Identification of U.S. Rice Cultivars by High-Performance Liquid Chromatography[1]

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ABSTRACT

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Reversed-phase high-performance liquid chromatography (HPLC) of prolamins was used to differentiate 29 rice cultivars including all major commercial long- (15), medium- (11), and short-grain (3) types produced in the United States. U.S. long-grain rice cultivars were segregated by characteristic peak patterns, positions, and intensities into one group of six and one of five cultivars plus four unique, nongrouped cultivars. Three of those long-grain types were international rices that also had unique prolamin HPLC patterns. Medium-grain cultivars were also separated into four groups consisting of two sets of three, one set of two, and three cultivars each with distinctly different prolamin patterns, whereas the HPLC patterns were unique for each of the three short-grain cultivars. Rice cultivars and genotypes separated into groups by HPLC had a common ancestor in their genetic backgrounds. However, all 29 were differentiated by their prolamin HPLC patterns and grain type.

Key words: *Oryza sativa*, Pedigree comparison

Cultivar identification of cereal grains is commercially important. In the field, cultivars are identified by their agronomic and morphological properties. Physical properties such as size, shape, and grain color are used by the Federal Grain Inspection Service (FGIS) to identify grain in commerce. However, the use of exotic germ plasms and interclass breeding to improve disease resistance, yield, and protein content has led to cultivars with heterogeneous kernel characteristics that are not easily identifiable.

Two methods, polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography (HPLC), have been developed and used in the last 10 years to accurately identify cultivars of cereal grains including wheat (Bushuk and Zillman 1978, Jones et al 1982, Lookhart et al 1982, Bietz 1984), oats (Robert et al 1983, Von Ohms 1981, Lookhart 1985, Lookhart and Pomeranz 1985), barley (Marchylo et al 1984), and corn (Wilson 1985, Smith and Smith 1986). In particular, separation of prolamin proteins by those methods produces characteristic genotypic fingerprints for most cultivars.

Identification of rice cultivars by applying various electrophoretic techniques to globulin and glutelin fractionations have been reported (Monod et al 1972; Du Cros et al 1979; Padhye and Salunkhe 1979; Shadi and Djurtoft 1979; Kim and Jo 1983; Kusama et al 1984; Sarkar and Bose 1984, 1985; Glaszmann 1986).

Three subspecies of Chinese rice have also been differentiated by the content and chemical structure of starch in the grain (Juliano 1972) and by electrofocusing of prolamins (Guo et al 1986). The subspecies investigated were Indica (long-grained, nonglutinous), Japonica (round-grained, nonglutinous), and glutinous. Iwasaki and co-workers (1982) investigated differences in the albumin and globulin extracts between long-, medium-, and short-grain rices. Differences in the storage proteins between round (Guang-Ji 9) and long (Au-Jian) rice seeds were found by SDS-PAGE analysis (Zhao and Boulter 1985). Milled rice grains contain about 80% starch and 7–8% protein, which is composed of about 80% glutelins, 10% globulins, 5% albumins, and less than 5% prolamins.

Genetic improvement of rice cultivars would be facilitated by cultivar identification through the analysis of the prolamins or other storage proteins. Consequently, the prolamins from milled rice of 29 major U.S. rice cultivars extracted with 70% ethanol were analyzed by reversed-phase HPLC to test the ability of this method to differentiate rice genotypes. All long-, medium-, and short-grained rices analyzed were differentiated from each other.

MATERIALS AND METHODS

Rice Samples

The 29 milled rice (*Oryza sativa* L.) samples analyzed were grown in 1983 in the Uniform Rice Performance Nursery at Beaumont, TX. They included all the major commercial long-, medium-, and short-grain types produced in the United States; 15 were long-grain types (Table I), 11 medium-grain (Table II), and three short-grain (CAMO, Nortai, and S-201). Three of the long-grain types were international rices.

The samples were hand-harvested at 18–22% moisture, cleaned, and dried slowly with heated air (30–38° C) for approximately 36 hr to a storage moisture content of 12.5 ± 0.5%. Rices from each cultivar were dehulled in a Satake laboratory rubber roll sheller and were milled on an experimental mill (Webb et al 1986).

Milling yield, protein, physical properties, and end-use properties for all cultivars were reported by Webb et al (1986).

TABLE I
Long-Grain Rice Samples Analyzed and Cultivar Subgroups Segregated on the Basis of Chromatographic Similarities

Grain Type and Cultivar	Subgroup
U.S. long-grain	
Bellemont	A
BN-73	A
Bond	C
Labelle	A
Leah	B
Lebonnet	B
Lemont	B
L-201	B
Newbonnet	A
Newrex	A
Skybonnet	B
Starbonnet	A
International long-grain	
CICA-6	D
CICA-8	E
IR-36	F

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Chemicals and Reagents

Acrylamide, *N,N'*-methylenebisacrylamide, ascorbic acid, Coomassie brilliant blue R-250, methyl green, and trichloroacetic acid were obtained from Sigma Chemical Company (St. Louis, MO), trifluoroacetic acid (TFA) from Sigma or from Pierce (Rockford, IL), lactic acid (USP grade) and ferrous sulfate heptahydrate (AR grade) from Mallinckrodt Chemicals (St. Louis, MO), and LC grade ethanol and acetonitrile from Burdick and Jackson Laboratories (Muskegon, MI). Hydrogen peroxide (3% practical grade) was purchased from a local pharmacy. Water was purified by passage, in series, through two mixed-bed ion-

TABLE II
Medium-Grain Rice Samples and Cultivar Subgroups Segregated on the Basis of Chromatographic Similarities

Cultivar	Subgroup
Brazos	A
LA-110	C
Mars	B
M-9	F
M-201	F
Nato	A
Nova 76	B
Pecos	D
RU 82011199	E
Saturn	B
Vista	A

TABLE III
Linear Gradient Program for Separation of Rice Prolamins by High-Performance Liquid Chromatography[a]

	Time (min)					
Solvent	0	5	10	17	18	19
% A[b]	25	35	50	75	85	25
% B[c]	75	65	50	25	15	75

[a] Using a 25 cm × 4.1 SynChropak RP-P column.
[b] Solvent A = Acetonitrile containing 0.1% trifluoroacetic acid.
[c] Solvent B = Water containing 0.1% trifluoroacetic acid.

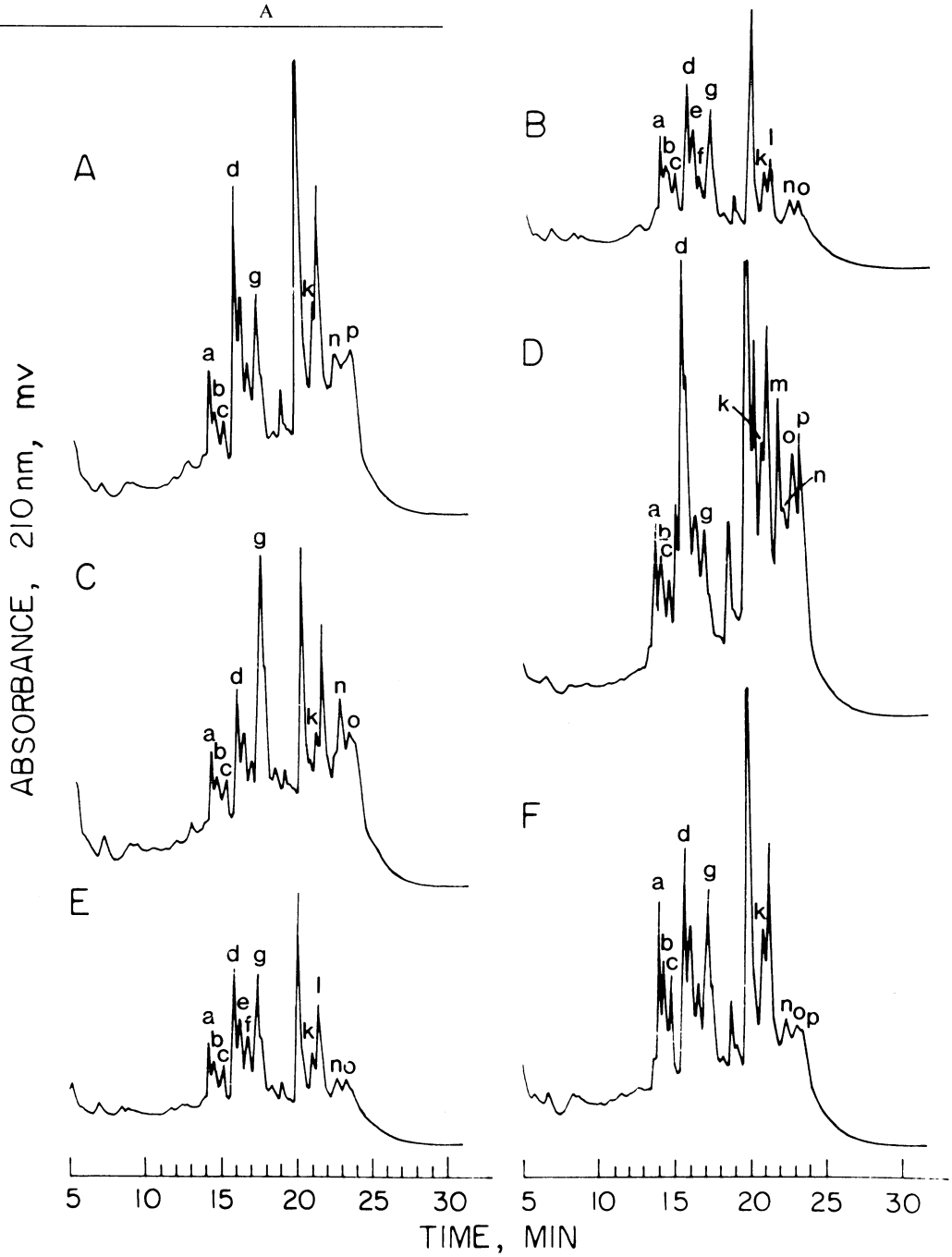

Fig. 1. High-performance liquid chromatographic prolamin patterns of long-grain rice cultivars in subgroup A, Table I: Bellemont (**A**), BN-73 (**B**), Labelle (**C**), Newbonnet (**D**), Newrex (**E**), and Starbonnet (**F**).

exchange cartridges, a charcoal bed, and a membrane filter. Aluminum lactate was from Fluka Chemicals (Hauppauge, NY).

Prolamin Extraction

Milled rice kernels were ground into flour using a Udy cyclone sample mill to pass a 0.5-mm sieve. Rice flour (250 mg) was extracted with (750 μl) 70% ethanol (Lookhart 1985).

HPLC

A modification of the HPLC procedure reported for oats (Lookhart 1985) was used. The HPLC system was composed of a Varian Associates (Walnut Creek, CA) model 5060 microprocessor-controlled pump, a Waters Associates (Milford, MA) model 710A autosampler, a Tracor model 970 variable wavelength detector (set at 210 nm), a SynChrom, Inc. (Linden, IN) SynChropak RP-P (C-18) 6.5-μm particle column (250 × 4.1 mm i.d.), and a Hewlett-Packard 3388 printer-plotter automation system.

Injections of 50 μl were made and prolamin peaks were eluted at 45° C, using the gradient defined in Table III, at 1.0 ml/min. The solvents were (A) acetonitrile + 0.1% TFA and (B) water + 0.1% TFA. All samples were extracted and analyzed at least twice.

RESULTS

The designated types—long-, medium-, and short-grain—were used as the first classification of rice cultivars. Chromatograms of extracts of each cultivar from each group were inspected for similarities and uniqueness. Peaks a–c, eluting between 13 and 15 min, were most useful for defining subgroups, and peaks d–g,

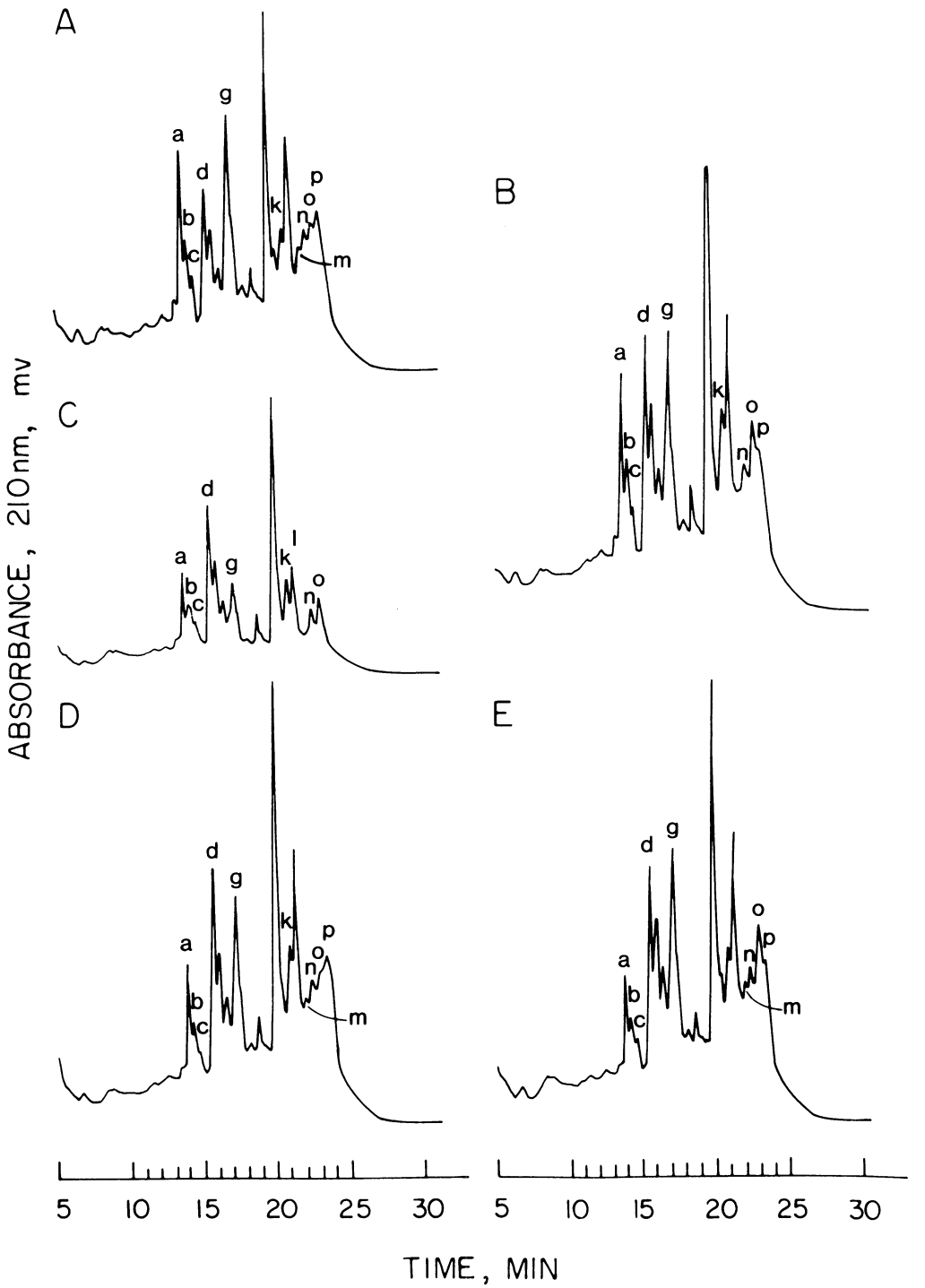

Fig. 2. High-performance liquid chromatographic prolamin patterns of long-grain rice cultivars in subgroup B, Table I: L-201 (**A**), Leah (**B**), Lebonnet (**C**), Lemont (**D**), and Skybonnet (**E**).

eluting from 16 to 24 min, were used for further differentiation within the subgroups. Peak time reproducibility was within the accuracy of the HPLC pump, ±1%, and the average percent deviation of peak heights on triplicate analysis of 10 peaks of various sizes was 4.70 ± 2.97. Small changes noted in this paper as differences among cultivars were not used unless repetitive analysis gave identical patterns within experimental error.

Long-Grain Rice

The long-grain rice cultivars were segregated into six subgroups, (A–F, Table I). Four subgroups (C–F) consisted of only one cultivar each with a unique HPLC pattern. The other two subgroups contained six (A) and five (B) members. The six-member subgroup (A) included Bellemont, Starbonnet, Newbonnet, Labelle, BN-73, and Newrex on the basis of the similar relative intensities of the triplet peaks marked a–c in Figure 1 (0.6 min separation between peaks b and c). Those members were differentiated from one another by characteristic peaks d and e, and k–p as discussed below (other quantitative differences in the 15–20 min range were found but not discussed).

The chromatograms A, B, E, and F appear quite similar to each other. However, the Bellemont pattern, A, was distinguished from patterns B–F by the broad peaks n and p. BN-73, pattern B, and Newrex, Pattern E, both had identical n and o patterns but differed in the relative intensities of peaks k–l and e–f. Pattern F, Starbonnet, has a characteristic set of peaks n, o, p. The Newbonnet pattern, D, had a characteristic m peak not found in the other patterns. The Labelle pattern, C, had a large n peak and very large g peak not found in the other cultivars of subgroup A.

Subgroup B, Table I (Skybonnet, Lebonnet, L-201, Lemont, and Leah) have similar peaks a, b, and c (the difference in elution times between b and c was 0.4 min) and intensities in the 13–15 min range, a–c, but differ in the 21–24 min range, k–p (Fig. 2).

Chromatograms were differentiated as follows: L-201 (Fig. 2A) contained a characteristic increasing stairstep quartet of peaks m–p in the 21–24 min range; although this is similar to the Figure 2D pattern, the peak height ratios of g to d are quite different. The Leah pattern (Fig. 2B) has a partially resolved triplet n, o, p in the 21–24 min range, with peak o being the highest. Two doublets consisting of peaks k, l and n, o and the small relative peak height of g to d were characteristic of Lebonnet (Fig. 2C). Lemont (Fig. 2D) has a characteristic quartet of rounded peaks m–p, with the third peak being a shoulder (unresolved) and the g-to-d peak height ratios smaller than those of the same peaks in Figure 2A. The

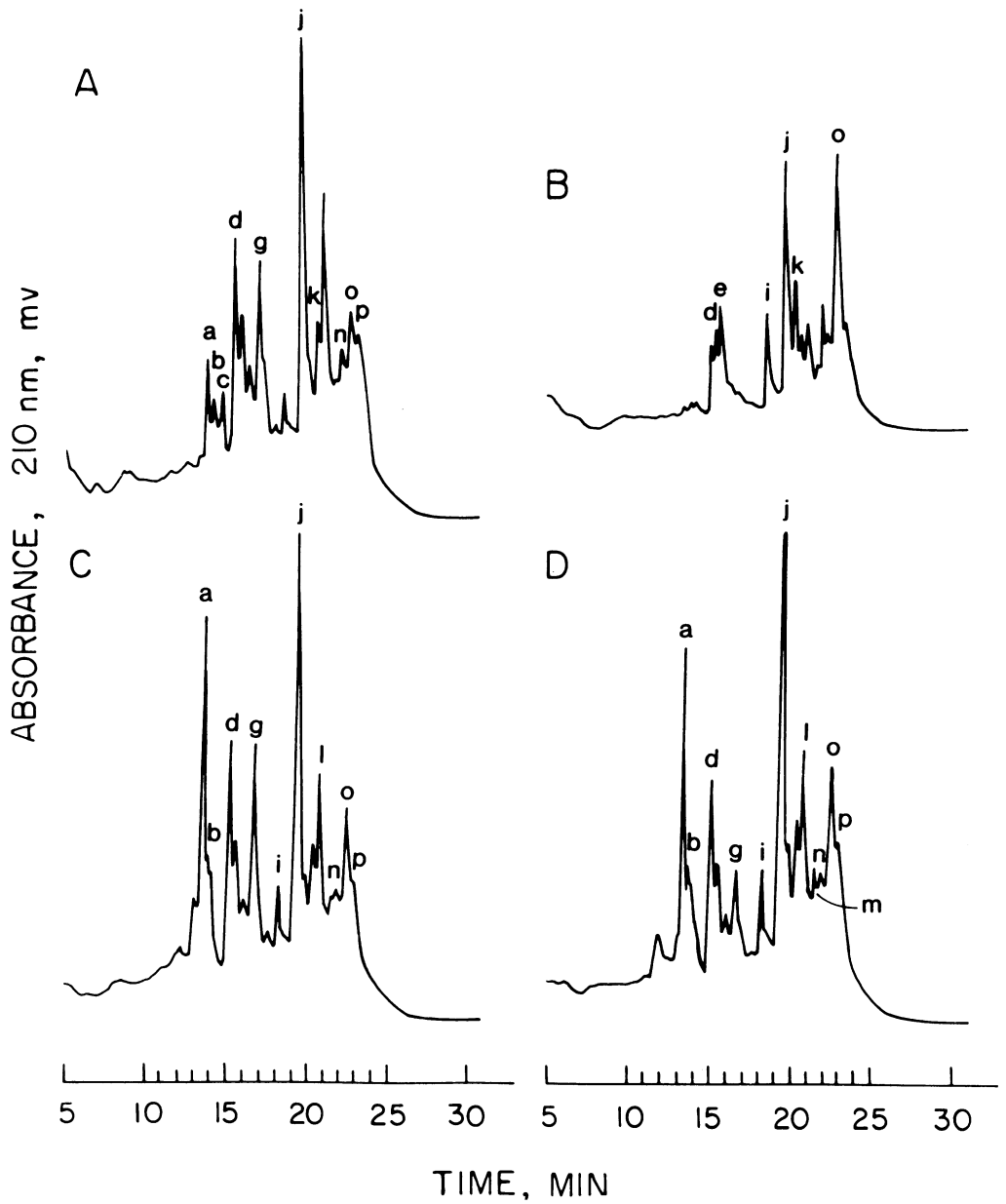

Fig. 3. High-performance liquid chromatographic prolamin patterns of long-grain rice cultivars in subgroups C–E, Table I: Bond (**A**), CICA-6 (**B**), CICA-8 (**C**), and IR-36 (**D**).

Skybonnet pattern (Fig. 2E) has a quartet pattern m–p in the 21–24 min range whose highest peak is o. Peaks d–k, between 16 and 21 min, were similar in position among the Figure 2 cultivars but quantitative differences were found.

The chromatogram for Bond (Fig. 3A) was similar to the patterns of Figure 1 cultivars but differed from them and other long-grain types by the nearly equal peak heights of the b and c peaks in the a–c triplet and by the n–p triplet in which the o peak

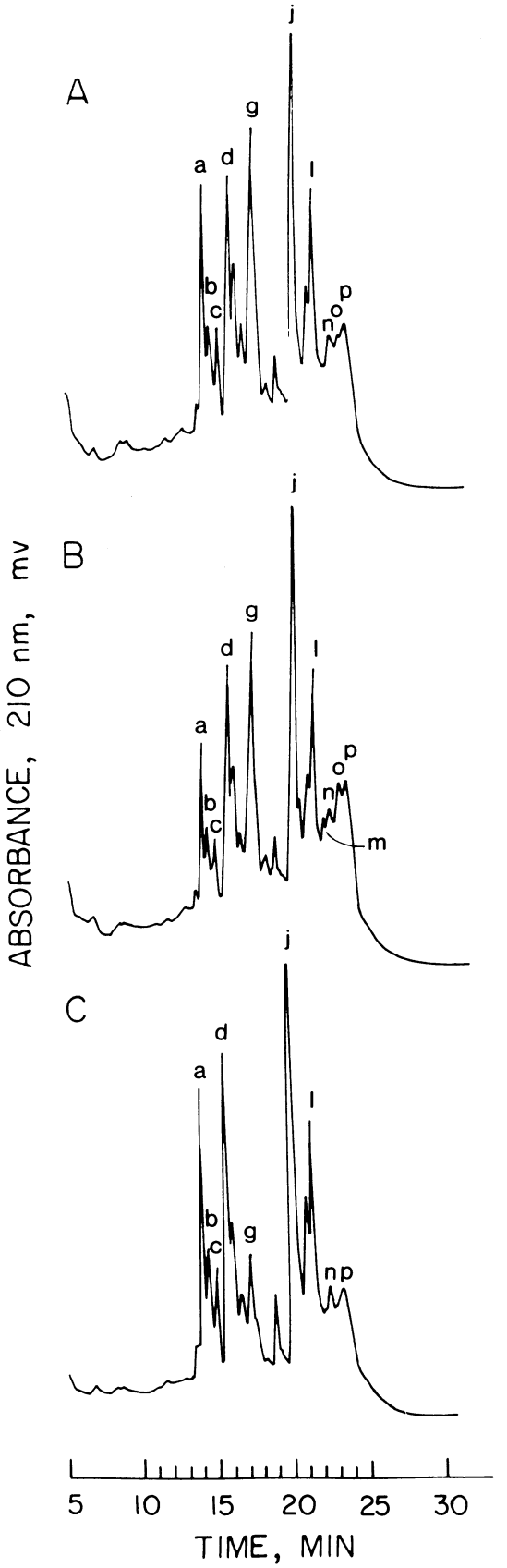

Fig. 4. High-performance liquid chromatographic prolamin patterns of medium-grain rice cultivars in subgroup A, Table II: Brazos (**A**), Nato (**B**), and Vista (**C**).

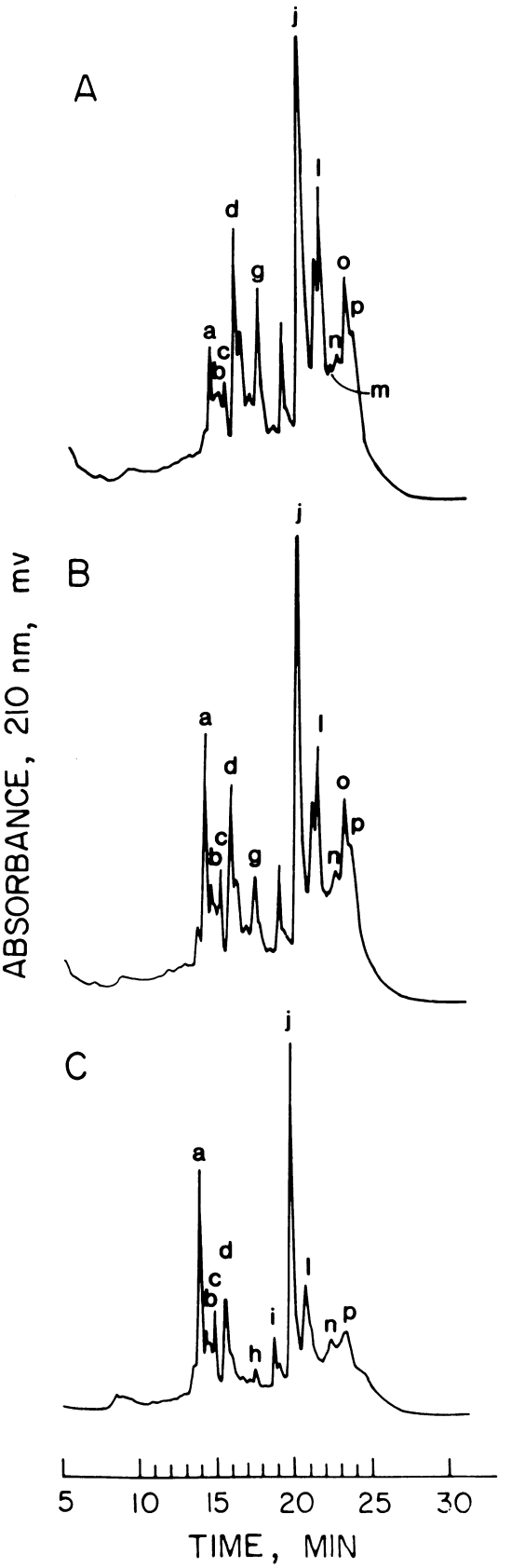

Fig. 5. High-performance liquid chromatographic prolamin patterns of medium-grain rice cultivars in subgroup B, Table II; Mars (**A**), Nova 76 (**B**), and Saturn (**C**).

was the largest. The remaining long-grain types were international samples and easily differentiated from the U.S. long-grain samples by differences in their patterns in the 12–16 min range, a–d (Fig. 3B, C, and D). Pattern 3B, CICA-6, did not have an a or b peak as did all other samples analyzed but did have o as its largest peak. CICA-8, pattern C, and IR-36, pattern D, had similar a, b, and i–p peaks but differed in their d-to-g peak ratios.

Medium-Grain Rice

The chromatograms of the prolamin extracts of the 11 medium-grain rice samples analyzed (Table II) were segregated into six subgroups by similarities of HPLC peaks a–g (elution time and relative intensity) between 12 and 18 min.

The first subgroup (A), containing cultivars Brazos, Nato, and Vista, was characterized by similarities of peaks a–c (relative intensity of and 0.6 min between peaks b and c) (Fig. 4A–C). The chromatograms of Brazos (Fig. 4A) and Nato (Fig. 4B) are very similar but differ in that Brazos has an n–p triplet where Nato has an m–p quartet. The Vista pattern (Fig. 4C) has a g peak that is about one-fourth the size of its d peak, whereas the height of the g peak for the other cultivars in this subgroup was larger than the height of their d peak. Vista also has a characteristic n, p pattern among the A subgroup of medium-grain rice cultivars.

Mars, Saturn, and Nova 76 comprise another subgroup (B, Table II) in medium-grain rice cultivars characterized by a split peak b found in the a–c region (Fig. 5A–C). Mars (Fig. 5A) and

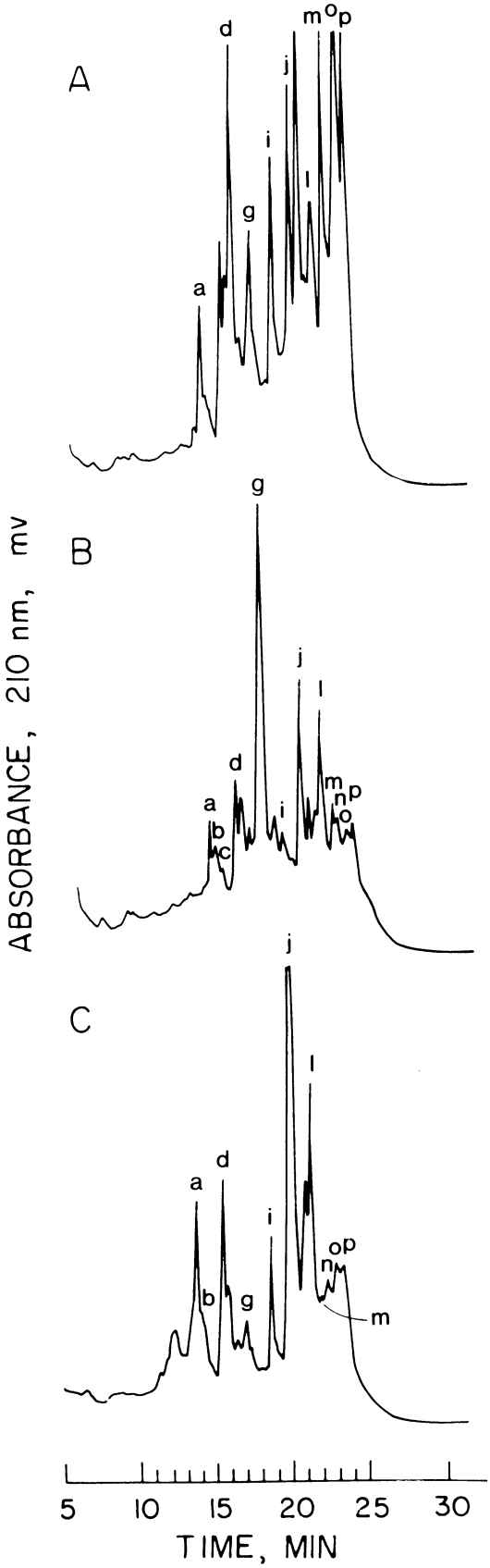

Fig. 6. High-performance liquid chromatographic prolamin patterns of medium-grain rice cultivars in subgroups C–E, Table II: Pecos (**A**), LA 110 (**B**), and RU 82011199 (**C**).

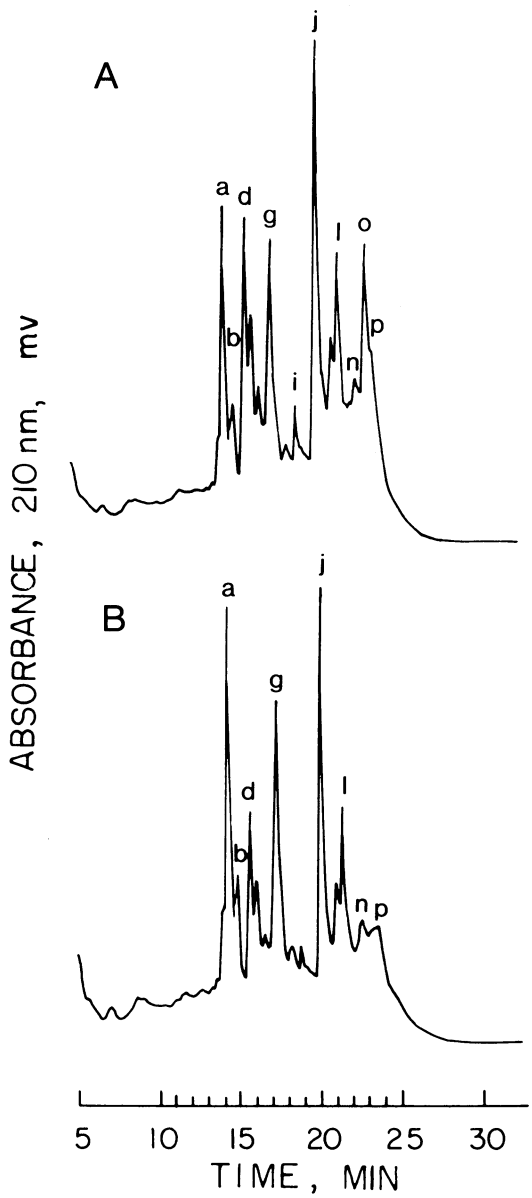

Fig. 7. High-performance liquid chromatographic prolamin patterns of medium-grain rice cultivars in subgroup F, Table II: M-9 (**A**), and M-201 (**B**).

Nova 76 (Fig. 5B) patterns were differentiated from Saturn (Fig. 5C) patterns by quantitative differences in heights of peak g, and by the lack of peak o in Saturn. They were distinguished from each other by the differences in their a-to-d peak height ratios and the presence of the m peak in Mars that is absent in Nova 76.

The Pecos cultivar's chromatogram (Fig. 6A) was unique to all the medium-grain rice samples in this study; its largest peak was o and it also contained a very large peak between j and l. Other medium-grain samples with unique patterns were LA-110 (Fig. 6B) and RU 82011199 (Fig. 6C). LA-110 was characterized by having g as its largest peak with a, d, and i small, whereas the genotype RU 82011199 had j as its largest peak with g very small and a, d, and i peaks of medium size.

The M-9 and M-201 samples, the final subgroup of medium-grain cultivars, were characterized by their a, b band patterns (Fig. 7), which consist of a large a peak and a small b peak. The patterns for M-9 (Fig. 7A) and M-201 (Fig. 7B) were differentiated by a large peak o in M-9 and rounded small peaks n and p in M-201.

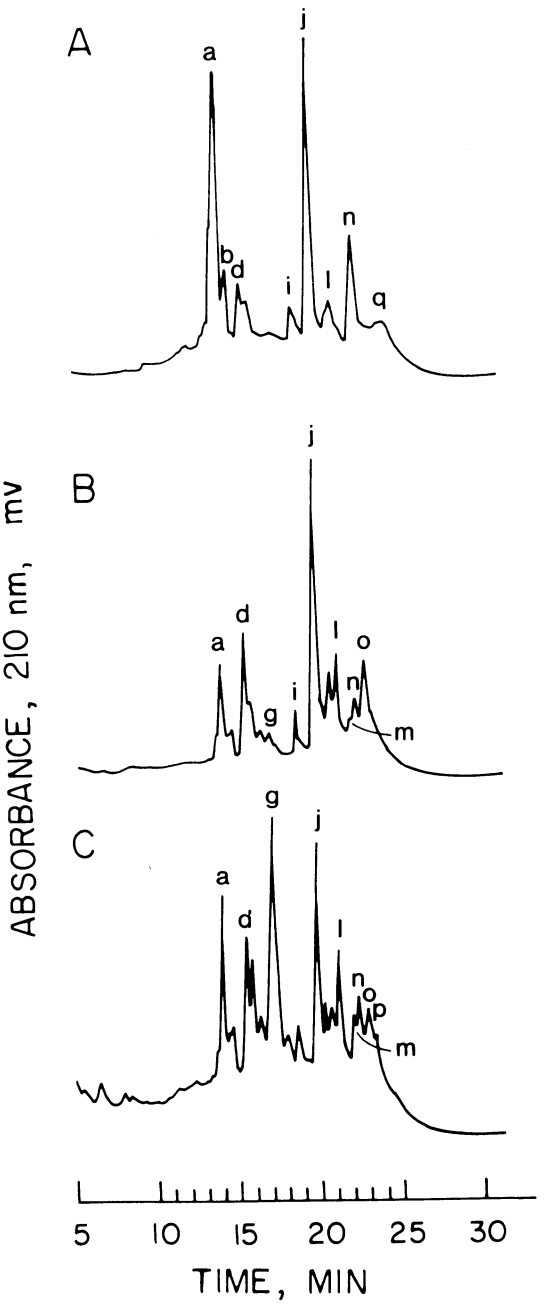

Fig. 8. High-performance liquid chromatographic prolamin patterns of short-grain rice cultivars: CAMO (**A**), Nortai (**B**), and S201 (**C**).

Short-Grain Rice

The prolamin HPLC patterns of the three short-grain rice samples (Fig. 8A–C) were all unique. The CAMO sample (Fig. 8A), a waxy (glutinous) rice, had a simple pattern of about 10 peaks and included a q peak, whereas all other samples had at least 18 peaks but no q peak. The Nortai and S-201 samples had similar a-to-d peak patterns, but the Nortai pattern had a major peak j (Fig. 8B) as compared to a major peak g for S-201.

DISCUSSION

As discussed subsequently, the cultivars in each subgroup of each grain type correlated well with their respective ancestry, as previously reported for wheat (Jones et al 1982, Lookhart et al 1983, Cox et al 1985). Dilday and Rutger (1986) reported that the gene pool for rice cultivars from Arkansas, California, Louisiana, and Texas consisted of 13, 15, 17, and 13 lines, respectively. Because 58 lines originated from 34 accessions, a large number of lines were common to each state.

Long-Grain Rice

The ancestry (R. H. Dilday, USDA, ARS, Stuttgart, AR, *personal communication*) of the rice cultivars correlates with the subgroups as follows: those cultivars in subgroup A (Table I) all have Bluebonnet in their pedigrees; those in subgroup B, Lemont, Lebonnet, and Skybonnet, have Bluebelle in their pedigrees, whereas L-201 and Leah go back to Rexoro for parents common to themselves and the other three cultivars. Subgroups C–F in Table I each contained one cultivar. The international cultivars' pedigrees are not available but are most likely different from U.S. rice cultivars. The Bond cultivar is a grandchild of Starbonnet in Table I-A and therefore its pattern looks somewhat like the I-A type patterns but not enough to group with them.

Medium-Grain Rice

The medium-grain types (Table II) were segregated into six subgroups, and the pedigrees of the cultivars within each subgroup were similar. Subgroup A, Table II, all had Blue Rose or Magnolia or both in their backgrounds. Subgroup B had Blue Rose and CI590 in their pedigrees. Subgroups C–E each contained one cultivar with a different pedigree: LA-110 (C), a Louisiana industrial rice with Sri Lanka and Taiwan background, Pecos (D), a Texas cultivar, with CI9545 and Gulfrose tracing back to Bluebonnet, and the foreign introduction RU 82011199 (E) whose pedigree was not available. Subgroup F contained two California cultivars, M-9 and M-201, both directly related through the M-3 cultivar as a common grandparent.

Short-Grain Rice

The three short-grain types had patterns different from each other and no similarities could be found in their pedigrees.

CONCLUSION

All rice cultivars examined were differentiated by HPLC of their prolamins. Cultivars with common ancestries tended to have similar chromatographic patterns. The ability to characterize prolamins of various cultivars and pick the most diverse for breeding purposes may be useful to rice breeders in the future. Furthermore, if associations between certain prolamin patterns and desirable or undesirable trait complexes are found among cultivars or subgroups of cultivars, chromatographic screening of accessions or plants before crossing could make breeders' work more efficient.

LITERATURE CITED

BIETZ, J. A., BURNOUF, T., COBB, L. A., and WALL, J. S. 1984. Wheat varietal identification and genetic analysis by reversed-phase high-performance liquid chromatography. Cereal Chem. 61:129.

BUSHUK, W., and ZILLMAN, R. R. 1978. Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature.

Can. J. Plant Sci. 58:505.
COX, T. S., LOOKHART, G. L., WALKER, D. E., HARRELL, L. G., ALBERS, L. D., and RODGERS, D. 1985. Genetic relationships among hard red winter wheat cultivars as evaluated by pedigree analysis and gliadin PAGE patterns. Crop Sci. 25:1058.
DILDAY, R. H., and RUTGER, J. N. 1986. Genetic contribution of ancestral lines to rice cultivars. Proc. 21st Rice Technical Working Group Meeting, February 24-26, 1986, Houston, TX. (Abstract).
DU CROS, D. L., WRIGLEY, C. W., and BLAKENEY, B. 1979. Fractionation of rice-grain proteins by gradient gel electrophoresis and gel isoelectric focusing characterization of rice genotypes. Riso 28(3):275.
GLASZMANN, J. C. 1986. Varietal classification of rice based on isozyme polymorphism—focus on U.S. rices. Proc. 21st Rice Technical Working Group Meeting, February 24-26, 1986, Houston, TX. (Abstract).
GUO, Y.-J., BISHOP, R., FERHNSTRÖM, H., YU, G.-F., LIAN, Y.-N., and HUANG, S.-D. 1986. Classification of Chinese rice varieties by electrofocusing. Cereal Chem. 63:1.
IWASAKI, T., SHIBUYA, N., SUZUKI, T., and CHIKUBU, S. 1982. Gel filtration and electrophoresis of soluble rice proteins extracted from long, medium, and short grain varieties. Cereal Chem. 59:192.
JONES, B. L., LOOKHART, G. L., HALL, S. B., and FINNEY, K. F. 1982. Identification of wheat cultivars by gliadin electrophoresis: Electrophoregrams of the 88 wheat cultivars most commonly grown in the U.S. in 1979. Cereal Chem. 59:181.
JULIANO, B. O. 1972. Physicochemical properties of starch and protein in relation to grain quality and nutritional value of rice. Pages 389-405 in: Rice Breeding. R. F. Chandler, ed. International Rice Research Institute: Los Baños, Philippines.
KIM, S.-I., and JO, D.-H. 1983. Fractionation and electrophoretic patterns of rice proteins. J. Korean Agric. Chem. Soc. 26:65-72.
KUSAMA, T., YANAGI, S. O., and IWAMOTO, M. 1984. Comparison of rice (*Oryza sativa* L.) seed proteins from several varieties. Agric. Biol. Chem. 48(6):1649.
LOOKHART, G. L. 1985. Identification of oat cultivars by combining polyacrylamide gel electrophoresis and reversed-phase high-performance liquid chromatography. Cereal Chem. 62:345.
LOOKHART, G. L., and POMERANZ, Y. 1985. Characterization of oat species by polyacrylamide gel electrophoresis and high-performance liquid chromatography of their prolamin proteins. Cereal Chem. 62:162.
LOOKHART, G. L., JONES, B. L., HALL, S. B., and FINNEY, K. F. 1982. An improved method for standardizing polyacrylamide gel electrophoresis of wheat gliadin proteins. Cereal Chem. 59:178.
LOOKHART, G. L., JONES, B. L., WALKER, D. E., HALL, S. B., and COOPER, D. B. 1983. Computer-assisted method for identifying wheat cultivars from their gliadin electrophoregrams. Cereal Chem. 60:111.
MARCHYLO, B. A., and KRUGER, J. E. 1984. Identification of Canadian barley cultivars by reversed-phase high performance liquid chromatography. Cereal Chem. 61:295.
MONOD, M., MARIE, R., FEILLET, P. 1972. Fractionation of the proteins from some rice varieties by polyacrylamide gel electrophoresis. C. R. Acad. Sci. 274(13):1957.
PADHYE, V. W., and SALUNKHE, D. K. 1979. Extraction and characterization of rice proteins. Cereal Chem. 56:389.
ROBERT, L. S., MATLASHEWSKI, G. J., ADELI, K., NOZZOLILLO, C., and ALTOSAAR, I. 1983. Electrophoretic and developmental characterization of oat (*Avena sativa* L.) globulins in cultivars of different protein content. Cereal Chem. 60:231.
SARKAR, R., and BOSE, S. 1984. Electrophoretic characterization of rice varieties using single seed (salt soluble) proteins. Theor. Appl. Genet. 68:414.
SARKAR, R., and BOSE, S. 1985. Identification of rice varieties using electrophoregram tracings of salt soluble seed protein and some of their genetical properties. 2nd Int. Symp. Cultivar Identification and Evaluation, May 1985, Braunschweig, Germany. (Abstract).
SHADI, A. I., and DJURTOFT, R. 1979. Studies of rice proteins byn crossed immunoelectrophoresis, gel electrophoresis, and isoelectric focusing. Cereal Chem. 56:402.
SMITH, J. S. C., and SMITH, O. S. 1986. Environmental effects on zein chromatograms of maize inbred lines revealed by reversed-phase high-performance liquid chromatography. Theor. Appl. Genet. 71:607.
VON OHMS, J. P. 1981. Identifizierung deutscher Hafersorten mittels Elektrophorese der Endospermproteine. Landswirtsch. Forsch. 34:88.
WEBB, B. D., POMERANZ, Y., AFEWORK, S., LAI, F. S., and BOLLICH, C. N. 1986. Rice grain hardness and its relationship to some milling, cooking, and processing characteristics. Cereal Chem. 63:27.
WILSON, C. M. 1985. A nomenclature for zein polypeptides based on isoelectric focusing and sodium dodecyl sulfate polyacrylamide gel electrophoresis. Cereal Chem. 62:361.
ZHAO, W., and BOULTER, D. 1985. Differences in the storage protein composition of different varieties or rice. Shiyan Shengwu Xuebao 18(1):119.

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