Discrimination of Sister-Line IR Rice Varieties by Polyacrylamide Gel Electrophoresis and Reversed-Phase High-Performance Liquid Chromatography[1]

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ABSTRACT

Methods developed to identify genetically diverse rice varieties were applied to semidwarf rice varieties from the International Rice Research Institute derived from the same cross, which are especially difficult to differentiate. Acid polyacrylamide gel electrophoresis (PAGE) and Supelcosil C_8 reversed-phase high-performance liquid chromatography (RP-HPLC) of 5*M* acetic acid extracts of brown and milled rice and Vydac C_{18} RP-HPLC of 60% 1-propanol (prolamin) extracts of milled rice differentiated sister lines IR36 and IR42 (but not sister lines IR28 and IR29); IR32, IR38, and IR40; IR52 and IR54; and IR56 and IR60. Vydac C_4 RP-HPLC of alkylated glutelins from milled rice differentiated IR36 from IR42 and IR52 from IR54 and showed slight differences between IR28 and IR29 and between IR38 and IR40. Vydac C_4 RP-HPLC patterns of prolamins differentiated IR28 from IR29 and IR38 from IR40. Thus, PAGE and RP-HPLC (especially the latter) are useful for differentiating sister-line rice varieties. The effect of location of growth on brown rice PAGE, Vydac C_{18} RP-HPLC of milled rice prolamin, and Vydac C_4 RP-HPLC of milled rice alkylated glutelins of IR36 and IR42 grown in three locations in the Philippines was quantitative rather than qualitative.

Recently, reversed-phase high-performance liquid chromatography (RP-HPLC) and polyacrylamide gel electrophoresis (PAGE) methods developed for cereal and legume variety identification were adapted to rice (Lookhart et al 1987, 1991; Hussain et al 1989; Huebner et al 1990). The methods differentiated genetically diverse varieties and the semidwarf sister-line varieties IR36 and IR42, developed by the International Rice Research Institute (IRRI) and designated as IR varieties.

Because more than 50% of the world rice area is planted in modern semidwarf varieties (Dalrymple 1986), the ability to differentiate such sister lines is especially desirable since 12 of the 34 IR varieties are sister lines. Sister lines are genetically similar and harder to differentiate than lines or varieties derived from different crosses and parents. Therefore, we analyzed proteins of additional IR sister-line varieties by various RP-HPLC and PAGE methods in three laboratories to determine how well these techniques can differentiate sister lines. The effect of location of growth in the Philippines on RP-HPLC and PAGE protein patterns of IR36 and IR42 was also studied.

MATERIALS AND METHODS

Rice Samples

Samples of rough rice of sister-line varieties IR28 and IR29; IR32, IR38, and IR40; IR36 and IR42; IR52 and IR54; and IR56 and IR60 were obtained from the Plant Breeding Department of the IRRI. Pedigrees of these varieties are summarized in Table I. IR36 and IR42 genotypes were also grown by the IRRI Agronomy Department in 1987 in the Philippines at the Maligaya Rice Research and Training Center, Muñoz, Nueva Ecija, and at the Visayas Agricultural Experiment Station, Jaro, Iloilo.

Rice was dehulled with a dehuller (THU-3SA, Satake Engineering Co. Ltd., Tokyo, Japan). Brown rice (100 g) was milled with a pearling mill (Satake TM-05), or 5 g was milled in a sample mill (Pearlest, Kett Electric Laboratory, Tokyo).

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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Acid PAGE

At Winnipeg, Ontario, brown and milled rices were ground into meals with a Udy cyclone mill with a 1-mm sieve. Meal (0.50 g) was extracted for 2 hr with 2 ml of 5*M* acetic acid containing 1% (w/v) sucrose, centrifuged at 8,850 × *g* for 10 min, and subjected to PAGE in 10 or 12% gels in aluminum lactate buffer, pH 3.1 (Hussain et al 1989).

RP-HPLC

At Winnipeg, brown and milled rice meal samples (0.3 g) were extracted with 0.5 ml of 5*M* acetic acid for 2 hr at room temperature, with occasional vortexing, and centrifuged twice at 8,850 × *g* for 10 min. The 0.2-ml extract was passed through a 0.45-μm filter before RP-HPLC on a 5-μm (300 Å pore) Supelcosil LC-308 C_8 column (250 × 4.6 mm, Supelco Canada Ltd., Oakville, ON) with a guard column (50 × 4.6 mm, Supelguard LC-308) (Hussain et al 1989). The unit was a liquid chromatograph (Hewlett Packard 1090M) incorporating a DR5 two-solvent reservoir delivery system, autoinjector, autosampler, heated column compartment (50°C), and diode-array detector. Analytical control and quantitation were provided by an HP-310 computer running HP 79994A software for the analytical workstation and HP 79995A operating software. Flow rate was 1.5 ml/min. Elution solvents were initially 22% aqueous acetonitrile (ACN) containing 0.1% trifluoroacetic acid (TFA), then 28% ACN at 18 min, and 50% ACN at 32 min.

At Manhattan, Kansas, milled rice was ground to a meal using

TABLE I
Pedigrees of the IR Sister-Line Rice Varieties[a]

Variety	Selection	Pedigree[b]
IR28	IR2061-214-3-8-2	IR833-6-1-1-1/IR1561-149-1//IR1737
IR29	IR2061-464-4-14-1	
IR32	IR2070-747-6-3-2	IR20*^{2}/*O. nivara*//CR94-13
IR38	IR2070-423-2-5-6	
IR40	IR2070-414-3-9	
IR36	IR2071-625-1-252	IR1561-228-1-2//IR1737//CR94-13
IR42	IR2071-586-5-6-3-4	
IR52	IR5853-118-5	Nam Sagui 19/IR2071-88
IR54	IR5853-162-1-2-3	//IR2061-214-3-6-20
IR56	IR13429-109-2-2-1	IR4432-53-3-3/Ptb 33//IR36
IR60	IR13429-299-2-1-3	

[a]Varieties developed by the International Rice Research Institute.
[b]Female and male parents are separated by a slash to indicate a single cross and by two slashes (//) to indicate the second cross. Asterisk (*) denotes backcrosses, and the number in the superscript indicates the dosage of the recurrent parent.

a Udy cyclone mill with a 0.5-mm sieve. Rice meal (0.25 g) was extracted in duplicate with 0.75 ml of 60% 1-propanol for 30 min at room temperature and analyzed twice at 1 ml/min by RP-HPLC on a Vydac C_{18} 5-μm (300 Å pore) column (218TP54, 250 × 4.6 mm, Separations Group, Hesperia, CA) (Lookhart et al 1991). The system included an HP 1090 chromatography data system and a 1040A diode-array detector. A data point was stored every 640 msec on an HP 9000-310 computer for subsequent integration, replotting, and comparison. Elution was performed with a multistep linear gradient beginning at 25% aqueous ACN containing 0.1% TFA, 35% ACN at 5 min, 50% ACN at 10 min, 75% ACN at 17 min, 85% ACN at 18 min, and back to 25% ACN at 19 min. Chromatograms were normalized to make the tallest peaks the same height for all patterns.

At Peoria, Illinois, milled rice (7–10 grains) was ground with a Wig-L-Bug (Crescent Dental Manufacturing Co., Lyons, IL) for 15–25 sec. Large particles that remained were removed by sieving (0.5 mm) and were reground for 10 sec; then 150 mg of the fine meal was defatted with 3 ml of a mixture of 1-butanol and ethanol (1:1, v/v) for 15 min by vortex mixing (Buchler Vortex-Evaporator, Buchler Instruments, Kansas City, MO). The residue was extracted sequentially with 1.2 ml of 60% 1-propanol for 30 min to solubilize prolamins, 1.2 ml of 1.0M NaCl in 0.05M sodium phosphate buffer, pH 6.5, for 30 min to extract albumins and globulins, and then with 1.3 ml of 0.05M sodium phosphate, pH 8, containing 3% sodium dodecyl sulfate (SDS) and 0.5% dithiothreitol for 1.0 hr at 40°C to extract glutelins (Huebner et al 1990). Extracts were centrifuged at 17,000 × g for 20–25 min at 25°C and filtered through a 0.2- or 0.45-μm filter before RP-HPLC on a 5-μm (300 Å pore) Vydac C_4 column (150 × 4.6 mm) with a 22 × 3.5 mm guard column (SynChrom RSC, Lafayette, IN) and a 0.5-μm in-line prefilter (A-103, Upchurch, Oak Harbor, WA) at 0.9 ml/min and 58°C (Huebner et al 1990). Glutelin extracts (600 μl) were alkylated with 9 μl of 30% 4-vinyipyridine in 60% ethanol for 1 hr to minimize reoxidation of reduced glutelins (Huebner and Bietz 1987) and were centrifuged before RP-HPLC. The HPLC apparatus included a solvent delivery system (SP8700, Spectra-Physics, San Jose, CA), an autosampler (SP8780XR), and a monitor (SF770 Spectroflow, Kratos Analytical, Ramsey, NJ) (Huebner et al 1990). Recording was done with an OmniScribe recorder (Houston Instruments, Austin, TX). Data were simultaneously stored in a computer system (Classic 7870, ModComp, Ft. Lauderdale, FL) for subsequent integration or replotting. HPLC conditions were modified from those previously used (Huebner et al 1990) to optimize separations without lengthening analysis time. Solvent A was 0.11% TFA in water, and solvent B was 90% ACN containing 0.09% TFA. The gradient began at 27% B (24% ACN) and increased linearly to 37% B (33% ACN) at 2 min, to 38% B (34% ACN) at 26 min, to 47% B (42% ACN) at 55 min, and to 49% B (44% ACN) at 59 min. Prolamins were also analyzed at 60°C, using a gradient beginning at 20% B (18% ACN) and increasing linearly to 37% B (33% ACN) at 12 min, to 41% B (37% ACN) at 20 min, and to 47% B (42% ACN) at 40 min, with a final 4-min hold at 47% B.

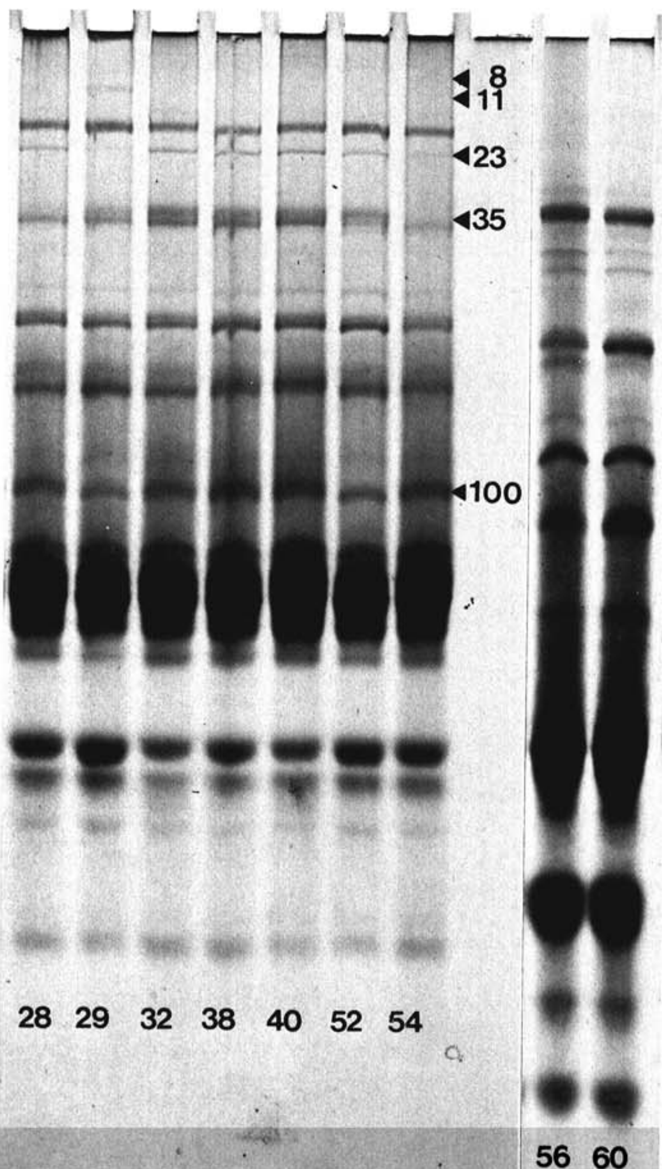

Fig. 1. Polyacrylamide gel electrophoretic patterns (12% gel) of acetic-acid extractable brown rice proteins of sister-line varieties IR28 and IR29; IR32, IR38, and IR40; IR52 and IR54; and (run separately) IR56 and IR60.

RESULTS AND DISCUSSION

The following sections describe the use of PAGE and RP-HPLC to differentiate IR sister-line varieties IR28 and IR29; IR32, IR38, and IR40; IR36 and IR42; IR52 and IR54; and IR56 and IR60.

IR28 and IR29

IR28 has a high amylose content, while IR29 has waxy starch. PAGE (12% gel) of brown rice extracts showed only minor qualitative differences between IR28 and IR29 (Fig. 1). Minor bands at 8 and 11 mm from the origin were visible only in IR29. The 23-mm band was more intense in IR28, and the 35-mm band was more prominent in IR29. The waxy starch of IR29 gelatinized in 5M acetic acid, used for protein extraction.

PAGE (10% gel) of IR28 and IR29 milled rice showed only minor differences (Fig. 2). The band at 51 mm was present only in IR29. Milled rice contained fewer protein bands than brown rice did, since milling removes embryo and bran layers (compare Figs. 1 and 2) However, some bands were absent in milled brown rice, particularly the band at 54 mm. The band at 100 mm was also more intense in milled rice. This is due to selective precipitation of glutelins by phytate at acidic pH; bands at 54 and 100 mm are glutelin bands (Juliano et al 1991).

SDS-PAGE patterns in 17% gels of proteins extracted from brown or milled rice varieties with 2% SDS + 5% 2-mercaptoethanol in 0.063M sodium phosphate, pH 6.8 (Ng and Bushuk 1987), were similar except for IR29 (waxy), which lacked the 54-kD band present in IR28 and other nonwaxy samples (results not shown).

Supelcosil C_8 RP-HPLC patterns of 5M acetic acid extracts of brown IR28 and IR32 rices were qualitatively similar before 26 min (Fig. 3). These patterns are also similar to those of IR36 and IR42 (Hussain et al 1989). In IR29 (not shown), the peak at 23.5 min was reduced to a shoulder compared to that in IR28. The total concentration of extracted protein decreased in IR29, probably due to interference of starch gelatinized by 5M acetic acid.

In the 29–32 min region (Fig. 4), sister-line varieties had similar

Supelcosil C_8 RP-HPLC patterns. Sister lines IR36 and IR42 were previously differentiated (Hussain et al 1989). From 29 to 31 min, IR28 and IR29 differed from IR32, IR38, IR40, IR52, and IR54 (Fig. 4) but were similar to IR36 (Hussain et al 1989).

Supelcosil C_8 RP-HPLC patterns of acetic acid extracts of milled rice genotypes were very similar up to 26 min except for relative heights of peaks at 21.3 and 21.5 min and the single vs. the double peak at 22.9 min (Fig. 5). Brown and milled rice had similar Supelcosil C_8 RP-HPLC patterns before 26 min except for the peak at 23.5 min, which was reduced to a shoulder in milled rice as in IR28 (Figs. 3 and 5). IR28 and IR29 milled rices had similar patterns before 26 min (not shown) but differed quantitatively from 26 to 32 min (Fig. 6). All milled rices had major peaks from 26.5 to 28.5 min that were absent in brown rice (Fig. 6). Most likely, as in PAGE (above), these correspond

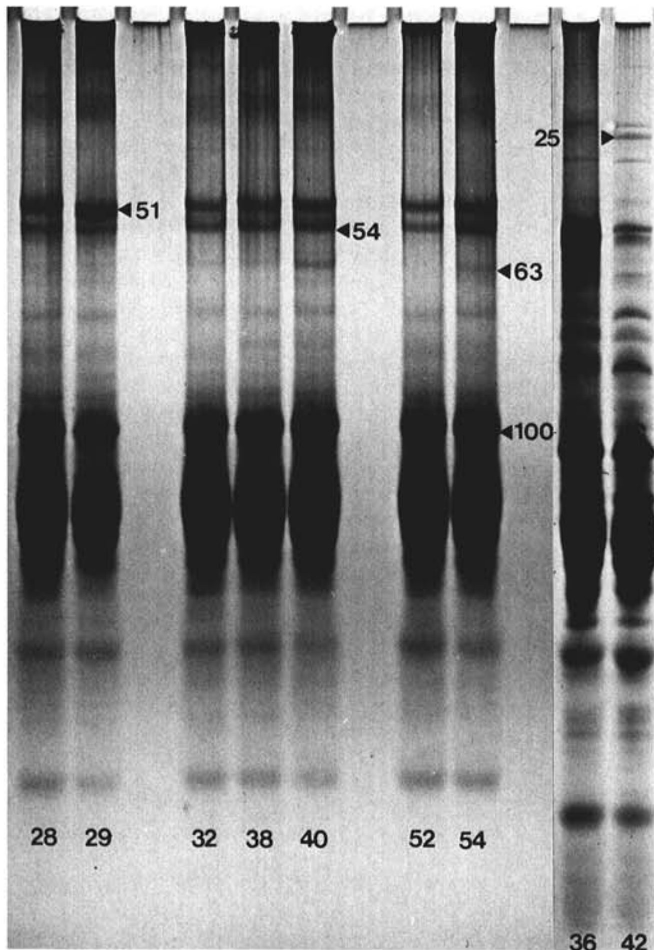

Fig. 2. Polyacrylamide gel electrophoretic patterns (10% gel) of acetic-acid extractable protein from milled rice of sister-line varieties IR28 and IR29; IR32, IR38, and IR40; IR52 and IR54; and (run separately) IR36 and IR42.

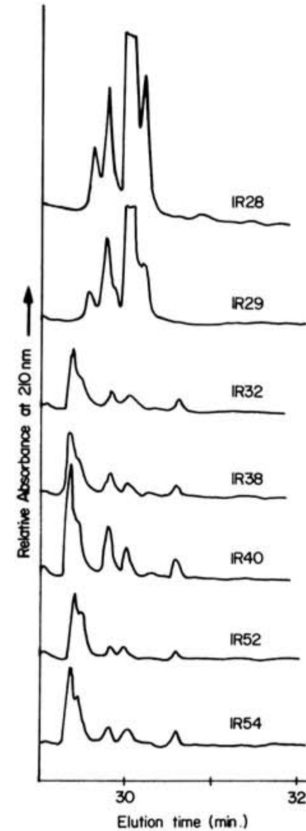

Fig. 4. Supelcosil C_8 reversed-phase high-performance liquid chromatographic patterns (29–32 min) of acetic-acid extractable brown rice proteins of sister-line varieties IR28 and IR29; IR32, IR38, and IR40; and IR52 and IR54.

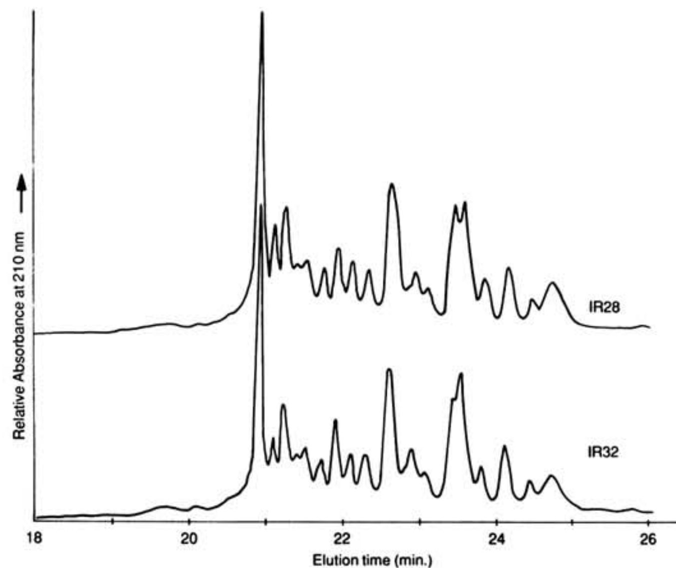

Fig. 3. Supelcosil C_8 reversed-phase high-performance liquid chromatographic patterns (18–26 min) of acetic-acid extractable proteins from IR28 and IR32 brown rices.

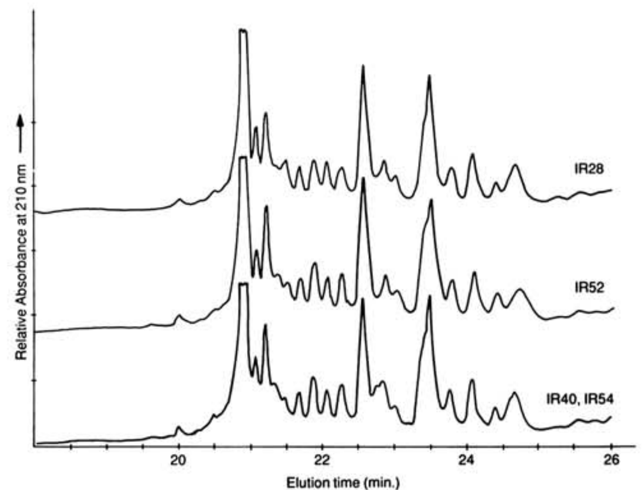

Fig. 5. Supelcosil C_8 reversed-phase high-performance liquid chromatographic patterns (18–26 min) of acetic-acid extractable proteins of milled rice of sister-line varieties IR28 (identical to sister-line IR29 and to IR32 and IR38) and varieties IR52 and IR54 (identical to IR40, sister-line of IR32 and IR38).

to brown rice glutelins that complex with bran phytate and precipitate in acetic acid (Juliano et al 1991). IR28 and IR29 were similar from 29 to 31 min, but IR28 had major peaks at 26.5, 26.9, and 27.3 min, whereas IR29 had peaks at 26.5 and 26.9 min. IR28 and IR29 both had a prominent peak at 29.9 min that was minor in IR32, IR38, IR40, IR52, and IR54.

Upon Vydac C_{18} RP-HPLC, prolamins extracted from milled IR28 and IR29 with 60% 1-propanol both had a major peak at 11.9 min but differed between 17 and 18 min (Fig. 7). Vydac C_4 RP-HPLC patterns of milled IR28 and IR29 alkylated glutelins (Fig. 8) differed by the moderately large peak at 45.5 min in IR29 that was absent in IR28, and by quantitative differences at 17–20, 22–23, 41–42, and 47–48 min. Alkylation slightly modified glutelin subunit hydrophobicity but stabilized samples for at least one month. The modified multistep linear gradient better resolved closely eluting early peaks and reduced chromatography time from 2–3 hr to 1 hr. The long blank area during the first 17 min was found to be necessary. When the gradient was adjusted higher to elute proteins sooner, the separation was impaired, preventing the separation of the proteins between 17 and 27 min. This phenomenon has not been noted in other cereal protein separations by RP-HPLC.

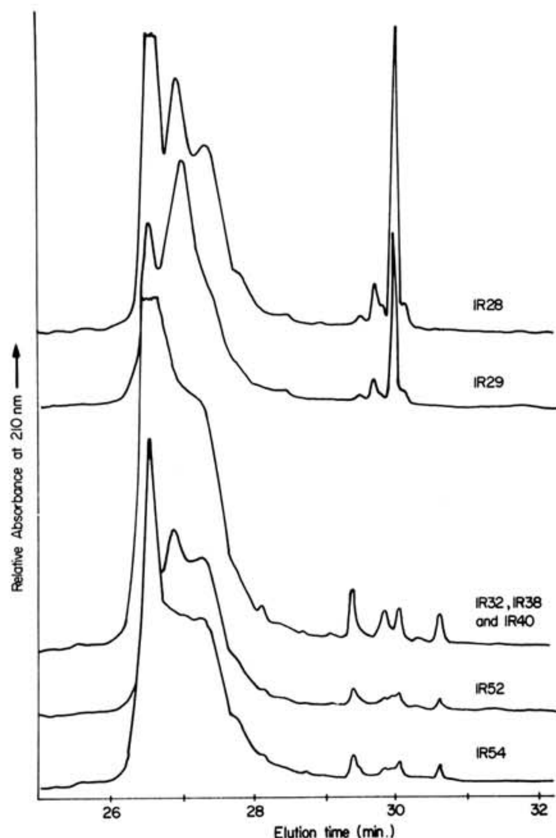

Fig. 6. Supelcosil C_8 reversed-phase high-performance liquid chromatographic patterns (26–32 min) of acetic-acid extractable milled rice proteins of sister-line varieties IR28 and IR29, IR40 (identical to IR32 and IR38) and varieties IR52 and IR54.

Vydac C_4 RP-HPLC patterns of milled IR28 and IR29 prolamins also differed between 34 and 35 min (Fig. 9). IR28 also had a doublet at 41.9–42.5 min, while a single 42.5-min peak was present in IR29. IR29 also had peaks at 35.0, 37.9, 38.7, 39.8, and 41.1 min that did not occur in IR28. Thus, among the various PAGE and RP-HPLC techniques tested, RP-HPLC of milled rice prolamins on a Vydac C_4 column best differentiated sister lines IR28 and IR29.

IR32, IR38, and IR40

PAGE patterns of proteins extracted from brown (Fig. 1) or milled (Fig. 2) IR32, IR38, and IR40 rice sister lines were similar. Supelcosil C_8 RP-HPLC patterns of acetic acid-soluble proteins from these sister lines were also nearly identical between 29 and 32 min, although peaks were higher in IR40 (Fig. 4).

Supelcosil C_8 RP-HPLC patterns of milled rice acetic acid extracts of IR32, IR38, and IR40 were identical to those of IR28 before 26 min (not shown) and from 26 to 29 min (Fig. 6) except for the more prominent shoulder at 23.6 min in IR40 (Fig. 5). IR40 peaks were again higher, particularly at 26.5 min. Between 29 and 31 min, patterns were similar to those of brown rice extracts

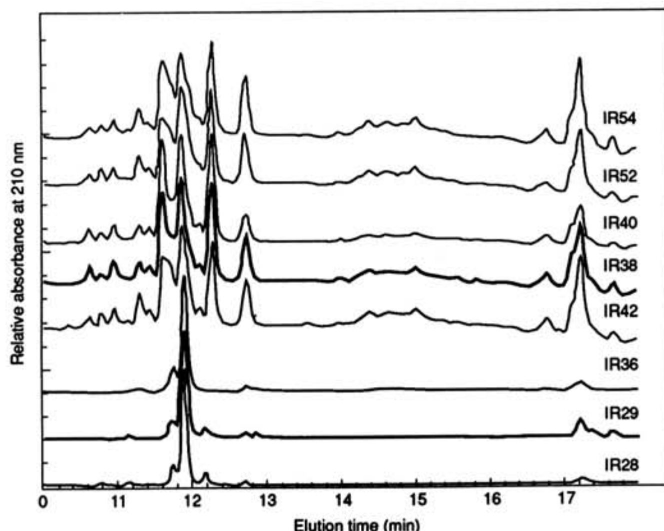

Fig. 7. Normalized Vydac C_{18} reversed-phase high-performance liquid chromatographic patterns of 60% 1-propanol-extracted prolamins of milled rice of eight sister-line varieties IR28 and IR29, IR36 and IR42, IR38 and IR40, and IR52 and IR54.

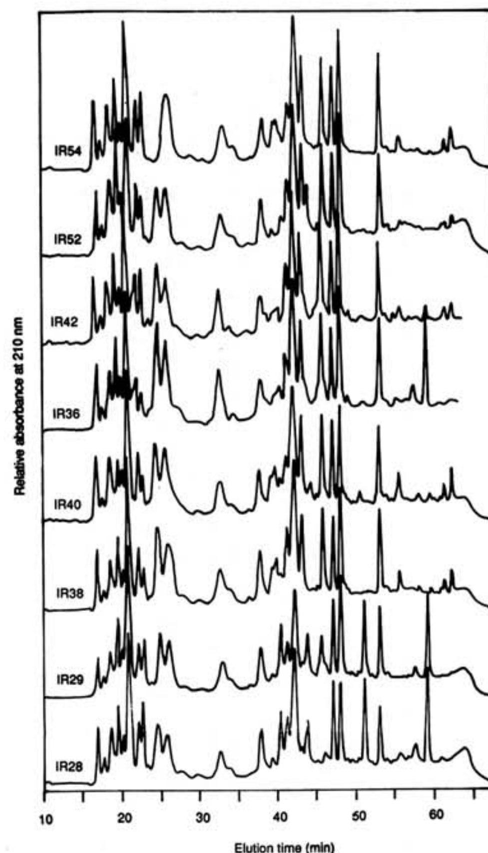

Fig. 8. Normalized reversed-phase high-performance liquid chromatographic patterns (Vydac C_4, 58°C) of alkylated glutelins from milled rice of four pairs of sister-line varieties (IR54 and IR52, IR42 and IR36, IR40 and IR38, and IR29 and IR28).

(Fig. 4). IR38 and IR40 also had identical Vydac C_{18} patterns of milled rice prolamins between 10 and 13 min but differed in intensity of peaks at 16–18 min (Fig. 7). Similarly, IR38 and IR40 had almost identical Vydac C_4 RP-HPLC patterns of milled rice alkylated glutelins (Fig. 8) except for prominent small peaks present at 44.3 and 50.4 min in IR40 but absent in IR38 and the broader IR38 peak at 26.5 min, suggesting heterogeneity.

IR36 and IR42 and Location Effects

Upon PAGE, bands at 0–20 and 23–26 mm in IRRI-grown IR36 brown rice were verified to be more intense or better separated than in IR42 (Hussain et al 1989). PAGE of brown rices grown at Muñoz and Jaro showed little location effect (data not shown). IR36 from Jaro had an extra-faint band at 85 mm and had less intense 30- and 48-mm bands than when grown at IRRI or Muñoz. In IR42 from Jaro, two bands at 43–47 mm had slightly better separation than did IR42 samples from the other locations. Differences in band intensity between IR36 and IR42 were less evident at Muñoz and Jaro than at IRRI, but separation of the bands at 43–47 mm remained better in IR36 than in IR42.

Differences between IR36 and IR42 brown rices upon PAGE (Hussain et al 1989) were also evident in milled rice (Fig. 2). The major difference was the prominent 25-mm band in IR42, which was faint in IR36.

Supelcosil C_8 RP-HPLC of milled IR36 and IR42 rice acetic acid extracts was not done. However, major differences at 29–31 min in brown rice (Hussain et al 1989) probably also occur in milled rice.

Vydac C_{18} RP-HPLC of milled rice prolamins differentiated IR36 from IR42 (Fig. 7), as reported previously (Lookhart et al 1991). IR36 had a prominent 11.4-min peak and lacked the small 12.1-min peak of IR28 and IR29. IR42 had a pattern identical to the patterns of IR38, IR40, IR52, and IR54. The effect of growing location (Muñoz, Jaro, and IRRI) on the patterns of IR36 and IR42 was minimal (data not shown) and did not affect the region of 11–13 min but only the region of 14–18 min. Minor differences were also reported for five U.S. varieties from five locations (Lookhart et al 1991).

IR36 and IR42 differed upon Vydac C_4 RP-HPLC of milled rice alkylated glutelins (Fig. 8). IR36 had a prominent peak at

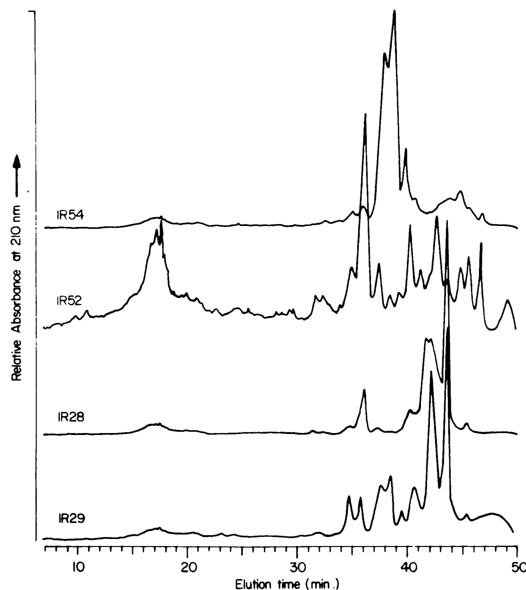

Fig. 9. Reversed-phase high-performance liquid chromatographic patterns (Vydac C_4, 60°C) of milled rice prolamins from sister-line varieties IR52 and IR54 and varieties IR28 and IR29.

Fig. 10. Normalized reversed-phase high-performance liquid chromatographic patterns (Vydac C_4, 58°C) of alkylated glutelins from milled rice of sister-line varieties IR36 and IR42 grown in the Philippines at Muñoz, Nueva Ecija, IRRI (Los Baños Laguna), and Jaro, Iloilo.

59 min that was minor in IR42. RP-HPLC patterns of alkylated glutelins of IR36 milled rice samples from three locations (Muñoz, Jaro, and IRRI) were qualitatively similar (Fig. 10). For IR42 samples from the three locations, alkylated glutelin RP-HPLC patterns were qualitatively identical, but there were quantitative differences for some peaks (Fig. 10).

IR52 and IR54

PAGE patterns of IR52 and IR54 brown rice acetic acid extracts were qualitatively similar, but bands at 23 and 35 mm were more intense in IR52 (Fig. 1). PAGE patterns of milled rice extracts were very similar for these sister lines, except for the band at 63 mm, which was absent in IR52 but present in IR54 (Fig. 2).

Supelcosil C_8 RP-HPLC patterns of 5*M* acetic acid extracts of brown IR52 rice showed a single peak at 22.8 min (not shown), as in IR28 and IR32 (Fig. 3), but showed a double peak for IR54. A double peak at 23.5 min in IR52 and IR54 (not shown) was similar to that of IR28 (Fig. 3).

The Supelcosil C_8 RP-HPLC patterns of milled rice acetic acid extracts were similar for IR52 and IR54 before 26 min except for the double peak at 22.8 min in IR54 vs. a single peak in IR52 (Fig. 5). From 26 to 32 min, IR54 lacked the distinct 26.8-min peak seen in IR52 (Fig. 6). RP-HPLC patterns of IR52 and IR54 were identical from 29 to 31 min. At 28.9–30.1 min they had a triplet vs. a doublet in IR32, IR38 and IR40.

Vydac C_{18} patterns of milled IR52 and IR54 prolamins were identical at 10–13 min but differed in peak heights at 16–18 min. They differed from IR38 and IR40 at 10–13 and 16–18 min, particularly by the broader peak at 11.7 min (Fig. 7).

Upon Vydac C_4 RP-HPLC of milled rice alkylated glutelins, IR52 had extra peaks at 24.5 and 43.8 min and larger peaks at 39–40.5 min than did IR54 (Fig. 8). Vydac C_4 RP-HPLC patterns of milled IR52 and IR54 prolamins also differed between 32 and 50 min (Fig. 9) in contrast to Vydac C_{18} results (Fig. 7). On the Vydac C_4 column, IR52 also had sharper prolamin peaks at 16–19 min than those of IR54.

IR56 and IR60

IR56 and IR60 had qualitatively similar PAGE patterns of brown rice extracts (Fig. 1). These sister lines were not analyzed further.

CONCLUSIONS

One would expect PAGE and RP-HPLC patterns of proteins from sister-line rice varieties to be more similar than those of most varieties since sister lines are genetically similar. In previous studies, differences among non-sister-line rice samples were readily evident upon RP-HPLC (Lookhart et al 1987, 1991; Huebner et al 1990). Sister lines IR36 and IR42 also differed in Supelcosil C_8 RP-HPLC patterns of acetic acid extracts of brown rice (Hussain et al 1989).

In the present study, these sister lines also gave different Vydac C_{18} RP-HPLC patterns of milled rice prolamins and Vydac C_4 RP-HPLC patterns of milled rice alkylated glutelins. PAGE patterns of acetic acid extracts of brown and milled rices differed only slightly, even for sister lines IR36 and IR42 (Figs. 1 and 2). Differences between IR36 and IR42 were greater than with the other sister-line IR varieties studied. SDS-PAGE was not effective in differentiating sister-line varieties except for IR28 (nonwaxy) and IR29 (waxy) rices. Environmental effects on RP-HPLC patterns of IR36 and IR42 milled rice prolamins and alkylated glutelins were minor.

Our results show clear advantages of RP-HPLC over PAGE for differentiation of closely related rice genotypes. This is facilitated by various changes introduced into the RP-HPLC methods. Various bonded phases, such as C_{18}, C_8, and C_4 columns from different manufacturers, differ significantly in selectivity, causing elution patterns to vary. (Only one type, a C_4, was used for the glutelin separation at Peoria). Solvent gradient modification can significantly affect resolution. For example, a multistep rather than a linear gradient better resolves early peaks while reducing analysis time. Using different organic solvents or a combination of solvents and different modifiers or quantities of modifiers (such as TFA), as well as different temperatures, will also change the elution profile. This could lead to many hundreds of other combinations of conditions for possible further improvements in separations. Alkylation of reduced glutelins is also desirable, since it prevents reoxidation during storage or analysis, thereby giving more reproducible results. Huebner and Bietz (1987) showed similar effects of these factors in differentiating wheat varieties by RP-HPLC. Thus, with such modifications, we have shown that RP-HPLC can differentiate all rice genotypes, including closely related sister lines. Our experience indicates that these pattern differences are due to genotype and not to variation associated with HPLC parameters or environment (Huebner and Bietz 1987; Scanlon et al 1989; Lookhart et al 1991).

LITERATURE CITED

DALRYMPLE, D. G. 1986. Development and Spread of High-Yielding Rice Varieties in Developing Countries. Agency for International Development: Washington, DC.

HUEBNER, F. R., and BIETZ, J. A. 1987. Improvements in wheat protein analysis and quality prediction by reversed-phase high-performance liquid chromatography. Cereal Chem. 64:15-20.

HUEBNER, F. R., BIETZ, J. A., WEBB, B. D., and JULIANO, B. O. 1990. Rice cultivar identification by high-performance liquid chromatography of the endosperm proteins. Cereal Chem. 67:129-135.

HUSSAIN, A., SCANLON, M. G., JULIANO, B. O., and BUSHUK, W. 1989. Discrimination of rice cultivars by polyacrylamide gel electrophoresis and high-performance liquid chromatography. Cereal Chem. 66:353-356.

JULIANO, B. O., HUSSAIN, A., RESURRECCION, A. P., and BUSHUK, W. 1991. Note: Interference of phytate with extraction of protein from brown rice using 5*M* acetic acid. Cereal Chem. 68:317-318.

LOOKHART, G. L., ALBERS, L. D., POMERANZ, Y., and WEBB, B. D. 1987. Identification of U.S. rice cultivars by high-performance liquid chromatography. Cereal Chem. 64:199-206.

LOOKHART, G. L., JULIANO, B. O., and WEBB, B. D. 1991. Effect of solvent extraction, environment, and genetic background on differentiating milled rices by reversed-phase high-performance liquid chromatography. Cereal Chem. 68:396-400.

NG, P. W. K., and BUSHUK, W. 1987. Glutenin of Marquis wheat as a reference for estimating molecular weights of glutenin subunits by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 64:324-327.

SCANLON, M. G., SAPIRSTEIN, H. D., and BUSHUK, W. 1989. Evaluation of the precision of high-performance liquid chromatography for wheat cultivar identification. Cereal Chem. 66:112-116.

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