Journal of Cereal Science 50 (2009) 388–391

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Rapid DNA-based identification of wheat and barley varieties

N. Tran-Dinh, M. Carole, S. Uthayakumaran, I.L. Batey, C.W. Wrigley*

CSIRO Food and Nutritional Sciences, Riverside Life Science Centre, 11 Julius Avenue, North Ryde, NSW 2113 Australia

ARTICLE INFO

Article history:
Received 16 June 2009
Received in revised form
6 August 2009
Accepted 11 August 2009

Keywords:
Micro-fluidic capillary electrophoresis
Lab-on-a-chip analysis
Grain DNA
Microsatellite analysis

ABSTRACT

Micro-fluidic capillary electrophoresis methodology was developed to analyse grain DNA composition, thus to provide unequivocal distinction between varieties of wheat (*Triticum aestivum* L.) and of barley (*Hordeum vulgare* L.). This 'Lab-on-a-chip' technology complements protein composition analysis by micro-fluidic capillary electrophoresis, which is already in routine use for variety identification. Whereas it had been difficult to distinguish between some varieties by protein analysis using the Lab-on-a-chip system, distinctions proved to be possible using a combination of DNA extraction and microsatellite analysis, taking advantage of the speed and convenience of DNA chips. Several combinations of micro-satellites permitted the DNA analysis system to provide distinction between two wheat varieties and between all but two (Chebec and Schooner) of the main eleven Australian barley varieties (Arapiles, Baudin, Barque, Chebec, Gairdner, Grimmett, Lindwall, Parwan, Schooner, Skiff and Sloop).

1. Introduction

The combination of micro-fluidics (Nguyen and Wereley, 2002) with capillary electrophoresis (Bietz and Schmalzried, 1992) has revolutionised the analysis of biological macromolecules, especially DNA, RNA and proteins. The resulting procedures are extremely rapid, while providing a high degree of resolution together with a quantitative record of the components separated. This approach to determining the composition of these macromolecules has been commercialised under the name 'Lab-on-a-chip' due to the ability of manufacturers to provide a small flat 'chip' with multiple apertures for applying sample solutions and reagents. This system has been demonstrated to be well suited to the requirements of identifying varieties of various grain species, based on analysing grain protein composition after reduction of disulfide bonds (as the fully reduced polypeptides) (Rhazi et al., 2009; Uthayakumaran et al., 2005).

The use of protein composition for this purpose is ideal, given the ease of extracting grain proteins and the proven reliability that protein composition provides for varietal identification (Bietz and Schmalzried, 1992; Lookhart and Wrigley, 1995). Furthermore, Lab-on-a-chip analysis of grain protein composition has been shown to be effective for on-the-spot identification of grain deliveries at the grain elevator or flour mill; the Lab-on-a-chip system allows capillary electrophoresis to be taken out of the laboratory and deployed under the cramped, rushed and even dirty conditions that usually accompany grain receival (Batey et al., 2007).

However, the analysis of grain protein composition has been ineffective in distinguishing between certain combinations of wheat (*Triticum aestivum* L.) varieties that are closely related. In seeking a solution to this problem, proteomics has been applied successfully (Skylas et al., 2005), thereby providing enhanced resolution of minor grain proteins that can be used for differentiation. However, proteomic mapping by two-dimensional gel electrophoresis is an exacting methodology, not suited for routine testing of multiple grain samples, even in a specialised laboratory.

Micro-fluidic capillary electrophoresis of DNA composition offers a promising alternative to analysing protein composition as it can be expected to provide greater discrimination between varieties, provided that suitable differences in base sequences can be identified. Therefore, analysis of grain DNA composition was investigated as a means of complementing grain protein analysis to provide distinction between specific combinations of varieties of both wheat and barley (*Hordeum vulgare* L.). The Australian wheat varieties Frame and Yitpi were studied as a combination that is difficult to distinguish on the basis of protein composition by micro-fluidic capillary electrophoresis.

In addition, we studied a set of eleven barley varieties currently grown in Australia. The Lab-on-a-chip analysis of protein composition has been effective in distinguishing between most (80%) of 27 Australian barley varieties. However, the protein-based procedure gave similar patterns for each of the following combinations of

* Corresponding author. Tel.: +61 2 9490 8401; fax: +61 2 9490 8499.
E-mail address: colin.wrigley@csiro.au (C.W. Wrigley).

0733-5210/$ – see front matter
doi:10.1016/j.jcs.2009.08.005

barley varieties, making these targets for developing a DNA-based procedure.

- Arapiles, Chebec, Gairdner, Schooner and Sloop
- Barque and Grimmett
- Baudin and Lindwall
- Parwan and Skiff

Therefore, analysis of grain DNA composition was investigated as a means of complementing grain protein analysis to provide distinction between varieties of wheat and barley that are difficult to distinguish on the basis of protein composition. The DNA alternative was considered to be especially attractive because the Lab-on-a-chip equipment, already in use by the industry for protein analysis, could also be deployed for DNA analysis. Furthermore, this approach to variety identification would not be affected by variations in growth and storage conditions. However, compared to protein extraction and fractionation, the extraction and amplification of DNA is a longer and more complex procedure, requiring the facilities of a well equipped laboratory.

DNA analysis may be expected to provide greater distinguishing power because a wide range of approaches and sequences may be used. Nevertheless, increased ability to distinguish may also introduce the complication that minor differences in genotype may be detected within a variety. That is, different grains (biotypes) of the same authentic variety sample may differ in DNA composition. Multiple biotypes of one variety have been described for protein analysis (Lookhart and Wrigley, 1995). The problem of multiple biotypes was already evident in the analyses of protein composition for the Australian barley varieties Franklin, Mackay and Wyalong.

Encouragement to follow the DNA line of research was provided by literature reports of successful distinction between cereal varieties, based on differences in DNA composition (Perry, 2004). Several molecular biology approaches are possible, including the analysis of Restriction Fragment Length Polymorphisms (RFLP; Kim and Ward, 1997, 2000; Paull et al., 1998; Siedler et al., 1994; Ward et al., 1998), Randomly Amplified Polymorphic DNA (RAPD; Devos and Gale, 1992; Joshi and Nguyen, 1993), Amplified Fragment Length Polymorphisms (AFLP; Barrett and Kidwell, 1998; Barrett et al., 1998), Sequence-Tagged Site markers (STS; Burkhamer et al., 1998; Chen et al., 1994) and microsatellite markers (Bryan et al., 1997; Perry, 2004; Prasad et al., 2000; Roder et al., 1995, 1998).

Microsatellite markers (Simple Sequence Length Polymorphisms (SSLP), Short Tandem Repeats (STR) or Simple Sequence Repeats (SSR)) are highly polymorphic and are PCR-based molecular marker technique. They are widely dispersed in eukaryotic, as well as prokaryotic genomes, and can be used as markers for differentiation of taxa to strain level. The use of microsatellite markers for differentiation of cereal varieties would call for a routine PCR-based technique that offers high-throughput. However, limitations might be expected; the isolation and characterisation of microsatellite markers is often labour-intensive and expensive, and optimisation of PCR protocols is time consuming. A set of seven microsatellite markers have been developed for a multiplexed PCR used to identify Canadian durum-wheat varieties (Perry, 2004). Macaulay et al. (2001) investigated a set of microsatellite markers for the differentiation of European and North American barley varieties. Following experimentation with various extraction methods and with a range of primer combinations, procedures were developed that provided effective distinctions to complement those that have proven difficult using the analysis of protein composition alone. In contrast to earlier reports, the methodology developed could be applied to the Lab-on-a-chip system, thereby providing rapid and automated DNA analysis and interpretation.

2. Material and methods

2.1. Grain sample

Authentic samples of wheat and barley varieties were obtained from the Australian Winter Cereals Collection, Tamworth, NSW. Additional commercial samples were provided by GrainCorp, an Australian grain handling company.

2.2. Equipment

Basic items of equipment included a coffee grinder to mill grain samples (although fineness of grind was not found to be critical), a vortex mixer to provide thorough mixing during extraction, and a small bench centrifuge to clarify extracts. The basic Lab-on-a-chip equipment for DNA analysis (the Agilent Bioanalyzer model 2100) is the same as was described for protein analysis (Uthayakumaran et al., 2005), except that DNA chips were used, each chip taking ten samples. The laptop computer usually placed beside it can alternatively be some other form of computer.

2.3. Genomic DNA extraction and PCR amplification

Genomic DNA was extracted from a single grain of either wheat or barley using the Qiagen DNeasy Plant Mini Kit (Qiagen, Australia) following the manufacturer's instructions. Starting material was disrupted by bead beating in a FastPrep FP120.

All microsatellite PCR amplifications were performed in an Eppendorf MasterCycler ep "S". The microsatellite marker DuPw004 (Perry, 2004) was used for differentiation of the wheat varieties Frame and Yitpi. The following primers were used for amplification of the microsatellite marker DuPw004: DuPw004.F (5'- GGT CTG GTC GGA GAA GAA GC-3') and DuPw004.R (5'- TGG GAG CGT ACG TTG TAT CC-3'). Amplification was performed with a mixture (25 μL total reaction volume) containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, dNTP (0.2 mM), forward and reverse primers (1.25 pmol each), *Taq* polymerase (0.5 U; Bioline, Australia), and 1 μL of template DNA. An initial denaturation (95 °C, 5 min) was followed by 40 cycles (95 °C, 15s; 58 °C, 30s; 72 °C, 30s). A final extension was performed at 72 °C for 5 min.

Barley variety differentiation was tested using a set of five microsatellite makers (Macaulay et al., 2001; Table 1). Amplification was performed with a mixture (25 μL total reaction volume) containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, dNTP (0.2 mM), forward and reverse primers (20 pmol each), *Taq* polymerase (0.5 U; Bioline, Australia), and 1 μL of template DNA. An initial denaturation (95 °C, 5 min) was followed by 40 cycles (95 °C, 15s; 58 °C, 30s; 72 °C, 30s). A final extension was performed at 72 °C for 5 min.

Table 1
Barley microsatellite primer sequences.

Primer	Forward primer (5'-3')	Reverse primer (5'-3')
Bmac 0156	AACCGAATGTATTCCTCTGTA	GCCAAACAACTATCGTGTAC
Bmag 0120	ATTTCATCCCAAAGGAGAC	GTCACATAGACAGTTGTCTTCC
Bmag 0135	ACGAAAGAGTTACAACGGATA	GTTTACCACAGATCTACAGGTG
HMV 03	ACACCTTCCCAGGACAATCCATTG	AGCACGCAGAGCACCGAAAAAGTC
Bmag 0173	CATTTTTGTTGGTGACGG	ATAATGGCGGGAGAGACA