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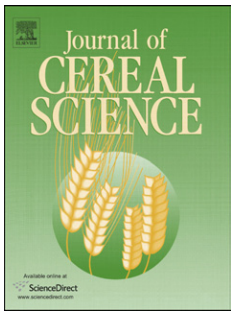

Optimization and development of capillary electrophoresis for separating and identifying wheat low molecular weight glutenin subunits

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PII: S0733-5210(12)00004-5

DOI: 10.1016/j.jcs.2011.12.005

Reference: YJCRS 1480

To appear in: *Journal of Cereal Science*

Received Date: 14 June 2011

Revised Date: 27 November 2011

Accepted Date: 13 December 2011

Please cite this article as: Li, J., Wang, S., Yu, Z., Li, X., Guo, G., Feng, S., Ma, W., Yan, Y., Optimization and development of capillary electrophoresis for separating and identifying wheat low molecular weight glutenin subunits, *Journal of Cereal Science* (2012), doi: 10.1016/j.jcs.2011.12.005

1 **Research Note**

2 Optimization and development of capillary electrophoresis 3 for separating and identifying wheat low molecular weight 4 glutenin subunits

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Since the 1990s, capillary electrophoresis (CE) has been used for protein separation and appeared to be a powerful tool for wheat storage protein analysis (Bietz and Schmalzried, 1995). CE has been successfully used to separate and characterize wheat seed proteins such as water-soluble proteins (Bean and Tilley, 2003), gliadins (Yan et al., 2003; Lookhart and Bean, 1996) and HMW-GS (Yan et al., 2004a,b; Zhang et al., 2008; Gao et al., 2010). It has been a versatile and robust tool for fast, efficient and automated separations and requires small sample volume and a low consumption of solvents (Miguel et al., 2010). Weegels (1995) developed a method for the separation of high molecular weight (HMW-) and low molecular weight (LMW-) glutenin subunits with CE back in the 1990's. However, until now, CE based identification of LMW-GS alleles has not been reported. In this work, we optimized and developed a rapid CE separation method for LMW-GS allele identification.

Seven Chinese bread wheat cultivars were used in this study, including three superior gluten quality ones, Jimai-20, Xiaoyan-6 and Zhongyou-9507, and four poor quality cultivars, Zhoumai-16, Jingdong-8, Jing-411, and CB037. All samples were obtained from the Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS). Two CB037 LMW-GS near-isogenic lines (NILs) including CB037-1 with *Glu-A3c*, *Glu-B3h,* and *Glu-D3d,* and CB037-2 with *Glu-A3c*, *Glu-B3null*, and *Glu-D3d*, were used in this study. Another two LMW-GS NILs, Ari124-3 with *Glu-A3c*, *Glu-B3d*, and *Glu-D3c*, and Ari127-6 with *Glu-A3c*, *Glu-B3g*, and *Glu-D3c*, obtained from the Institute of Crop Science, CAAS, were used as controls. Two durum wheat LMW-GS variants Lira 42 with LMW-1 type subunit and Lira 45 with

LMW-2 type subunit were kindly provided by Prof. Lafiandra, University of Tuscia, Italy. The Chinese wheat variety Chinese Spring was obtained from China Agricultural University. The glutenin proteins were extracted based on protocols outlined by Melas *et al.* (1994) and Zhang *et al.* (2008). A Beckman P/ACE 800 system with Gold Software for system control and data acquisition was used to separate LMW-GS. After a series of optimizing procedures, a rapid, high resolution and reproducible CE separation for LMW-GS were obtained by using 40.2 cm length (30 cm to detector) and 50 mm ID capillary at 38 ^{o}C and 11.0 kV under 0.1 M phosphate-glycine buffer (pH 2.5) containing 20% (v/v) acetonitrile and 0.05% (w/v) hydroxypropylmethyl. Generally, the separation of one sample can be completed in less than 20 min.

In order to obtain pure LMW-GS samples, different precipitation methods for the total extracted glutenins were tested. Results showed that 40% and 80% (v/v) cold acetone (-20^{o}C) can precipitate HMW-GS and most of the LMW-GS from the extracted glutenin subunits, respectively. Figure 1 shows three CE separation patterns of glutenin proteins from Chinese Spring precipitated using different concentrations of cold acetone (-20^{o}C), corresponding to LMW-GS (a), HMW-GS (b) and total glutenins (c). All samples were separated by CE for 20 min with the same electrophoresis conditions. Apparently, clear CE separation patterns were obtained for LMW-GS eluted at about 12-18 min without HMW-GS contamination after the two-step with cold acetone precipitation, in which 40% (v/v) cold acetone removed HMW-GS, and then 80% (v/v) cold acetone precipitated the supernatant which is

formed by LMW-GS (Melas *et al.*, 1994) and Zhang *et al.* (2008). Results demonstrated that no HMW-GS peaks were detected after adding two steps of cold (-20^{o}C) acetone (Figure 1), but the elution time of HMW-GS and LMW-GS were both changed. The elution time of LMW-GS (Figure 1a) was higher than that of HMW-GS (Figure 1b). Similarly, the elution time of HMW-GS was also slightly higher in total glutenin separation (Figure 1c) compared with the purified HMW-GS CE pattern (Figure 1b). Results indicated that the migration shift significantly increases the separation resolution of LMW-GS.

The CE separation results of LMW-GS from CB037 NILs and controls (Ari124-3, Ari127-6 and CS) are shown in Figure 2. It is obvious that all LMW-GS were well separated and their allele compositions at *Glu-A3*, *Glu-B3* and *Glu-D3* loci could be readily identified by specific peaks. Several typical LMW-GS alleles, such as *Glu-A3c*, *Glu-A3a*, *Glu-B3a*, *Glu-B3d*, *Glu-B3g*, *Glu-B3h*, *Glu-D3a*, *Glu-D3c* and *Glu-D3d*, displayed clearly different CE patterns, and thus can be easily discriminated. In particular, a characteristic peak at about 15 min for *Glu-B3h* was present in CB037 and CB037-2, but absent in CB037-1 (Figure 2).

In durum wheat, LMW-GS especially those associated at the *Glu-B3* locus have shown to be important for end-use quality. The best pasta-making characteristics are associated with the presence of the LMW-2 type while the LMW-1 type has a negative effect on pasta quality. The LMW-2 type subunits also exert a positive effect on gluten strength when present in hexaploid tritordeum, an amphiploid of hybridation between *Triticum durum* and *Hordeum chilense*. In order to fast identify

these two types of LMW-GS, both SDS-PAGE and the optimized CE were used to separate the LMW-1 type in Lira 42 and the LMW-2 type in Lira 45 and Simeto (Figure 3). Results revealed that, in addition to the same component (band 1 and peak 1), the LMW-2 type in both Lira 45 and Simeto had specific bands (Figure 3A) and CE peaks 2 and 5 (Figure 3B) while Lira 42 with the LMW-1 type contained characteristic bands and peaks 3 and 4. These characteristics could be used as reliable biochemical markers for rapid identification and screening superior quality LMW-2 type subunits in wheat quality improvement.

The reported protocol can clearly separate LMW-GS subunits. Characteristic peaks were also identified for most alleles and LMW-1 & 2 types, making it possible to rapidly identify LMW-GS alleles and types.

Acknowledgements

This research was financially supported by grants from the National Natural Science Foundation of China (30830072), the Chinese Ministry of Science and Technology (2009CB118303) and Key Projects of National Plant Transgenic Genes of China (2008ZX08002-004 and 2009ZX08002-017B).

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Figure legends:

Figure 1. Optimization of Sample Extraction

SDS-PAGE and CE separation patterns of glutenin subunits of Chinese Spring by different extraction methods. a: LMW-GS: 80% cold acetone (-20^{o}C) precipitation after removing HMW-GS (40% cold acetone precipitation of HMW-GS). b: HMW-GS: 40% cold (-20 ^{o}C) acetone precipitation. c: LMW-GS + HMW-GS: 80% cold (-20^{o}C) acetone precipitation.

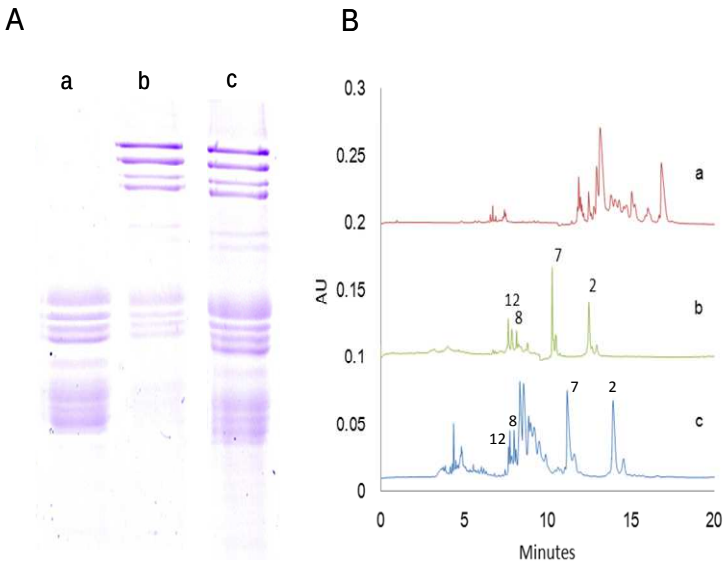
"c" represents the total glutenins not alkylated with 4vp while "a" represents LMWGS alkylated with 4vp. Comparison between the two patterns shows that 4vp causes slower migration of LMWGS but higher resolution of peaks.

Figure 2. Identification of LMW-GS alleles from wheat variety CB037 and its near-isogenic line (NIL) by CE.

1. Ari124-3 (*Glu-A3c*, *Glu-B3d*, *Glu-D3c*); 2. Ari127-6 (*Glu-A3c*, *Glu-B3g*, *Glu-D3c*); 3 Chinese Spring (*Glu-A3a*, *Glu-B3a*, *Glu-D3a*), 4. CB037 (*Glu-A3c*, *Glu-B3h*, *Glu-D3d*), 5. CB037-1 (*Glu-A3c*, *Glu-B3null*, *Glu-D3d*), 6. CB037-2 (*Glu-A3c*, *Glu-B3h*, *Glu-D3d*).

Figure 3. Identification of LMW-GS from 3 durum wheat cultivars by SDS-PAGE and CE. A: SDS-PAGE. B: CE separation of LMW-GS from Lira 45 (LMW-2), Lira 42 (LMW-1) and Simeto (LMW-2).

155 **Fig. 1**



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158 **Fig. 2**

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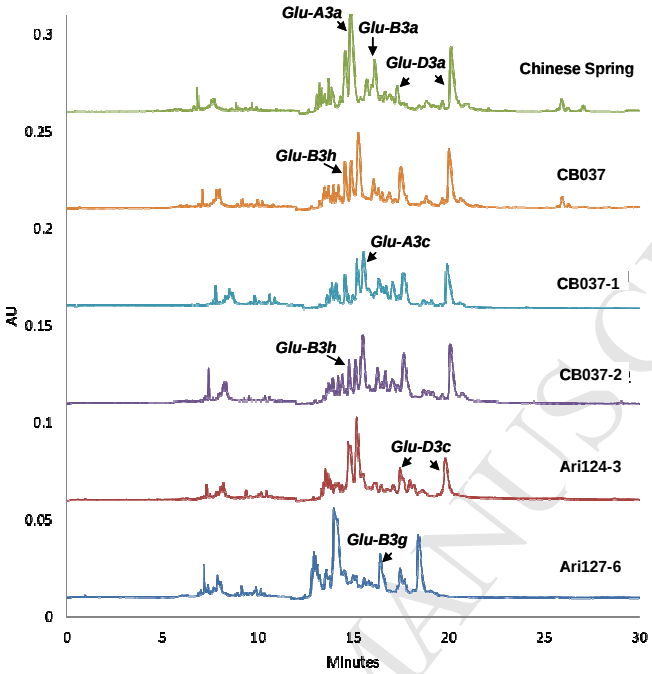

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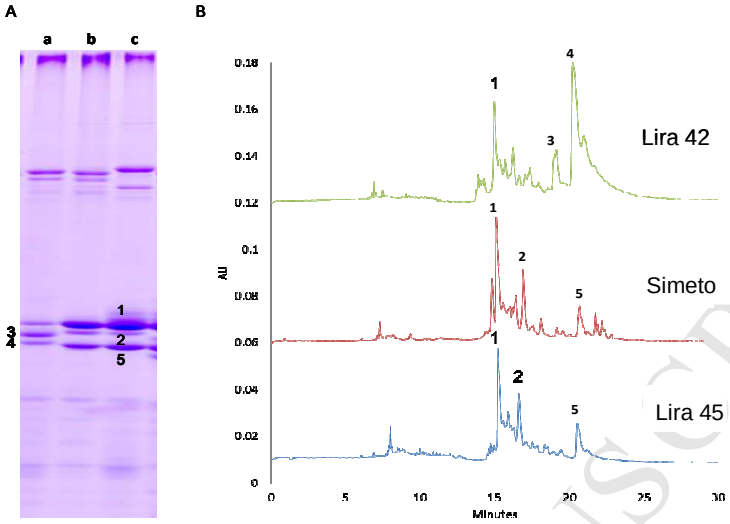
Fig. 3



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- We focus on capillary electrophoresis (CE) procedure,
- For the first time, CE procedure is successfully used for wheat LMWGS analysis;
- The CE analysis of LMWGS is used for wheat cultivar identification;
- We demonstrated the application of this procedure for wheat quality improvement;
- The CE method can be used as a new method complimentary to the existing ones.