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Effect of high molecular weight glutenins and D-zone gliadins on bread-making quality in German wheat varieties

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Summary
The composition of HMW-GS and D-zone gliadins was investigated in the German wheat gene pool. Gliadins and high molecular weight glutenin subunits (HMW-GS) from 153 German wheat varieties were analysed using. The most frequent protein combinations found, were 1, 7+9, 5+10 (HMW-GS) and N, d3, d11+d12 (D-zone gliadins) in spring wheat varieties and N, 6+8, 2+12 (HMW-GS) and d9, d2+d4, d11+d12 (D-zone gliadins) in winter wheat varieties. The score of the rapid mix test (RMT) was used to evaluate the influence of HMW-GS and D-zone gliadins on the baking quality. Correlation and multiple regression analyses were performed between the particular gliadins as well as the HMW-GS and the score of the rapid mix test. The baking quality was positively influenced by subunits 1, 2*, 7+9, 14+15, 17+18, 5+10 (HMW-GS), the null-allele of *Gli-A1* and *Gli-D1*, gliadins d2+d4, d3, and d7, whereas the other subunits and gliadins negatively effected the score of the RMT.

Key words: *Triticum aestivum* var. *aestivum*, high molecular weight glutenin subunits (HMW-GS), D-zone gliadins, SDS-polyacrylamide-gelelectrophoresis (SDS-PAGE), baking quality

Introduction

A major part of the variability of wheat cultivars in baking quality could be based upon the constitution of glutenins and gliadins (Bietz and Huebner 1980, Pomeranz 1988). Best described are the high molecular weight glutenin subunits (HMW-GS) concerning their influence on baking quality as well as their methodical handling (Payne et al., 1981b).

Glutenins form, besides intramolecular, also intermolecular disulfide bonds. An extensive three dimensional network of polypeptide chains is formed during polymerization (Payne et al. 1979, Graveland et al. 1985). These aggregated high-polymeric proteins are responsible for the elasticity and the retention of CO_2 of doughs (Khan and Bushuk, 1979).

Gliadins are monomeric molecules with intramolecular disulfide bonds. This is, however, not true for most of the ω-gliadins, which do not possess any cystein residues to form disulfide bonds (Shewry et al., 1986). They are mainly responsible for the viscosity and extensibility of the doughs (Payne et al., 1984).

Storage proteins are coded on chromosomes of the homoeologous groups 1 and 6. The gene loci of the HMW-GS are located on the long arm of the

chromosomes of homoeologous group 1 and are designated *Glu-A1*, *Glu-B1*, and *Glu-D1*, respectively (Payne and Lawrence, 1983). The gene loci of the gliadins are located on the short arm of the chromosomes of homoeologous groups 1 and 6 and are designated *Gli-A1*, *Gli-B1*, and *Gli-D1* on 1A, 1B and 1D and *Gli-A2*, *Gli-B2*, and *Gli-D2* on 6A, 6B and 6D (Payne et al., 1987b). The gene loci of the low molecular weight glutenins *Glu-A3*, *Glu-B3* and *Glu-D3* are closely linked with the *Gli-1* gene loci on the chromosomes of homoeologous group 1 (Singh et al. 1991). Every gene locus shows an allelic variation and is responsible for the cultivar specific protein quality and therefore for the baking quality (Gupta and MacRitchie 1994).

In this work the correlation between storage proteins and baking quality was confirmed by using German wheat cultivars. By adding a further protein fraction, the D-zone gliadins, this correlation is more firmly established and the variability of baking quality is better explained.

Material and methods

The constitution of HMW-GS and D-zone gliadins was determined in a total of 153 German spring (27) and winter wheat varieties (126). The material was delivered by the Bundessortenamt (Hannover, Germany) or grown at the Bayerische Landesanstalt für Bodenkultur und Pflanzenbau.

Total protein was extracted from crushed half wheat grains. After reduction with β-mercaptoethanol, HMW-GS were separated by SDS-PAGE, described by Ng and Bushuk (1987).

Gliadins were extracted from crushed half wheat grains with 30% (v/v) chlorethanol, as described by Branlard and Darvedet (1993), subjected to a 12% SDS-PAGE and separated for 2h30 at 10mA constant amperage, using Biometra electrophoresis chambers. Gels were stained with coomassie-blue overnight, destained with H_2O_{bid} and photos taken. Designation of the HMW-GS are according to Payne et al. (1983) and designation of the D-zone gliadins are according to Khelifi et al. (1992).

For evaluating the influence of the proteins, the scores of the baking volume (rapid-mix test) were used as a quality parameter. These scores were taken from the „Beschreibende Sortenliste", 1984 - 1997. Correlation and multiple regression analyses were performed to estimate the relation between the alleles of the HMW-GS as well as the D-zone gliadins and the scores of the rapid mix test (SAS Inst. 1990). To estimate the mutual influence of the alleles on the baking quality, the mean of the rapid mix test score from varieties with the same HMW-GS combination was calculated. This number was compared with the mean of the rapid mix test score from varieties, which differ in one allele. Rare subunit combinations were ommited in this calculation.

Results

Table 1 shows the HMW-GS alleles found. *Glu-B1* shows the highest variability in this study. It was found to have six alleles, *Glu-A1* and *Glu-D1* three, each. Spring-

and winter wheat varieties also showed the greatest difference regarding Glu-B1. Subunits 7+8, 6+8, and 17+18 exist only in winter wheat. The most common subunits found were subunits 7+9 with 40,5% and 6+8 with 42,1%. Subunits 14+15 were found to exist exclusively in spring varieties and they are the second most frequent subunits (29.6%), after subunits 7+9 (59.3%). At *Glu-A1*, the null-allele is much more frequent in winter varieties (75.4%) compared to spring varieties (14.8%). The occurrence of subunits 1 and 2* is higher in spring varieties. Subunits 5+10 in *Glu-D1* are distinctively more often found in spring varieties (74.1%) compared to winter varieties (52.4%). In winter varieties, the percentage of subunits 5+10 and 2+12 is roughly the same, 52.4% and 45.2%, respectively.

Table1: Alleles of the HMW-GS. Line 6 shows the effect of the alleles on the baking quality by correlating the single allele with the score of the RMT. Numbers reaches from -1 to +1. Negative numbers indicate a negative effect on baking quality.

	Glu-A1			Glu-B1						Glu-D1		
Subunits	1	2*	N	7	7+8	7+9	6+8	14+15	17+18	2+12	3+12	5+10
Alleles	a	b	c	a	b	c	d	h	i	a	b	d
Spring var.	19	4	4	3	-	16	-	8	-	7	-	20
Winter var.	28	3	95	9	10	51	53	-	3	57	3	66
Effect on baking quality	0.303	0.109	-0.340	-0.054	0.004	0.397	-0.517	0.198	0.067	-0.415	-0.082	0.435

In winter varieties 21 subunit combinations could be found. Combinations N, 7+9, 5+10 (23 varieties, 18,3%) and N, 6+8, 2+12 (22 varieties, 17.5%) were most frequent, followed by N, 6+8, 5+10 (19 varieties, 15,1%) and 1, 7+9, 5+10 (12 varieties, 9,5%). 26,9% of the varieties (7 out of 26), which have the 1BL/1RS-translocation possess the combination: N, 6+8, 2+12. In spring varieties eight combinations could be found. The most common combination in spring varieties is 1, 7+9, 5+10 (10 varieties, 37.0%) followed by N, 7+9, 5+10 (4 varieties, 14,8%) and 1, 7, 5+10 as well as 2*, 14+15, 5+10 (3 varieties, 11,1% each).

Five *Gli-B1* alleles, three *Gli-A1* and three *Gli-D1* alleles were detected among D-zone gliadins. Again, the B-genome showed the greatest differences between spring and winter varieties. Analogously to the HMW-GS, greatest variation was found in the B genome (Table 2). Varieties, which possess the 1BL/1RS-translocation, have Gli-R1 instead of Gli-B1. 48.1% of the spring wheat varieties have gliadin d3, which appears only in 2.4% of the winter wheat varieties. At *Gli-D1*, the null-allele (11.1%) and gliadin d7 (14.8%) were found to be distinctively more frequent in spring- than in winter wheat varieties. Typical *Gli-A1* gliadins of winter wheats are d9 (57,1%) and d8+d9+d10 (13.5%).

Gliadin combination d9, d2+d4, d11+d12 is the most common combination in winter wheat varieties (54.0%), followed by the 1BL/1RS-translocation varieties, which possess N, Gli-R1, d11+d12 (20.6%). Analogously to HMW-GS more allelic combinations exist in winter wheat. In spring wheat the distribution of the gliadin combination is more consistent. 37.0% of the analysed varieties possess combination N, d3, d11+ d12 and 29,6% d9, d2+d4, d11+d12 (Table 3).

Table 2: Alleles of the D-zone gliadins. Line 6 shows the effect of the alleles on the baking quality by correlating the single allele with the score of the RMT. Numbers reaches from -1 to +1. Negative numbers indicate a negative effect on the baking quality. Varieties which possess the 1BL/1RS Translocation, have Gli-R1 instead of Gli-B1.

	Gli-A1			Gli-B1			Gli-R1	Gli-D1		
Subunits	d9	d8+d9+d10	N	d2+d4	d3	d5		d7	d11+d12	N
Alleles	b	d	e	b	d	e		a	b	c
Spring var.	11	1	15	13	13	1	-	4	20	3
Winter var.	72	17	37	89	3	8	26	6	119	1
Effect on baking quality	-0.156	0.010	0.223	0.056	0.322	-0.009	-0.320	0.297	-0.301	0.085

Table 3: Gliadin combinations of the D-zone gliadins

Gli-A1	Gli-B1	Gli-D1	Spring wheat		Winter wheat	
N	-*	d11+d12	-		26	20.6%
N	d3	d7	3	11.1%	2	1.6%
N	d3	d11+d12	10	37.0%	1	0.8%
N	d5	d7	-		1	0.8%
N	d5	d11+d12	1	3.7%	5	4.0%
N	d2+d4	N	1	3.7%	-	
N	d2+d4	d7	-		2	1.6%
d9	d2+d4	N	2	7.4%	1	0.8%
d9	d2+d4	d7	1	3.7%	1	0.8%
d9	d2+d4	d11+d12	8	29.6%	68	54.0%
d9	d5	d11+d12	-		2	1.6%
d8+d9+d10	d2+d4	d11+d12	1	3.7%	17	13.5%
Total			27	99.9%	126	100.1%

* Gli-B1 is replaced by Gli-R1

A correlation analysis between the individual alleles for D-zone gliadins and HMW-GS and the rapid mix test score was performed (see Table 1 and 2). The influence of all other alleles was not taken into account in this calculation. This correlation analysis showed a positive association for HMW subunits 1, 2*, 7+9,

14+15, 17+18 as well as 5+10 and a negative association for the null-allele of *Glu-A1*, and for subunits 6+8, 2+12, and 3+12. Subunits 7+8 exhibited only a slight effect on the baking volume. The results for the D-zone gliadins, were a positive association for gliadins d3 (*Gli-B1*), d7 (*Gli-D1*), and the null allele of *Gli-A1* and a weak positive association for gliadins d2+d4 (*Gli-B1*), d8+d9+d10 (*Gli-A1*), and the null-allele of *Gli-D1*. A weak negative association was found for gliadin d5 (*Gli-B1*) and a strong negative association for *Gli-R1* and gliadins d9 (*Gli-A1*), and d11+d12 (*Gli-D1*). By multiple regression analysis, 43% and 25% of the variability of the baking volume could be attributed to HMW-GS and D-zone gliadins, respectively (α = 1%). This attribution can be raised to 50% by correlating HMW-GS and D-zone gliadins together with the score of the rapid mix test.

To estimate the mutual influence of the alleles on the baking quality, the mean of the rapid mix test score from varieties with the same HMW-GS combination was calculated (see Table 4). This number was compared with the mean of the rapid mix test score of varieties, which differ in one allele. In these considerations only HMW-GS combinations were included. D-zone gliadins were left out, because they exhibit only a small effect on baking quality ($r^2 = 0.25$).

Table 4: RMT-scores of subunit combinations

Glu-A1	Glu-B1	Glu-D1	varieties	RMT- score (mean)
N	6+8	2+12	22	3.3
N	6+8	5+10	19	5.2
N	7	2+12	6	4.0
N	7+8	2+12	6	5.5
N	7+9	2+12	14	5.5
N	7+9	5+10	27	6.7
1	6+8	2+12	5	5.2
1	7+9	5+10	22	7.2
1	7+9	2+12	4	7.0

The comparison of the rapid mix test scores show that the alleles contribute to baking volume with a quite different degree in different combinations. The baking volume is always higher, if subunit 1 is present at *Glu-A1*. The improvement of the baking quality by subunit 1 is, however, small, if this subunit is exchanged against the null-allele in a combination, which has already a good baking volume, e.g. the exchange of N, 7+9, 5+10 to 1, 7+9, 5+10 improves the RMT score by 0.5 points. The improvement is, however, as a rule greater if the null-allele is exchanged against subunit 1 in an allele combination with a lower RMT score, e.g. N, 6+8, 2+12 to 1, 6+8, 2+12 or N, 7+9, 2+12 to 1, 7+9, 2+12. The improvements for these exchanges are 1.9 or 1.5 points, respectively. The same is valid for subunits 7+9 and 5+10. This subunit pairs improve the baking quality, but the more, the lower the RMT score is in an allelic combination.

Discussion

Payne et al. (1987a) and Cerny et al. (1992) developed independently different scoring systems to evaluate the *Glu-1* alleles concerning their contribution to baking quality. They assign scores to the different alleles and the addition of the scores of the allelic combination is assigned to the specific variety. The results of our correlation of the HMW-GS and the baking volumes are consistent with these scoring systems: Subunits 7+9 and 5+10 have a more positive effect on baking quality compared to 6+8 and 2+12, respectively (Payne et al., 1981a). The better effect of subunits 5+10 compared to subunits 2+12 is explained by structural features of subunit 5. Subunit 5 possesses an additional cystein residue at the N-terminal end of the repetitive region (Shewry et al., 1997). This leads to further inter- and intramolecular disulfide bonds and resulting from this more highpolymeric glutenins. Subunits 5+10 and 7+9 occur more often in varieties with a high baking volume and none of the investigated varieties with a high baking volume has subunits 6+8 and 2+12. This suggests, that the breeders unconsciously selected subunits 5+10 and 7+9, when breeding for high baking quality.

These scoring systems, however, take only the single alleles into account and not the whole allelic combination. It should be kept in mind, that the effect of an allele on baking quality may not be considered separately from the alleles of the other genomes. There are always interactions between single loci (also of non homoeologic loci) of the gluten proteins (Rodriguez et al., 1996). Therefore, subunits contribute to the baking volume with a quite different degree in different combinations.

25% of the variability of the baking volume could be explained by the alleles of the D-zone gliadins. As the HMW-GS, these alleles contribute to the quality at a different degree. The allelic combinations are concentrated in three groups in spring wheat and two groups in winter wheat varieties, respectively. Similar results were obtained by E. Johansson (1996), who used Zeleny and specific Zeleny sedimentation volume as parameters for baking quality. Branlard and Darvedet (1993), found a positive effect of the Null *Gli-D1* allele on bread wheat quality. This result is also consistent with our correlation, using the German wheat varieties.

All investigated varieties, which possess the 1BL/1RS translocation, do not have *Gli-A1* gliadins. The reason for this is unknown. Some workers have shown, that the 1BL/1RS translocation is negatively associated with baking quality (Dhaliwal and MacRitchie, 1990). So, the negative correlation of these varieties could be due to the missing of *Gli-B1* and *Gli-A1* gliadins.

The mostly used method to analyse gliadins is A-PAGE together with the nomenclature of Metakovsky et al. (1984). This way, however, HMW-GS and D-zone gliadins can not be investigated simultaneously. Furthermore, the analysis of these gels is rather difficult because of the complexity of the whole gliadin patterns. By the combination of the used methods, HMW-GS and D-zone gliadins as well as the 1BL/1RS translocation can be analysed simultaneously on the same gel.

It has been discussed whether the gliadins or the low molecular weight glutenins (LMW glutenins), which are closely linked, have a similar or greater effect on baking quality (Payne et al. 1987b). As it is more costly, to extract LMW glutenins, to run them in a gel and to assign them to specific bands, the D-zone gliadins could be used as markers for certain LMW glutenins.

From the results to date the conclusion can be drawn, that it is important for a breeder to know the allelic combination of the D-zone gliadins and/or the LMW glutenins, because an important part of the variability of baking quality is caused by them. Further research is necessary to analyse LMW glutenins and to determine quantitatively all protein fractions, especially the HMW-GS.

The list with all the alleles and allele combinations of the investigated varieties will be delivered on request.

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Composition and concentration of proteins in Lithuanian wheat cultivars: relationships with bread-making quality

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Summary

The concentration and composition of storage proteins in Lithuanian-grown wheat cultivars were investigated. The protein concentration did not explain the differences in bread-making quality between the cultivars. Zentos and Sirvinta were considered as good bread-making quality wheats according to the Lithuanian millers and bakers. These cultivars showed the highest Glu-1 quality scores, contained high-molecular-weight (HMW) glutenin subunits and D-zone omega gliadins correlated with high gluten strength as well as a high percentage of large unextractable polymeric proteins in total large polymeric proteins.

The cultivars Jubilatka and Marabu were found to carry an 1BL/1RS rye translocation. This gave the lowest rye adjusted Glu-1 quality scores, and also the lowest percentage of large unextractable polymeric proteins in the total large polymeric protein.

The strong cultivars in this study, could be interesting as breeding material in the other Nordic and Baltic countries. Also, the D-zone omega gliadin d7, found to be relatively common in the Lithuanian wheats but uncommon worldwide, could be of interest to introduce in wheat cultivars in other Nordic countries. This gliadin is correlated with high gluten strength.

Key words: bread-making quality, protein composition, protein concentration, wheat cultivars.

Introduction

In determining the bread-making quality, both concentration and composition of proteins are of importance (Finney and Barmore 1948, Payne et al. 1987, Johansson 1996). A strong positive relationship has been demonstrated between grain protein concentration and volume and texture of the baked loaves (Finney and Barmore 1948). Correlations have also been found between specific gliadins (Branlard et al. 1994, Johansson 1996) and bread-making quality, as well as between particular HMW subunits of glutenin and bread-making quality (Payne et al. 1987; Uhlen 1990; Johansson et al. 1993). Cultivars can be given a Glu-1 quality score according to their HMW glutenin subunit composition (Payne et al. 1987). Also a rye-adjusted Glu-1 quality score has been calculated for cultivars containing the 1BL/1RS rye translocation (Payne et al. 1987).

Polymeric proteins play as well a critical role for wheat flour processing properties. Both amount and size distribution of the polymeric proteins have been shown to be important. They can be measured using size exclusion high-performance liquid chromatography (SE-HPLC) techniques after extraction of proteins using sonication (Gupta et al. 1993).

The dependence between bread-making quality, and protein concentration and composition can be taken advantage of by wheat breeders. In the Lithuanian wheat-breeding programme, high priority is given to the possibility to produce cultivars with good quality properties. The gluten strength of the Lithuanian cultivars should also fit the desire of many different users.

The aim of our work was to investigate the protein concentration and composition in commercially grown Lithuanian wheats. A better knowledge about this is of importance for Lithuanian breeders and also for breeders in the other Baltic and Nordic countries.