

Capillary Electrophoresis of Gliadins as a Tool in the Discrimination and Characterization of Hulled Wheats (*Triticum dicoccon* Schrank and *T. spelta* L.)

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ABSTRACT

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Twenty-two lines of emmer (*T. dicoccon* Schrank) and 10 of spelt (*T. spelta* L.) were analyzed using capillary electrophoresis for their gliadins. These proteins were separated on an uncoated fused-silica capillary (30 cm long, 22 cm to detector, 50 μm i.d.) using the isoelectric buffer 40 mM aspartic acid, 4M urea, 0.5% (w/v) HEC, and 20% (v/v) acetonitrile. Samples were run for 20 min at 22kV and 42°C. By using these conditions, gliadins were separated into 21–30 components (peaks and shoulders). The major peaks eluted between 4.5 and 8.5 min. Elec-

trophoregrams of tested lines showed qualitative and quantitative differences, including number of peaks, presence or absence of some major peaks, and areas of peaks. Lines belonging to the same species can be discriminated mainly on the basis of β - and ω -gliadin patterns. The γ - and ω -gliadins seem to be more useful in the differentiation of emmer from spelt. The comparison of electrophoregrams relative to hulled and unhulled species evidenced the high similarity between species with the same genome composition (durum wheat-emmer, and common wheat-spelt).

The hulled wheats *Triticum monococcum* L. (einkorn), *T. dicoccon* Schrank (emmer), and *T. spelta* L. (spelt) are among the oldest domesticated species. They have been staple crops for millennia (Feldman 1976). The progressive decline of these ancient wheats is strictly related to the diffusion of the modern free-threshing wheat species (Nesbitt and Samuel 1995). Nowadays, the hulled wheats cultivation is minimal and usually limited to marginal areas and subsistence farming systems. Recently, interest in these ancient wheats has increased. This is due to the adaptability to poor soils (Laghetti et al 1999), attractive nutritional attributes (Abdel-Aal et al 1998; Piergiovanni 1999), potential therapeutic properties (Strehlow and Herzka 1988), preparation of alternative and new foods (D'Antuono and Bravi 1996) and source of useful genes (Radic et al 1997). However, further investigations are needed for a complete knowledge of all these topics.

Several studies established that the end-use of one cultivar primarily depends on its technological quality which, in turn, appears to depend on the quantity and quality of gluten proteins present in the endosperm (Finney et al 1982). Gliadins comprise about half of the gluten proteins of wheat. They are a group of more than 50 proteins that share structural homology and similar physico-chemical properties. The significance of gliadins and their subgroups to the functionality of wheat flour has been the subject of considerable debate. Some researchers suggested that gliadins control the loaf volume potential (Khatkar et al 2002). Moreover, the study of these proteins is important to solve the open question of the safety of hulled wheats for celiac patients (Auricchio et al 1982; Lahdeaho et al 1995).

Because of the high heterogeneity of gliadins, powerful separation techniques are needed to their characterization. Several methods have been developed over the years, currently various types of chromatographic and electrophoretic techniques are used routinely. The most discriminating chromatographic technique is reverse-phase HPLC (RP-HPLC) (Wieser et al 1994a). However, electrophoretic techniques have remained the most popular procedures, acid-PAGE, and SDS-PAGE are the established methods. One of the newest electrophoretic techniques to be applied to the study of cereal storage proteins is capillary electrophoresis (CE) (Bean et al 1998). Several methods for successfully separating wheat gliadins have been developed (Bietz and Schmalzried 1995;

Lookhart and Bean 1995). CE usefulness in the identification and differentiation of wheat cultivars through the study of gliadin profile was also described (Werner et al 1994; Yan et al 1999; Bean and Lookhart 2000; Siriamornpun et al 2001).

The complex loci coding for gliadin proteins in hulled wheats species are closely homologous to those of bread and common wheat and only minor differences were observed in the electrophoretic profile among hulled and unhulled species (Harsch et al 1997). At the present, the genetic diversity of gliadins within and among hulled wheat species has been little investigated. The available data are scarce and based only on acid-PAGE (Metakovsky and Baboev 1992; Galterio et al 1994; Piergiovanni and Blanco 1999). Galterio et al (1994), analyzing three Italian emmer populations, observed an appreciable variation of gliadins and identified several new alleles attributed to the *Gli-A1*, *Gli-B1*, and *Gli-A2* loci. Piergiovanni and Blanco (1999), studying the γ -gliadin fraction in emmer and spelt samples, observed three patterns in spelt and no variation in emmer.

The aim of this work was to investigate the potential of capillary electrophoresis in the study of α -, β -, γ -, and ω -gliadins variation in hulled wheats species. The aptitude of CE in the varietal identification was also explored.

MATERIALS AND METHODS

Thirty-two samples, 22 of emmer (*T. dicoccon* Schrank), and 10 of spelt (*T. spelta* L.) were analyzed (Table I). The samples are homogeneous lines developed from the germplasm hulled wheat collection held at Germplasm Institute-CNR (Bari, Italy). Selection was mainly for agronomic traits (Piergiovanni et al 1996; Laghetti et al 1999). Farvento and Lucanica are emmer cultivars developed during the same selection (Perrino et al 1996). Commercial cultivars of *T.aestivum* L. (Autonomia and Manital) and *T.durum* Desf. (Norba, Simeto, and Venusia) were included as controls.

Gliadin extraction. Gliadins were extracted from whole flour (60 mg) with cold 70% (v/v) aqueous ethanol (500 μL) on the vortex for 30 min at room temperature. Samples were centrifuged for 15 min at 11,000 $\times g$ and 20°C. The supernatant was used for the capillary electrophoretic analysis. The albumins and globulins present in the supernatant were those extracted in 70% aqueous ethanol. Each flour sample was extracted in duplicate and analyzed on the day of extraction.

Capillary zone electrophoresis (CZE). Beckman P/ACE MDQ equipment (Beckman Coulter, Fullerton, CA) was used to separate gliadin extracts. Separations were achieved using uncoated fused-silica capillaries (30 cm long, 22 cm to detector, 50 μm i.d.). The separation buffer was 40 mM aspartic acid, 4M urea, 0.5% (w/v)

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HEC (M_v ca. 90,000) and 20% (v/v) acetonitrile (Capelli et al 1998). Samples were run for 20 min at 22kV and 42°C. Extracts were pressure injected at 0.6 psi for 5 sec and separated proteins were detected by UV absorbance at 214 nm. According to Capelli et al (1998), the capillary was rinsed only with separation buffer between the runs. An acceptable run-to-run reproducibility was obtained under the described conditions. The overall variation of migration time was 4% measured on the highest peak of γ -fraction. The overall difference in peak areas was never greater than $\pm 2\%$. Beckman Karat 32 software was used for the acquisition and elaboration of electrophoregrams. A-PAGE patterns of wheat controls were used for the determination of the migration time relative to each gliadin fraction in the electrophoregrams.

RESULTS

Previous studies showed the advantages of using acidic isoelectric buffers in the analysis of storage proteins from wheat, oats, rice, barley, and rye (Capelli et al 1998; Bean and Lookhart 2000). In this study, the separation of hulled wheat gliadins was performed in an isoelectric buffer based on aspartic acid. Under the conditions of analysis, an excellent resolution of gliadin fractions and short separation times were obtained. From 21 to 30 peaks were resolved in <15 min. Albumins and globulins present in the ethanolic extracts migrated the earliest (2–4 min), while all the major gliadin peaks eluted between 4.5 and 8.5 min. Gliadins are usually divided into four fractions α -, β -, γ - and ω -based on decreasing electrophoretic mobility in A-PAGE. Lookhart and Bean (1995) showed that the migration order of gliadin fractions in CE was the same as in A-PAGE.

The α -gliadins move the fastest, in this study between 4.5 and 5.5 min. They are followed by the β -gliadins (5.5–7 min), γ -gliadins (7–8 min) and ω -gliadins (>8.5 min). With respect to the pattern, both qualitative and quantitative differences, including

presence or absence, number, and height of peaks, were detected among the tested lines.

Emmer

The α -gliadin fraction appeared as an apparent quadruplet in 16 lines. The detailed analysis of this region revealed the existence of differences with a high individual character, a useful tool for distinguishing the lines from each other. First of all, the quantity of each α -gliadin peptide, derived from the area beneath the peaks, differed from line to line. For example, the first peak of a quadruplet (see arrows in Fig. 1) appeared as a very small shoulder in the electrophoregram of MG 5293/2, while it is a clear peak in the lines MG 5400/2 and MG 5400/5. The analysis of the shape of peaks belonging to the quadruplet revealed the presence of shoulders (11 lines showed this pattern) mainly associated to the second or third peak (Fig. 1, lines MG 5400/2 and MG 5400/5). The quadruplet was split in two couples of peaks, the second peak was replaced by a new one with the lowest migration time in two lines (MG 5281/1 and MG 5302/1). Finally, α -gliadins appeared as a cluster of peaks (5–7), in the lines Lucanica, Farvento, MG 4378/1, MG 5281/1A, MG 5282/2, MG 5380/1, and PLV 4378. These results suggest that more than four peptides usually constitute the α -fraction, though the quantity of some peptides was frequently so low that they appear as shoulders.

The β -gliadin fraction consisted of several peaks whose number and area varied remarkably from line to line. Ten peaks and shoulders (Fig. 2, migration time 5.5–7min) were observed in Lucanica, while only five peaks were detected in PLV 30832. The lack of or very low expression of the peptides migrating immediately after the α -fraction was observed in eight lines (Fig. 2, line PLV 30832). Farvento and MG 5302/1 did not show significant peaks immediately before the γ -fraction. Peaks with large areas belonging to the β -gliadins were observed in 10 lines. The over-expressed peptide (see arrows in Fig. 3) varied from line to line. The posi-

TABLE I

Identification Code and Cultivar Name of Analyzed Hulled Wheat Lines

<i>T. dicoccon</i>				
MG 4378/1	MG 5281/1	MG 5281/1A	MG 5282/2	MG 5285/1
MG 5285/7	MG 5293/2	MG 5302/1	MG 5302/4	MG 5333/1
MG 5380/1	MG 5400/2	MG 5400/5	MG 5507	MG 27201/4
MG 30782	MG 30833/2	MG 30835/1	PLV 4378	PLV 30832
Lucanica	Farvento			
<i>T. spelta</i>				
MG 4451/1	MG 5285/3	MG 5320/2	MG 15398/1	MG 15347/1
MG 15433/1	MG 15451/1	MG 15577/1	MG 27182/4	MG 27201/1

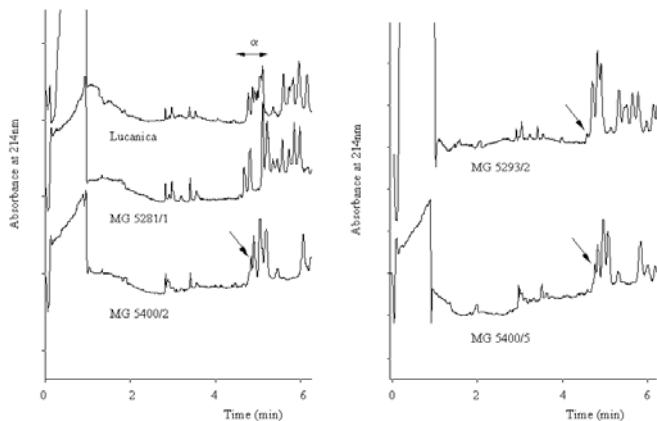


Fig. 1. Representative α -gliadin patterns observed in emmer lines. Arrow indicates characteristic peaks. Separation buffer was 40 mM aspartic acid, 4M urea, 0.5% HEC, and 20% acetonitrile. Separation conditions: 50 μ m i.d. \times 30 cm long (22 cm to detector) uncoated capillary at 22kV and 42°C.

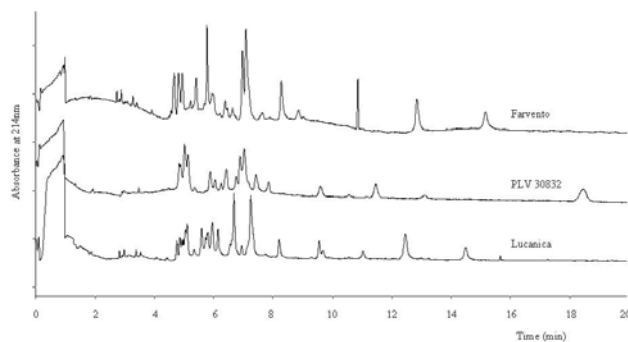


Fig. 2. Capillary electrophoretic pattern of gliadins extracted from emmer lines Farvento, PLV 30832, and Lucanica. Separation conditions as in Fig. 1.

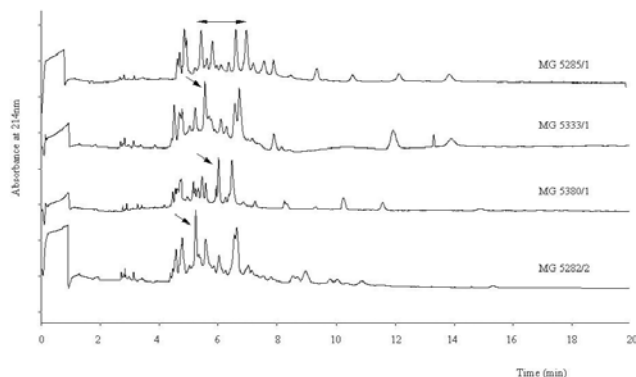


Fig. 3. Capillary electrophoretic pattern of gliadins extracted from emmer lines MG 5285/1, MG 5333/1, MG 5380/1, and MG 5282/2. Arrows indicate some characteristic peaks. Separation conditions as in Fig. 1.

tion in the electrophoregrams show them close to the α -gliadins in MG 5282/2 and MG 5507; close to the γ -fraction in MG 5293/2, MG 5380/1, and Lucanica; about in the middle of the β -fraction in MG 5333/1 and Farvento. More than one peak with large area was detected in the lines MG 5281/1, MG 5285/1, and MG 5281/1A.

The lowest variation was associated with the γ -fraction. The most frequent pattern, detected in 12 lines, consisted of three peaks sometimes partially overlapped; the third was prominent (Fig. 4). The lack of the first peak of this triplet was detected in the lines Lucanica and Farvento (Fig. 2), MG 5281/1, MG 5282/2, MG 5333/1, MG 5507, and MG 30782. These were almost the same lines showing peaks with large area in the β -fraction. Only one peak attributable to γ -gliadins was observed in MG 5285/1 (Fig. 3) and MG 5281/1A which, in turn, showed an over-expression of β -gliadins.

According to the mobility in A-PAGE, ω -gliadins are usually divided in two subgroups, $\omega 5$ and $\omega 1,2$ (slow and fast, respectively). In the present study, peptides belonging to ω -fraction were detected on a broad range of migration time. The ω -gliadins showed, along with the β -fraction, a very high individual character. An appreciable variation of numbers, migration times, and area of peaks was observed. Intense peaks appeared in the electrophoregram of MG 5302/1 (Fig. 4), MG 5281/1, and MG 5285/7; a series of small peaks, distributed on a broad range of migration time, was detected in MG 5282/2; peaks with very high migration time (>14 min), were observed in several lines (Figs. 2 and 3).

Spelt

A quadruplet of peaks was observed also for the α -gliadins of spelt (migration time 4.5–5.5), but its structure did not resemble that of emmer. Only the lines MG 4451/1 and MG 15577/1 showed a quadruplet similar to that described for emmer. The first peak of a quadruplet appeared isolated in the lines MG 15398/1 (see arrow in Fig. 5), MG 15433/1, MG 15451/1, and MG 27182/4. A cluster of peaks (5–8) was detected in the other lines (Fig. 5, lines

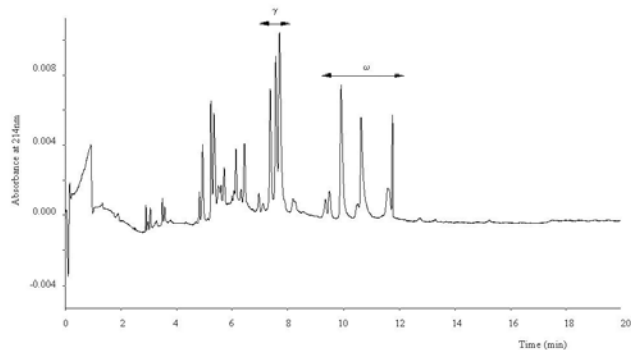


Fig. 4. Capillary electrophoretic pattern of gliadins extracted from emmer line MG 5302/1. Separation conditions as in Fig. 1.

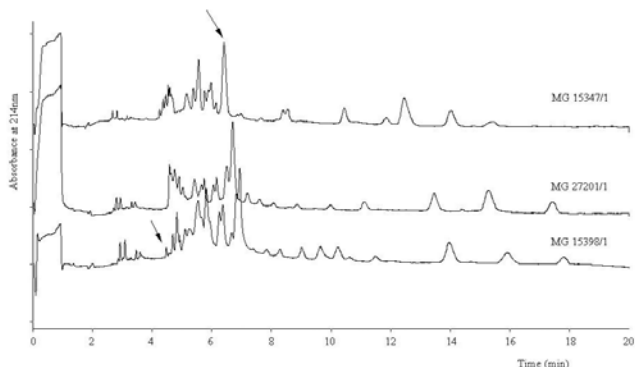


Fig. 5. Capillary electrophoretic pattern of gliadins extracted from spelt lines MG 15347/1, MG 27201/1, and MG 15398/1. Arrows indicate characteristic peaks. Separation conditions as in Fig. 1.

MG 15347/1 and MG 27201/1). Distinctive traits of the cluster were the height of first peak; the line MG 15347/1 was the exception, and the lack of the first peptide detected in the lines MG 15398/1 (see arrow in Fig. 5), MG 15433/1, MG 15451/1, and MG 27182/4.

As in emmer, the β -fraction showed individual characters. The tested lines can be divided in two subgroups. The first group consists of six lines characterized by peaks with large areas belonging to the β -fraction (Fig. 5, lines MG 15347/1 and MG 15398/1), the second group consists of lines without this kind of peak (Fig. 5, line MG 27201/1). The over-expressed peptides showed a lower variation respect to emmer being placed only in the middle of β -fraction. In the contrast to emmer, any relationship seems to exist between the over-expression of peptides belonging to the β -fraction and the pattern of γ -gliadins.

A very low variation of the γ -fraction was observed also in spelt. One peak with large area was observed in the electrophoregram of eight lines (see arrow Fig. 5, line MG 15347/1). Generally, very small peaks immediately before or after this peak were detected. The peak immediately before was relevant only in MG 5320/2. Finally, a triplet of peaks similar to that frequent in emmer was observed in MG 15398/1 (Fig. 5).

The ω -gliadin pattern was, as expected, different between tetraploid and hexaploid hulled wheats. Spelt ω -fraction was characterized by a higher number of peaks distributed almost over the entire range of investigated migration time (Fig. 5). Moreover, the peaks attributable to the slow ω -type represented a significant fraction (15–20% of the area). As for emmer, ω -gliadins greatly differed from line to line; as a consequence they can be useful in varietal identification.

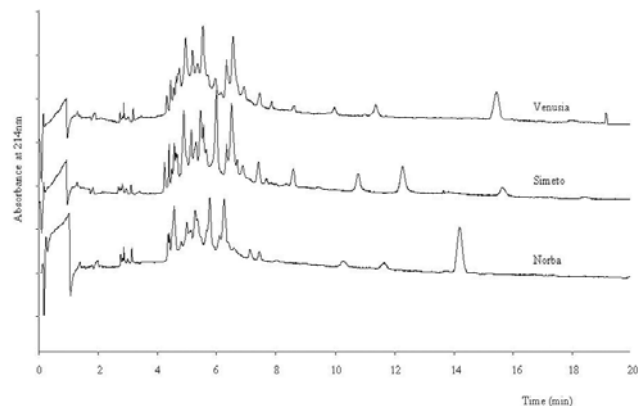


Fig. 6. Capillary electrophoretic pattern of gliadins extracted from durum wheat cultivars Norba, Simeto, and Venusia. Separation conditions as in Fig. 1.

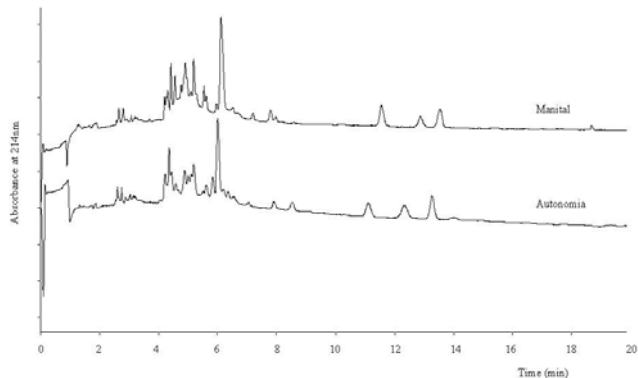


Fig. 7. Capillary electrophoretic pattern of gliadins extracted from common wheat cultivars Autonomia and Manital. Separation conditions as in Fig. 1.

DISCUSSION

The advantages of CE in gliadin analysis are well established (Lookhart and Bean 1995; Capelli et al 1998), but the aptitude of this technique to discriminate different cultivars is still not sufficiently investigated (Werner et al 1994; Yan et al 1999; Bean and Lookhart 2000; Siriamornpun et al 2001). The present work shows that CE allows to evidence major and minor qualitative as well as quantitative differences among lines. The elaboration of this information makes the technique suitable for the varietal identification as well as for the characterization of each sample. The potential of CE was emphasized by the absence of identical electrophoregrams among the 32 lines analyzed in the present study.

Bean and Lookhart (2000) reported that wheat cultivars could be differentiated using the main body of gliadin fraction. The present study shows that lines belonging to the same hulled wheat species can be differentiated mainly comparing the pattern of β - and ω -gliadins. γ - and ω -gliadins seems to be more useful in the discrimination of emmer from spelt. CE of gliadins is useless in the discrimination of hulled and unhulled species. The comparison of the electrophoregrams relative to durum (Fig. 6) and common wheat cultivars (Fig. 7) evidences the high similarity between species with the same genome composition (durum wheat and emmer; common wheat and spelt).

Qualitative and quantitative differences of gliadin fractions among wheat cultivars were related to the technological properties of flour (Khatkar et al 2002). Wieser (2000), using RP-HPLC on the gliadins from some wheat species, found that an α -fraction dominated in hulled wheats. The author grouped the α - and β -fractions in the same class simply designed α . This convention derived from the high similarity among the sequences of the proteins belonging to α - and β -fractions that, in the opinion of some authors, does not justify their separation in two classes. When α - and β -fractions were grouped together, the area observed in this study covered a broad range in emmer (29–61%, mean value 44.2%) and in spelt (34–53%, mean value 43.4%). This broad variation suggests that different technological properties will be associated with the tested lines. Similar quantities of (α - + β -), γ -, and ω -fractions such as MG 5302/1 (area percentage 29:35:36), and lines characterized by dissimilar composition such as Lucanica (area percentage 60:18:22) or MG 5400/5 (area percentage 32:27:41). According to Wieser (2000), the area percentage of (α - + β -) fractions was ever superior to that of γ -gliadin, exceptions were the lines MG 5302/1 and MG 5302/4 both derived from the same germplasm accession (MG 5302).

According to Piergiovanni and Blanco (1999), the γ -gliadins showed a very low variation both within and among the tested hulled wheats. The lack of one or two peaks in the γ -fraction was coupled in emmer to the detection of peaks with large area belonging to β -fraction. It is possible to speculate that a relationship exists among the genes coding for these peptides.

ω -Gliadins are a minor component of the alcohol-soluble proteins. A strong dependence of ω -fraction by genotype exists for common wheat (Wieser et al 1994b). Using RP-HPLC, Seilmeier et al (2001) studied the gliadins from one emmer cultivar and the spelt cultivar Schwabenkorn. They observed that the proportion and the pattern of ω -gliadins were strongly determined by the species and, within each species, by the genotype. The results of the present study agreed with this result. Qualitative differences in the ω -fraction affect both the technological properties of flour and the amino acid composition of gliadins. Branland and Felix (1994) suggested that *Gli-1* variants would be more profitable than the *Glu-3* variants to improve dough extensibility and dough swelling. Previous studies (Seilmeier et al 2001) on wheat showed that proteins belonging to the ω -subgroups significantly differed in the amino acid composition. The $\omega 5$ -subgroup is characterized by a high proportion of Gln, Pro, Phe, and its proteins contain more Ile than Leu. The $\omega 1,2$ -subgroup in comparison to the $\omega 5$ -fraction has

less Glu, Phe, and Ile, but much more Pro and Leu. The presence of both ω -subgroups in emmer was reported by Kasarda et al (1983). Conversely, Seilmeier et al (2001) did not observed peaks attributable to the $\omega 1,2$ -subgroup. The low number of samples analyzed by these authors, as well as the high variation of ω -fraction observed in this study, could justify this disagreement.

CONCLUSIONS

The potential of hulled wheats does not reside in their capacity to mimic attributes that are already present in the commercial cultivars of wheat, but in their specific characteristics. The gliadin variation observed in this study was that both emmer and spelt are a rich source of genome diversity for breeders. This is very important because a recent study on gliadin polymorphism in Italian durum wheat cultivars showed that many of them have identical electrophoretic pattern (Kudryavtsev et al 1996). The short time of CE analysis strongly suggests the use of this technique in the screening of large collections. Familiarity with the traits of hulled wheat germplasm is fundamental for its utilization.

The present study also shows the potential of CE of gliadins in the discrimination and characterization of hulled wheat lines. The presented electrophoregrams show the most divergent zones between species with different genome (hexaploid or tetraploid). The β - and ω -gliadins produced the most useful fractions in the line discrimination within each species. Moreover, CE, providing both qualitative and quantitative information about each gliadin fraction, could be useful for preliminary estimations of technological performances of each line.

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