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Application of a Rapid Electrophoresis Technique Analysing the Glutenin Subunit Composition of Wheat Genotypes

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The characterization of the old Hungarian varieties and landraces is an important part of Hungarian cereal research and breeding. Analysis of these germplasms with the most up-to-date methodologies results a broad scale of diversity of glutenin alleles, which proves their genetic heterogenicity. Exploitation of this attribute is an untapped possibility for developing modern varieties in our breeding programs. The previous research work revealed this diversity by SDS-PAGE analysis and MALDI-TOF technology. The powerful tool, the high throughput lab-on-a chip technique can facilitate the effectiveness of this function and decreases the cost of the analysis. This study demonstrates the application of this technique for analysing the old varieties. The allelic composition and their effects on bread making quality concluded by means of functional analysis.

Keywords: wheat, glutenin, lab-on-a-chip, MALDI-TOF-MS, SE-HPLC

Abbreviations: HMW GS – high molecular weight glutenin subunits; LMW GS – low molecular weight glutenin subunits; LOC – lab-on-a-chip; MALDI-TOF-MS – matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SDS-PAGE – sodium dodecyl sulphate polyacrylamide gel electrophoresis; SE-HPLC – size-exclusion high performance liquid chromatography; UPP – unextractable polymeric protein; BEU – Brabender Extensograph Unit

Introduction

The genetic make-up – the allelic composition on the three high molecular weight (HMW GS), the three low molecular weight (LMW GS) glutenin sub-unit, and the six gliadin coding loci – determines what kind of polypeptides are present in a wheat flour sample. The large level of polymorphism on certain loci (thus the number of possible alleles present) causes an extremely huge potential for biodiversity. The high level of polymorphism of wheat prolamins results in a special effect in relation to the overall functional properties of wheat dough. During dough formation when prolamin proteins are hydrated and form the gluten network, the numerous structurally similar, but slightly different proteins produce a

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mass in which several characteristics (such as size, polarity, charge distribution, solubility, and viscosity) show a continuous distribution over a relatively large interval. This structural feature is unique among protein complexes (Islam et al. 2012).

This qualitative aspect of protein composition is perturbed further by the expression levels of genes determining the absolute and relative amounts of different gene products in the sample. The variation in the relative proportions of gliadin and glutenin does occur between different wheat varieties and largely depends on the growing conditions of wheat. This ratio of monomeric to polymeric proteins affects the physical properties of dough, with higher relative proportions of glutenin imparting greater dough strength. While the gliadin fraction has been reported to contribute to the viscous properties and dough extensibility of wheat dough the polymeric glutenin fraction of wheat gluten has long been considered to have a prominent role in the elastic and strengthening of dough (Shewry et al. 2009).

The genetic potential of certain quality traits is manifested through the effects of growing conditions, where all of the agronomic treatments, soil and climatic parameters alter the final quality of the grain. The realization, that both qualitative and quantitative aspects of composition are important factors determining bread-making quality, led to strategies to work on gene (G) and gene product levels simultaneously. Because of the fact that the level of gene expression is highly dependent on growing conditions, these effects (E) and their interaction with the genetic factors (G × E), are essential parts of these strategies (Békés 2012).

In many countries, the genetic basis of good bread-making quality was represented by old varieties selected from landraces. In Hungary this role was played by populations from the Tisza and Bánát regions. Hungarian wheat production was always famous for its landraces, which developed over several centuries in various wheat-growing regions of the country. In response to unfavourable climatic effects (drought or frost damage) and to epidemics of various fungal diseases, these landraces underwent constant changes and their populations were genetically heterogeneous. The present study had two aims: i) the evaluation of a new research tool, the lab-on-a-chip (LOC) technique in relation to be utilized in the wheat breeding practice to identify the HMW and LMW glutenin alleles of germplasms; ii) to study genetic make-up of old Hungarian varieties and its relationship bread-making quality for the development of new genotypes with the quality type and protein composition of the old Hungarian varieties in order to improve the genetic variability of bread-making quality.

Materials and Methods

Plant material

Wheat lines of 48-old Hungarian cultivars and landraces (RMF population, Table 1) have been selected from the germplasm collections of the Agricultural Institute, Centre for Agricultural Research of the Hungarian Academy of Sciences (CAR-HAS), Martonvásár, Hungary and from the Institute of Agrobotany, Tápiószele, Hungary. Cultivars with

Table 1. Allelic composition of the RMF wheat lines determined by MALDI-TOF method

Samples*	*Glu1-A*	*Glu1-B*	*Glu1-D*	*Glu3-A*	*Glu3-B*	*Glu3-D*
AUSTRO-BANKUT-IHAR	a/c	b	a/d	b	c	b
AUSTRO-BANKUT-RCA	a	c	a	b	c	b
BANKUTI-1014-RCAT000106	a	c	a	b	c	b
BANKUTI-1014-TRI1307	a	c	a/d	b	b	b
BANKUTI-1201	b	al	a	f	i	c
BANKUTI-1204	a	c	a	c	c	d
BANKUTI-1205-RCAT000030	b	c	a	a	i	c
BANKUTI-1205-TRI3709	a	c	a/d	b	c	b
BANKUTI5-11-1	b	c	a	c	c	b
BETA-BANKUTI	a	c	d	c	b	b
BETA-BANKUTI-RIC	b	c	a	b	c	b
BETA-BANKUTI-W	a	c	a	b	c	b
DIOSZEGI-N12	a	u	a	a	f	m
FERTODI-293-24-5	a	c	a	c	c	d
FLEISCHMANN-481	b	u	d	b	b	b
LOVASZPATONAI-407	b	u	d	b	b	b
SZEKACS1055	a	c	d	b	a	d
SZEKACS1055-15-3	a	c/al	d	b	a	d
SZEKACS1242	a	c/al	d	b	a	d
SZEKACS1242-6-2	c	c/al	a/d	b	a	d
TF-BODROGOLASZI-RCAT000395	a	c	a	c	c	c
TF-ECS-RCAT002138	b	c	a	d	c	b
TF-ECS-RCAT002139	a/b	c	a/d	d	c	b
TF-ECS-RCAT002140	b	c	a	d	c	b
TF-GYULAVARI	c	u	a	c	c	c
TF-HOMOKSZENTGYORGY-RCAT001973	a	u	a	a	a	a
TF-KARCAG-RCAT003873	c	c	a	b	a	d
TF-KARCAG-RCAT003874	b	c	a	b	a	d
TF-KISZOMBOR-RCAT003784	b	c	a	d	c	b
TF-KOMADI-RCAT000402	a	c	a	b	c	c
TF-KOMADI-RCAT000403	b	c	a	b	a	d
TF-KOMORO-RCAT000060	a	c	a	b	b	b
TF-KOMORO-RCAT000061	a	c	a	b	b	c
TF-KOMPOLT-10	a/b	c	d	b	c	c
TF-NAGYKALLO-RCAT002131	a	c/u	a/d	a	b	d
TF-NAGYKALLO-RCAT002133	a	c	a	b	b	d
TF-NAGYKALLO-RCAT002134	a	c	a	a	b	d
TF-NOSZVAJ-RCAT000063	a	c	a	b	c	d
TF-NOSZVAJ-RCAT000064	a	u	a	f	b	d
TF-RAVAZD-RCAT002135	a	c	a	b	c	m
TF-RAVAZD-RCAT002137	a	c	a	b	c	m
TF-RETSAG-RCAT000054	b	c	d	b	c	m
TF-SZAJLA-RCAT000068	a/b	u	a/d	f	b	a
TF-SZAJLA-RCAT000069	c	c	a	c	c	b
TF-SZEGED-RCAT002154	a	c	a	b	b	m
TF-SZEGED-RCAT002155	a	c	a	b	b	m
TF-TIMAR-RCAT002150	a	c	a	c	c	c
TF-TIMAR-RCAT002151	a/b	c	a	c	c	c

* Samples are available in small amount for research application in gene bank of the CAR-HAS, Martonvásár. Lines indicated with bold letters have been selected for further LOC, compositional and quality analysis from samples derived from triplicate field trial.

known glutenin allelic composition, Chinese Spring and Miranovskaja (Békés et al. 2006a, b) were used as reference materials in LOC analysis.

Based on the field performance as well as on their glutenin allelic composition, twenty lines were selected (indicated bold letters in Table 1) and grown in the Plant Breeding Nursery of CAR-HAS in triplicate plots. For the functional and SE-HPLC analysis samples were tempered overnight to 15.5% moisture content and milled with a Chopin CD1 mill, provided white flour. For the LOC and MALDI-TOF analysis grain samples (n = 3 * 20 = 60) were milled with laboratory mill (FQC-2000, Metefém Ltd, Hungary), 3 single seed were analysed individually.

LOC analysis

Proteins were extracted from whole grains according to the sequential procedure of Uthayakumaran et al. (2005) with some modifications. The extracts (4 µl each) were applied with Agilent sample buffer to each of the 10 sample wells of a Protein 230 series II LabChip for analysis in the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) following the manufacturer's instructions. Each sample contained internal standards, upper marker of 240 kDa and lower marker of 4.5 kDa. The evaluation was performed with Agilent 2100 Expert software.

MALDI-TOF-MS

The dried mixtures of HMW GS samples were dissolved in 60 µl acetonitrile (ACN)/H_2O (v/v, 50:50) containing 0.05 v/v trifluoroacetic acid (TFA) for 1 h at room temperature. Sample preparation was carried out according to the dried droplet method (Kussmann et al. 1997), using sinapinic acid (SA) as matrix. A sample/matrix solution on mixture 1:10 v/v) was deposited (2 µl) on a 96-sample MALDI target, and dried at room temperature. MALDI-TOF mass spectrometric experiments were carried out using the same equipment, protocol and identification of HMW and LMW glutenin alleles as described by Baracskai et al. (2011).

SE-HPLC

Glutenin-, gliadin- and albumin/globulin content of samples were determined in triplicate by size exclusion high performance liquid chromatography (SE-HPLC) applying the extraction protocol of Singh et al. (1990) followed by the separation procedure of Batey et al. (1991). Results have been expressed as % of total protein content (relating the area under the peak of interest to the area of the total chromatogram) and % of flour (multiplying the above percentage with the protein content of the flour. Unextractable polymeric protein (UPP) percentages have been determined using the method of Gupta et al. (1993). The relative amounts of polymeric proteins (peak one) from the two extracts are expressed as UPP%. Identical HPLC separation parameters have been used for this procedure as for the above glutenin and gliadin separations.

Functional analysis

The following methods and instruments were used to determine the functional properties of the lines: protein content: Kjeltec 1035 Analyzer (AACC 46-10), wet gluten and gluten index: Perten Glucomatic System (AACC-38-12), Wet gluten spread: MSZ 6369/5-87 Hungarian standard, Zeleny sedimentation test (AACC 56-61.02), Brabender Farinograph (ICC115/1, MSZ6369/6-1988) and Chopin Alveograph (ICC 121). Gluten index (GI), a measure of dough strength, was determined as the gluten remaining on a sieve (g) * 100/total gluten (g) (ICC 155).

Statistical analysis

Dough strength (Rmax) and Extensibility (Ext) of samples have been estimated from protein content and the glutenin allelic composition applying the PSS mathematical model of Békés et al. (2006c). Statistical analysis was carried out using STATISTICA 9.0 (StatSoft, Inc., 2006, Tulsa, OK, USA). Significance in differences among samples and treatments were characterized by ANOVA method.

Results

Glutenin alleles in the RMF by MALDI-TOF analysis

The list of HMW GS and LMW GS alleles identified in the RMF population are tabulated in Table 1. A total of 9 HMW GS alleles were found among the set of 48 wheat lines investigated with 3, 4 and 2 alleles were identified at *Glu-A*1, *Glu-B*1 and *Glu-D*1 loci, respectively. A total of 15 alleles encoding LMW GS were found in the collection, with 5 alleles corresponded to each of the *Glu-A*3, *Glu-B*3 and *Glu-D*3 loci, respectively.

In the sample population, there are dominant alleles for each locus: *Glu-A1b* = 57.8, *Glu-B1c* = 75.0%, *Glu-D1d* = 68.9%, *Glu-A3b* = 58.3, *Glu-B3c* = 50.0% and *Glu-D3c* = 35.4%.

The most frequent allelic variants were b,a,d (15.5%) and a,c,a (33.9) for HMW and LMW glutenin alleles. The diversity in the glutenin alleles is rather high, theoretically allowing 24 (3*4*2) different HMW GS, 125 (5*5*5) different LMW GS combinations and 3000 (24*125) different combinations on the 6 glutenin loci, from which 14, 20 and 36 appear in this set, respectively. It is an indication of the tremendous potential to apply the existing biodiversity in the population through breeding (Fig. 1).

Comparing the HMW GS alleles in some RMF lines to previously published data only one slight difference can be observed. Since the publication of Bedő et al. (1998, 1999) it is realized that the x type of subunit in the Chinese Spring cultivar used as standard to identify the *Glu-B1b* allele by Payne and Lawrence (1983) (subunit 7) is a rarely appearing protein compared to it slightly larger version, subunit 7*. The slight difference in size between 7 and 7* cannot be distinguished by SDS-electrophoresis, like the very similar two y-type subunits (8 and 8*) coded by *Glu-B1* locus. However, the slight differences between the elements of these two pairs are clearly detectable by MALDI-TOF analysis. So the earlier findings for *Glu-B1b* now can be revised and be designated to *Glu-B1b* (7 + 8),

Glu-B1u (7* + 8), *Glu-B1ak* (7* + 8*) and *Glu-B1al* (7 + 8*) as it happened for the Canadian (Ng et al. 1989), Italian (Pogna et al. 1989) and Australian (Zhen and Mares 1992) germplasms as well as for the cultivars of breed in Martonvásár, Hungary (Baracskai et al. 2011).

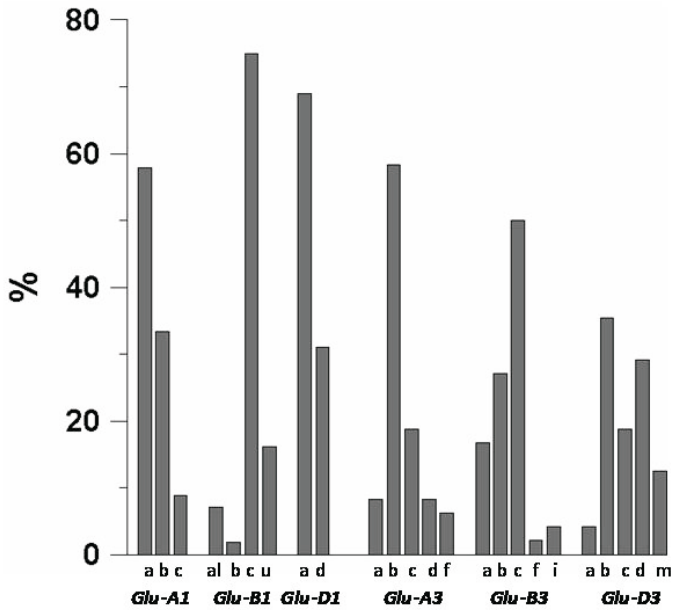

Figure 1. Distribution of the HMW and LMW GS glutenin alleles in the RMF population

In cases of biotypes, each allele have been included in the calculation

Similarly to the *Glu-A1x* the (containing the 2^{*B} subunit), found in some progenies of the old Hungarian cultivar, Bánkuti 1201 (Juhász et al. 2003), the *Glu-B1al,* appearing in the RMF sample set in Bánkuti 1201 and in 3 Székács lines (Table 1) has an already utilized potential to improve bread-making quality. When *Glu-B1al* is present, the Bx7 subunit is over-expressed (Marchylo et al. 1992) as a percentage of the total amount of HMW glutenin present, and this over-expression is associated with greater dough strength (Butow et al. 2003; 2004). D'Ovidio et al. (1997) presented evidence that the *Glu-B1al* allele includes two copies of its x-type glutenin gene followed by several reports with findings explaining the regulatory background of the overexpression (Butow et al. 2003; Radovanovic and Cloutier 2003).

Interesting characteristic of the allelic composition RMF population is that the *Glu-B1i* allele, containing subunits 17 + 18 is completely missing in the sample population. The *Glu-B1i* allele is one of the "best" alleles at maintaining gluten strength. The overwhelming majority of RMF lines contain the *Glu-D1d* (5 + 10) allele, so a further increase in

dough strength was usually not needed for the traditional bread-making technologies in those regions where these lines have been cultivated.

Application of LOC technique

Protein composition and functional properties of samples of twenty samples have been analysed by LOC technique. These studies reveal that the current LOC technology provides comparable results with the conventional SDS-PAGE and the MALDI-TOF techniques. By this method, the bands of glutenin subunits are also separated into two groups, for the HMW and the LMW subunits, however the order of polypeptides after separation are slightly different than found in the above two methods producing unexpected sequences.

In agreement with previous studies (Rhazi et al. 2009; Balázs et al. 2011) the order of the largest HMW GSs observed in this study is Ax2* < Ax1 < Dx2 compared to those found by the SDS-PAGE and MALDI-TOF (Ax1 < Dx2 < Ax2*), while in case of the y-type subunits the sequence of Dy9 < Dy8 < Dy10 was found compared to the expected By8 < By9 < Dy10 sequence. This unexpected sequence clearly indicate that principle of the separation using the LOC system is not clearly size-based separation but inherent characteristics of the polypeptides like hydrophobicity play important role (Balázs et al. 2011).

The technique, using the conditions recommended by the manufacturer, separates proteins in the 4–240 kDA molecular size intervals. In each sample two internal standards, an upper and a lower marker of 240 kD and 4.5 kDa is applied, respectively. Because of the relatively large size of HMW glutenin subunits, they can be clearly separated from the LMW GS, but the range of separation is rather narrow, the migration velocity of the upper marker and of some particular HMW polypeptides seems to be identical. Since the built-in software of the apparatus calculates the migration time of the samples using the internal standards migrations, running without internal standards does not result numeric data. So, using internal standards, the identification of Dx2, Ax2* and especially of the Ax1 polypeptides is ambiguous. In most cases HMW subunits were easily identified, except there were no significant difference between the elution times of subunits Bx7 and Bx7* and subunits Dx2 and Dx5. However based on the intensity of Bx polypeptide the *Glu-1Bu* and *Glu-1Bal* can be differentiated, while the presence of the Dy12 or Dy10 subunit clearly differentiate between the *Glu-1Da* or *Glu-1Dd* alleles.

As it was found in earlier studies the analysis of LMW glutenin subunits by the LOC technique is rather difficult. The repeatability is good (Balázs et al. 2011), however, fusion of the subunits is frequent. Several attempt have been evaluated in relation to improve the resolution comparing different extraction and sample derivation techniques by Balázs et al. (2012) with no significant improvement,

Because of the limited resolution of LMW GS, identification of individual proteins in this region as well as of LMW GS alleles is problematical. However, it was found that the LOC patterns of samples containing different combinations of LMW GS alleles are distinguishable. Manual or computer aided comparison of patterns from systematically executed LOC separations of 'unknown' and known samples on the same chip resulted in rea-

sonably good predictions for the allelic composition of LMW GS in the unknown samples (success rate: 87% and 81% for manual and computerized predictions, respectively).

In overall this new and fast technology was found to be capable to differentiate among the RMF wheat lines, The LOC can be an outstanding, easily executable, and relatively low cost, high-throughput tool for researchers and breeders.

Relationship between the glutenin alleles and functional properties of the flour

To investigate the effects of different aspect of protein composition on dough parameters in the RMF population, protein content and protein composition characterized by the distribution of total protein content among the glutenin-, gliadin- and albumin/globulin fraction, UPP% and the glutenin allelic composition were related to functional measurements.

Table 2. ANOVA analysis on quality attributes to investigate the individual and interactive effects of glutenin alleles

		HMW	LMW	HMW*LMW	Category
Water absorption	F	**0.24**	**0.19**	**0.19**	**1**
	p	0.99202	1.00000	1.0000	
Zeleny sedimentation	F	**3.24**	**3.62**	**2.74**	**2**
	p	0.00010	0.00000	0.0000	
Dough development time	F	**7.02**	**6.70**	**4.46**	**2**
	p	0.00000	0.00000	0.0000	
Farinograph stability	F	**2.41**	**3.80**	**3.56**	**2**
	p	0.00830	0.00000	0.0000	
Rmax	F	**17.81**	**9.46**	**4.27**	**3**
	p	0.00000	0.00000	0.00000	
Gluten index	F	**12.63**	**7.51**	**5.95**	**3**
	p	0.00000	0.00000	0.00000	
Alveograph W	F	**13.54**	**7.46**	**5.31**	**3**
	p	0.00000	0.00000	0.0000	
Ext	F	**0.89**	**7.46**	**3.87**	**4**
	p	0.04320	0.00000	0.01920	
Wet gluten spread	F	**7.17**	**12.32**	**5.87**	**4**
	p	0.00000	0.00000	0.00000	
Alveograph P/L	F	**0.77**	**1.56**	**2.07**	**4**
	p	0.57075	0.04331	0.00000	

Categories defined based on the F values: 1 – No significant relationship; 2 – Significant, effects of HMW and LMW GS alleles are comparable; 3 – Significant, effects of HMW GS > LMW GS; 4 – Significant, effects of LMW GS > HMW GS.

Table 2 contains relationships between the glutenin alleles and functional parameters. ANOVA F values and probabilities of significance (p) are given for the HMW and LMW glutenin allelic combination and their (HMW*LMW) interaction. Based on the results of ANOVA statistics (Table 2), the quality parameters monitored in this study fall into four groups (indicated in the last column of Table 2). While water absorption was found to be

not related to glutenin composition (Group 1), Farinograph dough development time, Farinograph stability and Zeleny sedimentation value showed significant dependency from both the HMW and LMW GS alleles on very similar level (Group 2). The rest of the parameters, investigated, also showed significant relationships to the glutenin alleles but in cases of the dough strength type of parameters (Rmax, Alveograph W and Gluten index) the effect of HMW GS was found significantly larger than those for LMW GS (Group 3), while in cases of the parameters describing the extensional characteristics of the dough (extensibility, gluten spread and Alveograph P/L) the extent of the effects were found to be the opposite (significantly larger effects for LMW GS alleles then for HMW Gs alleles (Group 4)).

The observation on the relationship of water absorption, one of the most important functional parameters of the flour, to protein composition is in agreement with previous *in vitro* studies (Tömösközi et al. 2002; Haraszi et al. 2004b), where the supplementation with the same amounts of gliadin or glutenin proteins derived from different varieties is resulted the same alteration in water absorption. It is important to note in Groups 2–4, the effects of the HMW*LMW GS interaction are strongly significant for each parameter, investigated. On structural level it means that on the top of the individual effects of the glutenin alleles there is large contribution of the interaction between the different alleles. In cases Rmax and extensibility this different effects have been estimated by using the PSS mathematical model (Békés et al. 2006c). It was found that the contribution of individual HMW GS alleles for dough strength and extensibility is 54 and 23%, respectively. The same values for the contribution of individual LMW GS alleles were 19 and 41%, while 21% and 34% were found for the contribution of HMW GS × LMW GS interactions.

The realization of the existence of large contribution of allele–allele interactions in wheat flour dough may alter our way of utilizing our knowledge of relating genetic/chemical information to quality attributes in wheat breeding, in the grain industry and in basic research. In breeding, the real value of a certain allele has to be investigated in several backgrounds to be able to realize its interaction-potential. So different allelic combinations, rather than certain individual glutenin alleles should be targeted in breeding situations to develop new lines with certain quality attributes, especially to improve extensibility.

The relationships between protein composition and functional properties of the dough

Linear correlation coefficients summarised in Table 3 indicate how the overall protein composition can alter the different dough properties. As expected, protein content showed signficant relationships with water absorption and with two from the three extensional parameters (extensibility and Alveograph P/L). Interestingly wet gluten spread was found to be not related to protein content. In agreement with the results of previous studies, glutenin content of the total protein in the flour had significant positive effects on dough strength, type of dough parameters and negative effects on extensional properties. However, the effects are just the opposite in case of the gliadin content (negative effect on dough strength and positive on extension). The glutenin to gliadin ratio, one of the most important protein parameters to describe dough properties (Wrigley et al. 2006) showed

Table 3. Relationships (r) between protein composition and functional data in the RMF population

	Protein [% flour]	Wet gluten [% flour]	Glutenin [% protein]	Gliadin [% protein]	Non-prolamins [% protein]	Glu/Gli	UPP [%]	Glutenin [% flour]	Non-prolamins [% flour]
Water absorption	0.505	0.010	–0.598	0.573	0.640	–0.226	–0.548	0.060	0.636
Dough development time	0.038	–0.439	0.697	–0.713	–0.596	0.320	0.614	0.558	–0.225
Farinograph stability	–0.390	–0.317	0.700	–0.696	–0.669	0.356	0.510	0.144	–0.568
Zeleny sedimentation	0.061	–0.316	0.525	–0.556	–0.383	–0.010	0.629	0.450	–0.114
Wet gluten spread	0.419	0.172	–0.581	0.580	0.545	–0.600	–0.428	–0.016	0.529
Gluten index	–0.298	–0.323	0.729	–0.733	–0.665	0.169	0.801	0.250	–0.496
Alveograph W	0.118	–0.415	0.838	–0.852	–0.763	0.249	0.586	0.633	–0.235
Farinograph P/L	0.094	–0.098	–0.497	0.493	0.501	–0.319	–0.349	–0.403	0.336
Rmax	–0.175	–0.253	0.562	–0.564	–0.517	0.256	0.386	0.251	–0.348
Ext	0.578	0.155	–0.563	0.536	0.615	–0.578	–0.226	0.152	0.680

The values for Rmax and Ext (in Tables 2–3) were derived from a mathematical model and not the results of functional dough testing.

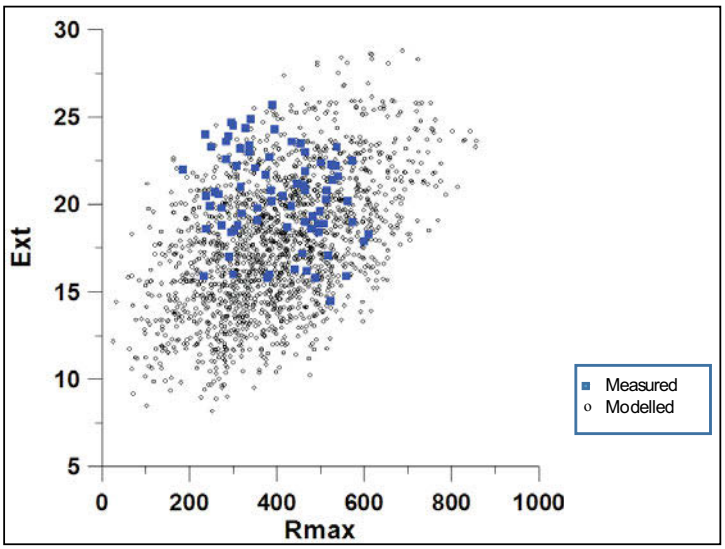

Figure 2. The genetic potential for dough strength (Rmax) and extensibility (Ext) derived from the possible variants of HMW and LMW GS alleles in the RMF population. Values indicated by small open circles (n = 3000) were calculated from each combination of the alleles on the 6 glutenin loci, filled squares show measured Rmax and Ext values

similar trends as the glutenin content. Since the pioneer work of Orth and Bushuk (1972) the relative amount of the very large glutenin polymers in the flour is used as the best predictor of dough strength. The application of the SE-HPLC based determination of these proteins, the percentage of the unextractable polymers (UPP%) is widespread both in breeding and in quality control in the industry. The results derived from this study also prove that dough strength is directly related to size distribution of the polymeric glutenin measured by UPP. Our data do not support, however, the recommendation of Bangur et al. (1997) to use the amount of total glutenin in the flour (glutenin % in the total protein multiplied by the protein content of the flour) to estimate the extensional properties of the dough.

Water absorption of the flour usually described as the function of the total protein and pentosan content as well as the level of starch damage caused by the milling process (Wrigley et al. 2009). Our results indicate that the amount of the non-prolamin (albumin and globulin) proteins might play also and important role determining water absorption (Table 3).

Discussion

On the basis of the results presented here, it can be stated that the selection basis for wheat varieties with good bread-making quality can be extended to include components from the old Hungarian varieties holding certain combinations of HMW and LMW glutenin alleles if the aim is to increase gluten content, combined with good rheological properties. The

large diversity of the glutenin alleles provide an excellent basis to select lines in the breeding program what satisfy the most demand of certain applications. The potential of such activity is well illustrated in Figure 2. The Rmax and Extensibility data of the RMF lines investigated in this study are shown here together with data of each theoretically possible glutenin allele combination in the RMF population calculated by the PSS model covering the 20-880 BEU and 8–29 cm intervals, respectively. Any combination of dough strength and extension values in these intervals can be achieved by crossing RMF lines containing the suitable glutenin alleles.

The glutenin allele related selection process requires fast, relatively cheap and reliable methodology to provide meaningful information about the allelic composition of large number of progenies. In the light of results of this study it seems that the LOC technique is capable to serve in this role helping the breeder to select the lines of interest to develop better bread-making cultivars.

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