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Detection of High Molecular Weight Glutenin Subunits in Triticale (× *Triticosecale* Wittm.) Cultivars by Capillary Zone Electrophoresis

BOLESŁAW P. SALMANOWICZ[†]

Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska Street 34, PL 60-479 Poznań, Poland

An improved method for separating and characterizing high molecular weight glutenin subunits (HMW-GS) in hexaploid triticale by capillary zone electrophoresis (CZE) was developed. A low-concentrate mixture of hydrophilic polymers, poly(vinylpyrrolidone) (PVP) and hydroxypropylmethylcellulose (HPMC), in an isoelectric buffer was employed for dynamic coating of the capillary inner wall. In separation buffer PVP with lower concentrated poly(ethylene oxide) (PEO) was replaced. The CZE electropherograms of HMW-GS showed two group peaks in accordance with x- and y-type subunits with migration times of 6.8−7.8 and 8.4−11.5 min, respectively. In total, 14 HMW subunits (2 subunits encoded by *Glu-A1* locus and 12 by *Glu-B1*) were identified. The CZE analyses revealed that each of the subunits Bx7 and By8 determined by SDS-PAGE makes up three subunits (Bx6.8, Bx7, and Bx7* and By8, By8*, and new By8**, respectively), with different migration times. It was also shown that the subunits By18 and By20 in triticale determined by SDS-PAGE have different migration times in comparison with the same subunits in bread wheat. For these new HMW-GS, the following names were assigned: By18* instead of By18 and By20* instead of By20. The presented CZE method is an efficient alternative to the SDS-PAGE procedure for early selection of useful triticale genotypes with good breadmaking quality.

KEYWORDS: Allelic variation; *Glu-1* loci; storage proteins; triticale; wheat

INTRODUCTION

Triticale (× *Triticosecale* Wittmack) is a synthetic species resulting from crossing wheat (*Triticum* ssp.) and rye (*Secale* ssp.). This species shows some agronomic advantages relative to its parents together with its high yield potential in a wide range of environmental conditions (*1*−*3*). Grains of that species contain high concentrations of the essential amino acid lysine, which makes it more nutritionally valuable than wheat (*4, 5*). However, in comparison with common wheat, most triticale flour has lower protein content and milling yield, higher ash content, and inferior loaf volume, which limit its breadmaking quality (*6*). Hexaploid triticale flour from many cultivars is suitable for the manufacture of products that may be prepared with gluten of lower tenacity (cookies, cakes, crackers, tortillas, and waffles) (*6, 7*) and can be used for breadmaking by mixing it with wheat flour at up to 70% triticale amount (*5*).

Commonly grown triticale is hexaploid (AABBRR) and differs from bread wheat (*Triticum aestivum* L.) (AABBDD) by the presence of the R genome of rye that replaces wheat genome D. The breadmaking quality of bread wheat is mainly determined by the composition and quantity of gluten-forming storage proteins, in particular the high molecular weight glutenin subunits (HMW-GS) (*8*−*11*). HMW-GS form large polymeric structures through disulfide bonds, which are related to molecular properties of dough. In bread wheat, HMW glutenin genes are encoded at the complex loci *Glu-A1*, *Glu-B1*, and *Glu-D1* on the long arms of homeologous group 1 chromosomes (*12*). Each locus includes two tightly linked genes encoding two types of HMW-GS, designated x-type and y-type. The absence of the wheat *Glu-D1* loci in triticale with their known positive effects on breadmaking quality causes deterioration in rheological properties and total gluten strength and an increase in dough viscosity, and thus breadmaking quality is poorer. Although genetic variability for gluten content exists in triticale, the highest gluten content is still 10−15% lower than in wheat (*12*). In triticale there are only four wheat HMW glutenin genes even though one to three polypeptide subunits are synthesized in any particular cultivar (*13*−*15*). The corresponding two rye HMW secalin genes are encoded at the *Glu-R1* locus, designated *Sec-3* (*16*), which is located on the long arm of chromosome 1R. The rye secalins are distinct from wheat storage proteins and not capable of forming gluten.

In *T. aestivum*, HMW glutenin subunit compositions and study of their effects on bread quality were analyzed by some separation techniques such as polyacrylamide gel electrophoresis (SDS-PAGE and/or A-PAGE), reverse-phase high-performance liquid chromatography (RP-HPLC), and capillary electrophoresis

[†] Telephone +48 61 65 50 232; fax +48 61 65 50 301; e-mail bsal@igr.poznan.pl.

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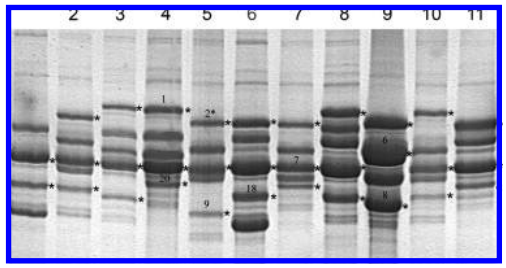

Figure 1. SDS-PAGE pattern of HMW glutenin subunits in triticale cultivars with characteristic subunit sets at the *Glu-A1* and *Glu-B1* loci. The bands marked with asterisks (∗) indicate the wheat HMW glutenin subunits. Lanes: 1, Kazo (N/7+18); 2, Moderato (2*/7+18); 3, Bogo (1/7+8); 4, Marko (1/7+20); 5, Mundo (2*/7+9); 6, Moreno (2*/7+18); 7, Sorento (2*/7+20); 8, Kitaro (1/7+18); 9, Janko (2*/6+8); 10, Disco (1/7+18); 11, Lasko (2*/7+20).

(CE) (*8, 9, 17*−*19*), whereas HMW glutenin subunits from triticale seeds were so far identified only by SDS-PAGE (*13*−*15, 20*) and using PCR-based DNA markers (*21*). The presence of both wheat and rye HMW proteins in triticale, which appear in different amounts and additionally often characterize similar electrophoretic mobilities, interfere to a large extent with the identification of individual HMW glutenin and secalin subunits using SDS-PAGE gels (*5, 13, 14*). For CZE separation of HMW proteins in wheat cultivars mainly low pH [sodium phosphate, phosphate−glycine, aspartic acid, iminodiacetic acid (IDA)] buffers with some additives have been used (*18, 22*−*26*). Several methods have been reported to control EOF, including polymer coating by covalent bonding and dynamic coating (*27*).

The present paper describes the exploitation of the capillary electrophoretic technique to identify and characterize European triticale cultivars carrying different HMW glutenin allelic combinations at *Glu-A1* and *Glu-B1* loci. The CZE method has been developed for the separation of wheat HMW-GS from triticale employing some hydrophilic polymers for dynamic coating of the capillary inner wall and optimization of resolution and ensured stability of the EOF. The HMW-GS compositions determined by the CZE and SDS-PAGE analyses were compared with literature SDS-PAGE data.

MATERIALS AND METHODS

Plant Materials. Grains of 86 European cultivars of both (68) winter and (18) spring complete hexaploid triticale were analyzed. The grain samples were obtained from DANKO Plant Breeding at Choryñ, Plant Breeding Companies at Strzelce (Branch at Mayszyn) and Szelejewo (western Poland), GEVES Surgeres (France), and Research Institute of Crop Production, Prague (Czech Republic).

Chemicals. Iminodiacetic acid (IDA), hydroxypropylmethylcellulose (HPMC), poly(vinylpyrrolidone) ($M_r \sim$ 40,000 and 360,000) (PVP), *N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate (SB3-12), poly(ethylene oxide) ($M_r \sim$ 8,000,000) (PEO) and polyethylenimine ($M_r \sim$ 750000) (PEI) were the products of Sigma Chemical Co. (St. Louis, MO). PVP ($M_r \sim$ 1,000,000) was purchased from Polysciences (CA). Sodium phosphate monobasic and dibasic and potassium phosphate monobasic were the products of J. T. Baker (Phillipsburg, NJ). 4-Vinylpyridine and poly(vinyl alcohol) (PVA) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Dextran and poly(vinylpyrrolidone) ($M_r \sim$ 160,000) were from Fluka (Riedel-deHaën, Germany). All solutions were filtered through a 0.5 μm Millipore (Bedford, MA) membrane filter before being injected into the capillary. Distilled water was deionized using a Milli-Q system (Millipore, Bedford, MA).

Instruments. CE experiments were performed on a Beckman-Coulter P/ACE System MDQ capillary electrophoresis instrument equipped with a diode array detector and Karate software, ver. 6.0, for system control and data handling. The separations were carried out using uncoated fused silica capillaries (Polymicro Technologies) with internal diameter of 50 μm, 31.2 cm in total length, and a detection window at 21 cm from the capillary inlet. Temperature in the capillary was controlled using a fluorocarbon-based cooling fluid.

Extraction of Total Triticale HMW Subunits. The extraction of total HMW subunits was based on the method of Wieser et al. (*9*) with some modifications. First, 25 mg of flour from the endosperm portion single seeds (the embryo portion removed with a knife) was pre-extracted twice with 0.25 mL of 0.4 M NaCl + 0.067 M $HKNaPO_4$ (pH 7.6) at room temperature to remove albumins and globulins (mixing each time on a vortex mixer for 10 min and centrifuging for 6 min at 13000*g*). The gliadins were next removed by extraction with 0.25 mL of 70% (v/v) ethanol and then twice from each kernel residue with 0.25 mL of 50% (v/v) 1-propanol (mixing each time on a vortex mixer for 20 min and centrifuging for 8 min at 15000*g*). The polymeric glutenins and secalins were twice extracted with 0.25 mL of 0.05 M Tris-HCl buffer (pH 7.5) containing 50% (v/v) 1-propanol, 2 M urea, and 1% (w/v) DTT at 60 °C under nitrogen, and next mixtures were centrifuged (10 min, 16000*g*). The joint supernatants were transferred into a new tube, and reduced total HMW proteins were precipitated by addition of 1-propanol to a final concentration of 62% (v/v); samples were left at 4 °C for the whole night. Precipitated HMW-GS were collected after centrifugation for 10 min at 13000*g* and next redissolved in 40 μL of a solution containing 40% v/v ACN + 0.1% (w/v) trifluoroacetic acid, vortexed for 30 min at 60 °C, and centrifuged again for 10 min at 16000*g*. All samples were used for CZE analyses within 24 h of extraction. Two protein separations were performed for each analytical assay.

Separation by SDS-PAGE. Reduced total triticale HMW subunits were separated by SDS-PAGE on a Protean II xi cell (Bio-Rad Laboratories, Hercules, CA) electrophoresis unit using the discontinuous buffer system of Laemmli (*28*). Ten microliter volumes of HMW-GS supernatants were loaded onto the upper 4.5% gel, and the proteins were separated on 11.5% (w/v) polyacrylamide in resolving solution at 240 V for 4.5 h. The gels were stained overnight with Coomassie Brilliant Blue. Designation of the wheat HMW glutenin and rye secalin subunits was performed according to the methods of Payne and Lawrence (*29*) and McIntosh et al. (*30*).

Separation by CZE. The separation of total HMW subunits was carried out according to the method of Bean et al. (*18*) with some modifications. A solution containing 50 mM IDA, 0.15% PEO with a molecular weight of 8,000,000, 0.05% 26 mM SB3-12, and 20% (v/v) acetonitrile (ACN) was used as separation buffer. As the polymer solution for dynamic coating of the capillary wall, a buffer containing 0.1 M IDA, 0.2% (w/v) PVP (M_r 360,000), 0.05% (w/v) HPMC, and 20% (v/v) ACN was used. The separation was performed with a constant voltage of 10.5 kV at 40 °C. The capillary was equilibrated with the running buffer for 4 min (0.3 MPa) before each sample injection. To ensure good repeatability, after each separation the capillary was rinsed stepwise with 0.1 M HCl (0.3 MPa for 4 min), with Milli-Q water (0.3 MPa, 1 min) and then with coating polymer solution (0.25 MPa, 3 min). For the first use on a day, the capillary was additionally flushed with 1 M HCl for 20 min, followed by a 5 min rinse with Milli-Q water and 15 min with separation buffer. Samples were injected hydrodynamically under low pressure [0.5 psi (3.447×10^{-3}) MPa]) for 6 s into the anodic end. The PVP and PEO solutions were stored in a refrigerator at 4 °C after being degassed with a vacuum system in an ultrasonic tank. All solutions and buffers were filtered through a 0.20 μm syringe filter. Proteins were detected by UV absorbance measurements at 200 nm. Three protein separations were performed by CZE for each analytical assay. The particular HMW-GS were identified by CZE through comparison of single and mixture samples by using standard wheat cultivars and results obtained from SDS-PAGE separations. The relative quantities of individual HMW-GS in total HMW extracts were determined by corrected peak areas A_{corr} according to formula: $A_{corr} = i \times A = (L_d \times A)/t$, where I is velocity, L_d is the capillary length to detector, A is the uncorrected peak area, and t is the migration time. In CE, early peaks migrate through the detector window more quickly than do later peaks. It creates a peak area bias, which can be eliminated by using corrected peak area.

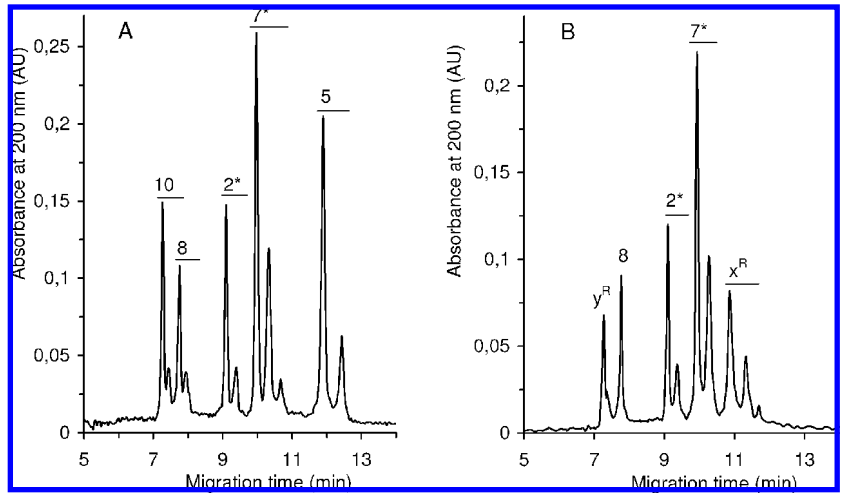

Figure 2. Separation of HMW glutenin and secalin subunits from the wheat cultivar Nadobna (**A**) and triticale cv. Clercal (**B**) with the same wheat subunit composition 2*/7*+8. Proteins were separated at 10.5 kV and 45 °C with a 50 mM isoelectric IDA buffer, containing 0.15% poly(ethylene oxide), 26 mM SB3-12, and 20% ACN. Prior to the separation, the capillaries (31.2/21 cm) were rinsed with 0.1 M IDA solution containing 0.2% PVP, 0.05% HPMC, and 20% ACN. HMW-GS are numbered according to accepted nomenclature (*30*). x^R and y^R indicate x-type and y-type HMW secalin subunits from triticale cv. Clercal, respectively.

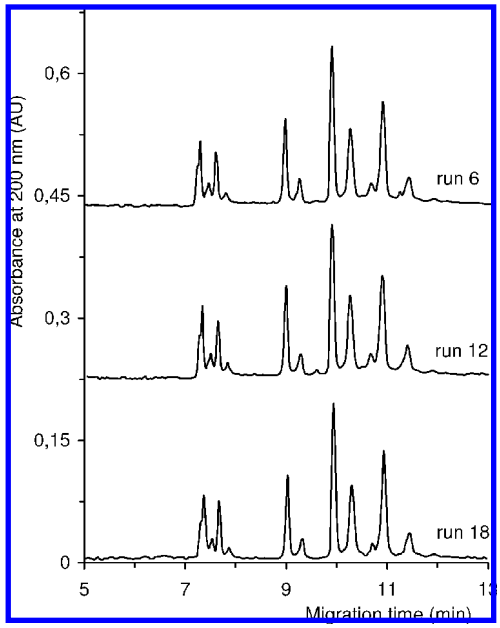

Figure 3. Reproducibility of sequential HMW protein fraction from triticale cultivar Todan. Runs 6, 12, and 18 are shown from series of 18 consecutive injections. Separation conditions were as described in **Figure 2**.

Statistical Analysis. All statistical analyses were conducted using Minitab statistical software (version 12, Minitab Inc., State College, PA).

RESULTS AND DISCUSION

SDS-PAGE Analysis. Reduced total triticale HMW extracts were used for identification of HMW-GS and HMW secalin subunits (HMW-SS) by one-dimensional SDS-PAGE. **Figure 1** shows electrophoretic patterns of some triticale varieties differing in their alleles at two wheat *Glu-1A* and *Glu-B1* loci and one rye *Glu-R1* locus. The analyzed accessions had two or three HMW-GS and two HMW-SS. HMW-GS extracts from six common wheat cultivars, Hope (1/6+8/5+10)-1Ax/1Bx+1By/1Dx+1Dy), Glenlea (2*/7+8*/5+10), Katepwa (2*/7+9/5+10), Lancota (1/13+16/5+10), Rapsodia (N/17+18/2+12), and Federation (1/20/5+10), were used as HMW-GS reference markers. In total, 11 HMW glutenin subunits were identified among triticale cultivars by SDS-PAGE; two typical wheat subunits (Ax1 and Ax2*) encoded by the *Glu-A1* locus, three x-type (Bx6, Bx7, Bx13), and five y-type (By8, By9, By16, By18, and By20) encoded by the *Glu-B1* locus, and additionally one typical triticale subunit Bx6.8. However, the presence of both wheat and rye HMW proteins with similar electrophoretic mobilities and molecular masses makes the identification of individual subunits difficult. Similar patterns of allelic variations were earlier described using the same method by Amiour et al. (*13, 14*). In the present study, 80 cultivars appeared to show genetic homogeneity, whereas 6 others were found to be heterogeneous with respect to the HMW-GS. In the analyzed material two wheat HMW-GS allelic compositions, 2*/6.8+20 and 2*/7+18, were the most frequent (24.4 and 22.1%, respectively).

Optimization of CZE Separation Conditions. HMW glutenin extracts from common wheat were used as model proteins for the developed separation method of wheat HMW proteins in triticale. The presence of both wheat and rye HMW proteins in triticale, which appear in different amounts and additionally often have similar molecular weights and electrophoretic mobilities, interfere to a large extent with the identification of individual subunits using both SDS-PAGE and earlier applied in CZE conditions. Until now, low-conductive phosphate−glycine buffer (pH 2.5) or isoelectric buffer system composed of iminodiacetic acid in conjunction with 20% ACN and 0.05% HPMC was the most popular as separation buffer of cereal storage proteins in uncoated fused-silica capillaries (*11, 18, 22*−*26*). After each separation the capillaries were rinsed only with separation buffer (*18, 22, 23*) or with phosphoric or hydrochloric acid and then separation buffer (*25, 26*).

In the present study, a low-concentrated PVP independently or in the mixture with HPMC resolved in isoelectric IDA buffer containing 20% ACN was employed as coating solvent for improvement of resolution, stabilization of baseline, and run-to-run separation reproducibility. Three types of PVP with different molecular masses (M_r 40,000, 360,000, and 1,000,000) and concentrations (0.1−2%) as additives to IDA buffer with different concentrations were examined. Dynamic coating of the capillary with inert molecules such as high molecular hydrophilic polymers is essential because it prevents the variation of EOF and reduces protein−capillary wall interactions

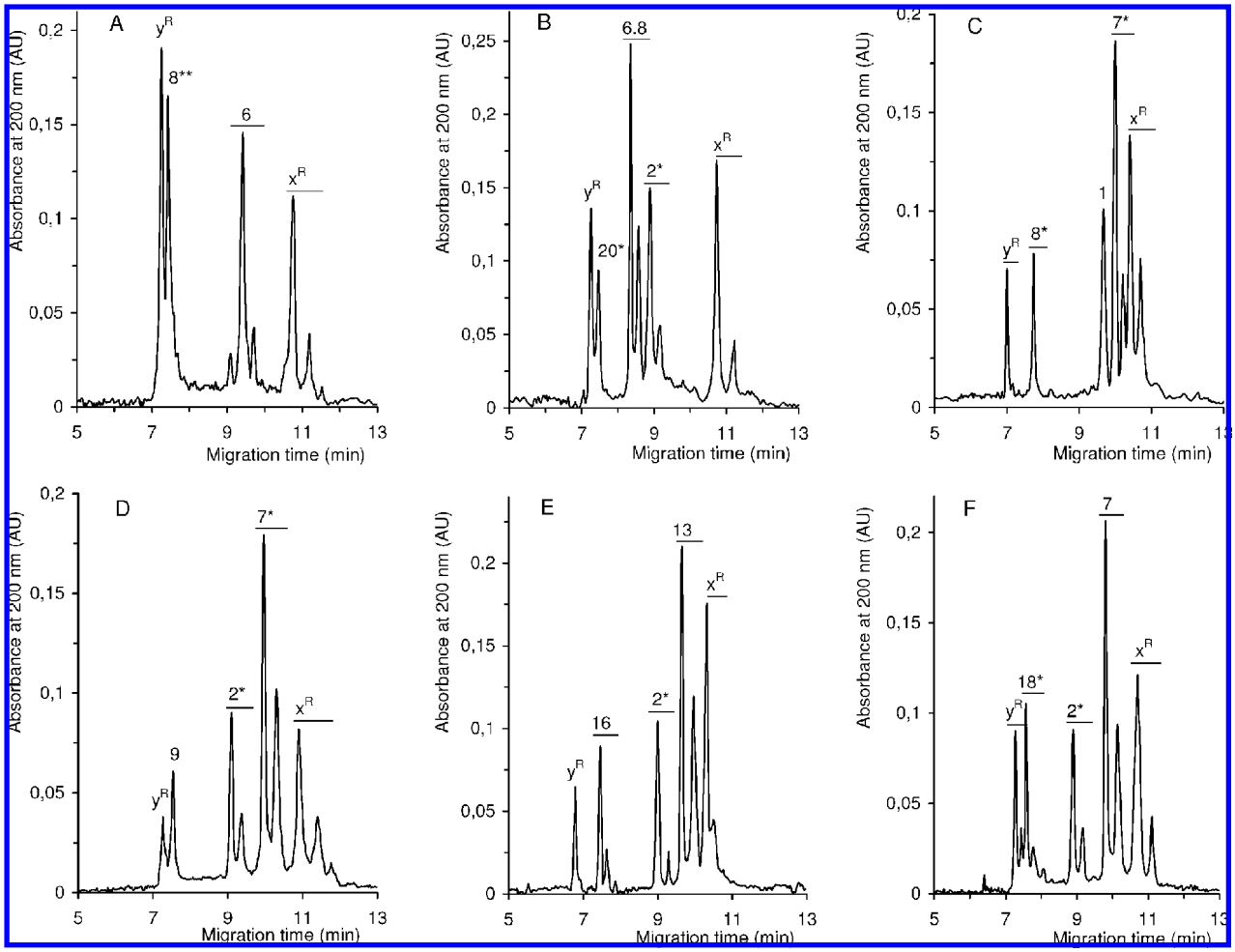

Figure 4. Electropherograms of HMW glutenin and secalin subunits from triticale cultivars with different subunits compositions: (**A**) Focus (N/6+8**); (**B**) Sorento (2*/6.8+20*); (**C**) Bogo (1/7*+8*); (**D**) Woltario (2*/7*+9); (**E**) Donatus (2*/13+16); (**F**) Dublet (2*/7+18). x^R and y^R indicate x-type and y-type HMW secalin subunits from particular cultivars, respectively. Separation conditions were as described in **Figure 2**.

(*27, 31*). It has been revealed that especially PVP, a low-viscosity polymer, has good dynamic coating ability in protein and DNA separation media (*24, 32, 33*). Due to them, its lower viscosity can be easily replaced in the capillary with fresh separation buffer. Until now, dynamic coating of the capillary has been accomplished by flushing 1−5% concentration of PVP with an aqueous solution (pH 6−8) and next filled with separation buffer containing another hydrophilic polymer. The application of mixture of low-concentrated PVP with molecular mass (M_r = 360,000) and HPMC for separation of total triticale HMW subunits provided better run-to-run repeatability of migration times and corrected peak areas. Optimal conditions were obtained by flushing (3 min) capillary with isoelectric (0.1 M IDA) buffer containing 0.2% (w/v) (PVP), 0.05% (w/v) HPMC, and 20% (v/v) ACN (data not shown). It indicates that mixtures of hydrophilic polymers with different chemical compositions can create multiple layers on the capillary surface, which provide better masking of the negative charger inner wall.

Further optimization of the separation efficiency and the resolution was performed replacing PVP in separated buffer with four low-concentrated (0.1−0.5%, w/v) hydrophilic polymers (PEO, PVA, PEI, PVP, and dextran). In recent years these polymers, possessing also self-coating and sieving capability, with 1−10% concentration have been tested independently for separation of cereal storage proteins and other biological important polymers by SDS-CGE methods (*27, 35, 36*). PEO, among the four examined polymers in this work, was the most effective additive for improving resolution and migration time reproducibility of total triticale HMW subunits by CZE. Some authors (*32−34*) also reported that DNA separations using capillary coated with PVP and filled with low-concentrated PEO provides the advantages of reproducibility of run-to-run separations, high resolution, and rapidity. Concentrations of PEO from 0.05 to 0.5% in isoelectric buffer with 50−150 mM IDA and 10−30% ACN were tested. Resolution was judged to be optimum at 0.15% PEO (data not shown). Two example CZE electropherograms of HMW-GS from wheat cultivar Nadobna and triticale cultivar Clercal showed that all characterized subunits can be accurately identified (**Figure 2**). Both wheat and triticale cultivars have subunit Ax2* encoding at the *Glu-A1* locus and subunit Bx7* and By8 encoding at the *Glu-B1*locus.

The addition of zwitterionic detergent SB3-12 at approximately its critical micelle concentration (26 mM) to separation buffer including PEO instead of HPMC in case either the separation of wheat and triticale HMW protein extracts additionally improves migration time reproducibility and there is better separation efficiency of more slowly migrating subunits. This compound was earlier recommended by Lookhart and Bean (*24*) as a factor improving resolution of cereal storage proteins, with the exception of use of them in IDA buffer in conjunction with ACN and HPMC (*18*).

To test the run-to-run reproducibility of HMW-GS separations 18 consecutive injections were performed. In **Figure 3** three

Table 1. Wheat HMW Glutenin Subunit Compositions for 86 European Triticale Cultivars Determined by SDS-PAGE and CZE

		Glu-B1		
no.	Glu-A1	SDS-PAGE	CZE	cultivars
1	N	6+8**	6+8**	Focus, Modus, Salvo, Trias, Ugo[a]
2	1	6+8	6+8**	Olimpus
3	2	6+8	6+8**	Alzo, Angus, Hewo, Janko, Prader, Prado, Lupus, (Ugo)[a]
4	N	7+20	6.8+20*	Gabo, Lamberto, (Mieszko)[a]
5	1	7+20	6.8+20*	(Marko)[a]
6	2*	7+20	6.8+20*	Carnac, Dato, Ego, Fidelio, Lasko, Legalo, Lukas, Matejko, Massimo, Mungis, Santop, Sekundo, Sorento, Tewo, Trigold, Trinidad, Tornado, Vision, Wanad
7	N	7+8	7*+8	Pronto,[a] Kargo, Mieszko[a]
8	1	7+8	7*+8	Pawo, (Kargo),[a] Magistral
9	2*	7+8	7*+8	Clercal, Tricolor, Trimaran, Witon
10	N	7+8	7*+8*	Prego
11	1	7+8	7*+8*	Bogo, Eldorado, Malno
12	N	7+9	7*+9	(Mundo)[a]
13	2*	7+9	7*+9	Mundo,[a] Woltario[a]
14	N	7+18	7+18*	Chrono, Kazo, Magnat, Modus, Pinokio, (Pronto),[a] Zorro
15	1	7+18	7+18*	Almo, Dagro, Disco, Kitaro
16	2*	7+18	7+18*	Aliko, Ampiac, Baltiko, Boreas, CHD503, Dinaro, Dublet, Gniewko, Grenado, Krakowiak, MAN4302, Moderato, Moreno, Nemo, Piano, Presto, Titan, Todan, Tropic, Vero, Vision, (Woltario)[a]
17	N	13+16	13+16	Meridial
18	1	13+16	13+16	Antares
19	2*	13+16	13+16	Binova, Donatus, Saka, Ticino

[a] Heterogeneous cultivars; in parentheses, genotype with smaller quantitative participation in the individual cultivar.

electropherograms were presented (runs 6, 12, and 18) from series of 18 HMW protein separations of triticale cultivar Todan. Five major peaks (three HMW-GS and two HMW-SS) were selected to assess the reproducibility of subunit migration times and corrected peak area quantification. Standard deviations for migration times of peaks representing high peaks of corresponding subunits were very low (0.01−0.06 min). The relative standard deviation (RSD) values for migration times of these subunits ranged from 0.16 to 0.68%. Also, the corrected peak area reproducibility was satisfactory; the RSD values for main peaks ranged from 1.64 to 2.46%. This modified procedure keeps satisfactory peak shapes and separation efficiencies for major peaks of wheat HMW-GS in range within 4.4×10^5−6.8×10^5 plates/m. The application of standard wheat protein extracts with each data set and comparison with previous runs are very important for ensuring good reproducibility and separation resolution.

CZE Analysis of HMW Glutenin Subunit. Six examples of CZE electropherograms of the presented different HMW-GS compositions in triticale genotypes are shown in **Figure 4**. These results indicate that satisfactory resolution of all HMW proteins can be achieved in 13 min. In general, HMW proteins were separated into two or three multiple peaks for individual HMW-GS and two for HMW-SS. In most cases, a high peak and a minor peak for particular peaks were observed. According to previous literature data (*25, 26*), these multiple peaks present different variants of posttranslational modifications of subunits. The individual HMW-GS and HMW-SS in triticale cultivars were separated in two time ranges, namely, y-type subunits with shorter migration time (6.8−7.8 min) and x-type subunits with longer migration times (8.4−11.5 min) at 10.5 kV. In most cases, the relative migration orders of the wheat HMW glutenin subunits were different in SDS-PAGE and CZE.

At the *Glu-A1* locus, the predominant part of the analyzed triticale samples has the subunit Ax2* or Ax1 with identical migration times as wheat HMW glutenin subunits, respectively. Subunits Ay encoded by alleles *Glu-A1−2* were not observed taking into consideration a sleeping gene, as in wheat (*8, 10*).

In total, at the *Glu-B1* locus 12 HMW-GS were distinguished in analyzed material, where 8 subunits were identical as in common wheat. Among five x-type HMW-GS, four subunits (Bx6, Bx7, Bx7*, and Bx13) have identical times of migration as standard wheat HMW-GS, which were also analyzed in this work. However, one subunit only appeared in triticale cultivars, and earlier was marked as Bx6.8 by Amiour and co-workers (*13*). This subunit characterizes the shortest migration time among the x-type subunits [**Figure 4B** (cv. Sorento)]. During separation on the SDS-PAGE gels electrophoretic mobility of this subunit is similar to that of subunits Bx7 and Bx7*, and it is a problem with correct identification (**Figure 1**). For this reason the subunit Bx6.8 in triticale cultivars was determined by Tohver and co-workers (*5*) as Bx7 and by Brzezinski (*20*) as Bx7*. Distinct from SDS-PAGE in the presented CZE method subunit Bx7 from allelic pair Bx7+By18 and subunit Bx7* from allelic pairs Bx7*+By8* and Bx7*+By9 were also easily distinguished (difference in migration times is 0.12 min at 10.5 kV). [**Figures 4F** (cv. Dublet), **4C** (Bogo), and **4D** (Woltario), respectively]. The relative migration orders of x-type HMW-GS encoded by genes at *Glu-A1* and *Glu-B1* loci were 6.8 < 2* < 13 < 6 < 1 < 7* < 7 on the basis of migration times (from the fastest to the lowest).

In European triticale cultivars seven y-type HMW-GS were identified, where five subunits (By8, By8*, By8**, By9, and By16) have identical migration times as wheat HMW-GS and two new subunits, By18* and By20*, were detected. These new subunits on the basis of SDS-PAGE patterns were earlier determined as By18 and By20 by Amiour et al. (*13*) or By26 and By25 or By19, respectively, by Brzezinski (*20*) and Tohver et al. (*5*). The migration times of the new subunits By18* and By20*, unique to triticale, differed from migration times of wheat subunits Bx20 and By18. The distinction of these subunits was earlier described using PCR-based DNA markers by Salmanowicz and Dylewicz (*21*). Besides, the CZE analyses revealed that subunit By8 determined by SDS-PAGE is made up of three subunits (By8, By8*, and the new By8**). The new subunit Bx8** in allelic compositions *Glu-B1d* (subunit pair 6+8) migrated more rapidly in the capillary than in subunit pairs

7*+8* (*Glu-B1ak*) and 7*+8 (*Glu-B1u*), differences in migration times of 0.18 and 0.21 min at 10.5 kV, respectively [**Figure 4A** (cv. Focus), **4C** (Bogo), and **2B** (Clercal), respectively]. For the fastest migrating subunit By8 encoded by *Glu-B1d*, the new name By8** was given. The discrimination between alleles encoding subunit pairs Bx6+By8** and Bx7+By8/By8* (not distinguished by SDS-PAGE) is particularly important, because these allelic compositions give differential effects on breadmaking quality in common wheat cultivars (*10*). The relative migration orders of y-type HMW-GS encoded by gene at *Glu-B1* locus were 20* < 8** < 16 < 9 < 8* < 8 < 18* on the basis of migration times (from the fastest to the lowest).

HMW-GS in European Triticale Cultivars. Eighty-six European cultivars of both winter and spring triticale were analyzed by CZE for detection of wheat HMW-GS encoded by genes at *Glu-A1* and *Glu-B1* loci. The analyzed accessions were divided into 18 different groups according to their HMW-GS compositions (**Table 1**). In total, 14 wheat HMW-GS (2 subunits encoded by *Glu-A1* locus and 12 by *Glu-B1* locus) in triticale were identified. Three allelic compositions of wheat HMW-GS with subunits pairs 6+8*, 6.8+20*, 7*+8, 7+18*, and 13+16 and two with pairs 7*+8* and 7*+9 were determined. Differences in the identification of wheat HMW-GS compositions for 16 triticale cultivars determined by SDS-PAGE (*5, 13, 14, 20*) in comparison with the CZE data for these cultivars (**Table 1**) should be explained by the diversity of intensity of both HMW glutenin and secalins bands in SDS-PAGE patterns, owing to considerable quantitative difference of protein subunits. Besides, these subunits often have similar molecular weight and electrophoretic mobilities in gels, which makes the identification of particular subunits difficult.

At the *Glu-A1* locus, subunit Ax2* was predominant (66.3%) in the analyzed samples, as among 134 European triticale cultivars described earlier by Amiour and co-workers (*13, 14*). At the same time the low frequency (12.8%) of subunit Ax1 was observed. Both of these subunits have a significant influence on breadmaking quality in common wheat.

As in wheat, high allelic variation at the *Glu-B1* was determined; 12 alleles were distinguished. The allelic compositions encoding subunit pairs Bx7+By18* and Bx6.8+By20* were the most frequently appearing (36.1 and 24.4%, respectively). Similar allelic distribution at this locus determined using SDS-PAGE was earlier described by some authors (*5, 13−15, 20, 21*).

In conclusion, the presented CZE method is capable of separating wheat and rye HMW proteins in triticale extracts with good reproducibility and separation efficiency. The use of the PVP and HPMC mixture for dynamic coating of the capillary wall and a low-concentration solution of PEO for the isoelectric separation buffer improved separation resolution between consecutive peaks of HMW-GS and HMW-SS subunits. Using selective preparation of HMW proteins precipitated by addition of 1-propanol to a final concentration of 62% (v/v), low molecular weight glutenin and secalin subunits from glutenin extracts were eliminated, which have similar migration times as HMW-GS and HMW-SS during the CZE separation. In comparison with other existing CZE methods of HMW glutenin separations the presented method offers significant improvements in terms of both run-to-run reproducibility and separation efficiency of faster-migrating subunits. The migration time reproducibility was 0.25−0.75% RSD, and corrected peak area reproducibility was in the order of 0.8−6% RSD. The method also allows quantification of individual subunits. This advantage is of great importance for HMW-GS, because both the quality and quantity of individual subunits influence to a large extent the dough functionality and breadmaking quality of triticale cultivars. All allelic pairs of wheat HMW-GS in triticale cultivars can be identified and characterized on the basis of their relative migration times and peak areas. In particular, extensive allelic variations wheat HMW-GS compositions encoded by Glu-B1 locus, including novel x-type and y-type HMW-GS, can be unambiguously determined. Identification and characterization of rye HMW secalin subunits in triticale cultivars will be the subject of the next work. At present, triticale breeding has aimed at searching for genotypes with relatively high grain yields. A deeper knowledge of wheat HMW glutenins enables breeders to choose genotypes that have a suitable composition of HMW proteins. Fast and more accurate characterization of wheat HMW-GS from triticale cultivars by CZE is an efficient alternative to identification of wheat *Glu-1* genes by PCR-based DNA markers and the standard SDS-PAGE method separation.

ABBREVIATIONS USED

EOF, electroosmotic flow; HMW-GS, high molecular weight glutenin subunit; HMW-SS, high molecular weight secalin subunit; LMW-GS, low molecular weight glutenin subunit; HPMC, hydroxypropylmethylcellulose; IDA, iminodiacetic acid; PEI, polyethylenimine; PEO, poly(ethylene oxide); SB3-12, *N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate.

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