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Assessment of genome relationships in the genus *Oryza* L. based on seed-protein profile analysis

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Summary. Cultivated and wild *Oryza* species belonging to different genomic groups were studied with regard to their soluble seed-protein profiles. There is an essential uniformity in the banding patterns within various genomes and the basic patterns are not species-specific but genome-specific. *O. meridionalis* contains a subgenome similar to the A genome of *O. rufipogon*. Certain specific bands present among A genome species have been found to be useful in tracing the phylogenetic affinity between the cultivated species and their presumed wild progenitors.

Key words: *Oryza* species – Seed-protein profiles – Variation – Genome-specific

Introduction

The genus *Oryza* contains about 22 diploid (2n = 2x = 24) and tetraploid (2n = 4x = 48) taxa all but two of which have been grouped into four complexes of closely related species (Tateoka 1962, 1963; Vaughan 1989). Morinaga (1943, 1959, 1964) and Morinaga and Kuriyama (1959a, b) devised a system of formulae for classifying the genomes of the species of *Oryza* based on a meiotic analysis of different combinations of hybrids between diploids, on the one hand, and between diploids and tetraploids, on the other hand. They have recognized six (A–F) distinct genome classes within the genus. The *Oryza sativa* complex, constituting the AA genome, comprises the cultivated species (*O. sativa* and *O. glaberrima*) and their close wild relatives (Morishima and Oka 1960). *O. officinalis* is the other major complex, and is made up of diploid and tetraploid taxa of various genomes. The genomic constitutions of two species in each of the remaining complexes (*Ridleyi, Meyeriana*) have yet to be determined.

The importance of seed-protein electrophoretic pattern analysis as a reliable approach to demarcating genome characteristics has been well recognised (Johnson and Hall 1965; Johnson et al. 1967). For example, while tracing phylogenetic affinities in the *Triticinae* by using seed-protein profiles, Johnson and Hall (1965) demonstrated that the analysis of *Triticum aestivum* (AABBDD) and its progenitors, corroborated the evidence obtained by other methods. Similarly, in *Gossypium* the variation in seed-protein profile pattern was found to be in accordance with the classification of the diploid species into six genomic groups (Johnson and Thein 1970).

The following investigation, which embraces 55 accessions belonging to 13 species (60% of the total) of the genus *Oryza* was designed to determine the pattern of the seed-protein profile variation between and within the species and, additionally, to analyze how the profile differences between species are distributed among the genomes recognized within the genus.

Materials and methods

A list of the species investigated, along with their accession numbers, is given in Table 1. The seeds of *O. sativa* accessions were obtained from the Central Rice Research Institute (CRRI), Cuttack, India. The remaining species accessions were provided by the International Rice Research Institute (IRRI), Manila, The Philippines.

Extraction and electrophoresis

For electrophoretic studies the mature and viable seeds were dehusked and finely ground. The powdered sample (0.05 g) for each accession was homogenised in 0.3 ml of 0.5 M Tris buffer

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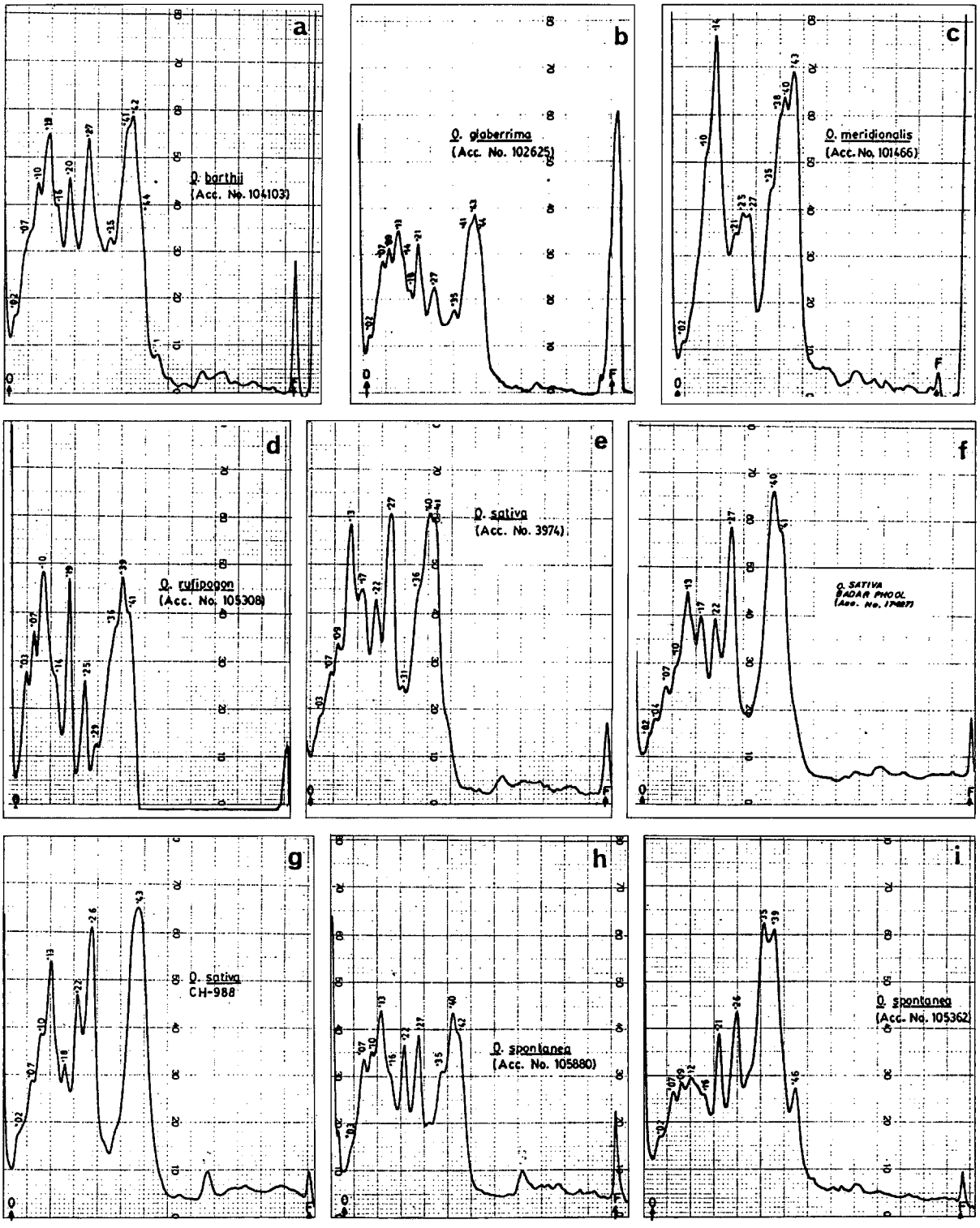

Fig. 1a–i. Densitograph profiles of soluble protein fractions of seeds of A genome species. *Figures* above the peaks refer to their RF. values. Migration is from anode to cathode. *O*, origin; *F*, front

Table 1. A list of *Oryza* species used in the present study together with their accessions and genomes

Species	2n	Genome	Accession number
Oryza sativa complex			
O. sativa	24	AA	845, 868, 889, 1016, 2079, 3974, 17427, 19358, CH-988, CTH-2
O, rufipogon	24	AA	105308, 105390, 105720, 105760, 105766, 105780, 105953
O. spontanea	24	AA	103406, 103833, 104967, 105362, 105784, 105791, 105880
O. glaberrima	24	A^gA^g	100139, 100984, 102625, 103229 103330, 103463, 104047
O. barthii	24	A^gA^g	100119, 100931, 104061, 104081, 104084, 104103, 104122, 104286, 105571, 105613
O. meridionalis	24	A^mA^m	101466
O. officinalis complex			
O. officinalis	24	CC	101074, 105085
O. eichingeri	24	CC	101426, 105412
O. latifolia	48	CCDD	103808, 105557
O. grandiglumis	48	CCDD	105560, 105669
O. meyeriana complex			
O. granulata	24		102117, 104503
O. ridleyi complex			
O. ridleyi	48		105366
Ungrouped			
O. brachyantha	24	FF	101233, 105151

(pH 7.6). The suspension was centrifuged at 4,000 g for 30 min, and 100 µl of the supernatant was loaded for electrophoresis. A cationic system of polyacrylamide disc-gel electrophoresis was employed according to the method of Davis (1964) and Ornstein (1964), with suitable modifications (Sarkar and Bose 1984), using a 10% gel and a β-alanine buffer (pH 4.5). Gels were stained in 0.1% amido black and destained in 9% acetic acid. Gels were scanned at 570 nm using a Gilson Holochrome with a gel-scanning attachment.

Results

The electrophoretic patterns of the soluble protein fractions were reproducible in all the species and accessions examined.

Oryza sativa complex

There exists a basic similarity in the protein profile for the species in this complex. In all of them, bands or peaks could be characteristically grouped into three distinct zones (slow, intermediate, fast) according to their relative mobility (Figs. 1, 2). The slow-moving zone consists of a group of major and minor bands distinguishable either as humps and notches or as prominent peaks in the densitograph with Rf. values ranging from 0.02 to 0.18. The zone of intermediate mobility has two prominent peaks, one slow (migrating at Rf. between 0.19 and 0.22) and the other faster (between 0.25 and 0.27). In *O. meridionalis* (Fig. 1 c), however, one accession (Acc. 101466) has three peaks (0.21, 0.25, 0.27) instead of the normal two.

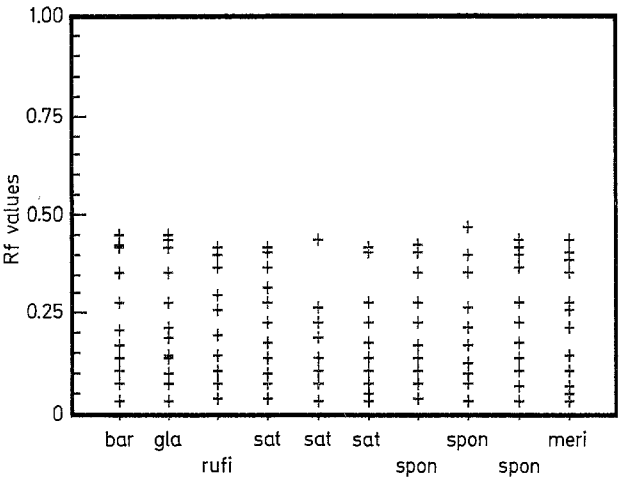

Fig. 2. Rf. values of the protein bands in the A genome species

In comparison, the fast-moving zone showed considerable variation, both between and within the species, with regard to number of bands and their Rf. values. All the accessions of *O. rufipogon, O. barthii* and *O. glaberrima* and three each of *O. sativa* (Acc. 3974, 868, 845) and *O. spontanea* (Acc. 105880, 105791, 103833), for example, have three closely placed peaks, appearing in shape like a 'trident' (Figs. 1 a, b, d, e, h). The component peaks of the trident, however, show variation in Rf. values. In the three other accessions (Acc. 105784, 105362, 104967) of *O. spontanea* (Fig. 1 i) the fastest component of the trident was found to be located at some distance from the other two peaks, while in one accession each of *O. spontanea* (Acc. 103406) and *O. meridionalis* (Acc. 101466) there are clusters of four peaks instead of three (Fig. 1 c). In a few accessions of *O. sativa* (Figs. 1 f, g) this may be replaced either by two peaks (Acc. 19358, 17427) or by one (Acc. 2079, 1016, 889, CTH-2, CH-988).

O. officinalis complex

In complete contrast to the *O. sativa* complex, all the accessions from the four species of *O. officinalis* complex exhibited extremely poor band yields and an increase in the sample dose did not result in any improvement.

The relative densitograph profiles (Figs. 3 a, b; 4 a, b) of *O. latifolia* (CCDD) and *O. grandiglumis* (CCDD), compared to those of *O. officinalis* (CC) and *O. eichingeri* (CC), clearly reveal the existence of considerable homology in soluble protein fractions within each pair of species. The Rf. values of the three major bands in *O. latifo-*

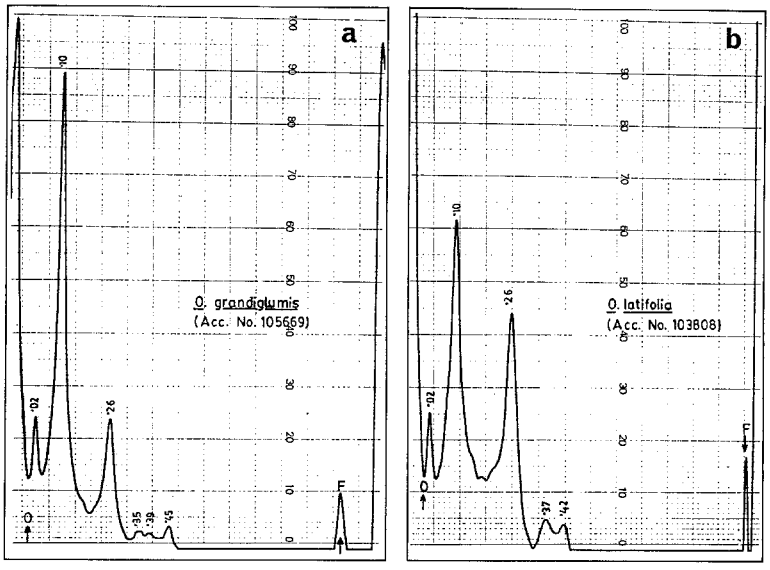

Fig. 3a, b. Densitograph profiles of soluble protein fractions of CD genome species

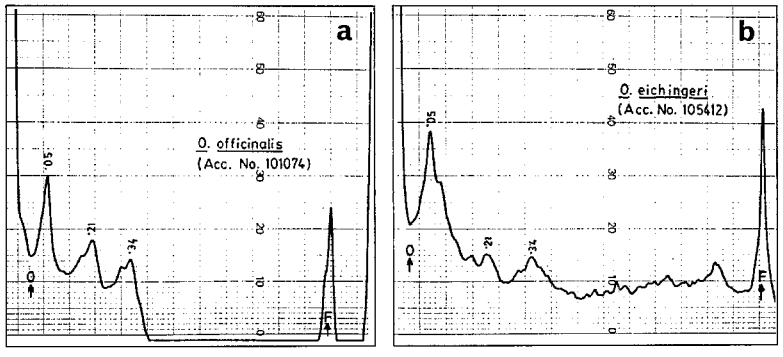

Fig. 4a, b. Densitograph profiles of soluble protein fractions of C genome species

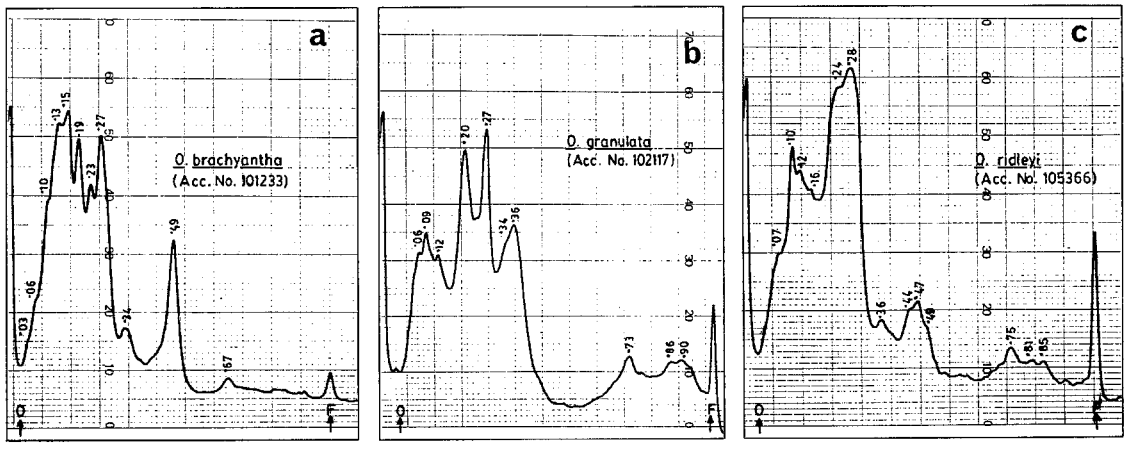

Fig. 5a–c. Densitograph profiles of soluble protein fractions of the F genome (**a**) and an unknown genome species (**b, c**)

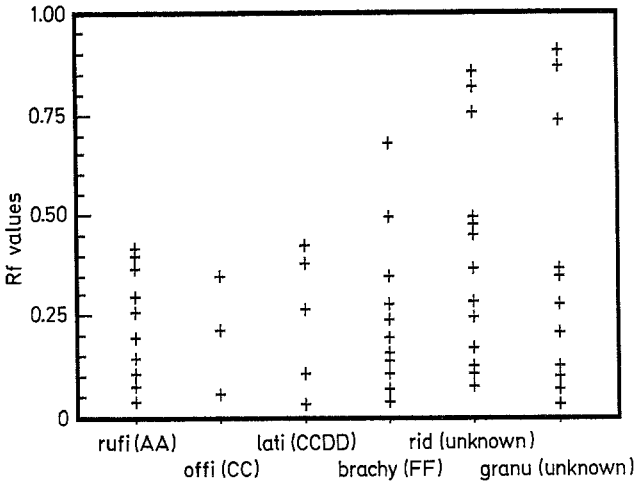

Fig. 6. Rf. values of the protein bands in species representative of various known and unknown genomes

lia and *O. grandiglumis*, for example, are identical. The same is true for *O. eichingeri* and *O. officinalis*. However, surprisingly, the banding character of the CC genome is not reflected in the profiles of the CCDD species.

Other complexes

The two species, *O. granulata* and *O. ridleyi* (belonging to the *O. meyeriana* and *O. ridleyi* complexes, respectively) and the ungrouped species, *O. brachyantha* (FF), are characterised by the presence of marked differences in profile pattern (Figs. 5 a, b, c) not only among themselves but also with respect to species in the *O. sativa* and *O. officinalis* complexes (Fig. 6).

Discussion

The results of seed-protein profile analysis involving 13 species clearly show that major differences between the patterns are not species-specific but genome-specific. The A genome species of the *O. sativa* complex, for example, are indistinguishable from one another on the basis of the existence of the three distinct zones in their banding patterns. Similarly, species with a CC genome have almost identical banding patterns. The same is true for species having a CCDD genome. The occurrence of a unique profile pattern in the F genome species (*O. brachyantha*) is in agreement with the conclusion reached by Li et al. (1961) and Yang et al. (1965) that this species has a different genome compared to other species in the genus. The genomic constitution of *O. granulata* and *O. ridleyi* is not known. Based on the present study there are good reasons to suggest that they are species with genomes other than those currently recognised in the genus (i.e., A, C, D, F, and B, E unpublished data).

The occurrence of three and four peaks in *O. meridionalis* in intermediate and fast-moving zones instead of the normal two and three peaks, respectively, found in the species enlisted in the A genome, indicates that this species (considered to be an Australian form of *O. rufipogon*; Ng et al. 1981) has a slight but consistent difference from the others and, therefore, deserves a special status. A similar view was made by Second (1985) who, on the basis of isozyme data, concluded that *O. meridionalis* shows genomic differences with respect to the Asian and American forms of *O. rufipogon*. The present study, and that of Second (1985), thus supports the view of the IRRI (1964) in designating the genome of *O. meridionalis* as A^mA^m within the AA group.

The 'trident'-shaped peaks present in the electrophoretic profiles serve as a useful marker in resolving the phylogenetic affinities between the species of the *O. sativa* complex. In an earlier study (Sarkar and Bose 1987), following crosses between trident and single-band strains of *O. sativa*, it was demonstrated that the trident band components were inherited en bloc, behaving as a single entity in F_2 and subsequent generations. The present analysis reveals that a close affinity exists between *O. rufipogon* and *O. sativa*, on the one hand, and between *O. barthii* and *O. glaberrima*, on the other. The trident peaks among the accessions in the former appear to be well defined and distinct while in the latter the bands are found rather compressed with indistinct peak tracings in the densitographs. This provides supporting evidence for the view proposed by earlier workers (Chang 1976; Second 1982) that *O. barthii* and *O. rufipogon* are the wild progenitors of *O. glaberrima* and *O. sativa*, respectively. The basic similarity in the profile character of the two wild species, also supports the proposal that they have arisen from a common ancestral stock. The existence of only one or two peaks in a few accessions of *O. sativa*, instead of the typical trident observed in all the accessions of *O. barthii, O. glaberrima, O. rufipogon* and some *O. sativa*, are best considered as derivatives of the trident type.

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