UDC 575:633.11:664.6
ISSN 0352-9193

preliminary communication

Genetic Basis of Breadmaking Quality of Croatian Wheat Cultivars

Genetička osnova pekarske kakvoće hrvatskog sortimenta pšenice

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Received: June 28, 1994
Accepted: June 19, 1995

Summary

The unique viscoelastic and cohesive properties of wheat dough are mainly due to its water-insoluble, storage proteins, gluten. The strongest effect on quality is due to a group of high-molecular-weight (HMW) subunits of glutenin, coded by the alleles on homocologous group I chromosomes: 1A, 1B and 1D. At present, 20 different multiple alleles coding HMW glutenins are known. Although hexaploid wheat probably could possess two genes at each of the chromosome mentioned, i.e. altogether 6, the known cultivars have only 3 to 5 subunits of HMW glutenin. Each of the subunits composition has shown a different effect on breadmaking quality. The subunits **7 + 8**, **7 + 9** *and* **17 + 18** *coded by* **Glu–B1b**, **Glu–B1c**, *and* **Glu–B1i**, *as well as the subunits* **5 + 10**, *coded by* **Glu–D1d** *allele have the greatest positive effect on breadmaking quality, while the subunits* **6 + 8** *and* **2 + 12** *coded by the multiple alleles* **Glu–B1d** *and* **Glu–D1a** *have a significant negative effect.*

The Croatian wheat cultivars are grouped according to the value of their HMW glutenin subunits and were related to their breadmaking properties. The strategy, results and prospects of Croatian wheat breeding program based on glutenin subunits are discussed.

Sažetak

Pšenični lijepak (gluten), u vodi netopljivi dio proteina endosperma zrna, daje tijestu dragocjeno svojstvo viskoelastičnosti. Dio lijepka čine glutenini velike molekularne mase (HMW) koji najjače utječu na fizičke osobine tijesta. Do sada je poznato ukupno 20 alela koji kodiraju sintezu podjedinica HMW glutenina. Geni za sintezu HMW podjedinica glutenina locirani su na duljem kraku kromosoma homeologne skupine I: kromosomi 1A, 1B i 1D. Premda pšenica na svakom od spomenutih kromosoma može imati 2 gena, dakle ukupno 6, većina sorata ima samo od 3 do 5 podjedinica HMW glutenina. Svaka podjedinica drukčije utječe na pekarsku kakvoću. Tako podjedinice **7 + 8**, **7 + 9** *i* **17 + 18** *kodirane alelima* **Glu–B1b**, **Glu–B1c** *i* **Glu–B1i** *kao i podjedinice* **5 + 10** *kodirane alelom* **Glu–D1d** *imaju signifikantan pozitivan utjecaj, dok multipli aleli istih lokusa* **Glu–B1d** *i* **Glu–D1a** *za podjedinice* **6 + 8** *i* **2 + 12** *imaju signifikantan negativan utjecaj.*

Hrvatski sortiment pšenice svrstan je prema vrijednosti HMW podjedinica glutenina i uspoređen s pekarskim pokazateljima. Izneseni su rezultati stvaranja novih sorata pšenice temeljeni na poznavanju genetičke kontrole osobina o kojima ovisi kakvoća kruha. Razmatrana je strategija i perspektiva oplemenjivanja pšenice prema podjedinicama HMW glutenina.

Introduction

Amount and quality of grain proteins are the main factors determining breadmaking suitability of wheat. A very strong environmental influence on the total protein content of wheat grain is well known. Also, generally, there is an inverse relationship between grain yield and grain protein content. The most difficult objective so far has been to breed economically successful wheat cultivars for breadmaking. Enhancing the breadmaking stability of wheat varieties is therefore a key objective of cereal breeders.

The unique viscoelastic and cohesive properties of wheat dough are mainly due to its storage proteins – gluten. Gluten proteins are the complex mixture of proteins that are subdivided into two groups: (i) gliadins,

Presented at the 2nd Croatian Congress of Food Technologists, Biotechnologists and Nutritionists, Zagreb, June 15–17, 1994

which allow viscous flow and therefore impart extensibility, and (ii) glutenins, which impart viscoelasticity to the dough. However, it is important to obtain a balance between elasticity end extensibility. The limiting factor in the quality of bread made in Croatia, as in most European countries, is dough elasticity which depends upon glutenins. It was proven by many investigators that the high-molecular-weight (HMW) glutenin subunits are the most important for breadmaking. HMW glutenin proteins are large, heterogeneous molecules, formed by up to 20 different subunits.

The subunit composition of glutenin is an inherited property, and it varies according to the wheat cultivar. Different combinations of subunits in different cultivars could account for the different properties of glutenin, and different breadmaking quality of flour *(1–4)*.

Table 1. Designation loci, alleles, subunits and quality scores of HMW glutenins *(5)*
Tablica 1. Obilježavanje lokusa, alela i podjedinica HMW glutenina te odgovarajuća ocjena kakvoće *(5)*

Locus	Allele	Subunits	Quality score
Glu–A1 (3 alleles)	a	**1**	3
	b	**2***	3
	c	*N*	1
Glu–B1 (11 alleles)	a	7	1
	b	**7 + 8**	3
	c	**7 + 9**	2
	d	*6 + 8*	1
	e	20	-
	f	13 + 16	-
	g	13 + 19	-
	h	14 + 15	-
	i	**17 + 18**	3
	j	21	-
	k	22	-
Glu–D1 (6 alleles)	a	*2 + 12*	2
	b	3 + 12	2
	c	4 + 12	1
	d	**5 + 10**	4
	e	2 + 10	-
	f	2.2 + 12	-

Remark: bold = »good-quality subunits«, italic = »poor-quality subunits«
Napomena: označene »dobre« (**poludebelo**) i »loše« (*italik*) podjedinice

Table 2. Frequency of »good-quality« (***7 + 9*** and ***5 + 10***) and »poor-quality« (***6 + 8*** and ***2 + 12***) HMW glutenin subunits among wheat cultivars of different countries *(20)*
Tablica 2. Učestalost »dobrih« (***7 + 9*** i ***5 + 10***) i »loših« (***6 + 8*** i ***2 + 12***) glutenina velike molekularne mase u sortama nekih zemalja *(20)*

Subunits	Frequency / %		
	Canada	France	Great Britain
7 + 9	65	18	8
5 + 10	83	44	19
6 + 8	8	14	45
2 + 12	19	49	65

In hexaploid wheat (*Triticum aestivum*) the genes or gene clusters responsible for control of HMW glutenin synthesis are located on the long arm of the homoeologous group 1 chromosomes (1A, 1B and 1D). In fact, the HMW glutenin synthesis is under control of multiple alleles. Each allele is manifested as one or two bands (subunits), numbered according to its mobility in the SDS-PAGE gel. All known wheat cultivars contain between three and five HMW glutenin subunits, although, hexaploid wheat could probably possess six *(4)*. Multiple alleles ***a*** to ***f*** from ***GLU–D1*** locus on ***1D*** chromosome control synthesis of HMW subunits ***2 + 12***, ***3 + 12***, ***4 + 12***, ***5 + 10***, ***2 + 10*** and ***2.2 + 12***. Multiple alleles ***a*** to ***k*** from ***GLU–B1*** locus on ***1B*** chromosome control synthesis of subunits ***7***, ***7 + 8***, ***7 + 9***, ***6 + 8***, ***20***, ***13 + 16***, ***13 + 19***, ***14 + 15***, ***17 + 18***, ***21*** and ***22***, and multiple alleles ***a*** to ***c*** from ***GLU–A1*** locus on ***1A*** chromosome control synthesis of subunits ***1***, ***2**** and ***N*** (null) *(3–5)*. A tight genetic linkage was observed between listed couples of subunits, so they always appear in the same combinations *(3)*. Alleles at these three loci have shown different effects on breadbaking quality *(6–9)*. Based on the relationship between certain HMW glutenin subunit and SDS sedimentation the quality score values from 3 to 10 were determined for complete HMW subunits composition *(11)* (Table 1).

It is important that only one allele from the multiple series can be present at the particular locus coding for one or two subunits. Only F_1 seeds and heterozygous segregants contain the HMW glutenin subunits characteristic for both parents *(10)*. Many authors *(7–17)* determined a large negative effect of the ***GLU–B1*** allele ***d*** (subunits ***6 + 8***), as well as the ***GLU–D1*** allele ***a*** (subunits ***2 + 12***) on breadmaking traits. However, an opposite strong positive effect of the ***GLU–B1*** alleles ***b***, ***c*** *and* ***I*** (subunits ***7 + 8***, ***7 + 9*** *and* ***17 + 18*** respectively), and the ***GLU–D1*** allele ***d*** (subunits ***5 + 10***) is observed. There was no significant difference in main effects of ***Glu–A1*** alleles alone, however ***Glu–A1*** *x* ***Glu–B1***, as well as ***Glu–A1*** *x* ***Glu–D1*** allele interactions are highly significant *(16)*.

Existence of »*good-*« and »*poor-quality*« subunits can be demonstrated by their frequencies in wheat cultivars of different countries known for good (Canada) or poor (Great Britain) quality wheat (Table 2). Some evidence indicates that in the US hard red spring wheat cultivars, the frequency of subunits ***5 + 10*** is even higher (98 %) than in Canadian wheats *(18)*.

Materials and Methods

Forty-five Croatian wheat cultivars released between 1967 and 1987 were studied.

HMW glutenin subunits determination – endosperm proteins for SDS-PAGE were isolated from a part of the wheat grain. The electrophoresis was carried out on 5 % and 10 % polyacrylamide gels. Standard cultivars were used for the determination of glutenin subunits *(20)*. The quality score (3 = bad, 10 = the best) based on the relationship between certain HMW glutenin subunits composition and SDS sedimentation value *(11)* was used for grouping wheat cultivars in clusters.

The 1B/1R chromosome translocation were determined by PAGE of wheat gliadins *(21)*.

Flour extraction of wheat was determined at laboratory (Bühler experimental mill) and industrial levels ("Mlinar", Križevci).

Rheological determinations of wheat dough quality were conducted by Brabender farinograph, extensograph and amylograph using standard methods.

Industrial experimental baking was performed by standard method for white bread.

Results

Based on the results of HMW glutenin subunits analysis performed on 45 Croatian wheat cultivars released between 1967 and 1986 *(20)*, a dendrogram was constructed (Fig. 1). Ten out of 45 cultivars showed heterogeneous HMW glutenin subunits picture, possessing 2 to 4 different genotypes. The number of heterogeneous genotypes is indicated in parenthesis after the name of cultivar. In that case, the specific cultivar is classified according to the most prevalent HMW glutenin subunits composition. Only 5 out of 45 cultivars (11 %) posses 1B/1R chromosome translocation. The quality score of listed Croatian cultivars varies from 4 (cvs. Nada and Moslavka) to 9 (cvs. Granka, Nova Marijana, Tena, Sivka, Pitoma and Poljarka). There are no cultivars with extremely high quality score 10, or extremely low quality score 3 values.

The most frequent subunit for ***Glu–A1*** is ***1***, for ***Glu–B1*** is ***7 + 9*** and for ***Glu–D1*** is ***2 + 12***. (Table 3). Therefore, cultivars released in that period possess very high proportion of low value subunits ***2 + 12***. To illustrate the oposite effect of subunits ***2 + 12*** versus ***5 + 10***, the best example could be the comparison of farinograms and extensograms of divergent cultivars Dukat and Cerera, having diverse breadmaking characteristics (Fig. 2). As both cultivars posses 1B/1R chromosome translocation, the differences are mainly due to the HMW glutenin subunits composition.

Table 3. Frequency of HMW glutenin subunits in Croatian wheat cultivars released between 1967 and 1986
Tablica 3. Učestalost podjedinica glutenina velike molekularne mase u hrvatskim sortama pšenice priznate od 1976. do 1986. godine

Glu–A1		*Glu–B1*		*Glu–D1*	
Subunit	Frequency/%	Subunit	Frequency/%	Subunit	Frequency/%
N	31	*6 + 8*	27	2 + 12	71
1	62	*7*	7	5 + 10	29
*2**	7	*7 + 8*	11		
		7 + 9	38		
		20	13		
		22	4		

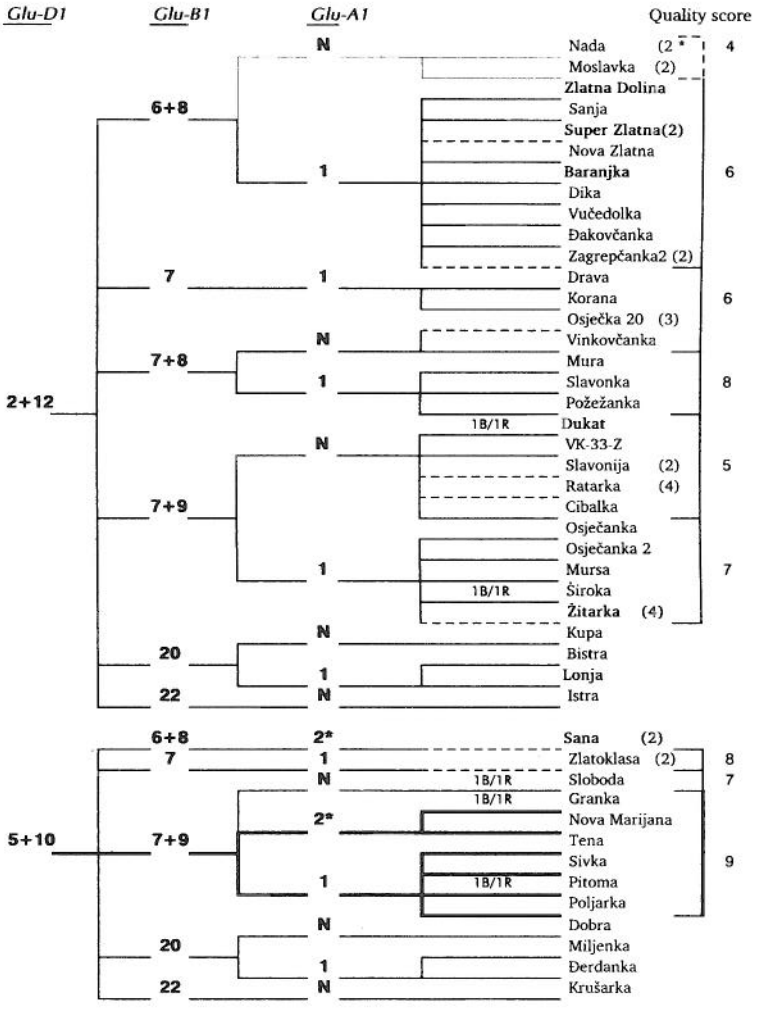

Fig. 1. Dendrogram based on HMW glutenin subunits composition of 45 wheat cultivars released in Croatia betwen 1967 and 1986
Slika 1. Dendrogram na temelju podjedinica glutenina HMW za 45 sorata ozime pšenice priznatih u Hrvatskoj od 1967. do 1986. godine

Discussion

As 1B/1R chromosome translocation is wide spread among modern wheat cultivars, its effect on bread-making quality is extremely important. The wheat lines possessing 1B/1R chromosome translocation were significantly superior in grain yield *(18)*, grain protein content and wet gluten, but not in sedimentation value and loaf volume *(8,17)*. It is observed that 1B/1R translocation in wheat, causes excessive dough stickiness when slightly overmixed *(19)*. Dough stickiness and a lack of mixing tolerance are the reasons why rye-derived wheat cultivars are not recommended for growing in some countries. Such cultivars have the secalins, which have replaced the gliadins and LMW glutenins normally encoded by the missing short arm of 1B chromosome. Should we restrict the presence of 1B/1R chromosome translocation in our wheat cultivars?

Now, the question is also: Could we based the selection on favorable HMW glutenin subunits and breed better quality wheat cultivars successfully? There is a general opinion that the potential value of an allele for improving a population depends partly on its present frequency which implies that the allele at ***Glu–D1d*** coding for ***5 + 10*** subunits has a positive effect on bread-making quality, and is at a relatively low frequency of 29 %. Therefore, the increase of its frequency could improve the population of Croatian wheats. Increasing the frequency of rare allele ***Glu–B1b*** (subunits ***7 + 8***), or introduction of a new allele ***Glu–B1i*** (subunits ***17 + 18***), which are not present in Croatian wheat gene–pool, could greatly increase loaf volume. Gluteninbased selec-

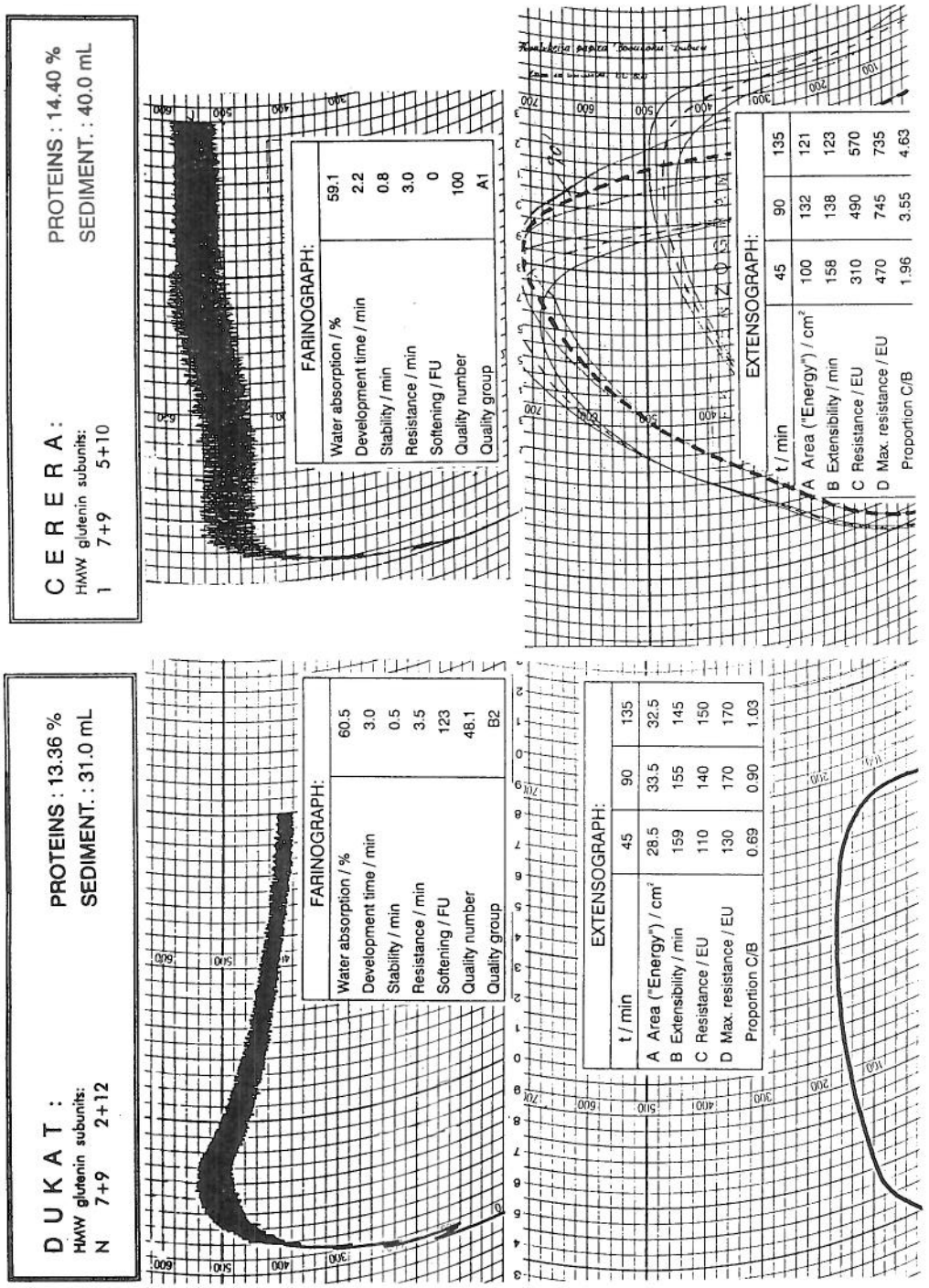

Fig. 2. Effect of different HMW glutenin subunits composition of cv. Dukat (N, 7 + 9, 2 + 12) and cv. Cerera (1, 7 + 9, 5 + 10) on rheological properties of wheat dough

Slika 2. Utjecaj različitih podjedinica glutenina velike molekularne mase sorata Dukat (N, 7 + 9, 2 + 12) i Cerera (1, 7 + 9, 5 + 10) na reološke značajke tijesta

tion could be very useful, and improvement could be rapid in early stages of wheat breeding. The best examples are the two extremes: (i) the well known poor quality wheat of Great Britain, has been improved recently due to favorable HMW glutenin subunit introduction, and (ii) good quality American hard red winter wheat having already a high frequency of favorable alleles for HMW glutenins but slow improvement rate. The quality of the US wheat cultivars depends more on the genetic variation at other loci coding for quality, as LMW glutenins, gliadins, lipids, starch or sugars. As Croatian wheat breadmaking quality is somewhere between the Great Britain and US wheats, the expected improvement could be promising, but not as fast as in Great Britain. The best

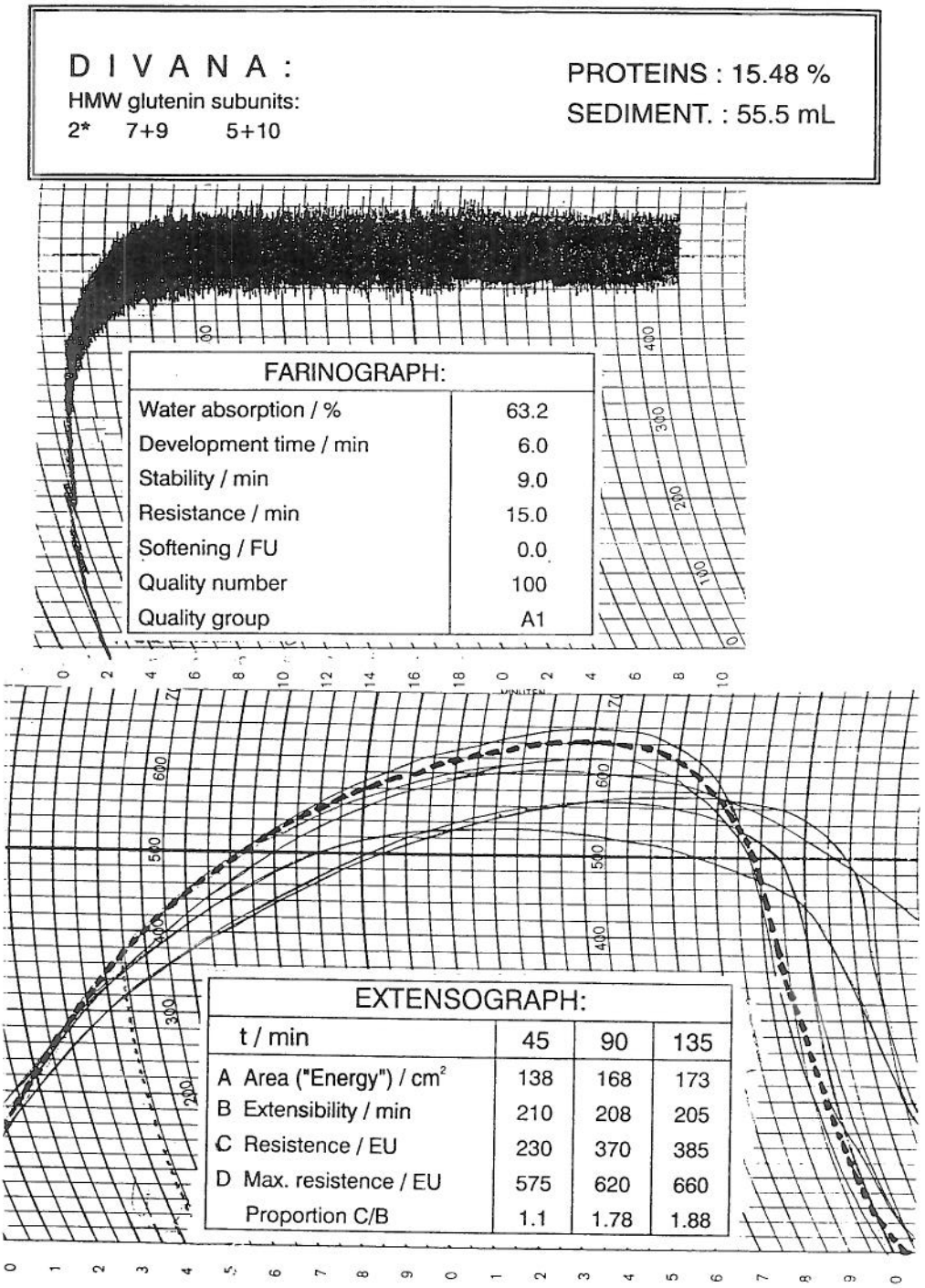

Fig. 3. Quality data of the new Croatian high quality wheat cultivar Divana (VG-90-HP)
Slika 3. Značajke kakvoće nove hrvatske visokokvalitetne sorte pšenice Divana (VG-90-HP)

Table 4. Average quality characteristics of cv. Divana (VG-90-HP) in comparison with the standard for quality – cv. Žitarka (Podravka, Koprivnica 1991–1994)
Tablica 4. Prosječni pokazatelji kakvoće sorte Divana (VG-90-HP) u usporedbi sa standardnom sortom Žitarka (Podravka, Koprivnica, 1991–1994.)

Character	Divana (VG-90-HP)	Žitarka (standard)
Hectoliter wt. /(kg/hL)	79.9	80.1
TKW /g	40.6	37.8
Grain protein/%	16.3	13.9
Sedimentation/mL	62.4	36.5
Wet gluten/%	39.2	30.3
Flour extraction/%	72.7	67.2
Water absorption /%	64.2	61.5
Quality class	I	II
Quality number/group	100/A1	64.8/B1

Table 5. Results of industrial milling of high quality wheat cultivar Divana (VG-90-HP) at »Mlinar«, Križevci in 1994
Tablica 5. Rezultati industrijske meljave visokokvalitetne pšenice Divana (VG-90-HP), »Mlinar«, Križevci, 1994.

Flour	Extraction	Ash	Water
		w/%	
Farina T400	0.41	-	-
Semolina T400 for paste	11.30	-	-
Flour T400	9.37	0.417	14.63
Flour T500	32.69	0.582	14.70
Flour T850	25.96	0.962	15.16
Total extraction	79.73		

Rheological characteristics of different flour types
Reološke značajke pojedinih tipova brašna

Character	T400	T500	T850
FARINOGRAPH:			
Water absorption/%	56.4	60.8	62.8
Development time/min	8.0	8.0	5.5
Stability/min	3.0	3.0	3.0
Resistance/min	11.0	11.0	8.5
Softening/FU	20.0	38.0	40.0
Quality number	85.3	84.2	78.0
Quality group	A–1	A–2	A–2
EXTENSOGRAPH:			
A Area (Energy) /cm^2	127	120	114
B Extensibility /mm	163	184	230
C Resistance /EU	360	270	210
D Max. resistance /EU	590	500	360
Proportion C/B	2.21	1.47	0.91
AMYLOGRAPH:			
Max. viscosity /AU	1005	560	370

proof is the new cultivar Divana (VG-90-HP), released recently (Fig. 3). At the moment, Divana is the best quality wheat ever grown in our wheat growing regions. The conclusion is based on several years of examinations: laboratory and industrial milling, as well as industrial bread baking (Tables 4, 5 and 6). It is certain that cv. Divana, besides favorable subunits of HMW glutenin, also incorporates a part of the remaining, earlier mentioned, genetic variation for quality.

Table 6. Results of experimental industrial bread baking (Podravka, Koprivnica, 1993)
Tablica 6. Rezultati pokusnog industrijskog pečenja kruha (Podravka, Koprivnica, 1993.)

Wheat flour	Bread yield	Specific volume mL/g	Loaf height cm	Gain score
Žitarka (standard for quality)	145.2	4.79	8	87
Divana (VG-90-HP)	150.1	5.24	10	100

Genes at ***Glu–A1*** locus of commercial wheat cultivars control synthesis of only one HMW glutenin subunit (many have a 'null'-subunit). Scientists are concentrated on the transfer of chromosomal segments carrying novel alleles in order to increase the total possible number of HMW glutenin coding alleles to six. A possible source could be wheat relatives listed as confident information in *Cereal genetransfer programme – Proposal for licences* of Agricultural Genetics Company Limited, Cambridge. So far, breadmaking tests of British scientists on the dough showed favorable increase in stability and decrease in stickiness associated with the alien 1R chromosome segment.

Besid known HMW glutenin coding genes, several glutenin genes have been purified by molecular cloning. To complete genetic engineering of wheat it will be necessary to learn how to insert genes into cells of the plant. The incorporation of new genes into tobacco and some other species has been reported recently, so perhaps it will not be too long before wheat can be similarly modified genetically *(4)*.

Conclusion

The potential value of an allele for improving population mean depends partly on its present frequency. For example, the allele at ***Glu–B1*** coding for HMW glutenin subunits ***7 + 8*** has a positive effect on mixing traits and is at relatively low frequency in Croatian wheat gene pool. On the other hand, ***6 + 8*** subunits coded by other allele from multiple series of the same gene locus has a very large negative effect on the same traits. Increasing frequency of the favorable allele will greatly improve mixing traits of Croatian wheats. The HMW glutenin subunits determination procedure is relatively simply, and does not require a big amount of seed (large limiting factor in early generation of selection) as for other phenotypic (rheological) evaluations. However, selection based solely on the average effects of glutenin alleles would exploit only a portion of the available genetic variance. Superior glutenin alleles could be fixed quickly in particular genotype, and the next step should be incorporating remaining genetic variation for quality traits, located at loci other than those coding glutenin, as it was achieved by breeding cv. Divana (VG-90-HP).

Acknowledgment

We are very grateful to dr. Ljiljana Vapa, Institute of Biology, for generously supplying us with analytical data of HMW glutenins in Croatian wheat cultivars.

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