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Associations among inbred lines of maize using electrophoretic, chromatographic, and pedigree data

1. Multivariate and cluster analysis of data from 'Lancaster Sure Crop' derived lines

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Summary. Associations among 18 'Lancaster Sure Crop' derived inbred lines of maize (*Zea mays* L.) were determined using multivariate and cluster analysis. Objectives were to assess the degree of unique characterization among lines afforded by reversed-phase high-performance liquid chromatography (RP-HPLC) and starch gel electrophoresis of allozymes and to compare associations among lines revealed by biochemical and pedigree data. RP-HPLC revealed 11 different chromatograms that uniquely identified 79% of lines that differed by more than isogenic or near isogenic segments. Allozymic data for 21 loci provided unique discrimination among 93% of non-isogenic lines. Chromatographic and allozymic data together provided unique characterization of all non-isogenic lines. Cluster and multivariate analyses of biochemical data associated lines into three groups that would have been expected on the basis of pedigree breeding records. More detailed associations were dependent upon the data set employed. Multivariate and cluster analysis of chromatographic, electrophoretic, and pedigree data could be useful in revealing more detailed associations among elite germplasm than hitherto available, thus providing data pertinent to line and hybrid development, plant variety protection, and germplasm security.

Key words: High performance liquid chromatography – Zeins – Allozymes – Plant variety protection – Germplasm security – Heterosis

Introduction

Accurate descriptions of inbred lines and of cultivated varieties are necessary in the production of pure foundation seed stocks, commercial varieties, and hybrids. Varieties and inbred lines must also be characterized to receive plant variety protection (PVP), thereby contributing to germplasm security. In addition, progress in plant breeding could be enhanced through a more complete knowledge of germplasm constitution and a more thorough understanding of relationships between lines (Hallauer and Miranda 1981). Such relationships can only be revealed by comparing descriptions of lines that are either genotypic per se or are directly reflective of the genotype.

Electrophoretic and chromatographic data have been shown to provide descriptions that are not significantly affected by environment and are, therefore, reflective of genotype (Batey 1984; Marchylo and Kruger 1984; Kruger and Marchylo 1985a, b; Smith and Smith 1986). In maize (*Zea mays* L.), electrophoresis of allozymes (Goodman and Stuber 1980, 1983; Stuber and Goodman 1983) and isoelectric focusing of zein storage proteins (Nucca et al. 1978; Wall et al. 1984; Wilson 1984) have allowed the unique characterization of at least 85% of widely used inbred lines (Stuber and Goodman 1983). Reversed-phase high-performance liquid chromatography (RP-HPLC) can also be used to reveal differences between inbred lines and hybrids of maize (Bietz 1983, 1985; Smith and Smith 1986). RP-HPLC has been reported to provide discrimination, at least between varieties of wheat (*Triticum aestivum* L., *T. durum* Desf.), that equals or even exceeds that obtained by other separation methods (Bietz 1983). Thus, RP-HPLC could also have great potential in characterizing inbreds and hybrids of maize. Furthermore, the discriminatory power of either electrophoresis or of RP-HPLC is enhanced when both methods are employed, thereby taking advantage of their complimentary modes of separation (Bietz 1983). The combined use of these techniques could perhaps provide discrimination between lines that are very closely related by pedigree. If such discrimination is possible, it is then feasible to investigate whether these biochemical data can be used to reveal associations among lines that are reflective of pedigree relationships. Associations revealed by biochemical data could then be of potential use in the more efficient choice of germplasm for breeding and hybrid development programs.

This study has three objectives. First, to assess the discriminatory power of RP-HPLC data alone. Second, to assess the degree to which discrimination is enhanced through the combination of RP-HPLC (zein) and allozymic data. Third, to compare associations revealed through comparisons of RP-HPLC (zein) and allozymic data with those to be expected on the basis of known pedigrees.

Materials and methods

For these initial studies, the analysis was done using inbred lines of current and historical importance which are related by pedigree. These inbreds were derived by selection directly from, or following hybridization to, lines selected from the open-pollinated variety 'Lancaster Sure Crop'. Ten publicly available inbred lines and eight lines that are proprietary to Pioneer Hi-Bred International, Inc. (Table 1) were surveyed by RP-HPLC and by isozyme electrophoresis. Protein extraction, chromatographic conditions, data acquisition, and normalization procedures for RP-HPLC were exactly those given in Smith and Smith (1986). Inbred Oh43t was injected thrice during the course of separations as a control. Following normalization of peak area data, 52 different peaks were revealed across the 18 lines. Identical peaks were defined as such if they eluted within 3sd units (7 s) as calculated from means of elution times for all control peaks. Comparisons between the chromatograms of each pair of lines resulted in two data matrices; one weighted according to peak area percent and the other unweighted. Associations among lines were examined using principal component and cluster analysis based on variance-covariance matrices from both weighted and unweighted data matrices.

For the inbred lines H98 and Oh40B, allozymic data for all loci (Table 2) except for *Dial* were taken from Stuber and Goodman (1983). All other allozymic data were determined from starch gel electrophoresis using methods similar to Cardy et al. (1980, 1983) with only minor modifications according to Smith (1984). Associations among lines were determined from principal coordinate (Gower 1972) and cluster analysis based on distance measures using modified Roger's distances calculated from allozyme allele frequencies. Similar treatments of allozymic data in maize have been performed by Doebley et al. (1983) and by Goodman and Stuber (1983).

Calculations of coefficients of parentage (s) were used to derive pedigree dissimilarity (1-s) (Kempthorne 1969; Delannay et al. 1983). Relationships between lines on the basis of pedigree dissimilarity or pedigree distance were then revealed by principal coordinate analysis and by cluster analysis.

Results

Representative examples of similar chromatographic profiles are presented in Fig. 1. These plots, which were not standardized for equivalent amounts of protein across inbreds, revealed only minor differences between the following near isogenic lines; Oh43 and Oh43t at approximately 5 min (Fig. 1 a), 247 and 247nonHt at approximately 27 min (Fig. 1 b), and O41wx and either

Table 1. Lines used in the analysis of pedigree, electrophoretic, and chromatographic data

Line	Background[a]
Group 1PED	
Oh40B	Lancaster Sure Crop[b]
Oh43	Oh40B × W8 (probably from Minnesota[b]; Baker, pers. commun.)
Oh43t	(Oh43 × Ht[c]) with multiple backcrosses to Oh43
Oh45	Oh40B × W8
247nonHt	Oh43 × Oh45
247	(247 × Ht[c]) with multiple backcrosses to 247nonHt
Va26	Oh43 × K155
A619	(A171 × Oh43) Oh43
H98	Oh43 × Hy
693	247 × Coker 616
Group 2PED	
O41nonHt	Oh43 × (Iodent[b] × Wf9)
O41	(O41 × Ht[c]) with multiple backcrosses to O41nonHt
O41wx	(O41 × wx[d]) with multiple backcrosses to O41nonHt
699	(O41 × 247) O41
Group 3PED	
C103	Noah Hershy Strain of Lancaster Sure Crop
CI.187-2	Krug[b]
Mo17t	187-2 × C103 (a source of Mo17 with Ht resistance)
375	Approx. 60% C103, 25% B14, 12% M3204

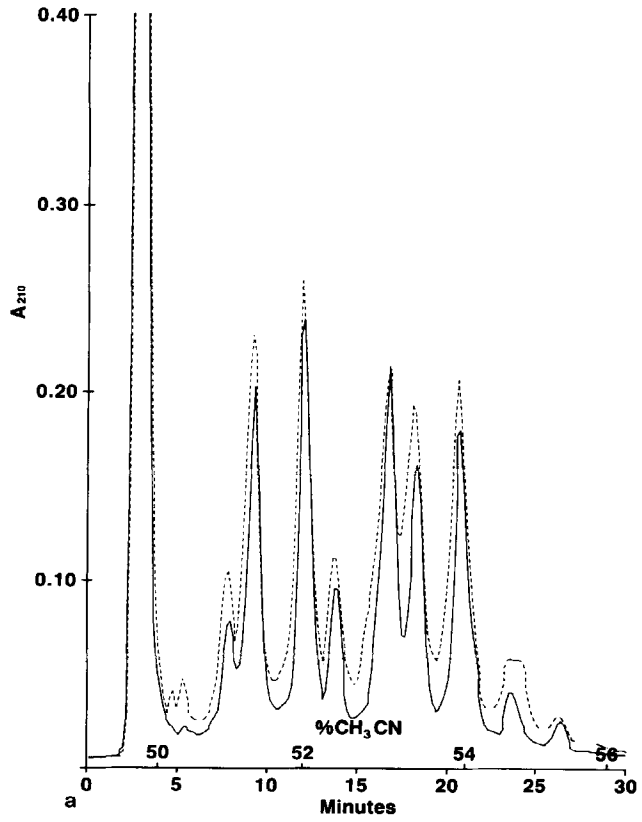
[a] Henderson (1976) unless otherwise stated
[b] Open-pollinated variety
[c] Source of resistance to *Helminthosporium turcicum*
[d] Source of waxy gene

Table 2. Allozyme genotypes of inbred lines used in the present study

Line	Allele[a] at locus																				
	Acp1	*Adh1*	*Cat3*	*Dia*	*E8*	*Glu1*	*Got1*	*Got2*	*Got3*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Mdh4*	*Mdh5*	*Mmm*	*Pgd1*	*Pgd2*	*Pgm1*	*Pgm2*	*Phi*
Oh40B	4	6	9	8	4	7	4	4	4	4	4	1	3	16	12	12	M	3.8	5	9	4	4
Oh43	4	4	9	12	4.5	6	4	4	4	4	6	6	6	16	12	12	M	3.8	5	9	4	4
Oh43t	4	4	9	12	4.5	6	4	4	4	4	6	6	6	16	12	12	M	3.8	5	9	4	4
Oh45	4	4	9	8	4.5	6	4	2	4	4	4	1	6	18	12	12	M	3.8	5	9	4	4
247nonHt	4	4	9	8	4.5	6	4	2	4	4	4	1	6	18	12	12	M	3.8	5	9	4	4
247	4	4	9	8	4.5	6	4	2	4	4	4	1	6	18	12	12	M	3.8	5	9	4	4
Va26	4	4	9	8 (12)	4.5	6	4	2	4	4	6	6	6	16	12	12	M	3.8	5	9	4	4
A612	4	4	9	12	4	6	4	4	4	4	6	6	3	16	12	12	M	3.8	5	9	4	4
H98	2	4	9	8	4.5	6	4	4	4	4	4	6	3.5	16	12	12	M	3.8	5	9	4	4
693	4	4	9	8	4.5	6	4	2	4	4	4	6	6	16	12	12	M	3.8	5	9	4	4
O41nonHt	4	4	9	12	4.5	6	4	4	4	4	6	6	6	16	12	12	M	2	5	9	4	4
O41	4	4	9	12	4.5	6	4	4	4	4	6	6	6	16	12	12	M	2	5	9	4	4
O41wx	4	4	9	12	4.5	6	4	4	4	4	6	6	6	16	12	12	M	2	5	9	4	4
699	4	4	9	12 (8)	4.5	6	4	4 (2)	4	4	6 (4)	6 (1)	6	16 (18)	12	12	M	2 (3.8)	5	9	4	4
C103	3	4	9	8	4	6	4	4	4	4	4	6	6	16	12	12	M	3.8	5	9	4	4
CI.187-2	2	4	9	8	4.5	6	4	4	4	4	6	6	6	16	12	12	M	3.8	5	9	8	4
Mo17t	2	4	9	8	4	6	4	4	4	4	4	6	6	16	12	12	M	3.8	5	9	8	4
375	3	4	9	8	4	6	4	4	4	4	6	1	6	18	12	12	M	3.8	5	9	4	4

[a] Numerical designations are those used by Stuber and Goodman (1983). All loci were fixed except for those where the allele of least frequency is given in parentheses. These frequencies (f) were: for Va26, *Dia1–12* f=0.08; for 699, *Dia1–8* f=0.17, *Got2–2* f=0.08, *Idh2–4* f=0.04, *Mdh1–1* f=0.17, *Mdh3–18* f=0.08, *Pgd1–3.8* f=0.17

Fig. 1. a Chromatograms of Oh43 (broken line) and Oh43t (solid lines). The solvent front elutes prior to 5 min in all chromatograms. **b** Chromatograms of 247nonHt (broken line) and 247 (solid line). **c** Chromatograms of O41wx (small broken line), O41t (large broken line), and O41 (solid line). **d** Chromatograms of 699 (broken line) and O41 (solid line)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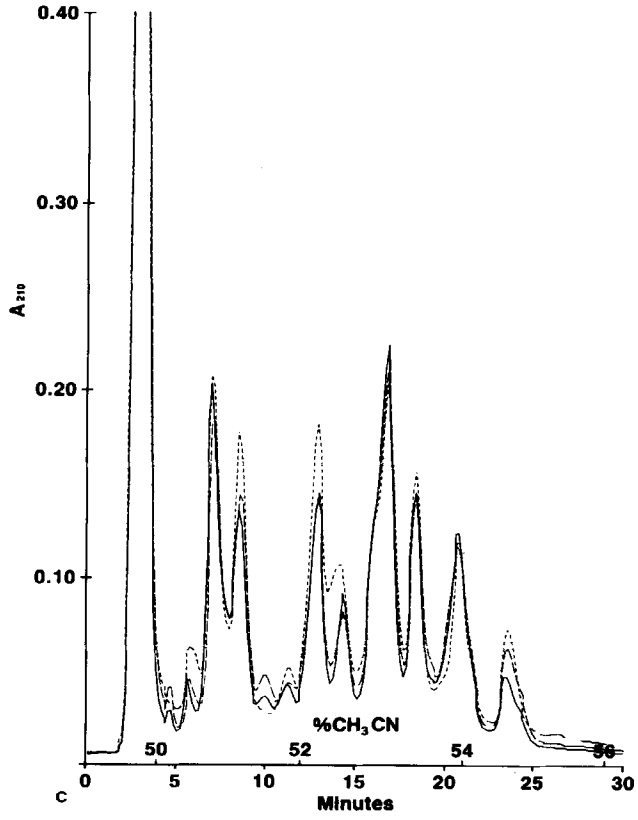
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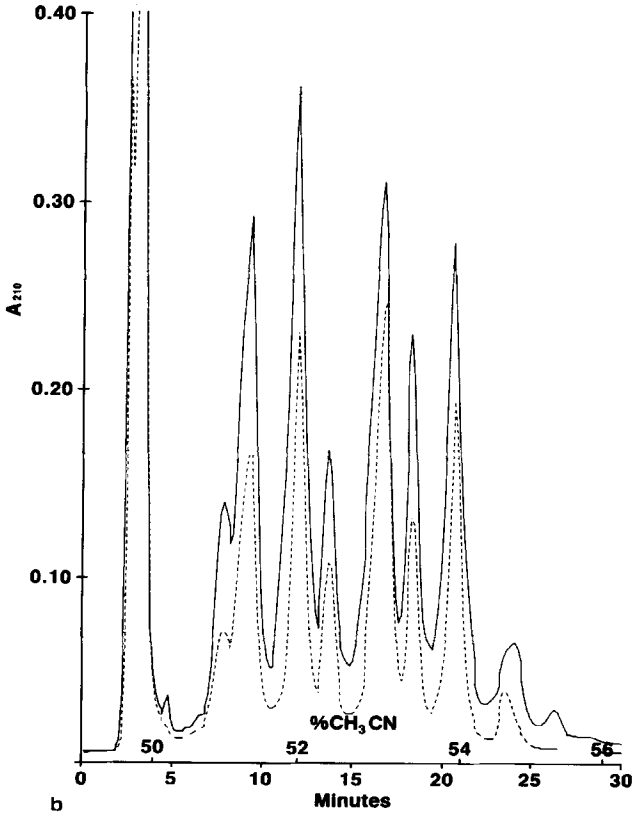
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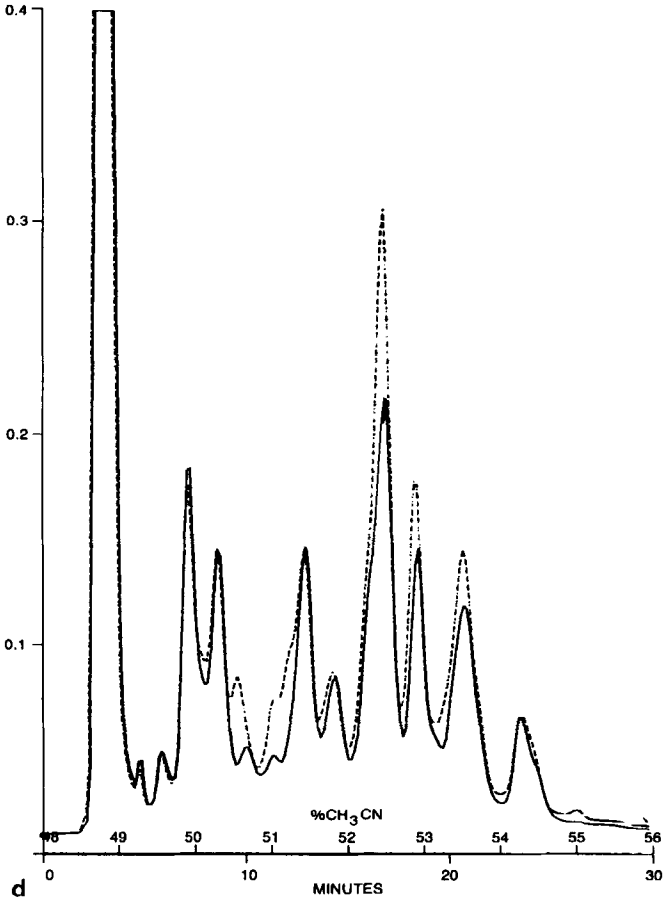
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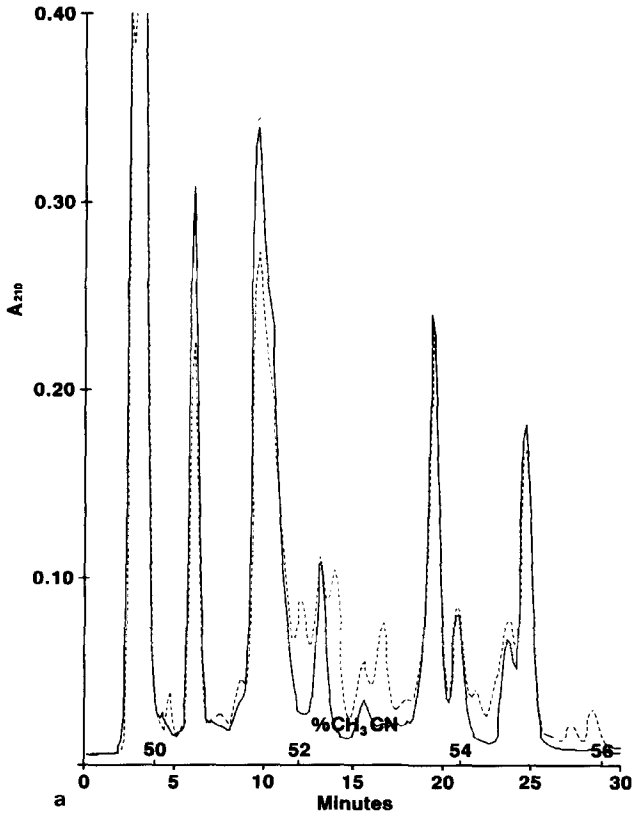
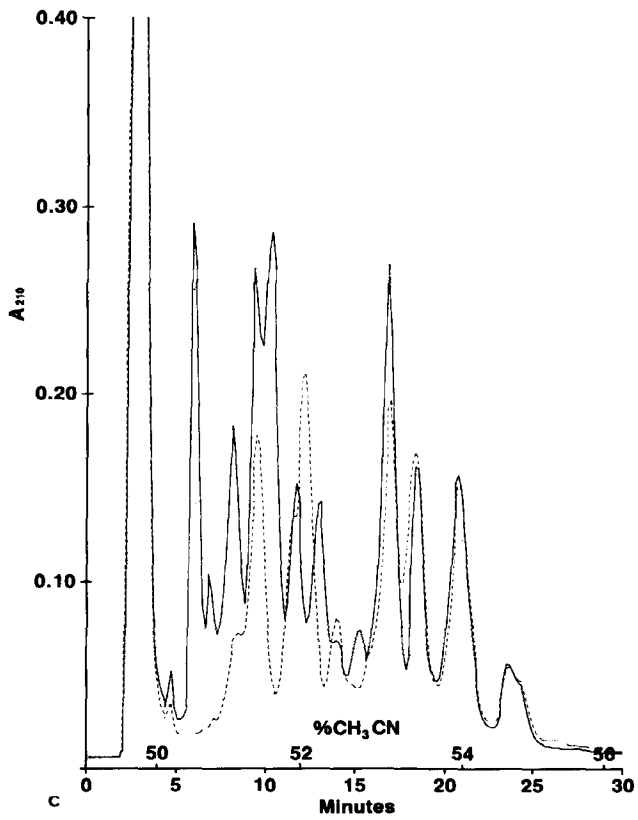
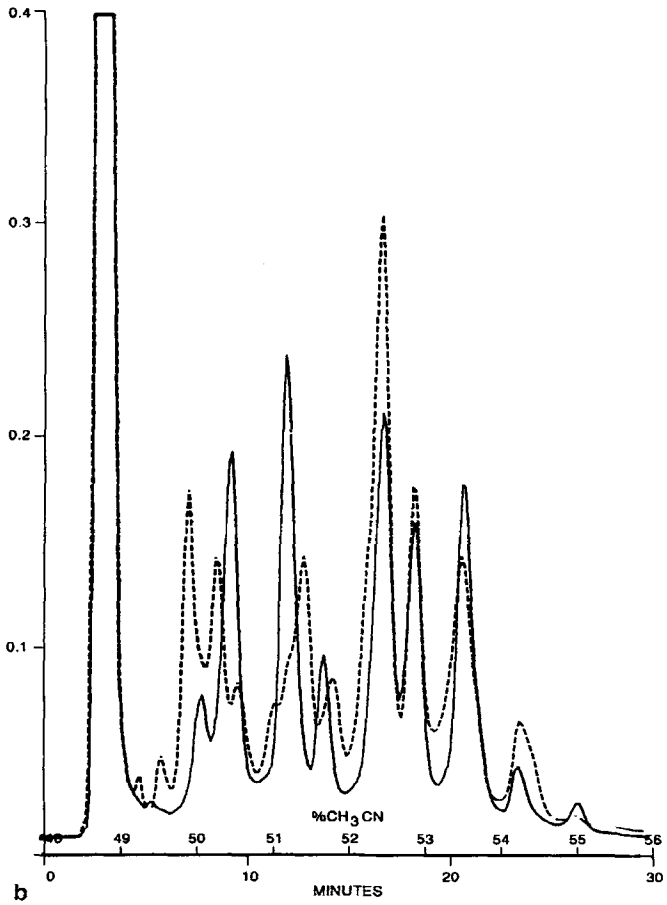






O41 or O41t at approximately 10 min (Fig. 1 c). The chromatograms of lines O41 and 699 (Fig. 1 d) were also similar but differed between 9 and 11 min.

Examples of different chromatographic profiles are presented in Fig. 2. Comparisons of the profiles of lines 375 and C103 (Fig. 2 a) revealed many similarities, particularly for the elution of major peaks; however, differences were obvious at approximately 12, 14, 17, 22, 27, and 28 min. Comparisons of the chromatograms of Oh43 and 699 (Fig. 2 b) and of Oh45 and 693 (Fig. 2 c) revealed totally different elution profiles from 0 to 15 min with strikingly similar profiles thereafter.

Visual inspections of all chromatograms revealed two sets of lines with closely similar, if not identical, profiles. The first set comprised 247nonHt, 247, A619, Oh43 and Oh43t. The second set comprised O41, O41t, O41wx and 699. Lines from among these two different sets revealed mutually distinct chromatograms as did all other lines.

Three groups of lines had identical allozyme profiles for the 22 loci that were surveyed: 1) O41nonHt, O41, O41wx; 2) Oh43, Oh43t; and 3) 247nonHt, 247 and Oh45.

Fig. 2. **a** Chromatograms of 375 (broken line) and C103 (solid line). **b** Chromatograms of 699 (broken line) and Oh43t (solid line). **c** Chromatograms of Oh45 (broken line) and 693 (solid line)

Pedigree backgrounds are presented in Table 1 and the expected relationships on the basis of these data are presented in Fig. 3. Principal coordinates 1, 2, and 3, encompassed 31.3, 15.1, and 10.7% of the variation, respectively (Fig. 3 b). Lines were included in three broad groups, the first two of which were related. Group 1PED included Oh45, Oh40B, 247, Va26, Oh43, Oh43t, A619, 247nonHt, 693, and H98. Group 2PED included O41nonHt, O41wx, O41, and 699. Group 3PED included Mo17t, C103, 375, and CI.187-2. Both cluster analysis (Fig. 3 a) and principal coordinate analysis (Fig. 3 b) separated H98 and 693 from other Group 1PED lines. Cluster analysis (Fig. 3 a) further subdivided Group 1PED lines into a) Oh45, Oh40B, 247 and b) Va26, Oh43, Oh43t, A619, and 247nonHt. Both analytical procedures clearly separated Group 3PED lines from Group 1PED and 2PED.

Associations among lines on the basis of the RP-HPLC data sets are presented in Fig. 4 (unweighted data set) and in Fig. 5 (weighted data set). Principal components 1, 2, and 3 encompassed 75.8, 6.3, and 4.4% of total variation for the unweighted RP-HPLC data and 52.4, 17.0, and 10.4% of the total variation for the weighted RP-HPLC data, respectively. On the basis of the unweighted RP-HPLC data set (Fig. 4) lines fell into three groups that were exactly those revealed on the basis of pedigree data except that H98 and Va26 clustered, albeit loosely, with Group 3PED lines rather than with Group 1PED lines. Like the pedigree data, the RP-HPLC unweighted data set revealed an association among the majority of Group 1PED lines and all group 2PED lines that was greater than either showed to Group 3PED lines.

Associations revealed on the basis of the weighted zein data set (Fig. 5) are similar to those described for the unweighted set (Fig. 4). Both weighted and unweighted sets revealed two clusters: a) Oh43t, 247, A619, 247nonHt, Oh43 (lines comprising Group 1PED) and b) O41nonHt, O41, O41wx, 699 (lines comprising Group 2PED). In the weighted RP-HPLC data set, Group 3PED lines were separated into a) CI.187-2, Mo17t and b) 375 and C103, the latter being more closely associated with Oh45, Oh40B, and Va26. These levels of association contrast with those revealed by both the RP-HPLC unweighted and pedigree data sets.

Associations among lines on the basis of allozyme data are presented in Fig. 6. Principal coordinates 1, 2, and 3 encompassed 36.0, 21.7, and 14.1% of the total variation, respectively. Group 2PED lines (O41wx, O41t, O41, 699) were tightly clustered (Fig. 6 a) and revealed a close association with Oh43, Oh43t, and A619 (3 lines of Group 2PED), (Fig. 6 a, b). Three other lines of Group 1PED (Oh45, 247, 247nonHt) were tightly clustered and were joined to two other Group 1PED lines (Va26, 693). Group 3PED lines (375, Mo17, C103, CI.187-2) were loosely clustered, also including H98 from Group 1PED. Group 3PED lines and H98 were not closely related to Group 2PED lines or to other Group 1PED lines.

Discussion

Reversed-phase high-performance liquid chromatography (RP-HPLC) revealed a high degree of protein heterogeneity (52 hydrophobically different proteins) among 18 lines that are at least somewhat, and often exceptionally, closely related by pedigree. RP-HPLC revealed 11 qualitatively different chromatographic profiles among the 18 lines; differences of a magnitude greater than that observed for purely nongenotypic effects (Smith and Smith 1986). Of the nine lines included in two classes with qualitatively similar chromatograms [a) A619, Oh43, Oh43t, 247nonHt, 247 and b) O41nonHt, O41, O41wx, and 699], all were related by pedigree. Oh43 and 247 share an approximately 50% pedigree relationship while Oh43 and A619 share an approximately 75% pedigree relationship (Table 1). The remaining lines are presumably more closely related to their respective and chromatographically similar recurrent parents through multiple backcrosses. Excluding the isogenic or near isogenic lines (Oh43t, 247, O41wx, O41), 11 different chromatograms were revealed among the 14 remaining lines. Thus, RP-HPLC alone provided unique distinction among 79% of 'Lancaster Sure Crop' and derived lines.

Allozymic data (Table 2) also revealed much heterogeneity among these inbred lines. Thirteen different allozyme profiles were revealed among the 18 lines, thus, uniquely identifying 72% of all lines. Once again, this is an underestimate of the discriminatory power of allozymes because of the inclusion of isogenic or near isogenic lines and their recurrent parent lines. Of the 14 lines that were not isogenic or near isogenic conversions, 13 revealed different allozymic profiles. Thus, allozymic data alone allowed the unique identification of 93% of 'Lancaster Sure Crop' and related lines.

Allozymic and chromatographic data were jointly able to provide unique characterization of all 14 lines that were more than isogenically different. Oh45 and 247nonHt, which revealed allozymic identicality at 22 loci, differed quantitatively in their chromatograms to an extent that at least equaled the maximum difference, and exceeded the majority of differences, found for nongenotypic effects on chromatograms (Smith and Smith 1986). Likewise, allozymic data revealed differences between lines that exhibited identical or nearly identical chromatograms, thereby distinguishing between individual lines of i) 247, A619, and Oh43 and ii) O41 and 699. Thus, the products of allozymic and zein gene loci were able to provide discrimination between all 'Lancaster Sure Crop' lines of major importance in U.S. maize production and breeding that were available for study and that differed by more than isogenic and closely linked gene segments.

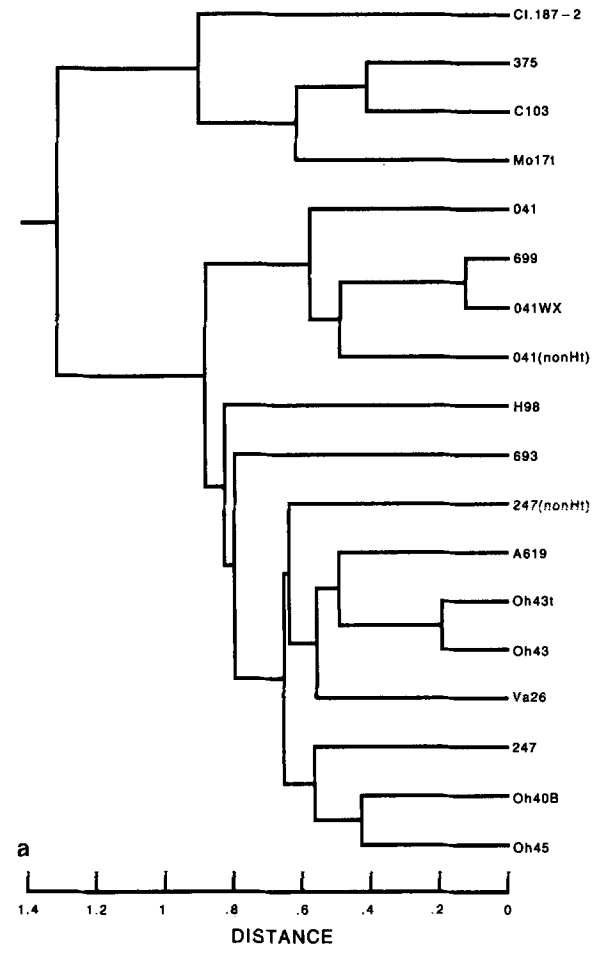
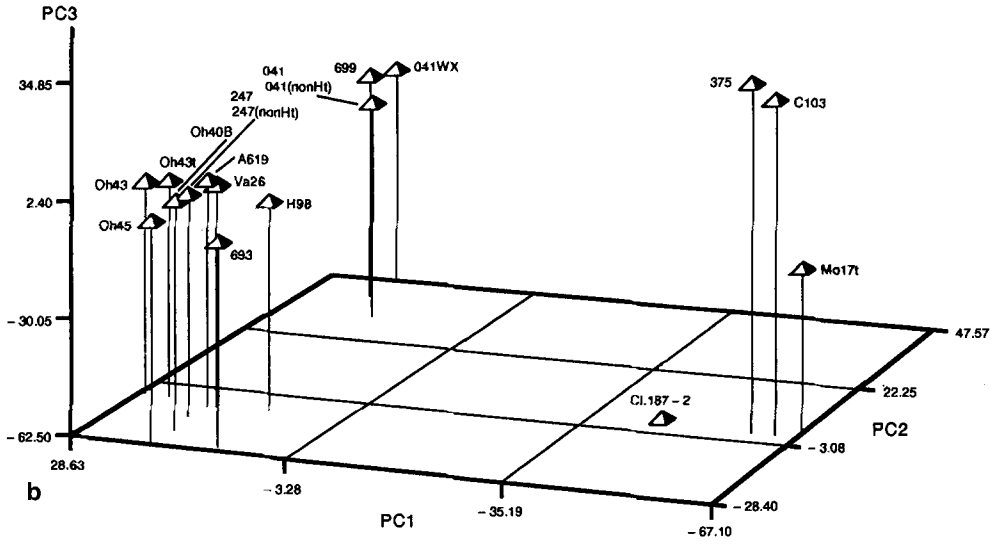


Fig. 3. a Dendrogram revealing associations between lines following cluster analysis of pedigree data. **b** Associations between lines on the basis of the first 3 principal coordinates from multivariate analysis of pedigree data



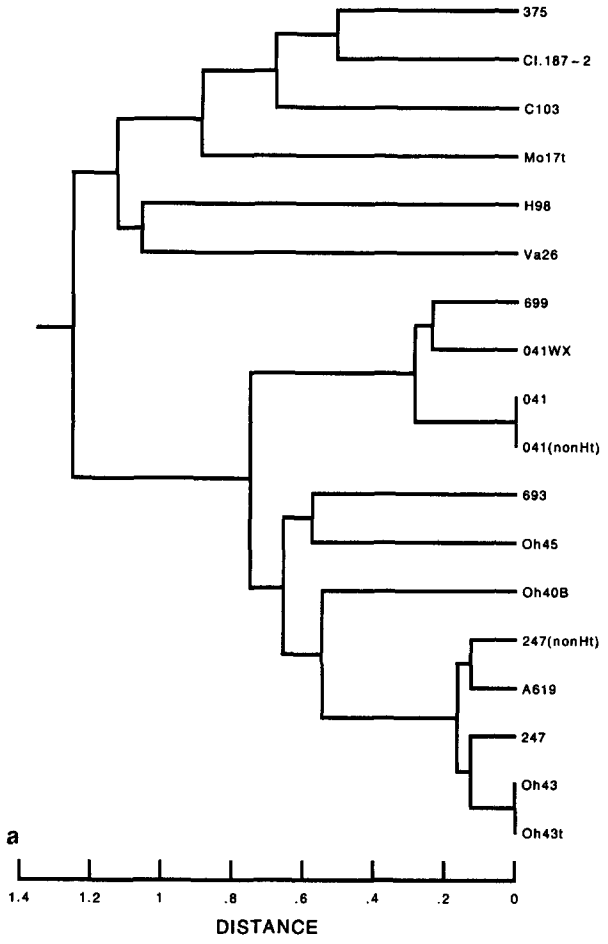
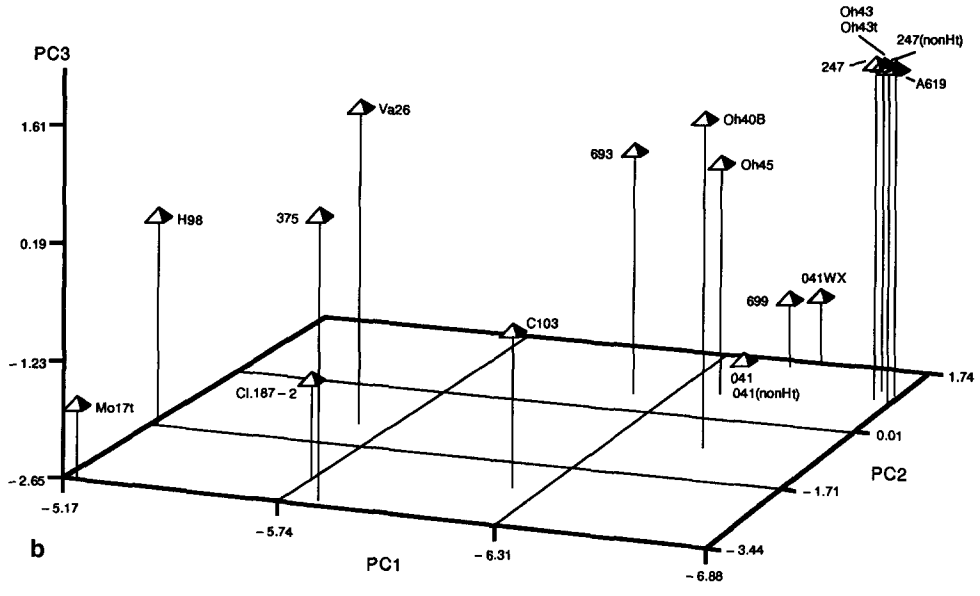


Fig. 4. a Dendrogram revealing associations between lines following cluster analysis of unweighted chromatographic data. **b** Associations between lines on the basis of the first 3 principal components from multivariate analysis of unweighted chromatographic data



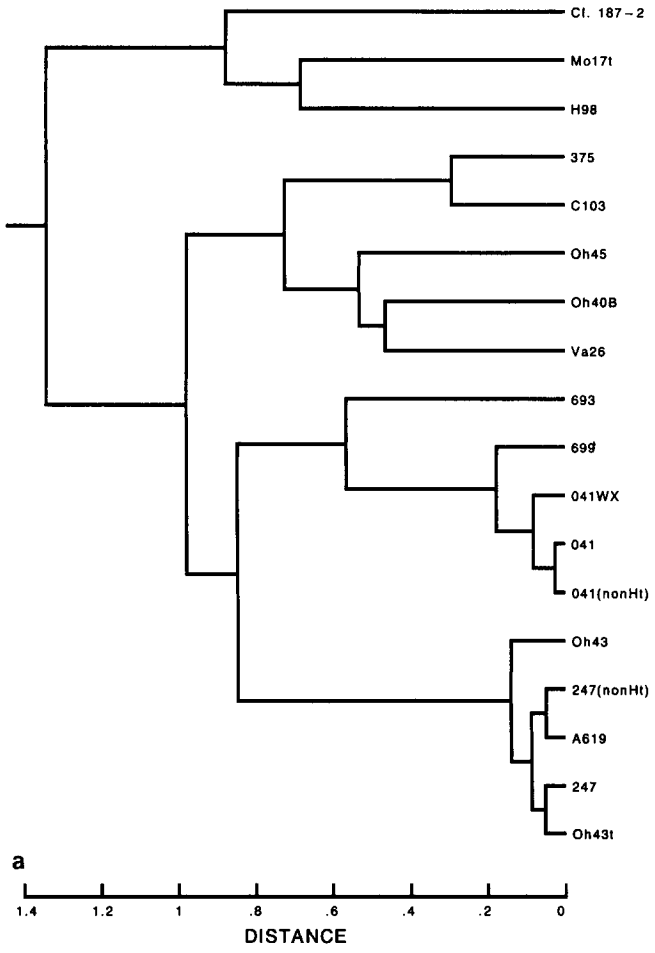
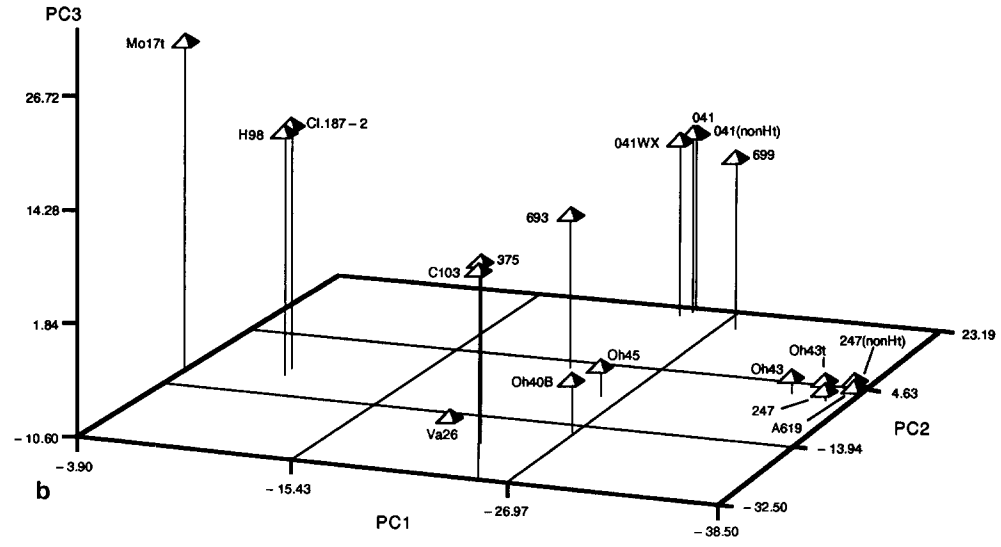


Fig. 5. **a** Dendrogram revealing associations between lines following cluster analysis of weighted chromatographic data. **b** Associations between lines on the basis of the first 3 principal components from multivariate analysis of weighted chromatographic data



Cluster and multivariate analyses of all data sets showed overall similarities in associations among lines. The majority of lines related by pedigree to Oh43 (Groups 1PED and 2PED) were shown to be closely associated among themselves and to be distantly associated with lines connected by pedigree to C103 and CI.187-2 (Group 3PED). Among the Oh43 related lines, all data sets partitioned O41nonHt, O41, O41wx, and 699 (Group 2PED) as a separate cluster. Closer examination of more detailed associations among lines revealed some differences according to the data set used for analysis. Pedigree and RP-HPLC unweighted data were unable to reveal a close association of O41 and 699 lines with any particular group 1PED line. However, both allozymic and RP-HPLC weighted data revealed an association of O41 and 699 with Oh43 and A619, and Oh43, A619, and 247, respectively. Analysis of pedigree data revealed the strict clustering of C103 and CI.187-2 related lines (Group 3PED) and their separation from all other 'Lancaster Sure Crop' lines. Analyses of allozymic and RP-HPLC unweighted data sets also revealed similar groupings but included H98, and H98 and Va26, respectively. The RP-HPLC weighted data set indicated a grouping of CI.187-2, Mo17t, and H98. However, in contrast to allozymic and RP-HPLC unweighted data, the RP-HPLC weighted data separated C103 and 375 from CI.187-2 with C103 and 375 instead being aligned more closely with Oh40B, Oh45, and Va26.

Comparisons of associations among lines revealed by allozymic and chromatographic data, which are essentially genotypic comparisons, therefore, support a broad perspective of

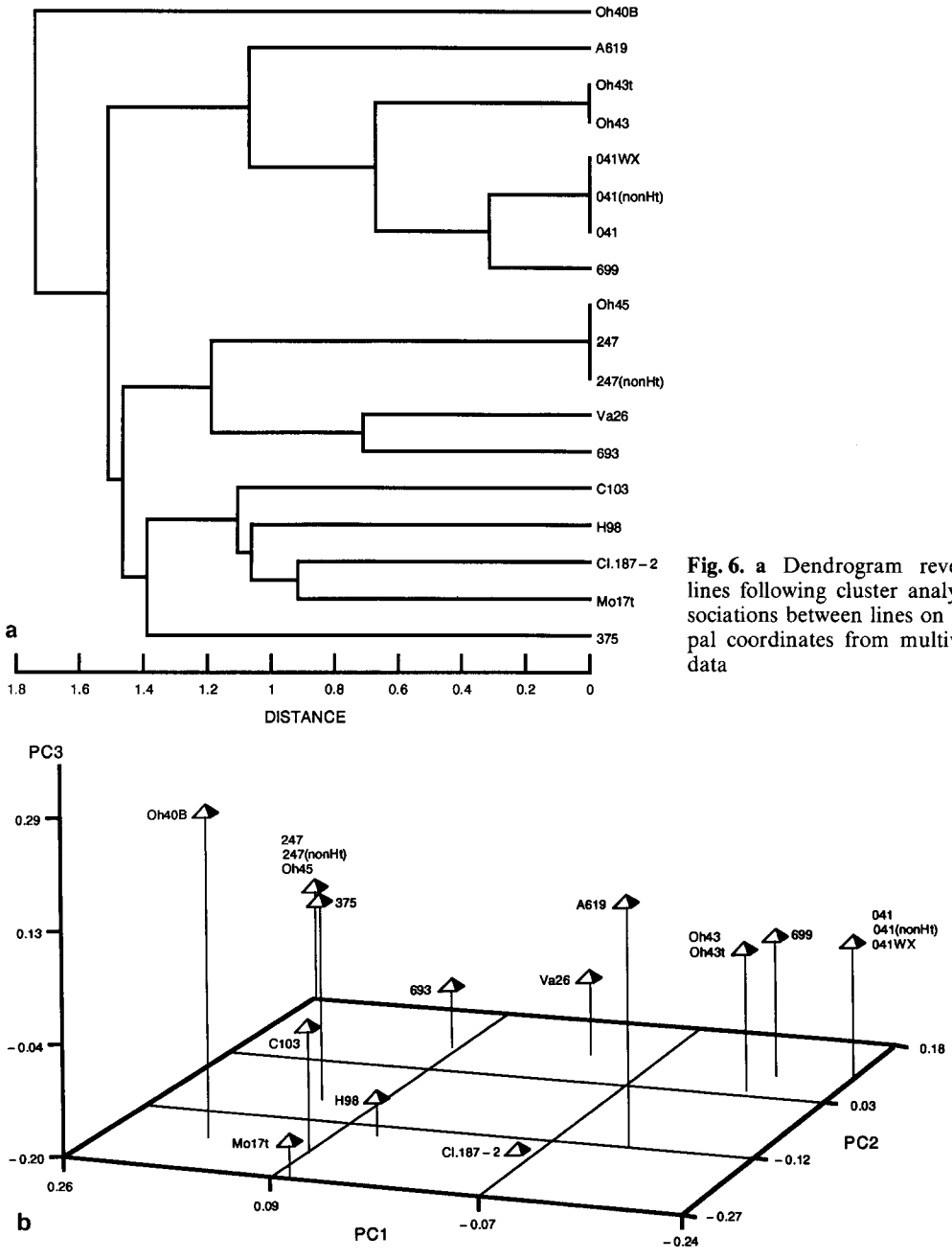


Fig. 6. **a** Dendrogram revealing associations between lines following cluster analysis of allozymic data. **b** Associations between lines on the basis of the first 3 principal coordinates from multivariate analysis of allozymic data

what could be expected in terms of relationships based solely upon a knowledge of pedigrees. For example, isozymic and chromatographic data consistently showed a separation of lines O41 and 699 (Group 2PED) from Oh43 and other group 1PED lines. Thus, biochemical data substantiated what could be expected from pedigree records that Group 2PED lines contain non 'Lancaster Sure Crop' germplasm and, thereby, constitute a distinguishable cluster of lines. A second example of associations revealed by all data sets was the separation of Group 1PED and Group 2PED lines from Group 3PED lines. On the basis of pedigree information alone, the lack of association among group 3PED lines could be, in part, an artifact simply because these lines have no recent connection through pedigree breeding. However, both isozymic and chromatographic data substantiate that there are differences between the C103 and Oh43 selections of 'Lancaster Sure Crop' germplasm.

The results presented herein revealed a level of congruence between allozymic, chromatographic, and pedigree data that allowed inbreds of the 'Lancaster Sure Crop' germplasm pool to be split further into at least three groups. Differences in more detailed associations among lines that are revealed by the various data sets are to be expected since each data set surveys a portion of the genome. Generally, the weighted RP-HPLC data set was able to provide greater discrimination and more detailed associations among lines than was the unweighted RP-HPLC data set. The critical importance or validity of such differences can only become apparent after additional comparisons in which a greater portion of the genome is surveyed either through tests involving genotype × genotype interaction (heterosis) or linkage studies involving vastly more genetic markers (e.g., restriction fragment length polymorphisms, RFLP's). Chromatographic, electrophoretic, and pedigree data could be useful as preliminary screens in tests of germplasm associations prior to these more detailed, time consuming, and costly studies.

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References

Batey IL (1984) Wheat varietal identification by rapid ion-exchange chromatography of gliadins. J Cereal Sci 2:241–248

Bietz JA (1983) Separation of cereal proteins by reversed-phase high-performance liquid chromatography. J Chromatogr 155:219–238

Bietz JA (1985) High performance liquid chromatography: How proteins look in cereals. Cereal Chem 62:201–212

Cardy BJ, Stuber CW, Goodman MM (1980) Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Department of Statistics, Mimeo Ser No 1317, North Carolina State University, Raleigh NC

Cardy BJ, Stuber CW, Wendel JF, Goodman MM (1983) Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Department of Statistics, Mimeo Ser No 1317 Revised, North Carolina State University, Raleigh NC

Delannay X, Rodgers DM, Palmer RG (1983) Relative genetic contributions among ancestral lines to North American soybean cultivars. Crop Sci 23:944–949

Doebley JF, Goodman MM, Stuber CW (1983) Isozyme variation in maize from the southwestern United States: taxonomic and anthropological implications. Maydica 28:97–120

Goodman MM, Stuber CW (1980) Genetic identification of lines and crosses using isoenzyme electrophoresis. Ann Corn and Sorghum Res Conf Proc 35:10–31

Goodman MM, Stuber CW (1983) Maize. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding, Part B. Elsevier, Amsterdam, pp 1–34

Gower JC (1972) Measures of taxonomic distance and their analysis. In: Weiner JS, Huizina J (eds) The assessment of population affinities in man. Oxford University Press, London, pp 371–380

Hallauer AR, Miranda FO (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames IA

Henderson CB (1976) Maize research and breeders manual No 8. Illinois Foundation Seeds Inc, Champaign IL

Kempthorne O (1969) An introduction to genetic statistics. Iowa State University Press, Ames IA

Kruger JE, Marchylo BA (1985a) Selection of column and operating conditions for reversed-phase high-performance liquid chromatography of proteins in Canadian Wheat. Can J Plant Sci 65:285–298

Kruger JE, Marchylo BA (1985b) Examination of the mobilization of storage proteins of wheat kernels during germination by high-performance reversed-phase and gel permeation chromatography. Cereal Chem 62:1–5

Marchylo BA, Kruger JE (1984) Identification of Canadian barley cultivars by reversed-phase high-performance liquid chromatography. Cereal Chem 61:295–301

Nucca R, Soave C, Motto M, Salamini T (1978) Taxonomic significance of the zein isoelectric focusing pattern. Maydica 23:239–249

Smith JSC (1984) Genetic variability within U.S. hybrid maize: multivariate analysis of isozyme data. Crop Sci 24:1041–1046

Smith JSC, Smith OS (1986) Environmental effects on zein chromatograms of maize inbred lines revealed by reversed-phase high-performance liquid chromatography. Theor Appl Genet 71:607–612

Stuber CW, Goodman MM (1983) Allozyme genotypes for popular and historically important inbred lines of corn, *Zea mays* L. USDA Agric Res Serv, No 16, 28 pp

Wall JS, Fey DA, Paulis JW (1984) Improved two-dimensional electrophoretic separation of zein proteins: application to study of zein inheritance in corn genotypes. Cereal Chem 61:141–146

Wilson CM (1984) Isoelectric focusing of zein in agarose. Cereal Chem 61:198–200