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A. Juhász · O. R. Larroque · L. Tamás · S. L. K. Hsam · F. J. Zeller · F. Békés · Z. Bedő

Bánkúti 1201—an old Hungarian wheat variety with special storage protein composition

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Abstract Bánkúti 1201, an old Hungarian wheat variety with special quality traits, was analysed to determine the relationships between its storage protein composition and superior quality-attributes for breadmaking. Based on the storage protein composition, the variety appears to have the nature of a population, containing several genotypes with different gluten protein alleles. Using molecular markers, a new mutant x-type HMW glutenin allele was identified, containing an extra cysteine residue and showing a moderate, positive-effect on gluten properties. In lines possessing subunits Bx7+By8 the overexpression of the Bx-type subunit could be detected, resulting in a higher unextractable polymeric protein (UPP) content and increased dough strength. It was found that the presence or absence of subunit Bx7 has an equilibrating effect on the dough extensibility, which is generally characteristic of the Bánkúti 1201 population. The complex good breadmaking quality of the variety, which has strong but highly extensible dough, is probably due to the balance between lines which express subunit Bx7 and those which do not.

Keywords Wheat · Storage protein alleles · Cysteine residue · Subunit overexpression · Quality

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A. Juhász (✉)
Agricultural Research Institute of the Hungarian Academy of Sciences, Brunszvik u 2, Martonvásár, H-2462, Hungary
e-mail: Angela.Juhasz@csiro.au
Tel.: 61-2-6246-4967, Fax: 61-2-6246-5000

Z. Bedő
Agricultural Research Institute of the Hungarian Academy of Sciences, Brunszvik u 2, Martonvásár, H-2462, Hungary

O. R. Larroque · F. Békés
CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

L. Tamás
Eötvös Loránd University, Department of Plant Physiology, Pázmány P. stny 1/c, Budapest H-1117, Hungary

S. L. K. Hsam · F. J. Zeller
Technical University Munich, Division of Plant Breeding and Applied Genetics, 51 Lange Point, Freising-Weihenstephan, 85350, Germany

Present address:
A. Juhász, CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

Introduction

The use of populations of old landraces and varieties in plant breeding has numerous advantages when the aim is to increase genetic variability. They may possess among other characteristics, special storage protein compositions with alleles or allele combinations that rarely occur in modern wheat cultivars. Selection from varieties with traditionally superior quality and the use of genetically heterogeneous populations as gene sources play an important role in developing and improving new genotypes.

The Eastern European traditional wheat germplasm has a unique breadmaking quality type, which is characterised by high protein and gluten contents, and excellent rheological properties. Red Fife, which was one of the parents of the Canadian spring wheat variety Marquis, and the Bánát landraces, which are found, for example, in the pedigree of Bezostaya 1, all originate from the eastern edge of the Carpathians. Bánkúti 1201, developed in the first half of the 20th century from a cross between Marquis and Bánkúti 5, enjoyed great popularity in Hungary, and variants of this variety were also used as a basic breeding stock in the neighbouring countries (Bedő et al. 1995; Vida et al. 1998).

In the early seventies, the old Hungarian varieties were gradually removed from cultivation and their populations are only preserved in gene banks. In 1994 experiments were started in the Agricultural Research Institute of the Hungarian Academy of Sciences to test the old Hungarian wheat varieties, especially the Bánkúti 1201 population. The experiments were primarily aimed at examining the HMW-glutenin subunit composition of the variety and at

revealing its relationship with technological properties. The superior technological parameters detected in the different Bánkúti 1201 lines could partly be explained by the heterogeneous high-molecular-weight glutenin subunit (HMW-GS) composition (Bedő et al. 1995).When the population of Bánkúti 1201 was divided into lines on the basis of HMW glutenin subunit composition (Bedő 1994) several allelic variants were discovered associated to the three different *Glu-1* loci present on the homeologous group-1 chromosomes. One of the most remarkable things about this variety is that despite having mainly subunits 2+12 (Bedő et al. 1995; Vida et al. 1998) or 3+12 (Kárpáti et al. 1995) on chromosome 1D, it has excellent technological properties. The old Hungarian wheat varieties generally have high protein contents (above 15%) with gluten contents of over 40%. Their rheological properties are also good and they can be classed in the A_1–A_2 Farinograph category based on the Hungarian quality standard (Bedő et al. 1995; Vida et al. 1998).

Besides the effects of the different storage protein alleles and allele combinations manifested in the genotypes, the relative amounts and ratios of the individual protein fractions affect the development of the gluten network and the dough properties. Based on preliminary results (Vida et al. 1998), the aim of this study was the qualitative and quantitative analysis of storage protein components, which could be related to the excellent technological properties of the Bánkúti 1201 variety.

Materials and methods

Plant material

In order to test the Bánkúti 1201 population maintained by the Agricultural Research Institute of the Hungarian Academy of Sciences, a field experiment was set up in the wheat-breeding nursery in Martonvásár. The population was divided into lines by means of spike selection based on the high-molecular-weight glutenin subunit composition (Vida et al. 1998). Fifty two spike progeny lines of the Bánkúti 1201 population were involved in the experiments. Grain material harvested in 1996–97 was used for the detailed analysis of storage-protein composition, for the molecular genetic studies and for quality testing. The quantitative composition and rheological parameters were measured on 18 lines representing the population harvested in 1999.

Storage protein composition

The HMW, LMW glutenin and gliadin allele compositions were determined and identified using the method of Jackson et al. (1996) for protein extraction, SDS and acid polyacrylamide gel-electrophoresis. The HMW and LMW glutenin subunits were separated on SDS-PAGE gels (T = 12.3%, C = 2.6%) under constant current conditions (8 mA/mm gel) for about 20 h. The gliadins coded by the *Gli-1* loci were separated using A-PAGE (pH 3.1) and classified according to the system of Metakovsky (1991).

Analysis of HMW glutenin genes using molecular markers

A primer pair specific to the N-terminal region of the 1Dx5 HMW glutenin gene published by D'Ovidio et al. (1994) was used for PCR testing. The amplified 1,289-bp fragment was cloned and sequenced. Since the sequence was 100% similar to that of the known 1Ax2* gene, another primer pair was designed to determine the sequence overlapping the 3′ end of the 1,289-bp fragment. The new PCR product – 300 bp in size – was cloned and the nucleotide sequence was determined (Juhász et al. 2001). The entire identified mutant gene, $1Ax2^{*B}$ was amplified using a primer pair specific to 1Ax-type genes, published by D'Ovidio et al. (1995).

Quantitative composition

A set of 18 lines, representing the Bánkúti 1201 population for storage protein composition, was analysed by size exclusion (SE) and reverse phase (RP)-HPLC. Total protein extracts were prepared (Batey et al. 1991) to determine the glutenin and gliadin contents and the amount of unextractable polymeric protein (UPP) (Gupta and MacRitchie 1994). The normalised gliadin contents in percentages were calculated as a product of protein contents and gliadin contents. The HMW and LMW glutenin subunit contents and the relative amounts of individual HMW GSs were determined with RP-HPLC (Marchylo et al. 1989). The molar percentages of the different HMW-GSs were calculated using Mr values estimated with the PEPSTATS software (Institute Pasteur, France) and using the results published by Cloutier and Lukow (2001).

Technological parameters

Gluten quality parameters were characterised by measuring protein (Tecator Kjeltec 1035 Autoanalyzer) and gluten contents, by calculating gluten indexes (ICC 137/1 and ICC 155) and by determining SDS sedimentation values (Soltek SDS system). Dough properties were characterised by using small-scale methods, involving a 2 g Mixograph (Rath et al. 1990), a Micro Extension Tester (Rath et al. 1994) and the Micro-Baking Technique (Gras and Békés 1996).

The statistical relationships between storage protein composition and functional properties were analysed using various correlation methods and analysis of variance (STATISTICA 6.0 program package, StatSoft Incorporated, USA).

Results and discussion

Genetic variability in the storage protein composition of the wheat variety Bánkúti 1201

All the HMW, LMW glutenin and gliadin loci showed high variability for allelic composition in the Bánkúti 1201 population (Table 1). More than 95% of the population contained the 2+12 allele at the *Glu-D1* locus; the presence of this allele is highly characteristic of landraces and old wheat varieties (Lagudah et al. 1987; Tahir et al. 1996; Igrejas et al. 1997). Subunits 7+8 (38.5%), 7*+9 (59.6%) and 6+8 (1.9%) were detected at the *Glu-B1* locus. This high level of heterogeneity in Bankúti 1201, is characteristic of a population. This can be explained by the pedigree of the variety: one of the parents of Bánkúti 1201, Bánkúti 5, was selected from the landrace Tiszavidéki. The other parent, Marquis, also showed heterogeneous properties; the breeding material classed in quality group *Number 1 Manitoba hard* was used in Hungary for autumnization, followed by its use as breeding material (Rajháthy 1961; Symko 1999).

Based on their relative mobility on SDS-PAGE gels, the subunits generally assigned as Bx7 could be sub-

Table 1 Alleles and the frequency with which they occur in the Bánkúti 1201 population

Locus	Allele	HMW GSs	Frequency	
			No.	%
Glu-A1	a	1	8	15.4%
	b	2*	5	9.6%
	new (#)	**2*^{B}** (#)	39	**75.0%**
Glu-B1	d	6+8	1	1.9%
	b	7+8	20	38.5%
	c	**7*+9**	31	**59.6%**
Glu-D1	a	**2+12**	50	**96.2%**
	d	5+10	2	3.8%
Glu-A3	a		17	32.7%
	d		1	1.9%
	f		34	**65.4**
Glu-B3	b		1	1.9%
	g		1	1.9%
	i		50	**96.2%**
Glu-D3	a		4	7.7%
	c		48	**92.3%**
Gli-A1	a		16	30.8%
	e		1	1.9%
	f		2	3.9%
	m		33	63.4%
Gli-B1	b		1	1.9%
	m		51	**98.1%**
Gli-D1	**a**		41	**78.9%**
	b		2	3.9%
	g		9	17.2%

The most characteristic alleles are labelled in bold

grouped further. Marchylo et al. (1992) found that different genotypes may possess Bx7 subunits which differ from one another in SDS-PAGE analysis, indicating the existence of at least two different Bx7 protein subunits in the different cultivars. Subunit 7, identified for example in cv Chinese Spring (CS), also differed in its nucleotide sequence from subunit 7*, found in the bread wheat cultivar Cheyenne (CNN) (Anderson and Greene 1989). The coding sequence of Bx7 (CS) is *at least 18-bp* longer than that of Bx7* (CNN), resulting in slightly lower electrophoretic mobility on SDS-PAGE gels. The Bánkúti 1201 lines possessing allele 7+8 all contained the

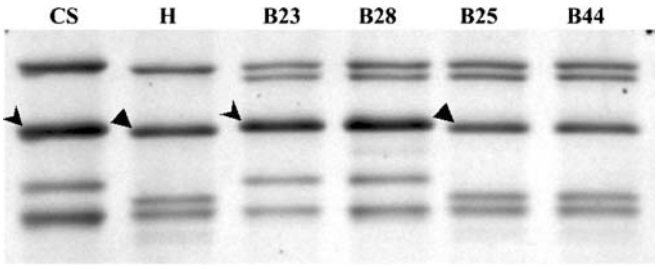

Fig. 1 Differences between subunits Bx7 and Bx7*, both occurring in the Bánkúti 1201 population. *CS* – Chinese Spring, *H* – Hereward, Bánkúti 1201 lines: *B23, B28, B25* and *B44*. *Arrowheads* indicate the subunit Bx7 identified in CS and found e.g. in lines B23 and B28. *Triangles* indicate subunit Bx7* identified in CNN, and found in cv Hereward and e.g. in Bánkúti 1201 lines B25 and B44

CS type of Bx7 subunit, while in lines containing allele 7*+9 the subunit Bx7* was detected (Fig. 1).

About 70% of the Bánkúti 1201 lines analysed possessed a specific allele at the *Glu-A1* locus. The allele, designated as *Glu-A1*x2*^{B}, was identified by molecular markers and is a mutant variant of the known 1Ax2* gene (Juhász et al. 2001) (Fig. 2). There is a C-G point mutation in the middle of the repetitive region of the gene (at 1,181 bp), resulting in a serine to cysteine amino-acid change. Due to this point mutation, the subunit 2*^{B} has an extra cysteine in its amino-acid sequence, similar to subunit 5.

High heterogeneity in the allelic composition of the LMW glutenin subunits and gliadin alleles was also observed. Certain alleles, such as *Glu-B3*i and *Gli-B1*m, not characteristic of modern European wheat varieties, were identified. However, these alleles were detected in higher frequency in Canadian cultivars (Wheat quality electrophoresis 1998; Cornish 2001). This similarity can be explained by pedigree analysis, which demonstrated relationships between some old Hungarian cultivars and Canadian wheat varieties, due to common ancestors such as Red Fife or Hard Red Calcutta. The allelic composition at the *Gli-1* loci confirmed the relationship between the *Glu-3*/*Gli-1* loci, reported by Jackson et al. (1996).

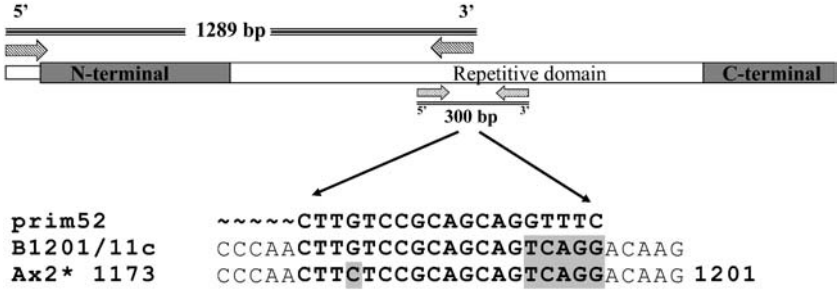

Fig. 2 Identification of a cysteine point mutation in wheat variety Bánkúti 1201. *Striped arrow* represents the prim51 – prim52 primer pair, published by D'Ovidio (1994), used to test the Bánkúti 1201 population. The approximate 1,300-bp fragment was sequenced and gave 100% homology to the known 1Ax2* gene sequence, except at the binding site of the two primers. Since the correct hybridization of the 3′-end primer (prim52) was not possible based on the sequence comparisons, another primer pair was designated to check this region. The primers designated by a *hatched arrow* amplified a fragment 300-bp in size

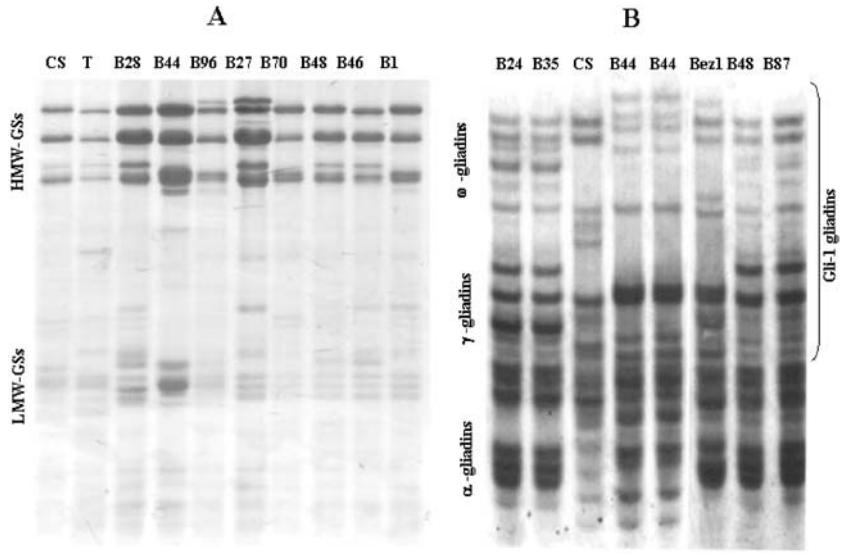

Fig. 3A, B The-most frequent HMW, LMW-GS and gliadin compositions in the Bánkúti 1201 population. **A** The most frequent HMW and LMW-GS compositions detected in the Bánkúti 1201 population: *CS* – Chinese Spring, *T* – Top, *B28* – 2*^{B} 7+8 2+12, f-i-c; *B44* – 2*^{B} 7*+9 2+12, a-b-c; *B96* – 1 7*+9 2+12, a-i-c; *B27* – 1 7+8 2+12, f-i-c; *B70* – 2*^{B} 7*+9 2+12, f-i-c, *B48* – 2* 7+8 2+12, a-i-c; *B46* – 2* 7+8 5+10, a-i-c; *B1* – 2*^{B} 7*+9 2+12, a-i-c. **B** Gli-1 gliadin alleles characteristic of the Bánkúti 1201 population: *CS* – Chinese Spring, *Bez1* – Bezostaya 1, Bánkúti 1201 lines: *B24* – m-m-a; *B35* – m-m-a; *B44* – a-b-g; *B48* – a-m-a, *B87* – a-m-a

Several alleles, like *Gli-A1*a/*Glu-A3*a, *Gli-B1*b/*Glu-B3*b, *Gli-B1*m/*Glu-B3*i and *Gli-D1*a/*GluD3*a, were expressed together in the different Bánkúti 1201 lines as result of the close linkage between *Gli-1* and *Glu-3* complex loci.

Altogether the presence of eight HMW-GS, five LMW-GS and eight *Gli-1* gliadin allele combinations were identified in the 52 Bánkúti 1201 lines analysed. The most frequent HMW-GS, LMW-GS and gliadin allelic compositions are shown in Fig. 3A and B. Twenty different allelic combinations were found, three of which covered 61.5% of the lines. The three most-frequent combinations were: 2*^{B} 7*+9 2+12, f-i-c, m-m-a (40.4%), 2*^{B} 7+8 2+12, a-i-c, a-m-a (11.5%) and 2*^{B} 7+8 2+12, f-i-c, m-m-a (9.6%).

The quantitative aspects of storage protein composition as determined by HPLC analysis also exhibited high variability in Bánkúti 1201. This variety can be characterised by moderate glutenin content (36.76%) and a high quantity of gliadin (52.74%), resulting in a relatively low glutenin to gliadin ratio (0.70 on average). This observation is confirmed by the results of Fullington et al. (1980) determining the relative amounts of the different storage protein fractions using quantitative SDS-PAGE. The Bánkúti 1201 lines contained large amounts of UPP (48.40% on average) in contrast to other varieties possessing allele 2+12 at the *Glu-D1* locus (Gupta and MacRitchie 1994; Ciaffi et al. 1996; Larroque et al. 1999). More than 1/3 of the studied lines can be characterised by relatively high Glu/Gli values and UPP content. In three cases, the UPP values detected (58.18, 58.89 and 60.07%) were even higher than that of the extra strong Canadian wheat variety Glenlea (57.8%). Generally, the higher UPP content was combined in these lines with the overexpression of the Bx-type subunit measured by RP-HPLC. As far as allelic and quantitative storage protein composition, lines possessing subunits 7+8 resulted in higher UPP% and Bx% values and calculated Bx/By values (Table 2) similar to the results of Butow and co-workers (personal communications). Due to the homogeneity found at the *Glu-D1* locus the effect of the 2+12 allele on qualitative and quantitative parameters could not be evaluated.

The high gliadin content characteristic of the Bánkúti 1201 population shows a strong, significant relationship with the alleles at the *Glu-A3* (r = 0.64, p = 0.1%) and *Gli-A1* (r = 0.64, p = 0.1%) loci. The most characteristic LMW glutenin (f-i-c) and *Gli-1* (m-m-a) allelic compositions showed a positive relationship with a higher monomer and a lower polymeric protein content, while alleles less common in Bánkúti 1201, such as a-b-c or d-g-a (*Glu-3*) and a-b-g or e-m-g (*Gli-1*), showed a positive relationship with gluten and UPP contents. The latter alleles were identified more frequently in modern, cultivated genotypes. A less-strong relationship could be established between the *Glu-3* (r = 0.55, p = 1%) and *Gli-1* (r = 0.55, p = 1%) allelic compositions and the LMW glutenin content measured by RP-HPLC.

The high heterogeneity in the allelic composition observed on each storage protein locus and the relatively small population size makes the proper statistical evaluation – such as a multiple analysis of variance considering all the nine related loci – impossible. Therefore the effects of only two HMW-GS loci (*Glu-A1* and *Glu-B1*) have been characterised quantitatively while only trends were identified in case of relating the LMW-GS and gliadin allelic composition to dough properties.

Table 2 Quantitative parameters measured by SE- and RP-HPLC

	Protein %		Gliadin %		Glu/ Gli		UPP[a] %		HMW/ LMW		Ax mol%		Bx mol%		Dx mol%		By mol%		Dy mol%		x/y		Bx/ By
Min	11.70		46.41		0.62		35.89		0.46		9.76		30.17		12.51		6.97		10.56		2.10		2.05
Max	15.20		56.65		0.88		60.07		0.87		17.89		52.04		23.40		14.77		18.10		4.70		7.47
Mean	14.17		52.51		0.71		48.40		0.61		14.48		38.63		19.30		12.14		15.44		2.71		3.40
Std Dev	0.80		2.76		0.08		5.36		0.09		2.42		7.32		2.268		2.07		2.02		0.63		1.37
2* 7+8 2+12	11.70	**A**	52.15	**B**	0.67	**A**	52.47	**C**	0.62	**AB**	11.54	**A**	46.48	**B**	18.76	**B**	9.33	**A**	13.89	**B**	3.31	**B**	4.98	**B**
2*[B] 7+8 2+12	14.16	**B**	50.00	**A**	0.79	**B**	52.45	**C**	0.64	**B**	12.43	**A**	48.71	**C**	15.74	**A**	10.35	**AB**	12.78	**A**	3.41	**B**	4.95	**B**
2* 7*+9 2+12	15.00	**C**	53.97	**C**	0.66	**A**	36.62	**A**	0.59	**A**	13.99	**B**	33.98	**A**	20.08	**C**	14.38	**C**	17.58	**D**	2.13	**A**	2.36	**A**
2*[B] 7*+9 2+12	14.32	**D**	53.55	**BC**	0.68	**A**	47.26	**B**	0.60	**A**	15.73	**C**	33.76	**A**	20.90	**C**	13.01	**B**	16.60	**C**	2.39	**A**	2.64	**A**
$LSD_{5\%}$[b]	*0.37*		*1.72*		*0.05*		*2.91*		*0.07*		*1.37*		*1.70*		*1.06*		*1.13*		*0.70*		*0.30*		*0.61*

[a] UPP – unextractable polymeric protein [b] $LSD_{5\%}$ – least significant difference at $p = 5\%$

Effect of the qualitative and quantitative composition of HMW glutenins on functional properties

Based on the Hungarian wheat standard, Bánkúti 1201 has very high quality and the rheological parameters indicate medium-strong or strong and highly extensible dough. Both gluten testing and small-scale testing confirmed the good bread-making quality of Bánkúti 1201 (Table 3). The results also indicated great heterogeneity of the population for the measured qualitative and rheological parameters.

To relate these parameters to protein composition, 18 lines containing the most-frequently appearing glutenin and gliadin alleles have been selected and further investigated. The statistical analyses highlight some interesting effects on technological properties. In a pair-wise comparison for the effects of alleles either of *Glu-A1* or *Glu-B1* loci (Table 4a), the presence of 2^{*B} and the 7+8 alleles resulted in larger average polymeric size (increased UPP%), increased mixing requirement (larger MT), stronger (larger Rmax), more stable (smaller RBD) and less extensible (smaller Ext) dough compared to those containing the corresponding 2* or 7*+9 alleles. When the combinative effects of the alleles on the two loci were compared it was found that the dough-strengthening effects of $Ax2^{*B}$ appears mostly in the weaker background, containing the 7*+9 allele (Table 4b). Multiple analysis of variance indicated a significant effect of *Glu-B1* for all parameters except PR, while the effect of *Glu-A1* was significant only for UPP%. Significant allelic interactions were also detected for UPP%, MT, PR and RBD.

The weaker than expected effects of the 2^{*B} allele on polymer size and dough properties may be explained by the primary and secondary structure of this mutant glutenin subunit. Similar to subunit 5 encoded on chromosome 1D, 2^{*B} contains five cysteine residues (Juhász et al. 2001), in contrast with the x-type HMW-GSs which generally contain four cysteine residues. However, there is an important difference between the two subunits in the localization of the extra cysteine. The extra cysteine in 2^{*B} was found in the middle of the repetitive region, whilst that in 1Dx5 is located at the beginning of this region. The different locations of the extra cysteine in 1Dx5 and $1Ax2^{*B}$ might result in differences in the role of the rest of the cysteine residues in the polypeptides forming inter- and intra-polypeptide disulphide bonds. In vitro experiments using synthetic peptides and glutenin analogue molecules with systematically altered numbers and positions of cysteine residues in dough systems, clearly indicated that both the number and position of cysteines are important factors modifying the size-distribution of the glutenin polymer and the rheological properties of the dough (Tamás et al. 1998, 2002). Although there are several softwares predicting the availability and reactivity of cysteine residues in a polypeptide, all of them are based on molecular data from investigation of globular proteins. Therefore the estimated results for prolamin proteins are less reliable (Solomon, personal communication).

Table 3 Characteristic values of functional properties measured in the Bánkúti 1201 population

Parameter	N	Minimum	Maximum	Mean	Std. dev.[a]
SDS sedimentation value	52	66.50	101.00	78.77	7.10
Protein (%)	52	15.10	18.55	17.24	0.63
Wet gluten (%)	52	36.80	51.65	46.27	3.17
MT (s)[b]	18	144.00	422.00	224.14	68.14
PR (AU)[b]	18	345.00	475.00	410.31	32.98
RBD (%)[b]	18	9.00	25.00	18.25	4.53
Extension (AU)	18	588.72	1,409.20	981.56	225.96
Rmax (AU)[b]	18	249.66	998.58	497.78	213.62

[a] Std. dev. – standard deviation
[b] MT – mixing time, PR – peak resistance, RBD – resistance breakdown, Rmax – maximal resistance to extension

Table 4a Comparative effects of Glu-A1 and Glu-1B alleles on different functional parameters using a *t*-test of the means

Locus	Allele	N	UPP[a]		MT[a]		PR[a]		RBD[a]		Ext[a]		Rmax[a]	
Glu-1A														
	2*	4	44.01	**A**	185.75	**A**	432.25	**A**	22.50	**B**	1,262.75	**B**	336.88	**A**
	2*^{B}	32	48.95	**B**	228.94	**B**	407.56	**A**	17.72	**A**	946.42	**A**	517.89	**B**
Glu-1B														
	7+8	12	52.45	**B**	263.92	**B**	434.42	**B**	16.67	**A**	833.67	**A**	653.92	**B**
	7*+9	24	46.37	**A**	204.25	**A**	398.25	**A**	19.04	**B**	1,055.51	**B**	419.71	**A**

[a] UPP – unextractable polymeric protein, MT – mixing time, PR – peak resistance, RBD resistance breakdown, Ext – extensibility, Rmax – maximal resistance to extension

Table 4b Combinative effects of Glu-A1 and Glu-1B loci on different functional parameters using the analysis of variance and a *t*-test of the means

ANOVA	UPP[a] F	MT[a] F	PR[a] F	RBD[a] F	Ext[a] F	Rmax[a] F
Glu-A1	11.53**	0.75	0.02	2.83	2.21	2.97
Glu-B1	63.56***	49.60***	0.05	14.31***	17.18***	17.17***
Glu-A1 × *Glu-B1*	11.63**	8.70**	11.60**	8.69**	2.73	1.29

t-test of the means

Glu-B1	*Glu-A1*	N	MT		PR		RBD		Ext		Rmax	
7+8	**2***	**2**	217.00	**B**	427.00	**AB**	23.00	**C**	1,170.50	**B**	357.00	**A**
7+8	**2*^{B}**	**10**	273.30	**C**	435.90	**B**	15.40	**A**	766.30	**A**	713.30	**B**
7*+9	**2***	**2**	154.50	**A**	437.50	**B**	22.00	**C**	1,355.00	**C**	316.75	**A**
7*+9	**2*^{B}**	**22**	208.77	**B**	394.68	**A**	18.77	**B**	1,028.29	**B**	429.07	**AB**
$LSD_{5\%}$[b]			*49.40*		*32.50*		*2.80*				*206.80*	

[a] UPP – unextractable polymeric protein, MT – mixing time, PR – peak resistance, RBD – resistance breakdown, Ext – extensibility, Rmax – maximal resistance to extension
[b] $LSD_{5\%}$ – least significant difference at $p = 5\%$, ** – significant at $p = 1\%$, *** – significant at $p = 0.1\%$

The significant strengthening effect of the *Glu-B1* locus both on gluten quality and rheological properties in lines expressing the 7+8 allele seems to be related to the mechanism and level of expression of subunit Bx7 rather than to the structure of the encoding genes. In these samples the presence of the subunits 7+8 was associated with the over-expression of subunit Bx7. Figure 4 illustrates the MT and Rmax values measured with a 2-g Mixograph and Micro Extension Tester respectively as a function of the relative amounts of the Bx-type subunit measured by RP-HPLC. Based on these results, the lines could be divided into two distinct groups: lines overexpressing the Bx-type subunit and resulting in significantly higher dough strength, each of them containing the allele 7+8 (grey symbols on Fig. 4), and lines expressing the subunit Bx7* to a lower extent (average value: 30–35%), associated with By9 (blocked-in on Fig. 4) and producing significantly weaker dough. Among other allelic forms of *Glu-B1*, such as subunits 17+18, the positive effect of subunits 7+8 on gluten quality is well known (Payne 1987). However, in most cases, as in case of cv Chinese Spring, the presence of the genes *Glu-B1*x7 and *Glu-B1*y8 does not result in the overexpression of subunit Bx7. There are several genotypes (Red River 68, TAA 36) which contain two copies of the gene *Glu-B1*x7 (Lukow et al. 1992; D'Ovidio et al. 1997). Conflicting results have been published about the possible duplication of the *Glu-1B*x7 gene in the overstrong Canadian variety, Glenlea. The high level of expression may result either from more effective transcription/more efficient translation (Lukow et al. 1992; Rampitsch et al. 2000), or from gene duplication (Cloutier and Lukow 2001) or both. In the

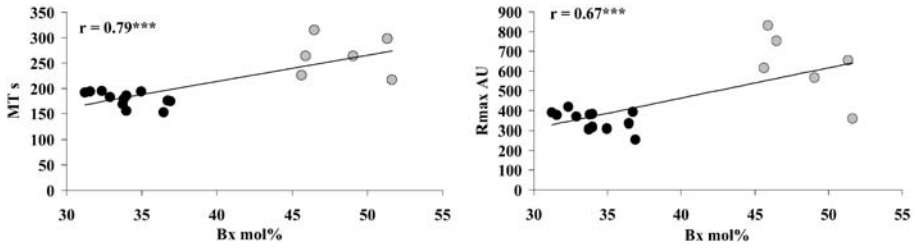

Fig. 4 Differences between MT and maximal resistance to extension (Rmax) values as a function of Bx% content in Bánkúti 1201 lines. *Blocked-in symbols* represent the lines containing allele 7*+9, *grey symbols* represent lines containing allele 7+8

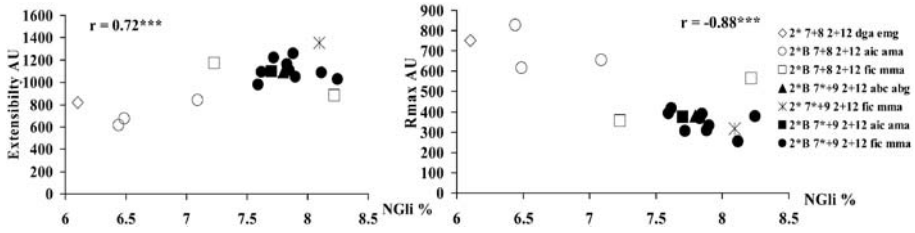

Fig. 5 Effects of gliadin content and allelic composition on extensibility and maximal resistance to extension (Rmax). Extensibility and Rmax values are presented as a function of normalized gliadin content [corrected to the protein content of the individual samples – (NGli)]. Lines with different storage protein composition are designated by different symbols

case of Bánkúti 1201 both RP-HPLC and densitometric evaluations indicate a 140–170% overexpression of subunit Bx7 compared to that of subunit Bx7* in the population and 30% higher expression compared to subunit Bx7 in general (Marchylo et al. 1992; Butow, personal communications). Further experiments will be needed to explain the reasons for this phenomenon.

Due to the homogeneity found at the *Glu-D1* locus the effect on quality associated with this locus could not be estimated.

Qualitative and quantitative effects of LMW glutenin and gliadin alleles

The population is also characterised by high extensibility which might be related to the unusual glutenin to gliadin ratio and the presence of specific LMW glutenin and gliadin alleles. These alleles (*Glu-A3*f/*Gli-A1*m, *Glu-B3*i/*Gli-B1*m and *Glu-D3*c/*Gli-D1*a) are present in most of the samples and largely responsible for the strong correlations observed between dough extensibility and maximum resistance with wet gluten content (data not shown) and gliadin content (Fig. 5). The *Glu-A3*a, d and *Glu-B3*b alleles, manifested in lower incidences, have a contrasting effect on dough parameters. As the distribution of data points on Fig. 5 indicate, lines possessing these alleles resulted in stronger, less-extensible dough. The allelic composition at the *Glu-3* and *Gli-1* loci and the above average amount of gliadin proteins emphasise the importance of the low-molecular-weight storage proteins on dough properties. It is an interesting coincidence that the high gliadin content (as shown in Table 1) and extensibility characteristics of all the individual lines of the Bánkúti 1201 population were inversely related to the HMW glutenin subunit composition (the presence or absence of alleles 7+8 and 2^{*B}).

The balancing effects of two subpopulations of lines on dough properties

Lines containing the dominating 2^{*B} allele in the Bánkúti 1201 population can be divided into two subpopulations based on the alleles present at the *Glu-B1* locus. These two groups differ from each other in both storage-protein composition and technological properties. The differences emerging from the presence or absence of subunits 7+8 have superior effects on dough strength and a decreasing effect on extensibility. The first group (about 67% of the lines) contains 7*+9 subunits on chromosome 1B, in addition to the *Glu-A3*f, *Glu-B3*i and *Gli-A1*m alleles. The cumulative effect of these alleles and their interactions results in high protein and gluten contents (originating from the higher gliadin content) and good extensibility. The second group contains allele 7+8 (about 33% of the lines), resulting in higher dough strength and dough stability due to the overexpression of subunit Bx7 and the higher UPP content. The complex good quality of the variety is thought to be derived from the 60% to 40% ratio of the two subpopulations, i.e. from the optimal balance between stable, strong dough properties and good extensibility.

In conclusion, the importance of identifying and isolating storage protein genes has been emphasized. Landraces and old varieties may serve as an important gene reservoir, for the discovery of novel or mutant genes. In addition to the effect of the encoding genes and their combinations, their expression apparatus and the resulting amounts of individual storage proteins might be respon-

sible for the size distribution of the glutenin macropolymer and dough properties. The heterogeneity in storage protein composition, involving the coincidental expression of different subunits coded on the same locus, such Bx7 and Bx7*, may result in a well-balanced quality system.

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