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IDENTIFICATION OF *GLU-A1* AND *GLU-D1* HIGH MOLECULAR WEIGHT GLUTENIN SUBUNITS OF COMMON WHEAT (*TRITICUM AESTIVUM* L.) USING GENETIC MARKERS

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*High molecular weight glutenin subunits (HMW-GS) of wheat are important factors in the determination of bread-making quality. They are responsible for elasticity and polymer formation of wheat dough. In the present study, 43 winter and 40 spring common wheat (*Triticum aestivum *L.) cultivars originated from Estonia, Belarus, Finland, Denmark, France, Germany, the Great Britain, Latvia, Lithuania, the Netherlands, Norway, Poland, Russia, Sweden, and New Zealand were characterised for* Glu-A1 *and* Glu-D1 *allelic composition using PCR method. Analyses were conducted with one DNA marker for identification of Glu-D1 allele encoding subunit Dx5, three DNA markers for Glu-A1 Ax1, Ax2* and AxNull subunits. It was determined that 32 (74.4%) winter and 35 (83.3%) spring cultivars had allele* Glu-D1d*, and 23 (53.5%) winter and 33 (78.6%) spring —* Glu-A1a *or* Glu-A1b *alleles, which have positive effect on dough properties. Polymorphism at* Glu-A1 *locus was detected in 15 cultivars, and 9 cultivars were polymorphic for locus* Glu-D1*. The obtained results were compared with published SDS-PAGE data. Complete or partial agreements were found for 78.1% of* Glu-A1 *and 70.6% of* Glu-D1 *alleles. Rapid and accurate identification of wheat Glu-1 alleles by molecular markers can be used for selection of wheat genotypes with good bread-making potential.*

Key words: *wheat cultivars, HMW-GS, DNA markers,* Glu-1 *loci, allelic variation.*

INTRODUCTION

Wheat is one of the major food crops providing staple food for the world population. A large portion of wheat is processed as flour and used in bread baking. The bread-making quality of common wheat (*Triticum aestivum* L.) is attributed to grain protein content and gluten quality. Gluten is a composite of grain storage proteins: high molecular weight glutenin subunits (HMW-GS), low molecular weight glutenin subunits (LMW-GS) and gliadins. HMW-GS, which constitute about 30% of the glutenin fraction, have particular importance in determining the viscoelastic properties of dough. They form a continuous elastomeric polymer network which becomes a backbone for glutenin-gliadin interactions and is responsible for the dough strength and elasticity (Kasarda, 1989; Shewry *et al*., 2002).

The HMW-GS composition is controlled by pairs of tightly linked loci on the long arm of the group 1 of homeologous chromosomes in A, B and D genomes (*Glu-A1, Glu-B1* and *Glu-D1*) of hexaploid wheat (Payne and Lawrence, 1983; Payne, 1987). The locus pair contains genes that encode two types of HMW glutenin subunits, one of greater molecular weight, designated the x-type, and the other of lower molecular weight, designated the y-type. The y-type gene(s) of A genome are silent in hexaploid bread wheats (Payne, 1987). Allelic variation exists at each of the HMW glutenin loci (Payne and Lawrence, 1983; McIntosh *et al*., 2013), and different alleles variably affect dough properties. The subunit Ax1 or Ax2* controlled by the locus *Glu-A1* and the subunits Dx5+Dy10 controlled by the locus *Glu-D1* are positively associated with bread-making quality compared with Axnull and Dx2+Dy12 subunits (Payne, 1987; Pogna *et al*., 1987; Gupta *et al*., 1994; Kasarda, 1999; Branlard *et al*., 2001; Luo *et al*., 2001; Tohver *et al*., 2001; He *et al*., 2005; Kocourková *et al*., 2008; Liang *et al*., 2010; Aktaş, Baloch, 2017; Langner *et al*., 2017). Thus, the relationship between glutenin subunit composition of wheat grain storage proteins and baking quality could be used as a selection criterion in breeding programmes. In particular, one of the

aims of wheat breeding in Estonia is to produce superior cultivars, suitable for production of yeast leavened bread preferred by local consumers. Breeding for baking quality could be facilitated by creation of databases, which may offer the breeders a tool for choosing the right parental cultivar combinations with suitable HMW glutenin subunit composition (Anonymous, 2017; Tohver, 2007; Békés, Wrigley, 2013).

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) of proteins has been used routinely for the detection of large number of HMW-GS alleles in wheat collections in different countries (Payne, 1987; Gupta *et al*., 1994; Branlard *et al*., 2001; Luo *et al*., 2001; Johansson *et al*., 2003; He *et al*., 2005; Tohver, 2007; Liang *et al*., 2010; Abugalieva *et al*., 2016). However, this method is time consuming and requires equipment and expertise to successfully identify the protein subunits. Another shortcoming of protein-based glutenin analysis is that it is based on protein mobility, depending on protein size and charge, but not on protein amino acid sequence, which negatively affects specificity of analysis and reliability of the results.

At the present time, the availability of the nucleotide sequences of the HMW glutenin subunit genes enables the development of a set of DNA markers for the glutenin loci and the designing of allele specific primers for PCR analysis (Anonymous, 2018; Gale, 2005; Liu *et al*., 2012). Thus, information about alleles of the genes encoding glutenin proteins can be obtained by applying PCR on samples from the early stages of plant development to plant flowering, and using any part of the plant. This enables selection of the necessary genotypes without analysing grain proteins. Another benefit of this method is that DNA markers are not affected by cultivation practices and the plant growth environment. Application of DNA markers also allows discrimination between homo- and heterozygous plants. Use of DNA markers increases the efficiency and speed of the development of improved cultivars, and the method is becoming a standard and reliable technology in breeding for higher baking quality (Ahmad, 2000; Ma *et al*., 2003; Gale, 2005; Kocourková *et al*., 2008; Liang *et al*., 2 010; Polishchuk *et al*., 2010; Jin *et al*., 2011; Liu *et al*., 2012; Rasheed *et al*., 2014; Zamani *et al*., 2014; Tian *et al*., 2015; Khlestkina *et al*., 2017).

The aim of this study was to characterise the combinations of *Glu-A1* and *Glu-D1* HMW-GS alleles present in 7 old and 75 modern European wheat cultivars and 1 cultivar from New Zealand using DNA markers and to compare results of the two genotyping methods, PCR and protein SDS-PAGE, for 18 winter and 16 spring cultivars. The results could be applied in wheat breeding for the improvement of baking quality.

MATERIAL AND METHODS

Plant materials. In total, 43 winter and 40 spring cultivars of bread wheat (*Triticum aestivum* L.) from the Estonian Crop Research Institute (ECRI) genetic collection and additionally winter wheat cultivars 'Ritmo' from the Gene Bank of the Crop Research Institute (CRI) (the Czech Republic) and 'Bjørke' from NordGen (Norway) were analysed. There were three different periods of release of the tested cultivars: very old, bred in 1930s; old, bred in the period of 1950s–1970s; and modern, bred after 1980s. The cultivars originated from 13 European countries: Germany (DEU – 21), Estonia (EST – 12), Sweden (SWE – 9), Denmark (DNK – 7), Poland (POL – 6), Finland (FIN – 5), Belarus (BLR – 4), the Netherlands (NLD – 4), Latvia (LVA – 3), Norway (NOR – 3), the Great Britain (GBR – 3), Lithuania (LIT – 2), and France (FRA – 1), two Lithuanian–Estonian (LIT/EST) cultivars and one cultivar originated from New Zealand (NZL).

DNA extraction. Genomic DNA was extracted using the sorbent method from leaf material collected from 7–10-day-old wheat seedlings (5–10 plants per sample) grown under the control conditions in the laboratory. DNA extraction was conducted with NucliSENS easyMAG (bioMereux) device and proprietary reagents.

PCR analysis. PCR primers were synthesised according to published data (Table 1), and obtained from DNA Technology A/S (Denmark).

Amplifications were carried out on a SeeAMP thermocycler (Seegene) in 20-µl reaction volumes containing 4 µl plant genomic DNA (not quantified), 1x FIREPol Master Mix Ready to Load, 1.5 mM of $MgCl_2$ (Solis BioDyne), 0.5 µM of each primer (for the markers *Axnull*, *Ax1+Ax2** and *x2**) or 0.3 µM of DxF, 0.1 µM of Dx5F and 0.4 µM of DxR for the marker *5+10*, and ddH_2O (up to 20 µL). The PCR conditions were as described in literature (see Table 1). The amplified fragments were separated using a horizontal electrophoresis chamber and PowerPac Basic Power Supply (BioRad) on 1–2.5% agarose gel with 1x TAE buffer, stained with ethidium bromide and visualised using UV light. Molecular mass markers were 100 bp DNA Ladder (Solis BioDyne) and O'RangeRuler 50 bp DNA Ladder (Thermo).

RESULTS

The genotyping results are presented in the Tables 2 and 3. Among 83 bread wheat cultivars a 2625-bp PCR fragment was amplified with the *x2** marker in 33 (39.8%) samples, suggesting they have the *Glu-A1b* allele (protein subunit Ax2*). In 34 cultivars (41.0%) a 529-bp PCR product was amplified using the marker *Axnull,* suggesting they have the *Glu-A1c* allele (subunit Axnull). Thirty-two genotypes (38.6%) carried the *Glu-A1a* allele (subunit Ax1). At the *Glu-D1* locus, 67 (80.7%) cultivars contained subunit Dx5, generating 343- and 320-bp bands with the marker *5+10*.

Polymorphism at *Glu-A1* locus was observed in 15 cultivars from the ECRI collection (18.1%), whereas 9 cultivars (10.8%) were polymorphic for locus *Glu-D1*.

Table 1

PCR PRIMERS OF THE SPECIFIC MOLECULAR MARKERS USED FOR IDENTIFICATION OF HMW-GS ALLELES

Locus	Marker	Primer sequences (5'-3')	Allele (subunit)	Expected size of DNA fragments (bp)	References
Glu-D1	*5+10*	DxF: TTTGGGGAATACCTGCACTACTAAAAAGGT Dx5F: AAAAGGTATTACCCAAGTGTAACTTGTCCG DxR: AATTGTCCTGGCTGCAGCTGCGA	*d* (Dx5) others	320, 343, 361	Ishikawa and Nakamura, 2007
Glu-A1	*Axnull*	F: ACGTTCCCCTACAGGTACTA R: TATCACTGGCTAGCCGACAA	*c* (null)	~ 920	Lafiandra *et al*., 1997
	*Ax1+Ax2**	F: CCATCGAAATGGCTAAGCGG R: GTCCAGAAGTTGGGAAGTGC	*a* (Ax1), *b* (Ax2*)	~1500	
	*x2**	F: CCGATTTTGTTCTTCTCACAC R: CACCAAGCGAGCTGCAGAT	*b* (Ax2*)	~ 2652	De Bustos *et al*., 2000

Among the 43 winter and 40 spring bread wheat cultivars, there were large differences in frequency distributions of HMW-GS for Axnull (49.1 and 16.7%), Ax2* (20.6 and 47.9%) and Dx5 (66.7 and 74.5%, respectively) (Fig. 1). Frequency of Ax1 in winter and spring cultivars was 30.2 and 35.4%, respectively.

The majority of investigated winter wheats from Germany ('Anthus', 'Ararat', 'Brilliant', 'Compliment', 'Flair', 'Leiffer', 'Olivin') and Denmark ('Ambition', 'Audi', 'Jensen', 'Maserati', 'Skagen') were homozygous for the allele *Glu-A1c*, which is correlated with weaker gluten, and a few tested samples originated from Poland ('Korweta', 'Muza', 'Turnia'), Norway ('Bjørke' (ECRI collection)), the Netherlands ('Ritmo' (ECRI collection)) and Great Britain ('Dorota', 'Ebi'). Our results showed that the *Glu-A1c* allele was found in the modern winter cultivar 'Ada' (LIT), which is also the quality standard in the ECRI winter wheat collection trial (Ingver and Koppel, 2014).

Among spring wheat only six cultivars, originated from Germany ('Munk', 'Taifun', 'Trappe'), the Netherlands ('Hamlet', 'Tybalt') and New Zealand ('Amethyst'), showed amplification exclusively with marker *Axnull*.

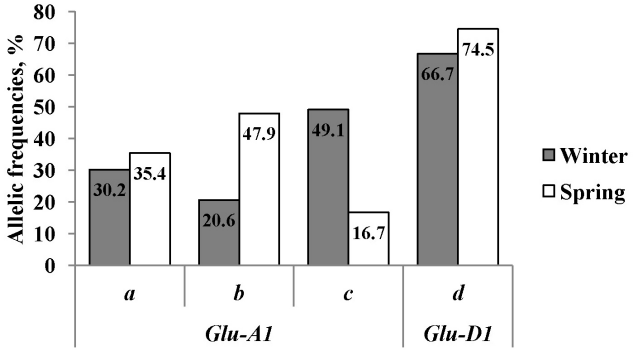

Fig. 1. Frequencies of *Glu-A1* and *Glu-D1* alleles in winter and spring wheat cultivars.

In contrast, exclusively Ax1 and Ax2* subunits were found in all cultivars presented in this study from Belarus (spring 'Darja', 'Razevet', 'Rostan', 'Viza'), Latvia (winter 'Fredis' and spring 'Robijs', 'Uffo') and Finland (spring 'Anniina', 'Aune', 'Kruunu', 'Mahti', 'Manu'). 'Manu' is a ECRI quality standard in spring wheat baking quality (Ingver and Koppel, 2014). Cultivars 'Bjørke' (NOR) received from NordGen and 'Ritmo' (NLD) from the CRI Gene Bank carried *Glu-A1b* and *Glu-A1a* alleles, respectively (Fig. 2, A).

Polymorphism at *Glu-A1* locus was revealed by DNA markers in 7 (16.3%) winter wheat cultivars from the ECRI collection — modern 'Portal', 'Tarso' (DEU), 'Joni', 'Sani' (EST) and very old cultivars 'Jõgeva 22', 'Kehra', 'Ümarik' (EST) (Fig. 3). There were eight (20.0%) modern spring wheat cultivars originated from Finland ('Anniina', 'Kruunu', 'Mahti'), Great Britain ('Azurite'), Belarus ('Darja', 'Viza') and Germany ('Picolo', 'Triso') also heterozygous for *Glu-A1* locus.

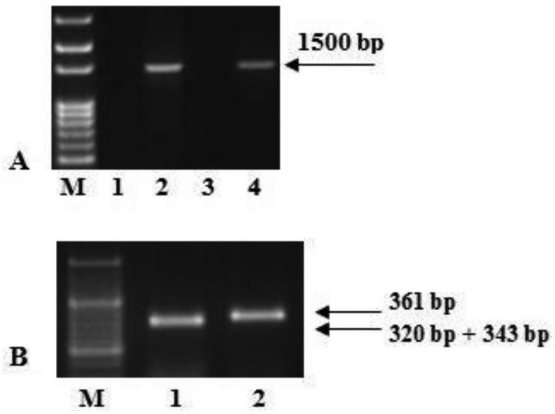

Fig. 2. The results of *Ax1+Ax2** (A) and *5+10* (B) marker amplification in Bjorke and Ritmo cultivars. (A) – 1, Bjørke (ECRI collection); 2, Bjørke (NordGen); 3, Ritmo (ECRI collection); 4, Ritmo (CRI Gene Bank); M, marker (100 bp DNA Ladder (Solis)). (B) – 1, Ritmo (ECRI collection); 2, Ritmo (CRI Gene Bank); M, marker (O'GeneRuler 50 bp (Thermo)).

Table 2

ALLELIC VARIATIONS AT HMW-GS *GLU-A1* AND *GLU-D1* LOCI IN INVESTIGATED WINTER WHEAT CULTIVARS BASED ON DNA MARKERS

Cultivar	Period of release[2]	Origin	*Glu-A1*		*Glu-D1d* allele (subunit Dx5)[1]
			allele	subunit	
Ada[3]	C	LIT	*c*	null	+
Akteur	C	DEU	*a*	Ax1	+
Ambition	C	DNK	*c*	null	-
Anthus	C	DEU	*c*	null	-
Ararat	C	DEU	*c*	null	-
Audi	C	DNK	*c*	null	-
Bjørke (ECRI collection)	C	NOR	*c*	null	+
Bjørke[3,4] (NordGen)	C	NOR	*b*	Ax2*	+
Brilliant[4]	C	DEU	*c*	null	+
Compliment[4]	C	DEU	*c*	null	+
Dorota	C	GBR	*c*	null	+
Ebi[3]	C	GBR	*c*	null	+
Eka[3]	C	EST	*b*	Ax2*	+
Flair[3,4]	C	DEU	*c*	null	+
Fredis	C	LVA	*a*	Ax1	+
Gosmer	C	DNK	*b*	Ax2*	+
Gunbo[3]	C	SWE	*b*	Ax2*	-
Jensen	C	DNK	*c*	null	+
Joni[3]	C	EST	*b/c*	Ax2*/null	-
Jõgeva [22]	A	EST	*a/b*	Ax1/Ax2*	-
Kallas	C	LIT/EST	*c*	null	+
Kalvi[3]	C	EST	*b*	Ax2*	+
Kehra	A	EST	*a/b*	Ax1/Ax2*	-
Korweta[3]	C	POL	*c*	null	+
Kuusiku[3]	A	EST	*a*	Ax1	-
Lars[3]	C	DEU	*a*	Ax1	+
Leiffer	C	DEU	*c*	null	+
Luunja	A	EST	*a*	Ax1	+/-
Maribo	C	DNK	*a*	Ax1	+
Maserati	C	DNK	*c*	null	+
Mulan[4]	C	DEU	*a*	Ax1	+
Muza	C	POL	*c*	null	+/-
Nemunas	C	LIT/EST	*c*	null	+
Olivin[4]	C	DEU	*c*	null	+
Portal[3]	C	DEU	*a/c*	Ax1/null	+
Puuk[3]	B	EST	*a*	Ax1	-
Ramiro[3]	C	DEU	*a*	Ax1	+
Ritmo (ECRI collection)	C	NLD	*c*	null	+
Ritmo[3,4] (CRI Gene Bank)	C	NLD	*a*	Ax1	-
Sani[3]	C	EST	*a/b*	Ax1/Ax2*	+/-
Širvinta [13]	C	LIT	*a*	Ax1	+
Skagen	C	DNK	*c*	null	+
Tarso[3,4]	C	DEU	*b/c*	Ax2*/null	+
Turnia	C	POL	*c*	null	+/-
Ümarik	A	EST	*a/b/c*	Ax1/Ax2*/null	-

[1] + *d* allele; - *a* or *a*-like allele; [2] A, very old, bred in 1930s; B, old, bred in the period of 1950s–1970s; C, modern, bred after 1980s; [3] cultivars were previously tested with SDS-PAGE; [4] cultivars were previously tested with allele-specific DNA markers.

Table 3

ALLELIC VARIATIONS AT HMW-GS *GLU-A1* AND *GLU-D1* LOCI IN INVESTIGATED SPRING WHEAT CULTIVARS BASED ON DNA MARKERS

Cultivar	Period of release[2]	Origin	*Glu-A1*		*Glu-D1d* allele (subunit Dx5)[1]
			allele	subunit	
Amaretto	C	DEU	*a*	Ax1	+
Amethyst	C	NZL	*c*	null	+
Anniina	C	FIN	*a/b*	Ax1/Ax2*	-
Aune	C	FIN	*b*	Ax2*	+/-
Azurite	C	GBR	*b/c*	Ax2*/null	+
Baldus[3]	C	NLD	*a*	Ax1	+
Bjarne[3,4]	C	NOR	*b*	Ax2*	+
Bombona	C	POL	*b*	Ax2*	+
Canon	C	SWE	*b*	Ax2*	-
Darja	C	BLR	*a/b*	Ax1/Ax2*	+/-
Hamlet	C	NLD	*c*	null	+
Helle[3]	C	EST	*b*	Ax2*	-
Katoda	C	POL	*b*	Ax2*	+
Kruunu	C	FIN	*a/b*	Ax1/Ax2*	+
Łagwa	C	POL	*a*	Ax1	+
Mahti[3]	C	FIN	*a/b*	Ax1/Ax2*	+
Manu[3]	C	FIN	*b*	Ax2*	+
Meri[3]	C	EST	*b*	Ax2*	-
Monsun[3]	C	DEU	*a*	Ax1	+
Munk[3]	C	DEU	*c*	null	+
Picolo	C	DEU	*a/c*	Ax1/null	+
Razevet	C	BLR	*b*	Ax2*	+
Robijs	C	LVA	*a*	Ax1	+
Rostan	C	BLR	*a*	Ax1	+
Runar[3]	B	NOR	*b*	Ax2*	+
Satu[3]	C	SWE	*b*	Ax2*	+/-
Specifik	C	FRA	*b*	Ax2*	+
SW Estrad[3]	C	SWE	*b*	Ax2*	-
SW Kadrilj	C	SWE	*a*	Ax1	+
SW Kaliber	C	SWE	*a*	Ax1	+/-
Taifun[3]	C	DEU	*c*	null	+
Tjalve[3,4]	C	SWE	*b*	Ax2*	+
Trappe	C	DEU	*c*	null	+
Triso[3]	C	DEU	*a/b*	Ax1/Ax2*	+
Tybalt	C	NLD	*c*	null	+
Uffo	C	LVA	*a*	Ax1	+
Vanek	C	DEU	*a*	Ax1	+
Vinjett[3]	C	SWE	*b*	Ax2*	+
Viza	C	BLR	*a/b*	Ax1/Ax2*	+/-
Zebra[3,4]	C	SWE	*b*	Ax2*	+

[1] + *d* allele; - *a* or *a*-like allele; [2] A, very old, bred in 1930s; B, old, bred in the period of 1950s–1970s; C, modern, bred after 1980s; [3] cultivars were previously tested with SDS-PAGE; [4] cultivars were previously tested with allele-specific DNA markers; N/A, data were not available.

The majority of the winter and spring wheat cultivars had subunit Dx5. The frequencies of Dx5 were higher in spring wheat cultivars than in winter wheats (Fig. 1). The marker

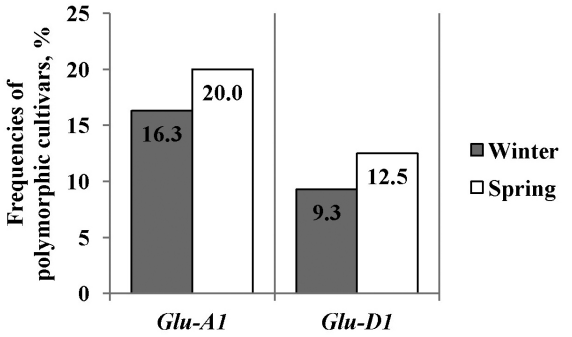

Fig. 3. Frequencies of polymorphic winter and spring wheat cultivars for *Glu-A1* and *Glu-D1* loci.

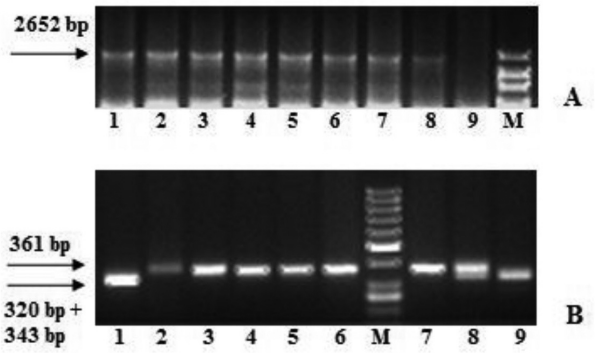

Fig. 4. Gel elecrophoresis of PCR products amplified with cv. 'Viza' DNA using *x2** (A) and *5+10* (B) markers. (A) – 1–8, Ax2* subunit present; 9, Ax2* subunit absent; M, marker (100 bp DNA Ladder (Solis BioDyne)). (B) – 1, 9, Dx5 subunit present; 2–7, Dx5 subunit absent; 8, heterozygous sample; M, marker (O'GeneRuler 50 bp (Thermo)).

of this subunit was detected in genotypes of all countries of origin, including ECRI winter and spring wheat trial quality standards 'Ada' (LIT) and 'Manu' (FIN). As for samples of 'Ritmo' (NLD) and 'Bjørke' (NOR) from different genetic collections, the marker of Dx5 protein subunit was amplificated in both 'Bjørke' samples and in 'Ritmo' from the ECRI genetic collection (Fig. 2, B).

Four (9.3%) winter wheat cultivars, the very old 'Luunja' (EST) and modern 'Sani' (EST), 'Muza' and 'Turnia' (POL), and five (12.5%) modern spring wheat cultivars 'Darja', 'Viza' (BLR), 'SW Kaliber', 'Satu' (SWE) and 'Aune' (FIN) from ECRI stock were polymorphic for the *Glu-D1* locus (Fig. 3).

Thus, modern cultivars 'Sani' (EST), 'Darja' and 'Viza' (BLR) (Fig. 4) are polymorphic for both *Glu-A1* and *Glu-D1* HMW-GS loci. However, the total amount of polymorphic samples among modern wheat cultivars was not great — 14.5% for *Glu-A1* and 10.5% for *Glu-D1* loci.

From all the investigated Estonian cultivars, there were included six old and very old ('Jõgeva 22', 'Kehra', 'Kuusiku', 'Luunja', 'Puuk', 'Ümarik') and 6 modern ('Eka', 'Helle', 'Joni', 'Kalvi', 'Meri', 'Sani'). As for the old cultivars, it was found that four (66.7%) of them were polymorphic ('Jõgeva 22', 'Kehra', 'Ümarik' — for *Glu-A1*; 'Luunja' — for *Glu-D1*) (Fig. 5). Among modern

cultivars, only two (33.3%) showed polymorphism: 'Joni' — for *Glu-A1*, and 'Sani' — for both *Glu-1* loci.

Among the 83 wheat cultivars used in this study, we found that 34 had been previously tested with SDS-PAGE (Anonymous, 2017; Tohver, 2007; Békés and Wrigley, 2013) and 11 with allele-specific DNA markers (Jin *et al*., 2011) (Tables 2 and 3). Comparison of our results with previous HMW-GS SDS-PAGE (Anonymous, 2017; Tohver, 2007; Békés and Wrigley, 2013) indicated complete or partial agreements of 78.1% and 70.6% for allelic variants in *Glu-A1* and *Glu-D1* loci, respectively. Disagreement in results of DNA and protein SDS-PAGE of HMW-GS genotyping is presented in Table 4.

DISCUSSION

We used PCR markers to select good quality donors and apply in marker-assisted selection in wheat breeding in Estonia.

For the locus *Glu-D1* we used a marker that detects the Dx5 subunit, because the major difference between *Glu-D1d* and *Glu-D1a* alleles lies in their 1Dx subunits, since there are only minor differences in structure between subunits 1Dy10 and 1Dy12 (Shewry *et al*., 2002; Dong *et al*., 2013). It was shown that hierarchical arrangement of *Glu-D1* glutenin subunits in relation to their proposed contributions to dough strength is described as: Dx5 > Dx2 > Dy10 ≥ Dy12 (Pogna *et al*., 1987; Lafiandra *et al*., 1993; Kasarda, 1999). The experiments with transgenic wheats with elevated levels of subunits Dx5 and Dy10 have shown that a comparatively small increase in Dx5 had significant positive effect on dough properties and polymeric protein content, whereas increase of Dy10 had smaller effect (Blechl *et al*., 2007).

Table 4

CULTIVARS WITH COMPLETE OR PARTIAL DISAGREEMENT OF DNA GENOTYPING AND SDS-PAGE OF HMW-GS (BY TOHVER, 2007; BÉKÉS, WRIGLEY, 2013)

Cultivar	*Glu-A1* subunit (x)		*Glu-D1* subunits (x+y)	
	SDS-PAGE	PCR	SDS-PAGE	PCR
Winter wheat				
Ada	1	null	5+10	5+10
Bjørke (ECRI collection)	2*	null	5+10	5+10
Gunbo	1	2*	5+10	2+12
Lars	2*/null	1	5+10	5+10
Eka	2*	2*	2+12/5+10	5+10
Kuusiku	1	1	2+12*	2+12
Mulan	N/A	1	2+12	5+10
Puuk	N/A	1	2+12*	5+10
Ritmo (ECRI collection)	1	null	2+12	5+10
Širvinta 1	1/2*	1	5+10	5+10
Sani	1/2*/null	1/2*	5+10	2+12/5+10
Turnia	N/A	null	5+10	2+12/5+10
Spring wheat				
Baldus	null	1	2+12	5+10
Helle	2*/null	2*	2+12	2+12
Mahti	1	1/2*	5+10	5+10
Monsun	1	1	2+12	5+10
Munk	1/null	null	5+10/2+12	5+10
Satu	2*	2*	2+12	5+10/2+12
SW Estrad	2*	2*	5+10	2+12
Taifun	1	null	2+12	5+10
Triso	1/2*	1/2*	2+12/5+10	5+10
Zebra	2*	2*	3+12	5+10

Bold, disagreement; N/A, data were not available.

It is likely that in HMW-GS, differences in the number of cysteine residues are associated with variation in baking quality. The primary structure of HMW-GSs is composed of a signal peptide (removed from mature subunit), a N-terminal domain, a central repetitive domain, and a C-terminal domain. Subunit Dx5 differs from all other Dx subunits, which sequences are known, in a single additional cysteine located at the N-terminal part of its repetitive domain (reviewed by Shewry *et al*., 2002). The remaining Dx subunit variants in common wheat are all Dx2-like according to their structure and effect on bread baking properties (Dong *et al*., 2013). Cysteine residues provide intermolecular disulphide bonds between high molecular weight and low molecular weight glutenin subunits to form protein polymers with a range of different sizes that can reach up to 10 million Daltons (reviewed by Shewry *et al*., 2002; Anjum *et al*., 2007). Relative to Dx2, the presence of extra cysteine in Dx5 is frequently associated with superior baking quality (Shewry *et al*., 2002; Dong *et al*., 2013; Rasheed *et al*., 2014).

Although about 29 alleles at the *Glu-D1* locus have been reported in bread wheat, two allelic variants *Glu-D1a* and *Glu-D1d* are most common (Tohver, 2007; Jin *et al*., 2011; Békés, Wrigley, 2013). Two rare subunit pairs were found previously by SDS-PAGE method only in four cultivars from our study: 2+12* was in very old Estonian cultivars 'Kuusiku' and 'Puuk' and 3+12 was in modern cultivars 'Zebra' and 'Ritmo' (Johansson *et al*., 2003; Tohver, 2007; Békés, Wrigley, 2013). Based on these findings we assume that investigated wheat genotypes that showed Dx5 subunit amplification possessed the *Glu-D1d* allele and the other genotypes carried *Glu-D1a*.

Ten analysed winter wheat cultivars ('Akteur', 'Eka', 'Fredis', 'Gosmer', 'Kalvi', 'Lars', 'Maribo', 'Mulan', 'Ramiro', 'Širvinta 1') and 22 spring wheat cultivars ('Amaretto', 'Baldus', 'Bjarne', 'Bombona', 'Robijs', 'Katoda', 'Kruunu', 'Łagwa', 'Mahti', 'Manu', 'Monsun', 'Razevet', 'Rostan', 'Runar', 'Specifik', 'SW Kadrilj', 'Tjalve', 'Triso', 'Uffo', 'Vanek', 'Vinjett', 'Zebra') carried desirable alleles *a* (Ax1) or *b* (Ax2*) in the locus *Glu-A1* and *d* (Dx5+Dy10) in *Glu-D1* simultaneously. Less desirable combination of subunits Axnull and Dx2 (or alike), was detected only in four winter wheats ('Ambition', 'Anthus', 'Ararat', 'Audi').

It should be noted that the ECRI quality standard cv. 'Ada' carries *Glu-A1c* allele encoding Axnull HMW-GS (in combination with *Glu-D1d* (Dx5)) by DNA marker analysis, but according to baking tests carried out in ECRI, 'Ada' had very good baking quality based on protein content, dough stability and final baking tests (Koppel and Ingver, 2010; Ingver and Koppel, 2014; Koppel and Kangor, 2017).

The differences in allelic variations between winter and spring wheats (Fig. 1) may be explained by the largely different sets of parents used in winter and spring wheat breeding programmes in different countries (Tohver, 2007; Jin *et al*., 2011).

Old Estonian winter wheat cultivars (Table 1) may be a source of valuable Ax1 and Ax2* subunits.

Among 34 common wheat cultivars that were tested with SDS-PAGE previously for the locus *Glu-A1*, complete discrepancies between SDS-PAGE and PCR results were found for 'Ada', 'Bjørke' (ECRI collection), 'Gunbo', 'Lars', 'Ritmo' (ECRI collection), 'Baldus' and 'Taifun' (Table 4). For the locus *Glu-D1*, 5 cultivars ('Mulan', 'Puuk', 'Ritmo' (ECRI collection), 'Baldus', 'Monsun', 'Taifun', 'Zebra') contained Dx5 based on the DNA markers, but were not identified with SDS-PAGE, and 2 cultivars ('Gunbo', 'SW Estrad') with Dx5 identified using SDS-PAGE were not identified by the PCR.

Presumably overestimated molecular masses and low resolution was the main reason for the discrepancy. Different subunits were identified by the mobility on SDS-PAGE, but there was not always consistent agreement between molecular weight and mobility in SDS-PAGE method, and because of close position of Dx2 and Dx5 on the gel, accurate identification was complicated. The identification of these alleles using specific PCR primers was precise and straightforward (Jin *et al*., 2011; Zamani *et al*., 2014). However, functional markers were not developed for the majority of rare HMW-GS alleles, such as *Glu-D1j*, *Glu-D1b*, which can be determined by SDS-PAGE. Another possible reason for discrepancy might be cultivar polymorphism, as samples from different plants were taken for each study, or the possibility of errors in labelling and cross contamination that occurred in seed collections during seed handling or propagation (Gewin, 2011).

Old cultivars were bred by different methods compared to modern cultivars. There were no strict rules for homogeneity and stability before 2000 when Estonia became a member of the International Union for the Protection of New Varieties of Plants (UPOV). Polymorphism and non-homogeneity that appear more often in older varieties (Fig. 5) could be explained by the reasons mentioned above.

Concerning previous DNA genotyping results (Jin *et al*., 2011), discordance was detected for the locus *Glu-A1* in cv. 'Bjørke' (ECRI collection) and for *Glu-D1* in 'Mulan' and 'Ritmo' (ECRI collection). It is interesting to note that our results of cv. 'Zebra' genotyping with DNA markers for the

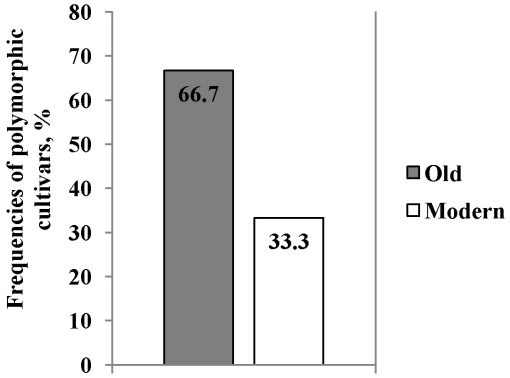

Fig. 5. Frequencies of polymorphic old and modern Estonian wheat cultivars.

locus *Glu-D1* (Dx5) correspond with those by H. Jin *et al*. (2011) but differ from SDS-PAGE data (Dx3+Dy12) (Tohver, 2007).

For testing of the presumption that the samples of the same cultivar from different gene banks may have differences in their genotypes, we conducted analysis of genetic material from the ECRI genetic collection and from gene banks of NordGen and CRI using HMW glutenins DNA markers (Table 2). The amplification of the *Glu-A1*, *Glu-D1* allele markers showed that 'Bjørke' and 'Ritmo' winter cultivars from the ECRI collection and other gene banks varied in HMW glutenin allelic composition (Fig. 2). ECRI and NordGen 'Bjørke' samples had *Glu-A1c*, *Glu-D1d* and *Glu-A1b*, *Glu-D1d* alleles in their genotypes, respectively. Cv. 'Ritmo' from ECRI and CRI carried *Glu-A1c, Glu-D1d* and *Glu-A1a, Glu-D1a* (or *Glu-D1a*-like) alleles, respectively. Consequently, although the HMW glutenin allelic composition historical data exists (Anonymous, 2017; Tohver, 2007; Jin *et al*., 2011; Békés, Wrigley, 2013), it is still necessary to analyse genetic material also from own collection.

CONCLUSIONS

Functional DNA markers for *Glu-D1* and *Glu-A1* genes were used in the ECRI for the first time to characterise spring and winter wheat cultivars. This method helps to avoid misinterpretation of results from SDS-PAGE that were used earlier for predicting baking quality.

The agreement of the PCR and SDS-PAGE data for most of the cultivars showed the utility of molecular markers in wheat breeding programmes for early selection of desirable HMW glutenin subunits, without the need of carrying out an analysis of storage proteins. It is possible to conclude that molecular-genetic analysis based on HMW glutenin subunit markers can discriminate the HMW-GS alleles, providing an efficient, accurate and reliable diagnostic technique for rapid genotyping assay. Thus, the present study was initiated to apply an advanced DNA-based technology to assess genetic polymorphism of the genes encoding storage proteins in wheat cultivars used in Estonia. The obtained results are important for the evaluation of crop ge-

netic resources and subsequent utilisation in wheat breeding programmes to improve wheat quality and accelerate the breeding process.

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AUGSTA MOLEKULĀRĀ SVARA GLUTENĪNA SUBVIENĪBU *GLU-A1* UN *GLU-D1* IDENTIFIKĀCIJA MĪKSTIEM KVIEŠIEM (*TRITICUM AESTIVUM* L.) AR MOLEKULĀRO MARĶIERU PALĪDZĪBU

Augsta molekulārā svara glutenīna subvienībām ir liela nozīme kviešu maizes cepšanas kvalitātes noteikšanā. Šajā pētījumā ar polimerāzes ķēdes reakcijas (*PCR*) metodes palīdzību tika noteikts lokusu *Glu-A1* un *Glu-D1* alēļu spektrs 43 ziemas un 40 vasaras mīksto kviešu šķirnēm ar dažādu ģeogrāfisko izcelsmi. Ātra un precīza *Glu-1* alēļu noteikšana ar PCR metodi var tikt efektīvi izmantota selekcijas procesā vērtīgāko kviešu genotipu identifikācijai.