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Diversity of Monomeric Prolamins in Triticale Cultivars Determined by Capillary Zone Electrophoresis

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Capillary zone electrophoretic (CZE) analysis of monomeric prolamins (wheat gliadins and rye secalins) covered 28 hexaploid triticale (*Triticosecale* × Wittm.) cultivars. The ethanol-soluble proteins were separated on an uncoated fused-silica capillary using the isoelectric 60 mM iminodiacetic (IDA) buffer in conjunction with 20% (v/v) acetonitrile and 0.075% (w/v) polyvinylpyrrolidone (PVP). For each separation, dynamic coating of the capillary wall with a buffer containing 0.1 M IDA and 0.05% (w/v) hydroxypropylmethylcellulose (HPMC) was performed. Separations of prolamins provided very good resolution and high reproducibility (<0.8% RSD). Prolamin profiles of all analyzed cultivars showed both qualitative and quantitative differences, including number of peaks, presence or absence of peaks, and area of peaks. The number of prolamin peaks detected in particular triticale cultivars varied from 22 to 28; in total, 56 components were distinguished. The CZE electropherograms of prolamins showed five main groups of protein peaks, in order of mobility α-prolamins, β-prolamins, γ-prolamins, ω1-prolamins, and ω2-prolamins, with migration times of 6.8−7.7, 7.8−10.4, 10.5−12.2, 12.3−17.4, and 17.5−25.6 min, respectively. Triticale seeds in comparison with wheat contained fewer α-prolamins and higher quantity of ω-prolamins. Hierarchical clustering of the investigated cultivars was based on Bhattacharyya distances calculated from the CZE data. The cultivars grouped in four main clusters. The obtained CZE results were compared with A-PAGE data.

KEYWORDS: Capillary electrophoresis; gliadins; isoelectric buffer; prolamins; secalins; triticale

INTRODUCTION

The hexaploid triticale is an interesting new crop due to its wide soil adaptation, high yield potential, good disease resistance, and tolerance to environmental stress. The biological value of triticale makes it suitable for use as both animal fodder and human food (*1*−*3*). The significance of this species in world breeding programs has significantly increased during the past two decades. Triticale grain has a higher lysine content than wheat and offers excellent feed potential for both ruminant and nonruminant animals (mainly swine) as well as for poultry (*4*). It can also be used to make biscuits, cookies, and unleavened breads such as tortillas and chapattis (*5*, *6*). Triticale may have the same potential for making bread as wheat over the long-term (*1, 7*−*9*).

Due to increased numbers of triticale varieties, interest in new research methods for both the detection of genetic variability and varietal identification has become especially important. Knowledge of genetic distances between initial materials for crossing is essential for the rationalization of such breeding programs. A number of protein markers and their isoenzyme variants and molecular markers are accessible for genetic differences. Gliadins and secalins, which make up approximately 50% of all storage proteins of triticale seeds, are characterized by high heterogeneity, and they are in insignificant degree depending on environmental conditions (*10*−*12*). Gliadins and secalins are monomeric proteins that form only intradisulfide bonds, whereas both HMW and LMW glutenins and HMW secalins are polymeric proteins stabilized by interchain disulfide bonds. The monomeric prolamins are encoded by large multigene families and are inherited in blocks.

Substitution of the wheat D genome for the rye R genome in triticale has excluded (among other things) the loci *Gli-D1* and *Gli-D2* encoded wheat gliadins and LMW glutenins and has brought the rye loci *Sec-1* and *Sec-2* (*9, 13*). The complex *Sec-1* locus encodes two types of rye prolamins, γ-secalins (M_r range from 36 to 40 kDa) and ω-secalins (M_r from 40 to 48 kDa). The locus *Sec-2* encodes typical rye γ-secalins with M_r from 74 to 78 kDa. The bread wheat and rye possess two homologous monomeric prolamins: γ-gliadins/γ-secalins (40 kDa) and ω1,2-gliadins/ω-secalins (*13, 14*). However, the ω-secalins are more water-soluble than γ-secalins and most wheat gliadins (*15*)

The triticale prolamins were characterized more frequently using acid− (A) or sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) (*16*−*18*) and reverse-phase high-performance liquid chromatography (RP-HPLC) (*19*). A-PAGE

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Table 1. Cultivar Names of Analyzed Samples and Division of CZE γ-Prolamin Profiles

Triticale (× *Triticosecale* Wittm.)			
Algoso (γ-2)[a]	Grenado (γ-2)	Lamberto (γ-4)	Sekundo (γ-2)
Aliko (γ-1)	Hewo (γ-2)	Legalo (γ-3)	Sorento (γ-2)
Baltiko (γ-4)	Hortenso (γ-2)	Magnat (γ-2)	Trismart (γ-2)
Disco (γ-1)	Janko (γ-1)	Matejko (γ-3)	Todan (γ-4)
Dublet (γ-3)	Kazo (γ-2)	Moderato (γ-1)	Witon (γ-4)
Fidelio (γ-2)	Kitaro (γ-2)	Pawo (γ-1)	Woltario (γ-2)
Gniewko (γ-2)	Krakowiak (γ-2)	Prado (γ-2)	Zorro (γ-2)
Bread Wheat (*Triticum aestivum* L.)			
Clever	Slade	Nadobna[b]	Satyna[b]
Rye (*Secale cereale* L.)			
Bosmo	Diament	Motto	

[a] CZE profile notation of triticale γ-prolamin fraction in parentheses. [b] Wheat cultivars carrying 1BL/1RS wheat/rye chromosomal translocation.

patterns of prolamins in hexaploid triticale registered in Poland were presented by Brzeziński (*20*) and SDS-PAGE ones by Rzepka-Plevneš et al. (*17*). However, electrophoretic methods are slow and difficult to quantify and lack the ability to distinguish between some triticale cultivars. Besides, due to the complexity of triticale proteins, for example, the presence of both wheat and rye prolamins, which appear in different amounts and additionally often characterize similar electrophoretic mobilities, efficient electrophoretic techniques are required for their study.

One of the newest electrophoretic techniques applied to the study of proteins is capillary electrophoresis (CE). CE offers the benefits of very rapid, high-resolution separation and reproducibility and fully automated analyses of cereal storage proteins (*21*−*23*). The usefulness of this technique in the identification and differentiation of cultivars from different species of *Triticum* through the study of gliadins and glutenins was extensively described (*24*−*27*). At present, genetic diversity of prolamins from triticale using capillary zone electrophoresis (CZE) has been very little investigated. Siriamornpun and co-workers (*11*) characterized prolamins from only five Australian triticale varieties by CZE and indicated the potential of this method for the identification of cultivars of these species. They affirmed that the CZE gliadin profiles of particular cultivars grown in different localizations are similar.

This paper presents new CZE data concerning diversity of seed monomeric prolamins in triticale and is aimed at further contributing to the characterization of storage proteins of these species. The CZE profiles of monomeric prolamins were compared with electrophoretic patterns of these proteins obtained by A-PAGE method. The high resolution and separation efficiency of the presented CZE method make rapid triticale cultivar identification and monomeric prolamin characterization possible.

MATERIALS AND METHODS

Plant Materials. Samples of 28 Polish cultivars of both winter (24) and spring (4) hexaploid triticale were analyzed (**Table 1**). An additional four bread wheat cultivars and three rye cultivars were used as standard samples. The grain samples were obtained from four Polish plant-breeding stations (DANKO Plant Breeding at Choryn and Plant Breeding Companies at Strzelce, Smolice, and Szelejewo).

Chemicals. Iminodiacetic acid (IDA), acetonitrile, hydroxypropylmethylcellulose (HPMC), poly(vinylpyrrolidone ($M_r \approx$ 40000 and 360000) (PVP) were products of Sigma Chemical Co. (St. Louis, MO). Sodium phosphate monobasic and dibasic, potassium phosphate monobasic, and sodium chloride were products of J. T. Baker (Phillipsburg, NJ). All solutions were filtered through a 0.45 μm Millipore (Bedford, MA) membrane filter before being injected into the capillary. Distilled water was deionized using a Milli-Q system (Millipore, Bedford, MA).

Apparatus. CE experiments were performed on a Beckman-Coulter P/ACE System MDQ capillary electrophoresis instrument equipped with a built-in 0−30 kV high-voltage power supply and a diode array detector and Karate software, ver. 8.0, for system control and data handling. The separations were carried out by using uncoated fused silica capillaries (Polymicro Technologies) with internal diameter of 50 μm, 31.2 cm in total length, and a detection window was created at 21 cm from the capillary inlet.

Extraction of Monomeric Prolamins. The extraction of prolamins was performed by three methods (differences in the first stage of protein separation). The first method was based on the method of Wieser et al. (*28*), as typical for wheat gliadins, with some modifications. In brief, in this method 25 mg of flour from endosperm portion single seeds (the embryo portion removed with a knife) was pre-extracted twice with 0.25 mL of 0.2 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 and secalins were extracted twice from the pellet with 125 μL of cold 70% (v/v) ethanol in the first extraction and 75 μL in the second one. The material was mixed on a vortex mixer for 30 min and after centrifugation for 8 min at 13000*g*. The supernatants were transferred into a new tube and next were filtered through a 0.45 μm membrane filter prior to use in CE analysis. In the second method, a preliminary extraction of albumins and globulins was performed using 0.4 M NaCl solution instead of phosphate-buffered NaCl solution based on a procedure for extraction of gliadin presented by van Eckert et al. (*25*). The pre-extraction was carried out twice for 10 min, at room temperature and centrifuging for 10 min at 15000*g*. In the third method, the prolamins were extracted directly from triticale flour; 25 g of flour was extracted twice with behavior of the same quantity of cold 70% (v/v) ethanol using extraction conditions as in both above-mentioned methods. All samples were used for CZE analyses within 24 h of extraction. Two protein separations were performed for each analytical material.

Separation by A-PAGE. Monomeric prolamins were separated by A-PAGE using a Protean II xi cell (Bio-Rad Laboratories, Hercules, CA) electrophoresis unit according to the procedure of Bushuk and Zillman (*29*) with some modifications. The proteins were separated in an 8.0% (w/v) acrylamide gel (C: 2.67%). The upper electrode buffer contained 0.0048 M potassium sulfate and 0.023 M formic acid, and the lower buffer contained 0.109 M formic acid. Electrophoresis was performed at 100 V for 15 min and then at 450 V at 18 °C. Gels were fixed and stained with 0.25% Coomassie Brilliant Blue R250 in 45% (v/v) methanol and 10% (v/v) acetic acid and destained with 10% (v/v) methanol and 10% (v/v) acetic acid.

Separation by CZE. The monomeric prolamin separations were carried out according to the method of Bean and Lookhart (*23*) with some modifications. In the separation buffer HPMC was substituted for PVP with a molecular weight of 40000. A solution containing 60 mM IDA, 0.075% (w/v) PVP, and 20% (v/v) acetonitrile (ACN) was applied as separation buffer. As the polymer solution for dynamic coating of the capillary wall for each separation, a buffer containing 0.1 M IDA and 0.05% (w/v) HPMC was used. The separation was performed with a constant voltage 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 N 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 rinse 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 solutions were stored in a refrigerator at 4 °C after being degassed with a vacuum

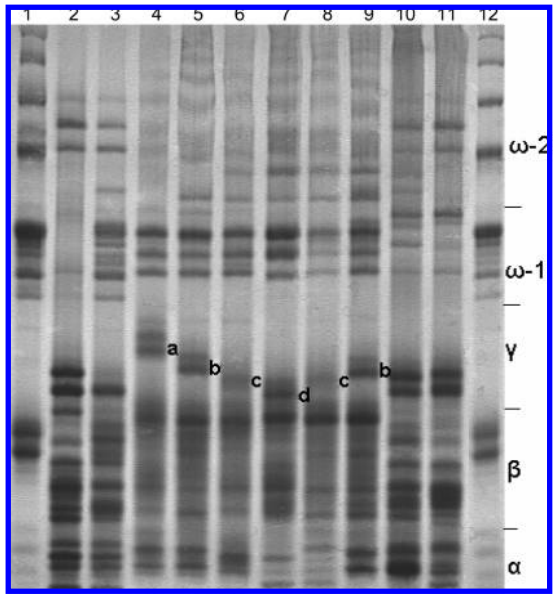

Figure 1. A-PAGE patterns of monomeric prolamins distinguished in the investigated triticale cultivars (lanes 4−9) and wheat and rye as standards. Marked bands a−d indicate the particular patterns of γ-prolamin fraction. Lanes: 1 and 11, rye (cv. Bosmo and Motto); 2, wheat (cv. Slade); 3 and 10, wheat carrying 1BL/1RS wheat/rye chromosomal translocation (cv. Nadobna and Satyna, respectively); 4, Moderato; 5, Woltario; 6, Legalo; 7, Witon; 8, Matejko; 9, Sorento.

system. All solutions and buffers were filtered through a 0.20 μm syringe filter. Three protein separations were performed by CZE for each analytical assay.

Statistical Analysis. A statistical analyses of CZE data was performed as described in Mardia et al. (*30*).

For the 28 investigated cultivars and all 56 distinguishable CZE protein peaks the frequencies of individuals falling into four classes of peak areas were calculated. The classes were defined using a gradual arbitrary scale of four peak areas [(1, trace (0−5% relative quantity of peak area); 2, small (5−35%); 3, medium (35−65%); 4, high (<65%)]. For all pairs of cultivars, distances between pairs were calculated using the formula $d_{ij}^{(k)} = \sum d_{ij}^{(k)}$, where $d_{ij}^{(k)}$ denotes the Bhattacharyya distance between cultivars *i* and *j*, calculated for the *k*th peak. For the *k*th peak, the Bhattacharyya distance $d_{ij}^{(k)}$ between cultivars was calculated from the frequencies of four peak area classes, $x_{i1}^{(k)}$, ..., $x_{i4}^{(k)}$ and $x_{j1}^{(k)}$, ..., $x_{j4}^{(k)}$, according to the formula $d_{ij}^{(k)} = \sum_{r=1}^{4} [(x_{ir}^{(k)})^{1/2} - (x_{jr}^{(k)})^{1/2}]^2$. In this way, the distance used in the analysis measures differences in the frequencies of individuals characterized by different peak areas, treating all four classes equally. The final measure of distance was calculated as a sum of distances resulting from all peaks. On the basis of the calculated distances, a hierarchical clustering of triticale cultivars was performed using the group average method, and a dendrogram was constructed.

RESULTS AND DISCUSSION

A-PAGE Analysis. The ethanol-soluble fractions from 28 seed triticale cultivars were analyzed for their prolamin compositions by using the A-PAGE method. Selected electrophoretic prolamin patterns of individual cultivars are presented in **Figure 1**. Additional electrophoretic profiles of prolamins from rye, wheat, and wheat carrying 1AL/1RS or 1BL/1RS wheat/rye chromosomal translocation (lanes 1 and 11, 2 and 10, and 3, respectively) used as standards are shown. Generally, in A-PAGE gels the triticale gliadin and secalin bands are partly overlapping. For this reason, in the present study no attempt was made to assign unambiguously the recorded proteins to the gliadin and secalin class. Fundamentally, the analyzed cultivars were homogeneous with respect to A-PAGE patterns; only three cultivars (Prado, Sorento, and Woltario) showed two electrophoretic profiles. Generally, the numbers of major prolamin bands recorded in particular cultivars were similar, and from 18 bands in cv. Matejko to 22 bands in cv. Dublet were observed. The monomeric prolamins of triticale migrated in order of mobility from α-gliadins + α-secalins (α-prolamins), β-gliadins + β-secalins (β-prolamins), γ-gliadins + γ-secalins (γ-prolamins) to the slowest ω-gliadins + ω-secalins (ω-prolamins). As for wheats, triticale β- and γ-prolamins made up the major protein subgroups, whereas triticale α-prolamins are present in smaller number and smaller quantities. The γ-prolamin bands showed considerable variability in electrophoretic mobility, and four (1−4) characteristic patterns can be distinguished. Pattern b (lanes 5 and 9) appeared the most frequently in investigated material (53.5%). Pattern a (lane 4) produced six cultivars and pattern c (lanes 6 and 8), three cultivars; however, pattern d (lane 7) has four cultivars (**Table 1**). A-PAGE patterns of triticale prolamins showed two zones of ω-protein bands designated ω1- and ω2-prolamins (**Figure 1**). Prolamin bands of a quickly migrated subfraction classified as ω1-prolamins had composition and electrophoretic mobilities similar to those of some bands of ω-prolamins in wheat with rye/wheat 1B/1R translocation. However, quantities of the most slowly migrated subfraction designated ω2-prolamins differed significantly in particular triticale cultivars and often produced weakly stained bands, sometimes even ill-defined (**Figure 1**, lanes 4−9).

The A-PAGE patterns of triticale prolamins extracted using the three methods described under Materials and Methods revealed only insignificant qualitative and quantitative differences (data not shown). Mainly more intense bands in the γ- and ω-regions of electropherograms were observed when the prolamins were extracted directly with aqueous 70% ethanol. This indicated that during preliminary elimination of albumin and globulin proteins using phosphate buffer and/or NaCl solution, some γ- and ω-prolamins (mainly secalins) were partially coextracted together with these proteins. Earlier, Graybosch et al. (*31*) and Hussain and Lukow (*15*) have affirmed that rye γ-secalins present in wheat cultivars carrying 1AL/1RS or 1BL/1RS wheat/rye chromosomal translocation were partly dissolved in low concentrate (0.04 M) NaCl solution.

Influence of Extraction Method on Composition of Monomeric Prolamins. The advantages of using acid isoelectric buffers for CZE separation of cereal storage proteins have been widely presented (*22*−*24*, *27*). In the case of CZE analyses of wheat gliadin and glutenin extracts it is recommended that a preliminary fractionation of proteins for removal of albumins and globulins be performed (*23, 24*). These proteins, as shown by Bean and Lookhart (*24*), bind strongly to the capillary wall, which makes elimination of all proteins from the capillary before the next separation considerably difficult. A phosphate-buffered NaCl solution (*28*) or only 0.4% NaCl solution (*25*) was most often used to eliminate the albumins and globulins. Preliminary study by CZE confirmed that in the case of triticale flour during removal of the albumins and globulins before the extraction of prolamins with ethanol solution, some secalins were coextracted together with these proteins. In **Figures 2** and **3** are compared CZE electropherograms of monomeric prolamin fractions from triticale and rye, which were isolated by three extraction methods. Depending on the applied solution for the extraction of albumins and globulins the composition of triticale prolamins considerably changed. During preliminary extraction with phosphate-buffered NaCl solution or 0.4% NaCl solution large amounts of γ- and ω-secalins were coextracted (**Figure 2**). Similarly, in the case of rye samples considerable loss of these

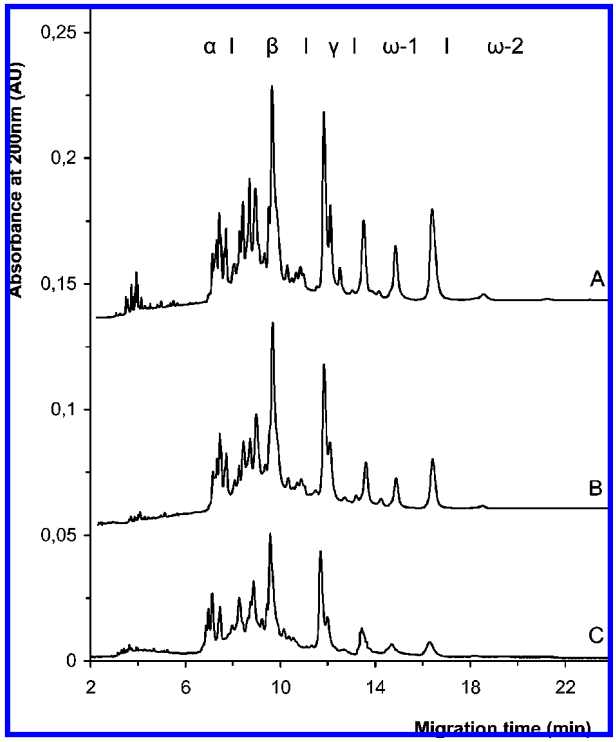

Figure 2. Comparison of CZE profiles of seed monomeric prolamins from triticale (cv. Moderato) obtained by different extraction methods: (**A**) direct extraction with 70% ethanol; (**B**) pre-extraction with phosphate-buffered NaCl solution; (**C**) pre-extraction with NaCl solution. Marked peak zones indicate the particular prolamin subfractions. Proteins were separated at 10 kV and 40 °C with a 60 mM isoelectric IDA buffer, containing 0.075% PVP, and 20% ACN. Prior to the separation, the capillaries (31.2/21 cm) were rinsed with 0.1 M IDA solution containing 0.05% HPMC.

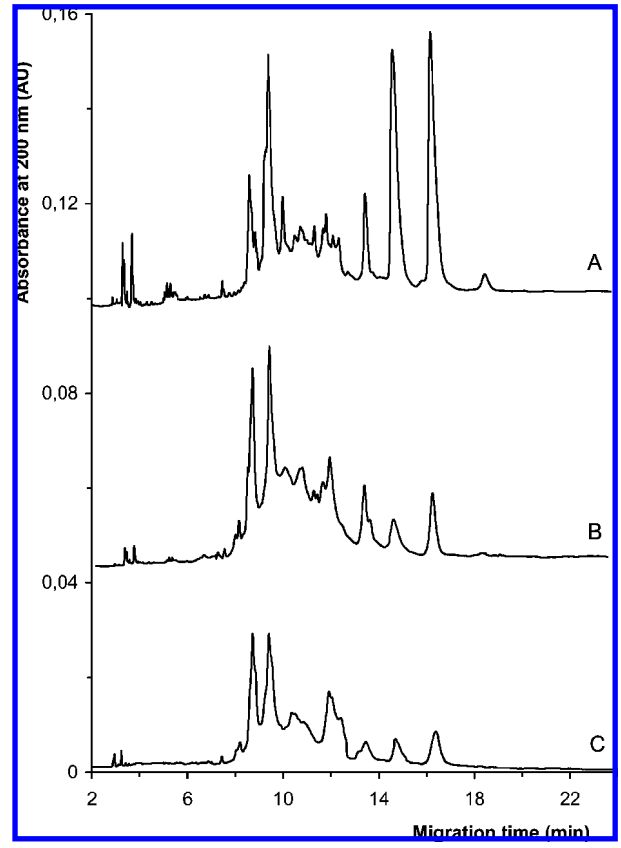

Figure 3. Comparison of CZE profiles of seed monomeric prolamins from rye (cv. Bosmo) obtained by different extraction methods: (**A**) direct extraction with 70% ethanol; (**B**) pre-extraction with phosphate-buffered NaCl solution; (**C**) pre-extraction with NaCl solution. Separation conditions were as described in **Figure 2**.

proteins was observed (**Figure 3**). In this connection in detection of prolamin polymorphism in triticale the ethanol-soluble fraction was obtained by direct extraction of flour with 70% ethanol. It required modification of the separation buffer composition and polymer solution for dynamic coating of the capillary wall for each separation.

Optimization of CZE Conditions. In the case of CZE separation of triticale the ethanol fraction consisted of monomeric prolamins and albumins and globulins, a commonly applied running buffer containing IDA, HPMC, and AcN (*23, 24, 27*) did not give satisfactory reproducibility of protein migration times. In this connection for dynamic coating of the capillary wall during separation, PVP instead of HPMC was used, whereas HPMC as a component of polymer solution for preliminary dynamic coating of the capillary before each separation was applied. Some authors (*32*−*35*), for improvement of reproducibility of run-to-run separations and resolution during DNA separation, have used two different polymers for dynamic coating of the capillary, first, for preliminary coating before the separation run and, second, in low concentration in separation buffer. PVP, a low-viscosity polymer, had good dynamic coating ability in protein and DNA separation media (*32, 34*). In the initial study, concentration of PVP from 0.05 to 2% in isoelectric buffer with (50−150 mM) IDA and 10−30% AcN was tested. At the same time a buffer containing 0.1 M IDA and 0.05% (w/v) HPMC as the polymer solution for preliminary dynamic coating of the capillary wall for each separation was used. Separation efficiency, resolution, and peak shapes were satisfactory at 0.075% PVP, 60 mM IDA, and 20% AcN. All proteins were separated in the presented conditions in <26 min. The migration order of triticale prolamin fraction in capillary was the same as in A-PAGE gel. The obtained CZE protein profiles were consistent with those of Siriamornpun et al. (*11*) and Lookhart et al. (*36*), who presented a potential of capillary electrophoresis for identification of Australian triticale varieties and wheat lines carrying either 1DL/1RS or 1BL/1RS wheat/rye chromosomal translocations, respectively.

To test the run-to-run reproducibility of prolamin separations 24 consecutive injections in optimal conditions were performed (data not shown). Four major protein peaks (major peak for each with characteristic zone) were selected to assess the reproducibility of protein migration times and corrected peak area quantification. Standard deviations for migration times of peaks representing high peaks of corresponding protein were very low (0.02−0.08 min). The relative standard deviation (RSD) values for migration times of these protein peaks ranged from 0.28 to 1.34%. The corrected peak area reproducibility was also satisfactory; the RSD values for main peaks ranged from 1.84 to 2.68%. This modified procedure kept satisfactory peak shapes and separation efficiencies for prolamin peaks.

Detection of Prolamin Polymorphism in Triticale by CZE. The two monomeric prolamin classes, gliadin and secalin, make up ca. 50% of the major storage proteins in triticale grains. Four selected electropherograms of triticale prolamins obtained by direct extraction of triticale flour with 70% ethanol are presented in **Figure 4**. The albumins and globulins, which were extracted in considerable amounts together with the prolamins, migrated first (<6 min). The profiles of these proteins generally differed very little for all triticale cultivars, in agreement with what was previously described for wheat (*24, 27*). The monomeric prolamins migrated in the broad range of times of 6.8−25.6

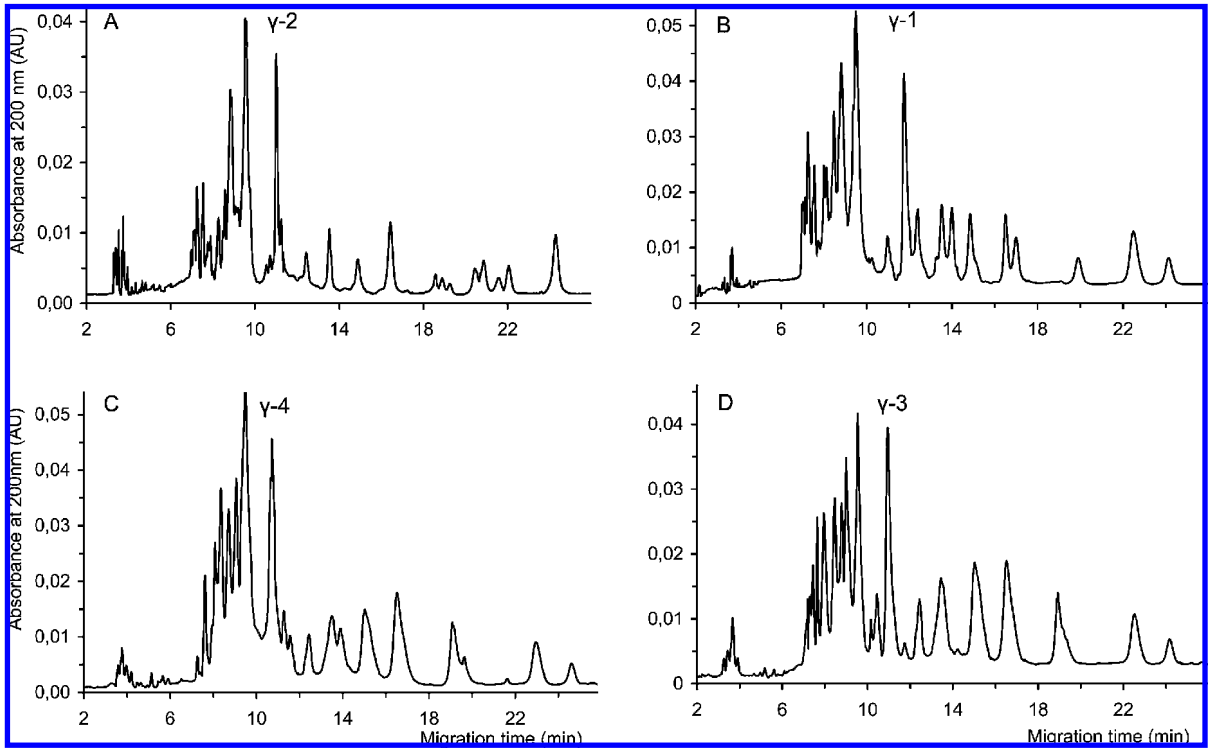

Figure 4. CZE electropherograms of monomeric prolamins from four triticale cultivars with different γ-protein compositions: (**A**) Fidelio; (**B**) Aliko; (**C**) Witon; (**D**) Matejko. Marked γ-1, γ-2, γ-3, and γ-4 zones indicate particular γ-prolamin profiles for individual cultivars. Separation conditions were as described in **Figure 2**.

min and were separated into five fractions (α-, β-, γ-, ω1-, and ω2-prolamins). The α-fraction migrated the earliest in the range of migration times of 6.8−7.7 min. Next moved β-prolamins (7.8−10.4 min), γ-prolamins (10.5−12.2 min), ω-1 prolamins (12.3−17.4 min), and, the most slowly moving, ω-2 prolamins (>17.5 min). CZE triticale prolamin electropherograms showed from 22 to 28 protein peaks. A similar number of prolamin peaks was detected by Siriamornpun et al. (*11*). Regardless of qualitative differences (presence or absence and number of protein peaks), considerable quantitative differences (values of peak areas or high of peaks) during analyses of individual cultivars were also observed.

The α-prolamin fraction consisted of five to eight protein peaks, which were mainly α-gliadins. Areas of individual peaks varied remarkably in particular cultivars. Cultivars Dublet and Moderato characterized the highest amount of proteins belonging to the α-prolamin fraction; however, Krakowiak and Prado possessed the smallest amount. Analysis of the sharp peaks belonging to this fraction revealed the presence of shoulders mainly associated with the first and fifth peaks. Twelve analyzed triticale cultivars showed this richer pattern.

From 6 to 12 peaks and shoulders were detected in the β-prolamin fraction including both β-gliadins and β-secalins. This fraction showed a high individual character. A high qualitative and quantitative variation in this region of the profiles was noticed. A low expression of the β-prolamin migrating immediately after the α-zone was observed especially in five cultivars (Fidelio, Grenado, Prado, Lamberto, and Sekundo). In all cultivars appeared a characteristic doublet peak with the largest area among all peaks. The last peak was dominant and had a migration time of 9.6 min, whereas one or two remaining peaks appeared as small shoulders. Additionally, in four cultivars (cv. Moderato, Grenado, Pawo, Gniewko) from three to six small or middle peaks after the major doublet were observed immediately in the profiles of γ-secalin fraction.

In the γ-prolamin fraction a low number of peaks with very diverse mobilities were detected. Generally, the γ-gliadins were a quantitatively significant fraction and appeared as the triplet or quadruplet. The observed patterns of this fraction can be divided in four subgroups on the basis of the migration time (t_R) of the dominant peak, as in the case of separation by A-PAGE. In the most frequent quadruplet profile (16 cultivars), the dominant peak had a migration time of 11.21 min (**Figure 4A**; **Table 1**; notation, γ-2). In five cultivars the dominant peak of γ-prolamins migrated the most slowly and had t_R of 11.73 min (**Figure 4B**; **Table 1**; γ-1); in four cultivars (**Figure 4C**; **Table 1**; γ-4) one migrated the fastest and had t_R of 10.69 min and in the remaining three cultivars (**Figure 4D**; **Table 1**; γ-3) had a t_R of 10.94 min.

Like A-PAGE patterns, CZE profiles of triticale ω-prolamins showed two zones of peaks with different migration mobilities and values of peak areas and were designated ω1-prolamins and ω2-prolamins (**Figures 2** and **4**). In the ω1-prolamin zone a low variation of protein peaks was observed. The proteins of this fraction made up a significant quantity of total monomeric prolamins (20−35% of total the peak area) and consisted of three major peaks with high peak areas and some minor peaks. The main peaks had migration times of 12.41, 13.54, and 16.46 min. Similar CZE profiles of ω-secalins presented in wheat cultivars carrying 1AL/1RS or 1BL/1RS wheat/rye chromosomal translocation were published by Lookhart and co-workers (*36*).

Like the β-prolamin fraction, the ω2-prolamin zone of analyzed cultivars differed significantly both quantitatively and qualitatively, and they can be useful in cultivar identification (**Figure 4**). This fraction consisted of two to eight protein peaks, which were distributed on the broad range of migration time from 17.5 to 25.6 min. In part of the analyzed material (cv. Gniewko, Lamberto, Moderato, and Pawo) a very small amount of these subfractions was evident; other cultivars (cv. Fidelio, Hortenso, Krakowiak, Legalo, and Witon) showed peak area

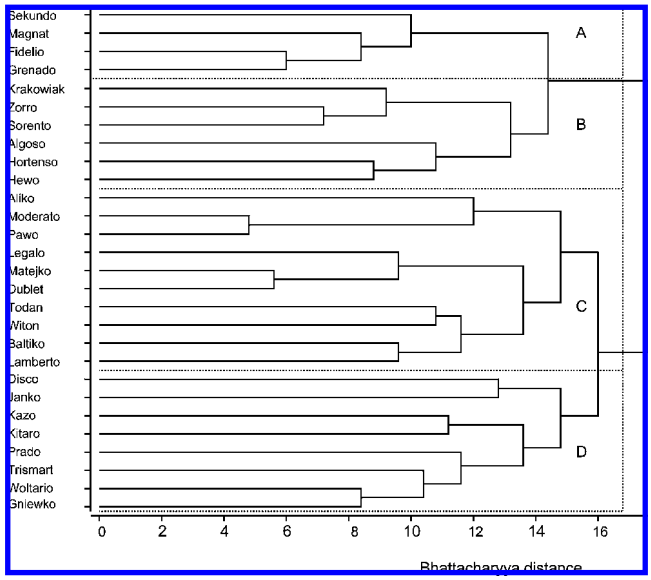

Figure 5. Dendrogram of 28 triticale cultivars based on Bhattacharyya distances calculated from CZE prolamin profile data.

with high values. Distinct from A-PAGE patterns of the ω2-prolamin zone, which are to a large extent illegible, CZE triticale profiles enabled precise quantity and quality characterization of this fraction to be carried out.

Triticale Cultivar Differentiation. The polymorphism of monomeric prolamins detected by CZE allowed the differentiation of individual triticale varieties on the basis of migration times of particular peaks and their peak areas. The results of the statistical analysis covering 56 gliadin and secalin peaks detected in 28 CZE electropherograms taking the value of their peak areas in a four-gradual scale into consideration are summarized in a diagram (**Figure 5**). The dendrogram indicated a division of the examined cultivars into four main clusters (A−D). Adjoining clusters A and B comprised 10 cultivars containing the most popular profile of the γ-prolamin fraction with dominant peak migrating in 11.21 min. The remaining five cultivars with the same profile of the γ-prolamin zone together with two cultivars with the fastest migration zone made up cluster D. However, cluster C was formed of the three remaining cultivars with the most quickly migrating γ-prolamins and all cultivars having the most slowly migrating γ-prolamins.

In conclusion, the present study shows the potential of CZE analysis of monomeric prolamins in the characterization and identification of triticale cultivars. The use of the CZE method allowed a fast (26 min) and qualitative determination of particular monomeric prolamins. The high resolution and separation efficiency of the presented method make it possible to detect differences between particular monomeric prolamin (wheat gliadin and rye secalin) profiles of closely related triticale genotypes. The recent study on prolamin polymorphism in triticale cultivars showed that many of them presented identical electrophoretic and chromatographic profiles. The full detection of prolamin polymorphism is a fundamental source of knowledge about genomic diversity for breeders. The short time of analysis enables the use of the CZE method in the screening of large numbers of genotypes for early selection of triticale with good breadmaking quality.

The indication of triticale genotypes characterizing low content of rye secalins shows useful triticale strains with good breadmaking quality and favorable feed potential for both animals and poultry. Furthermore, providing both qualitative and quantitative data about individual prolamins by CZE, it could be useful for preliminary estimations of technological properties of the particular genotypes/strains/cultivars.

ABBREVIATIONS USED

EOF, electroosmotic flow; HPMC, hydroxypropylmethylcellulose; IDA, iminodiacetic acid.

LITERATURE CITED

(1) Lelley, T. Triticale: a low-input cereal with untapped potential. In *Genetic Resources, Chromosome Engineering, and Crop Improvement, Cereals*; Singh, R. J., Jauhar, P. P., Eds.; CRC Press, Taylor & Francis Group: Boca Raton, FL, 2006; Vol. 2, pp 395−430.

(2) Oettler, G. The fortune of a botanical curiosity—triticale: past, present and future. *J. Agric. Sci.* **2005**, *143*, 329–346.

(3) Pena, R. J. Factors affecting triticale as a food crop. In *Triticale Today and Tomorrow*; Guedes-Pinto, A., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; pp 753−761.

(4) Bruckner, P. L.; Cash, S. D.; Lee, R. D. Nitrogen effects on triticale grain yield, amino acid composition, and feed nutritional quality for swine. *J. Prod. Agric.* **1998**, *11*, 180–184.

(5) Pérez, G. T.; Leŏn, A. E.; Ribotta, P. D.; Aguirre, A.; Rubiolo, O. J.; Aňón, M. C. Use of triticale flours in cracker-making. *Eur. Food Res. Technol.* **2003**, *217*, 134–137.

(6) Serna-Saldivar, S.; Guajardo-Flores, S.; Viesca-Rios, R. Potential of triticale as a substitute for wheat in flour tortilla production. *Cereal Chem.* **2004**, *81*, 220–225.

(7) Martinek, P.; Vinterova, M.; Burešova, I.; Vyhnánek, T. Agronomic and quality characteristics of triticale (× Triticosecale Wittmack) with HMW glutenin subunits 5 + 10. *J. Cereal Sci.* **2008**, *47*, 68–78.

(8) Tohver, M.; Kann, A.; Täht, R.; Mihhalevski, A.; Hakman, J. Quality of triticale cultivars suitable for growing and bread-making in northern conditions. *Food Chem.* **2005**, *89*, 125–132.

(9) Lukaszewski, A. Cytogenetically engineered rye chromosomes 1R to improve bread-making quality of hexaploid triticale. *Crop Sci.* **2006**, *46*, 2183–94.

(10) Tatham, A. S.; Shewry, P. R. The S-poor prolamins of wheat, barley and rye. *J. Cereal Sci.* **1995**, *22*, 1–16.

(11) Siriamornpun, S.; Wootton, M.; Schulthesis, J. B. Potential of capillary electrophoresis for identification of Australian triticale varieties. *Austr. J. Agric. Res.* **2004**, *55*, 595–598.

(12) Metakovsky, E. V.; Branlard, G. P.; Graybosch, R. A. Gliadins of common wheat: polymorphism and genetics. In *Gliadin and Glutenin. The Unique Balance of Wheat Quality*; Wrigley, C., Békés F., Bushuk, W., Eds.; AACC Internattional: St. Paul, MN, 2006; pp 35−84.

(13) Shewry, P. R.; Bradberry, D.; Franklin, J.; White, R. P. The chromosomal locations and linkage relationships of the structural genes for the prolamin storage proteins (secalins) of rye. *Theor. Appl. Genet.* **1984**, *69*, 63–69.

(14) Gerlich, C.; Schieberle, P.; Wieser, H. Biochemical characterization and quantification of the storage protein (secalin) types in rye flour. *Cereal Chem.* **2003**, *80*, 102–109.

(15) Hussain, A.; Lukow, O. M. Characterization of the 1B/1R translocation in wheat using water extractable protein concentrate. *Euphytica* **1994**, *78*, 109–113.

(16) Vyhnánek, T.; Bednář, J. Detection of the varietal purity in sample of harvested wheat and triticale grains by prolamin marker. *Plant Soil Environ.* **2003**, *49*, 95–98.

(17) Rzepka-Plevneš, D.; Smolik, M.; Krupa, M. Electrophoretic variability of storage proteins in different triticale (× Triticosecale Wittm.) cultivars (in Polish). *Biul. IHAR* **2003**, *226/227*, 181–189.

(18) Rozinek, B.; Gunther, T.; Hesemann, C. U. Gel electrophoretic investigations of prolamins in eu- and alloplasmatic octoploid primary triticale forms. *Theor. Appl. Genet.* **1998**, *96*, 46–51.

(19) Kubiczek, R. P.; Huebner, F. R.; Bietz, J. A. RP-HPLC for varietal identification in cereals and legumins. Rye and triticale. In *High-*

Performance Liquid Chromatography of Cereal and Legume Proteins; Kruger J. E., Bietz J. A., Eds.; AACC: St. Paul, MN, 1994; pp 121−134.

(20) Brzezinski, W. Electrophoretic patterns of wheat, triticale, barley and oat cultivars registered in 2004−2006. *Wiad. Odmianoznawcze* **2006**, 1–81 (COBORU, Słupia Wielka).

(21) Lookhart, G. L.; Bean, S. R. A fast method for wheat cultivar differentiation using capillary zone electrophoresis. *Cereal Chem.* **1995**, *72*, 42–47.

(22) Capelli, L.; Forlani, F.; Perini, F.; Guerrieri, N.; Cerletti, P.; Righetti, P. G. Wheat cultivar discrimination by capillary electrophoresis of gliadins in isoelectric buffer. *Electrophoresis* **1998**, *19*, 311–318.

(23) Bean, S. R.; Lookhart, G. L. Ultrafast capillary electrophoretic analysis of cereal storage proteins and its application to protein characterization and cultivar differentiation. *J. Agric. Food Chem.* **2000**, *48*, 344–353.

(24) Bean, S. R.; Lookhart, G. L. Faster capillary electrophoresis separation of wheat proteins through modifications to buffer composition and sample handling. *Electrophoresis* **1998**, *19*, 3190–3198.

(25) van Eckert, R.; Berghofer, E.; Ciclitira, P. J.; Chirdo, F.; Denery-Papini, S.; Ellis, H. J.; Ferranti, P.; Goodwin, P.; Immer, U.; Mamone, G.; Mendez, E.; Mothes, T.; Novalin, S.; Osman, A.; Rumbo, M.; Stern, M.; Thorell, L.; Whim, A.; Wieser, H. Towards a new gliadin reference material—isolation and characterization. *J. Cereal Sci.* **2006**, *43*, 331–341.

(26) Siriamornpun, S.; Wootton, M.; Cox, J. M.; Békes, F.; Wrigley, C. W. Capillary electrophoresis of wheat gliadin proteins and its potential for wheat varietal identification using patter matching software. *Aust. J. Agric. Res.* **2001**, *52*, 839–843.

(27) Piergiovanni, A. R. General experimental aspects for capillary electrophoresis analysis of gliadins in *Triticum* species. *Cereal Res. Commun.* **2004**, *32*, 395–402.

(28) Wieser, H.; Antes, S.; Seilmeier, W. Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography. *Cereal Chem.* **1998**, *75*, 644–650.

(29) Bushuk, W.; Zillman, R. R. Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. *Can. J. Plant Sci.* **1978**, *58*, 505–515.

(30) Mardia, K. V.; Kent, J. T.; Bisby, J. M. *Multivariate Analysis*; Academic Press: London, U.K., 1979.

(31) Graybosch, R. A.; Peterson, C. J.; Hansen, L. E.; Mattern, P. J. Relationships between protein solubility characteristics, 1BL/1RS, high-molecular weight glutenin composition and end-use quality in winter wheat germplasm. *Cereal Chem.* **1990**, *67*, 342–349.

(32) Kaneta, T.; Ueda, T.; Hata, K.; Imasaka, T. Suppression of electroosmotic flow and its application to determination of electrophoretic mobilities in a poly(vinylpyrrolidone)-coated capillary. *J. Chromatogr., A* **2006**, *1106*, 52–55.

(33) Chiu, T.-Ch.; Chang, H.-T. Comparison of the separation of large DNA fragments in the presence and absence of electroosmotic flow at high pH. *J. Chromatogr., A* **2002**, *979*, 299–306.

(34) Chu, B.; Liang, D. Copolymer solutions as separation media for DNA capillary electrophoresis. *J. Chromatogr., A* **2002**, *966*, 1–13.

(35) Lookhart, G. L.; Bean, S. R. Improvements in cereal protein separation by capillary electrophoresis: resolution and reproducibility. *Cereal Chem.* **1996**, *73*, 81–87.

(36) Lookhart, G. L.; Bean, S. R.; Graybosch, R.; Chung, O. K.; Morena-Sevilla, B.; Baenziger, S. Identification by high-performance capillary electrophoresis of wheat lines containing the 1AL.1RS and the 1BL.1RS translocation. *Cereal Chem.* **1996**, *73*, 547–550.

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