 column (300 Å pore size, 5 µm particle size, 4.6 × 250 mm i.d.). Solvents were composed of water and acetonitrile (ACN), containing 0.1% (v/v) trifluoroacetic acid (TFA). Twenty µl samples were injected for analyses. Glutenin fractions were eluted with a linear gradient from 24% to 58% ACN over 30 min at 1 ml/min, using a column temperature of 50ºC. All samples were detected by UV absorbance at 210 nm in two replicates. The obtained chromatograms were analyzed by Total-Chrom software package (Perkin Elmer Instruments, USA). The peak areas (expressed in arbitrary units = AU) under albumins-globulins, gliadins (results not shown) and glutenin chromatograms were summed and used as a direct measure of total extractable wheat proteins content and consequently the proportions (%) of total as well as HMW and LMW glutenins were calculated.

Statistics

Statistical analysis was performed using STATISTICA 8.0 (StatSoft Inc., USA) software. The general analysis of variance (ANOVA, p<0.05) and least significant difference (LSD) pair wise comparisons of means were used to determine significant differences within two cultivars data set. Principal component analysis (PCA) was used to study associations among quality and glutenins properties and to establish similarities among cultivars.

Results

The quality parameters data for two sets of cultivars were summarized in Table 1. The mean values of gluten strength parameters (GI, DDT, DS, E, R and R/EXT) varied between weak to very strong level, which implies significant differences among dough rheology properties. Two sets of cultivars significantly differed in several quality attributes: GI, WA, DDT, DS and R/EXT.

Table 1. Wheat end-use quality parameters

Parameter	Croatian (n = 13)			Serbian (n = 13)		
	Mean	Range	SD	Mean	Range	SD
P*	13.9^{ns}**	12.0–16.4	1.3	13.5	12.7–14.3	0.5
WG	29.9^{ns}	25.7–37.0	4.2	31.8	24.6–40.0	5.3
GI	97^{b}	89–100	3.1	93^{a}	81–100	6.6
WA	60.4^{a}	56.9–64.4	2.3	64.0^{b}	60.5–68.3	2.5
DDT	3.2^{ns}	1.5–10.3	2.6	2.7	2.0–3.5	0.6
DS	56^{a}	26–125	24.7	72^{b}	30–110	27.3
E	84^{ns}	42–126	31.3	82	47–126	26.4
R	252^{ns}	149–350	69.3	223	140–360	55.8
EXT	165^{ns}	132–197	17.5	171	134–209	23.6
R/EXT	1.6^{b}	0.8–2.2	0.4	1.3^{a}	0.7–2.5	0.4

* P = protein content on dry matter (%); WG = wet gluten content (%); GI = gluten index; WA = water absorption (%); DDT = dough development time (min); DS = degree of softening (FU); E = energy (cm^2); R = dough resistance (EU); EXT = extensibility (mm); R/EXT = resistance to extensibility ratio; ns = not significantly
** significantly ($p < 0.05$) different values are marked with various alphabet letters

Allelic variations at *Glu-1* loci in wheat samples separated by SDS-PAGE were shown in Table 2. It was found that *Glu-1* allele combinations *bbd* and *bcd* responsible for synthesis of HMW subunits 2* 7 + 8 5 + 10 and 2* 7 + 9 2 + 10, respectively, have occurred in most Croatian cultivars (23.1%) while the *Glu-1* alleles *bcd* (2* 7 + 9 5 + 10) appeared in 38.5% of Serbian cultivars.

Regarding the quantitative results of glutenin proteins obtained by RP-HPLC method a large variability of analyzed parameters was noticed (Table 3). Two sets of cultivars sig-

Table 2. Composition of HMW glutenins in wheat cultivars

GLU-1 allele	GLU-A1	GLU-B1	GLU-D1	GLU-1 score	Frequency%	
					Croatian (n = 13)	Serbian (n = 13)
ccd	N	7 + 9	5 + 10	7	0.0	30.8
acd	1	7 + 9	5 + 10	9	7.7	0.0
bbd	2*	7 + 8	5 + 10	10	23.1	0.0
bcd	2*	7 + 9	5 + 10	9	23.1	38.5
bid	2*	17 + 18	5 + 10	10	7.7	0.0
cca	N	7 + 9	2 + 12	5	7.7	30.8
aca	1	7 + 9	2 + 12	7	7.7	0.0
cba	N	7 + 8	2 + 12	6	15.4	0.0
bda	2*	6 + 8	2 + 12	6	7.7	0.0

nificantly differed in the content of total and LMW glutenins as well as in their proportions in total extractable wheat proteins. Significant difference was also found for GLI/GLU ratio.

Table 3. Glutenins content (AU) and proportions (%)* in wheat cultivars

Glutenins	Croatian (n = 13)			Serbian (n = 13)		
	Mean	Range	SD	Mean	Range	SD
T GT**	30.5^{b}***	21.9–42.4	5.7	26.0^{a}	21.5–32.1	3.2
T HMW	8.1^{ns}	4.2–13.5	2.2	7.30	5.5–9.1	1.0
T LMW	22.4^{b}	16.8–32.8	4.3	18.7^{a}	14.8–23.0	2.4
% GT	33.6^{b}	28.5–37.1	2.9	31.7^{a}	28.2–38.5	2.8
% HMW	8.9^{ns}	5.5–12.9	1.6	8.9	7.4–10.9	1.0
% LMW	24.7^{b}	20.4–28.9	2.7	22.8^{a}	18.5–27.6	2.3
GLI/GLU	1.55^{a}	1.2–1.9	0.2	1.73^{b}	1.3–1.9	0.2

* % = proportion in total extractable wheat proteins
** T GT = total glutenins (AU) as sum of HMW and LMW content; T HMW = total HMW glutenins (AU); T LMW = total LMW glutenins (AU); GLI/GLU = gliadins to glutenins ratio; AU = arbitrary units (AU.s/mg flour); ns = not significantly
*** significantly ($p < 0.05$) different values are marked with various alphabet letters

Principal component analysis (PCA) was applied to effectively reduce large set of data into lower dimension of latent variables amenable for analysis (Kurtanjek et al. 2008). The first three PCA components accounted for 75.80% of the total data variance. The plot of PC1 vs. PC2 shows (Fig. 1) that the PC1 axis is determined by quantitative RP-HPLC glutenins data together with some dough strength parameters (GLU-1, R, GI, R/EXT, GLI/GLU), while the PC2 axis is the most defined by P, WG and DDT.

The plot of PC1 vs. PC2 loadings shows along the PC1 axis close relation between HMW glutenins composition (Glu-1 score) and dough rheology properties: GI, E, R and R/EXT, while WG, WA and DS are on opposite side and negatively correlated with this parameter. P content is positively related to WG, DDT and EXT and negatively with DS.

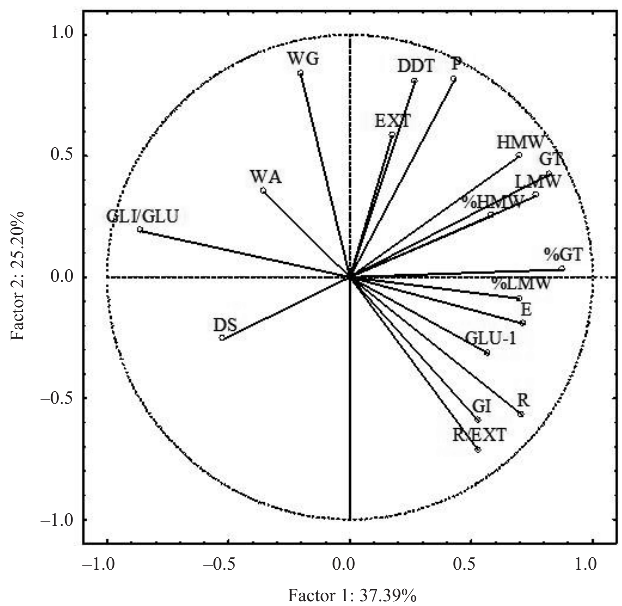

Figure 1. Projections of the variables onto the first two principal components. Cumulative contributions of the first three projections are: 37.39, 64.05 and 75.50%

Observing the quantitative RP-HLPC glutenins data, the P and DDT are strongly positively affected by HMW and total glutenins content, while the DS is negatively influenced by these parameters. Dough E is most affected by LMW glutenins proportion. The ratio of GLI/GLU was negatively associated with GI, E, R and R/EXT.

Comparing the two sets of wheat cultivars, several groups of related quality attributes and quantitative glutenins data were separated (Fig. 2). Cultivars Žitarka, Ljiljana and Zvezdana belong to the first group. These cultivars showed, on average, disturbed rheological properties due to low Glu-1 score and high GLI/GLU ratio (1.9). The second one consists of cultivars NS 40S, Felix, Srpanjka and Seka. The flour of these cultivars belongs to the medium strong class with GLI/GLU ratio of 1.5.

Third group includes cultivars Zlata, Soissons and Vojvodina. The flours of these cultivars are characterized as very strong due to HMW subunits 2* and 5 + 10 and the lowest GLI/GLU ratio (1.3). Cultivar Divana (2* 7 + 9 5 + 10) is the Croatian bread improver and has a distinct position in Fig. 2. Divana obtained the best quality attributes regarding the gluten elasticity properties and the highest HMW proportion (12.9%). In contrast to Divana, cultivar Sana, Croatian yield standard, is positioned on the opposite side. Due to unfavourable HMW composition (2* 6 + 8 2 + 12) and very low total (28.5%) and HMW glutenins (5.5%), this cultivar obtained characteristics of very weak flour. Cultivar Pobeda is Serbian quality and yield standard and in spite of favourable HMW subunits 2* 7 + 9 5 + 10 and higher HMW proportion (10.0%) did not show the excellent dough characteristics. The particular cultivars data were not shown.

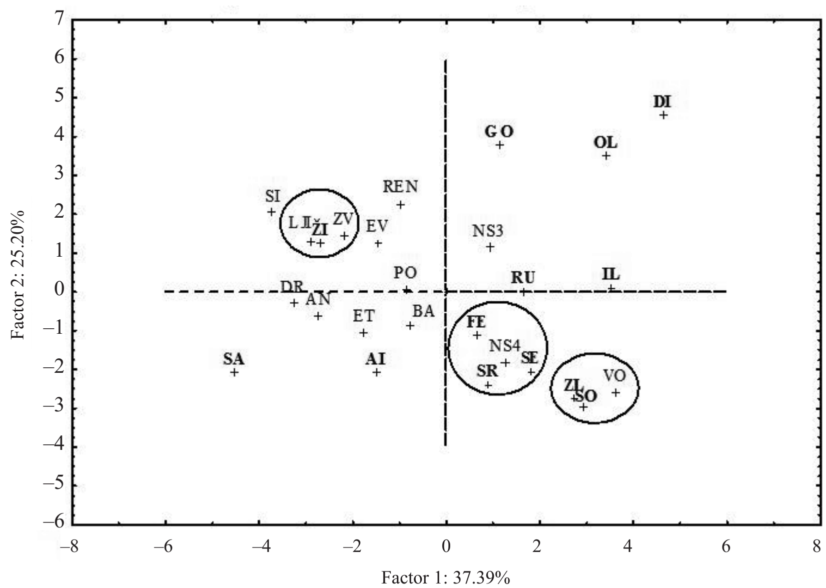

Figure 2. Cultivar clusters on the plane of the two first principal components. Quality attributes, HMW glutenins composition and RP-HPLC quantitative glutenins data determine the clusters. Cultivars are marked with letters: SR = Srpanjka, ŽI = Žitarka, DI = Divana, AI = Aida, FE = Felix, ZL = Zlata, IL = Ilirija, RU = Ružica, SA = Sana, SE = Seka, GO = Golubica, SO = Soissons, OL = Olimpija, VO = Vojvodina, AN = Angelina, DR = Dragana, LJI = Ljiljana, PO = Pobeda, BA = Bastijana, EV = Evropa 90, NS4 = NS 40S, SI = Simonida, ET = Etida, REN = Renesansa, ZV = Zvezdana, NS3 = NS3-5299/2. Croatian cultivars are bolded

Discussion

The domination of subunits 5 + 10 at the Glu-D1 locus and high frequency of subunit 2* at the Glu-A1 locus in Croatian and Serbian cultivars implies that wheat breeders of Agricultural Institute Osijek and Institute of Field and Vegetable Crops Novi Sad have made a considerable effort towards creation of new cultivars with improved gluten strength characteristics in the last decade (Denčić et al. 2008; Horvat et al. 2009). The domination of HMW glutenins combinations 2* 7 + 9 5 + 10 and N 7 + 9 5 + 10 in European winter wheat cultivars have been reported by Tohver (2007), Denčić et al. (2008), Tsenov et al. (2009).

The obtained positive influence of HMW glutenins composition (Glu-1 score) on dough strength parameters are in accordance with other authors (Wieser and Kieffer 2001; Horvat et al. 2006; Qury et al. 2010). The obtained significantly positive effects of total and HMW and LMW glutenins contents on dough rheological parameters and negative correlation of GLI/GLU ratio with these parameters were similar to those published in recent time (Tang et al. 2008; Kurtanjek et al. 2008; Anderson et al. 2011). On average, Serbian cultivars had significantly higher GLI/GLU ratio compared to Croatian ones. Vegetation season 2008/2009 in Croatia was characterized by extremely limited rainfall in March and April and with a 2ºC higher temperature during grain development compared to multi-year average. Torbica et al. (2010) analyzed several heat-stress years in the last decade in Serbia, including 2008/2009 season, and reported that changes in gluten complex structure under unfavourable weather conditions were highly genotype dependent. Triboi et al. (2003) and Dupont and Altenbach (2003) reported that high temperatures after anthesis increased the gliadins to glutenins ratio, while Balla et al. (2011) found that heat stress in combination with drought had an extremely negative influence on grain quality due to decreasing values of unextractable polymeric protein fraction as well as increasing values of gliadins to gliadins ratio.

In conclusion, the PCA analysis indicated that HMW glutenins composition accompanied with quantity aspect of glutenin proteins alone were not sufficient to explain the variability of wheat end-use quality. The obtained results give very useful information regarding the comparison of two sets of cultivars belonging to close wheat production regions and should be considered in both wheat breeding programs for further improvement of wheat quality.

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