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Quality of wheat cultivars created at the Agricultural Institute Osijek in relation to high molecular weight glutenin subunits (HMW – GS) composition

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Abstract

Background and Purpose: *The objective of this study was to estimate the effect of high molecular weight glutenin subunits (HMW-GS) composition of the Glu-1 loci on the quality of winter wheat cultivars created at the Agricultural Institute Osijek.*

Materials and Methods: *Ten winter wheat cultivars were grown in 4 different locations during two years in a randomized complete block (RCB) experimental design with three repetitions at each location. Samples were collected and subjected to quality analysis and determination of HMW-GS composition by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).*

Results: *Considering the HMW-GS composition, the most frequent subunit at the Glu-A1 locus was N, at the Glu-B1 locus 7+9 and at the Glu-D1 locus 2+12. The analyzed cultivar Demetra, with subunits 5+10 at the Glu-D1 locus, has shown the best bread-making quality. Among cultivars with subunits 2+12 at the Glu-D1 locus, the cultivars with subunits 7+8 at the Glu-B1 locus have shown on average better characteristics of gluten strength with regard to higher values of Gluten Index and resistance to exstensibility ratio (R/Ext). The results of the linear correlation between quality parameters and HMW-GS composition, expressed as the Glu-1 score, have shown the most significant ($P<0.05$) influence of HMW-GS on Gluten Index ($r=0.42$), dough energy ($r=0.48$) and dough maximum resistance ($r=0.47$).*

Conclusions: *These results indicated that the presence of HMW-GS 5+10 and 7+8 contributes to higher bread-making quality of analyzed cultivars. The HMW-GS composition has predictive value in quality analysis, therefore, the composition of HMW-GS at the Glu-1 loci has to be taken into consideration when parents for a bread wheat breeding programme are chosen.*

INTRODUCTION

The glutenin subunits (GS) of wheat (*Triticum aestivum L.*) are wheat endosperm proteins known to be responsible for the unique technological properties of flour (*1, 2, 3*). Glutenins, traditionally defined according to the Osborne classification as the kernel proteins unextractable in 70% ethanol but extractable in dilute acid or alkali, are a complex mixture of the high molecular weight glutenin subunits

TABLE 1

Correlation between distinguishable bands of glutenin subunits and quality status

QUALITY SCORE	QUALITY STATUS	GLU-1 LOCI		
		Glu-A1	Glu-B1	Glu-D1
4	Excellent	—	—	5+10
3	High	1 and 2	17+18 and 7+8	—
2	Medium	—	7+9	2+12 and 3+12
1	Low	Null	7 and 6+8	4+12

(HMW-GS) and the low molecular weight glutenin subunits (LMW-GS). Bread wheat varieties (cultivars) consist of 3-5 HMW-GS and approximately 15 LMW-GS. HMW-GS are encoded by polymorphic genes at Glu-1 loci (Glu-A1, Glu-B1 and Glu-D1 locus) present on the long arms of the group 1 chromosomes. The molecular weight of HMW-GS varied from 80000 to 120000 Da and they are one of the most important genetic factors in determining the dough-forming properties although accounting for only 10% of the wheat storage proteins. HMW-GS 5+10 and 2+12 at the Glu-D1 locus have a major effect on dough strength (*4, 5*). Payne and Lawrence (*6*) ranked the alleles of the three Glu-1 loci for their contribution to bread-making quality after dertermination of the quality of the lines of segregating progenies by the SDS-sedimentation test.

The agronomic and climate conditions largely contribute to the quantitative variation of HMW-GS which in turn affects the size distribution of glutenin polymers while electrophoretic «fingerprints« of the HMW-GS composition of a variety generally appear constant for samples from different locations (*7*).

The gluten proteins quality has been taken into consideration by wheat breeders of the Agricultural Institute Osijek in order to create a new cultivars with optimal gluten strength (*8, 9*).

MATERIALS AND METHODS

Materials. 10 wheat samples differing in bread-making quality were collected during the 2003/2004 and 2004/2005 seasons from experimental fields of the Agricultural Institute Osijek. Cultivars Demetra, Žitarka, Srpanjka, Zrnka, Alka, S. Žitarka, Golubica, Panonka, Ševa and Janica are bread winter wheats created at the Agricultural Institute Osijek. The experiment was set up at four locations (Osijek, Tovarnik, Nova Gradiška and Požega) as a randomized complete block experimental design (RCBD) field trial with three repetitions.

Methods. The crude protein content and grain hardness of samples was measured by NIT technology (*Infratec 1241, Foss Tecator*). All samples were milled into flour using a Brabender Quadrumat Sr. Mill, and flour was standardised at ash content 0.55%. The following wheat quality parameters were determined: Zeleny sedimentation value (ICC No 115/1), wet gluten content and Gluten Index according to Gluten Index method (ICC No 155), farinograph (ICC No 115/1) and extensograph (ICC No 114/1) properties. Composition of HMW-GS was analyzed by electrophoresis (SDS-PAGE) using Phast System, Pharmacia LKB, under the following conditions: 40 min, 250 V, 10 mA, 15 °C and 120 Vh. Gels were stained using 0.1 % Coomassie Brilliant Blue R. The electrophoresis was carried out on PhastGel gradi-

TABLE 2

HMW-GS composition and Glu-1 quality scores.

Cultivar	Year of realese	HMW-GS			Glu-1 score
		Glu-A1	Glu-B1	Glu-D1	
Demetra	1991	1	7+9	5+10	9
Žitarka	1985	N	7+8	2+12	6
Srpanjka	1989	N	7+8	2+12	6
Zrnka	2003	N	7+8	2+12	6
Alka	2003	N	7+8	2+12	6
S. Žitarka	1997	N	7+9	2+12	5
Golubica	1998	N	7+9	2+12	5
Panonka	2001	N	7+9	2+12	5
Ševa	2002	N	7+9	2+12	5
Janica	2003	N	7+9	2+12	5

ent 4-15 gels (43 x 50 x 0.45). Prior to electrophoresis, glutenins were extracted from 50 mg of whole-meal flour with SDS-PAGE sample buffer (2X stock buffer: 0,125 M Tris-HCl, 4% SDS, 20% glycerol, 0,2 M DTT, 0,02% Bromphenol Blue, pH 6,8). Identification of HMW-GS was made by comparison to known standards. Calculation of Glu-1 scores was made according to Payne and Lawrence (*6*) (Table 1).

Data analysis. Data analysis was performed using software Statistica 5.0 (StatSoft, Inc.). The following statistical parameters for quality traits of samples pooled over repetitions were analysed: the mean value, standard deviation and LSD test.

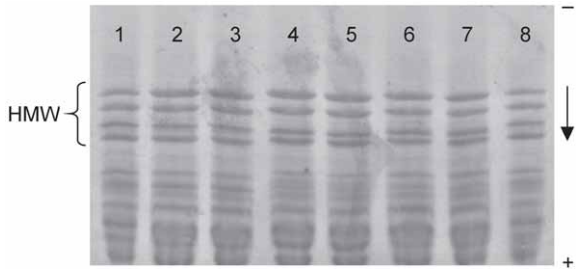

Figure 1. *SDS-PAGE electrophoregram of wheat glutenins. 1&8 cultivar Chinese Spring as standard with HMW subunits 2,7,8,12 in the arrow directions; 2&3 cultivar Srpanjka (2,7,8,12); 4&5 cultivar Golubica (2,7,9,12) and 6&7 cultivar S. Žitarka (2,7,9,12)*

RESULTS

At the investigated cultivars 4-5 HMW-GS differences electrophoretic mobility were found. The HMW-GS composition and Glu-1 quality scores are presented in Table 2. At the Glu-A1 locus 2 alleles out of 3 possible were detected; allele *a* which encodes synthesis of subunit 1 and allele *c* which encoded synthesis of subunit N (Null). At the Glu-B1 locus 2 alleles out of 11 possible were identified; allele *b* responsible for encoding subunits 7+8 and allele *c* responsible for encoding subunits 7+9. At the Glu-D1 locus 2 alleles out of six possible were identified; allele *a* responsible for encoding subunits 2+12 and allele *d* responsible for encoding subunits 5+10. The most frequent HMW-GS at the Glu-A1 locus was N, at the Glu-B1 locus 7+9 and at the Glu-D1 locus 2+12 (Table 2). Typical SDS-PAGE electrophoregram of the three investigated samples (cultivars Srpanjka, Golubica and S. Žitarka) is shown in Figure 1.

The mean values of indirect quality parameters for 10 wheat cultivars are presented in Table 3. Protein content ranged from 13.3 % (cultivars Demetra and Alka) to 14.6 % (cultivar Golubica), while sedimentation value varied between 43 cm^3 (cultivar Ševa) and 63 cm^3 (Golubica). Cultivars with protein content higher than 13 % and sedimentation value above 40 cm^3 are considered to have better quality according to the official Croatian Regulations (*10*). Content of gluten, expressed as wet gluten, varied between 29.3 % (cultivar Srpanjka) and 39.6 % (cultivar Golubica). Concerning wheat quality it must be mentioned that protein and wet gluten content are not a measure for gluten quality. Gluten Index and rheological dough properties are the most important indicators of wheat flour quality. These parameters define both the degree of gluten elasticity and extensibility. Gluten Index varied between 74 (cultivar Ševa) and 98 (cultivar Demetra). Values for grain hardness ranged from 29.8 (cul-

TABLE 3

Indirect quality parameters of wheat cultivars.

Cultivar	P[a]	SED	WG	GI	H	FY
	Mean[b]±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd
Demetra	13.3±1.0	56±8.9	30.1±4.2	98±1.7	29.8±13.2	72.1±1.4
Žitarka	14.4±1.1	54±7.3	38.7±6.50	76±9.9	63.1±10.7	68.8±1.9
Srpanjka	13.8±1.2	49±9.9	29.3±5.0	97±2.1	37.8±15.4	73.1±2.6
Zrnka	13.5±0.9	40±5.3	31.9±3.4	85±6.4	51.6±13.7	69.5±1.7
Alka	13.3±1.2	49±8.9	33.5±5.8	79±6.5	35.1±14.1	72.5±2.8
S. Žitarka	13.6±1.1	51±9.5	34.2±5.4	85±8.8	57.1±10.5	69.1±2.4
Golubica	14.6±1.2	63±6.5	39.6±6.6	82±10.7	59.2±12.3	72.2±1.8
Panonka	14.2±1.0	46±9.4	38.5±6.6	73±10.2	45.5±14.2	71.1±2.2
Ševa	14.3±1.3	43±9.3	36.2±5.0	74±9.5	49.6±11.8	71.9±2.3
Janica	13.9±0.9	60±11.5	33.4±4.6	91±6.9	53.8±9.5	70.9±2.2
Total	13.9±1.2	51±11.0	34.5±6.28	84±11.5	48.2±16.1	71.1±2.5
$LSD_{0.05}$	0.19	3.11	1.10	2.91	9.25	1.58

[a]P=Protein content (%); SED=Sedimentation value (cm^3); WG=Wet gluten (%); GI=Gluten Index; H=Grain hardness; FY=Flour Yield (%)

[b]Mean values of two years and 4 locations

TABLE 4

Rheological properties of wheat cultivars.

Cultivar	DDT[a]	R	DS	E	Ext	Rmax	R/Ext
	Mean[b]±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd
Demetra	2.0±0.5	3.5±2.3	71±34.4	107±19.0	163±15.6	517±112.4	2.0±0.8
Žitarka	3.0±0.7	4.2±0.9	80±19.3	59±16.0	158±10.3	265±72.1	1.3±0.3
Srpanjka	2.4±1.6	3.0±2.1	59±23.7	89±29.4	148±11.5	455±148.9	2.2±0.7
Zrnka	3.9±1.0	5.3±0.9	77±16.1	44±6.1	132±10.0	234±43.7	1.6±0.4
Alka	2.3±1.1	3.0±1.6	132±49.5	53±14.0	163±19.3	241±93.1	1.2±0.7
S. Žitarka	2.4±0.8	3.4±1.0	80±21.4	71±16.6	138±10.7	382±91.4	2.2±0.7
Golubica	4.0±1.1	5.5±1.2	67±20.6	66±13.4	176±11.0	266±61.4	1.1±0.3
Panonka	2.9±0.6	3.6±0.8	101±21.7	35±9.9	165±11.0	143±43.4	0.8±0.3
Ševa	4.0±0.5	4.8±0.8	103±16.7	35±11.1	156±14.0	152±47.7	0.8±0.3
Janica	2.9±1.0	4.2±1.5	55±12.7	81±16.5	160±8.8	379±81.9	1.7±0.4
Total	3.0±1.2	4.1±1.6	82±33.2	64±27.4	156±17.4	303±144.5	1.49±0.7
$LSD_{0.05}$	0.68	1.03	18.72	11.94	9.21	62.5	0.37

[a]DDT=Dough development time (min); R=Resistance (FU); DS=Degree of softening (FU); E=Dough energy (cm^2); Ext=Extensibility (mm); Rmax=Maximum resistance (EU); R/Ext=Resistance to Extensibility ratio

[b]Mean values of two years and 4 locations

tivar Demetra) to 63.1 (cultivar Žitarka), while flour yield varied between 68.8 (cultivar Žitarka) and 73.1 (cultivar Srpanjka).

Rheological dough properties are presented in Table 4. Concerning the mixing behavior of dough, the results obtained by farinograph analysis showed that dough development time varied between 2.0 min (cultivar Demetra) and 4.0 min (cultivars Golubica and Ševa), while dough mixing resistance varied from 3.0 min (cultivars Srpanjka and Alka) to 5.5 min (cultivar Golubica). The lowest degree of softening, as a measure of mixing tolerance index, was found for cultivar Janica (55 FU) and the highest cultivar Alka (132 FU).

According to extensographic parameters measured after 135 minutes of dough maturing, cultivars Panonka and Ševa (35 cm^2) had the lowest dough energy and cultivar Demetra (107 cm^2) the highest . Dough extensibility varied between 132 mm (cultivar Zrnka) and 176 mm (cultivar Golubica). Dough maximum resistance to extension ranged from 143 EU (cultivar Panonka) to 517 (cultivar Demetra). The lowest ratio between dough resistance and extensibility (R/Ext) as a measure of dough elasticity was calculated and it was determined that cultivars Panonka and Ševa had the lowest value (0.8), while cultivar Srpanjka (2.2) had the highest value (Table 4).

TABLE 5

Correlation coefficients (r) between HMW-GS composition and wheat quality parameters.

Parameters	Glu-1 score
Protein content (%)	–0.21*
Sedimentation value (cm^3)	0.05
Wet gluten (%)	–0.30*
Gluten Index	0.42*
Hardness	–0.43*
Flour Yield (%)	0.11
Dough development time (min)	–0.06
Resistance (FU)	–0.11
Degree of softening (FU)	–0.04
Dough energy (cm^2)	0.48*
Extensibility (mm)	0.03
Maximum resistance (EU)	0.47*
Resistance to extensibility ratio (R/Ext)	0.28*

* Statistical significance: $P<0.05$

DISCUSSION

HMW-GS as (a) polymeric glutenin fractions of wheat proteins have the most important role in defining bread-making quality (5). The grain samples were different in the HMW-GS composition (Table 2) and their crude protein content varied between 13.3% (cultivars Demetra and Alka) and 14.6% (cultivar Golubica) (Table 3).

From this study we observed that the analyzed cultivars did not vary widely in composition of HMW-GS at the Glu-1 loci, which is in agreement with our previous investigations of cultivars carried out at the Agricultural

TABLE 6

Quality parameters of cultivars differing in HMW-GS at the Glu-B1 locus.

Cultivar	P[a]	SED	WG	GI	H	FY
	Mean[b]±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd
Žitarka, Srpanjka, Zrnka, Alka	HMW-GS 7+8 at Glu-B1 locus					
	13.7±1.1	48±9.2	33.0±6.0	85±9.9	46.8±17.4	71.0±2.9
S. Žitarka, Golubica, Panonka, Ševa, Janica	HMW-GS 7+9 at Glu-B1 locus					
	14.1±1.1	53±12.0	36.1±5.9	82±10.4	53.0±12.4	71.1±2.4
Total	14.0±1.1	51±11.2	34.7±6.1	83±10.3	50.2±15.1	71.0±2.6
$LSD_{0.05}$	0.38	3.57	2.01*	3.60*	5.13*	0.91

[a]P=Protein content (%); SED=Sedimentation value (cm^3); WG=Wet gluten (%); GI=Gluten Index; H=Grain hardness; FY=Flour Yield (%)

[b]Mean values of two years and 4 locations

*Statistical significance: $P<0.05$

TABLE 7

Rheological properties of cultivars differing in HMW-GS at the Glu-B1 locus.

Cultivar	DDT[a]	R	DS	E	Ext	Rmax	R/Ext
	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd
Žitarka, Srpanjka, Zrnka, Alka	HMW-GS 7+8 at the Glu-B locus						
	2.9±1.3	3.9±1.7	87±39.7	61±24.5	150±17.7	298±130.9	1.56±0.7
S. Žitarka, Golubica, Panonka, Ševa, Janica	HMW-GS 7+9 at the Glu-B locus						
	3.3±1.0	4.8±4.8	81±26.4	58±23.7	159±16.4	264±124.1	1.31±0.7
Total	3.1±1.2	4.4±3.7	84±32.9	59±24.0	155±17.5	279±127.7	1.4±0.7
$LSD_{0.05}$	0.40	1.30	11.4	8.36	5.90*	44.19	0.23*

[a]DDT=Dough development time (min); R=Resistance (FU); DS=Degree of softening (FU); E=Dough energy (cm^2); Ext=Extensibility (mm); Rmax=Maximum resistance (EU); R/Ext=Resistance to Extensibility ratio

*Statistical significance: $P<0.05$

Institute Osijek (*11, 12*). The most frequent HMW-GS at the Glu-A1 locus was N, at the Glu-B1 7+9 and at the Glu-D1 2+12 (Table 2). A previous study of HMW-GS composition of Croatian cultivars realesed between 1967 and 1987 (*13*) already had shown that the range and distribution of the HMW-GS found in Croatian bread wheats did not vary widely in comparison with cultivars grown in other European regions. In a study by Jost *et al.* (1995) of Croatian wheat cultivars, the most frequent HMW-GS at the Glu-A1 locus was 1, at the Glu-B1 7+9 and at the Glu-D1 2+12.

According to the HMW-GS composition (Table 2) the cultivars were classified into several groups: cultivar Demetra (1, 7+9, 5+10), cultivars Žitarka, Srpanjka, Zrnka and Alka (N, 7+8, 2+12) and cultivars S. Žitarka, Golubica, Panonka, Ševa and Janica (N, 7+9, 2+12).

The mixing properties and baking performance of wheat flours are closely correlated with composition of HMW-GS. In particular, HMW-GS 5+10 and 2+12 at the Glu-D1 locus have a major effect on dough strength (*14, 15, 16*). Cultivar Demetra with HMW-GS 1, 7+9, 5+10 and with the highest Glu-1 quality score have shown the best technological characteristics concerning values of Gluten Index and extensographic parameters as a direct measure of gluten strength (Table 3, 4). The results of the linear correlation ($P<0.05$) analyses between wheat quality parameters and HMW-GS composition are presented in Table 5. HMW-GS composition (Glu-1 score) showed significant positive influence on Gluten Index ($r=0.42$), dough energy ($r=0.48$), maximum resistance to extension ($r=0.47$) and resistance to extension ratio ($r=0.28$). A significant negative influence was noticed on protein content ($r = -0.21$), wet gluten ($r = -0.30$) and grain hardness ($r = -0.43$).

Regarding the HMW-GS composition at the Glu-B1 locus, cultivars could be divided into two classes. Cultivars Žitarka, Srpanjka, Zrnka and Alka at the Glu-B1 locus had subunits 7+8, while cultivars S. Žitarka, Go-

lubica, Panonka, Ševa and Janica had subunits 7+9 (Table 6, 7). In accordance with Payne and Lawrence (*6*), the subunits 7+8 controlled by genes at the Glu-B1 locus generally had a more positive contribution to bread-making quality than GS 7+9 at the same locus (Table 1). The results of LSD test indicated that cultivars with HMW-GS 7+8 showed , based on average values, stronger gluten characteristics with regard to higher value of Gluten Index and resistance to extensibility ratio as measure of dough elasticity (Table 6, 7). The positive affect of HMW-GS 7+8 on bread-making quality observed in this study are comparable with other research (*17, 18, 19*).

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