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Multiplex polymerase chain reaction analysis of *Glu-1* high-molecular-mass glutenin genes from wheat by capillary electrophoresis with laser-induced fluorescence detection

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Abstract

The unique bread-making properties of wheat are closely correlated with composition and quantity of high-molecular-mass (HMW) glutenin subunits encoded by the *Glu-1* genes. We report the development of a multiplex polymerase chain reaction (PCR) method to identify bread wheat genotypes carrying HMW glutenin allele composition of *Glu-1* complex loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) by capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection. Two triplex primer sets of HMW glutenin subunit genes were examined. An automated and rapid CE–LIF technique is helpful in the multiplex PCR optimization process. Two fluorescent intercalating dyes (EnhanCE, and YO-PRO-1) are compared for detection of DNA fragments. Amplified DNA fragments of HMW glutenin *Glu-1* genes were well separated both by agarose slab-gel electrophoresis and CE, and revealed minor differences between the sequences of *1Ax2**, *1Axnull*, *1Bx6*, *1Bx7*, *1Bx17* and *1Dx5* genes. Moreover, CE technique requires samples of smaller volumes in comparison to slab-gel electrophoresis, and data can be obtained in less than 20 min. There was a very high concordance in the assessment of the molecular size of PCR-generated DNA markers. Fast and accurate identification of molecular markers of *Glu-1* genes by CE–LIF can be an efficient alternative to standard procedure separation for early selection of useful wheat genotypes with good bread-making quality.

Keywords: *Triticum aestivum*; Molecular markers; Food analysis; Wheat; Proteins; Glutenin; DNA

1. Introduction

Wheat flour bread-making quality is mainly determined by the composition and quantity of endosperm storage proteins, in particular the high-molecular-mass (HMW) glutenin subunits [1,2]. In hexaploid wheat, the HMW glutenin proteins, formed through intermolecular disulfide linkage, make up 5–10% of the total seed protein. They are encoded by homologous loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, which are located on the long arms of homologous group-1 chromosomes, 1A, 1B and 1D, respectively. Each locus includes two tightly linked genes encoding two types of HMW glutenin subunits, designated as *x*- and *y*-types. Therefore, there are six HMW glutenin genes even though only three to five subunits are synthesized in any particular cultivar [3]. The allelic variation of these subunits is associated with flour quality. In particular, the HMW glutenin genes *1Ax1* and *1Ax2** encoded by *Glu-1A* locus, and the *1Dx5* and *1Dy10* subunit pair at *Glu-1D* locus have been suggested to be associated with stronger dough and better baking properties [4–6]. Traditionally, polyacrylamide gel electrophoresis [PAGE; sodium dodecylsulfate (SDS)-PAGE and/or A-PAGE] and RP-HPLC are the most widely used techniques for identification of HMW glutenin composition in wheat. Over the last few years, capillary electrophoresis, as the newest instrumental method for fast and reliable analysis of proteins, has also been introduced to reveal biochemical variation among *Glu-1* HMW glutenin subunits [7,8]. It incorporates speed, high resolution, minimal sample requirement, and direct quantitative digital analysis.

Polymerase chain reaction (PCR) techniques have been used as alternative forms of analysis of HMW glutenin subunits on the DNA level. Several PCR analyses of specific primers for single genes [9–11] or allele-specific (AS-PCR) markers [12–14] of genes coding at the *Glu-1*. One PCR variation that has become popular is the simultaneous amplification of two or more regions of DNA molecule and is commonly referred to as multiplex PCR or multiplexing

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[15,16]. The development of capillary electrophoresis (CE) has led to a number of advantages including the ability to automated separation and quantitation of complex mixtures such as DNA restriction fragments and PCR products with high reproducibility and efficiency [17,18]. This technique offers superior resolution and improved sensitivity, thus representing a significant improvement of traditional gel electrophoresis techniques in these regards. Additionally, the introduction of laser-induced fluorescence (LIF) detection in CE improves dramatically both the limit of detection and obtainable linear dynamic range in comparison to that of UV detection [18].

The goal of this work was to develop an ultrasensitive CE–LIF method to identify bread wheat genotypes carrying major HMW glutenin allelic combinations by multiplex PCR-generated DNA markers. Two triplex primer sets of *Glu-1* genes were examined to detect minor differences between the sequences of *1Ax2** and *1Axnull* genes of *Glu-A1* locus; *1Bx6*, *1Bx7* and *1Bx17* of *Glu-B1*; and *1Dx5* of *Glu-D1* locus. The simultaneous separation of multiple markers by CE–LIF offers an increase in the amount of information obtained per unit time of analysis, a reduction of the cost of analyses through amplifications involving one set DNA marker, high throughput, and automated DNA analysis. In case of bread-making quality of wheat, a shorter time of analysis is very important from technological and breeding point of view. This quick, sensitivity and non-destructive PCR-based method is an efficient alternative to standard procedures for early selection of desirable wheat genotypes with good bread-making characteristics.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical reagent grade and used as received. Hydroxypropylmethylcellulose (HPMC) for CE with a viscosity of ~4000 cP for a 2% aqueous solution at 25 °C was obtained from Sigma (St. Louis, MO, USA) and 2-hydroxyethylcellulose (HEC) (average M_w 720 000) was from Aldrich (Milwaukee, WI, USA). Chloroform, isopropanol and phenol were from Fluka (Germany). DNA markers used in agarose gel electrophoresis and CE were obtained from Promega (USA) and Beckman-Coulter (USA), respectively. YO-PRO-1 (Molecular Probes, Leiden, The Netherlands) and EnhanCE (Beckman, Fullerton, CA, USA) were used as intercalating dyes to CE running buffers. LIF text mixture (1×10^{-7} M fluorescein sodium salt in water) was obtained from Beckman-Coulter. All other chemicals for prepared buffer solutions were from Sigma. Deionized and purified water (18.2 MΩ), obtained by water purification system (Brandford, USA), was used in all reactions and solutions.

PCR primer oligonucleotides were purchased from Integrated DNA Technologies (Coralville, USA). Oligonucleotides were constructed on the basis of the published by other authors [9,10,12] primer sequences of PCR molecular markers for particular specific alleles of *Glu-1* genes (Table 1). Potential primer cross-reactions for the used multiplex PCR primer sets were examined via Oligo 6.0 program (Molecular Biology Insights, Cascade, USA).

2.2. Plant material

Analyses were carried out on seeds from nine Polish wheat cultivars/strains [Henika (*1Axnull*/*1Bx17* + *y18*/*1Dx5* + *y10*—in abbreviation $N/17 + 18/5 + 10$); Izolda ($N/6 + 8/2 + 12$), Jasna ($1/7 + 9/5 + 10$), Mobela ($1/7 + 9/2 + 12$), HEC 2288 ($2^*/7 + 9/5 + 10$), STH 996 ($2^*/7 + 9/2 + 12$), Hezja ($N/7 + 9/2 + 12$), Juma ($1/6 + 8/5 + 10$) and AND 2298 ($2^*/6 + 8/5 + 10$)], representing major quality allelic compositions of bread wheat HMW glutenin subunits determined early by SDS-PAGE.

Table 1
Sets of primers for identification of individual wheat *Glu-1* alleles used with positive result in the development of multiplex PCR

Design of the multiplex set	Gene/allele composition	Set of primers (forward and reverse primers)	Reference
ABD	*1Axnull* (1)	F1: ACGTTCCCCTACAGGTACTA R1: TATCACTGGCTAGCCGACAA	[10]
	1Bx[a] (2)	F2: ATGGCTAAGCGCCTGGTCCT R2: TGCCTGGTCGACAATGCGTCGCTG	[12]
	1Dx5 (3)	F3: GCCTAGCAACCTTCACAATC R3: GAAACCTGCTGCGGACAAG	[9]
A2B	*1Axnull* (1)	F1: ACGTTCCCCTACAGGTACTA R1: TATCACTGGCTAGCCGACAA	[10]
	1Bx[a] (2)	F2: ATGGCTAAGCGCCTGGTCCT R2: TGCCTGGTCGACAATGCGTCGCTG	[12]
	*1Ax2** (4)	F4: CCGATTTTGTTCTTCTCACAC R4: CACCAAGCGAGCTGCAGAT	[10]

[a] This primer set was used for identification of *1Bx7* gene in [12].

2.3. DNA extraction

Genomic DNA from wheat cultivars/strains was extracted from lyophilized leaves of single plants about 2–3-weeks old as described by Horn and Rafalski [19] with small modifications. In brief, plant material was extracted with 100 mM Tris–HCl buffer (pH 8.0) containing 50 mM EDTA, 0.5 M NaCl, 10 mM 2-mercaptoethanol. After homogenization, 20% SDS was added and the mixture was incubated at 65 °C for 10 min and then mixed with 1/3 volume of 5 M potassium acetate and left on ice for 2 h. After centrifugation (16 000 × *g*, 10 min, 4 °C) the supernatant was precipitated with ice–isopropanol (1:1) over night at −20 °C. The pellet was resolved in TE with 100 μg/ml ribonuclease and the solution was incubated for 90 min at 37 °C. Phenol/chloroform (1:1) was added twice and the mixture was gently mixed at room temperature for 5 min. After centrifugation for 10 min at 12000 g, a higher (aqueous) phase was transferred to a fresh tube where DNA was precipitated by adding 1/10 volume of 3 M sodium acetate and −20 °C isopropanol (1:1). The DNA pellet was washed with 70% ethanol, air-dried and re-suspended in 50 μl TE. The isolated DNA was stored at 4 °C until use.

2.4. PCR conditions

PCR analyses were performed in MJ PTC-200 thermal cycler with heated lid in the final volume of 25 μl. PCR reactions were carried out for two primer sets of triplex PCR molecular markers of *Glu-1* genes (designated ABD and A2B; compositions are shown in Table 1). Multiplex PCR reaction conditions were established by optimization of two types (Taq and HotStart Taq) of DNA polymerases from Qiagen in the concentration range from 1.5 to 3.0 U/100 μl, $MgCl_2$ concentration (1.5–3.5 mM), each of primer concentration (0.1–0.4 μM) and each of deoxyribonucleotide concentration (150–350 μM). The next step was checked annealing temperature in the range 58–62 °C at the time from 30 s to 2 min, extension time in the range of 1.5–3 min at 72 °C, and number of cycles ranging from 35 to 45.

The final multiplex amplifications of HMW glutenin genes were performed in the reaction mixture which contained 1× buffer (Qiagen), 2 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 50 ng genomic DNA and 0.5 U/25 μl HotStar Taq DNA polymerase. Optimal thermal cycling conditions were at 95 °C for 15 min, followed by 40 cycles at 94 °C–1 min, 60.5 °C–40 s and 72 °C–2 min 15 s. After 40 cycles, the extension temperature was kept at 72 °C for 10 min.

The PCR products were also separated in ethidium bromide-stained 1.0% (w/v) agarose gels run in 1× TBE buffer and exposed to UV light to visualize DNA fragments.

2.5. Capillary electrophoresis

CE separations were performed on a P/ACE-MDQ system of Beckman-Coulter (Fullerton, CA, USA) equipped with a laser-induced fluorescence detector system module 488 (3 mW air-cooled argon-ion laser). Prior to detection by a photomultiplier the emission light was passed through both a 488 nm notch filter and long-pass filter (cut-on wavelength, 520 nm). The temperature of the cartridge holding the separation capillary was thermostated at 20 °C ± 0.1 by active liquid cooling.

Bare fused-silica capillaries with 75-μm internal diameters were used from Composite Metal Service (Worcester, UK). The sieving buffers consisted of TBE (89 mM Tris, 89 mM boric acid, 1 mM EDTA, pH 8.5) and 0.5–1.2% HPMC or 1–2% HEC were prepared by dissolving polymers in the TBE buffer using magnetic stirring (12 h). The buffers were filtered through a 0.45 μm membrane filter (Schleicher and Schell, Dassel, Germany) before use. The two intercalating dyes—1–2.5 μl/ml EnhanCE (Beckman-Coulter) or 0.1–1 mM YO-PRO-1—were added into separation buffer directly before analysis.

Before the first use, uncoated capillaries were preconditioned by rising with 0.1 M HCl for 60 min and next water for 10 min. Prior to each run, the capillary was rinsed with water for 1 min, and new sieving buffer for 8 min at 30 psi (1 psi = 6894.76 Pa). At the end of the day, the capillary was rinsed with deionized water for 10 min and stored overnight with water inside.

DNA samples were prepared for the CE by adding 5 μl of PCR products to 40 μl deionized water and then were injected hydrodynamically (2 psi, 10 s). Separations were carried out in the reversed polarity mode (negative potential at the capillary inlet) at 10 or 15 kV for 25 min. An eCAP dsDNA 1000 Text Mix (ΦX174/*Hae*III) containing 11DNA fragments ranging from 72 to 1353 base pairs and an eCAP dsDNA 20000 Test Mix (λ DNA/*Hin*dIII) containing eight DNA fragments ranging from 125 to 23 130 base pairs (bp) from Beckman-Coulter were used as standards to calibrate the systems. The data were acquired and evaluated by Karat version 5 software (Beckman-Coulter). Data points were plotted in Microsoft Excel 2000.

3. Results and discussion

3.1. Optimization of multiplex PCR amplification and of dsDNA fragment separations

The development of multiplex PCR involved examination of various combinations of selecting primer sets as well as different reaction components, and thermal cycling conditions. In this case the optimization is more challenging than a singleplex reaction because so many primer annealing events must occur simultaneously without interfering with each other [16,20]. As a preliminary part of multiplex PCR assay development, we performed the singleplex reactions for each primer pair and compared the results to the multiplex reaction where all of the primers are combined. These experiments help determine which, if any, primer pairs

produce non-specific products. Finally, we performed multiplex PCR studies with two triplex sets of molecular markers for identification of wheat *Glu-1* alleles (Table 1) used earlier in the singleplex PCR by other authors [9,10,12].

A laser-induced fluorescence detection was used because UV absorbance detection appeared to be unsatisfactorily sensitive in case of a small quantity of obtained multiplex PCR amplicons. As molecular sieves for double stranded (ds) DNA fragment separations were selected the cellulose-derive polymers HPMC and HEC. Stable solutions in TBE buffer of this type of polymer in the concentration 0.5–1.5% can be prepared reproducibly [21,22]. Preliminary studies revealed that pretreatment of new fused-silica capillaries with sequential flushes of deionized water and HCl solution prior to used two polymer-containing buffer filling allows to reduce electroosmotic flow (EOF) and is favorable to reproducible and effective dsDNA fragment analysis. The observations are in line with earlier reports on dsDNA separations using polymer network as sieving matrix [22,23].

We compared two fluorescent intercalators EnhanCE and YO-PRO-1 for on-capillary DNA labeling. It is known that concentration of intercalating dye is important for obtaining an optimum fluorescence signal from the dye-dsDNA complexes in used CE method [24]. The study was carried by preparing separation buffers containing different quantities of intercalating dyes and using DNA test mixture (ΦX174/*Hae*III DNA restriction fragments from Beckman) at total concentrations of 2, 10, 50 and 250 μg/ml (data not shown). The increase independently from DNA concentration was observed only up to a concentration of 600 ng/ml for YOPRO-1 and a dilution of 10^{-3} for EnhanCE. For DNA fragments in the range of 450–2500 bp, the optimum separation was obtained using TBE buffer with 1.0% HPMC and EnhanCE in 1.5×10^{-3} dilution yielding favorable plate number ranging from 0.4 to 0.8×10^{6}.

3.2. Analysis of multiplex PCR products

3.2.1. Analysis by agarose slab-gel electrophoresis with ethidium bromide

Post-amplification analyses of DNA fragments produced by multiplex PCR are routinely carried out by agarose gel electrophoresis. Fig. 1 shows electrophoretic patterns of multiplex PCR amplified products of nine wheat varieties differing in their alleles at three *Glu-1* loci using two triplex sets of primers (ABD and A2B). ABD multiplex set allowed identifying three *1Bx* and additionally *1Axnull* and *1Dx5* genes simultaneously, whereas A2B multiplex set identified all *1Ax* and three *1Bx* genes. If the amplification of DNA fragments for *Ax2** and *Axnull* genes in the case of using A2B multiplex set did not occur, that would indicate the presence of third gene of *Glu-A1* locus, i.e. *1Ax1*. On the other hand, the lack of amplifitation of DNA fragment for *Dx5* gene in the case of using ABC multiplex set may point to at the occurrence of other genes of *Glu-1D* locus, i.e.

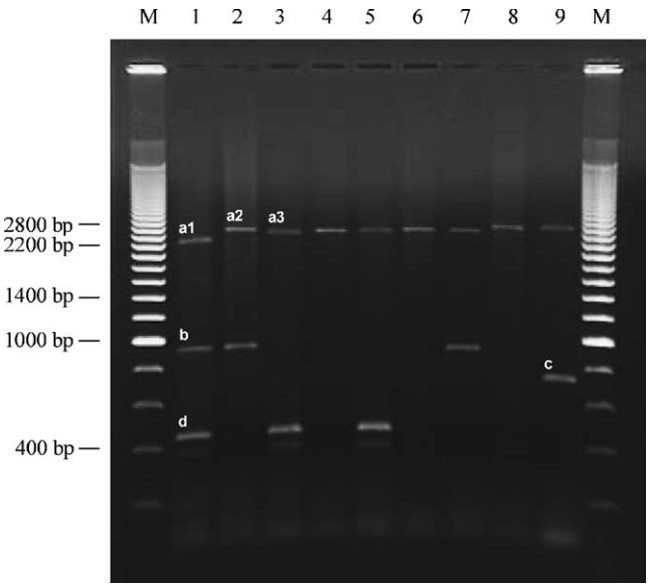

Fig. 1. Separation of triplex PCR amplified products on ethidium bromide-stained agarose gel using ABD and A2D (lanes 1–5 and 6–9, respectively) multiplex sets. Lanes: M–200 bp DNA ladder (Promega); (1) Henika (N/17 + 18/5 + 10); (2) Izolda (N/6 + 8/2 + 12); (3) Jasna (1/7 + 9/5 + 10); (4) Mobela (1/7 + 9/2 + 12); (5) HEC 2288 (2^*/7 + 9/5 + 10); (6) STH 996 (2^*/7 + 9/2 + 12); (7) Hezja (N/7 + 9/2 + 12); (8) Juma (1/6 + 8/5 + 10); (9) AND 2298 (2^*/6 + 8/5 + 10). Designation of DNA fragments: (a1) 2260 bp (*1Bx17*); (a2) 2490 bp (*1Bx6*); (a3) 2373 bp (*1Bx7*); (b) 920 bp (*1Axnull*); (c) 780 bp (*1Ax2**); (d) 450 bp (*1Dx5*).

1Dx2 or *1Dx3* or *1Dx4*, which are connected with a poor bread-making quality.

Using both triplex sets in all electrophoretic patterns similar band (Fig. 1, a1–a3), the size of which ranged between 2250 and 2500 bp, was observed. We have revealed that primer set described by Ahmad [12] and designed to detect the presence of subunit 7, allow to distinguish three genes of *Glu-1B* loci. DNA of wheat genotypes possessing *1Bx17* gene produced an approximately 2260 bp fragment specific of this gene (Fig. 1, band a1), *1Bx7* gene—2373 bp fragment (band a2) and 1*Bx6*—approximately 2500 bp fragment (band a3). An amplified product of about 920 bp (Fig. 1, band b), which is characteristic of *1Axnull* gene, was observed using ABD multiplex set, as reported previously by Lafiandra et al. [10]. However, the lack of this band in the analyzed samples showed the presence of one of two genes *1Ax1* or *1Ax2** which are crucial from the bread-making point of view [2,26]. The presence of a 450 bp band, showed the occurrence of *1Dx5* gene using ABD multiplex set (Fig. 1, traces 1, 3, and 5).

A 2652 bp DNA fragment, which is characteristic of *1Ax2** gene in a single PCR reaction [13], was not amplified in A2B multiplex set. The observed band with approximately 780 bp (Fig. 1, band d) was non-specific of each of these primer pairs individually and was obtained only with genotypes possessing *1Ax2** gene. In order to explain its occurrence, single PCR reactions with combination of one

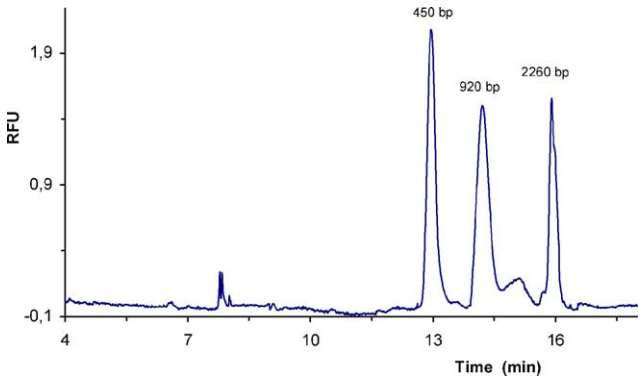

Fig. 2. Electropherogram of multiplex PCR amplified products using ABD multiplex set for analysis of DNA fragments from Henika cultivar (*1Axnull*/*1Bx17* + *y18*/*1Dx5* + *y10*) by CE–LIF in 89 mM tris base, 89 mM boric acid, 2 mM EDTA, 1.0% HEC, pH 8.5, and EnhanCE in 1.5×10^{-3} dilution. Separations conditions: uncoated fused silica capillary of 40.2 cm (30 cm effective length) × 75 μm i.d.; sample injection for 10 s at 1 psi, separation voltage 10 kV (reversed polarity). The numbers denote fragment sizes in base pairs.

primer for *1Ax2** gene and one primer for *1Bx7* gene were performed. The analysis showed that only combination of primers F2 (forward for *1Bx7*) and R4 (reverse for *1Ax2**) gave the expected band of 780 bp in size.

3.2.2. Analysis by CE–LIF

The described results made it possible to select the CE–LIF system for HMW glutenin subunit genotyping and comprising a uncoated fused-silica capillary (30 cm for detector), a separation buffer of TBE with 1.0% HPMC and 1.5 μl/ml EnhanCE, and operated at 20 °C using a separation voltage of −10 kV. Using this system the PCR products after eight-fold dilution were analyzed directly without any sample pretreatment. Optimizations of multiplex PCR amplifications for two triplex primer sets (ABD and A2D) were performed using genomic DNA isolated from wheat Henika cultivar. This cultivar includes four HMW subunits; $1B17x + 18y$ and $1D5x + 10y$, according to numbering system of Payne and Lawrence [25]. Fig. 2 presents electropherogram of multiplex PCR amplified products for above sample using ABD multiplex set by CE–LIF. On the basis of the obtained peak profile molecular markers of HMW glutenin subunit genes *1Axnull*, *1Bx17* and *1Dx5* can be easily determined. Three DNA fragments amplified with sizes 450 bp (*1Dx5*), 920 bp (*1Axnull*) and 2260 bp (*1Bx17*) were well separated by this system in time 12.96, 14.28 and 15.91 min, respectively. The untreated sample contained small amounts of nonspecific compounds (like, e.g. remaining primers, and non-specific PCR products), which migrate before desirable DNA fragments. The differences in the relatively intensities of the fragments are primarily caused by the fact that the fragments belong to different alleles and differ by the size of base pairs.

The possibility to obtain quantitative information in the form of peak heights or peak areas by CE–LIF was helpful in balancing the amplification yield from multiple PCR products. PCR products of *1Bx* genes amplified using equal concentration all primer pairs showed lower in peak height relative to the other amplicons in the multiplex. Analysis of multiplex peak patterns obtained at different concentration of reaction components and under different thermal cycling conditions permitted a better balance of the amplification yield of DNA fragment of this gene (final amplification conditions see Section 2.4). The precision of our CE system has been determined by injecting six replicate samples of both ladder and calculating for DNA fragments with sizes in the range of 600–2500 bp. The results demonstrated 2.1% relative standard deviation for migration times. This corresponds to a drift of ∼10 bp. To determine the sizes of amplified products they were mixed equally with one of the two text mixture markers and separated together.

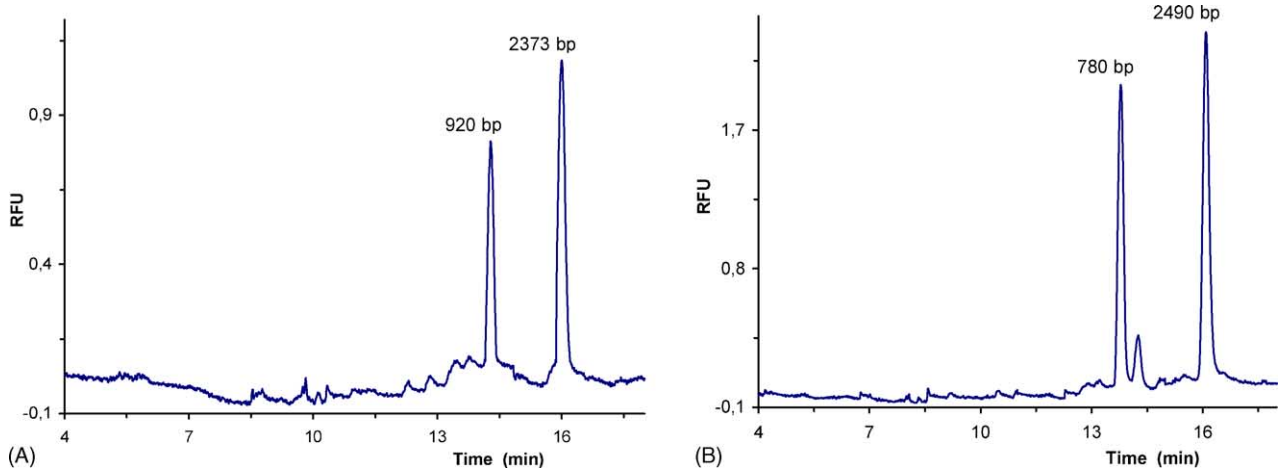

Fig. 3. Electropherograms of multiplex PCR amplified products using ABD (A) and A2B (B) multiplex sets for Hezja (*1Axnull*/*1Bx7* + *y9*/*1Dx2* + *y12*) and AND 2298 (*1Ax2**/*1Bx6* + *y8*/*1Dx5* + *y10*) cultivars by CE–LIF in 89 mM tris base, 89 mM boric acid, 2 mM EDTA, 1.0% HEC, pH 8.5, and EnhanCE in 1.5×10^{-3} dilution. Separation conditions as in Fig. 2. The numbers denote fragment sizes in base pairs.

Table 2
Identification of major allelic composition of wheat HMW glutenin subunits using two triplex sets of primers detected by CE–LIF

Multiplex set	Number of peaks of multiplex PCR products					
	$N/7+8/5+10$	$N/6+8/2+12$	$1/6+8/5+10$	$1/7+9/2+12$	$2^*/7+9/5+10$	$2^*/6+8/2+12$
ABD	3	2	2	1	2	1
A2B	2	2	1	1	2	2

3.3. Wheat HMW glutenin subunit variety testing by CE–LIF

To verify the applicability of the presented method to distinguish alleles of HMW glutenin related to bread wheat quality, multiplex PCR analyses were performed on nine wheat cultivars/strains. Separation of the PCR products by CE–LIF performed on the genomic DNA from the same materials as in the case using agarose gel electrophoresis gave the identical results. Fig. 3A and B present selected electropherograms of multiplex PCR amplified products of HMW glutenin subunits for the wheat cultivar Hezja and strain AND 2298 using appropriate multiplex primer sets. The composition of these cultivars/strains includes HMW glutenin subunits $N/7+9/2+12$ and $2^*/6+8/5+10$, respectively. In the case of Hezja cultivar, the peak with 14.28 min of migration time conform to DNA fragment for *1Axnull* gene (920 bp), and peak with 16.01 min of migration time—fragment for *1Bx7* gene (2373 bp). On the other hand, electropherogram for the AND 2298 strain shows two peaks with the migration times of 18.81 and 16.12 min conform molecular markers for *1Ax2** (780 bp) and for *1Bx6* (2490 bp), respectively. Table 2 was presents the number of peaks of multiplex PCR products detected by LIF–CE for major allelic composition of wheat HMW glutenin subunits.

4. Conclusions

One of the general aims in cereal science is to relate the wheat protein composition to its flour functional properties. This knowledge can then be used in breeding to improve the processing quality of wheat varieties. Useful single molecular markers of HMW glutenin alleles obtained by PCR technology and then analyzed by agarose gel electrophoresis were presented previously [9–13]. We have demonstrated that CE–LIF method can efficiently be used for analyzing amplified multiplex PCR products of HMW glutenin subunit genes. The simultaneous separation of several dsDNA fragments of *Glu-1* genes by CE–LIF in uncoated fused-silica capillary filled non-gel sieving offers an increase in the amount of information obtained per unit time of analysis, reduction of the cost of analysis of amplifications involving one set DNA marker, high throughput, and automated DNA analysis. This method revealed differences between the sequences of *1Ax1*, *1Ax2**, *1Axnull*, *1Bx6*, *1Bx7*, *1Bx17* and *x5* of *Glu-D1* genes. Moreover, CE–LIF method requires samples of a smaller size in comparison to those in the case of slab-gel electrophoresis, and data can be obtained in less than 20 min. It is also a very high concordance in the assessment of the molecular size of PCR-generated DNA markers. The fact that no pretreatment of PCR samples and no internal standards are needed, adds further advantages to the simplicity of the method. This fast, sensitive and non-destructive PCR-based method can be an efficient alternative to standard procedures for early selection of desirable wheat genotypes with good bread-making characteristics.

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