

SIMILARITY AND DIVERSITY OF SEED PROTEINS IN RICE VARIETIES¹

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Abstract

The whole seed protein profile of dehulled rice grains was analyzed by one dimensional SDS-PAGE among 118 rice accessions including 26 native varieties in Taiwan, 28 breeding strains, 32 improved cultivars and 32 introduced foreign cultivars or strains. Electrophoregrams of most accessions indicated similarity in major polypeptide groups (14-17KD, 20-22KD and 31-34KD), but variations in number of bands and relative intensity of bands were frequently observed. Several accessions with low content of some of the above major polypeptides were noted. The nomenclature system used in wheat gliadin electrophoregram together with molecular weight markers was adopted in this study for assigning band number and variety Taichung native No. 1 was used as reference. Two minor bands assigned band No. 56 and 57, which might be served as phenotypic marker, were found in 25-27KD region. From total 32 improved cultivars, all the *japonica* types showed Band 56 while the *indica* types exhibited Band 57. Whether this can be served as an indicator for distinction between *japonica* and *indica* rice required further investigation. Two dimensional electrophoregrams of 5 accessions indicated that in spite of the general common features each accession possessed its own specific separation pattern, which is very useful in analysis and identification of the genetic background of rice variety.

Key words: Rice seed proteins, similarity and diversity, 1-D and 2-D gel electrophoresis.

Introduction

Seed protein electrophoresis has been utilized as a tool for resolving specific taxonomic and evolutionary problems, species and cultivar identification in many crops (Ladizinsky and Hymowitz, 1979; Moustakas *et al.*, 1986; Ferguson and Grabe, 1986). Specific protein electrophoregram can be used as genetic marker, e. g. isozyme electrophoregram for inheritance study in crop plants (Kahler and Lay, 1985; Tanksley and Orton, 1983). By polyacrylamide gel electrophoresis (PAGE),

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Hymowitz and Hadley (1972) reported a trypsin inhibitor variant in seed protein of soybean was controlled by two codominant alleles in a single locus. A codominant multiple allelic system was also noted by Orf and Hymowitz (1977) in a second trypsin inhibitor variant in seed protein of soybean. Orf *et al.* (1978) also utilized PAGE to analyze soybean seed lectin (SBL) and pointed out that SBL is controlled by a single dominant gene. In durum wheat (*Triticum turgidum* L.), gliadin electrophoregrams were used for the study of its relationship with wheat quality (Kosmolak *et al.*, 1980; du Cros *et al.*, 1982) and its association with glume color (Leisle *et al.*, 1985). Two dimensional PAGE has been employed by Sozinov *et al.* (1984) for genetic analysis of gliadin components in winter wheat. As indicated by Ladizinsky and Hymowitz (1979), stability, uniformity and additive nature are the main features of seed protein profile. They concluded that uniformity and uniqueness of the seed protein profile are typical of many groups of plants. However, variations in number of bands and their position in the profile has been reported, especially when a greater number of accessions were examined.

Rice (*Oryza sativa* L.) seed protein can be grouped into four fractions according to the solubilities: albumin, globulin, prolamin and glutelin with the major storage protein being glutelin (Juliano, 1972). Previously, most studies concerning rice protein involved isolation, characterization and biosynthesis (Juliano and Boulter, 1976; Yamagata *et al.*, 1982; Villareal and Juliano, 1978; Perdon and Juliano, 1978; Mondac and Juliano, 1978; Cagampang *et al.*, 1976; Damardjati *et al.*, 1985; Chen and Cheng, 1986). Very few information is available for varietal difference in electrophoretic pattern of seed protein among rice cultivars. Nakai (1977) reported variation in soluble protein profile between diploid and induced autotetraploid rice. Studies on aminograms and SDS-PAGE of milled rice glutelin of 12 *Oryza sativa* samples by Villareal and Juliano (1978) indicated little possibility of finding out variants of rice glutelin. Damardjati *et al.* (1985) evaluated protein quality and properties in six varieties of Indonesian rice and found nearly identical electrophoretic patterns of whole protein extract among four varieties tested. However, variation in some minor subunits was noted in their studies. Sarkar and Bose (1984) revealed qualitative and quantitative differences of salt soluble protein fraction of seeds in eight rice varieties. Chen and Cheng (1986) also observed variation of electrophoretic patterns in some minor bands of endosperm protein extract from three *indica*, two *japonica* and a Taiwan wild rice. In this study, we are interested in finding out specific rice seed protein profile from electrophoresis of total seed protein, which might be further characterized and used as genetic markers for inheritance study. One dimensional PAGE patterns of various rice accessions were analyzed. A more detailed analysis by two dimensional gel electrophoresis was also carried out in some accessions. Types of variations and potential phenotypic markers were discussed in this report.

Materials and Methods

Seeds lots from 118 rice accessions, including 26 native rice varieties in Taiwan, 28 breeding lines from seed laboratory of Department of Agronomy in National Taiwan University, 32 improved cultivars and 32 varieties or strains originated from different countries, were obtained for this study. The 32 improved cultivars include 11 *indica* types, 12 *japonica* types, four upland rice varieties and five glutinous rice accessions.

Chemicals and reagents obtained from the commercial sources were listed below: Tris base, EDTA, Nonidet P-40, sodium dodecyl sulfate (SDS), acrylamide, N,N'-methylene-bisacrylamide, ammonium persulfate, ammonium sulfate, bovine plasma albumin, Coomassie Brilliant Blue R-250, bromophenol blue, glycerol, glycine, molecular weight standard kit (SDS-7), sodium chloride (Sigma Chemical Co., USA); methanol, acetic acid, sucrose, phosphoric acid, NaOH (E. Merck); carrier ampholytes-Biolyte 3/10, 6/8 (Bio-Rad Laboratories, USA).

Preparation of Rice Samples and Extraction of Total Seed Proteins

Three to four grams of mature rice grains from each accession were dehulled with a hand-cracker. The dehulled rice grains (including endosperm, embryo, pericarp and aleurone layer) were then ground to very fine powder and stored at -20°C until use.

The buffer for extracting total seed proteins consisted of 50 mM Tris-HCl, 2% sodium dodecyl sulfate (SDS), 0.6% 2-mercaptoethanol and 4 M urea at pH 7.5. One ml of buffer was added to 200 mg of rice meal in a centrifuge tube. The sample was extracted by shaking vigorously at room temperature for three hours. The extract was collected by centrifugation at $13,500 \times g$ for 15 minutes. The extraction was repeated three times. Protein extracts were combined and submitted to SDS-PAGE analysis.

Analysis of Rice Protein by SDS-PAGE

SDS-PAGE was conducted by the method of Laemmli (1970) with slight modification. The running gel contained 14% of acrylamide while the stacking gel contained 5.0% acrylamide. The electrode buffer was 25 mM Tris, 192 mM glycine and 0.1% SDS at pH 8.3. The sample contained total seed protein extract, 8% glycerol, 8% 2-mercaptoethanol and trace amount of bromophenol blue, which was preheated at 100°C for 3-5 minutes before loading. The sample size in each lane was 20 μl containing approximately 30-40 μg protein. The applied starting current was 7.5 mA and increased to 25 mA when the tracking dye reached the running gel. Bovine serum albumin (66 KD), egg albumin (45 KD), glyceraldehyde-3-phosphate dehydrogenase (36 KD), carbonic anhydrase (29 KD), trypsinogen (24 KD), soybean trypsin inhibitor (20.1 KD), and α -lactalbumin (14.2 KD) were served as molecular

weight standard. For each run, the check variety-Taichung Native No. 1 (TCN 1) was always included. After electrophoresis, the gels were stained with 0.12% (w/v) Coomassie Brilliant Blue R-250, 50% methanol and 10% acetic acid for two hours, then destained in 30% methanol and 10% acetic acid overnight with several changes. The gel was dried on the filter paper under vacuum with low heat. For gel analysis, the nomenclature of the band number followed the method of Bushuk and Zillman (1978) by assigning a reference (TCN 1) and defining a major band in the middle of gel with an arbitrary mobility of 0.50. All the other bands of TCN 1 and other accessions are identified on the basis of electrophoretic mobility relative to the reference band.

Two Dimensional Gel Electrophoresis

The preparation and running of isoelectric focusing (IEF) gels for the first dimension followed the procedures of O'Farrell (1975). The second dimension was SDS-PAGE as described above.

For IEF, 2% of ampholyte (pH 3.5-10) in the cylindrical gel was used. The sample was prepared by mixing protein extract (100-200 μ g) with equal volume of sample buffer containing 9.95 M urea, 4% NP-40, 2% ampholyte (pH 6-8), 5% 2-mercaptoethanol and 0.3% SDS. The upper (cathode) reservoir solution was 20 mM NaOH and the lower (anode) reservoir solution was 10 mM phosphoric acid. All samples were applied at the top of the gels. IEF was completed in 8000 Vh. The first-dimensional gels were soaked in equilibrium buffer containing 62 mM Tris-HCl (pH 6.8), 2.3% SDS, 10% glycerol and 5% 2-mercaptoethanol for 1 h. The equilibrated gels were then subjected to second dimensional SDS-PAGE (14%, 1.5 mm thick) at current of 35 mA. The slab gels were stained for 3 h with Coomassie Blue R-250 and then destained as described above.

To measure pH gradient, an IEF gel was cut into 5-mm slices. Each slice was placed in a small test tube containing 0.2 ml of degassed 25 mM KCl. The tubes were sealed and shaken at room temperature for 2 h; then the pH was measured with a pH meter.

Results

Rice seed protein profile of SDS-PAGE showed certain variations among different cultivar accessions (Tables 1 and 2). Although most of the accessions showed a greater similarity rather than dissimilarity among the major band patterns, variations in number of bands, band intensity, and band patterns were observed in some accessions. Presences of crowded band patterns as well as some diffuse bands cause the difficulty in counting the exact band number. In general, three major polypeptide groups, i.e., 14-17 KD, 20-22 KD and

Table 1. Summary of major variations in rice seed protein profile of SDS-PAGE

Zone	A					B					C				
	MW ^a	I	II	III	MW	I	II	III	MW	I	II	III	IV	V	
Band Type	66KD	████							████						
		▨▨▨▨	▨▨▨▨	▨▨▨▨											
		▨▨▨▨	▨▨▨▨	▨▨▨▨											
No. of Accession	90	1	27			54	55	9		32	27	41	7	11	
(%)	Start point (76.3)(0.8)(22.9)				(45.8)(46.6)(7.6)				(27.1)(22.9)(34.8)(5.9)(9.3)						
Band Type															
	24KD	████													
	29KD	████													
		▨▨▨▨	▨▨▨▨	▨▨▨▨											
No. of Accession	43	67*	1	7		100	10	8							
(%)	(36.4) (56.8) (0.8) (5.9)				(84.7) (8.5) (6.8)										

Note: Bar types referred to different band intensity.

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*: Including two accessions with band intermediate between Type I and II.
 a: MW - Molecular weight standards

Table 2. Band pattern classification referring to Table 1 of rice seed protein profiles among accessions examined

Accession ^a No.	Name	Zone				
		A	B	C	D	E
1	Taichung native No 1	I	I	I	II	I
2	Ti-chueh-wu-chien a	I	I	I	II	I
3	Ti-chueh-wu-chien b	I	II	II	III	I
4	Eu-mang-tsao-keng	I	II	II	III	I
5	Yu-kao-tzu	I	II	III	II	I
6	Ti-chueh-liu-chou	I	II	II	II	I
7	A-lun-ch'ung	I	I	I	II	I
8	Shuang-chiang	I	I	II	II	I
9	Liu-tou-tzu	I	I	III	II	I
10	Kung-erh-ai	I	II	IV	II	I
11	Chiu-kuo-tsao	I	I	III	II	I
12	Taichung-pai-ko	I	II	III	I	I
13	Taipei-wu-ko	I	II	I	II	I
14	Chiu-ko	I	I	I	II	I
15	Yu-tzu	I	II	III	II	I
16	Hua-lou	I	II	II	I	I
17	Sheng-li-shien	I	II	III	II	I
18	Tuan-kung-hua-lou	I	II	II	II	I
19	Ai-chueh-chien	III	I	I	II	I
20	Pai-ko-hua-lou	I	II	II	II	I
21	Kao-chueh-liu-chou	I	II	II	II	I
22	Tai-li-ching-yu	I	II	II	I	I
23	Wu-ko	I	II	II	II	I
24	<i>O. perennis</i> var. <i>formosana</i>	I	II	III	II	II
25	Pai-mi-fen	I	I	III	II*	I
26	Tsai-yuan-ch'ung	I	I	II	I	I
27	Hung-chueh-no	I	I	I	II	II
28	Chai-nung shien 8	I	II	I	II	I
29	Chai-nung shien 11	III	I	I	II	I
30	Kaohsiung shien 2	I	I	I	II	I
31	Taichung shien 2	III	III	V	II	III
32	Taichung shien 3	I	II	I	II	I
33	Taichung shien 5	I	I	II	II	I
34	Taichung shien 10	I	I	IV	II	I
35	Taichung shien 17	I	I	I	II	I
36	Tainung shien 18	I	I	III	II	I
37	Tainung shien 19	I	I	I	II	I
38	Hsin-chun-no 4	III	I	I	I	III
39	Tai-chung-no 70	III	III	V	I	III

Accession ^a No.	Name	Zone				
		A	B	C	D	E
40	Tai-nung selected 2	I	I	II	I	I
41	Tainung-shien-no 1	I	I	III	II	II
42	Tainung-shien-no 2	I	I	I	II	II
43	Tainan 1	I	I	III	I	I
44	Tainan 2	I	II	III	I	I
45	Tainung selected 1	I	I	III	I	I
46	Tung-lu 2	III	III	V	IV	III
47	Kwang fu 1	III	I	I	I	I
48	Kao-hsiung 18	I	II	III	I	I
49	Taichung 65	III	II	IV	I	I
50	Taichung 153	I	II	I	I	I
51	Taichung 179	III	II	I	I	I
52	Taichung 181	I	II	II	I	I
53	Taichung 189	I	II	III	I	I
54	Tainan 5	I	II	III	I	I
55	Tainung 61	I	II	I	I	I
56	Tainung 67	I	I	II	I	I
57	Tainung 69	I	I	I	I	I
58	Yoshino 1	III	I	I	I	I
59	C 4-63	I	III	I	II	I
60	C 46-15	I	III	I	II	I
61	Dawn	III	I	I	I	III
62	Della	I	II	III	I	II
63	Dular a	II	II	III	II	II
64	Dular b	I	II	III	II	II
65	Fukutomi	I	I	III	II*	II
66	Hokuriku 100	I	II	II	I	I
67	Huan-sen-goo	I	II	II	II	I
68	IRAT-13	III	III	V	IV	III
69	IR-8	I	II	III	II	II
70	IR-9-60	I	II	II	I	I
71	IR-22	I	II	III	II	I
72	IR-28	I	II	III	II	I
73	IR-29	I	II	II	II	II
74	IR-30	I	I	IV	II	I
75	IR-1529-680-3	I	I	I	II	I
76	IR-1820-210-2	I	I	I	II	I
77	IR-1905-pp11-4-61	I	II	III	I	I
78	IR-2153-338-3	I	I	IV	II	I
79	IR-4547-2-1-2	III	III	V	I	I
80	Joyanti	I	I	III	II	I
81	Kameji	I	II	III	I	I

Accession ^a No.	Name	Zone				
		A	B	C	D	E
82	Kanto 51	I	II	IV	I	I
83	Koshihikari	III	III	V	IV	III
84	Lomello	III	II	IV	I	I
85	N 22	III	I	I	II	I
86	P-738-97-3-1	I	II	III	II	I
87	Peta	I	I	I	II	I
88	Tetep	I	II	III	I	I
89	Toyonishiki	I	II	II	I	I
90	Yoneshiro	III	I	I	I	I
91	C 266	I	II	II	I	I
92	CC1-3	I	II	II	I	I
93	CC1-7	I	II	II	I	I
94	CC1-10	I	II	II	I	I
95	CC1-13	I	II	II	I	I
96	CC1-16	I	II	II	I	I
97	CC1-17	I	II	II	I	I
98	Tai-ta-yu 20	I	I	III	II	I
99	Tai-ta-yu 36	I	II	I	II	I
100	Tai-ta-yu 43	III	I	III	II	I
101	Tai-ta-yu 45	III	I	III	II	I
102	Tai-ta-yu 57	I	I	III	II	I
103	Tai-ta-yu 65	I	I	III	II	I
104	Tai-ta-yu 105	III	I	V	IV	I
105	Tai-ta-yu 139	I	I	III	II	I
106	Tai-ta-yu 146	I	I	III	II	I
107	Tai-ta-yu 161	III	I	V	IV	I
108	Tai-ta-yu 179	I	I	III	II	I
109	Tai-ta-yu 187	I	I	III	II	I
110	Tai-ta-yu 26-5	III	I	III	II	I
111	Tai-ta-yu 2-1-3	I	I	V	II	I
112	Tai-ta-yu 43-3-6	I	II	I	II	I
113	Tai-ta-yu 43-3-9	I	II	III	II	I
114	Tai-ta-yu 78-3-1	III	I	V	IV	I
115	Tai-ta-yu 98-5-5	I	I	III	II	I
116	Tai-ta-yu 108-3-3	III	I	I	II	I
117	Tai-ta-yu 4-3-3-12	III	III	V	IV	III
118	Tung-lu-yu 78	III	I	I	I	I

^a: Accession No. 1: check variety; 2-27: Taiwan native varieties; 28-58: improved varieties, 28-37 are *indica* type, 38-42 are glutinous rice, 43-46 are upland rice, 47-58 are *japonica* type; 59-90 are plant introduction accessions; 91-118 are breeding lines.

*: Mobility of this band type is slightly slower than type II.

31-34 KD, can be easily identified from electrophoregram in most accessions (Fig. 1). The electrophoregram was dissected into five zones for the convenience of analysis. Zones A is from the start point of the running gel to 66 KD region (Band 0-21), zone B from 66 KD-36 KD (Band 22-44), zone C from 36 KD-29 KD (Band 45-54), zone D from 29 KD-24 KD (Band 55-60) and zone E from 24 KD-14KD (Band 61-80). In zone A, most variation was referred to difference in band intensity, however, some accessions with very weak or without these high molecular weight bands were observed (Fig. 1; lane H). A variant with an extra band (Band 11), which was not observed in any other accessions was found in a Pakistani accession (Dular a). However, seeds of the same variety (Dular b) maintained in Taiwan Agricultural Research Institute did not show this extra band. Seeds of Dular a was originated from IRRI but propagated in Nankang. We believed that the extra band of Dular a accession in zone A probably was originated from some wild rice species. Because Band 11 was frequently observed in several wild rice accessions (data not included). In zone B, variations in band number, relative band intensity as well as band patterns between 63 to 53 KD region (Band 28-32) (Fig. 1, lane A-L) were frequently observed. In zone C, a crowded band pattern made the distinction a little more difficult. From the overall band pattern, most difference in this zone laid between 34 KD-30 KD (Band 48-53). Types of variations can be viewed from lane A to lane H of Fig. 1. One of the three major polypeptide groups was in this zone. A most distinct difference which may serve as a pheno-

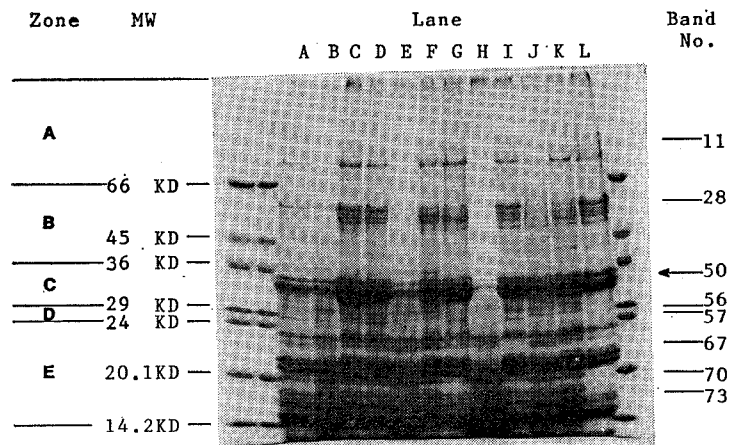


Fig. 1. Electrophoregram of rice seed protein from 12 accessions showing different types of variations.

Lane A: Taichung native No. 1; B: Chai-nung-shien 11;
 C: Ti-chuen-wu-chien b; D: Yu-kao-tzu; E: Dawn;
 F: Tai-nung 61; G: IR-29; H: Tung-lu-yu 314;
 I: Dular a; J: Taichung 65; K: Hokuriku 100;
 L: Taiwan wild rice (*Oryza perennis* var. Formosana).

typic difference for most varieties was in zone D. Two types of band, assigned Band 56 (slower band) and 57 (faster band), was observed between 24-29 KD (Fig. 1, lane I and J). Although the mobilities of these two bands were very close, most accessions possessed one or the other except the accession Ti-chueh-wu-chien *b* which had both bands (Fig. 1, lane C). From our recent study, Ti-chueh-wu-chien *b* was proved to be an accession contaminated by out-cross.

Of total 118 accessions examined, 67 accessions (56.8%) possessed Band 57 (faster band), 43 (36.4%) with Band 56 (slower band), 1 (0.8%) with both 56 and 57 bands, and 7 accessions (5.9%) with diffuse bands at this region which could not be distinguished. From 32 improved cultivars all *indica* rice possessed Band 57 while those of *japonica* types had Band 56. Whether these two particular bands could be used for genetic study demands further examination. Furthermore, two varieties possess band slightly slower than Band 57 was noted. Two other major polypeptide groups of most varieties are in zone E. One group is in 17-14 KD region (Band 73-77) and the other is in 22-20 KD region (Band 67-70). Variations in this zone was lack of some major bands or difference in intensity of some major bands (Fig. 1, lane F-H). For example, four varieties [IR-29, Della, Dular (Fig. 1, lane I) and the wild type rice (Fig. 1, L)] had very weak band 70 and 73 or even lack these bands compared to other varieties. Some accessions had relatively weaker intensity in Band 67, 68 and 70 than others (Fig. 1, lane E and H). The major types of variations were summarized in Tables 1 and 2.

The two dimensional electrophoregrams of rice seed proteins from five accessions demonstrated a level of complexity-particularly in the relatively scarce proteins of pI's between 4.5 and 6.5. Figure 2 shows two examples of the electrophoregrams: TNC 1 and Dular a. The major polypeptide group 31-34 KD was resolved into more than 10 spots in which pI's ranged mainly from pH 6.2 to 7.2. Due to the basic property of the major polypeptide group 20-22 KD (Wen and Luthe, 1985), a large amount of the protein was focused at the top of the first-dimension gel. More than five spots could be visualized in this group in spite of the poor resolution. It is believed that some of the basic proteins have moved into the cathode reservoir due to the general phenomenon of cathodic drift-slow electrophoresis of basic ampholytes (Lei, *et al.* 1983). The electrophoregrams of five rice accessions reveal that each accession has its own specific two dimensional separation pattern with some common spots.

Discussion

The nomenclature of band number in SDS-PAGE electrophoregram followed the method of Bushuk and Zillman (1978), which required a reference variety. Since TCN 1 was the check variety in gel analysis, it was used as the reference

variety. The major band located between 29 and 36 KD region (Fig 1, arrowed) of TCN 1 was assigned with an arbitrary mobility of 0.50 (band number 50) and all the other bands were identified by a mobility relative to this reference variety. The employment of this nomenclature is for the convenience of pointing out band difference along the electrophoregram. Otherwise, variation in number of bands in different accessions will cause difficulties in assigning number to a specific band. The results in Table 1 and Fig. 1 indicated varietal differences in number of bands, band pattern and relative band intensity of rice protein SDS-PAGE profile. Although the band patterns have difference among the rice accessions, most cultivars seem to have similar pattern with variation in band intensity.

Several accessions exhibited remarkable variation in the major polypeptide. The bands 48-52 (34-32 KD region) were weak in Tung-lu-yu 314, Koshihikari, IRAT-13 and IR 4547-2-1-2 compared with other accessions. The higher molecular weight bands (< 45 KD) in these varieties were also very faint. To our knowledge, no report has been published regarding the large difference in rice seed proteins among varieties. Whether this is due to varietal specificity or some other factors has been under study. Present evidence shows that seed sources contaminated with defective seeds, which were probably infected with certain pathogen, tend to lose those higher molecular weight bands.

Robinson and Megarrity (1975) reported that seed proteins are mainly storage proteins and are not likely to be changed in dry mature seeds of different age. This phenomenon is confirmed by Ferguson and Grabe (1986) studying ryegrass. However, the composition of seed proteins is affected slightly by environmental conditions and seasonal fluctuations (Lee and Ronald, 1967; Gray *et al.* 1979). From our study, same variety obtained from different sources frequently exhibited slightly different SDS-PAGE patterns, especially variation in relative band intensity. Whether this is due to environmental effect, evolved land race, or different management systems requires more study. From our further investigation, it was indicated that different cropping seasons and stored seeds had effects on the relative band intensity but did not change the electrophoregram patterns.

As mentioned by Ladizinsky and Hymowitz (1979) that uniformity and uniqueness of seed protein profile are typical of many groups of plant, to find variation in the number of bands and their position in the profile frequently requires analyzing a large number of accessions. In SDS-PAGE analysis, 118 accessions including domestic and foreign varieties were used. Due to the limited number and lack of enough informations on general characteristics of these accessions, we are not able to correlate specific band patterns with certain agronomic characteristic at present. Nevertheless we believe some of the bands are inherited qualitatively, like that of 42 and 45 bands in gliadin of durum wheat (Leisle *et al.*, 1855).

Two dimensional electrophoregrams of Fig. 2 show that rice seed proteins are a very complex mixture, with several hundred detectable polypeptides. They vary widely in PI's, in molecular weights and in abundance in the extract. Heterogeneities of major polypeptide groups 31-34 KD and 20-22 KD were clearly observed. For obtaining better resolution of the basic proteins in the extract, nonequilibrium pH gradient electrophoresis (NEPHGE) as the first dimension (O'Farrell, 1977) may be used. However, poor resolution of the acidic components will be obtained. Therefore, no single set of conditions for two dimensional electrophoresis can provide a comprehensive, high-resolution analysis of all the polypeptides in the rice seeds. Examination of five rice accessions by two dimensional electrophoresis indicated that each accession possessed its own specific separation pattern with

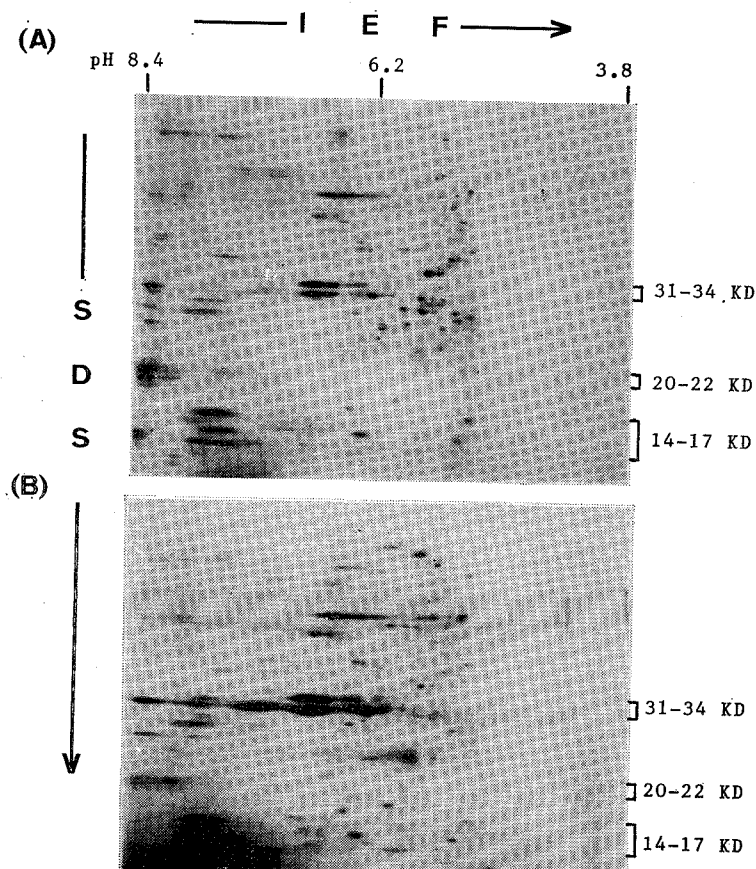


Fig. 2. Two dimensional gel electrophoregrams of total seed protein from two rice accessions.

First dimension: IEF, 8000 Vhr; Second dimension: SDS-PAGE (14%)

(A): Taichung native No. 1 (100 μ g protein)

(B): Dular (200 μ g protein)

some common spots.

This study suggests that analysis of rice seed proteins by one dimensional SDS-PAGE and two dimensional gel electrophoresis is very useful in identifying the rice accessions and investigating their genetic backgrounds in the future.

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水稻品種間種子蛋白質之同異性

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利用 118 個水稻品系（包括 26 個本省在來品種，28 個育種品系，32 個改良品種以及 32 個國外引入之品種或育種品系）從事水稻去殼穀粒全蛋白質剖面之 SDS-PAGE 膠板電泳分析。由電泳圖顯示，大部分品系具有很相似的主要勝肽鏈羣（14-17 KD, 20-22 KD 及 31-34KD），惟在部分品系中，電泳帶的數目以及帶與帶之間相對濃度亦發現時有不同。有些試測品系並僅具少量的上述主要勝肽鏈。有關電泳帶之命名係採用小麥麩朊蛋白質電泳圖系統輔以標準蛋白質分子量標幟因子，並利用臺中在來一號為參考品種。在電泳圖上 25-27 KD 之間有二條特出的非主要勝肽鏈帶（帶 56 及 57）其很有可能被利用來當一種外表型之記號。在所有檢試過的 32 個改良品種中，所有日本型均具有帶 56（較慢者），而印度型則具帶 57（較快者），是否這兩帶可利用來分辨印度型及日本型稻有待進一步之研究。此外由五個品系之雙向電泳圖分析顯示，除了其一般特性外每一品系均具有其特殊之分離型式，此將非常有助於鑑定水稻品種之遺傳背景。