World Applied Sciences Journal 4 (3): 384-395, 2008
ISSN 1818-4952

Distinction Between Cereal Genotypes Based on the Protein and DNA Composition of the Grain by Capillary Electrophoresis

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Abstract: Cereal grains are widely used for human food and animal feed throughout the world. Distinction between the various genotypes of any cereal species is important to segregate grains according to utilization type. DNA analysis indicates genotype (variety), whereas protein composition provides information about both variety and likely processing properties, reflecting the contributions of both genotype and growth/storage conditions. Standard methods of protein and DNA analysis involve gel electrophoresis in various formats. Capillary electrophoresis (CE) is one of the newer techniques to be so used. CE is a valuable addition to other methods of cereal protein and DNA analysis and should, in time, be applicable to analyzing DNA and all protein classes from all cereal grains. This review focuses on methods for distinguishing genotypes based on the protein and DNA composition of the grain by capillary electrophoresis. Microfluidic capillary electrophoresis (Lab-on-a-chip), a newly advanced and rapid technique, is also discussed.

Key words: Capillary Electrophoresis (CE) · Cereal · Genotypes · DNA · Protein

INTRODUCTION

Cereal grains are major foods in every country, either directly as human food or indirectly as animal feed [1]. The ability to distinguish among different varieties of grain crops is especially important to the agricultural sector because of the differences among varieties in their quality and other agronomic properties [2]. There are many methods available to discriminate between varieties. Reversed-phase high performance liquid chromatography (RP-HPLC) and polyacrylamide gel electrophoresis (PAGE) have been frequently used in cereal protein characterization. However, these methods are often labor intensive and neither cost effective nor reproducible (Table 1) [3]. Capillary electrophoresis (CE) is one of modern analytical techniques used for cereal variety identification. It has proved to be rapid and sensitive and it can be fully automated, providing high resolution and reproducibility [4]. Furthermore, the availability of advanced commercial instrumentation has led to the use of CE for the analysis of various compounds such as proteins, sugars, oligosaccharides, amino acids and soluble vitamins [5]. The use of CE for rice varietal identification has been reported over the past decade [4, 6, 7]. At present, the use of CE for variety identification has so far involved the use of sophisticated equipment that has been of considerable size and cost, warranting a place in a central laboratory. However, CE technology has recently been further improved upon older systems. The most recent approaches involve Lab-on-a-chip equipment for protein and DNA analysis. In addition, micro-array technology has been introduced to determine DNA composition efficiently for large numbers of samples [8]. The need to identify varieties and species of grains arises because they may be significantly different in their genetic traits with respect to grain-quality attributes and according to their agronomic potential, e.g., resistance to pathogens [9]. So this review focuses on distinction between genotypes of cereals, based on the protein and DNA composition of the grain by capillary

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Table 1: The comparative effectiveness of routine methods of variety identification based on protein analysis. Adapted from Wrigley and Bekes [3]

Factors	PAGE	PRE-CAST GELS	RP-HPLC	Conventional CE	Micro-fluidic CE
Time to set gel or regenerate column	60 min	10 min	10 min	2 min	2 min
Sample extraction	20 min	20 min	20 min	20 min	20 min
Sample run time	240 min	10-90 min	30 min	10 min	1 min
Protein visualization	Overnight	20 min or overnight	Instant	Instant	Instant
Data interpretation	10 min	10 min	Instant	Instant	Instant
Through-put in 24 h	20/gel	10/gel	30	100	>400*
Health risk to operators	Moderate	Low	Low-medium	Low	Low
Costs-equipment	Low	Low	High	High	Medium
Costs-consumables	Low	Medium	Medium	Medium	Medium
Costs-labor	High	Medium	Low	Low	Low

*Assuming sample extraction proceeds during CE analysis and that operations continue on a 24-hour basis

electrophoresis. The review describes the methods and applications of CE for cereals proteins and DNA analysis cover the period from 1995 to 2008.

SIGNIFICANCE OF VARIETY IDENTIFICATION

It is well known that there are different species of grain crops, with varying end uses. Knowledge of varietal identity goes beyond verification of grain-quality attributes. New requirements for Plant Breeders' Rights legislation in some countries provide the additional requirement of identification at the time of grain delivery to the silo. This verification may be performed subsequent to delivery, if subsamples of truck-loads are retained and available for checking at a central laboratory. However, ensuring that varietal declarations are correct before binning is a basic requirement of segregation for quality uniformity. Once the truck-load of grain is binned, it is obviously too late to reverse this step if the variety declaration is subsequently found to be wrong and thus inappropriate for the designated quality class. Variety identification by visual examination is subjective and poor in its ability to discriminate, whereas analysis of grain-protein composition has proved to be effective. Traditional methods of protein analysis, such as gel electrophoresis and HPLC, are slow and confined to the laboratory and because of the complexity of their use, back-up resources and trained operators are needed. The Lab-on-a-chip version of size-based capillary electrophoresis promises to overcome these difficulties, thereby offering a platform for quickly identifying grain varieties beyond the confines of the traditional laboratory [10].

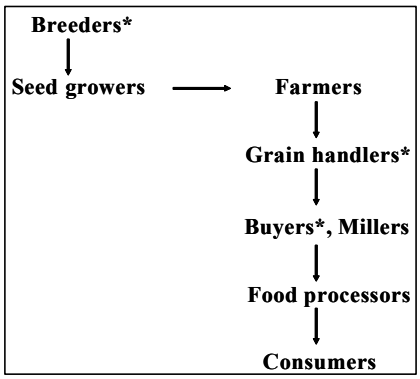

Fig. 1: Quality assurance (QA) in the grain production system. Adopted from Wrigley *et al.* [8].
* indicates critical stages from QA testings.

One step up the "grain chain" from the food processor in Fig. 1, the responsibility for quality assurance lies with the flour miller. Assurance of flour quality requires the miller to know the quality requirements specific to the flour customer, especially the type of dough strength and extensibility needed, the degree of starch damage, the desirable water absorption and more. These attributes depend partly on the miller's skill, but mainly they require the appropriate type of grain entering the mill and this is the buyer's responsibility. It is thus left to the grain-handling corporations to grade grain into the various quality classes, based on physical quality (test weight, screenings, etc.), on protein content and on variety [11, 12]. This last specification (variety) is critical for class determination because the genotype (G) indicates the set of genes that the breeder has "built into" the variety, right back at the start of the grain chain [13].

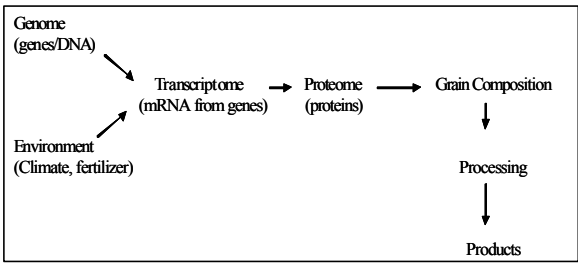

Fig. 2: The sequence of events from genes, through protein synthesis to the final determination of product quality. Analysis of varietal identity is generally directed at the DNA or protein levels. Reproduced with permission from Wrigley [9]

However, this genetic potential for quality type is progressively modified when the seed is planted, grown, harvested and stored (Fig. 1). The extent and nature of these environmental influences (E) are largely beyond the influence of the breeder, but there is some potential for the breeder also to "build in" tolerances to the effects on quality of adverse aspects of environmental factors, due to the genotype × environment (G×E) combination (Fig. 2). Subsequent to the breeder, the consequences of G×E must be considered throughout the grain chain, especially their effects on the critical attribute of dough quality [8].

CAPILLARY ELECTROPHORESIS FOR GRAIN PROTEINS SEPARATION

The quality of wheat and flour and thus the quality of the cereal products are mainly dependent on the protein composition [10, 14-22]. The composition of the gliadin fraction also changes during the maturation process and varies from cultivar to cultivar. So monitoring the fine structure of gliadin fractions can provide information regarding the wheat maturation process and also varietal identification [14]. Storage proteins (tertiary semantides), especially prolamins, have long been used for varietal identification because of the consistency of prolamin (gliadin) composition. This consistency has been demonstrated despite a wide range of variations in growth and treatment conditions. This aspect of phenotype for the genetic material is extremely stable under variable environmental conditions, such as growth location, growth season and soil conditions. Bean and Lookhart, reported [7] that rice cultivars were consistently differentiated in less than 15 min by capillary electrophoresis (CE). Capillary electrophoresis is a comparatively modern form of identification, with good potential to distinguish between cereal varieties. It has been used to characterize cereal proteins, demonstrating excellent resolution and reproducibility. Additionally, CE is a very fast method and does not necessarily require extensive skilled manpower [4].

During the past decade, capillary electrophoresis has developed as an alternative to gel electrophoresis, with similar or better resolution of zones [7, 23]. The use of CE for variety identification has generally involved the analysis of gliadin composition. The basis of fractionation by CE appears to differ from that of PAGE, so CE promises to provide different distinctions between varieties compared to PAGE. Comparative analyses in different laboratories with different brands of CE equipment have established that similar procedures can be applied in different situations [24]. CE offers the advantages of speed, automatic loading and instant interpretation of results. The latest advances in methodology offer an analysis time of less than ten minutes, with a little longer required for regeneration before application of the next sample.

CE has been reported to be suitable for analysis of not only grain but also flour and dough proteins [25]. In the early phase of dough preparation, mostly albumins were obtained; later gliadin proteins dominated the extract [18]. Barley seed protein, namely hordeins can be used to characterize barley. CZE has separated and characterized three barley species [26]. Barley extracted with 40% aqueous ethanol provided hordeins B and C whereas diploid species showed relatively similar pattern, tetraploid species showed hordein pattern significantly different [26].

Reports of the use of CE for variety identification have so far involved the use of sophisticated equipment that has been of considerable size, warranting a place in a central laboratory. However, CE offers the further promise of moving out of the laboratory with the emergence of small, portable equipment that would provide the ideal combination of convenience, speed of analysis and resolution, together with portability and consequently on-site use [3].

DNA ANALYSIS BY CAPILLARY ELECTROPHORESIS

As DNA composition (primary semantide) is the basis of genotype, it is not affected by environmental conditions, such as growth location, growth season and soil conditions. Identification of the genes present in the grain can be used in determining phenotypic

characteristics, such as the fragrance and cooking quality of rice. These represent examples of genomics strategies that are highly relevant to cereal chemists [27]. Differences in the sequence of the gene in different cultivars or differences in the level of expression of the gene may be used to explain differences in processing or end-use quality characteristics. Rice quality traits such as fragrance and gelatinization temperature (cooking temperature) can be explained by DNA sequence differences in specific genes identified using genomic approaches [27]. Rapid and reliable species and cultivar identification based on DNA analysis using genomics tools can be applied to grain and to food products. This technology has special advantages in the analysis of complex mixtures of cereals. Technologies for very high throughput and very low-cost analysis of large numbers of samples are now available. These may be applied by cereal chemists at many levels, namely, for selection in cereal breeding, in optimizing processing and to analyze the identity and composition of grain or food samples. Genomic tools for the major cereals are expanding rapidly. A complete DNA sequence is already available for rice and will become available for other cereals over the next few years [27].

Aspects of protein composition have long been used for variety identification because of the absence of variations in composition, despite changes in growth conditions. Most prominent in this respect are the gliadin proteins, whose electrophoretic patterns have been shown to very little with growth environment [28] with the possible exception of sulfur deficiency [29]. Nevertheless, analysis at the DNA level is the undisputed ultimate level at which to analyze for genotype, with no possibility for interference from growth conditions.

New technologies at the genome level now offer the possibility of conducting varietal identification by DNA analysis efficiently and economically. These new approaches involve the choice of specific markers that would provide adequate distinction. Protein markers are becoming identified for whose genes microsatellite-based PCR methods are available [30]. High throughput micro-plate colorimetric assays are under development to provide routine identification based on these genes [3, 31].

Species identification of animals or plants is a difficult task when they have lost their morphological characteristics (e.g., shape, size and appearance) during food processing. For that reason, species-identification methods are generally based on detection of species-specific compounds, such as protein and DNA [32]. Genomic tools for the major cereals are expanding rapidly. New technologies at the genome level offer the possibility of conducting varietal identification by DNA analysis efficiently and economically. Analysis at the genome level excludes the possibility of interference from fluctuations in the growth conditions, because it occurs at the start of the sequence of events shown in Fig. 2. These events extend from the genes through protein synthesis to the formation of all components of the grain [9].

MICROCHIP CAPILLARY ELECTROPHORESIS FOR GRAIN VARIETY IDENTIFICATION

There has been increasing interests in recent years in the application of miniaturized electrophoresis on an etched quartz chip (micro-fluidic CE or "lab-on-a-chip" CE), which is a potentially rapid method in biological research for protein and DNA analysis. CE on microchip is a promising technology since it offers an easy integration of many steps including sample preparation, derivatization and coupling of several separation procedures together [33, 34]. Several published papers have indicated that crucial advantages may overcome the inherent difficulties in achieving reproducibility, sample manipulation and automation at the sub-nanoliter level [35-40]. Uthayakumaran *et al*. reported [10] on-the-spot identification of grain variety and wheat-quality type by Lab-on-a-chip capillary electrophoresis. This form of CE has been used for identification of wheat variety and quality type. Analysis of each chip takes 30 min for 10 samples and distinction has been made between members of a set of 40 commonly grown Australian wheat varieties (Fig. 3 and 4). Quality type could be predicted by analysis of HMW and LMW glutenin subunits [41]. The technique has also been applied to the separation of protein from other grain and legumes and may also be useful for identifying variety and/or quality type in these crops [10]. Lab-on-a-chip CE of the full range of grain polypeptides offers a rapid means of testing grain for variety and also quality type in the case of wheat, using simple equipment of modest cost, that is convenient to use and small enough (footprint of 15×40 cm) to be operated remote from the conventional laboratory [10].

A new opportunity for obtaining variety information quickly and beyond the laboratory is now provided by the Lab-on-a-chip concept in equipment for analyzing protein composition. The procedure provides the protein composition of a grain extract in a one minute analysis, giving a digital output that is ready for matching against a library of patterns for authentic variety samples. The heart of the equipment is the "chip", about 5 cm square,

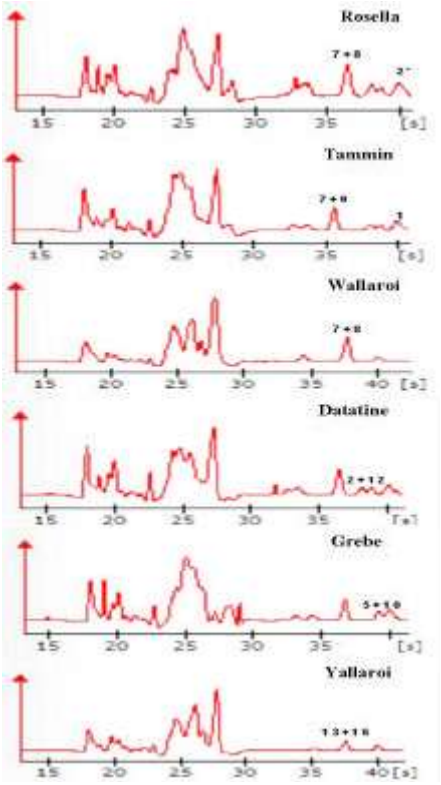

Fig. 3: Lab-on-a-chip capillary electrophoresis of wheat-flour proteins extracted under reducing conditions (1% SDS + 1%DTT), and exhibited as elution profiles. Some of the HMW subunits of glutenin are labeled by subunit numbers. Reproduced with permission from Uthayakumaran *et al*. [10]

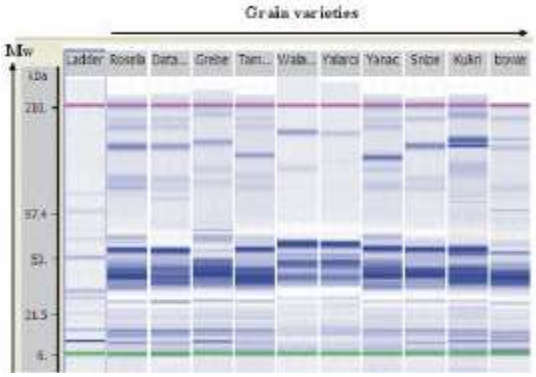

Fig. 4: Lab-on-a-chip capillary electrophoresis of wheat-flour proteins extracted under reducing conditions (1% SDS + 1%DTT). Elution profiles have been computer manipulated to simulate gel-electrophoresis patterns. The HMW subunits of glutenin occupy the top third of the patterns. The pattern at the extreme left is for molecular weight standards. Reproduced with permission from Uthayakumaran *et al*. [10]

which accepts ten sample extracts plus reagents. Although each analysis is very fast, it takes about an hour for the grinding, extraction and analysis of a 'chip-full' of ten samples [10, 42].

The Lab-on-a-chip system uses the capillary electrophoresis principle for analyzing protein composition. The full set of polypeptides is used for variety identification, using a single extraction with a combination of detergent (sodium dodecyl sulfate, SDS)

Table 2: Application of CE for analysis of cereal proteins and DNA (from 1995-2008)

Cereals	Compounds	CE Mode	Detection	Buffer	Separation condition	Ref.
Wheat	Protein (prolamins or glutelin)	CE	UV/200 nm	0.06 mol/L sodium borate, pH 9.0	10 kV, 40°C, 40 cm × 50 μm i.d	[43]
		CE	UV/200 nm	0.1 mol/L phosphate buffer, pH 2.5	7 kV, 40°C, 20 cm × 50 μm i.d.	[43]
		HPCE	UV/200 nm	0.1 M phosphate buffer, pH 2.5 containing HPMC	45°C and 22 kV, 27 cm (20 cm)×20 μm i.d.	[4]
		CE	UV/200 nm	0.1 M phosphate buffer pH 2.5	40°C, 10 kV, 27 cm (20 cm) × 50 μm i.d.	[44]
		CE	UV/200 nm	100 mM phosphate buffer, pH 2.5, containing 0.05%	15 kV, 45°C, 27 cm (20 cm) ×20 μm i.d.	[45]
		CZE	UV/200 nm	100 mM phosphate buffer, pH 2.5, containing 0.05% HPMC, 20% ACN	15 kV, 45°C, 27 cm (20 cm) × 20 μm i.d.	[46]
		FZCE	UV/200 nm	100 mM phosphate buffer, pH 2.5 containing 20% ACN and 0.05% HPMC	12 kV, 45°C 27cm × 25 μm i.d. 7 kV, 40°C,	[47] [6]

Table 2: Continued

Cereals	Compounds	CE Mode	Detection	Buffer	Separation condition	Ref.
		CE	UV/200 nm	0.1 Mol/L phosphate buffer, pH 2.5	27 cm × 50 μm i.d.	
		CE	UV/214 nm	ProSort® kit was modified by the addition of 5%methanol	8.1 kV, 30°C, 27 cm × 55 μm	[48]
		CZE	UV/214 nm	40 mM Asp buffer, 7 M urea and 0.5% 0.5% short-chain hydroxyethylcellulose, pH 3.9	1000 V/cm at room temperature, 50 mm i.d., 30 cm	[49]
		CZE	UV/214 nm	0.06 M sodium borate, pH 9.0 containing 20% ACN and 1% SDS	15 kV and 30°C, 47 cm ×50 μm i.d	[50]
		SDS-CE	UV/214 nm	Bio-Rad CE-SDS run buffer + 15% ethylene glycol	30°C, 8 kV, 27 cm (20 cm) × 75 μm i.d.	[51]
		CE	UV/200 nm	0.1 M phosphate pH 2.5	17 μA, 40°C, 24 cm (20 cm) × 20 μm	[52]
		CE	UV/200 nm	0.1 M phosphate pH 2.5	17 μA, 40°C, 24 cm (20 cm) × 20 μm	[53]
		CZE	UV/214 nm	100 m*M* phosphate buffer containing 0.05% MHPC, pH 2.5	15 kV, 25°C, 40 (30) cm ×350 mm i.d.	[54]
		CZE	UV/200 nm	50 mM IDA + 20% ACN and 0.05% HPMC	45°C, 30 kV, 27 cm (20 cm) × 50 μm i.d	[7]
		CZE	UV/214 nm	100 mM phosphoric acid/β Ala, pH 2.5 containing 1 M urea, 0.05% HPMC, 20 % ACN	14.3 kV, 40°C 20 cm × 50 μm i.d	[55]
		HPCE	UV/214 nm	100 mM phosphoric acid /â-Ala (pH 2.5) containing 1M urea, 0.05% HPMC and 20% ACN	14.3 kV, 40°C, 20 cm × 50 μm i.d.	[14]
		FZCE ,8 kV, 30°C, 75 μm i.d. uncoated silica-fused capillary 50 cm 214 nm.	UV/214 nm	16 mM boric acid, 16 mM sodium tetraborate, 33 mM phosphoric acid, 1% (w/v) PEG 400, and 10% (v/v) ethanol	8 kV, 30°C, 50 cm × 75 μm i.d.	[15]
		Lab-on-a-chip	-	Agilent sample buffer	1% SDS+DTT, proteine 200+ chip, Agilent 2100 Bioanalyzer	[10]
	Protein subunits	Lab-on-a-chip	-	-	reference proteins of 9, 14.5, 28, 46, 63.5, 95, 158 kDa, Agilent 2100 Bioanalyzer	[41]
		CZE	UV/214 nm	0.1 M phosphoric acid/β-alanine, pH 2.5, containing urea (1 M), 0.05% HPMC and 20% ACN	15 kV, 35°C, 50 μm i.d. × 27 cm (20 cm to detector)	[56]
		CZE 200 nm	UV/200 nm	50 mM IDA containing 20% ACN and 0.05% HPMC,	30 kV, 45°C, 50 μm × 27 cm	[57]

Table 2: Continued

Cereals	Compounds	CE Mode	Detection	Buffer	Separation condition	Ref.
	Chip Electrophoresis		fluorescent detection	-	Pro260 analysis Kit,	[58]
		CZE	UV/200 nm	100 mM phosphate buffer, pH 2.5, containing 0.05% HPMC	10 kV, 40°C, 27 cm (20 cm) ×20 µm i.d.	[45]
		CZE	UV/200 nm	0.1 M phosphate buffer, pH 2.5 containing 0.05% HPMC	10 kV, 40°C 27 cm(20 cm) ×20 µm i.d.	[4]
		CZE	UV/200 nm	sodium phosphate buffer, pH 2.5 containing 20% ACN and 0.05 % HPMC	12.5 kV, 40°C, 27 cm ×50 µm i.d.	[59]
		CZE	UV/200 nm	50 mM IDA buffer pH 2.5 containing 20 % acetonitile and 0.05% HPMC	8 kV, 40°C, 24 cm (20 cm) ×50 µm i.d.	[60]
		CZE	UV/200 nm	50 mM IDA + 20% CAN, 0.05% HPMC and 26 mM SB 3-12	30 kV, 45°C, 50 µm i.d × 27 cm (20 cm)	[7]
Rice	DNA (PCR product)	CE (ABI PRISM 3100)	Fluorescent	-	0.3 µL of GeneScan-500 ROX size standard and 12 µL of Hi-Di Formamide, viewed with GeneScan 3.7 and Genotyper 3.7 software	[61]
Rice	RNA	CE	-	-	RNas backing at 200°C or treated by diethylpyrocarbonate (DEPC), separated within 15 min using 1.0%T, 0%C linear polyacrylamide, 7 mol/L urea as denaturant..	[62]
Oat	Proteins	CZE	UV/200 nm	100 mM phosphate buffer, pH 2.5, containing 0.05% HPMC	15 kV, 45°C, 27 cm (20 cm))×20 µm i.d.	[45]
		CZE	UV/200 nm	0.1M phosphate buffer, pH 2.5 containing 0.05% HPMC	22 kV, 45°C, 27 cm (20 cm) ×50 µm i.d.	[4]
		CZE	UV/200 nm	50 mM IDA + 20% ACN and 0.05% HPMC	30 kV, 45°C, 50 µm i.d × 27 cm (20 cm)	[7]
Triticale	Protein	CE	UV/200 nm	0.1 M phosphate pH 2.5	17 µA, 40°C, 24 cm (20 cm) × 20 µm	[63]
Barley	Protein(barley malting)	FZCE	UV/200 nm	50 mM phosphate-glycine, pH 2.5, containing 20% ACN and 0.05% HPMC	12.5 kV, 45°C, 50 µm i.d. x 31 cm	[64]

Table 2: Continued

Cereals	Compounds	CE Mode	Detection	Buffer	Separation condition	Ref.
	Protein	CZE	UV/200 nm	50 mM IDA + 20% ACN and 0.05% HPMC	30 kV and 45°C, 50 µm i.d × 27 cm (20 cm)	[7]
	Malts and roast barley	HPCE	diode array detection (Detection: 200 and 360 nm)	30 mM phosphate buffer, pH 2.5	30 kV, 25°C 48.5 cm (40 cm) × 50 µm i.d.,	[65]
					50 mM carbonate buffer pH 9.5 20 kV, 25°C, 48.5 cm (40 cm) × 50 µm i.d.,	[65]
		HPCE	UV/200 nm	0.1 M phosphate-glycine buffer, pH 2.5, containing 20% ACN and 0.05% HPMC	12.5 kV, 40°C, 27 cm (20 cm) × 25	[21]
Barley	Protein	FZCE	UV/200 nm	100 mM phosphate-glycine buffer containing	20% ACN 0.05% HPMC -	[66]
Rye	Protein	CZE	UV/200 nm	50 mM IDA + 20% ACN and 0.05% HPMC,	30 kV and 45°C, 50 µm i.d× 27 cm (20 cm)	[7]
Maize	Protein	FZCE	UV/200 nm	80 mM phosphate-glycine buffer, pH 2.5 containing 60% CAN and 0.05% HPMC	12.5 kV, 45°C, 25 µm i.d. ×27 cm (20 cm)	[67]
Sorghum	Protein	FZCE	UV/200 nm	80 mM phosphate-glycine buffer, pH 2.5 containing 60% CAN and 0.05% HPMC,	12.5 kV, 45°C, 25 µm i.d. ×27 cm (20 cm)	[68]

(Abbreviation; HPMC: Hydroxypropylmethylcellulose, ANC: acetonitrile, SDS: sodium dodecyl sulfate, IDA: iminodiacetic acid)

Table 3: The relative suitability of methods of three distinct situations where variety identification might be required. Adapted from Wrigley and Bekes [3].

Examples of these situations	1. On-the-spot Grain receival at mill or elevator Export terminal	2. Regional lab Back-up to elevator Breeder or seed lab	3. Central lab Contract lab for many post-harvest samples
Requirements	Speed	Over-night	Efficiency for large numbers
Visual examination			
Image analysis	□□		□
Phenol test		□	
PAGE		□	□
RP-HPLC		□□	□
Conventional CE (protein)		□□	□□
Micro-fluidic CE (protein)		□□	□
DNA analysis	□	□□	
Micro-fluidic CE			
Micro-array		□	□□

and dithiothreitol (DTT) which breaks the inter-chain disulfide bonds of glutenin. Alternatively, a more complex extraction procedure involves prior removal of non-glutenin proteins, so that only glutenin-subunit composition is provided, both subunits of high-and low-molecular-weight (HMW and LMW, respectively). The Lab-on-a-chip system is especially suited to on-the-spot applications, such as the regional laboratory, or even for use at the grain-receival station [41].

Examples of cereal proteins and DNA that are recently isolated and characterized by different CE methods are listed in Table 2 [43-67].

STRATEGY FOR VARIETY DISTINCTION

Techniques for the identification of cereals are important for quality control at many points in the production and utilization of grain. Genomics data now allows any cereal cultivar to be identified by comparisons of DNA sequences. DNA analysis methods based upon genomics approaches offer opportunities for any desired level of purity testing or confirmation of identity [27]. Faced with such a range of methods of identification, it is difficult to decide what the most appropriate approach is. This decision is partly based on the range of factors listed in Table 3 in relation to the constraints of the situation in which the identification must be conducted. Examples of such situation, listed in Table 3, include testing needs throughout the wheat business chain. Table 3, shows that there are relatively few possibilities for effective identification of variety at delivery, given the short time-frame that is likely to be required. There is thus an obvious need for further research to provide methods suited to this requirement [3].

CONCLUSIONS

CE has rapidly taken its place as one of the more powerful and promising techniques available for the fractionation of complex mixtures of storage proteins from cereal grains. It is versatile, rapid, easily automated, readily quantified and offers high resolution separations that can help identify genotypes and relate structure with functionality [1]. Breeding applications most obviously involve DNA micro-arrays, but also methods involving gels and protein chips to predict properties such as dough quality, based on gluten composition. Selection for other aspects of grain quality involves antibody-based kits for starch-related enzymes and for defects. At harvest, gels, capillaries and chips provide the means of checking for varietal identity as well as assisting in the prediction of dough properties. Similar test systems assist the grain buyer in selecting grain consignments that will suit the flour customers of domestic millers. Extended application to the full range of diagnostic methods to the wheat crop has the potential to further increase the value of the harvest for export purposes [8].

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Colin W. Wrigley, Food Science Australia and Wheat CRC, Sydney, Australia, for his generosity in providing constructive comments. We also thank Commission on Higher Education for providing Ph.D. scholarship.

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