Electrophoretic Characterization of Spring Spelt Wheat Gliadins

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Gliadin compositions of 95 spring spelts, 5 winter spelts, and Neepawa Hard Red Spring (HRS) wheat were evaluated by acid−polyacrylamide gel electrophoresis (A-PAGE). Five spring spelts grown in three environments over 2 years were compared to the HRS cultivar Katepwa using A-PAGE and sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE). On the basis of the scoring of 13 bands, the spring and winter spelts diverged widely from Neepawa HRS wheat. None of the spring spelts evaluated had a gliadin protein profile identical to that of commercially available winter spelt. Spring and winter spelts were differentiated from Katepwa HRS wheat based on ω-gliadin composition, in which common wheat was characterized by the presence of a slow-moving ω-gliadin and a strong-staining fast-moving ω-gliadin. Common wheat and spring spelt accessions SK0505, SK0263, and RL5407 (black-hulled spelts) had a distinct strong band in the γ-gliadin region with MW of about 64K which was absent in winter spelts and the spring spelts SK0021 and PGR8801 (white-hulled spelts). The molecular weights of gliadin proteins were in the range of 34K−75K.

Keywords: *Spelt; wheat; gliadin; electrophoresis; A-PAGE; SDS-PAGE, NTSYS-PC*

INTRODUCTION

Wheat products are widely consumed, but a small proportion of individuals cannot tolerate wheat proteins. Glutenins and gliadins are recognized as the major wheat storage proteins, constituting about 80−85% of the total grain proteins with a ratio of about 1:1 in common or bread wheat. Glutenin proteins are divided into high molecular weight (HMW) (100−140K) and low molecular weight (LMW) (30K−50K) protein subunits by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) (Bietz and Simpson, 1992), while gliadins are categorized into α-, β-, γ-, and ω-gliadins based on their mobility on acid−polyacrylamide gel electrophoresis (A-PAGE) (Wall, 1979) with a molecular weight range of 44K−78K as determined by SDS-PAGE (Tatham and Shewry, 1990). These storage proteins have an important role in protein quality that affect wheat end-use product qualities (Miflin et al., 1983). For instance, HMW glutenins and ω-gliadins encoded by Glu-1 and Gli-1 loci, respectively, are associated with strong breadmaking quality (Payne, 1987). But α-gliadins coded by the Gli-2 locus (Payne, 1987) are thought to be responsible for gluten intolerance (Skerritt et al., 1990). MacRitchie et al. (1991) also investigated wheat protein composition in relation to breadmaking quality, indicating that dough strength increased as the proportion of HWM glutenin subunits in the parent gluten increased.

A-PAGE and SDS-PAGE have been used frequently to fractionate wheat proteins for identification of genotypes, establishment of chromosomal control, selection of desirable wheat lines in breeding programs, and/or the correlation of individual proteins with end-use quality. Recently, reversed-phase high-performance liquid chromatography (RP-HPLC) (Bietz, 1983; Courcoux et al., 1992) and capillary electrophoresis (Bietz and Schmalzried, 1995; Werner et al., 1994) have also been used for analyzing wheat storage proteins. Bietz and Simpson (1992) reviewed the electrophoretic and chromatographic procedures that have been applied to fractionate, characterize, and identify wheat proteins.

In the present study, A-PAGE and SDS-PAGE methods were used to characterize the gliadins in experimental spring and commercial winter spelt wheats, in comparison with Katepwa and Neepawa Hard Red Spring (HRS) wheats. Because winter spelt grain is being marketed as being less allergenic than conventional wheat and is currently available in health food stores for that purpose, the main objective of this study was to determine whether the gliadin profiles of HRS wheat, spring spelt genotypes, and those of winter spelts differ.

MATERIALS AND METHODS

Wheat Samples. Ninety accessions of spring spelt (*Triticum aestivum* subsp. *spelta* [L.] Thell) obtained from the USDA collection at Aberdeen, ID, were grown in unreplicated plots at Saskatoon in 1991. In additions, accessions designated as SK0021, SK0505, SK0263, RL5407, and PGR8801 obtained from the Agri and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, were grown at Saskatoon, Kernen, and Elrose in central Saskatchewan in 1992 and 1993 along with Katepwa HRS wheats (*T. aestivum* subsp. *vulgare* [Vill. Host] Mackey), as checks. All spelt accessions were selected on the basis of their ability to mature at Saskatoon when spring-sown. The five spelt wheat accessions were grown in a three-replicate randomized complete block experiment. Six samples of winter spelt were obtained from several sources, including one sample from the retail market in Saskatoon, SK, one sample from Nunweiler's Flour Co., Penticton, BC (both referred to as common winter spelt), and four samples (Lueg, Hubel, Oberkulm, and Ostro cultivars) from the Swiss Federal Research Station for Agronomy, Zurich, Switzerland. The winter spelts were grown in unreplicated plots at Saskatoon during the winter of 1993/1994. Spelt samples were dehulled by passing the spikelets between rubber-coated rolls and by

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Table 1. Protein and Gliadin Contents of Spelt and Common Wheats Grown at Three Sites in 1992 and 1993 (% Dry Basis)[a]

wheat subspecies	no. of samples[b]	protein (N × 5.7) mean	range	SE	gliadin (N × 5.7) mean	range	SE
winter spelt	6	17.4a	13.6−19.1	0.5	6.4a	5.1−7.1	0.2
spring spelt	30	16.5b	12.9−18.7	0.2	5.9b	4.9−6.9	0.1
common wheat	6	15.4c	13.2−17.1	0.3	5.3c	4.9−5.9	0.1

[a] Means with the same letters are not significantly different at $p < 0.05$. [b] Each sample was analyzed in duplicate.

aspirating to remove the hulls. The dehulled grain was then ground to pass a 1 mm screen on an Udy Cyclone sample mill (Udy Co., Fort Collins, CO).

Analytical Tests. The meals were analyzed by standard AACC procedures (1995) for moisture (Method 44−15A) and protein (Method 46−13). Gliadin proteins were quantified in 70% ethanol extract, following extraction with 0.5 M NaCl solution, by the Kjeldahl method. The sample to solvent ratio was 1:20 (w/v). For electrophoresis experiments, a meal sample containing about 6 mg gliadins (i.e. about 0.1 g of sample based on 6% wet basis gliadin in the sample) was extracted with 70% ethanol for up to 3 h at room temperature, after prior extraction with 0.5 M NaCl for 1 h to eliminate albumin and globulin proteins. The dissolved gliadins were then mixed with 0.85 times its volume of 60% (v/v) glycerol and 0.05% methyl green and centrifuged at 13000*g* for 5 min. Gliadin proteins were then separated by A-PAGE using a vertical slab gel apparatus (Bio-Rad Laboratories, Richmond, CA) according to the procedure of Ng et al. (1988). A 15 μL aliquot of extract was loaded into a gel slot. The gel was 8% (w/v) acrylamide and was run at 20 °C and a current rate of 25 mA/gel for 4 h and for 2 h to separate small molecular weight polypeptides. Aluminum lactate buffer, adjusted to pH 3.1 with lactic acid, was used as the electrode buffer. After electrophoresis, the gel was washed with 12% (w/v) TCA for 5 min and stained with Coomassie Blue G-250 overnight with gentle stirring.

SDS-PAGE was carried out according to the procedure of Ng et al. (1988) with modifications to standardize the procedure for gliadin proteins. A 100 μL aliquot of gliadin extract was heated in a boiling water bath for 2.5 min with 100 μL of 10% (w/v) SDS and 25 μL of mercaptoethanol and then mixed with 0.85 times its volume of 60% (v/v) glycerol and 0.05% (w/v) bromophenol blue dye. Then 15 μL of the reduced proteins was loaded into a gel slot. The separation gel was 17.3% (w/v) acrylamide, and the stacking gel was 3% (w/v) acrylamide.

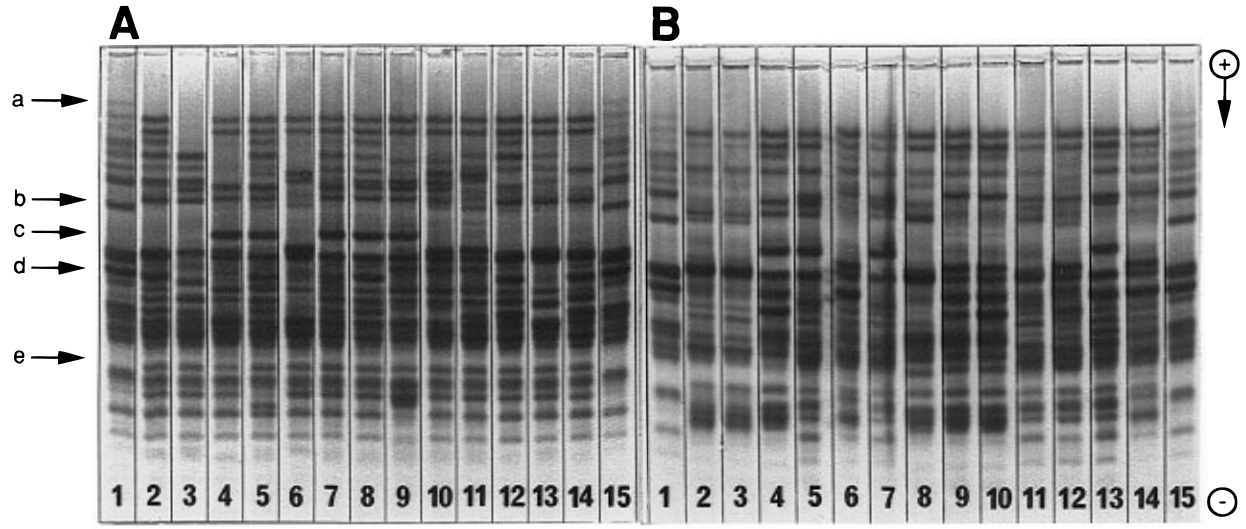

Figure 1. A-PAGE gliadin patterns of (A) Neepawa (1,15) and spring spelt (2−14) and (B) Neepawa (1,15), common winter spelt (2,3), and spring spelt (4−14) wheats grown in 1991.

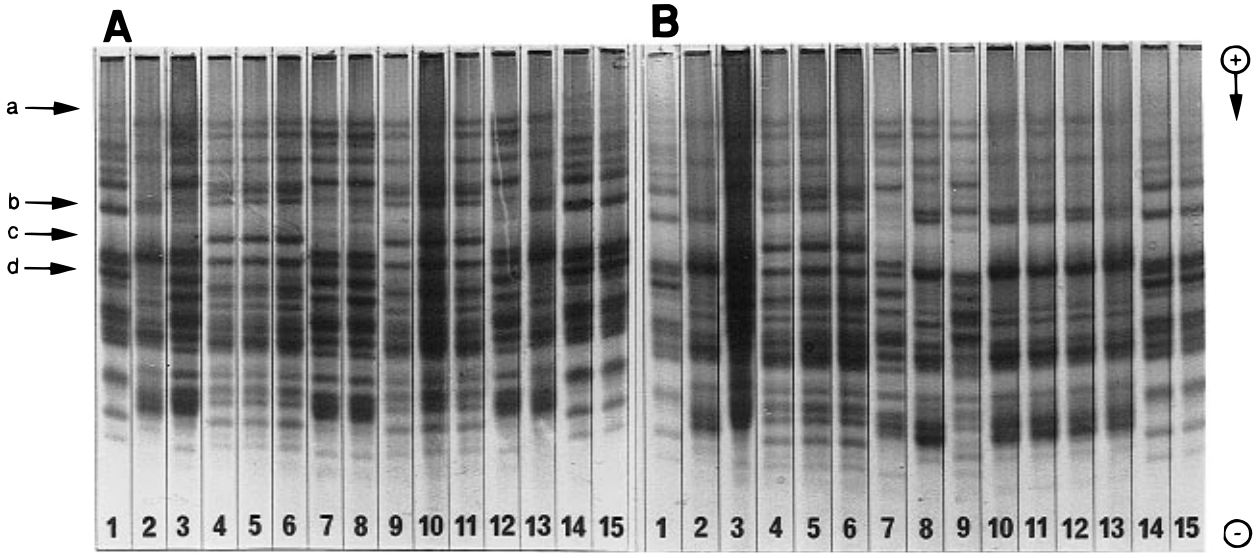

Figure 2. A-PAGE gliadin patterns of (A) Katepwa (1,14), winter spelt (2,13), spring spelt SK0021 (3,8), spring spelt SK0263 (4,9), spring spelt SK0505 (5,10), spring spelt RL5407 (6,11), spring spelt PGR8801 (7,12), and Neepawa (15) and (B) Katepwa (1,14), winter spelt (2,8−13), spring spelt SK0021 (3), spring spelt SK0263 (4), spring spelt SK0505 (5), spring spelt RL5407 (6), spring spelt PGR8801 (7), and Neepawa (15) wheats grown at three sites (Saskatoon, Kernen, and Elrose) in 1992.

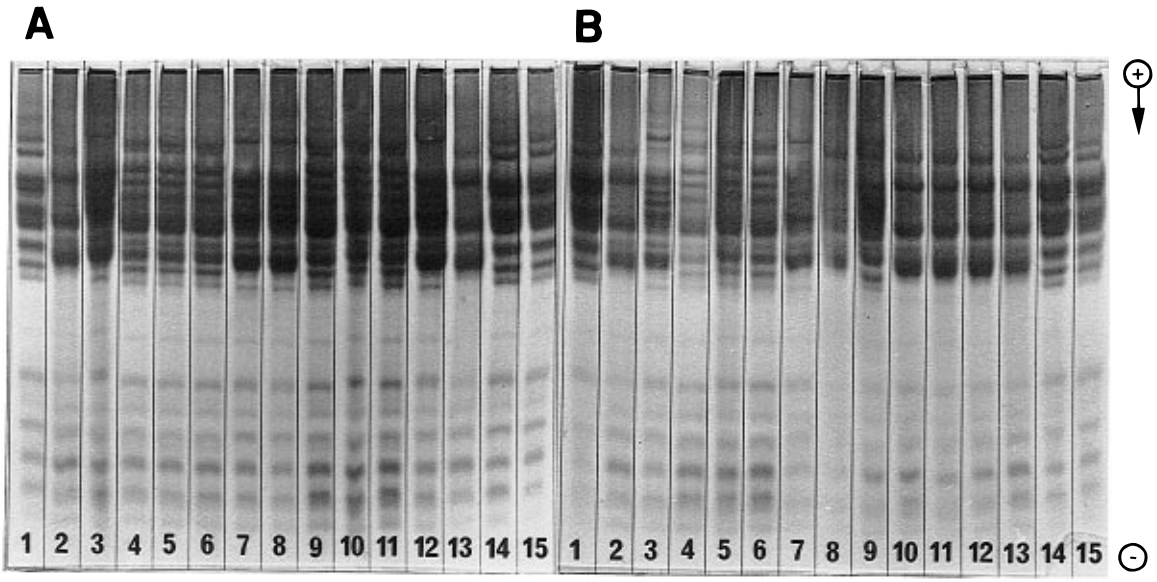

Figure 3. A-PAGE gliadin patterns of (A) Katepwa (1,14), winter spelt (2,13), spring spelt SK0021 (3,8), spring spelt SK0263 (4,9), spring spelt SK0505 (5,10), spring spelt RL5407 (6,11), spring spelt PGR8801 (7,12), and Neepawa (15) and (B) Katepwa (1,14), winter spelt (2,8−13), spring spelt SK0021 (3), spring spelt SK0263 (4), spring spelt SK0505 (5), spring spelt RL5407 (6), spring spelt PGR8801 (7), and Neepawa (15) wheats grown at three sites (Saskatoon, Kernen, and Elrose) in 1992 (2 h run).

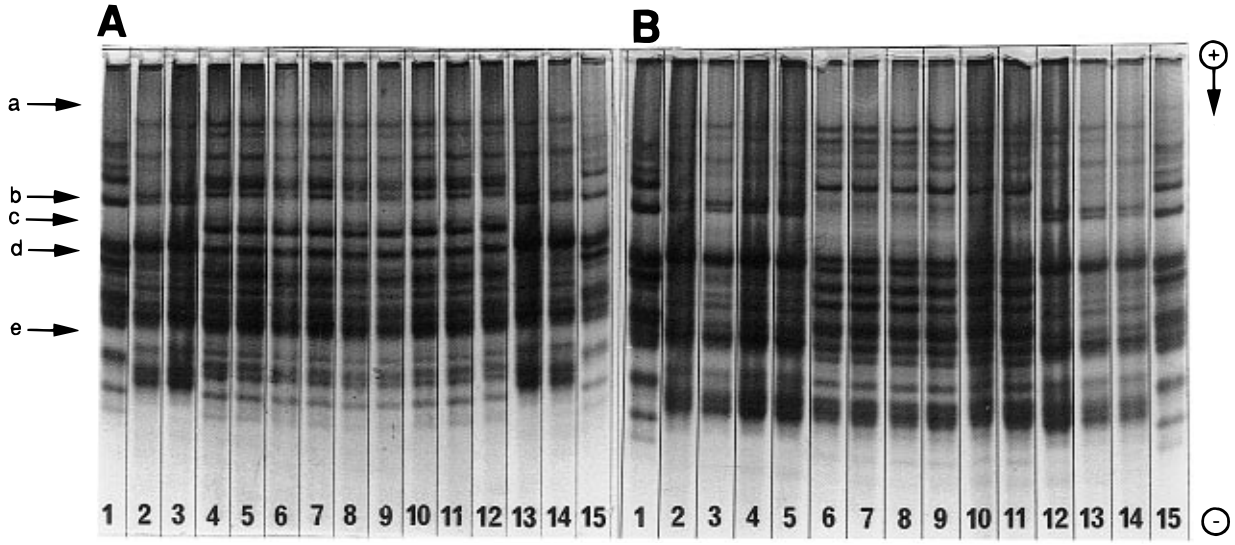

Figure 4. A-PAGE gliadin patterns of (A) Katepwa (1,15), winter spelt (2,3,13,14), spring spelt SK0263 (4−6), spring spelt SK0505 (7−9), and spring spelt RL5407 (10−12) and (B) Katepwa (1,15), winter spelt (2−5,12−14), spring spelt SK0021 (6−8), and spring spelt PGR8801 (9−11) wheats grown at three sites (Saskatoon, Kernen, and Elrose) in 1992.

The gel was electrophoresed on the above mentioned apparatus at 20 °C at a constant current of 5 mA/gel for 2 h, followed by 13 h at 10 mA/gel, in which the indicator dye was close to the end edge of the gel. After electrophoresis, the gel was rinsed with rinsing solution containing 100 mL of 100% (w/v) TCA, 330 mL of methanol, and 570 mL of water with gentle agitation for 1 h, and the rinsing process was repeated two more times. The gel was stained with Commassie Blue G-250 for 12−18 h with gentle agitation. All chemicals were of analytical reagent grade, and water was distilled and deionized by the NANOpure purification system (Barnstead Corp., Dubuque, IA). The HRS cultivar Neepawa was also used as a control in each gel.

Data Analysis. A set of 13 distinctly staining gliadin bands were scored from the highest to the lowest molecular weight band. The presence of a particular band in a wheat accession was assigned a value of "1", whereas the absence was designated by a "0". In this way, the 90 spring spelt wheats grown in 1991, 5 spring spelts from 1992−1993 tests, 5 winter spelts from 1993/1994, and Neepawa common wheat were scored for gliadin composition. The resulting data matrices were analyzed using SIMQUAD (similarity for qualitative data) routine of NYSYS-PC (Numerical Taxonomy and Multivariate Analysis System, version 1.80) to generate Jaccard similarity coefficients. The similarity coefficients were used to construct a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA) employing the SAHN (sequential, agglomerative, hierarchied, and nested) clustering routine from NTSYS (Joshi and Nguyen, 1993).

RESULTS AND DISCUSSION

There were significant differences between the five spring spelts and Katepwa HRS wheat in the 1992 and 1993 samples, based on their protein and gliadin contents (Table 1). In general, spelts showed higher protein and gliadin contents than those of Katepwa HRS wheat, with winter spelt having the highest concentrations. The winter spelts were, however, not grown under the same test conditions as the other wheats. On the basis of the gliadin content in each wheat subspecies, a sample weight containing about 6 mg of gliadin was used in each electrophoresis experiment to minimize the effect of protein content on wheat protein

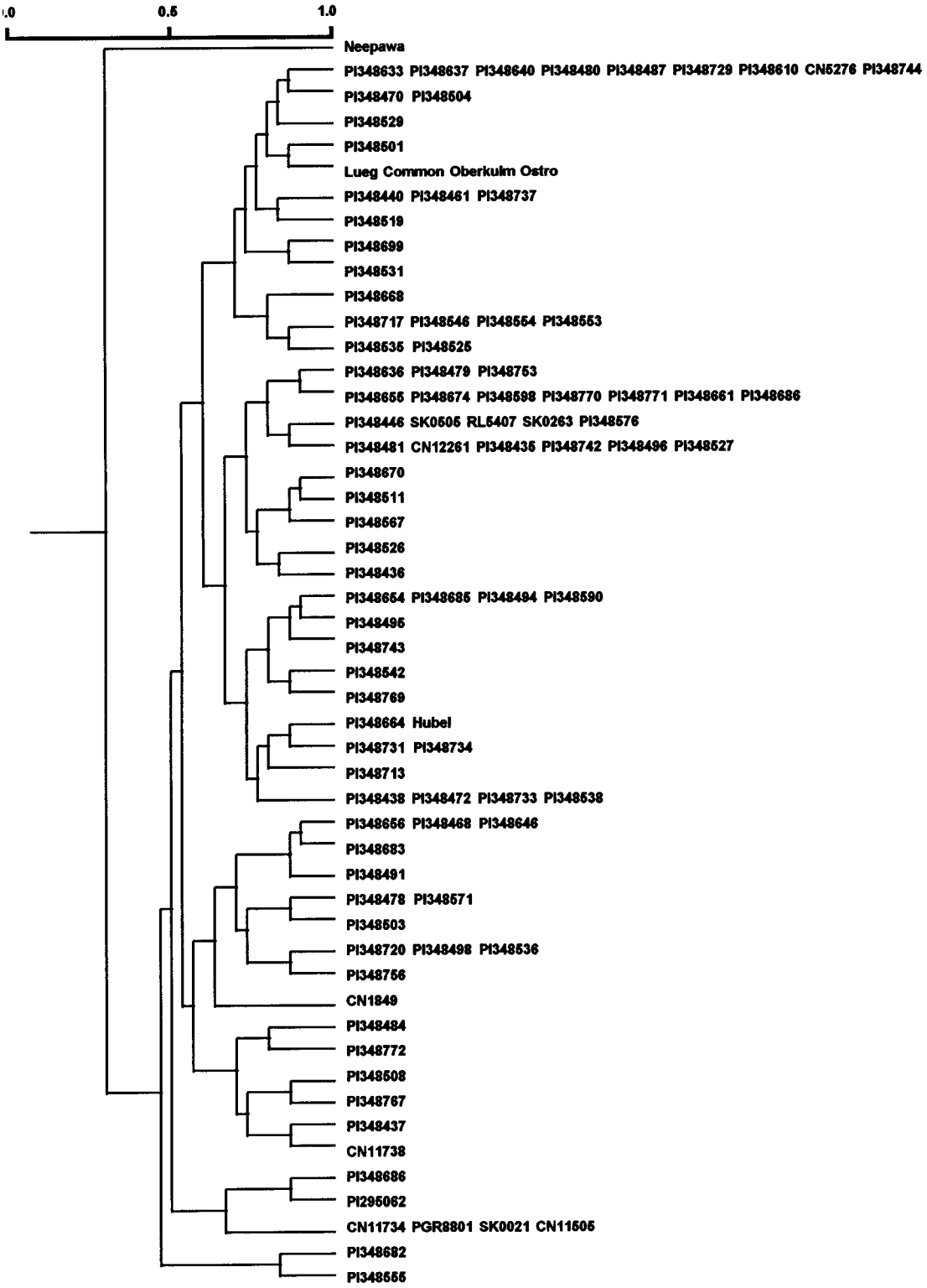

Figure 5. Dendrogram of gliadin compositions of 95 spring spelts, the 5 named winter spelts, and Neepawa HRS wheat. The scale indicates the similarity index.

profiles. Fullington et al. (1983) indicated that the proportion of gliadins increased significantly with an increase in protein content, while the proportion of low molecular weight albumins and globulins decreased significantly with use of high- and low-resolution SDS-PAGE. In addition, when equal amounts of protein were applied to the gel, the differences in the intensities of bands in the patterns of samples from a given subspecies might reflect the relative concentrations of these bands or protein subunits in this subspecies.

The 90 accessions of spring spelt grown in 1991, common winter spelt, and Neepawa were analyzed for their gliadin composition by the A-PAGE method. A random sample of A-PAGE gliadin profiles is shown in Figure 1A,B. It was obvious that there were differences in the number of ω-gliadin bands and their relative mobilities between Neepawa HRS wheat and the spelts. Common wheat was characterized by the presence of the slow-moving ω-gliadin band "a" with weak intensity. This band was absent in all spelt wheats. This finding

agrees with Federmann et al. (1992) who reported that slow-moving ω-gliadins were the most suited for discrimination between spelt and common wheat varieties. Neepawa HRS wheat also showed a strong-staining band "b" which was a fast-moving ω-gliadin. This band was not displayed by most spelt lines. This also confirms the results of Federmann et al. (1992) who detected the adulteration of spelt flour with common wheat flour by these bands. Another variation between common wheat and spelt wheat was the number of α-gliadin bands. Spring and winter spelts were characterized by a large number of bands in the α-gliadin region and the presence of a slow-moving α-gliadin labeled as "e".

Spring spelts had two types of gliadin pattern, one was characterized by the presence of a strong-staining band or polypeptide chain labeled as "c" (Figure 1A, B). This polypeptide chain was in the γ-gliadin region. This band was absent in common wheat, winter spelt, and some of the spring spelts. Therefore, this γ-gliadin band might be used to differentiate the two types of spring spelts, and spring spelt from common wheat and winter spelt. There was another strong-staining band "d" that appeared in common wheat and some of spring spelt lines (Figure 1A,B) but was absent in the winter spelt and some spring spelt lines. This band was also in the γ-gliadin region.

Lines of spelt selected to show the γ-gliadin band in later tests were SK0505, SK0263, and RL5407 (black-hulled spelts), while SK0021 and PGR8801 (white-hulled spelts) lines were selected to show the absence of band "c". Winter spelt, however, had a lower number of ω-gliadin subunits (Figure 2A,B). In the 1992 experiments, the five selected accessions of spring spelt were grown along with Katepwa HRS wheat. The electrophoregrams for these accessions are presented in Figure 2A,B. Again, the Katepwa HRS wheat gliadin pattern was characterized by the presence of the slow-moving ω-gliadin "a" and the strong staining fast-moving ω-gliadin "b" and the absence of the strong staining γ-gliadin "c". As expected, SK0505, SK0263, and RL5407 exhibited the γ-gliadin band, while SK0021 and PGR8801 did not and were similar to winter spelt for that band. In other words, winter spelt and spring spelts SK0021 and PGR8801 gliadin patterns did not display the major γ-gliadin band.

In order to examine the fast-moving α-gliadins of spring spelt accessions, the samples were run for only 2 h or half of the running time (Figure 3A,B). Owing to the short running time, the resolution of slow-moving gliadin proteins was low. All wheat subspecies had similar very fast-moving α-gliadin bands.

With regard to environmental influences on spring spelt gliadin profiles at three sites, each type (i.e. SK0505, SK0263, and RL5407 versus SK0021 and PGR8801) was run separately in comparison with both Katepwa HRS and winter spelt wheats (Figure 4A,B, respectively). Spelt gliadin patterns within groups were identical across environments. Winter spelt from different environments also showed identical gliadin patterns, having a distinct doublet protein subunit in the fast-moving ω-gliadin region. This band and band "b" in common wheat were absent in all spring spelt accessions. These results also confirm that SK0505, SK0263, and RL5407 accessions are characterized by the presence of a γ-gliadin band that was absent in SK0021 and PGR8801 gliadin profiles.

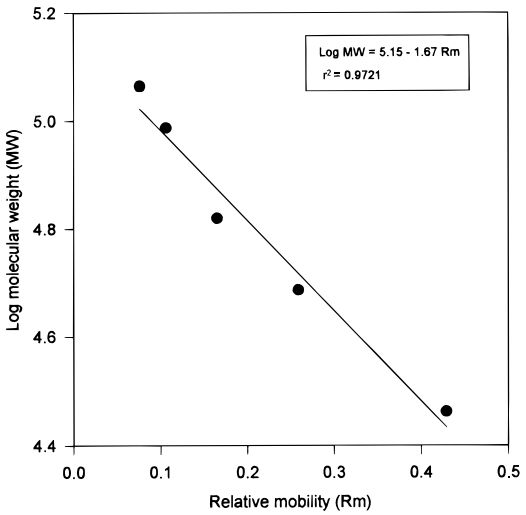

Figure 6. Calibration curve of molecular weight vs mobility relative to bromophenol blue on SDS-PAGE.

The dendrogram constructed on the basis of gliadin bands (Figure 5) illustrates that the winter and spring spelt wheats formed a cluster separate from the HRS wheat cultivar Neepawa. Thus, it would appear that the spelt wheats sampled are distinct from standard western Canadian spring wheats in gliadin protein composition. The spelt wheats grouped into three subclusters. One of these subclusters contained four of the winter spelt accessions. The commercial ("common") product purchased in a local store was identical to the Swiss cultivars Oberkulm, Ostro, and Lueg. Oberkulm and Ostro are derived from Swiss land races (Weilenmann, 1995). Thus, the winter spelt being marketed in Canada at the present time likely traces its origins to a Swiss winter spelt land race. Two of the Swiss winter spelt cultivars, Lueg (Winzeler et al., 1991) and Hubel, are derived from crosses with common wheat. Lueg was identical in the gliadin profile to the land race derivatives, while Hubel was not. None of the spring spelts had a gliadin profile identical with that of Oberkulm or common winter spelt. However, 26 spring spelts were in the same sub-cluster as Oberkulm and Common winter spelt. The black-hulled accessions SK0263, SK0505, and RK5407 were more similar to the winter spelts in gliadin profile than were the white-hulled accessions PGR8801 and SK0021 with Jaccard genetic similarity coefficients of similarity of 0.78 and 0.46, respectively. All the plant introduction accessions beginning with the digits "348" were collected in Spain, according to the available USDA germplasm passport information. Despite the restricted geographic origin of these spring spelt wheats, significant diversity in gliadin composition was observed.

The molecular weights (MW) of the spelt gliadins were estimated from the regression equation between log MW and relative mobility of protein markers on SDS-PAGE electrophoregrams (Figure 6). The MW ranged from 34K to 113K, indicating that gliadin extracts contained HMW non-gliadin components which could be polypeptide chains of glutenin (Figure 7A,B). Chakraborty and Khan (1988) have shown by SDS-PAGE that some glutenins are extracted with 70% aqueous ethanol. These non-gliadin components consisted of 4−5 sharp, thin bands with MW's of 94K−113K. The MW of gliadins ranged from 34K to 75K. This range was in agreement with values (44K−78K) reported by Tatham and Shewry (1990). In addition, Ewart (1973) estimated the polypeptide chain weights

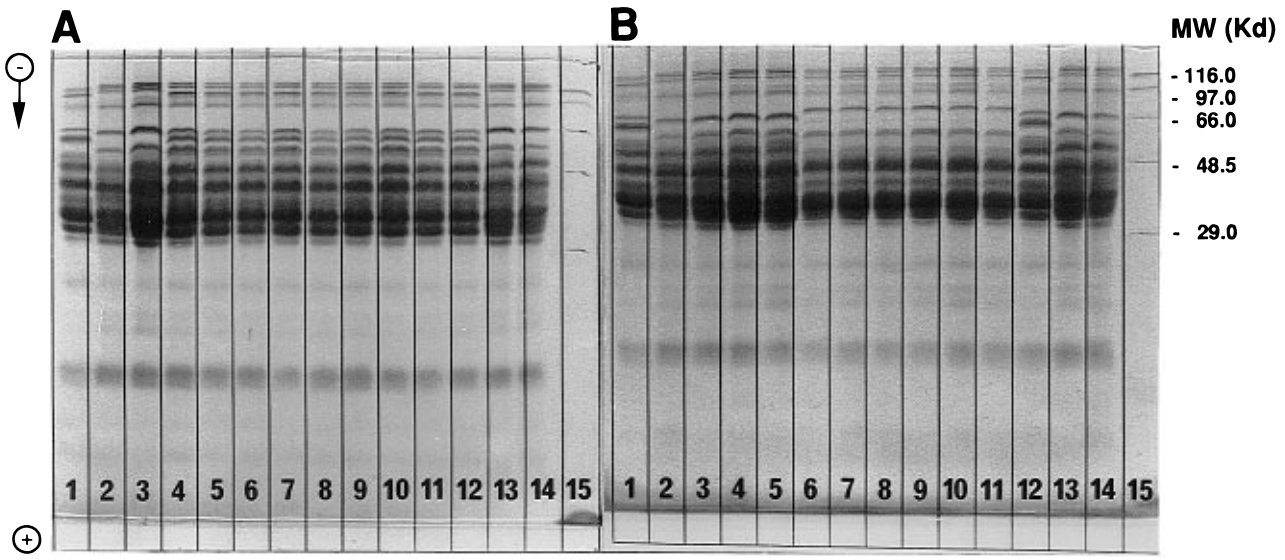

Figure 7. SDS-PAGE gliadin patterns of (A) Katepwa (1), winter spelt (2,3,13,14), spring spelt SK0263 (4−6), spring spelt SK0505 (7−9), spring spelt RL5407 (10−12), and protein marker (15) and (B) Katepwa (1,12), winter spelt (2−5,13,14), spring spelt SK0021 (6−8), spring spelt PGR8801 (9−11), and protein marker (15) wheats grown at three sites (Saskatoon, Kernen, and Elrose) in 1992.

of α-, β-, and γ-gliadin fractions that were in the range of 32K−44K.

Spring spelt accessions SK0505, SK0263, and RL5407 had identical protein subunit patterns based on SDS-PAGE (Figure 7A). These accessions and Katepwa HRS wheat showed a distinct strong band having a MW of 64K, which was absent in winter spelts and spring spelt accessions SK0021 and PGR8801 (Figure 7B). In other words, spring spelts SK0021 and PGR8801 and winter spelt SDS-PAGE patterns were characterized by the absence of the 64K band.

When 1993 samples were analyzed by A-PAGE and SDS-PAGE for their gliadin compositions, they gave identical electrophoretic patterns as the 1992 samples. Thus, only the electrophoregrams of 1992 samples are presented in the present study.

CONCLUSION

On the basis of the accessions screened in the present study, spring spelt had a gliadin profile distinct from that of the HRS wheat cultivar Neepawa. Furthermore, none of the spring spelts were identical to the commercial winter spelts. Five experimental spring spelt accessions studied in detail could be divided into two major types differing by a single γ-gliadin protein. The first type was characterized by the presence of a strong-staining band in the γ-gliadin region, as shown in black-hulled accessions SK0505, SK0263, and RL5407. These accessions exhibited large kernels with soft texture but high flour yields, sedimentation values, mixograph characteristics, loaf volumes, and bromate responses (Abdel-Aal et al., 1995). The second group which consisted of the white-hulled SK0021 and PGR8801 did not contain this γ-gliadin band which was also absent in winter spelt. In a previous study (Abdel-Aal et al., 1995) the latter accessions were inferior in the sedimentation value, mixograph characteristics, and loaf volume but were comparable to common wheat in kernel hardness and flour yield.

Spelt can be differentiated from common wheat based on ω-gliadin composition, in which common wheat was characterized by the presence of a slow-moving ω-gliadin and a strong-staining fast-moving ω-gliadin. The molecular weights of gliadin proteins were in the range of 34K−75K. In addition, common wheat and black-hulled spring spelt accessions SK0505, SK0263, and RL5407 had a distinct strong band with MW of 64K which was absent in winter spelt and white-hulled spring spelt accessions SK0021 and PGR8801.

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