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ALLELIC VARIATION AT HIGH MOLECULAR WEIGHT GLUTENIN LOCI ASSOCIATED WITH SUPERIOR BREADMAKING CHARACTERISTICS IN HEXAPLOID INTROGRESSIVES OF *TRITICUM AESTIVUM* L. AND *TRITICUM DURUM* DESF.

ABSTRACT

The aim of this study was to determine the composition of HMW glutenin alleles at the loci *Glu-A1*, *Glu-B1* and *Glu-D1* in introgressives derived from interspecific hybrids of various strains and varieties of *Triticum aestivum* L. and *Triticum durum* Desf. For locus *Glu*-A1, the following subunits were detected: 1 (16.9%), 2* (20.3%), and the null subunit N (62.7%). These subunits are coded for by the alleles a, b and c, respectively. For locus *Glu*-B1, the following subunits and subunit combinations were detected: 7 (18.6%), 7+8 (15.2%), 7+9 (47.4%), 6+8 (15.2%), 6+7+8 (1.7%) and 6+7+8+9 (1.7%). The first four subunit pairs are coded for by alleles a, b, c and d, respectively. The alleles that code for the last two subunit combinations have not yet been identified. For locus *Glu*-D1, the following subunit combinations were detected: 2+12 (35.6%), 5+10 (44.1%), 5+12 (6.8%), 2+5+10 (1.7%) and 2+5+10+12 (11.9%). The first two subunit pairs are coded for by alleles a and d, respectively. The alleles that code for the last three subunit combinations have not yet been identified. Three to seven HMW-glutenin subunit bands were found in each introgressive. Twenty two different combinations of subunits coded at the three loci were identified.

Key words: grain quality, introgressives of wheat *T. aestivum* L./*T. durum* Desf., loci *Glu*-1, HMW-glutenin subunits, *Triticum aestivum* L., *Triticum durum* Desf.

INTRODUCTION

Over the last twenty years, significant progress has been made in elucidating the genetics of glutenin proteins in hexaploid wheat (*Triticum aestivum* L.). This was made possible by improved methods of protein fractionation and by the greater availability of suitable gene donors.

High-molecular-weight (HMW) glutenins make up only a small proportion of the total storage proteins in wheat. Nevertheless, they play a very important role in bread-making.

When HMW glutenin is analyzed using SDS-PAGE, three to five distinct bands are visible. These bands represent different glutenin subunits, which are coded for by genes at the loci *Glu*-A1, *Glu*-B1 and *Glu*-D1 on the long arms of the homologous chromosomes 1A, 1B and 1D (Payne and Lawrence, 1983). Each of these loci

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is multiallelic, so that hundreds of genotypic combinations are possible in *T. aestivum*varieties.

The combination of HMW glutenin subunits plays an important role in determining grain quality in wheat (Payne *et al.*, 1981, 1983; Marchylo *et al.*, 1992; Branlard and Dardevet, 1995; Nakamura 2000 a, b, 2001; Wieser and Zimmermann, 2000; Branlard *et al.*, 2001; Shevry *et al.*, 2001; Gianibelli *et al.*, 2002). Recently, an important goal in wheat breeding has been the development of new, high-yielding varieties with improved bread-making properties. Wheat for making bread is requred to meet certain standards in terms of protein content and protein quality (Klockiewicz-Kamińska and Brzeziński, 1997).

In traditional breeding, genetic combinations of high-quality HMW glutenin subunits are obtained by crossing parental strains carrying different HMW subunits and selecting the progeny with the help of SDS-sedimentation and SDS-PAGE. However, improving grain quality in wheat with traditional techniques can be very difficult because of the limited availability of hexaploid donor candidates. This has prompted research in molecular techniques based on inserting and introgressing new genes to obtain high-quality combinations of HMW glutenin subunits (Flavell and Payna, 1987; Alvarez *et al.*, 2000; Lukaszewsky *et al.*, 2000; De Pace *et al.*, 2001; Lafferty and Lelley, 2001; Shevry *et al.*, 2001; Ballesteros *et al.*, 2003).

Among the most promising sources of new genes that may be useful in improving quality in wheat are species of the family Poaceae (Branlard *et al.*, 1989; D'Ovidio *et al.*, 1992; Ahmad *et al.*, 1997; Pilch *et al.*, 1999; Pilch 2002 b, 2005; Mesfin *et al.*, 2000).

The aim of this study was to determine the composition of HMW glutenin alleles at the loci *Glu-A1*, *Glu-B1* and *Glu-D1* in introgressives derived from interspecific hybrids of various strains and varieties of *Triticum aestivum* L. and *Triticum durum* Desf. The introgressives chosen for the study exhibited a high degree of grain quality.

MATERIALS AND METHODS

The study was conducted on sixty-four introgressives of *T. aestivum* and *T. durum.* Thirty-seven of the introgressives were F6 progeny, twenty-four were F9 progeny, and three were F13 progeny. The introgressives were provided by the Plant Breeding and Acclimatization Institute in Cracow.

The introgressives were interspecific generative hybrids of monosomic (2n=41) varieties of *T. aestivum* and *T. durum*. The *T. aestivum* varieties used were 'Favorit' and 'Chinese Spring', and the *T. durum* varieties used were 'Mirable', 'Khapli' and 'Fuensemiduro' (Pilch, 1996). All of the introgressives had improved spike traits because of the introgression of alien genes from *T. durum* (Pilch, 2000 b). Pedigrees, generations and the number of lines for each cross-combination are presented in Table 1.

The introgressives were cultivated using standard agrotechnical techniques, including fertilization with nitrogen and mineral supplements. Herbicides and pesticides were not used.

Table 1

Pedigrees of 64 introgressives investigated

No.	Cross-combination and generation	Number of lines
1.	*T. aestivum* L. v.TAW 125974/84 × /(T.aesivum L v.ChS - *T. durum* Desf. v. Mirable) T aestivum L. v. CHD 661/, F_6	4
2.	*T. aestivum* L. v. SMH 7843 × (*T. aestivum* L. v.ChS - *T. durum* Desf. v. Fuensemiduro), F_6	2
3.	*T. aestivum* L. v. OLH 2925/17 × (*T. aestivum* L. v. ChS - *T. durum* Desf. v. Mirable), F 6	2
4.	*T. aestivum* L. v. OLH 3095/3 × / (*T. aestivum* L. v. ChS - *T. durum* Desf. v. Fuensemiduro) *T. aestivum* L. v. M. Marksmann /, F 6	2
5.	*T. aestivum* L. v. STH 3432 × /(*T. aestivum* L. v. ChS - *T. durum* Desf. v. Mirable) *T. aestivum* L. v. CHD 661 /, F 6	1
6.	/(*T. aestivum* L. v.ChS - *T. durum* Desf. v. Fuensemiduro) *T. aestivum* L. v. M.Marksmann/ × *T. aestivum* L. v. SMH 2843, F 6	1
7.	(*T. aestivum* L. v. mono-5B Favorit - *T. durum* Desf. v. Fuensemiduro), F 6	4
8.	(T.aestivum L. v.mono-5BFavorit-*T. durum* Desf.v. Fuensemiduro) *T. aestivum* L. v. AND 166, F 6	2
9.	(T.aestivum L. v. mono-5B Favorit - *T. durum* Desf. v. Fuensemiduro) *T. aestivum* L. v. OLH 3095/3, F 6	1
10.	*T. aestivum* L.v.STH 290 × (*T. aestivum* L.v ChS -*T. durum* Desf.v.Khapli), F6	2
11.	*T. aestivum* L. v. STH 290 × /(*T. aestivum* L. v ChS - *T. durum* Desf. v. Fuensemiduro) *T. aestivum* L. v. M. Marksmann/, F 6	2
12.	*T. aestivum* L. v. STH 5576 × /(*T. aestivum* L. v ChS - *T. durum* Desf. v. Fuensemiduro) *T. aestivum* L. v M.Marksmann/, F 6	1
13.	*T. aestivum* L. v. STH 5576 × /(*T. aestivum* L.v.ChS - *T. durum* Desf. v. Mirable) *T. aestivum* L. v. CHD 661/, F 6	1
14.	*T. aestivum* L. v. STH 5576 × /(*T. aestivum* L. v. ChS - *T. durum* Desf. v. Mirable) *T. aestivum* L. v. M.Marksmann/, F 6	1
15.	*T. aestivum* L. v. STH 7430 × /(*T. aestivum* L. v.ChS - *T. durum* Desf. v. Mirable) *T. aestivum* L. v. M.Marksmann/, F 6	1
16.	*T. aestivum* L. v. STH 8663 × /(*T. aestivum* L. v. ChS - *T. durum* Desf. v. Fuensemiduro) *T. aestivum* L. v. M.Marksmann/, F 6	2
17.	*T. aestivum* L. v. STH 8663 × /(*T. aestivum* L. v. ChS - *T. durum* Desf. v. Mirable) *T. aestivum* L. v. CHD 661/, F 6	1
18.	*T. aestivum* L. v. Milan x(*T. aestivum* L. v. ChS - *T. durum* Desf. v. Fuensemiduro), F 6	5
19.	*T. aestivum* L. v. OLH 535 × (*T. aestivum* L. v. ChS - *T. durum* Desf. v. Mirable), F 6	1
20.	*T. aestivum* L. v. AND 103/84 × /(*T. aestivum* L. v. ChS - *T. durum* Desf. v. Mirable) *T. aestivum* L. v. CHD 661/, F 6	1
21.	(*T. aestivum* L. v. mono-5B Favorit - *T. durum* Desf . v. Mirable), F 9	24
22.	(*T. aestivum* L. v. ChS - *T. durum* Desf. v. Mirable) × *T. aestivum* L. v. CHD 661, F 13	1

After harvest, the grain from each introgressive was analyzed in accordance with the standard procedures for hexaploid wheat described by Pilch *et al.* (1999). The following data were recorded: protein content, Zeleny sedimentation volume and falling number. The introgressives were categorized into quality groups as described by Klockiewicz-Kamińska and Brzeziński (1997). The *T. aestivum* variety 'Begra' was used as the standard.

HMW glutenin subunits were matched with the corresponding alleles at loci *Glu*-A1, *Glu*-B1 and *Glu*-D1 as described by Payne and Lawrence (1983). The subunits were separated and identified using SDS-PAGE in accordance with the method described by Zillman and Bushuk (1979). The standards used were the

hexaploid *T. aestivum* varieties 'Favorit' and 'Chinese Spring' and the tetraploid *T. durum* varieties 'Mirable', 'Khapli' and 'Fuensemiduro'.

RESULTS AND DISCUSSION

Breadmaking quality in *T. aestivum* was improved by introgressive hybridization with wild relatives. The sixty-four introgressives evaluated in this study were assigned to quality groups E and A because of their high values for protein content, Zeleny sedimentation volume and falling number. In all cases, the values for these parameters were the same as or higher than in 'Begra' (Tables 2 and 3). This agrees well with previous reports that genes from *T. durum* can be used to improve grain quality in *T. aestivum* (Pilch *et al.*, 1999; Pilch, 2002, 2005).

Table 2.

Critical limits of the quality classes for 64 introgressives, in relation to the check variety Begra

Class	Protein content [%]	Sedimentation [ml]	Falling number [s]
E	> 14.3	> 57.0	>271.0
A	13.2 - 14.2	43.0 - 56.9	231.0 - 270.9
B	12.6 - 13.1	29.0 - 42.9	191.0 - 230.9
C	< 12.5	< 28.9	< 190.9
Begra	13.7	50.0	360.0

Table 3

Number of the introgressives in the several quality classes

Class	Protein [%]	Sedimentation [ml]	Falling number [s]
E	47	20	62
A	17	44	2
B	0	0	0
C	0	0	0

The composition of HMW glutenin and its effect on bread-making quality have been studied in practically every major wheat-producing country. Data on HMW glutenin subunits from 1,150 medium-quality and low-quality varieties has been gathered in many countries, including Japan, China, Argentina, Canada, Germany, the Soviet Union, Australia and Poland (Nakamura, 2000 a, 2001; He Zhong-hu *et al.*, 1992; Gianibelli *et al.*, 2002; Marchylo *et al.*, 1992; Rogers *et al.*, 1989; Morgunov *et al.*, 1990; Ma *et al.*, 2003; Brzezinski, 2003). Analysis of these data has helped researchers determine the diversity of HMW glutenin in *T. aestivum* varieties.

SDS-PAGE was used to analyze 367 superior-quality *T. aestivum* varieties cultivated in Argentina, China, Germany, the Soviet Union and Slovakia. All of the subunits coded at the locus *Glu*-A1 were detected. The following subunits and subunit pairs coded at the locus *Glu*-B1 were detected: 7, 7+8, 7+9, 6+8, 20, 13+16 and

17+18. The following subunit pairs coded at the locus *Glu*-D1 were detected: 2+12, 3+12, 4+12 and 5+10 (Rogers *et al.*, 1989; Morgunov *et al.*, 1990; He Zhong-hu *et al.*, 1992; Nakamura, 2000 b; Gianibelli *et al.*, 2002; Galova *et al.*, 2002

Of the subunits coded by *Glu*-A1, 2* was detected in 49% of the varieties, and 1 was detected in 39% of the varieties. Of the subunits and subunit pairs coded by *Glu*-B1, 7+9 was detected in 50% of the varieties, and 7+8 was detected in 39% of the varieties. Of the subunits and subunit pairs coded by *Glu*-D1, 5+10 was detected in 57% of the varieties, and 2+12 was detected in 42% of the varieties. The subunits and subunit pairs most frequently detected were 2* and 1 for locus *GLU*-A1, 7+9 and 7+8 for locus *Glu*-B1, and 5+10 and 2+12 for locus *Glu*-D1.

The varieties from the Soviet Union carried either the allele a or the allele *c* at *Glu*-A1. The allele a codes for subunit 1, and the allele *c* is the null allele. At locus *Glu*-D1, they carried the alleles for either of two subunit pairs: 5+10 or 2+12. These two pairs have been reported to have opposing effects on bread-making quality (Morgunov *et al.*, 1990).

The varieties from the Hokuriku area in Japan had a unique combination of glutenin subunits: 1, 17+18 and 5+10 (Nakamura 2001). This subunit composition was found in modern high-quality varieties, which indicates that genetic material from other countries had been introduced into the local variety.

In one study on high-quality *T. aestivum* varieties, the frequency of HMW glutenin subunits was determined (Brzeziński, 2003). The following subunits and subunit pairs were found:

– Locus *Glu*-A1: 1, 2* and N (null). The most common subunit was the N subunit, which was found in 60% of the varieties examined;
– Locus *Glu*-B1: 7+8, 7+9 and 6+8. The most common subunit was 7+9, which was found in 60% of the varieties examined; and
– Locus *Glu*-D1: 2+12 and 5+10. The most common subunit was 5+10, which was found in 70% of the varieties examined.

Five different combinations of subunits and subunit pairs were found (here and elsewhere in this paper, combinations of subunits and alleles are presented in the following order: *Glu*-A1, *Glu*-B1, and *Glu*-D1):

– N, 7+8, 5+10;
– N, 7+9, 5+10;
– 1, 7+9, 5+10;
– 1, 6+8, 2+12; and
– 2*, 7+9, 2+12.

The first two combinations listed above were the ones most frequently found in the varieties examined (Brzeziński, 2003).

In the present study, the following subunits and subunit combinations were found (Table 4):

– Locus *Glu*-A1: 1 (16.9%), 2*(20.3%), and N (62.7%). These subunits are coded for by the alleles *a*, *b* and *c*, respectively. The most common subunit was the N subunit, which was found at about the same frequency as in high-quality varieties from Germany, Slovakia and Poland varieties (Rogers *et al.*, 1989; Galova *et al.*, 2002; Brzeziński, 2003).

– Locus *Glu*-B1: 7 (18.6%), 7+8 (15.2%), 7+9 (47.4%), 6+8 (15.2%), 6+7+8 (1.7%) and 6+7+8+9 (1.7%). The first four subunit pairs are coded for by alleles *a* , *b*, *c* and *d*, respectively. The alleles that code for the last two subunit combinations have not yet been identified. The most common subunit was 7+9, which was found at about the same frequency as in high-quality varieties from China, Germany, the Soviet Union, Slovakia and Poland (He Zong-hu *et al.*, 1992; Rogers *et al.*, 1989; Morgunov *et al.*, 1990; Galova *et al.*, 2002; Brzeziński, 2003).
– Locus *Glu*-D1: 2+12 (35.6%), 5+10 (44.1%), 5+12 (6.8%), 2+5+10 (1.7%) and 2+5+10+12 (11.9%). The first two subunit pairs are coded for by alleles *a* and *d*, respectively. The alleles that code for the last three subunit combinations have not yet been identified. The most common subunit was 7+9 which was found at about the same frequency as in high-quality varieties from Argentina, Germany, the Soviet Union, Slovakia and Poland (Gianibelli *et al.*, 2002; Rogers *et al.*, 1989; Morgunov *et al.*, 1990; Galova *et al.*, 2002; Brzeziński, 2003).

Table 4

Allelic frequencies for the loci *Glu-1*

Allele	Subunit	Frequency [%]
	Glu-A1	
a	1	16.9
b	2*	20.3
c	N	62.7
	Glu-B1	
a	7	18.6
b	7+8	15.2
c	7+9	47.4
d	6+8	15.2
?	6+7+8+9	1.7
?	6+7+8	1.7
	Glu-D1	
a	2+12	35.6
d	5+10	44.1
?	5+12	6.8
?	5+10+2+12	11.9
?	2+5+10	1.7

In the present study, the following alleles were found in the introgressives investigated using SDS-PAGE (Table 4):

– Locus *Glu*-A1: *a*, *b* and *c*, which code for 1, 2* and N, respectively;
– Locus *Glu*-B1: *a*, *b*, *c* and *d*, which code for 7, 7+8, 7+9 and 6+8; and
– Locus *Glu*-D1: *a* and *d*, which code for 2+12 and 5+10.

Three to seven HMW-glutenin subunit bands were found in each introgressive. Twenty two different combinations of subunits were identified (Table 5). Each combination was found in one to eleven of the sixty-four introgressives. The com-

binations and frequencies observed in the introgressives differed from those reported for all of the high-quality *T. aestivum* varieties from the major wheat-producing countries discussed above, but were very similar to those reported for varieties registered in Poland.

Table 5

Allelic compositions at *Glu-1* loci in introgressives and parental varieties

Alleles at loci			Subunit combinations			Frequency [%]
A1,	B1,	D1				
c	c	d	N	7+9	5+10	11.9
c	a	a	N	7	2+12	11.9
c	d	d	N	6+8	5+10	8.5
c	c	a	N	7+9	2+12	6.8
c	b	a	N	7+8	2+12	6.8
a	c	d	1	7+9	5+10	6.8
b	c	d	2*	7+9	5+10	5.1
a	c	a	1	7+9	2+12	5.1
b	c	?	2*	7+9	5+12	5.1
c	b	?	N	7+8	5+10+2+12	3.4
b	b	d	2*	7+8	5+10	3.4
c	d	a	N	6+8	2+12	3.4
c	d	?	1	6+8	5+10+2+12	3.4
c	c	?	N	7+9	5+10+2+12	3.4
b	a	d	2*	7	5+10	3.4
c	?	d	N	6+7+8+9	5+10	1.7
a	a	?	!	7	2+5+10	1.7
c	b	d	N	7+8	5+10	1.7
c	a	?	N	7	5+10+2+12	1.7
b	c	a	2*	7+9	2+12	1.7
b	?	d	2*	6+7+8	5+10	1.7
c	c	?	N	7+9	5+12	1.7
			Parents			
c	b	a	N	7+8	2+12	Chinese Spring
b	c	d	2*	7+9	5+10	Favorit
?	Mirable	d	Mirable	6+8		Mirable
?	Khapli	?	Khapli	?		Khapli
?	Fuensemiduro	d	Fuensemiduro	6+8		Fuensemiduro

Designations: ? the alleles and subunits unidentified

In a previous study on British varieties, the combination 1, 17+18, 5+10 was found to be correlated with superior bread-making characteristics (Payne *et al.* 1987). This combination was not found in any of the introgressives examined in the present study.

Four of the five combinations previous found in high-quality Polish varieties by Brzeziński (2003) were found in the introgressives examined in this study. The only combination not found was 1, 6+8, 2+12. In this study, the combinations most

frequently found were N, 7+9, 5+10 (*c*, *c*, *d*) and N, 7, 2+12 (*c*, *a*, *a*). This agrees well with the study on high-quality Polish varieties, in which the combination most frequently found was N, 7+9, 5+10.

The subunits found and their relative frequencies may be correlated to the high level of grain quality in the introgressives examined in this study. However, other factors may also have played a role in determining the distribution of alleles at the three loci, including:

– the gene pool of the parental genotypes;
– selection pressure in favor of specific quality traits such as breadmaking characteristics;
– linkage with particular traits such as spike morphology and kernel morphology which had been continuously selected over generations of breeding; and
– linkage to genes of adaptive value which are advantageous in a particular geographical area.

T. durum varieties have generally been bred to exhibit a high frequency of high-quality alleles because of the demands of the food-processing industry. The introgressives chosen for this study had a particularly favorable distribution of high-quality genes derived from the paternal *T. durum* varieties (Pilch, 2002, 2005).

Some high-quality Polish varieties are heterogeneous and consist of two to three genotypes with different gluten protein alleles?) (Brzeziński, 2003).

Heterogeneity and superior bread-making characteristics are therefore not mutually exclusive. In fact, some of the introgressives examined in this study were heterogeneous and had superior bread-making characteristics.

Two factors determine the proportion of heterogeneous wheat varieties in a particular country. In some countries, stringent requirements for homogeneity compel breeders to develop homogeneous varieties. In countries without such requirements, opinion is divided on whether heterogeneity is desirable, and on which protocol should be used in a particular breeding program (Morgunov *et al.*, 1990).

When early generations are selected without regard to pedigree, there will be variability in various traits in later generations. Some breeders believe that a variety consisting of different biotypes gives more consistent yields because it can better adapt to changing environmental conditions. These breeders therefore do not aim at creating varieties that are completely homogeneous.

The relative frequencies of glutenin biotypes within a given variety may change drastically in response to environment conditions. This has been observed in ‘Saratovskaya 29’. Heterogeneity can be exploited to study and improve grain quality. Different biotypes of the same variety, especially those with identical gliadin patterns, can be considered to be almost isogenic. They provide useful material for research on the effect of different subunits on breadmaking characteristics and other traits.

Heterogeneity can also be used to directly improve quality in some varieties. For example, the Soviet varieties ‘Diana 3’ and ‘Saratovskaya 49’ both express the valuable subunit pair 17+18. However, they also express subunit pairs of lesser value: 6+8 in the case of ‘Diana 3’, and 7+9 in the case of ‘Saraovskaya 49’.

In the introgressives examined in this study, heterogeneity was primarily due to introgressive generative hybridization effects, namely, the instability of the introgressed genes from *T. durum*. This may be because the high-quality alien genes were associated with other superior traits such as spike morphology and kernel morphology which had been continuously selected over generations of breeding. Associations of this sort are possible because many of these genes are located on the long arms of chromosomes 1A, 1B and 1D (Pilch, 2003).

In a study on the geographical distribution of HMW glutenin alleles in populations of the wild tetraploid wheats in Israel, there was a significant correlation between the molecular weight of the HMW subunits encoded by Glu-A1-1 and the altitude, average temperature and rainfall of the region from which a particular population originated (Levy and Feldman, 1988). Similar correlations were found in hexaploid wheat varieties growing in different environments (Zhu and Khan, 2002).

In five of the introgressives examined in this study, some HMW glutenin subunits and subunit pairs corresponding to alleles of *Glu*-B1 and *Glu*-D1 were not identifiable with SDS-PAGE, even though they had been easily identified in the maternal *T. aestivum* varieties 'Favorit' and 'Chinese Spring' (Table 5). Their alleles may have been deleted, substituted or mutated so that they could not be expressed.

The same was true for some subunits and subunit pairs which had been identified in the paternal *T. durum* varieties 'Mirable', 'Khapli' and 'Fuensemiduro' (Tab. 5). This suggests that modified alleles had been transfered from 'Favorit' and 'Chinese Spring' to the *Glu*-A1 locus of 'Mirable', 'Khapli' and 'Fuensemiduro', and to the *Glu*-B1 locus of 'Khapli'. In some of the introgressives, this improved grain quality.

Modifications of this kind are possible in alleles coding for HMW glutenin subunits. Mutations of the allele *Glu*-B1a have been identified in the *T. aestivum* varieties 'Chinese Spring' and 'Cheyenne', in the *T. durum* variety 'Bidi', and in the wild species *T. turgidum*ssp. *dicoccoides* L. The mutated alleles contained either a duplication of a 54 bp sequence or the insertion of a 185 bp sequence in the "cereal-box" Bx promoter (Anderson *et al.*, 1998).

Another mutation containing a deletion of an 85 bp sequence in *Glu*-A1b has also been identified in the *T. aestivum* variety 'Cheyenne' (Forde *et al.*, 1985). In the Hungarian wheat variety 'Bankuti-1201', a point mutation was identified at bp 1181 in *Glu*-A1b. This caused a substitution of serine for cysteine in the 2* subunit. In *Oryza sativa*, a mutation in the *Glu*-A1allele *glu 4* produced a new allele, *glu 4a*, which codes for a new polypeptide, p16.50/a-1 (Qu *et al.*, 2003).

In the introgressives examined in this study, introgressions of alleles from *T. durum* were found only at *Glu*-A1 and *Glu*-B1. Transferability of subunit alleles in the *Glu*-1 loci during intervarietal, interspecific and intergeneric generative hybridization has been well documented.

In studies on lines with single-chromosome substitutions, genetic activity was detected not only on the chromosomes carrying the HMW glutenin alleles, but also on other chromosomes. In one study, bread loaf volume in 'Chinese Spring' was improved when the following chromosomes were replaced with their counterparts

from 'Cheyenne': 1A, 1B, 1D, 3A, 3B, 7A and 7B (Mansur *et al.*, 1990). In a similar study, bread loaf volume in 'Cappelle Deprez' was improved when the following chromosomes were replaced with their counterparts from 'Bezostaya 1': 1A, 1D, 4D, 4A, 5D, 6B and 6D (Krattiger *et al.*, 1987).

In a study on reciprocal substitutions between 'Cheyenne' and 'Wichita', flour quality was especially affected by substituting the chromosomes in Group 1, although other substitutions also had a significant effect. There was some evidence for interactions among genes on different chromosomes (Zemetra *et al.*, 1987). The introgression of chromosomes 1A, 1B, 1D from the high-quality variety 'Cheyenne' into the low-quality variety 'Chinese Spring' improved protein content, SDS-sedimentation volume, bread mixing time, and loaf volume (Rousset *et al.*, 2001).

Improvements in breadmaking quality have also been obtained by introgressing the alleles for several HMW glutenin subunits instead of whole chromosomes. In one study on crosses between *T. aestivum* varieties, the alleles for the following subunits and subunit pairs were introgressed: 8 and 7+8, which are coded at *Glu*-B1, and 2 and 2+12, which are coded at *Glu*-D1 (Rogers *et al.*, 2001). In another study, the alleles for subunits Ax and Ay at *Glu*-A1 were introgressed into *T. durum* from the diploid species *T. urartu* L. and *T. boeoticum* Boiss., and from the tetraploid species *T. dicoccoides* Tell. and *T. araraticum* L. There were significant improvements in protein content, gluten strength, and SDS-sedimentation volume (Dhalival *et al.*, 2002).

In one study in which alleles from the diploid species *T. boeoticum* Boiss. were introgressed into Swedish bread wheat, the introgressives produced a y-type subunit coded at Glu-A1 (Rogers *et al.*, 1997).

In another study, the alleles *Glu*-A1r and *Glu*-A1s were introgressed from the diploid species *T. boeoticum* Boiss. ssp. *thaoudar* into the *T. aestivum* variety 'Sicco'. *Glu*-A1r codes for the subunit pair 39+40, and *Glu*-A1s codes for the subunit pair 41+42. The subunit composition of 'Sicco' is 1, 7+9, 5+10. When *Glu*-A1a was replaced by *Glu*-A1r, there was a decrease in dough stickiness and an increase in stability during mixing. When *Glu*-A1a was replaced by *Glu*-A1s, there was a small improvement in gluten strength (Rogers *et al.*, 1997).

In another study, alleles for HMW gluten subunits were transfered from *Aegilops tauschii* Coss. to *T. aestivum.* There were significant changes in bread loaf volume, gluten index, maximum resistance, SDS-sedimentation volume and dough surface (Hsam *et al.*, 2001).

Breadmaking characteristics in high-yielding, poor-quality *T. aestivum* varieties can therefore be improved by introducing alleles for high-quality HMW glutenin subunits from other *T. aestivum* varieties or from closely related species in the family *Poaceae*. In other studies, breadmaking characteristics were improved when *Glu*-A1d, which codes for the subunit pair 5+10, was introgressed into varieties of rye and triticale (Łukaszewski and Curtis, 1992, 1994; Łukaszewsky *et al.*, 2000; Lafferty and Lelley, 2001).

In studies on synthetic wheat lines (AA BB DD), breadmaking quality was improved after the introgression of *Glu*-D1a (2+12) and *Glu*-D1*d* (5+10) (Peña *et al.*, 1995). However, when alleles of *Glu*-D1 from *Triticum tauschii* (Coss.) Schmal

were introgressed together with alleles of *Glu*-B1 and *Glu*-B3 from *Triticum turgidum* L., gene expression was very weak in comparison to the parental genotype. Gene silencing has also been observed in other studies in which genes were introgressed or substituted.

Interspecific generative hybridization of *T. aestivum* with *T. durum* has been used to improve grain quality in tetraploid species. In crosses of the type (4x x 6x) x 4x, *Glu*-1Dd was transferred to *T. durum* with the translocation 1AL/1DL (Vitellozzi *et al.*, 1997). In another study, *Glu*-1Dd was transferred from *T. aestivum* to tritordeum (Ballesteros *et al.*, 2003).

In other crosses between *T. aestivum* and *T. dicoccoides*, improvements in grain quality in *T. dicoccoides* were due the following subunit substitutions (Grama *et al.*, 1987):

– *Glu*-A1: 2*, 1;
– *Glu*-B1: 7+8, 17+18, and 13+16, 7+9; and
– *Glu*-D1: 5+10, 2+12.

In the present study, the novel subunit pair 5+12 coded by *Glu*-D1 was found in four of the introgressives examined (Table 4). This subunit pair has never been detected before in either *T. aestivum* or *T. durum*. This subunit pair improved grain quality, and all of the introgressives with this subunit had superior technological parameters.

The allele for this novel subunit pair probably arose as the result of breakage in other alleles at *Glu*-D1 in *T. aestivum*, such as those coding for the subunit pairs 2+12, 3+12, 4+12, 2.2+12 and 5+10. However, the mechanism at work in this case is still unknown. This change may have been caused by the effect of the *T. durum* chromosomes in the F1 generation during interspecific generative hybridization. *T. durum* chromosomes are known to cause changes in heterochromatic DNA in rye chromosomes in hexaploid triticale (Pilch, 1981 a, b; Pilch, 1987).

CONCLUSIONS

1. Grain quality was high in the introgressives examined in this study. This was evident for the improvement in protein content, Zeleny sedimentation volume and falling number in the introgressives from later generations.
2. The introgressives did not vary widely in terms of HMW glutenin subunit composition. Some had unique subunit combinations. Nine alleles in all were identified for the loci *Glu*-A1, *Glu*-B1 and *Glu*-D1. The frequency of subunits related to good bread-making quality was high. For example, the subunit pair 5+10 (*Glu*-D1*d*) was found in many of the introgressives. However, the subunits and subunit pairs 2+12 (*Glu*-D1*a*), 7+9 (*Glu*-B1*c*) and N (*Glu*-A1c) were also found in about the same proportion. It was therefore impossible to correlate good breadmaking characteristics with specific HMW-glutenin subunits using SDS-PAGE.
3. Not all of the introgressives in which the subunit pair 5+10 was found had good breadmaking characteristics. The presence of this subunit pair was correlated with high grain quality only in combination with certain subunits and subunit pairs coded at *Glu*-A1 and *Glu*-B1. In the literature, the subunit

pair 2+12 is often considered to be an indicator of poor breadmaking characteristics. In the introgressives examined in this study, the presence of 2+12 was correlated with superior technological parameters.

4. Grain quality is low in the maternal *T. aestivum* varieties 'Favorit' and 'Chinese Spring'. Therefore, high grain quality in the introgressives examined in this study was usually contributed by high-quality alleles from the paternal *T. durum* varieties. Some of the subunit combinations detected could not be identified with a corresponding allele with the help of SDS-PAGE. Furthermore, allelic changes at *Glu*-D1 increased the level of allelic variation, thereby improving the values for the technological parameters tested.
5. The *T. durum* varieties 'Mirable', 'Khapli' and 'Fuensemiduro' are promising donors of high grain quality in breeding programs aimed at improving *T. aestivum* by introgressive generative hybridization.

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