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Józef Pilch

Plant Breeding and Acclimatization Institute (IHAR), 30-420 Cracow, 4, Zawiła, Poland

IMPROVING GRAIN QUALITY IN WINTER WHEAT (*TRITICUM AESTIVUM* L) BY INTROGRESSING ALIEN HMW GLUTENIN GENES FROM TETRAPLOID *TRITICUM* AND DIPLOID *AEGILOPS* SPECIES:

A REVIEW

ABSTRACT

Significant progress has recently been made in elucidating the genetics of high molecular weight (HMW) glutenins and how they affect bread-making quality. There are hundreds of genotypes of *T. aestivum* with different combinations of alleles at loci *Glu-A1*, *Glu-B1* and *Glu-D*. The combination of alleles has a major effect on bread-making quality. Improving grain quality in *T. aestivum* is difficult with conventional methods. However, novel alleles and allele combinations from alien species can be inserted and introgressed into *T. aestivum* by using genetic engineering techniques.

This review describes variation in HMW-glutenin alleles in selected tetraploid *Triticum* species and diploid *Aegilops* species, and how introgressing these alleles into *T. aestivum* affects bread-making quality. The species included in this review are *T. durum* Desf., *T. dicoccum* Schubl., *T. turgidum* L., *T. dicoccoides* Schweinf., *Ae. squarrosa* L., *Ae. umbellulata* Zhuk., *Ae. comosa* Sibth. et Sm., and *Ae. markgrafii* L. All of the *Aegilops* species included in this review carry genes that code for HMW glutenins that are structurally homologous to those encoded by the locus *Glu-D1* in *T. aestivum*.

Key words: *Ae. squarrosa* L., *Ae. umbellulata* Zhuk., *Ae. comosa* Sibth. et Sm., *Ae. markgrafii* L., bread making quality, HMW glutenin subunits, introgression, *T. aestivum* L., *T. durum* Desf.., *T. dicoccum* Schubl., *T. turgidum* L., *T. dicoccoides* Schweinf.

INTRODUCTION

High molecular weight (HMW) glutenins are minor components that serve as storage proteins in *T. aestivum* L. and related species. They provide germinating seeds and growing seedlings with carbon, nitrogen and energy.

The composition of HMW glutenins affects the baking properties of bread wheat. Therefore, considerable attention has been focused on understanding the structure, function and genetics of HMW glutenins, and on using this un-

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derstanding to improve quality in wheat varieties. However, the molecular basis for differences in quality between HMW glutenin subunits is still unclear.

HMW glutenins consist of non-repetitive N-terminal and C-terminal amino acid sequences flanking a large central repetitive domain. X-type subunits contain tri-peptide repeat units, whereas y-type subunits contain hexa-peptide and nona-peptide repeat units.

The N-terminal region usually contains three to five cysteine residues, whereas the C-terminal region usually contains only one. These residues can form intermolecular di-sulfide bridges, thereby forming large polymers. The nature of these polymers determines the visco-elastic properties of the dough made from a particular wheat variety.

The central repetitive domain has a beta-spiral structure, which makes the protein molecule elastic (Gianibelli *et al.*, 2001). In addition to di-sulfide bridges, di-tyrosine cross links may also play an important role in determining the structure and function of glutenin polymers.

There are more consensus-type repetitions in subunit 1Dy10 than in subunit 1Dy12. Therefore, the pattern of repetitive beta-turns is more regular in subunit 1Dy10.

In the N-terminal region of subunit 1Bx20, two cysteine residues are replaced by tyrosine. This reduces the number of di-sulfide bridges and changes the pattern of di-sulfide bridges in the polymers. The dough is weaker than dough made from wheat containing the subunit 1Bx7, in which the cysteine residues are not replaced by tyrosine (Shewry *et al.*, 2003).

Subunit 1Dy12.1^{s} of *Aegilops squarrosa* is very similar in sequence and structure to subunit 1Dy10 of *Triticum aestivum*, which is associated with superior bread-making properties. Both subunits contain seven cysteine residues and a similar proportion of hexa-peptide and nona-peptide repeats (Yan *et al.*, 2004). Subunit 1Dy12.1^{s} of *Ae. squarrosa* may therefore prove useful in improving bread-making quality.

In hexaploid *Triticum aestivum*, there are three to five main bands of HMW glutenins. These bands correspond to the loci *Glu-A1*, *Glu-B1* and *Glu-D1*, which lie on the long arms of the homologous chromosomes *1A*, *1B* and *1D*. Each locus consists of two genes. One gene codes for the x-type subunit, which has a low electrophoretic mobility. The other gene codes for the y-type subunit, which has a high electrophoretic mobility (Payne and Lawrence, 1983).

There is a high degree of multi-allelism at each of the three loci. In diploid, tetraploid and hexaploid varieties of wheat, there are 22 alleles at *Glu-A1*, 56 alleles at *Glu-B1*, and 65 alleles at *Glu-D1*. There are therefore thousands of possible combinations of HMW glutenin subunits that determine grain quality (Mclntosh *et al.*, 2003; Payne *et al.*, 1981, 1983; Marchylo *et al.*, 1992; Branlard and Dardevet, 1995; Nakamura, 2000a, 2000b, 2001; Wieser and Zimmermann, 2000; Branlard *et al.*, 2001; Shevry *et al.*, 2001; Gianibelli *et al.*, 2002a, b).

The combination of alleles affects HMW glutenin both quantitatively and qualitatively. First, the total amount of HMW glutenin depends on whether there are three, four or five subunits. Second, the structure and properties of a particular subunit depend on the genetic sequence of the allele which codes for it.

Glu-D1 subunit 5+10 is associated with high gluten visco-elasticity and good dough properties (Redaelli *et al.*, 1997). This subunit pair is more consistently associated with good bread-making quality than other *Glu-D1* subunit pairs such as 2+12, 3+12 and 4+12 (Payne *et al.*, 1987).

In common wheat varieties from the the United States, subunit pair 5+10 is the most important factor that determines dough mixing properties (Dong *et al.*, 1991). Most Canadian western red spring wheat varieties have this subunit pair (Bushuk, 1998). In Germany, nine of the eleven best wheat varieties on the National List of Commercial Cultivars (BSA 1999) also have this subunit pair (Wieser and Zimmermann, 2000). Subunit pair 5+10 is also often found in superior bread wheat cultivars grown in the United Kingdom, Norway and Syria (Payne *et al.*, 1987; Uhlen, 1990; MirAli *et al.*, 1999).

The HMW glutenin subunits that are associated with superior quality in bread wheat varieties have been identified. Since then, there has been much research on HMW glutenin subunits in gene banks of wheat and related wild species aimed at identifying novel alleles that may be used in future breeding and genetic engineering programs. Nevertheless, much research remains to be done on orthologous HMW glutenins in species other than *T. aestivum*.

In barley, D-hordeins are encoded by the locus *Hor 3* on Chromosome 5. These proteins may be structurally related to HMW glutenins in wheat (Halford, 1992). In rye, locus *Glu*-R1 codes for one x-type subunit and one y-type subunit, and is thus similar to locus *Glu-1* in wheat (de Bustos *et al.*, 2001). HMW glutenin subunits are encoded by genes located on chromosome IE in *Elytrigia elongata*, and on chromosome IV in *Dasypyrum villosum* L. (Dvorak *et al.*, 1986; Blanco *et al.*, 1991; De Pace *et al.*, 2001).

In many species of the genus *Aegilops*, HMW glutenin subunits are encoded by chromosomal loci that are similar to locus *Glu-1* in *T. aestivum* (Williams *et al.*, 1993; Pfluger *et al.*, 2001 a; Rodriguez-Quijano *et al.*, 2001; Wan *et al.*, 2002; Yan *et al.*, 2003). The primary structure of the *Aegilops* HMW glutenin subunit is similar to that of wheat subunits, but has novel modifications that are not found in wheat subunits.

For example, *Aegilops cylindrica* has three novel HMW glutenin gene sequences that are expressed as the subunits 1Cx, 1Cy and 1Dy. The HMW glutenin molecule also has an additional cysteine residue near the end of its repetitive domain (Wan *et al.*, 2002). Subunit 1Dx 5 is highly associated with superior dough properties.

Structural variants of HMW glutenin subunits from *Aegilops* species may be useful in improving bread wheat varieties. This may be accomplished by inserting and introgressing promising alleles into the wheat genome in order to

obtain new combinations of HMW glutenin subunits (Flavell and Payne, 1987; Ceoloni *et al.*, 1998; Alvarez *et al.*, 2000; Lukaszewsky *et al.*, 2000; De Pace *et al.*, 2001; Lafferty and Leiley, 2001; Shewry *et al.*, 2001; Ballesteros *et al.*, 2003a, b).

Diploid, tetraploid and hexaploid species of *Triticum* and *Aegilops* may also provide many alleles that can be used to improve quality in varieties of hexaploid *T. aestivum* (Branlard *et al.*, 1989; D'Ovidio *et al.*, 1992 a, b; Ahmad *et al.*, 1997; Mesfin *et al.*, 2000).

Technological parameters in winter wheat have been improved by interspecific and intergeneric hybridization (Pilch *et al.*, 1999; Pilch, 2002, 2005a, 2005b, 2006b).

The aim of this paper is to review the literature on HMW glutenin alleles in selected tetraploid *Triticum* species and diploid *Aegilops* species in order to identify those alleles that can be recommended for use in breeding programs designed to improve bread-making properties in winter wheat.

The tetraploid (AABB) *Triticum* species included in this review are *T. durum* Desf., *T. dicoccum* Schubl., *T. turgidum* L. and *T. dicoccoides* Schweinf. The diploid *Aegilops* species included in this review are *Ae. squarrosa* L. (DSDS), *Ae. umbellulata* Zhuk. (UU), *Ae. comosa* Sibth. et Sm. (MM), and *Ae. markgrafii* L. (CC). All of the *Aegilops* species included in this review carry genes that code for HMW glutenins that are structurally homologous to those encoded by locus *Glu-D1* in *T. aestivum*.

ALLELIC VARIATION IN TETRAPLOID SPECIES OF *TRITICUM*

Tetraploid wheats with strong gluten properties are generally used for making pasta products with superior cooking characteristics. A strong elastic gluten is essential for preserving the integrity of pasta during cooking so that it remains firm and resilient. Variation in gluten elasticity and strength is usually attributed to differences in aggregative behavior among glutenins. These proteins include high molecular weight fractions encoded by the genomes A and B. In diploid, tetraploid and hexaploid wheat species, twenty-two alleles (a to v) have been identified at locus *Glu-A1*, and fifty-six alleles (a to bd) have been identified at locus *Glu-B1*. Not all are found in tetraploid wheat species (McIntosh *et al.*, 2003).

T. durum, *T. dicoccum*, *T. turgidum* and *T. dicoccoides* represent new sources of alleles at loci *Glu-A1* and *Glu-B1* (Tabs. 1 and 2). These species lack some of the alleles that are commonly found in *T. aestivum*, probably because they have different origins than present-day bread wheat varieties and because of the extensive use of the A and B genomes in breeding programs. Some alleles in *T. aestivum* may also represent mutations which arose only in that species.

On the other hand, some subunits found in tetraploid species are not found in *T. aestivum*. For example, subunits and subunit pairs 1", 2* *, 2* * *, 7+15 and 6+16 are found in *T. durum*, but not *T. aestivum*. Allelic diversity is correlated with bread-making quality, which indicates that the high frequency of desir-

able alleles such as *Glu-A1* a, *Glu-A1* b and *Glu-B1* c may be the result of more than a century of breeding guided by conventional technological tests.

T. durum

T. durum is a major crop in Mediterranean countries, but can also grow in more extreme climate zones. *T. durum* is used in making pasta, semolina, macaroni and couscous. The make-up of the storage proteins gliadin and glutenin determine quality in these products.

There are seven alleles at locus *Glu-A1* in *T. durum*. The most frequent is allele c. Four of these alleles are not found in *T. aestivum*: III, IV, V and VI. Alleles V and VI are also not found in *T. dicoccum*, *T. turgidum* and *T. dicoccoides* (Table 1; Vallega, 1988; Branlard *et al.*, 1989).

Table 1

Allelic variation at *Glu-A1* locus HMW-GS of *T. aestivum* L. and tetraploid species of *Triticum* L.

Allele	Subunit composition	*T. aestivum* L.	*T. durum* Desf.	*T. dicoccum* Schubl	*T. turgidum* L.	*T.dicoccoides* Schweinf.
a	1	+	+	+	+	
b	2*	+	+	+	+	+
c	Null	+	+	+	+	
j	III			+		
VII	VII			+		
I	(a)			+		
II	(a)			+		
III	1'		+	+		
IV	1"		+		+	
V	2**		+			+
VI	2***		+			+
w	2.1*					+
h	I					+
i	II					+
II	Null					+
III	III					+
IV	1					+
VI	1					+
v	VII					
ay	Null					
az	III					

Glu-A1 allele III codes for subunit 1', and allele IV codes for subunit 1''. Both of these alleles are rare and have been found only in varieties from Spain and Portugal.

Allele *Glu-A1* V codes for subunit 2**. This allele has a low frequency (2.9%) and has been found in varieties from Turkey, the Soviet Union, Yugoslavia, Ethiopia and IndiAe. Subunit 2** has a mobility between subunits 2* and 5.

Table 2.

Allelic variation at *Glu-B1* locus HMW-GS of *T. aestivum* L. and tetraploid species of *Triticum* L.

Allele	Subunit composition	*T. aestivum* L.	*T.durum* Desf.	*T. dicoccum* Schubl	*T. turgidum* L.	*T.dicoccoides* Schweinf.
1	2	3	4	5	6	7
a	7	+	+	+		
b	7+8	+	+	+	+	
c	7+9	+				
d	6+8	+	+	+	+	
e	20	+	+	+	+	
f	13+16	+	+		+	
g	13+19	+	+	+		
h	14+15	+		+	+	
i	17+18	+	+			+
j	21	+				+
k	22	+		+		+
				+		+
m	I					+
n	II			+		
o	III			+		
p	IV			+		
q	V			+		
r	VI			+		
XV	XV			+		
XVI	XVI			+		
XVII	XVII			+		
I	(a)			+		
II	(a)			+		
III	(a)			+		
IV	23+18			+	+	
V	(a)		+	+		
VI	19			+		
XII	7+15		+	+		
XIII	6+16		+			
XIV	23+22		+			
VII	?		+		+	
VIII	?		+		+	+
IX	?		+			+
X	?		+			+
XI	?		+			+

Table 2.

Allelic variation at *Glu-B1* locus HMW-GS of *T. aestivum* L. and tetraploid species of *Triticum* L. (continued)

Allele	Subunit composition	T. aestivum L.	T.durum Desf.	T. dicoccum Schubl	T. turgidum L.	T.dicoccoides Schweinf.
1	2	3	4	5	6	7
			+			+
m	Null					+
v	7+8					+
II	II					+
III	6+8					+
IV	IV					+
VI	VI					+
ay	XVI					
az	XVII					
be	?					
bf	?					

Glu-A1 allele VI codes for subunit 2***. This allele is very rare and has been found only in varieties from RussiAe. Subunit 2*** has a mobility that is higher than that of subunit 2**.

There are seventeen alleles at locus *Glu-B1* in *T. durum*. The most frequent are alleles b, d and e. Seven of these alleles are also found in *T. aestivum*: a, b, d, e, f, h and g. Ten are not found in *T. aestivum*: III, IV, VII, VIII, IX, X, XI, XII, XIII and XIV. Alleles XII, XIII and XIV are also not found in *T. dicoccum*, *T. turgidum* and *T. dicoccoides* (Table 2; Vallega, 1988; Branlard *et al.*, 1989).

Glu-B1 allele IV codes for subunit pair 23+18. It has a low frequency (4.3%). Subunit pair 23+18 has the same mobility as subunit pair 13+19, which is encoded by the allele g, which has a very low frequency (0.4%).

Glu-B1 allele XII codes for subunit pair 7+15. This allele has a frequency of 6.8%, and has been found in varieties from France and RussiAe. Subunit pair 7+15 has a mobility that is similar to subunit 7, which is encoded by allele a, and to subunit pair 14+15, which is encoded by allele h.

Glu-B1 allele XIII codes for subunit pair 6+16. It has a very low frequency (2.1%), and has been found in varieties from Spain, France, Bulgaria, Turkey and Russia.

Glu-B1 allele XIV codes for subunit pair 23+22. It has a very low frequency (0.2%).

T. dicoccum

T. dicoccum is also known as emmer wheat or farrum, and is a primitive hulled species that was once widely cultivated. Currently, this species survives in marginal farming areas in Spain, Italy, the Balkans, and Turkey, where it is associated with traditional agriculture. *T. dicoccum* is used for both livestock feed and human consumption.

T. dicoccum is vastly inferior in quality to *T. durum* and *T. aestivum*. Analysis of the glutenins and gliadins in *T. dicoccum* has revealed that there is a considerable amount of allelic variation at the loci that code for these proteins. *T. dicoccum* therefore promises to be a useful and diverse source of genes for breeding *T. durum* and *T. aestivum* varieties that are more readily digestible and that can be consumed by people with celiac disease.

There are eight alleles at locus *Glu-A1* in *T. dicoccum*. The most frequent is allele a, which codes for subunit 1. Three of these alleles are also found in *T. aestivum*: a, b and c. Five are not found in *T. aestivum*: j, I, II, III and VII. Alleles j, II, III and VII are also not found in *T. durum*, *T. turgidum* and *T. dicoccoides* (Table 1; Vallega, 1988; Branlard *et al.*, 1989; Pfluger *et al.*, 2001 b).

There are twenty-three alleles at locus *Glu-B1* in *T. dicoccum*. The most frequent are alleles b, d, IV and VI. Eight of these alleles are also found in *T. aestivum*: a, b, d, e, g, h, j and k. Fifteen are not found in *T. aestivum*. Thirteen are also not found in *T. durum*, *T. turgidum* and *T. dicoccoides* (Table 2; Vallega, 1988; Branlard *et al.*, 1989; Pfluger *et al.*, 2001 b).

Glu-B1 allele XV codes for subunit XV, which has a slightly higher mobility than subunit VI, which is encoded by allele r.

Glu-B1 allele XVI codes for subunit XVI, which has a slightly lower mobility than subunit VI.

T. turgidum

In *T. turgidum*, there are only sixteen different combinations of *Glu-A1* and *Glu-B1* alleles (Tabs. 1 and 2; Vallega and Mello-Sampayo, 1987). The combinations with the highest frequency are:

Glu-A1 a and *Glu-B1* b;
Glu-A1 a and *Glu-B1* d;
Glu-A1 a and *Glu-B1* e; and
Glu-A1 c and *Glu-B1* d.

There are four alleles at locus *Glu-A1* in *T. turgidum*. In cultivars from Portugal, the most frequent are alleles a and c. Three of these alleles are also found in *T. aestivum*: a, b and c. One is not found in *T. aestivum*: IV. Allele IV is also found in *T. durum*, but not *T. dicoccum* and *T. dicoccoides* (Table 1; Vallega and Mello-Sampayo, 1987).

There are eight alleles at locus *Glu-B1* in *T. turgidum*. In cultivars from Portugal, the most frequent are alleles b, c and d. Five of these alleles are also found in *T. aestivum*. Three are not found in *T. aestivum*: IV, VII and VIII. Alleles VII and VIII are also not found in *T. durum*, *T. dicoccum* and *T. dicoccoides* (Table 2; Vallega and Mello-Sampayo, 1987).

T. DICOCCOIDES

T. dicoccoides is also know as "wheat DIC", and can produce more protein per hectare than contemporary varieties of *T. aestivum*. Flour made from *T. dicoccoides* contains more lysine than flour made from *T. aestivum.* The proportion of lysine in the total protein is also greater. These are important factors in the manufacture of pasta and bread, and also in human nutrition (Ahmad *et al.*, 1997).

Increased protein content in *T. dicoccoides* is determined primarily by a single locus on chromosome 6B called *Gpc-6B1* (Olmos *et al.*, 2003). This locus lies 1.5 cM proximal to *Xcdo365* and 1.2 cM distal to *Xucw67*. These markers can be used to reduce the size of chromosome 6B1 and the linkage drag that occurs when *Gpc-6B1* is transferred into commercial varieties of *T. durum* and *T. aestivum*.

The glutenin proteins of *T. dicoccoides* are encoded by a unique set of alleles at loci *Glu-A1* and *Glu-B1*. At *Glu-B1*, three novel alleles have been found: be, bf and bg (Tabs. 1 and 2; Xu *et al.*, 2004).

Glu-B1 allele *be* codes for a subunit pair with a lower mobility than the subunit encoded by allele p. The 1Bx subunit has a mobility similar to that of subunit 14 of the cultivar 'Sappo'. The 1By subunit has a mobility similar to that of subunit 16 of the cultivar 'Norquay'.

Glu-B1 allele *bf* codes for a subunit pair that has a motility higher than that of the subunit pair encoded by *Glu-B1 p*, and similar to that of subunits 1 and 5. The 1Bx subunit has a motility between that of subunit 7 and that of subunit 17. The 1By subunit has a motility similar to that of subunit 8.

Glu-B1 allele *bg* codes for an unusual subunit pair. The 1Ax subunit has a higher mobility than the 1Bx subunit. The general rule is that 1Ax subunits have a lower mobility than 1Bx subunits, and that 1Ay subunits have a higher mobility than 1By subunits.

Ten alleles were identified at locus *Glu-A1*, none of which are found in *T. aestivum* or the other tetraploid varieties included in this study, except for allele j, which is also found in *T. dicoccum* (Table1; Xu *et al.*, 2004). Allele j codes for subunit III.

Thirteen alleles were identified at locus *Glu-B1*, none of which are found in *T. aestivum* or the other tetraploid varieties included in this study, except for alleles n, o, p, q and r, which are also found in *T. dicoccum* (Table 2; Xu *et al.*, 2004). Allele n codes for subunit II, allele o codes for subunit III, allele p codes for subunit IV, allele q codes for subunit V, and allele r codes for subunit VI.

Levy and Feldman (1988) identified seventeen alleles (*a* to *m*) at locus *Glu-A1* and twenty alleles (*a* to *k*) at locus *Glu-B1* in 466 accessions of *T. dicoccoides*. The alleles at each locus were identified on the basis of the molecular weight of the subunits for which they code.

The ranges for the molecular weights of the subunits encoded by each gene are:

Glu-A1-1: 103.5 kDa (allele *b*)to 114.0 kDa (allele *m*);
Glu-A1-2:71.5 kDa (allele *b*) to 76 kDa (allele *d*);
Glu-B1-1:93.0 kDa (allele *b*) to 99.5 kDa (allele *i*); and
Glu-B1-2:82.0 kDa (allele *b*) to 90.5 kDa (allele *k*).

The alleles with the highest frequency are:

Glu-A1-1: a (null);
y (108.0 kDa);
Glu-A1-2:a (null);
Glu-B1-1:i (99.5 kDa);
e (93.0 kDa);
f (96.0 kDa);
Glu-B1-2:*f* (88.0 kDa); and
b (82.0 kDa).

These alleles are associated with the ability to adapt to a wide range of ecological conditions (Levy and Feldman, 1988).

Among the alleles with the lowest frequency are:

Glu-A1-1: d, f, h, and *m*;
Glu-A1-2:*b*;
Glu-B1-1:a, *d* and *h*; and
Glu-B1-2:c, d, i and *k*.

In *T. dicoccoides*, it is difficult to associate the frequency of many HMW glutenin alleles with ecological conditions because some alleles are specific to certain regions, populations or transects. However, *Glu-A1-1* and *Glu-B1-1* subunits of high molecular weight are associated with high/low altitude, high/low temperatures and high/low rainfall, whereas *Glu-A1-1* alleles of low molecular weight are associated with low altitude. These correlations indicate that the molecular weight of HMW glutenins is under selective pressure and can be directly selected for in breeding programs aimed at improving grain quality.

The silencing of HMW glutenin genes in *T. dicoccoides* is not random, and affects genes at locus *Glu-A1* more so than those at locus *Glu-B1* during introgressive breeding.

ALLELIC VARIATION IN DIPLOID SPECIES OF *AEGILOPS*

Ae. squarrosa (D^sD^s), *Ae. umbellulata* (UU), *Ae. comosa* (MM), and *Ae. markgrafii* (CC) carry genes that code for HMW glutenins that are structurally homologous to those encoded by locus *Glu-D1* in *T. aestivum* (Rodriguez *et al.*, 2001; Liu *et al.*, 2003). In *T. aestivum, s*ixty-five alleles (a to bn) have been identified at locus *Glu-D1*, not all of which are found in the *Aegilops* species included in this study (McIntosh *et al.*, 2003). Novel alleles have been identified at loci *Glu-*D^S*1, Glu-U1, Glu-M1* and *Glu-C1*

AE. SQUARROSA

Ae. squarrosa (DsDs) is a special species within the genera *Aegilops*. Based on genetic evidence, this species hybridized with tetraploid *T. dicoccum* (AABB) to produce hexaploid *T. aestivum* (AABBDD) (Dvorak *et al.*, 1998). However, examination of the variability in glutenin alleles in *Ae. squarrosa* revealed that only certain genotypes from a restricted geographical range were involved (Lagudah and Halloran, 1988).

HMW glutenin genes from *Ae. squarrosa* significantly reduce mixing time and improve baking properties in *T. aestivum* (Lagudah *et al.*, 1988; Hsam *et al.*, 2001; Wieser *et al.*, 2003).

The D genome of *Ae. squarrosa* is the main contributor to desirable bread-making properties in common wheat cultivars, especially because of the HMW glutenin genes it provides (Lorenzo *et al.*, 1987; Payne *et al.*, 1987; Redaelli *et al.*, 1997; Lagudah *et al.*, 1988; Lawrence *et al.*, 1988; Ng and Bushuk, 1988; Odenbach and Mahgoub, 1988; Dong *et al.*, 1991; Branlard and Dardevet, 1995; Pena *et al.*, 1995; Mir-Ahli *et al.*, 1999; Hsam *et al.*, 2001; Rogers *et al.*, 2001; Deng *et al.*, 2005; Zhu *et al.*, 2005).

There is a significantly higher level of allelic variation in HMW glutenin genes and a higher number of HMW glutenin isozymes in *Ae. squarrosa* than in cultivated varieties of *T. aestivum* (Lagudah and Halloran, 1988; Williams *et al.*, 1993; Gianibelli *et al.*, 2001; Pfluger *et al.*, 2001a). *Ae. squarrosa* is therefore an important genetic resource for improving cultivars of *T. aestivum*.

Locus *Glu*-D^S 1 lies on the long arm of chromosome 1D, and codes for forty-two HMW glutenin subunit pairs (Yueming *et al.*, 2003). The subunit pairs with the highest frequency are: 3+12, 2+10, 4+12, 5+10, 2+12. Only five of the forty-two subunit pairs are common in *T. aestivum*: 2+12, 3+12, 4+12, 5+10 and 2+10 (Table 3; Yan *et al.*, 2003; Mclntosh *et al.*, 2003).

Each subunit pair found in *Ae. squarrosa* pairs has one x-type subunit with a lower mobility, and one y-type subunit with a higher mobility. They are therefore similar to the subunit pairs found in *T. aestivum* (Payne *et al.*, 1981). All of the x-type subunits found in *T. aestivum* are also found in *Ae. squarrosa*, except for subunits 2.2 and 2.2*, which have the lowest mobility and the highest molecular weight. This suggests that subunits 2.2 and 2.2* arose by crossing over between HMW glutenin genes in *T. aestivum* (Payne *et al.*, 1983).

There is a high level of allelic variation at *Glu*-D^S 1 in *Ae. squarrosAe.* Based on relative mobility, nine x-type subunits have been identified: 2.1, 1.5, 1.5*, 2, 3, 4, 5.1, 5 and 5*. Thirteen y-type subunits have also been identified: 10, 10.1, 10.2, 10.3, 10.4, 11, 12, 12.1*, 12.2*, 12.3, 12.4*, 12.5 and T_2 (Lagudah and Halloran, 1988; Gianibelli *et al.*, 2001).

Several novel 1Dy subunits have been identified which are not found in *T. aestivum*, including 1.5, 1.5*, 10.1, 10.2, 10.3, 10.4, 10.5, 12.1*, 12.2, 12.3, 12.4, and 12.5 (Lagudah and Halloran, 1988; Gianibelli *et al.*, 2001; Yan *et al.*, 2003; Yan *et al.*, 2004).

Table 3

Allelic variation at *Glu-D1* locus HMW-GS of *T. aestivum* L. and diploid species of *Aegilops* L.

Allele	Subunit composition	*T. aestivum* L. *Glu-D1*	*Ae .squarrosa L. Glu-D1*	*Ae. umbellulata* Zhuk *Glu-U1*	*Ae.comosa* Sibth.et Sm *Glu-M1*	*Ae. markgrafii L Glu-C1*
1	2	3	4	5	6	7
a	2+12	+	+			
b	3+12	+	+			
c	4+12	+	+			
d	5+10	+	+			
e	2+10	+	+			
f	2.2+12	+				
	Null+121*		+			
	1.5*+T2		+			
	1.5+10		+			
	1.5+10.3* 1.5+12.1*		+			
	1.5+12.3		+			
	1.5+12.4*		+			
	1.5+12.5		+			
	2+10.1		+			
	2+11		+			
	2+T2		+			
	2+12.4*		+			
	2.1+10.1		+			
	2.1+10.4		+			
	3+10		+			
	3+10.1		+			
	3+10.2		+			
	3+11		+			
	3+12.1*		+			
	3+T2		+			
	4+12.4*		+			
	4+10		+			
	4+10.1		+			
	4+10.2		+			
	4+10.3		+	+		
	4+12.1		+	+		
	5+12.3		+	+		

Table 3

Allelic variation at *Glu-D1* locus HMW-GS of *T. aestivum* L. and diploid species of *Aegilops* L. (continued)

Allele	Subunit composition	*T. aestivum* L. *Glu-D1*	*Ae .squarros*a L. *Glu-D1*	*Ae. umbellulat*a Zhuk *Glu-U1*	*Ae.comosa* Sibth.et Sm *Glu-M1*	*Ae. markgrafii L Glu-C1*
1	2	3	4	5	6	7
	5+10.1		+	+		
	5+10.2		+	+	+	
	5+12		+	+		
	5+12.1		+	+	+	
	5+12.2		+	+	+	
	5.1+10		+		+	
	5.1+10.2		+		+	
	5.1+12.1		+		+	
	5+10.2		+		+	
	5+12.1		+		+	
			+		+	
	1+8				+	
a	2+8				+	
b	3+8					
c	4+9					
d	5+10					
e	5+11					
f	6+8					
g	7+11					
h						
	1+9					
a	2+10					
b	3+11					
c	4+8					
d	4+9					
e	6+10					
g	7+8					
h	7+11					
i	1					
j	5+9					
k						
	1+5					
a	2+4					+
b	3+4					+
c	3+6					+
d						+

Subunit 12.1* has a slightly higher mobility than Dy subunit 12 in *T. aestivum* (Yan *et al.*, 2004). The gene that codes for it contains a 2,807 bp sequence consisting of a 1,950 bp open reading frame and an 857 bp upstream sequence. A perfectly conserved enhancer sequence lies from 209 to 246 bp upstream of the ATG start codon. The -300 element lies from 424 to 447 bp upstream of the start codon.

The mature protein of subunit 12.1* contains 648 amino acid residues, and has a molecular weight of 67.5 kDa. This is slightly less than that of subunit Dy 12 in *T. aestivum* (68.7 kDa), but is more than that of subunit 10 of *T. aestivum* (67.5 kDa). Subunit 12.1* is very similar to subunit 10 of *T. aestivum*, differing only by seven amino acid substitutions. This suggests that this subunit may be associated with good bread-making properties.

A dendrogram based on nucleotide sequences shows that the x-type and y-type subunit genes were clustered, and that the gene for subunit 12.1 in *Ae. squarrosa* is closely related to y-type subunit genes in genomes B and D in *T. aestivum*.

Subunit 12.4 is a small y-type subunit in *Ae. squarrosa*, and contains deletions in the central repetitive motifs (Gianibelli *et al.*, 2001; Gianibelli and Solomon, 2003).

Subunit 10.4 has the same mobility as subunit 8 in *T. aestivum*, which is encoded at locus *Glu-B1*.

Based on SDS-PAGE, subunit Dx 5 in *Ae. squarrosa* has a lower mobility than subunit 5 in *T. aestivum*, whereas subunit Dy 10 in *Ae. squarrosa* has a higher mobility than subunit 10 in *T. aestivum.* Subunit 10 in *Ae. squarrosa* also has a different isoelectric point than subunit 10 in *T. aestivum* (Lagudah and Halloran, 1988).

Subunit T_2 has a mobility that is close to that of subunit 12. Therefore, some of the HMW glutenin subunits in *Ae. squarrosa* are encoded by different genes and have different sizes than subunits with the same mobility in *T. aestivum*.

AE. UMBELLULATA (UU)

Based on SDS-PAGE, there are eleven HMW glutenin subunits in *Ae. umbellulatAe.* They are numbered from 1 to 11 in order of increasing mobility (Table 3; Rodriguez-Quijano *et al.*, 2001). Six of these subunits are not found in *T. aestivum*: 1, 6, 7, 8, 9 and 11.

Subunit 1 has a mobility that is similar to that of subunit 2.2 in *T. aestivum*. Subunits 2, 3, 4, 5, 6 and 7 have mobilities between those of subunits 1 and 2.2 in *T. aestivum*. Subunits 8, 9, and 10 have mobilities that are slightly lower than that of subunit 12 in *T. aestivum.* Subunit 11 has a motility that is slightly higher than that of subunit 12 in *T. aestivum*.

There are eight subunit pairs in *Ae. umbellulatAe.* They are encoded by eight alleles (a to h) at *Glu-U1*. None except subunit pair 5+10 is found in *T. aestivum*.

The subunit pair 5+10 is very rarely found in accessions of *Ae. umbellulata*, and is determined by allele e instead of allele d as it is in *T. aestivum*.

The *Glu-U1* alleles with the highest frequency are:

a (1+8);
f (5+11)
g (6+8)
c (3+8)
d (4+9); and
h (7+11).

AE. COMOSA (MM)

Based on SDS-PAGE, there is a high level of allelic variation in HMW glutenin genes in *Ae. comosAe.* There are eleven HMW glutenin subunits numbered from 1 to 11 in order of increasing mobility. The subunits are encoded by eleven alleles (a to k) at locus *Glu*-M1 (Table 3; Rodriguez-Quijano *et al.*, 2001). Six of these subunits are not found in *T. aestivum.*

Subunits 1 through 7 have mobilities that are between those of subunits 1 and 2.2 in *T. aestivum*. Subunits 8, 9, and 10 have mobilities that are slightly lower than that of subunit 12 in *T. aestivum*. Subunit 11 has a motility that is slightly higher than that of subunit 12 in *T. aestivum*. The subunits are encoded by eleven alleles (a to k) at locus *Glu-M1*.

Subunit pair 5+11 is similar to that of *Ae. umbellulata*.

The *Glu-M1* alleles with the highest frequency are g (6+10) and c (3+11). The other alleles are all rare.

AE. MARKGRAFII (CC)

There are six HMW glutenin subunits in *Ae. markgrafii*. Two subunits are not found in *T. aestivum*: subunits 1 and 6. Four different patterns are found which are encoded by four alleles (a to d) at locus *Glu-C1* (Table 3; Rodriguez-Quijano *et al.*, 2001).

The *Glu-C1* alleles with the highest frequency are b (2+4) and d (3+6). The other alleles are rare, including allele a (1+5).

INTROGRESSION OF ALIEN HMW GLUTENIN GENES INTO *T. AESTIVUM*

Cultivated varieties of *T. aestivum* are characterized by allopolyploidy and are tolerant to aneuploidy. There are many wild related species that represent a rich source of new genes that can be used in breeding programs directed at improving the quality of cultivated varieties. It is therefore possible to use chromosome manipulation to introduce alien chromatin carrying genes of interest into the genome of *T. aestivum.*

In wide hybridization of hexaploid *T. aestivum*, the pairing of homologous chromosomes during meiosis is suppressed by the dominant *Ph* (pairing homologous) genes, which ensure that the chromosomes pair up in a diploid-like manner. This blocks the introgression of alien chromosomes. Non-homologous chromosomes rarely pair and recombine in the presence of the *Ph1* and *Ph2* genes (Pilch, 2005 c).

It is therefore more difficult to transfer genes from alien genomes that are not homologous to that of *T. aestivum* than to transfer genes from homologous or closely related genomes. Special techniques of chromosome manipulation are needed to introduce genes from non-homologous genomes into *T. aestivum* (Pilch, 2006a).

Alien chromatin can be introduced into *T. aestivum* by producing amphiploids of *T. aestivum* and alien species, or by producing hybrids with substituted or translocated *T. aestivum* and alien chromosomes (Pilch, 2005a). However, the usefulness of these techniques in breeding programs is limited by chromosome instability, meiotic instability, and linkage drag on the alien chromosomes. Furthermore, alien chromosomes rarely pair and recombine with *T. aestivum* chromosomes in the presence of the *Ph* genes.

Homologous pairing can, however, take place in hybrids which lack the *Ph1* gene, have a mutation in the *Ph1* gene, or lack either one or both of the chromosomes 5B (Chen *et al.*, 1994; Sears, 1977; Feldman, 1966). Translocations between *T. aestivum* chromosomes and alien chromosomes are also possible in hybrids carrying gametocidal chromosomes, which induce structural changes in chromosomes (Endo, 1988).

Linkage drag on the alien chromosome makes it difficult to directly use such amphiploids in breeding programs. The most effective approach for introducing alien genes into *T. aestivum* therefore involves inducing translocations between homologous *T. aestivum* and alien chromosomes in order to minimize linkage drag.

Wild species can be successfully hybridized with *T. aestivum* by using immature embryo culture techniques or genetic systems based on *Ph* homologous pairing genes and *Kr* crossability genes from *T. aestivum* (Pilch, 2005a, b, c, 2006a).

Introducing alien chromatin into *T. aestivum* often reduces spike fertility and grain yield. This is sometimes caused by genetic linkages. The original yield can be recovered by subsequent genetic manipulation.

For example, the gene for resistance to eyespot (*Pseudocercosporella herpotrichoides* Fron.) was introduced into *T. aestivum* from chromosome 7D of *Ae. ventricosa* Tausch. Yield was restored to its former level by reducing the size of the introduced chromosomal segment (Carillo *et al.*, 1990).

In *T. aestivum*, the *Glu*-1 loci are associated with grain yield, which suggests that disrupting these loci can reduce yield (Carillo *et al.*, 1990). This accounts for the reduction in yield observed after introducing the *Glu-A1* allele r from *T. boeoticum* Boiss. into *T. aestivum* (Rogers *et al.*, 1997).

Interspecific and intergeneric hybridization has been used to introgress genes associated with superior bread-making properties into *T. aestivum*. However, these techniques are most often used to introgress genes that confer resistance to disease (Rong *et al.*, 2000; Ma *et al.*, 2001; Aghaee-Sarbarzeh *et al.*, 2002; Dhaliwal *et al.*, 2002b; Liu *et al.*, 2002; Hsam *et al.*, 2003; Leonova *et al.*, 2004; Cai *et al.*, 2005; Li *et al.*, 2005; Marais *et al.*, 2005; Mohler *et al.*, 2005; Oliver *et al.*, 2005; Schoenenberger *et al.*, 2005; Jakobson *et al.*, 2006; Pestsova *et al.*, 2006).

Some introgressions can improve spike characteristics (Pilch, 2005 a).

HMW glutenin genes have been introgressed in order to improve grain quality in diploid rye (*Secale cereale*), hexaploid secondary triticale (x *Triticosecale* Witt.), hexaploid tritordeum, and hexaploid *T. aestivum*.

INTROGRESSIONS FROM TETRAPLOID SPECIES OF *TRITICUM*

Bread-making quality in *T. aestivum* and pasta-making quality in tetraploid *Triticum* species are known to be largely associated with high molecular weight glutenins, which are encoded by the *Glu*-1 genes. The tetraploid species *T. durum*, *T. dicoccum*, *T. turgidum*, and *T. dicoccoides* carry HMW glutenin alleles at loci *Glu-A1* and *Glu-B1* that can affect bread making quality in *T. aestivum* (Payne *et al.*, 1981, 1987). *T. aestivum* cultivars usually contain from three to five HMW glutenin subunits, of which one or none are encoded by alleles at locus *Glu-A1*, and one or two are encoded by alleles at locus *Glu-B1*.

The *Glu-A1* allele a, which codes for subunit 2*, is associated with higher gluten strength than the null allele c (Payne *et al.*, 1981, 1987). Genotypes with allele c are far inferior in terms of rheological dough properties (Lawrence *et al.*, 1988).

In Swedish cultivars of *T. aestivum*, the presence of the alien subunit 2.1* is associated with better bread-making properties than is the presence of the *T. aestivum* subunits 1 and 2* (Johansson and Svensson, 1995). Introducing alien HMW glutenin alleles into *T. aestivum* may therefore improve quality.

Introgressives of *T. aestivum* and the *T. durum* cultivars ‘Mirable’, ‘Khapli’ and ‘Fuensemiduro’ have superior protein contents, Zeleny sedimentation indices and falling numbers. (Pilch *et al.*, 1999; Pilch, 2002). Based on SDS-PAGE, there were changes in the frequencies of several HMW glutenin subunits and in subunit composition. These changes reflect changes in the alleles at loci *Glu-A1*, *Glu-B1* and *Glu-D1* (Pilch 2006b). The introgressives therefore differed from other high-quality varieties of *T. aestivum*.

Five of the introgressives lack the HMW glutenin subunits that are encoded by alleles at loci *Glu-A1*, *Glu-B1* and *Glu-D1* in *T. aestivum*. These alleles may have been missing, substituted or mutated, thereby blocking gene expression. Nevertheless, technological parameters were superior in all five introgressives.

This suggests that “modified” alleles at locus *Glu-A1* in the cultivars ‘Mirable’, ‘Khapli’ and ‘Fuensemiduro’ and at locus *Glu-B1* in ‘Khapli’ were

transferred into the hybrid genotypes when these cultivars were crossed with the *T. aestivum* cultivars 'Chinese Spring' and 'Favorit'. They were subsequently distributed into some of the introgressives, in which they improved grain quality.

Modifications of this sort of the alleles coding for HMW glutenins at the *Glu*-1 loci are possible. Mutations of the *Glu-B1* allele a have been found in the *T. aestivum* cultivars 'Chinese Spring' and 'Cheyenne', in the *T. durum* cultivar 'Bidi', and in the wild species *T. turgidum* ssp. *dicoccoides* (Anderson *et al.*, 1998). The mutated allele a codes for a subunit 7 which contains a duplication of 54 bp or an insertion of 185 bp in the "cereal-box" Bx promoter sequence. In 'Cheyenne', there is also a deletion of 85 bp in the *Glu*-A 1 allele b, which codes for subunit 2* (Forde *et al.*, 1985).

The Hungarian *T. aestivum* cultivar 'Bankuti-1201' has superior with superior technological parameters. It also carries a mutated *Glu-A1* allele b in which a point mutation at 1,181 bp results in the substitution of serine for cysteine in subunit 2* (Juhasz *et al.*, 2001, 2003).

In rice (*Oryza sativa*), a mutation was found in the *Glu-A1* allele *glu 4*, which codes for subunit *a-2*. The mutated allele, *glu 4a*, codes for a new polypeptide, *p16.50/a-1* (Qu *et al.*, 2003).

In four other introgressives of *T. aestivum* and *T. durum*, the *Glu-D1* subunit pair 5+12 was identified. This subunit pair represents a new combination that has never been found in either *T. aestivum* or *T. durum* (Pilch, 2006 b). It improved grain quality in all of the introgressives carrying it, as evidenced by the high values for the technological parameters tested. This subunit pair probably arose as the result of breakage of alleles for other *Glu-D1* subunit pairs that contain subunits 5 and 12, such as 2+12, 3+12, 4+12, 2.2+12 and 5+10.

The mechanism by which this happened in this case remains unknown. One possibility is that the allelic breakage was caused by the influence of *T. durum* chromosomes. The breakage took place in the F1 generation during interspecific generative hybridization. In hexaploid triticale (X *Triticosecale* Witt.), *T. durum* chromosomes have long been known to induce changes in the heterochromatic DNA of the chromosomes donated by rye (Pilch, 1981a, b; Pilch, 1987).

Increasing the number of HMW glutenin subunits in *T. aestivum* improves dough properties (Payne *et al.*, 1984; Rogers *et al.*, 1997). Alleles that code for subunit pairs improve quality more so than alleles that code for a single subunit. For example, *Glu-B1* alleles b (7+8), c (7+9) and i (17+18) improve quality more than allele a (7) (Payne *et al.*, 1984).

Glu-D1 allele d (5+10) likewise improved quality more than alleles k (2) and p (36). *Glu-B1* allele c (7+9) also improved quality more than allele aj (8) (Rogers *et al.*, 1991).

Quality is worse with *Glu-A1* allele c (null) than with alleles a (1) or b (2*) (Payne *et al.*, 1984). Quality was worse with *Glu-B1* allele a h (null) than with

allele i (17+18), and with the *Glu-D1* allele i (null) than with allele d (5+10) (Lawrence *et al.*, 1988; Payne *et al.*, 1987).

The number of subunits encoded by a particular allele is not the only factor that determines its effect on grain quality. Different alleles that code for the same number of subunits can differ widely in terms of their effect (Payne *et al.*, 1984). For example, *Glu-D1* alleles d (5+10) and a (2+12) both code for subunit pairs, yet allele d is vastly superior.

Studies have been carried out on lines carrying deletions or duplications in chromosome segments containing loci that code for endosperm storage proteins. The deletions and duplications were directly responsible for increasing or decreasing grain quality to varying degrees (Rogers *et al.*, 1990). The simple addition of a HMW glutenin subunit does not always improve quality.

Introgression of alien genetic material can improve quality by introducing alleles that code for an increased number of subunits. For example, SDS-sedimentation volume is far better in lines in which a double-banded *Glu-U1* allele from *Ae. umbellulata* is introduced into chromosome 1A than in lines which carry the *Glu-A1* alleles a (1) or c (null).

Introducing a double-banded *Glu-A1* allele from *T. dicoccoides* into tetraploid *T. durum* carrying the *Glu-A1* allele c (null) improved gluten strength. The double-banded *Glu-A1* allele from *T. dicoccoides* improved many bread-making properties more than a single-banded *Glu-A1* allele carried by a tetraploid breeding line derived from a *T. durum* x *T. aestivum* cross (Ciaffi *et al.*, 1990, 1995).

In two Swedish bread wheat lines that were selected from interspecific crosses with wild wheat species, both x-type and y-type subunits were expressed at locus *Glu-A1*. These are the only two bread wheat lines that have an allele for a subunit pair at locus *Glu-A1*, except for those lines carrying the alleles for subunit pairs 39+40 and 41+42 (Margiotta *et al.*, 1995).

Biotypes of *T. monococcum* represent another source of double-banded *Glu-A1* alleles. However, SDS-sedimentation tests carried out at the diploid level were not promising, in that the number of HMW glutenin subunits carried by an accession was not correlated with the sedimentation volume (Saponaro *et al.*, 1995).

The *Glu-A1* alleles r (39+40) and s (41+42) were introgressed from *T. boeoticum* Boiss. into the cultivar 'Sicco' of *T. aestivum*. This slightly increased gluten strength and dough strength, decreased dough stickiness, and improved stability during mixing. The introgression also increased the number of HMW glutenin subunits in "Sicco" from five to six. The introgression of these alleles may be more effective if carried out in conjunction with selection for alleles at other loci that code for endosperm storage proteins and other grain components that are known to determine grain quality (Rogers *et al.*, 1997).

Although introgressing the *Glu-A1* alleles r and s increased gluten strength, it did not improve loaf volume or loaf score. The effect may have been too small to have been detected. The fact that 'Sicco' is already a high quality

cultivar would also make it difficult to detect the effect of introgressed alleles. Improvements in bread-making quality are also often difficult to detect in experiments of manageable size, even when sedimentation testing reveals improvements in gluten and dough properties.

Synthetic hexaploid wheat was produced using the *T. turgidum* variety 'Altar 84' as a parental component. Alleles lying on the chromosome arms 1AS, 5AL, 7AS and 1BS of *T. turgidum* increased dough tenacity, extensibility and elasticity. Alleles lying in and near the gliadin locus *Gli-B1* of *T. turgidum* also improved dough strength, dough viscosity, mixing tolerance and mixing time (Nelson *et al.*, 2006).

Quality in *T. durum* and *T. aestivum* was also improved by introgressing alleles from the diploid species *T. urartu* L. and *T. boeoticum* Boiss., from the tetraploid species *T. dicoccoides* and *T. araraticum* L., and from *Ae. speltoides* Taush. The introduced alleles significantly increased gluten strength, SDS-sedimentation index and protein content (Zeven and Waninge, 1986; Rogers *et al.*, 1997; Dhaliwal *et al.*, 2002 a).

The effect of *Glu-A1* alleles on grain quality in introgressives can be intensified by linkage with the gene for polyphenol oxidase (PPO). This gene is located on the long arm of chromosome 2A in *T. durum* (Jimenez and Dubcovsky, 1999). Reducing PPO activity in cultivars with other high-quality attributes helps preserves freshness. The SSR marker *Xgwm31a 2A* can facilitate selection of genotypes with lower PPO activity such as the *T. durum* cultivars 'Jennah', 'Khetifa' and 'Cham 1' (Watanabe *et al.*, 2006).

Introgressed *Glu-B1* alleles from tetraploid *Triticum* species can significantly affect bread-making quality in *T. aestivum*. These alleles represent a rich source of glutenin subunit diversity that can be exploited once their functional properties have been tested. *Glu-B1* allele b (14+15) from *T. durum* increased SDS sedimentation index and mixing development time more than alleles b (7+8) or e (20) (Turchetta *et al.*, 1995; Liu and Shepherd, 1996; Brites and Carrillo, 2001).

In 202 high-quality genotypes of *T. durum* from Turkey and Italy that had high SDS sedimentation indices, four *Glu-A1* alleles (a, b, c and III) and eight *Glu-B1* alleles (b, c, d, e, f, h, i and XII) were identified. SDS sedimentation index was higher in genotypes with the *Glu-A1* allele a than with allele c (null). SDS sedimentation index was also higher in genotypes with the *Glu-B1* alleles b and d than with allele e. The *Glu-B1* allele XII (7+15) also increased SDS sedimentation index (Turchetta *et al.*, 1995).

In thirty *T. durum* genotypes from Ethiopia, two *Glu-A1* alleles (*b* and *c*) and six *Glu-B1* alleles (*b*, *d*, *e*, *f*, *h*, and *i*) were identified. SDS sedimentation index and loaf volume were highest in the genotypes that carried both the *Glu-A1* allele c (null) and the *Glu-B1* allele b (7+8). 98% of the genotypes carried the *Glu-A1* allele c. Of the *Glu-B1* alleles, allele b was most common, followed by alleles e and d (Dessalegn *et al.*, 2003).

Specific y-gliadin components encoded by alleles at locus *Gli*-1 have been found in high-quality tetraploid *Triticum* species. They may prove useful in improving gluten visco-elasticity and other technological properties in *T. aestivum* (D'Ovidio *et al.*, 1992 b).

In *Agropyron elongalum* (Host) Nivski, subunits h1Bx and h1By have mobilities similar to those of subunits 1Bx 13 and 1By 16 in *T. aestivum*. When subunits h1Bx and h1By were introduced into *T. aestivum*, they improved flour quality (Feng *et al.*, 2004).

INTROGRESSIONS FROM DIPLOID SPECIES OF *AEGILOPS*

In *Ae. squarrosa, Ae. umbellulata*, *Ae. comosa* and *Ae. markgrafii*, various alleles for x-type and y-type subunits have been found, some of which are also found in *T. aestivum*, and some of which are not. All of the x-type subunits in *Ae. umbellulata* have a very high molecular weight (about 130 kDa). In *Ae. comosa,* there were only two x-type subunits with a very high molecular weight.

Subunits of very high molecular weight are very rare in cultivars of *T. aestivum*. Only one such subunit has been found in some Japanese cultivars: subunit 2.2, which is encoded by an allele at locus *Glu-D1* (Nakamura *et al.*, 1999).

The other x-type subunits from *Ae. comosa* and *Ae. markgrafii* have mobilities that are similar to that of subunit 1 in *T. aestivum*, which is encoded by an allele at locus *Glu-A1*. Subunit 3 in *Ae. comosa* and subunit 2 in *Ae. markgrafii* have identical mobilities.

In *Ae. umbellulata*, y-type subunits generally have higher molecular weights than y-type subunits in *Ae. comosa* and *Ae. markgrafii*.

Comparison of the amino-acid sequence of 1Ux and 1Uy in *Ae. umbellulata* with that of subunits in *T. aestivum* revealed various substitutions, insertions or deletions of one or more amino acid residues. In spite of these changes, the four proteins all function as HMW glutenin subunits.

The variation in HMW glutenin subunits found in species of the genus *Aegilops* may prove valuable in improving breadmaking quality in *T. aestivum*. Somatic hybridization with *Aegilops* species can be used to introduce new subunits or increase the number of subunits in *T. aestivum*.

Introgression of subunits 1Dx 2.1 and 1Dx 2 from *Ae. squarrosa* is promising because these subunits have a sequence that is very similar to subunit 1Dx 2 in *T. aestivum*. Subunit 1Dx 2 in *Ae. squarrosa* differs from subunit 1Dx 2 in *T. aestivum* in that it lacks internal duplicated regions (Wan *et al.*, 2005

Synthetic hexaploid wheats (AABBDD) were created using *T. turgidum* (AABB) and three *Ae. squarrosa* (DsDs) parental lines. The genetic material contributed by *Ae. squarrosa* had a clear effect on bread loaf volume, gluten index, maximum resistance, SDS-sedimentation index, dough surface and other quality parameters (Hsam *et al.*, 2001; Nelson el al., 2006).

Crossing *T. aestivum* with species of the genus *Aegilops* improved kernel hardness by introducing locus Ha, which is the main determinant of hardness. This locus lies on chromosome arm 5DS, and contains two tightly linked puroindoline alleles: *Pin* A and *Pin* B. Kernel hardness is lower with *Pin* B than with the null allele *Pin* Ae. Nevertheless, flour yield is higher in crosses that carry *Pin* B (Martin *et al.*, 2001).

Hardness affects bread-making quality mainly by increasing the degree of starch damage during milling. This increases water absorption and hydrolysis of starch into fermentable sugars that contribute to loaf volume (Pomeranz and Williams, 1990). Analysis of QTL wheat quality traits has shown that locus Ha has a strong effect on flour yield, starch damage, dough water absorption, cookie diameter and governed alveogram dough strength (Perretant *et al.*, 2000).

The synthetic hexaploid wheat 'WPI 219' (*T. turgidum* var. 'Altar 84' × *Ae. tauschii* Coss.) was crossed with the cultivar 'Opata' of *T. aestivum*. In the cross, the alleles at locus Ha were correlated with kernel hardness, kernel texture, alkaline water retention capacity, flour yield, and all traits related to the mechanics of kernel fracturing and to starch damage during milling. The alleles at locus Ha also affected dough strength and some mixing traits. Kernel hardness was positively correlated with protein content and gluten strength parameters including alveogram viscosity, SDS sedimentation index, Zeleny sedimentation index, Pelshenke index and lactic acid retention. Kernel strength was negatively correlated with alveogram extensibility (Nelson *et al.*, 2006).

High kernel hardness increases energy requirements during milling, but also increases flour yield and improves flowing and sifting properties during milling (Pomeranz and Williams, 1990).

Crossing with *Ae. squarrosa* improved protein concentration by up to 20% in *T. aestivum*. It also improved viscosity and value traits at and to right of the mixogram peak. This was attributed to the introgression of alleles at or near locus *Gli-D2*, which lies on chromosome arm 6DS (Nelson *et al.*, 2006).

In Mexico, the effect of crossing with *Ae. squarrosa* on grain protein and flour protein was attributed to alleles on chromosome arm 2DS. The fact that introgression of genes from *Ae. squarrosa* improved these traits suggest that genes from other wild accessions may improve quality. This is supported by a study of backcross progeny of *Ae. squarrosa* amphiploids to elite soft red winter wheats. Several recombinant lines were superior to either parent in terms of protein content and quality traits (Nelson *et al.*, 2006).

Studies have also be conducted on introgressing genes from *T. aestivum* into related species. The *Glu-D1* alleles from *T. aestivum* effectively improved quality in the crosses. In one study on *T. durum*, some lines were produced that possessed chromosome 1DL, the result of the translocation of 1AL and 1DL. This chromosome carries *Glu-D1* allele d of *T. aestivum.* Dough quality was greatly improved without affecting spike fertility (Vitellozzi *et al.*, 1997).

Introgressing allele 1Dx 5 into maize improved protein quality (Sangtong *et al.*, 2002).

CONCLUSIONS

Novel HMW glutenin alleles and allele combinations from tetraploid *Triticum* species and diploid *Aegilops* species may prove useful in improving bread making quality in *T. aestivum*.

HMW glutenin genes from alien species can effectively be introgressed into cultivars of *T. aestivum* by interspecific and intergeneric generative hybridization. The best approaches involve the pairing of homologous chromosomes or chromosome manipulations that lead to translocations.

Introgressing alien HMW glutenin genes into triticale, rye and *T. aestivum* effectively improved technological grain parameters. In *T. aestivum*, the expression of these genes may be controlled by regulatory genes.

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