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Differentiation of rice varieties by electrophoresis of embryo protein

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Summary. Variations in the embryo proteins separated by SDS-PAGE have been observed in 43 cultivated varieties of *Oryza sativa* L. Cluster and discriminant analysis applied to both protein components and morphological characters indicate that knowledge of the differences in embryo proteins can improve our understanding of genetic affinity and make it easier to differentiate between varieties of similar genetic backgrounds.

Key words: *Oryza sativa* – Embryo-proteins – SDS-PAGE – Genetic affinity – % Similarity-equation

Introduction

The systematic methodology mainly based on morphology has been improved by the incorporation of physiological, ecological or biochemical characters. Several biochemical analyses, especially of proteins, make it possible to establish differences at various taxonomic levels (Vaughan 1983). One of the biochemical methods more extensively used for taxonomic purposes has been the electrophoretic analysis of the proteins found in seeds and storage organs. These proteins are physiologically stable and easy to handle (Ladizinsky and Hymowitz 1979).

Serological and electrophoretic studies and the comparison of globulins and albumins in three species of *Brassica* were carried out by Vaughan et al. (1966). Boulter et al. (1967) found a correlation between the globulins in several tribes of the leguminous family. Sarkar and Bose (1984) observed variation in fractions of albumin and globulin in eight rice varieties. Vaughan and Denford (1968) studied the reciprocal relationship between the protein spectra of nine *Brassica* and *Sinapsis* species using a percentage similarity equation converted into a three dimensional model. Other studies comparing the percentage similarity have been carried out in *Suaeda* (Ungar and Boucard 1974) and in *Acer saccharum* (Ziegenfu and Clarkson 1971). Also, numerous studies have been accomplished in wheat and barley cultivars (Shewry et al. 1979; Zillman and Bushuk 1979; Marchylo and LaBerge 1980). Electrophoretic protein separation techniques in *Zea* (Smith and Lester 1980), *Gossypium* (Johnson and Thein 1970), *Hordeum* (McDaniel 1971), *Umbelliferae* (Crowden et al. 1969), *Coffea* (Centi-Grossi et al. 1969; Paine et al. 1973) and *Oryza* (Siddiq et al. 1972; Inocencio et al. 1980) have been used.

Electrophoretic analysis in the identification of varities is useful in plant breeding, commerce and distribution of seeds, as well as for legal reasons and patent protection. This study is directed towards obtaining more objective, stable and reproducible measures for identifying varieties and studying intervarietal affinity of cultivated rice (Primo and Barber 1976).

Materials and methods

Forty-three rice varieties (*Oryza sativa* L.) (Table 1), one-half representing advanced lines of improved varieties, were cultivated under identical conditions. Direct sowing and the usual cultivation practices were followed. The plants were fertilized with 720 kg/ha ammonium sulfate (21% N) and 300 kg/ha superphosphate (18% P_2O_5). The sowing density was of 25 plants/m^2. Biometric measurements of 47 quantitative morphological characters and 26 qualitative ones were taken according to earlier descriptive studies made in rice varieties (Tinarelli and Ravasi 1963; Chang 1976). Proteins were extracted from embryos of rice priviously dissected from the dry seed (14% humidity) and triturated as described by Culiáñez et al. (1981). In each case 0.1 g plant material was homogenized with 5 ml 0.05 M tris-HCl buffer (pH 8), 0.25 M sucrose, 0.001 M $MgCl_2$, 0.001 M $NaHSO_3$, 0.001 M 2-mercaptoethanol and 2% sodium dodecyl hydrogen sulfate (SDS), using a Polytron MOD-PT 10/35 (Kinematica). The homogenate was heated to 100 °C with a thermostat bath for 4 min, and then it was centrifuged at 4,000 × g for 30 min. Proteins in the supernatant were quantified by the method of Lowry et al.

Table 1. Rice varieties grouped according to embryo protein contents

Variety	Subspecies	Obtaining	Ancestors	Country
BALILLA (BAL)	Japonica	Selection	Chinesse Originario	Italy
MATUSASKA	Japonica	Selection		Japan
FRANCES	Japonica	Selection	Precoce Corbeta	France
GIRONA	Japonica	Seelction	Stirpe 136	Spain
BAL × SOLLANA (BALSOLL)	Japonica	Artificial crossing	Balilla and Sollana	Spain
NIVA	Japonica	Artificial crossing	Balilla and Razza 77	Spain
JUCAR	Japonica	Artificial crossing	Balilla and Stirpe 136	Spain
BETIS	Japonica	Artificial crossing	Gema and Sequial	Spain
CARRASQUER	Japonica	Selection	Bahia	Spain
BAHIA (B)	Japonica	Artificial crossing	Balilla and HI 2	Spain
SEQUIAL (S)	Japonica	Artificial crossing	Stirpe 136 and Balilla	Spain
BAL × LOMELLO (BALLOM)	Japonica	Artificial crossing	Balilla and Lomello	Spain
SB 414	Japonica	Artificial crossing	Sequial and Bahia	Spain
SB 428	Japonica	Artificial crossing	Sequial and Bahia	Spain
SB 541	Japonica	Artificial crossing	Sequial and Bahia	Spain
SB 551	Japonica	Artificial crossing	Sequial and Bahia	Spain
SB 553	Japonica	Artificial crossing	Sequial and Bahia	Spain
SB 597	Japonica	Artificial crossing	Sequial and Bahia	Spain
SB 1086	Japonica	Artificial crossing	Sequial and Bahia	Spain
S × DOSEL 223 (SD 223)	Japonica	Artificial crossing	Sequial and Dosel	Spain
SD 260	Japonica	Artificial crossing	Sequial and Dosel	Spain
SD 390	Japonica	Artificial crossing	Sequial and Dosel	Spain
SD 537	Japonica	Artificial crossing	Sequial and Dosel	Spain
SD 561	Japonica	Artificial crossing	Sequial and Dosel	Spain
BAL × STIRPE 605 (BALSTR)	Japonica	Artificial crossing	Balilla and Stirpe	Spain
B × IR 52 (BIR 52)	Japonica	Artificial crossing	Bahia and IR 52	Spain
S × B × S 58 A (SBS 58)	Japonica	Artificial crossing	Sequial and Bahia	Spain
SBS 128	Japonica	Artificial crossing	Sequial and Bahia	Spain
SBS 148	Japonica	Artificial crossing	Sequial and Bahia	Spain
SBS 172	Japonica	Artificial crossing	Sequial and Bahia	Spain
SBS 174	Japonica	Artificial crossing	Sequial and Bahia	Spain
S × HI 2 × S 21 (SHIS 21)	Japonica	Artificial crossing	Sequial and HI 2	Spain
SHIS 69	Japonica	Artificial crossing	Sequial and HI 2	Spain
SHIS 106	Japonica	Artificial crossing	Sequial and HI 2	Spain
ITALPATNA	Japonica	Artificial crossing	Agostano, P6, Blue Rose	Italy
RIBELLO	Japonica	Artificial crossing	Bersani and Ribe	Italy
BERSANI	Japonica	Selection	Sesia	Italy
RUBINO	Japonica	Artificial crossing	Nano and Carnaroli	Italy
M 7	Indica	Mutation	Like CS M3	USA
M 9	Indica	Artificial crossing	IR 8	USA
BLUEBELLE	Indica	Artificial crossing	CI 9214 and CI 8993	USA
BLUEBONNET	Indica	Artificial crossing	CI 9214 and CI 8993	USA
MARS	Indica	Artificial crossing	CI 9214 and CI 8993	USA

BAL: Balilla; BALSOLL: Balilla × Sollana; B: Bahia; S: Sequial; BALLOM: Balilla × Lomello; SD: Sequial × Dosel; BALSTR: Balilla × Stirpe; BIR: Bahia × IR 52; SBS: Sequial × Bahia × Sequial; SHIS: Sequial × HI × Sequial

(1951) and electrophoretically separated according to Conejero and Semancik (1977) in SDS-PAGE. Fifty microgram of protein were applied to each slot and run at 4 °C under a constant intensity of 2 mA per slot, for 3.5 h reaching a final voltage of 300 V. Gels were stained with Coomassie brilliant blue.

Protein characters were measured by a Beckman CDS-200 densitometer which delimited bands and measured their relative area automatically. Molecular weight was estimated with the kit marker Pharmacia Fine Chemicals LMW. The comparative analysis between varieties was carried out with three replicates of each variety by means of the Keuls test.

A number X_{ij} (i: character, j: variety) was assigned to both morphological and protein characters. The similarity of the varieties in the original data matrix was estimated by means of a distance coefficient defined in n-dimensional space, whose axes were the characters. Each variety is the end of a vector whose compounds are the morphological and protein characters used in the varietal description. The dendrogram of the concentration of points (clusters) expresses the percentage similarity between varieties. The statistical programmes used in the data processing were those corresponding to the cluster analysis and discriminant analysis available in biomedical computer programs (BMDP).

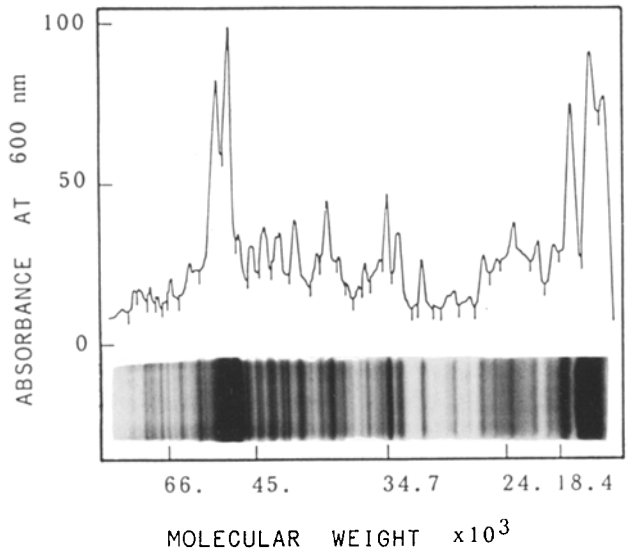

Fig. 1. A typical SDS-PAGE and densitometric tracing of rice embryo proteins ('BalLom'). The direction of migration was from left to right. The values of 37 protein components were expressed in percent of relative area. Values were means of three determinations. The maximum standard deviation was 0.70 with a variation coefficient of 14%

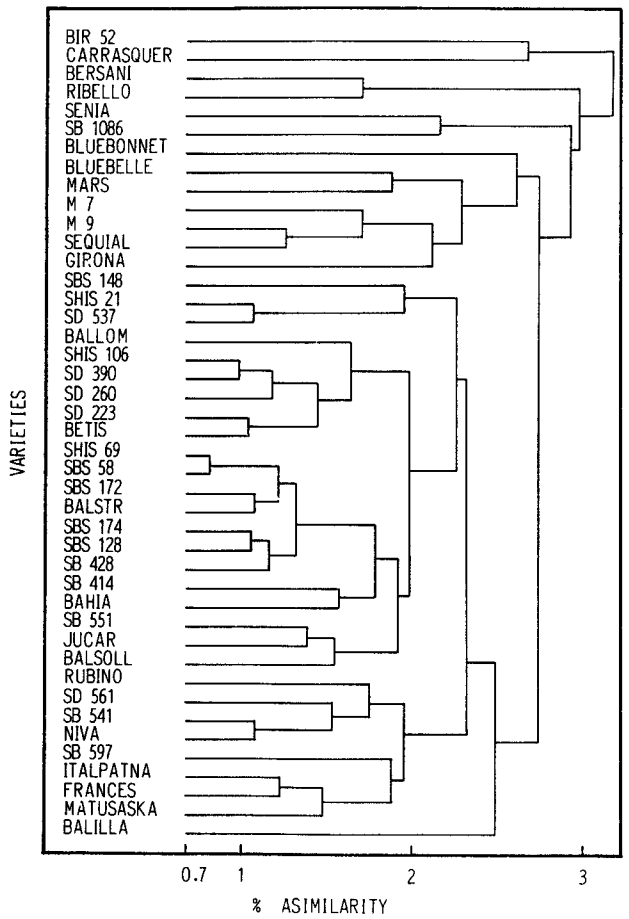

Fig. 2. Dendrogram of the quantitative morphological characters indicating taxonomic affinity with the different varieties of rice studied: 7 groups of affinity are shown. The seven variety groupings vary from 3.4 to 7.5%

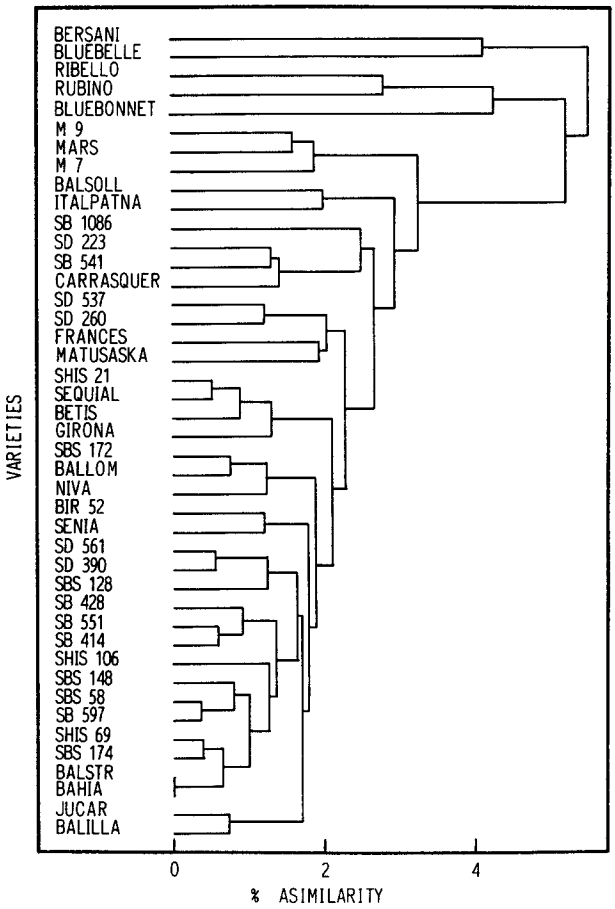

Fig. 3. Dendrogram of the qualitative morphological characters. The taxonomic affinity distinguishes six groups and they are included with in the range 4.0 to 6.5%

Results

a) Taxonomic affinity from morphology characters

Figure 2 shows the taxonomic affinity between different varieties of rice quantitative characters. The dendrogram made of these characters presents seven variety groupings at a percentage asimilarity lower than 1.5:1) 'BalStr'; SB 428; SBS 58, 128, 172, 174; SHIS 69. 2) 'Betis'; SD 223, 260, 390; SHIS 106. 3) SD 537; SHIS 21. 4) 'Niva'; SB 541; SD 561; 5) 'Matusaska'; 'Francés'; 'Italpatna'. 6) 'Sequial'; M 9. 7) 'Júcar'; SB 551.

Figure 3 shows taxonomic affinity based on qualitative characters. The dendrogram for these characters distinguishes six groupings at a percentage asimilarity lower than 1.0: 1) 'BalStr'; 'Bahía'; SB 597; SHIS 69; SBS 58, 148, 174. 2) 'Balilla'; 'Júcar'. 3) SB 414, 428, 551. 4) SD 390, 561. 5) 'BalLom'; SBS 172. 6) 'Sequial'; 'Betis'; SHIS 21.

Table 2. Variation coefficient of the proteinic characters (P.C.) corresponding to 37 electrophoretic protein groups

Varieties \ P.C.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
BALILLA	3.3	8.3	8.3	11.1	14.0	14.0	7.1	3.5	2.0	1.8	4.7	5.8	2.5	2.7	2.4	4.7	3.2
MATUSASKA	3.0	9.0	9.0	11.1	14.0	14.0	7.7	3.2	1.2	2.1	4.3	5.0	2.8	3.3	2.5	4.7	3.6
FRANCES	3.9	11.0	11.1	14.0	14.0	14.0	8.3	4.5	1.8	2.1	5.5	3.2	2.9	3.3	3.0	4.5	2.5
GIRONA	3.2	9.0	9.0	14.0	14.0	13.0	8.3	3.2	1.0	1.5	5.2	4.7	3.2	3.1	2.4	5.0	3.1
BALSOLL	5.5	13.2	11.1	13.7	11.2	12.2	11.0	5.8	0.9	2.1	7.7	5.8	3.6	3.6	1.7	4.3	4.3
NIVA	3.0	9.0	6.6	12.5	11.0	13.3	9.0	3.2	0.9	2.0	4.7	4.5	3.2	3.5	2.9	4.1	3.2
JUCAR	3.9	7.1	11.0	11.7	12.0	12.3	8.3	3.2	1.2	1.5	5.8	5.2	3.6	3.5	2.6	5.5	3.2
BETIS	3.2	10.0	11.0	9.0	12.3	11.4	9.0	2.7	0.8	1.8	5.5	5.0	3.5	3.5	2.9	6.2	3.0
CARRASQUER	3.3	10.0	12.0	12.3	13.3	12.0	11.0	3.7	1.0	2.1	6.2	5.0	3.9	3.6	2.8	6.6	2.8
BAHIA	5.0	8.3	9.0	12.5	13.2	11.7	6.6	3.1	0.9	1.6	4.7	4.5	3.3	3.2	2.5	5.4	2.4
SEQUIAL	3.7	10.0	11.0	11.0	13.7	11.4	7.7	3.5	1.1	1.5	6.6	4.5	3.2	3.2	2.4	5.8	2.6
BALLOM	2.2	6.6	9.0	11.0	12.0	13.0	7.7	1.1	1.0	1.8	8.3	5.5	3.2	4.1	2.6	2.5	2.0
SB 414	3.0	13.7	9.0	9.0	11.7	12.0	6.0	4.7	1.0	1.6	5.8	4.7	3.5	3.3	3.3	12.0	3.2
SB 428	6.6	12.5	11.7	9.0	13.2	11.0	13.4	3.6	0.9	1.5	6.6	5.8	4.3	4.3	4.3	11.0	3.2
SB 541	3.2	7.1	9.0	11.0	11.4	12.1	9.1	3.9	0.8	1.4	7.7	4.3	3.3	3.1	2.4	5.5	3.2
SB 551	5.8	6.6	9.0	10.0	12.0	13.8	5.8	3.4	1.1	1.4	6.6	4.5	3.1	3.0	2.5	5.0	3.6
SB 553	6.2	11.0	11.3	13.8	13.2	12.7	6.6	3.2	1.2	1.3	7.7	4.5	3.2	3.1	2.6	5.0	3.9
SB 597	3.0	13.3	9.0	8.3	13.2	11.0	12.0	3.0	0.9	1.8	6.6	5.2	3.6	3.9	3.3	2.8	0.8
SB 1,086	3.2	11.0	8.0	8.3	11.7	12.3	10.0	3.2	1.1	1.6	7.1	5.8	3.7	3.7	2.9	2.7	0.7
SD 223	3.6	10.0	9.0	8.3	11.4	13.7	5.8	3.9	1.0	1.6	6.2	4.7	3.7	3.1	2.8	2.4	1.3
SD 260	9.0	13.2	9.0	11.0	12.7	12.0	13.2	3.9	1.2	1.5	6.4	4.3	3.6	3.5	3.1	2.6	0.8
SD 390	5.2	8.3	11.0	7.5	13.2	12.1	7.1	3.7	0.9	1.5	5.6	4.5	3.3	3.2	3.1	2.5	1.4
SD 537	9.0	12.5	13.7	12.2	13.1	11.2	9.0	5.5	0.9	1.5	5.1	3.2	2.9	3.2	2.9	4.5	2.3
SD 561	9.0	13.2	12.5	12.6	13.1	11.9	12.3	5.0	0.8	1.5	6.5	4.7	4.7	3.2	2.8	11.7	2.5
BALSTR	2.0	11.0	13.2	5.5	13.1	11.7	6.6	3.3	1.1	1.6	5.5	12.0	3.0	6.2	3.1	13.0	2.4
BIR 52	6.2	11.1	13.2	6.6	12.0	11.0	9.0	4.1	1.0	2.0	5.4	4.7	3.2	3.5	2.8	11.0	2.6
SBS 58	5.5	8.3	13.1	11.6	13.3	11.4	7.1	3.2	1.0	1.6	7.0	4.3	3.7	3.7	2.5	5.5	2.7
SBS 128	4.7	8.3	11.1	13.2	11.7	6.0	5.0	10.0	1.1	1.5	5.6	4.1	3.0	3.0	2.7	4.1	2.6
SBS 148	4.5	7.1	11.0	12.0	13.2	5.2	3.7	7.1	2.0	2.1	5.2	4.1	3.0	3.3	1.8	4.2	2.5
SBS 172	4.3	7.7	12.5	12.1	8.0	7.1	5.0	12.2	0.8	1.5	3.0	3.2	4.5	4.6	1.0	4.3	2.7
SBS 174	9.1	11.3	12.5	13.1	13.2	6.6	4.3	11.7	0.7	1.5	5.1	4.6	2.6	2.8	3.7	4.1	3.2
SHIS 21	8.4	11.4	13.2	11.7	12.4	11.0	11.4	4.7	1.1	1.6	3.7	4.1	2.9	3.1	1.8	2.5	0.7
SHIS 69	9.2	10.1	8.3	9.3	13.2	6.0	5.5	4.7	2.2	1.7	6.3	6.5	4.1	4.0	2.4	2.3	0.8
SHIS 106	9.4	8.3	9.0	13.2	12.1	13.1	6.2	3.6	2.3	1.6	7.5	3.3	3.7	3.2	2.4	4.5	3.2
ITALPATNA	4.1	8.3	0.4	11.4	9.4	11.2	10.0	3.9	0.5	1.6	5.0	3.9	4.7	2.3	2.9	4.6	3.3
RIBELLO	3.9	8.4	0.9	11.4	13.1	12.7	7.1	4.5	0.8	1.6	5.3	4.7	2.9	3.2	3.0	4.0	3.1
BERSANI	6.2	9.4	13.2	12.5	9.4	6.6	5.2	13.0	1.3	1.9	5.1	5.0	3.6	3.4	3.1	13.0	4.3
RUBINO	3.5	6.4	13.8	11.0	13.1	5.5	3.5	6.1	1.1	0.6	7.5	5.2	3.7	3.0	2.1	11.0	4.0
M 7	6.7	10.3	13.6	12.0	13.4	9.0	10.0	2.1	1.4	1.3	8.1	5.8	4.1	4.5	0.9	5.8	2.1
M 9	5.0	13.2	7.5	12.0	13.1	13.9	10.1	5.2	1.1	1.7	7.5	5.8	3.7	5.5	0.7	2.9	1.3
BLUEBELLE	4.1	10.0	9.1	14.0	12.1	12.5	3.7	4.3	1.3	2.1	7.0	4.3	3.5	4.3	2.1	5.5	2.7
BLUEBONNET	3.6	11.0	9.8	12.5	13.2	13.0	9.9	3.2	1.0	2.1	5.5	5.2	3.7	3.9	3.3	5.0	3.1
MARS	4.0	11.2	7.6	11.3	12.1	11.3	8.2	2.1	0.7	2.1	4.3	5.1	2.7	1.2	0.7	3.1	1.1

(continued overleaf)

b) Taxonomic affinity from protein characters

A total of 37 protein components were observed among the 43 varieties (Fig. 1). Table 2 shows the variation coefficient of the protein characters. The maximum variation coefficient was 14%.

Figure 4 shows the taxonomic affinity obtained from protein characters. The dendrogram, shows only two groupings at a percentage asimilarity lower than 4.2: 1) 'Balilla'; 'BalSoll'; 'Bahía'; 'BalLom'; 'Sequial'; 'Carrasquer'; 'Betis'; 'Júcar'; SD 551. 2) 'Matusaska'; 'Francés'. No groupings are found below 3.2%.

The seven variety groupings described from the quantitative morphological characters appear here differentiated with a percentage asimilarity at 4.7, 4.3, 6.5, 6.4, 3.4, 7.5 and 4.2%, respectively. The six defined groupings established with the qualitative morphological characters appear differentiated with a percentage asimilarity at 4.8, 4.1, 5.2, 6.5, 5.6 and 4%, respectively.

Table 3 shows the variance analysis (test Keuls) of the protein characters. A letter was assigned to each grouping of varieties from the same character. The coincidence of any letter means that the varieties cannot be differentiated in this character. Even in the

(**Table 2** continued)

18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
8.3	8.3	11.1	14.0	13.0	10.2	2.5	1.0	2.3	5.5	5.2	3.3	3.8	3.3	5.0	2.9	14.0	10.0	3.2	1.0
9.0	9.0	11.1	14.0	14.0	7.1	3.5	2.0	1.8	4.7	5.8	2.5	2.7	2.4	4.7	3.2	14.0	7.1	3.5	2.0
11.0	11.1	14.0	14.0	13.0	7.7	3.2	1.2	2.1	4.3	5.0	2.8	3.3	2.5	4.7	3.6	13.0	7.7	3.2	1.2
9.0	9.0	14.0	13.0	13.0	8.3	4.5	1.8	2.1	5.5	3.2	2.9	3.3	3.0	4.6	2.5	13.1	8.3	4.5	1.8
12.7	11.1	12.3	13.1	13.7	8.1	3.2	1.0	1.5	5.2	4.7	3.3	3.1	2.4	5.0	3.1	11.0	8.3	3.2	1.0
9.0	6.6	12.5	11.0	13.0	12.0	5.6	0.6	2.1	7.7	5.8	3.6	3.1	1.7	4.3	4.1	12.0	11.0	5.8	0.9
7.1	11.1	12.0	11.0	13.0	9.0	3.2	0.8	2.0	4.7	4.5	3.2	3.5	2.9	4.1	3.2	13.3	9.3	3.3	0.9
10.0	13.3	9.0	12.2	13.2	8.3	3.2	1.2	1.5	5.8	5.2	3.6	3.5	2.6	5.5	3.2	13.0	8.3	3.2	1.2
10.0	12.0	11.3	13.4	13.0	9.5	2.7	0.8	1.8	5.5	5.0	3.5	3.5	2.9	6.2	3.8	13.1	9.0	2.6	0.7
8.2	9.0	12.4	12.7	12.2	11.3	3.4	1.1	2.1	6.2	5.0	3.9	3.6	2.8	6.6	2.8	11.4	10.8	3.7	1.0
10.0	11.1	11.0	14.0	13.8	6.6	3.1	0.9	1.6	4.7	4.5	3.3	3.0	2.5	5.5	2.4	11.2	6.4	3.1	0.9
6.6	9.0	11.0	11.3	14.0	7.7	3.5	1.1	1.4	6.3	4.3	3.2	3.0	2.4	5.8	2.6	11.0	7.7	3.5	1.1
11.7	9.0	9.0	12.0	12.8	7.5	1.1	1.6	1.6	8.3	5.5	3.2	4.1	2.6	3.5	2.0	12.4	7.5	1.1	1.0
12.5	13.2	9.0	12.9	12.2	6.0	4.7	1.0	1.6	5.8	4.7	3.5	3.3	3.0	9.3	3.4	11.4	6.0	4.7	1.4
7.1	9.0	11.0	13.5	11.0	12.1	3.6	0.9	1.5	6.6	5.8	4.3	4.1	4.0	11.0	3.2	10.9	13.4	3.6	0.9
6.6	9.0	10.0	11.2	12.0	9.1	3.9	0.8	1.4	7.7	4.3	3.3	3.0	2.4	5.5	3.1	11.4	9.0	3.9	0.8
11.1	11.3	13.4	12.3	13.2	5.8	3.3	1.1	1.4	6.6	4.5	3.3	3.1	2.5	5.0	3.6	13.0	5.8	3.4	1.1
12.4	9.0	8.3	13.1	11.8	6.6	3.2	1.2	1.3	7.7	4.5	3.3	3.1	2.6	5.0	3.9	13.2	3.6	3.2	1.2
11.4	8.0	8.3	13.0	11.4	12.1	3.0	0.9	1.8	6.6	5.2	3.6	3.9	3.3	2.8	0.8	12.1	12.8	3.0	0.9
10.0	9.0	8.3	13.1	11.8	10.0	3.2	1.1	1.6	7.1	5.8	3.7	3.7	2.9	2.7	0.7	13.0	10.4	3.2	1.1
11.7	9.0	11.0	11.8	12.3	5.8	3.9	1.0	1.6	6.2	4.7	3.7	3.1	2.8	2.4	1.3	12.4	5.8	3.9	1.0
8.3	11.1	7.7	13.0	12.4	13.8	3.9	1.2	1.5	6.4	4.3	3.6	3.6	3.1	2.1	0.8	11.4	12.3	3.9	1.2
12.5	11.7	13.2	12.2	12.0	7.1	3.7	0.9	1.5	1.6	4.5	3.3	3.2	3.3	2.5	1.4	12.0	7.1	3.7	0.9
13.2	12.5	13.2	12.2	13.2	9.3	5.1	1.2	1.7	5.1	3.2	2.9	3.2	2.9	4.6	2.3	13.3	9.0	5.5	0.8
11.0	13.3	5.5	13.0	12.4	9.1	5.0	0.8	1.5	6.5	4.7	4.5	3.2	2.8	11.0	2.5	12.0	11.8	5.0	0.8
11.1	13.3	6.6	12.2	11.6	6.4	3.7	1.2	1.6	5.7	12.3	3.0	6.2	3.1	11.4	2.4	11.0	6.8	3.5	1.1
8.3	12.7	11.0	13.2	13.4	11.2	8.3	0.7	1.6	6.5	5.5	3.1	3.2	2.7	12.0	2.3	11.0	10.8	8.3	0.9
8.3	11.1	13.2	12.3	11.4	9.0	4.1	1.0	2.0	5.4	4.7	3.1	3.5	2.8	11.2	2.6	11.4	9.0	4.1	1.0
7.1	11.0	11.7	13.8	12.0	7.1	3.2	1.0	1.6	7.0	4.3	3.7	3.7	2.5	5.5	2.7	11.0	7.1	3.2	1.0
7.7	12.5	12.0	13.0	6.0	5.0	10.0	1.1	1.5	5.6	4.1	2.0	3.0	2.7	4.1	2.4	6.0	5.2	10.1	1.1
11.2	12.3	13.3	12.8	5.2	3.7	7.1	2.0	2.0	5.2	4.2	3.1	3.2	1.8	4.2	2.5	5.2	3.7	7.1	2.0
11.4	13.2	13.4	12.3	7.1	5.0	12.0	0.9	1.5	3.1	3.2	4.5	4.6	1.2	4.3	2.7	7.1	5.0	12.2	0.9
10.3	8.3	9.0	12.1	6.6	4.3	12.0	0.8	1.4	5.1	4.6	2.6	2.8	2.4	4.0	3.2	6.5	4.3	12.1	0.8
8.3	9.3	13.1	12.4	11.0	10.8	4.7	1.1	1.6	3.7	4.1	2.9	3.1	1.9	2.5	0.7	11.0	9.0	4.7	1.1
8.2	0.5	11.4	11.8	6.0	5.5	4.7	2.2	1.7	6.5	6.6	4.1	4.1	2.6	2.1	0.8	6.1	5.5	4.7	3.2
8.4	0.7	13.2	12.0	13.1	6.2	3.6	2.3	1.6	7.5	3.3	3.7	3.2	2.4	4.5	3.2	11.1	6.2	3.6	2.3
9.1	11.6	12.4	11.8	12.4	10.3	3.9	0.9	1.6	5.0	3.9	4.7	2.3	2.9	4.5	3.3	12.0	10.1	3.9	0.9
6.6	13.2	11.0	12.1	13.8	7.1	4.5	0.8	1.6	5.3	4.7	2.9	3.2	2.0	4.3	3.1	13.2	7.1	4.5	0.8
10.4	12.9	12.0	12.1	6.6	5.2	12.0	1.3	1.9	5.1	5.0	3.6	3.6	3.1	12.1	4.3	6.6	5.2	12.0	1.3
12.4	7.4	11.3	12.0	5.5	3.5	6.6	1.2	0.9	7.5	5.2	3.7	3.0	2.1	10.1	4.0	5.5	3.5	6.6	1.2
10.0	9.1	14.0	11.8	10.4	10.0	2.0	1.4	1.3	8.1	5.8	4.1	4.5	0.8	5.8	2.1	13.1	10.0	5.2	1.1
10.1	9.0	11.1	12.8	10.4	3.7	4.3	1.1	2.1	7.0	6.4	5.3	4.3	1.2	5.5	2.5	5.5	7.3	3.4	1.0
10.3	9.6	10.1	12.8	10.4	3.7	2.1	1.7	1.1	5.2	3.3	4.2	2.1	1.0	4.3	4.0	3.5	6.2	4.9	1.3

closest varieties ('Balilla' and 'Bahia'), a varietal distinction is possible, since there exists in this case significant differences in 14 protein compounds (1, 8, 9, 13, 15, 20, 21, 23, 24, 25, 28, 31, 33, 36).

Discussion

The commercial and agronomic necessity to precisely define cultivated rice varieties is hampered by the great morphological similarity caused by the strong genetic relationship between varieties. Even the use of the highest possible number of morphological characters is often insufficient for distinguishing some varieties.

Seed material or powder is relatively easy to handle with respect to protein extraction and, more important, the seed may be regarded as a fixed physiological state. In taxonomic studies, it is critical to compare organs at the same stage of development and this applies to chemistry as well as morphology. In this sense, the seed, and its proteins, may be regarded as a "conservative" unit, little affected by the environment, geographic origin, seasonal fluctuations and chromosomal rearrangements (Ladizinsky and Hymowitz 1979;

Table 3. Variance analysis (Keuls test) of protein characters. Coincidence in letters means there is no difference among varieties (reference to P.C.). Different letters mean differences in 99%

Varieties \ P.C.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
BALILLA	def	cd	cd	cde	ab	de	ijk	bcd	g	de	cde	ghi	ij	hi	mn	fgh	hij	gh
MATUSASKA	bc	bcd	cde	cde	ab	cde	efg	cde	g	cde	ab	ij	a	a	d	efg	kl	cd
FRANCES	a	cd	ed	cde	ab	cde	fgh	bcd	p	e	a	fgh	bc	cde	de	efg	m	c
GIRONA	fgh	ed	cde	ede	ab	e	ghi	ijk	q	de	cde	a	cd	bed	jkl	def	def	i
BALSOLL	ab	ed	cd	cde	ab	ede	ghi	ab	ij	bcde	bed	fg	fg	cde	def	fgh	jkl	cde
NIVA	lm	de	cde	cde	ab	cd	jk	l	b	de	gh	ij	hij	k	a	de	n	efg
JUCAR	a	cd	a	cde	a	de	hij	ab	bc	de	ab	efg	fgh	fgh	ijk	d	kl	cd
BETIS	fgh	ab	cde	cde	ab	ef	def	ab	op	bcde	de	hi	hij	ghi	jgh	hij	kl	cde
CARRASQUER	ab	cd	cde	abc	ab	e	ghi	a	a	ede	cde	fgh	ghi	efg	ijk	jk	ijk	fgh
BAHIA	bc	cd	cde	cde	ab	cde	jk	efg	h	de	ef	fgh	f	jgh	hij	k	ghi	fgh
SEQUIAL	jkl	bcd	cd	cde	ab	ef	def	a	be	bcde	bcd	efg	fg	cde	de	hij	de	fgh
BALLOM	efg	cd	cde	cde	ab	de	fgh	cde	mnñ	bcde	efg	efg	ef	def	def	ijk	efg	cde
SB 414	a	a	bc	cde	ab	cde	fgh	a	kl	cde	ab	hi	fgh	ijk	jgh	c	a	efg
SB 428	a	g	cd	abc	ab	cd	ghi	jk	j	bcde	efg	fg	ghi	cde	mn	lmn	kl	a
SB 541	n	de	cde	abc	ab	c	k	def	cd	bcde	h	ij	ghi	ji	ñ	mnñ	hij	a
SB 551	bcd	ab	cd	cde	ab	cd	hij	a	de	bcde	de	def	fg	cde	def	hij	de	b
SB 553	cde	a	bc	bcd	ab	de	bcd	fgh	nñ	bcde	efg	efg	fg	abc	efg	fgh	m	def
SB 597	mn	ed	cd	cde	ab	ef	def	bcd	kl	bc	gh	efg	fgh	abc	fgh	fgh	ghi	cd
SB 1,086	a	ef	cde	ab	ab	cde	jk	jk	ij	cde	efg	ghi	hij	hi	mn	bc	a	i
SD 223	ab	cd	de	ab	ab	de	ijk	bcd	kl	bcde	fgh	ij	ij	ghi	klm	abc	a	j
SD 260	def	cd	cde	ab	ab	ef	bcd	fgh	j	bcde	ef	fg	ij	abc	hij	a	a	i
SD 390	o	ef	d	cde	ab	cde	k	fgh	o	bcde	efg	c	hif	fgh	klm	ab	a	j
SD 537	kl	bcd	cde	a	ab	cde	efg	cde	a	bcde	de	efg	fg	bcd	ghi	a	a	j
SD 561	o	de	de	cde	ab	ef	hij	k	hi	bed	bcd	bc	k	bcd	hij	def	bc	h
BALSTR	ñ	ef	cde	cde	b	de	k	k	a	bcd	efg	j	k	bcd	nñ	ñ	c	a
BIR 52	a	cd	de	a	b	de	def	abc	mnñ	bcde	de	j	de	k	ñ	mnñ	bc	a
SBS 58	fgh	ef	cde	ab	ab	c	ghi	ijk	b	bcde	efg	hi	fg	efg	mn	lm	bc	a
SBS 128	mn	cd	d	a	ab	c	hij	ghi	j	de	de	fg	fg	efg	lm	mnñ	d	a
SBS 148	lm	bcd	d	cde	ab	f	efg	bcd	k	bcde	fgh	def	k	ghi	efg	hij	fgh	b
SBS 172	jk	bcd	cde	cde	ab	b	a	m	l	cde	de	efg	bcd	ab	ghi	d	m	cde
SBS 174	ij	ab	cde	cde	ab	a	a	l	q	de	bc	efg	de	jk	c	de	n	b
SHIS 21	hi	abc	cde	cde	a	b	a	m	ij	bcde	efg	fg	de	a	fgh	de	fgh	fgh
SHIS 69	o	cd	cde	cde	ab	b	a	m	a	bcde	cde	efg	ab	ab	ghi	d	kl	b
SHIS 106	o	cd	d	e	ab	j	jk	jk	mn	bcde	a	de	cd	abc	b	nñ	m	a
ITALPATNA	ñ	cd	a	abc	ab	b	abc	jk	a	bcde	efg	j	cd	k	def	abc	a	i
RIBELLO	ñ	bcd	bc	e	ab	cde	cde	def	q	bcde	gh	ab	bcd	bcd	d	def	l	cd
BERSANI	ghi	bcd	cd	cde	ab	de	ijk	fgh	fg	bcde	bc	d	k	a	ijk	mnñ	ñ	a
RUBINO	fgh	abc	cd	cde	ab	e	efg	ijk	bc	bcde	cde	fg	cd	bcd	jkl	lm	ñ	a
M 7	mn	cd	cde	cde	ab	b	ab	m	q	de	bcd	fgh	hij	fgh	klm	l	n	a
M 9	cde	a	cde	cde	ab	a	a	l	p	o	gh	ghi	ij	ab	c	ghi	a	j
BLUEBELLE	n	cd	de	cde	ab	e	ijk	a	ñ	b	h	ij	k	ijk	a	ijk	b	j
BLUEBONNET	jkl	f	b	cde	ab	e	ijk	l	l	bcde	gh	ij	ij	k	b	c	a	j
MARS	ghi	cd	cde	cde	ab	cd	ghi	hij	m	de	fgh	de	k	kj	b	hij	fgh	gh

(continued overleaf)

Table 3 (continued)

19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
cde	cd	efg	def	m	cd	a	hij	hi	f	def	cde	ghi	bc	ef	mn	c	a	a
cde	bc	cde	cde	ijkl	b	ab	ghi	gh	nñ	cd	cde	efg	a	fgh	ml	m	h	ed
cde	bc	cde	cde	fgh	fg	ab	fgh	ghi	lmn	cd	cde	efg	a	d	ij	m	c	efg
a	ab	hi	cdc	ijkl	cd	ab	ijk	g	j	cd	def	fgh	a	a	jk	hij	b	a
def	ab	hij	bed	ijkl	de	b	k	ghi	lmn	def	ijk	fgh	fgh	ejk	kl	hij	ef	a
bc	bc	efg	ghi	klm	fg	b	k	f	a	cd	cd	jkl	b	hij	mnñ	de	h	i
cde	bc	hij	cde	ijkl	de	b	jk	hi	lm	fgh	jkl	cd	efg	m	o	e	a	hi
bcd	bc	b	ghi	ghi	b	b	def	e	g	abc	c	efg	ghi	d	kl	kl	i	a
cde	bc	hi	efg	ijkl	ij	b	jk	ij	lm	ghi	hij	ghi	k	jkl	ño	bc	a	b
cde	ab	hi	bcd	igkl	fg	b	hij	ghi	jk	cd	cd	jkl	cde	d	mnñ	c	c	a
cde	bc	gh	abc	ijkl	cd	b	fgh	ij	jk	def	ghi	a	m	hij	nño	ghi	n	cde
cde	abc	fgh	a	ijkl	d	b	def	hi	jk	bcd	fgh	efg	b	e	k	ghi	gh	def
bc	abc	fgh	abc	lm	cd	b	cde	e	f	bcd	hij	cde	efg	efg	k	kl	n	m
cde	bc	gh	abc	jklm	de	a	jk	cd	f	bcd	hij	cde	ghi	jkl	h	nñ	n	k
def	bc	jk	cde	m	j	ab	def	e	d	efg	m	hij	k	a	e	k	i	m
cde	bc	efg	ab	lm	hij	ab	cde	gh	jk	cd	fgh	efg	def	klm	lm	ij	i	fgh
cde	abc	bed	ijk	efg	de	ab	c	bc	ño	cd	b	b	ñ	cd	h	ño	j	bc
def	bc	efg	abc	ijkl	ef	ab	ghi	ghi	lm	efg	c	a	ñ	cd	fg	k	m	a
f	d	hij	def	klm	ef	a	efg	e	e	def	m	a	lm	cd	a	a	n	a
ef	d	bc	ghi	efg	d	ab	c	a	e	hij	hij	c	lm	ijk	bc	qr	n	k
cde	bcd	def	hij	def	de	b	fgh	ab	f	cd	jkl	klm	k	efg	g	a	n	l
bc	bcd	gh	abc	jklm	fg	b	cd	cd	c	cd	m	klm	lm	j	e	oc	j	a
cde	bcd	hij	n	cd	gh	b	ghi	e	d	j	kl	lm	l	klm	f	s	n	jk
b	bc	hi	n	ab	cd	b	ijk	j	lmn	fgh	lm	a	k	ijk	mn	ab	f	a
cde	bcd	ijk	lm	ijkl	ghi	b	ghi	d	ab	fgh	n	ijk	mn	ijk	a	mn	i	j
cde	abc	k	lm	ghij	hij	ab	fgh	bc	bc	cd	n	m	nñ	jkl	b	s	m	m
bcd	e	efg	m	lm	de	b	ghi	bc	c	hij	n	jkl	nñ	hij	d	r	n	b
cde	a	fgh	n	def	bc	a	b	ab	f	j	n	def	ñ	lm	cd	q	kl	k
f	e	a	n	a	d	b	cde	gh	i	efg	c	efg	fgh	cd	h	f	d	cde
cde	cd	hi	n	ab	d	b	cde	hi	kl	bcd	efg	b	fgh	c	mn	ij	b	cde
cde	abc	cde	lm	de	de	b	cd	f	e	a	a	m	a	cd	mn	l	k	ghi
cde	bc	hi	n	a	de	b	def	hi	kl	ij	ijk	ijk	k	ijk	k	l	e	jk
ef	bcd	gh	kl	d	ef	b	ijk	j	o	ghi	ghi	cde	cde	cd	jk	ghi	g	b
a	abc	hij	def	cd	cd	b	hij	f	d	ab	c	def	cd	cf	nño	d	gh	m
cde	cd	bed	n	hijk	ij	b	a	cd	a	abc	cd	jkl	efg	ed	a	p	b	m
a	e	bc	jkl	efg	ghi	b	ab	ab	lmn	a	def	def	a	cd	i	ij	ef	b
a	cd	jk	ghi	bc	de	b	cde	hi	h	fgh	efg	fgh	hi	efg	o	gh	g	ghi
a	bc	jk	hij	ab	de	b	cd	hi	hi	fgh	ghi	cde	efg	hij	lm	j	gh	efg
bc	a	hij	cde	klm	de	b	cde	f	g	cd	ghi	m	j	ijk	ij	g	ef	a
b	e	a	ijk	ab	a	b	def	cd	lmn	de	a	b	nñ	ed	hi	s	c	m
bc	e	bcd	jkl	de	de	ab	hij	a	o	def	ed	m	b	cd	o	a	m	l
cde	a	gh	n	ab	gh	b	def	e	a	abc	cd	ljk	ij	cd	a	s	lm	m
cde	abc	fgh	n	cd	de	b	hij	g	a	bcd	ab	ijk	def	cd	lm	f	d	l

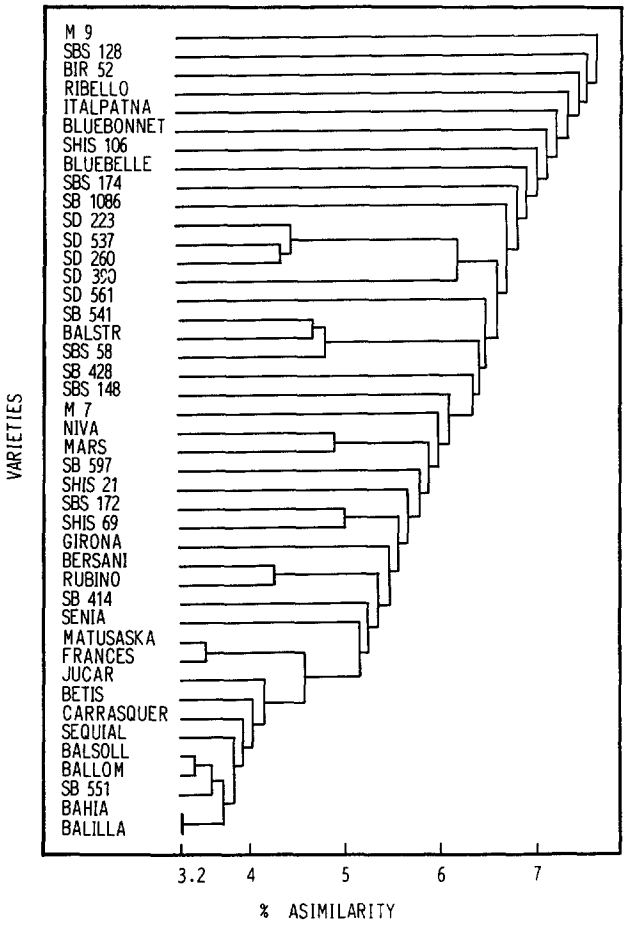

Fig. 4. Taxonomic affinity obtained from protein characters. The dendrogram shows only two groupings at a percentage asimilarity lower than 4.2%

Vaughan 1983). The biochemical characters complement the morphological ones ensuring more reliability and stability. In addition, morphological characters are not simultaneous in time, hence their comparison requires a prolonged period of time.

The results obtained in this work suggest that analysis of embryo protein components provides better knowledge of the genetic affinity of the studied varieties than does that obtained from the morphological characters. The high morphological proximity (1.0 and 1.5% asimilarity) of the variety groupings diminishes in the protein characters dendrogram where the previously grouped varieties can be differentiated for higher percentage asimilarity (3.4 to 7.5% asimilarity). All this suggests that embryo proteins are a good reflection of the genotype of the individual and that they are a useful tool in the study of taxonomy.

The electrophoretic analysis of proteins for the identification of varieties has been previously carried out, due to commercial necessities, in other cereals. The application of these investigations to other crops will follow the pace that legislation demands.

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