Classification of Chinese Rice Varieties by Electrofocusing

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ABSTRACT

Examination of the prolamin fractions by electrofocusing allowed categorizing 25 varieties of Chinese rice into four major groups. Two bands (pI 5.3 and 7.7) are constitutive. Four major protein bands allow differentiation of Indica, Japonica, and glutinous rices and their hybrids and also indicate the application of physicochemical treatment. Minor bands allow further differentiation within each group.

On the basis of the content and chemical structure of the starch in the grain (Juliano 1972), three subspecies of Chinese rice can be distinguished, namely Indica (polished, long grained, nonglutinous rice) Japonica (polished, round grained, nonglutinous), and glutinous. Rice grains have an approximate composition of 80% starch, 7–8% protein (occasionally 14%), and 12% water. The proteins comprise 80% globulins, 10% glutelin, 5% albumin, and less than 5% prolamin.

Rice varieties need to be distinguished and identified so that rice husbandry can be improved by genetic and other research. A simple means of visualizing changes in protein composition, such as that related to spoilage during storage, would also be valuable. For these reasons, we analyzed the albumins and prolamins from 25 varieties of Chinese rice by high resolution electrofocusing, which differentiates proteins on the basis of the isoelectric point (pI). The albumin patterns are complex, but differences in the prolamin patterns allow four classes of rice and individuals within each class to be distinguished.

Electrofocusing was used to examine proteins of Texas long-grain rice (Padhye and Salunkhe 1979b) and of an Egyptian rice (Shadi and Djurtoft 1979). Each of these studies examined only one variety, and electrofocusing was used only to determine the range of isolectric points of the extracted proteins. Other major cereals such as wheat (Wrigley and McCausland 1977), barley (McCausland and Wrigley 1977), corn (Valentini et al 1979), and rye (Wrigley 1977) have been subjected to varietal analysis by electrofocusing.

MATERIALS AND METHODS

Rice milled from mature grains of 25 varieties was ground with a Moulinex electric coffee grinder. Before extraction, the rice was defatted by shaking for 24 hr with two changes of *n*-hexane (6 ml of hexane per gram of flour). The defatted flour was dried in a vacuum desiccator and stored at 4° C.

Acrylamide and *N,N'*-methylenebisacrylamide (bis), urea, mercaptoethanol *N,N,N',N'*-tetramethylethylenediamine (TEMED), and ammonium persulphate (Ultrograde, for electrophoresis), and carrier ampholytes (Ampholine) were from LKB. All other chemicals were analytical grade.

Separation of Albumin and Prolamin

The extraction procedure for rice proteins (Fig. 1) was a modification of that used by Padhye and Salunkhe (1979a) for black gram proteins. The fractionation procedure for prolamins was followed as far as dialysis before freeze-drying. For each rice variety, equal weights of flour were extracted under standardized conditions. We assumed that the same final protein concentration in the dialysate was obtained, and therefore equal volumes (30 μl) of the dialysate were applied to each track of the electrofocusing gel.

Electrofocusing of Rice Protein Extracts

Thin-layer polyacrylamide electrofocusing gels (0.5 mm thick) at pH 3.5–9.5 were prepared according to instructions (LKB note 1818-P) but modified to include 6*M* urea in the gel; 7.2 g of urea was added to 3.5 ml of 29.1% (w/v) acrylamide, 3.5 ml of 0.9% (w/v) bis, and 1.5 ml of carrier ampholytes, pH 3.5–9.5, and the volume was made up to 20 ml with double-distilled water. After deaeration for 10 min, 0.5 ml 1% (w/v) ammonium persulphate was added, and the mixture was injected into the prepared mold. Polymerization was complete in about an hour.

Urea and mercaptoethanol were added to the protein samples to give final concentrations of 6*M* and 2% respectively. (Urea is not necessary for electrofocusing of albumins, but it gives clearer and sharper bands.) To avoid possible carbamylation of the proteins and thus a change in isolectric point (Righetti and Chillemi 1978), gels and samples were used immediately after preparation, the gels were prefocused, and the sample was applied near the anode because no cyanate is formed and no carbamylation occurs below pH 5.0.

Electrofocusing was performed in an LKB 2117 Multiphor Electrofocusing Unit with an LKB 2197 power supply and LKB 2209 Multitemp thermostatic circulator. The electrolytes were 1*M* NaOH at the cathode and 1*M* phosphoric acid at the anode. The runs were all performed at a constant power of 25 W, with voltage and current limited to maxima of 2,000 V and 45 mA, respectively. The gels were prefocused for 15 min; then the samples were applied, and the experiment was run for 2.5 hr. The pH gradient across each gel was measured as described by Guo and Bishop (1982).

The separated proteins were simultaneously fixed and stained by using a colloidal suspension of Serva Blau G (equivalent to Coomassie Brilliant Blue G-250) in trichloroacetic acid/H_2SO_4 (Righetti and Chillemi 1978). In one experiment, the proteins were fixed and stained simultaneously by use of a solution of 0.2% bromophenol blue in ethanol, water, and acetic acid (50:45:5) (Awdeh 1969). The normal method of fixation with trichloroacetic and sulphosalicylic acids, followed by staining with Coomassie Brilliant Blue dissolved in ethanol, acetic acid, and water was unsuitable for the alcohol-soluble prolamins.

RESULTS AND DISCUSSION

Fractionation of Rice Proteins

With only a few modifications, Padhye and Salunke's method (1977a) worked successfully. Initial experiments showed that only the albumins and prolamins were profitably extractable for our purposes; Figure 1 shows only the necessary steps for these classes of proteins. The albumins, obtained in aqueous solutions, were stored at −20° C. Because the ethanol in which the prolamins were extracted interfered with electrofocusing, we either removed the ethanol by dialysis of the prolamins or reduced the concentration of ethanol by placing the ethanol-containing solutions in a vacuum desiccator for 24 hr.

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Extraction of the prolamins was much simpler and more rapid than extraction of the albumins. The electrofocusing band patterns of the prolamins are also less complicated than those of the albumins and allow easier identification of variety.

Ultraviolet Spectra

The spectra produced by the two classes of protein extracted from the Chinese rice are more typical of pure protein spectra than those of the Texas rice (Padhye and Salunkhe 1979b). The spectra from the Chinese rice albumins were similar for all varieties, with a trough at 248 nm and a peak at 270 nm. The prolamin spectra were also similar for all the varieties, with a trough at 258 nm and a peak at 275 nm. A small shoulder was also observed at 282 nm, suggesting high tryptophan content.

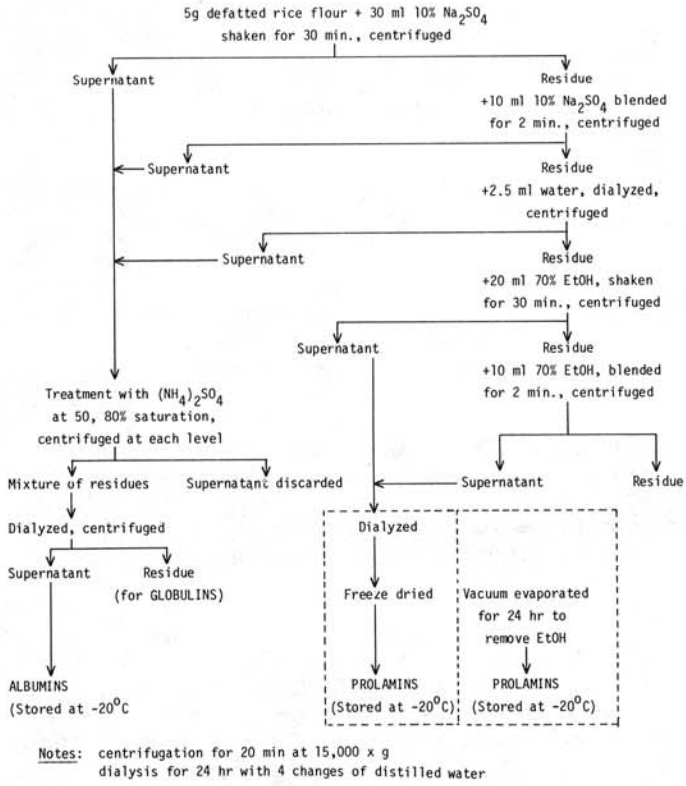

Fig. 1. Extraction procedure for Chinese rice proteins. (After Padhye and Salunkhe 1979a.)

Electrofocusing of Albumins

When albumin fractions of Chinese rice were examined by electrofocusing, more than 50 protein bands appeared, spread fairly evenly throughout pH range 3.5–9.5. Because of the complexity of the patterns and the involved extraction procedure, we considered the albumin fractions to be unsuitable for classifying the Chinese rice varieties.

The number of protein bands we found in the albumin fraction sharply contrasts with that observed in Texas long-grain rice (Padhye and Salunkhe 1979b). On electrofocusing, only one protein band was seen, although six were found after two-dimensional electrophoresis in sodium dodecyl sulfate. The differences are probably not entirely related to the lower sensitivity of the bromophenol blue they used, because the same number of albumin bands appeared in Chinese rice whether the stain was an equivalent to Coomassie Blue or bromophenol blue. Perhaps the total protein concentration in the Texas rice extracts was lower than that in our study.

Electrofocusing of Prolamins

We observed as many as 20 protein bands in the prolamin fractions, compared with 50 or more in the albumin fraction. In Texas long-grain rice, Padhye and Salunkhe (1979b) found only five bands in the prolamin fraction.

Based on the pI and staining intensity of the protein bands, the 25 varieties of Chinese rice can be divided into four groups. We assigned Roman numerals to the intensely stained bands as follows: I (pI 9.3), II (pI 8.2), III (pI 8.0), IV (pI 7.7), V (pI 7.4), and V (pI 5.3).

Group 1. The 12 hybrid species of Indica rice (A–L in Fig. 2) all exhibit intensely stained bands I and V, with about 10 moderately or weakly stained bands in the alkaline region of their band patterns.

The two types of prolamin band patterns are related to the different parentages. The first hybrid types (A–F, Fig. 2) are sterile

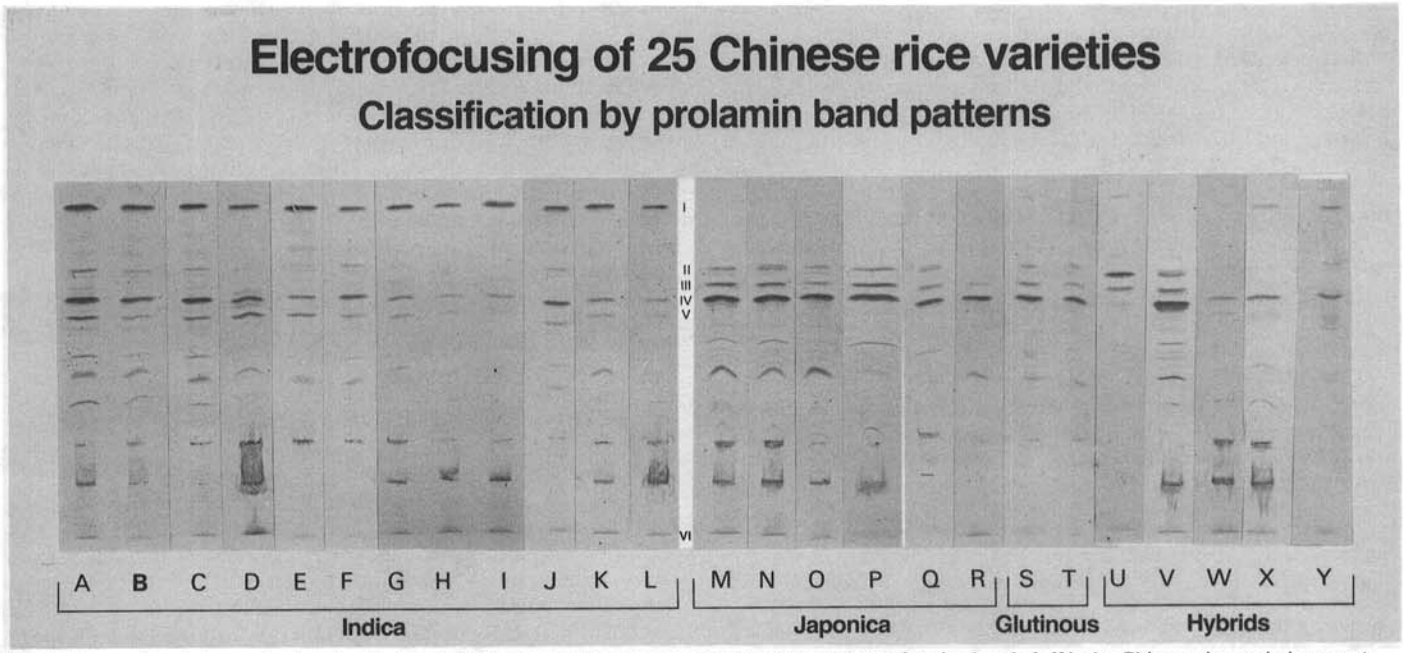

Fig. 2. Composite photograph of prolamin bands. Sorted according to presence or major absence of major bands I–IV, the Chinese rice varieties are: **A,** Shanyou 2; **B,** V-you-3; **C,** Gangchao 24; **D,** Zhongxian 2134; **E,** Shanyou 6; **F,** Shanyou 3; **G,** Qinglian 16; **H,** Zhuxi 26; **I,** Xiangaizhao 9; **J,** Shuangwan 105; **K,** Nanjing 11; **L,** Zhuguang 29; **M,** Wugeng 7412; **N,** Dangxuanwan 2; **O,** Nonghu 6; **P,** Huxuan 19; **Q,** Nangeng 33; **R,** Wangeng 74-24; **S,** Quinuo 55; **T,** Quihuanuo; **U,** Chuanxinnuo; **V,** Nanjing 34; **W,** Nanjing 35; **X,** Quichao 2; **Y,** Quichao 4.

lines and come from the crossing of Zhenshan 97A or V20A varieties of Chinese rice with IR8 or IR20 from the International Rice Research Institute. These hybrids have in common a greater number of bands and a more obvious band II than the second type of hybrid in this group.

The second type of hybrid (G–L, Fig. 2) consists of six consanguineous varieties of a Chinese dwarf strain. These hybrids have fewer bands than the first type; have weaker bands III, IV, and V; and lack band II in all but variety J.

We think that the differences between the weakly staining bands in the neutral and basic regions of the prolamin band patterns indicate possible interspecific differences within the Indica rices.

Group 2. The prolamin band patterns from six varieties of Japonica rice (M–R, Fig. 2) differ markedly from those of the Indica rices. The Japonica species all lack bands I and V but exhibit bands II, III, and IV more intensively than the Indica rices do. The weaker bands in the neutral and acid regions of the patterns are more numerous from the Japonica than from the Indica rices. The weakly stained bands appear to be species specific. Parent species of the Japonica rices include Nongken from Japan and a dwarf strain from China.

Group 3. Two glutinous rices (S and T, Fig. 2) exhibit the same prolamin band pattern, which is most similar to that in Japonica rices: absence of bands I and V and presence of an obvious band III.

Group 4. The prolamin band patterns of five hybrid rices (U–Y, Fig. 2) contain one characteristic common to Indica, Japonica, and glutinous rices. These five hybrid rices all have the major band IV, band VI of *Oryza,* and band I, although it is less intensely stained than in the Indica rices. (As mentioned, band I is absent from the prolamin patterns of Japonica rices.) Some special differences appear among the hybrids in this group, perhaps because they arise from several different parents or as a result of multiparent compound hybridization between Indica and Japonica rice. Other causes may be chemically induced or effects of climate and geography (e.g., temperature, humidity, and illumination) during the long periods that these species have been cultivated.

The protein band pattern of Nanjing 34 (V, Fig. 2) seems to show some characteristics of Indica and Japonica rices, of which it is a hybrid. Presence of bands II, III, and IV is a Japonica characteristic, but the obvious band V is similar to that in Indica rice (the very weak band I is not obvious in the figure).

With Indica IR26 and the offspring of a compound hybridization of the "jing" subspecies as parents, Nangeng 35 (W, Fig. 2) is also an Indica-Japonica rice. Quichao 2 and Quichao 4 (X and Y, Fig. 2) are sister strains of hybrid rices with the same parents; they exhibit prolamin band patterns that are similar to each other and to that of Nangeng 35.

Chuanxinnuo (U, Fig. 2), a hybrid of Indica and glutinous rices, has bands similar to those of glutinous rice in the acidic and neutral regions, an intense band III as in Japonica rice, and a weak band I as in Indica rice. Perhaps the greater intensity of band III than of band IV is because the major band IV of Japonica rice has moved to the more alkaline location of band III.

In summary, the prolamin band patterns include well-stained bands IV and VI, band IV usually being more intensely stained. Band VI is constitutive. Bands I and V are characteristic of Indica rice and are absent from Japonica and glutinous rices. Other differences in prolamin band patterns reflect interspecific differences.

CONCLUSIONS

Japonica rices are thought to have evolved from Indica rices, and the direct ancestor of cultivated species of *Oryza sativa* is probably O.S.L.F. Spontane (Ding 1964). In the electrofocusing band patterns of the prolamin fractions, bands I and V appear to change from "present" to "absent" from Indica to Japonica, with the intensity of band I in an Indica-Japonica hybrid being intermediate to that of the parents. Bands IV and VI, which determine the phenotype, are stable.

Presumably, changes in the proteins of rice induced by environmental factors and gene recombinations have equal effects on the amino acid composition and the ratio of protein fractions in the rice grain. The starches differ, however; Indica rice contains only amylose, Japonica contains branched amylopectins, and the glutinous rices contain more greatly branched amylopectins (Juliano 1972). The regularity that we observed in the electrofocusing band patterns of prolamins, especially in bands I and V, may perhaps have some relationship to the differences in the starches. The electrofocusing band patterns of rice prolamins could thus be used as one biochemical indicator for checking the quality of rices and for studying the evolution of rice.

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