

Identification by High-Performance Capillary Electrophoresis of Wheat Lines Containing the 1AL.1RS and the 1BL.1RS Translocation¹

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ABSTRACT

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High-performance capillary electrophoresis (HPCE) was used to analyze grain proteins from U.S. wheat cultivars carrying 1AL.1RS from Amigo or 1BL.1RS from Kavkaz wheat-rye chromosomal translocations. To date, these are the only 1RS translocations known to occur in U.S. cultivars. Chromosome specific patterns were observed among released cultivars and experimental lines. The method was verified by analyzing

protein extracts of the heterogeneous cultivars Nekota and Rawhide and their homogeneous, homozygous non-1RS progeny. The purified 1AL.1RS and 1BL.1RS progenies were derived from Nekota and Rawhide, respectively. HPCE provides a rapid and efficient method for detection of flour or grain derived from 1AL.1RS and 1BL.1RS wheats, and can differentiate the two types of translocations.

Wheat-rye chromosomal translocations have been a common means to transfer desirable genes from rye (*Secale cereale* L.) to wheat (*Triticum aestivum* L.) (Zeller 1973). The most common of those translocations in North American wheat breeding programs are 1BL.1RS, found in derivatives of the Russian cultivars Kavkaz and Aurora, and 1AL.1RS, derived from the wheat germplasm line Amigo (Zeller and Hsam 1983). Genes for stem, stripe and leaf rust resistance and powdery mildew resistance are carried on 1BL.1RS, and genes for stem and stripe rust, greenbug, and powdery mildew resistance are carried on 1AL.1RS. In addition, 1BL.1RS confers a grain yield advantage under environmental stress (Morena-Sevilla et al 1995). 1BL.1RS translocation lines are cultivated worldwide, while 1AL.1RS is more common among U.S. cultivars. 1RS continues to be used in wheat breeding programs. In the 1993, 1994, and 1995 USDA-ARS Southern Regional Performance Nurseries, a regional testing program for advanced breeding lines, approximately 20% of the entrants carried either 1AL.1RS or 1BL.1RS (R. Graybosch, unpublished results).

Deleterious effects of 1BL.1RS on wheat end-use quality have also been identified (Zeller and Hsam 1983; Moonen and Zeven 1984; Dhaliwal et al 1987, 1988). The most common difficulties with 1BL.1RS are reduced gluten strength (diminished mixing tolerance), dough stickiness, and reduced loaf volume per unit protein. Poor internal loaf characteristics are often found in breads made from 1AL.1RS lines. These problems must be overcome if 1RS wheats are to gain acceptance in the hard red winter and spring wheat growing regions. Future use of 1AL.1RS or 1BL.1RS will depend on the identification of genetic backgrounds in which these deleterious effects are diminished. Therefore,

simple and rapid means of identifying wheat lines carrying 1AL.1RS or 1BL.1RS translocations must be developed.

Previous methods used to identify grain or flour derived from wheat-rye translocation lines have been based on analysis of 70% ethanol-soluble prolamin proteins (gliadins of wheat, secalins of rye). Howes et al (1989) reported the use of a monoclonal antibody to a wheat γ -gliadin to identify 1BL.1RS wheat lines. However, this method is based on the absence of a wheat gene product. Such an absence could also arise from mutations or chromosomal deletions independent of 1BL.1RS. Lookhart et al (1991) reported the use of high-performance liquid chromatography (HPLC) and electrophoresis to identify lines containing 1AL.1RS and 1BL.1RS. However, HPLC could not differentiate between those lines. Berzonsky et al (1991) reported the use of an electrophoretic method (acid polyacrylamide gel electrophoresis [A-PAGE]) to differentiate 1AL.1RS from 1BL.1RS translocations in single kernels of four soft wheat lines. That method required considerable time, and the protein band resolution was poor. Johansson et al (1994) compared the use of electrophoretic methods (A-PAGE and sodium dodecyl sulfate [SDS] PAGE) to identify 1BL.1RS rye translocations in wheat. They characterized the rye secalins utilizing a two-dimensional procedure that is highly labor intensive. They concluded that SDS-PAGE was the best electrophoretic method to identify 1BL.1RS translocations, since the resolution was adequate and high-molecular weight glutenin subunit composition could be obtained from the same gel system. Both Graybosch et al (1993) and Andrews et al (1996) used secalin-specific monoclonal antibodies that reacted to protein extracts of both 1RS wheats. Again, the two types of translocations were not differentiated.

High-performance capillary electrophoresis (HPCE) complements other analytical methods and has high resolution and reproducibility, fast analysis times, easy quantification, and minimum sample size (Lookhart and Bean 1995). Commercial HPCE instruments have only been available for six years. It is a welcome addition for protein characterization and identification. In this article, HPCE is used to identify and differentiate lines carrying 1AL.1RS or 1BL.1RS.

MATERIALS AND METHODS

Plant Samples

Thirty-one experimental lines and seven released wheat cultivars were studied. The experimental lines were developed by breeders in various hard red winter (HRW) wheat states and as part of a joint USDA-ARS/University of Nebraska study to develop

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HRW wheat germplasm with improved endosperm protein quality and quantity. Several of the experimental lines had pedigrees that contained either a 1AL.1RS or 1BL.1RS-carrying parent. Released cultivars included in the study were: Aurora, Kavkaz, Nekota, Rawhide, TAM 105, TAM 107, and TAM 200. TAM 107 and TAM 200 are homogenous for 1AL.1RS, Aurora and Kavkaz are homogeneous for 1BL.1RS, while Nekota and Rawhide are heterogeneous for 1AL.1RS and 1BL.1RS, respectively. In addition, homogeneous, homozygous sister lines, with or without the rye translocations, selected from both Nekota and Rawhide were studied. TAM 105 is a parent of TAM 107 and does not contain the rye translocation. A rye cultivar, Rymin, was used as a type standard for rye.

Pedigrees of Wheat Cultivars

TAM105 = 'short wheat' / Sturdy composite bulk selection; TAM107 = 'TAM105' *4/ Amigo; TAM200 = ((Sinvalocho / Wichita /2/ Hope / Cheyenne /3/Wichita /4/ Seu Seun 27 /TX391-56-D8) /5/ Tascosa /6/ Centurk, TX71A1039-V1)*3 /7/ Amigo; Rawhide = Warrior*5 /Agent /2/ Kavkaz /4/ Parker *4 /Agent /2 Keloterkovskaia 198 / Lancer /3/ Vona; Nekota = Bennett / TAM107.

Protein Extraction

Wheat proteins (mainly gliadins) were extracted for HPCE as previously described (Lookhart and Bean 1995a) following manual grinding of the grain in a mortar and pestle.

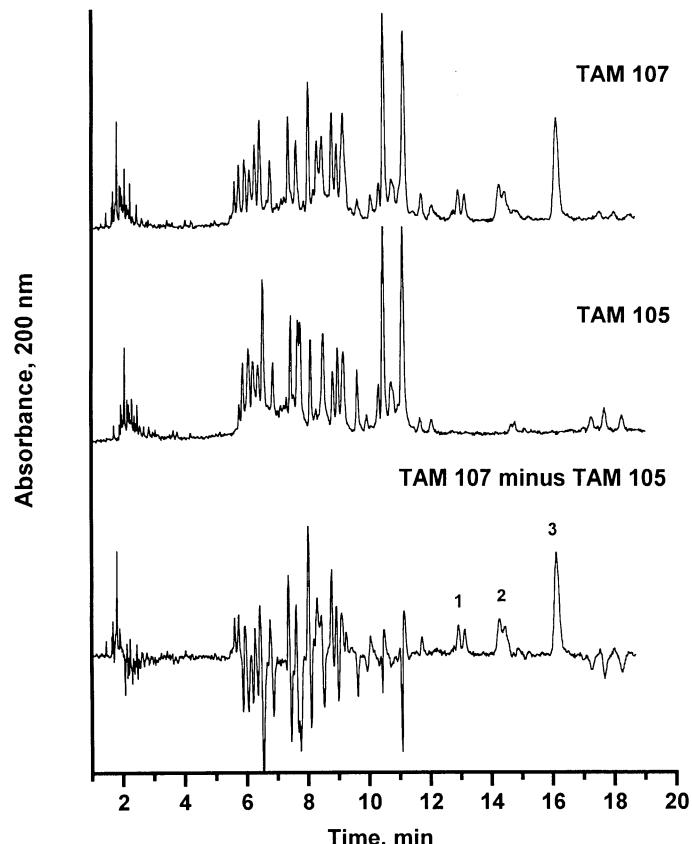


Fig. 1. Gliadin high-performance capillary electrophoresis (HPCE) patterns of wheat cvs. TAM 107 and TAM 105 and the difference of their electrophoretic patterns. TAM 105 does not contain the 1AL.1RS translocation. The most positive peaks in the difference pattern are rye proteins. That material was not present in the line without the rye translocation. HPCE gliadin patterns were produced by separation in 27 cm (20 cm separation length) \times 20 μ m i.d. capillaries at 15 kV (+ to -) and 45°C with 100 mM phosphate buffer, pH 2.5, containing 0.05% hydroxypropyl-methyl cellulose plus 20% acetonitrile. Samples were pressure injected (5 sec).

High-Performance Capillary Electrophoresis

A Beckman P/ACE 2100 system was used to separate all extracts. The method was essentially as described in Lookhart and Bean (1995b). Capillaries used were uncoated fused-silica, 27 cm long (20 cm separation length) with 20 μ m i.d. The buffer was 100 mM phosphate (pH 2.5) containing 20% acetonitrile (ACN) and 0.05% hydroxypropyl-methyl cellulose (HPMC). Constant voltage (15 kV) and temperature (45°C) were used. All samples were pressure injected for 5 sec (0.31 nl). Proteins were monitored by UV absorption at 200 nm.

RESULTS AND DISCUSSION

The gliadin HPCE patterns of TAM 107, TAM 105, and their difference pattern are shown in Figure 1. In the 13–18 min range, three major differences between TAM 107 and TAM 105 electrophoregrams are labeled (1, 2, and 3). Those proteins suspected to be of rye origin occurred at time periods of 13, 14.4, and 16 min. In fact, the HPCE prolamin pattern of a rye cultivar Rymin exhibited peaks in the same time periods (data not shown). The $\sec-1$ locus found on 1RS is known to be complex, encoding several proteins (Gupta and Shepherd 1992). The HPCE pattern of TAM 200, another 1AL.1RS wheat, exhibited the same pattern of proteins in this region (data not shown) as did TAM 107. The two even-sized peaks of the 13-min doublet typify the 1AL.1RS translocation pattern. The HPCE pattern of the aqueous extract of Rymin rye showed the 1AL.1RS doublet (data not shown). Lookhart et al (1991) and Johansson et al (1994) previously reported that rye secalins in 1RS translocations electromigrated with α -gliadins, by gel electrophoresis. The slowest HPCE electromigrating proteins were also ω -gliadins (Lookhart and Bean 1995b).

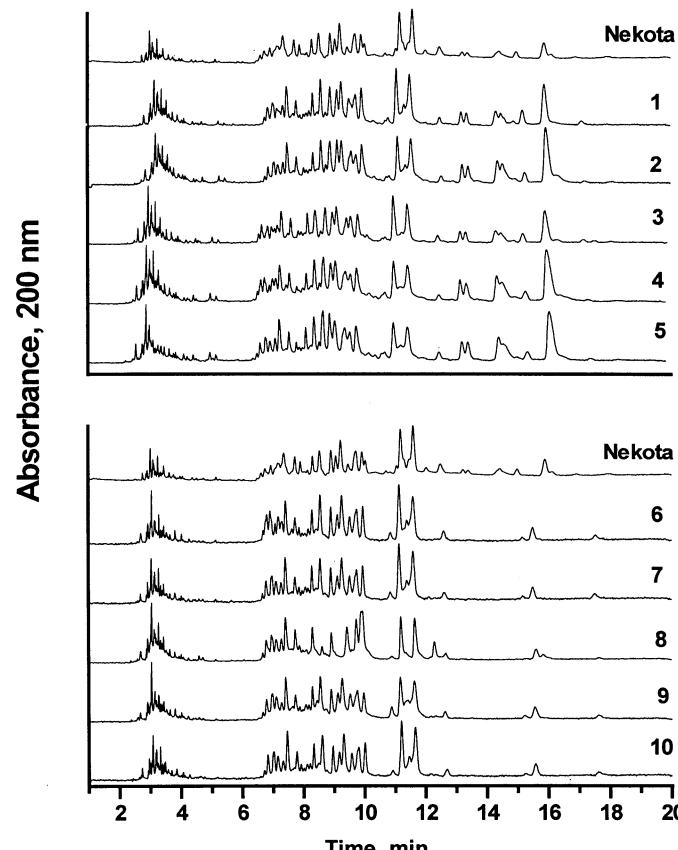


Fig. 2. Gliadin high-performance capillary electrophoresis (HPCE) patterns of the heterogeneous rye translocation cv. Nekota and 10 homogeneous, homozygous sister lines. Lines 1–5 contain 1AL.1RS and lines 6–10 do not. Extraction and analysis conditions as given in Fig. 1.

Therefore the proteins migrating in the 12–16 min range on HPCE, using the ACN-Phosphate buffer, would be ω -gliadin-like. This agrees with our present finding that differences between rye translocation and nontranslocation lines is in that region.

Nekota, a recently released wheat cultivar, is heterogeneous for the 1AL.1RS translocation. Gliadin HPCE patterns of Nekota and homogeneous 1AL.1RS sister lines selected from Nekota are shown in Figure 2 (lines 1–5). Non-1RS selections from Nekota are shown in Figure 2 (lines 6–10). The Nekota lines with the rye translocation show the three peaks noted in Figure 1 for the 1AL.1RS rye proteins. The intensity of the peaks in the Nekota pattern for the rye proteins appears less than that found in the 1AL.1RS sister lines. This is to be expected since Nekota is heterogeneous and only \approx 50% of the kernels extracted would carry the rye proteins. The HPCE gliadin patterns of Nekota and sister lines 1–5 were all similar. All samples were normalized to make the largest peak the same size for all samples. This eliminates differences due to protein content or extraction method. Difference in peak intensity reflect Nekota heterogeneity. Thus, it is easy to note the doublet at \approx 13 min, which is consistent with the 1AL.1RS translocation.

The Nekota-derived sister lines without the rye proteins are shown in Figure 2 (lines 6–10). They are homogeneous only with respect to the absence of the rye translocation and lack the peaks at 13, 14.4, or 16 min, that are characteristic of the rye proteins. Gliadin HPCE patterns of the sister lines 6, 7, 9, and 10 (Fig. 2), were similar with minor differences in the 6–12 min range. The HPCE pattern of line 8 (Fig. 2) was different from the others however, especially in the 8–13 min range. The earliest electromigrating wheat proteins are primarily α - and β -gliadins (with this

buffer, in the 6–10 min range), while the slowest electromigrating proteins (those electromigrating after 12 min) are ω -gliadins (Lookhart and Bean 1995b). Several Nekota-derived sister lines (40) were analyzed for 1AL.1RS translocations by SDS-PAGE and antibodies (Graybosch et al 1993), and the results were in total agreement.

The HPCE pattern of the wheat cultivar Rawhide, heterogeneous for 1BL.1RS, is shown in Figure 3. Sister lines selected from Rawhide that carry the translocation are shown in lines 1–5 and those without the rye proteins are in lines 6–10. HPCE patterns of Aurora and Kavkaz showed the same secalin pattern as each of these 1BL.1RS lines (data not shown). Like the 1AL.1RS lines, the 1BL.1RS rye proteins electromigrate between 12 and 16 min. The most characteristic peaks for 1BL.1RS electromigrate at \approx 13 min, very similar in time to the 1AL.1RS peaks. However, the shape of the 1BL.1RS peaks are different. There are two peaks; the first peak is about twice as large as the second. The 1BL.1RS characteristic rye protein group also was larger than that of the 1AL.1RS group. The heterogeneous Rawhide HPCE pattern contained peaks at 8 and 11 min not found in the 1BL.1RS lines. However, those peaks were found in the nonrye translocation Rawhide-derived lines (lines 6–10). HPCE gliadin patterns for lines 1–5 were uniquely similar to each other, whereas the patterns between 8 and 11 min of lines 6–10 were similar to both themselves and to Rawhide.

This HPCE data was in complete agreement with previous data on Rawhide biotypes identified by SDS-PAGE, antibodies, and leaf rust screening from 94 selected Rawhide lines (Morena-Sevilla et al 1995 a,b).

Gliadin HPCE 1AL.1RS patterns of six different experimental lines are shown in Figure 4. Three 1AL.1RS experimental lines

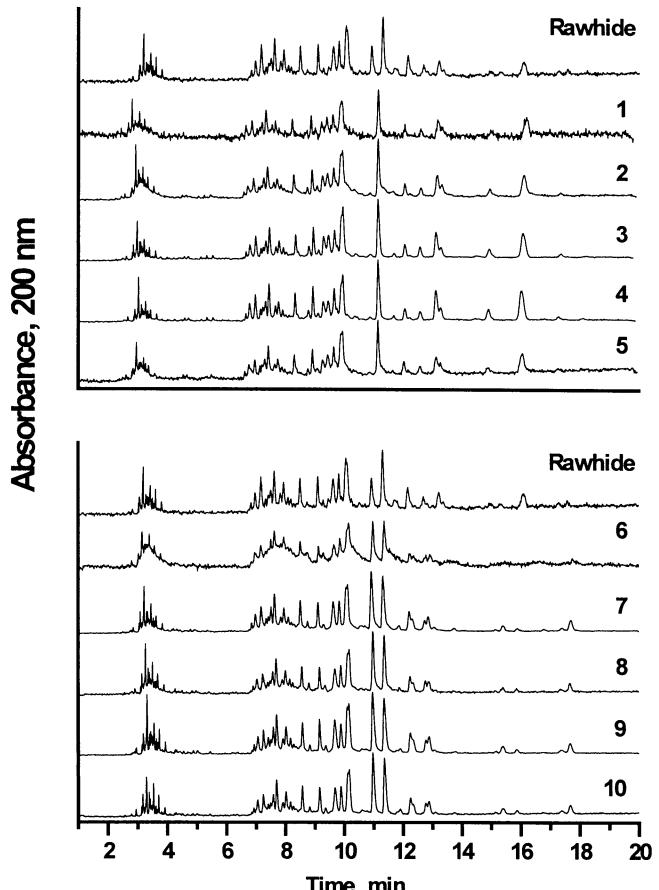


Fig. 3. Gliadin high-performance capillary electrophoresis (HPCE) patterns of the heterogeneous rye translocation cv. Rawhide and 10 near-isogenic sister lines. Lines 1–5 contain 1BL.1RS and lines 6–10 do not. Extraction and analysis conditions as given in Fig. 1.

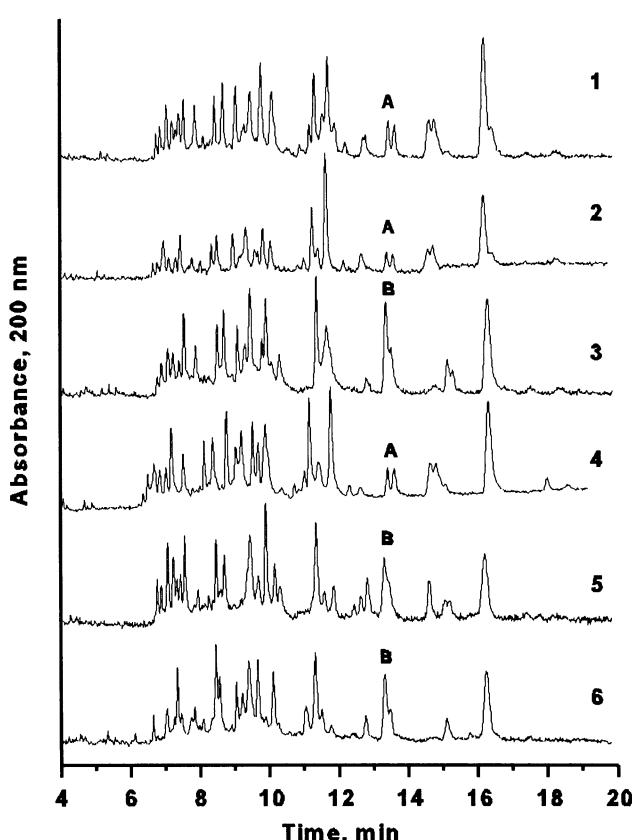


Fig. 4. Gliadin high-performance capillary electrophoresis (HPCE) patterns of experimental lines containing either 1AL.1RS (A) or 1BL.1RS (B). Lines 1–6 were Nebraska experiment station lines 1703, 1715, 1797, 1081, 1087, and 2121, respectively. Extraction and analysis conditions as given in Fig. 1.

(1, 2, and 4) have characteristic peaks, labeled A. Three 1BL.1RS lines (lines 3, 5, and 6) have peaks labeled B. Differences in the overall gliadin HPCE patterns are noted for each line. However, consistency was found in the presence of the characteristic protein peaks electromigrating at \approx 13 min as a small doublet for 1AL.1RS and as a larger 2:1 doublet for the 1BL.1RS lines. Exact times may vary slightly with capillary, instrument, and buffer, but these peak differences are consistent. Gliadin HPCE patterns of 25 other experimental lines from across the HRW wheat growing region were also consistent with these protein patterns (data not shown).

CONCLUSIONS

Several methods including A-PAGE and SDS-PAGE can confirm the presence of 1RS genes in wheat lines. HPLC can identify 1AL.1RS and 1BL.1RS translocation lines but not differentiate them. HPCE is capable of rapidly and efficiently differentiating grain or flour derived from the Kavkaz 1BL.1RS translocation and the Amigo 1AL.1RS translocation. Those translocations are the only translocations presently found in cultivars released in the United States. However, if other novel translocations utilizing other rye sources are released, additional research would be required to identify and differentiate them. HPCE should, thus, be a valuable tool in identifying and developing wheat-rye translocation lines with improved processing quality.

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