Theor. Appl. Genet. 50, 201-210 (1977)

Study of Interrelationship among A-Genome Species of the Genus *Oryza* through Isoenzyme Variation

K. Palanichamy and E.A. Siddiq
Division of Genetics, Indian Agricultural Research Institute, New Delhi (India)

Summary. The interrelationships among ten different A-genome species of the genus *Oryza* were studied based on variations in the electrophoretic pattern of isoenzymes of two non-specific enzymes, esterase and peroxidase. There were 16 isoenzymes of esterase and 14 of peroxidase. The esterase pattern could be classified into 3 different Zymograms 1e, 2e & 3e based on the presence and/or absence of bands at particular Rf values. The pattern 1e was found exclusively among the species and varietal groups of *sativa* complex, whereas 2e and 3e were distributed exclusively among the species of the *glaberrima* complex and related wild forms. The peroxidase pattern also fell into 3 different zymograms viz. 1p, 2p and 3p. Unlike esterase, all three zymograms were present in both the *sativa* and *glaberrima* complexes.

The similarity indices (S) between the different pairs of entries were computed taking into account the presence as well as the relative intensity of the corresponding isoenzyme bands. The varieties and sub-species of *O. sativa* showed very high similarity values with the Asian *perennis (O. perennis* sub sp. *balunga)*, lending evidence for the probable differentiation of the former from the latter. The African cultivated species *O. glaberrima* showed very high similarity to the African *perennis* form *O. perennis* sub sp. *barthii*, *O. breviligulata* and *O. stapfii*. The only *cubensis* form studied had the same esterase and peroxidase pattern as that of the species of the *glaberrima* complex and also a very high similarity with this group. Thus, the entire A-genome species could be broadly grouped into the *sativa* and *glaberrima* complexes, and within the group there was a lot of overlapping in similarity values making it difficult to identify and pin-point species or subspecies based on their isoenzyme patterns and similarity values.

Key words: A-genome - Oryza - Isoenzymes - Esterase - Peroxidase - Species Interrelationship

Introduction

In spite of extensive studies on morphological and physiological differences, distributional features, crossability and cytogenetic behaviour of interspecific hybrids, the nature of phylogenetic relationships and evolutionary dynamics, especially in the 'A' genome species of the genus *Oryza*, still remains an unsettled question. (IRRI Symposium on Rice Genetics and Cytogenetics, 1963). In the recent past, extent of homology in the electrophoretic pattern of proteins has been extensively used as a criterion of intra and interspecific relationships in many plant species (c.f. Johnson 1967; Johnson and Hall 1966; Johnson et al. 1967; and Siddiq et al. 1972). It was suggested by Wall and Whitaker (1971) that electrophoretic variation in isoenzymes can reveal a much more detailed picture of chromosome blocks and loci differences than can genome analysis using storage proteins. An attempt, therefore, has been made to study the isoenzyme pattern of two non-specific enzymes, namely esterase and peroxidase in representative 'A' genome species of the genus *Oryza*, to gain a better insight into the species interrelationships with particular reference to the origin of the cultivated rices *O. sativa* and *O. glaberrima*.

Materials and Methods

Sixty strains representing ten different 'A' genome species of the genus *Oryza* were included in the present study. The seedlings were raised under uniform conditions of temperature (30 ± 2°C) and moisture supply. For extraction of esterase enzymes, 0.5 to 1.0 g fresh weight of five-day-old shoots were ground with three times the volume of 0.2 M Tris-HCl buffer (pH 6.0) containing 0.006 M β-mercaptoethanol in an ice cold pestle and mortar kept in an ice bath. For peroxidase enzymes, the whole of the seeds, 72 hours after germination, was ground with the same buffer containing no mercaptoethanol. The paste obtained was centrifuged at 20,000x g for 20 min. at 4°C and the clear supernatent was used for electrophoresis. The protein content in the supernatent was determined by the method described by Lowry et al. (1951).

Polyacrylamide gel electrophoresis with 7.5% acrylamide gel was employed, following the method outlined by Davis (1964) and Ornstein (1964) for anionic systems, with 0.2 M Tris-glycine (pH 8.3) as tray buffer. Crude extract containing 150 mg of protein was applied over the spacer gel and electrophoresis was carried out with a constant current supply of 3 mA per gel tube using bromophenol blue as the tracking dye. As soon as the electrophoresis was over, the gels were removed from the tubes and stained for the enzymes as follows:

Esterases: The gels were incubated in the staining mixture containing, per ml of phosphate buffer (pH 5.9), 0.4 mg of fast blue RR salt and 5 mg alpha naphthyl acetate in 0.5 ml of 50% acetone, for 30 min. at room temperature.

Peroxidases: The gels were kept immersed in the staining solution containing, per ml of distilled water, 0.1 ml of 0.2 M acetate buffer (pH 5.1) and 0.17 mg 0-dianisidine for 30 min. at room temperature. The gels were then transferred to a staining tube containing 0.1 M H_2O_2 and stained for 5 min. In both cases the reaction was stopped with 7% acetic acid and the gels were also stored in 7 per cent acetic acid.

The isoenzyme bands in each variety were characterised by their respective Rf values, where

$$Rf = \frac{\text{Distance travelled by the band from the tip of running gel}}{\text{Distance travelled by the tracking dye}}.$$

The homology or otherwise of the bands between strains was confirmed by subjecting the 'mixed extracts' of pairs of varieties to coelectrophoresis. The gels were scanned in Joyce Lobell chromoscan at a wave length of 490 nM for esterase and 465 nM for peroxidase to obtain the densitograph patterns.

Two different methods were used to classify the sixty strains. One was the method of zymogram analysis (Hunter and Markert 1957), where the isoenzyme patterns are classified into different 'Zymograms' based on the presence and/or absence of different bands at particular Rf values.

The second approach was the computation of similarity indices (S), using the formula of Sokal and Sneath (1963) modified to take the intensity of the bands into account. Intensity of the band was taken as the area enclosed by a band in the densitograph.

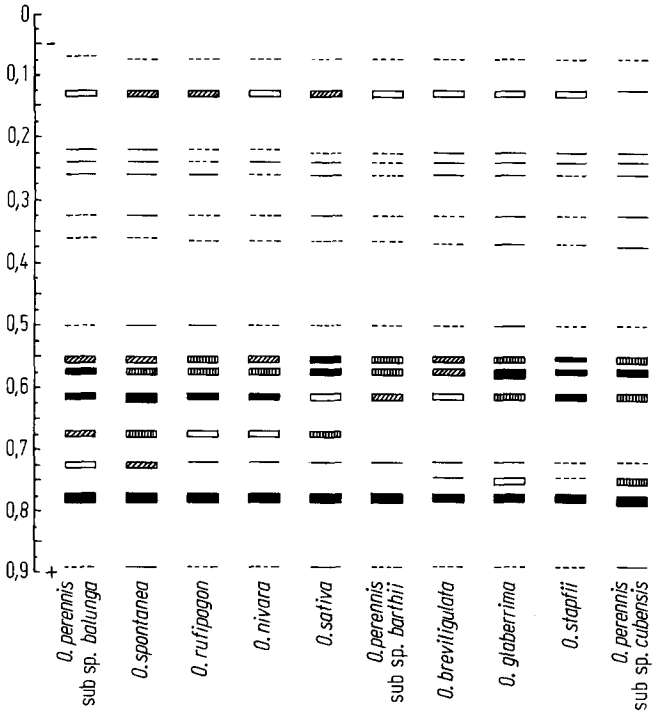
$$S = \frac{m}{m+d} \frac{ai}{Ai} \text{ where,}$$

m = the number of similar bands
d = the number of dissimilar bands
ai = Minimum area of the ith band
Ai = Maximum area of the ith band between any pair of varieties.

Results

Zymogram Analysis

The sixty strains were classified on the basis of their zymogram patterns. There were 16 isoenzymes of esterase, and esterase pattern fell into 3 different



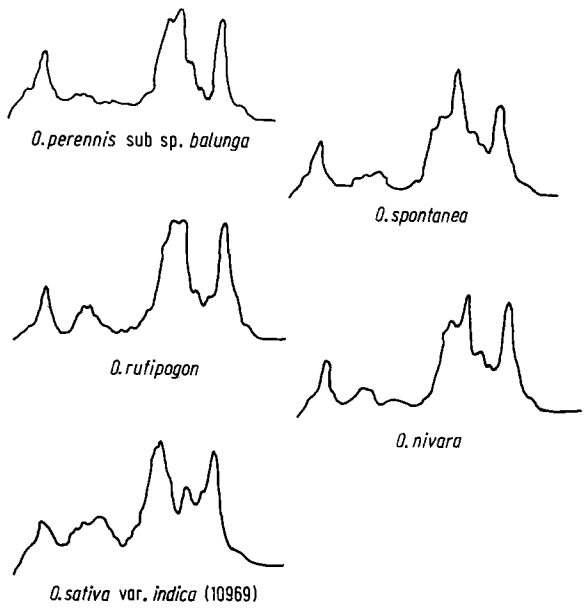


Fig. 1. Zymogram and densitograph tracings of esterase isoenzymes in the representative A-genome species of the genus *Oryza*

zymograms, namely 1e, 2e and 3e. 1e differed from 2e by the presence of band 12 and absence of band 14 respectively at Rf 0.670 and 0.745, whereas 2e differed from 3e by the absence of band 14 only. All the species of the *sativa* complex had zymogram 1e exclusively, whereas 2e and 3e were

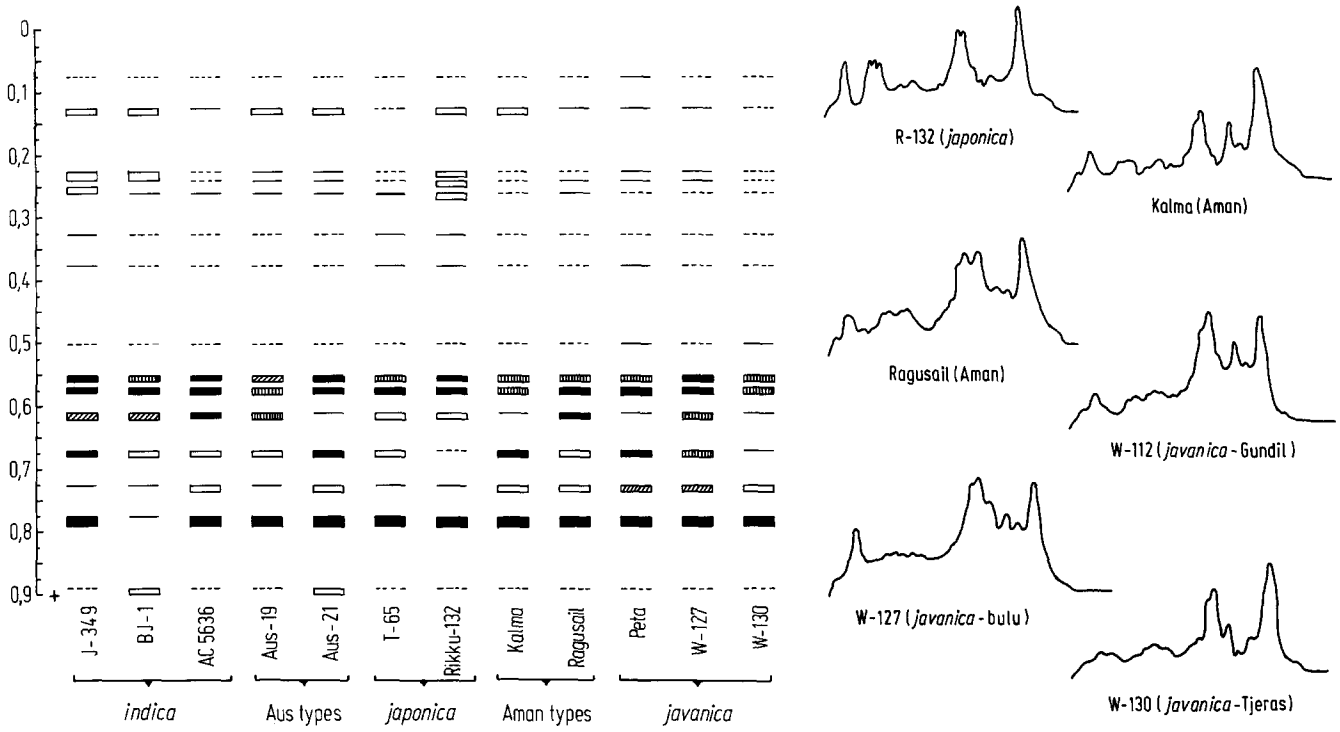


Fig. 2. Zymogram and densitograph tracings of esterase isoenzymes in the subspecies *indica*, *japonica* and *javanica* of *Oryza sativa* L.

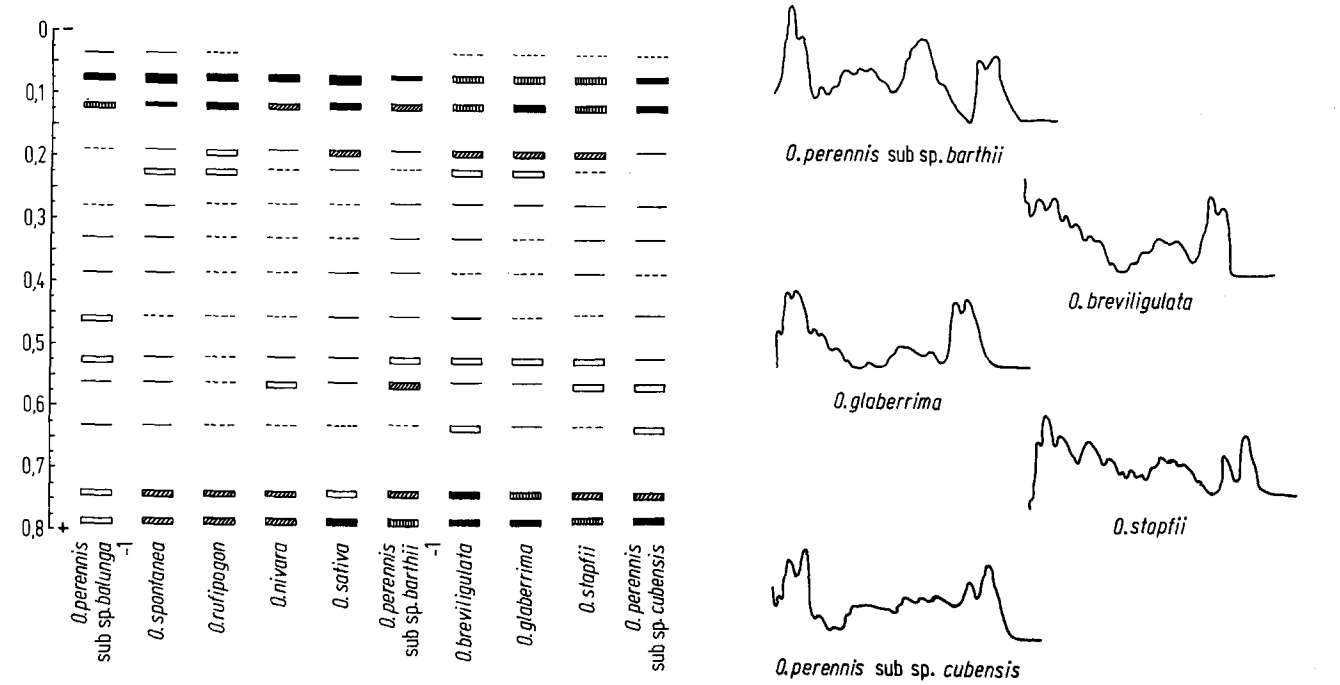


Fig. 3. Zymogram and densitograph tracings of peroxidase isoenzymes in the representative A-genome species of the genus *Oryza*

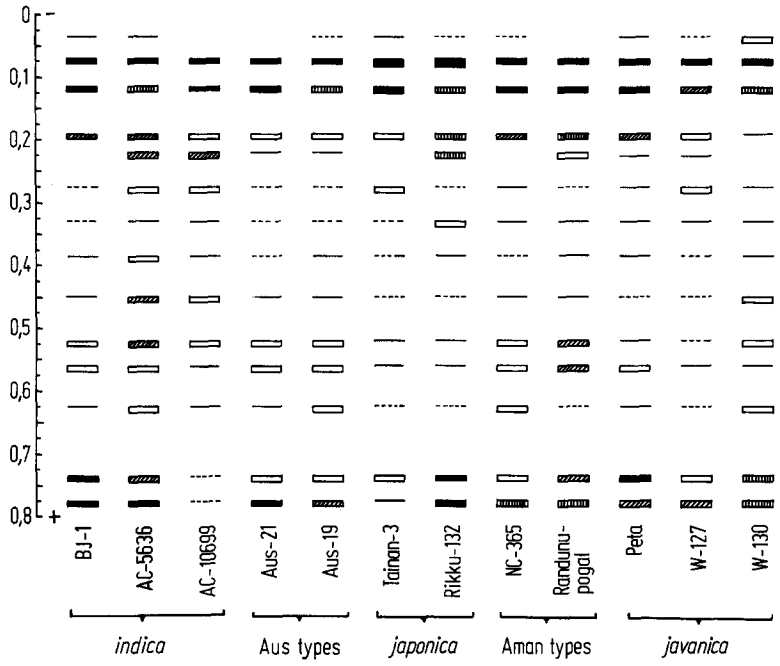


Fig.4. Zymogram of peroxidase isoenzymes in the subspecies *indica*, *japonica* and *javanica* of *Oryza sativa* L.

found exclusively among the species of *glaberrima* complex and related wild species (Figs.1, 1a, 2 & 2a).

The peroxidase pattern had 14 isoenzymes falling into zymogram classes 1p, 2p and 3p. 1p differed from 3p in the absence of band 5 at Rf 0.225, while 2p differed from 3p in the absence of band 1 at Rf 0.03. However, unlike the situation with esterases, the *sativa* and *glaberrima* complexes did not show any specific zymogram pattern of their own. *O.sativa* and *O.glaberrima* strains shared all three zymograms, whereas *O.perennis* sub sp. *balunga* and *O.breviligulata* included 1p and 3p. *O.perennis* sub sp. *barthii* had 2p, while *O.perennis* sub sp. *cubensis* and *O.stapfii* had 3p and 1p respectively (Figs.3, 3a & 4).

Similarity indices and Species Relationships

Relationship among Wild 'A'-Genome Species

Similarity indices (S) within and between the 3 subspecies of *O.perennis* are presented in Table 1. The three strains of Asian *perennis (balunga)* exhibited close similarity among themselves, their 'S' values ranging from 83.50 to 87.26%, whereas the two African strains *(barthii)* had a lower similarity of 76.80 and 76.14%, with the Asian *perennis*, the only *cubensis* type, showing an intermediate similarity of 80.03%. The two African *(barthii)* types had a very high similarity of 92.22%, and 84.0% with *cubensis*.

Relationships between the Cultivated Types and Related Wild Species

It can be seen (Table 2) that the Asian cultivated type *O.sativa* exhibited a high similarity of 86.09% with *balunga*, and was comparatively more divergent from *barthii* with a lower similarity of 79.14%. On the other hand, the African cultivated type *O.glaberrima* had a high similarity of 85.27% with *barthii* compared with a low similarity of 78.30% with *balunga*.

Interrelationships among the Species of the *sativa* Complex

Data on the cumulative average similarities and the respective range values are summarised in Table 3.

Table 1. Similarity indices between three sub-species of *O.perennis*

Name of species	*O.p. balunga*-1	*O.p. balunga*-2	*O.p. balunga*-3	*O.p. barthii*-1	*O.p. barthii*-2	*O.p. cubensis*
O.perennis sub sp. *balunga*-1	-	87.26	84.14	76.50	76.14	80.03
O.perennis sub sp. *balunga*-2		-	83.50	76.54	77.33	77.46
O.perennis sub sp. *balunga*-3			-	78.46	80.02	73.92
O.perennis sub sp. *barthii*-1				-	92.22	84.74
O.perennis sub sp. *barthii*-2					-	84.23
O.perennis sub sp. *cubensis*						-

Table 2. Average similarities between the cultivated forms and their probable progenitors in the Genome-A

Sr. No.	Name of species	*O.sativa* Cumulative average percentage similarity	*O.glaberrima* Cumulative average percentage similarity
1.	*O.perennis* sub sp. *balunga*	86.09	78.30
2.	*O.perennis* sub sp. *barthii*	79.14	85.27

The four wild species of the complex, naley *O.perennis* sub sp. *balunga*, *O.spontanea*, *O.rufipogon* and *O.nivara*, exhibited high similarity among themselves, ranging from 85.63 to 87.80%. All the subspecies and varietal groups of *O.sativa* also showed high similarities with *O.perennis* sub sp. *balunga*, ranging from 85.59% for *javanica* to 85.83% for *indica*. The extent of similarity between *O.spontanea* and the *sativa* varietal groups ranged from 80.86 to 83.83%.

Among the sub-species groups of *O.sativa*, *japonica* was the closest one to *indica* with a high similarity of 89.92%. Similarly the 'aus' and 'aman' types were closer to *javanica*, with 'S' values of 86.78 and 88.17%, respectively. However, it can be seen in general, that there is a considerable overlapping in similarity values between the different groups as seen in the wide range of 'S' values. For instance, one *japonica* strain was more divergent from *indica*, with a similarity of only 84.38%, though in general, *japonica* was closest to *indica* as seen from high average similarity values.

In the same way, though 'aus' and 'aman' and *javanicas* as a group were divergent from *indica*, there were some types which were closer to *indica*, as evident from the high similarity of 89.59% between the 'aman' type and an *indica* strain and 90.39% between one of the 'aus' types and an *indica* strain.

The intra-group similarity was maximum in the *japonica* and *nivara*, as seen from the highest average similarities of 92.07 and 93.22% with narrow ranges of 89.40 to 95.10% and 92.22 to 94.24, respectively. The *javanica* and 'aman' types of Bengal showed the maximum intragroup variation as revealed by the comparatively wide range of similarity values of 78.29 to 93.93% for *javanica* and 81.28-93.24% for 'aman' types. *O.perennis* sub sp. *balunga* also exhibited considerable intra-group variation. The magnitude of intra-group variation in 'S' values in the remaining species and varietal groups was intermediate between those of *japonica* and *javanica*.

Interrelationships among the Species of *glaberrima* Complex

Data on the similarity indices among the species of the *glaberrima* complex are presented in Table 4. The two *barthii* types between them had the highest similarity of 92.22%. On the ohterhand there was a wide intra-group variation in the *breviligulata* and *glaberrima* groups with the similarity ranging from 81.86 to 92.15% in the former and 81.72 to 93.62% in the latter. The similarity between the two *O.stapfii* types was also of a lower order of 84.36%. The similarity between *barthii* and *breviligulata*

Table 3. Cumulative average similarity indices between the wild and cultivated species of the *'Sativa'* complex

Name of species	*O. perennis* sub sp. *balunga*	*O. spontanea*	*O. rufipogon*	*O. nivara*
O. perennis sub sp. *balunga*	84.97 (83.50-87.26)	86.53 (82.86-90.88)	85.86 (83.90-87.98)	86.63 (82.42-89.70)
O. spontanea		88.53 (86.71-91.26)	87.80 (83.80-91.17)	87.43 (84.13-91.00)
O. rufipogon				85.86 (84.16-86.78)
O. nivara				93.22 (92.22-94.24)
O. sativa sub sp. *indica*				
O. sativa sub sp. *japonica*				
O. sativa sub sp. *javanica*				
O. sativa ('Aus')				
O. sativa ('Aman')				

Figures in parenthesis are range of the values

Table 4. Percentage similarity among the species of the *'glaberrima'* complex

Name of species	*O. perennis barthii*-1	Sub sp. *barthii*-2	*O. breviligulata* 1	2	3
O. perennis sub sp. *barthii*-1	-	92.22	85.90	83.77	83.80
O. perennis sub sp. *barthii*-2	-	-	84.31	80.12	84.68
O. breviligulata-1			-	92.15	83.98
O. breviligulata-2				-	81.86
O. breviligulata-3					-
O. glaberrima-1					
O. glaberrima-2					
O. glaberrima-3					
O. glaberrima-4					
O. glaberrima-5					
O. glaberrima-6					
O. glaberrima-7					
O. stapfii-1					
O. stapfii-2					

types varied from 80.12 to 85.90% and that of *glaberrima* and *barthii* ranged from 80.02 to 90.06%. In this case also, a lot of overlapping in similarity values was observed. For instance, one of the *glaberrima* strains (glab. 1) was very close to *barthii* showing 90.06% similarity, whereas few other types (glab. 3) exhibited the very high similarity of 92.32% with *O. breviligulata*. Some of the *glaberrima* strains were also closely related to *O. stapfii* with a similarity value as high as 92.40%.

O. sativa sub sp. *indica*	*O. sativa* sub sp. *japonica*	*O. sativa* sub sp. *japonica*	*O. sativa* ('Aus')	*O. sativa* ('Aman')
86.83 (81.63-90.85)	86.62 (84.21-88.09	85.59 (81.61-90.28)	85.73 (80.66-91.03)	85.70 (81.49-88.36)
83.44 (78.98-86.45)	83.83 (80.07-85.75)	83.79 (78.97-88.24)	82.54 (78.41-86.35)	80.86 (78.71-83.27)
87.83 (83.49-93.10)	88.24 (86.44-89.46)	86.54 (80.37-90.31)	84.69 (80.01-88.49)	83.69 (82.00-87.21)
85.55 (83.87-88.80)	83.53 (79.83-85.15)	82.37 (80.40-85.00)	85.93 (81.74-90.40)	82.91 (79.10-89.29)
86.37 (80.80-91.09)	89.92 (84.38-92.39)	87.15 (77.97-90.35)	86.31 (81.40-90.39)	87.49 (85.85-89.59)
	92.07 (89.40-95.10)	86.30 (77.00-90.43)	85.16 (80.30-89.04)	85.48 (82.83-88.72)
		85.56 (78.29-93.93)	86.78 (82.54-92.43)	88.17 (83.45-92.33)
			87.73 (83.87-91.62)	84.54 (83.58-85.51)
				87.10 (81.28-93.24)

O. glaberrima							*O. stapfii*	
1	2	3	4	5	6	7	1	2
90.06	84.60	85.84	83.27	88.49	87.42	87.97	89.16	90.47
87.78	80.02	82.19	80.75	88.29	88.58	87.23	86.37	89.19
84.02	88.25	91.59	89.19	87.28	85.92	86.88	39.34	87.46
83.09	91.00	92.32	86.75	84.78	82.41	86.41	85.77	84.37
81.28	80.45	83.02	87.09	86.92	86.87	85.61	79.90	88.90
-	84.57	84.64	81.72	85.26	87.68	87.61	86.41	84.66
	-	93.62	86.87	84.86	85.84	87.19	87.27	85.55
		-	88.85	86.10	86.23	88.60	88.22	87.06
			-	86.06	84.77	86.32	87.22	86.26
				-	90.10	91.68	84.16	91.80
					-	92.74	83.12	92.80
						-	83.64	92.40
							-	84.36
								-

Discussion

Genome Analysis

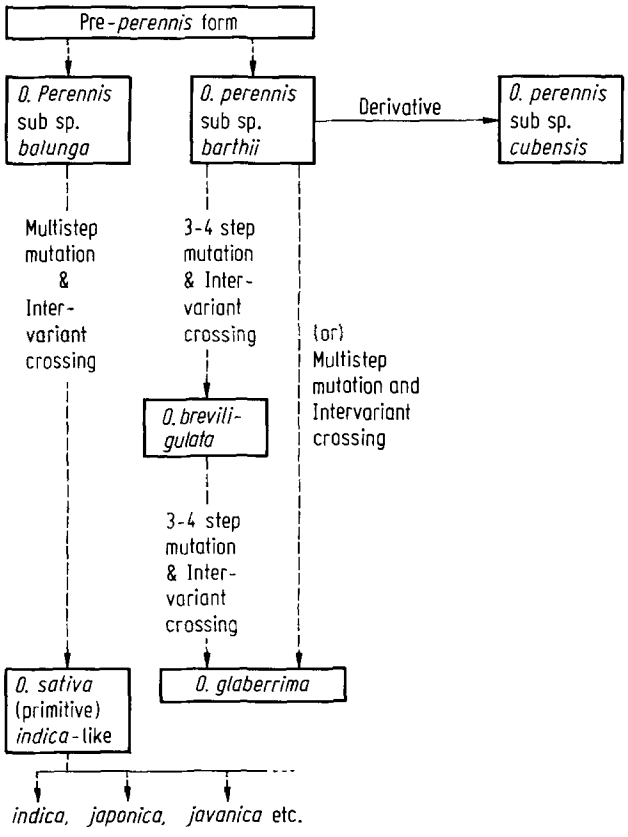
Electrophoretically variant enzymes have provided suitable material for studying genetic homologies and genome relationships. For instance, in *Triticinae* Bhatia (1968) reported that zymorgrams of species having the same genome do not differ much from one another. The presence of basically identical zymogram patterns of esterase and peroxidase in the *sativa* and *glaberrima* complexes corroborate

the suggestion of a single genome symbol 'AA' for this group by Morinaga and Kuriyama (1960). However, though basically similar, *sativa* and *glaberrima* complexes had specific zymograms for esterase, which adds further evidence in support of the modified genome symbol for 'AA', with suitable super or subscripts to differentiate the genomes of *sativa* and *glaberrima* (A^iA^i) (c.f. Richharia 1960; Yeh and Henderson 1961, 1962; Bouharmont 1962). In the symposium on Rice Genetics and Cytogenetics held in the Philippines in 1963, all the species of the *sativa* complex were assigned the genome 'AA', whereas in the *glaberrima* complex, the genomes of the different species were differentiated by superscripts such as A^gA^g for *O. glaberrima*, *O. breviligulata* and *O. stapfii*, A^bA^b for *O. perennis* sub sp. *barthii*, and $A^{cu}A^{cu}$ for *O. perennis* sub sp. *cubensis*. However, based on the presence of the same zymograms of esterase and peroxidase in all these species and the higher similarity and overlapping in 'S' values observed, it seems reasonable to conclude that all these species can be given a single genome designation, A^gA^g or A^bA^b. The observations of Bhatia (1968) lend evidence to this proposal. The presence of similar zymograms within species of the *sativa* and *glaberrima* groups further confirms the earlier observations of some workers (c.f. Shahi et al. 1969; Chu 1967), that it is difficult to identify and pinpoint species based on their zymogram patterns. However, zymogram analysis provides additional taxonomic criteria for broadly grouping the species into different complexes, for instance, into *sativa* and *glaberrima* groups based on esterase zymograms.

Origin of the Cultivated Species

It has been demonstrated that closely related plant species share more isoenzymes in common than species which evolved divergently (West and Garber 1967; Bhatia 1968; Sheen 1970, 1972). It is obvious that the higher the similarity values the higher will be the genetic homology and the closer the phylogenetic relationship. On this basis, the presence of identical zymograms in the cultivated species *O. sativa* and the Asian wild form *O. perennis* sub sp. *balunga* and the higher similarity of 86.09% lend additional evidence to the proposal of some of the earlier rice workers (c.f. Sampath and Rao 1951; Richharia 1960; Yeh and Henderson 1962; Sampath 1962; and Oka and Chang 1962) that *O. perennis* sub sp. *balunga* is the probable ancestor of *O. sativa*. In the same way, though the similarity between *O. glaberrima* and *O. perennis* sub sp. *barthii* is very high, this study does not subscribe to the earlier view that the latter is the progenitor of the former. This is mainly due to the fact that the *glaberrima* types exhibited very high similarities with both *barthii* as well as *O. breviligulata* (90.06 and 93.75% respectively). It may be possible that *O. breviligulata* could also have been the immediate progenitor of *O. glaberrima*. This view is substantiated by earlier reports of very close similarity among these three species. Moreover, the recovery of *glaberrima*-type mutants from *breviligulata* by Sampath and Jachuck (1969) also points to a similar conclusion.

Taking into account the earlier proposals (Sampath and Rao 1951) that *O. perennis* sub sp. *balunga* is the oldest species, and the close similarity be-



tween the *glaberrima* and *sativa* complexes observed on the basis of isoenzyme studies, the following scheme may be proposed to explain the probable mode of origin of both *O.sativa* and *O.glaberrima* from a pre-*perennis* form which should have closely resembled the sub-species *balunga*.

A single mutation affecting the mobility and activity of band 16 of esterase of the *sativa* complex might explain the origin of the zymograms of 2e or 3e found in *barthii*, which might have given rise by multistep mutations to *glaberrima* either directly or through the intermediate step of *O.breviligulata*.

Interrelationships among the Species of the *Glaberrima* and *Sativa* Complexes

As pointed out earlier, the species of the *glaberrima* complex, namely *O.glaberrima*, *O.breviligulata*, *O.stapfii* and *O.barthii* types, exhibited higher inter- and intra-specific similarities and a lot of overlapping, besides sharing the same zymograms for both esterases and peroxidases, rendering impossible the separation into distinct species groups. The earlier observations of Bardenas and Chang (1966), that *O.stapfii* is a synonym of *O.breviligulata*, the reports of Yeh and Henderson (1962) and Chevalier (1932) that *O.stapfii* is a synonym of *O.breviligulata*, that of Chevalier (1932) and Porteres (1956) that *O.glaberrima* is a cultivated form of *O. breviligulata* and the reports of Yeh and Henderson (1962) and Chevalier (1932) that *O.stapfii* is a variety of *O.glaberrima* also support the present proposal that grouping them into distinct species may be far from reality.

A similar situation could be observed in the *sativa* complex too, where the different species exhibited higher intergroup similarities and overlapping apart from having identical zymogram patterns. Moreover, in some groups the intra-group variation transcended the limits of the intergroup variation in the other group and hence classifying them into distinct species becomes almost impossible. The entire *sativa* complex seems to be a single complex. It should be mentioned that in the *sativa* complex, only *O.sativa* has been recognised as a valid species at the International Rice Genetics and Cytogenetics Symposium held in the Philippines in 1963.

Sub-specific Differentiation in *O.sativa*

Another controversial aspect of the phylogeny of *Oryza* is the probable mode of subspecific differentiation in *O.sativa*. Japanese workers (c.f. Morinaga 1955, 1956; Morinaga and Kuriyama 1955, 1960; and Nakao 1957) considered that *japonica* varieties of China and Japan and 'bulu' varieties of Indonesia are derived from 'aus' types, and 'tjerah' varieties of *javanica* are the derivatives of the 'aman' types of Bengal. On the other hand, Sampath and Seetharaman (1962) regarded *japonica* varieties as derivatives of hybrids between *indica* varieties and Asian *O.perennis* forms found in South China and Taiwan.

The closest similarity of *japonica* to *indica* and the higher intra-group homogenity in *japonica* observed in the present study might indicate the possible differentiation of the former from the latter, as reported earlier by Siddiq et al. (1972) based on electrophoretic variation in seed proteins. The closest similarity between 'aman' and *javanica* further suggests the possible differentiation of *javanica* from 'aman' rices of Bengal, as reported by the Japanese workers (c.f. Morinaga 1955, 1956; Morinaga and Kuriyama 1960; and Nakao 1957). However, the extensive overlapping in similarity values between 'aus', 'aman' and *indica* types brings out the fact that 'aus' and 'aman' are nothing but forms of *indica*, and it seems reasonable to assume that the sub-species, namely *japonica* and *javanica*, have differentiated from predominantly *indica* - like forms. The homogeneity of the *japonica* forms may be due to the fact that they are of recent origin, as suggested by earlier workers like Ramiah and Ghose (1951), whereas the wide variation among the different *javanica* strains may be either due to their origin from different *indica* forms or to the process of parallel evolution in the newer habitats after their initial differentiation from *indica*.

Acknowledgement

The financial support of the Indian National Science Academy (Basic Sciences Scheme: study of species relationship in the genus *Oryza* using variability in protein fraction and enzyme patterns) for the investigation is gratefully acknowledged.

Literature

Bardenas, E.A.; Chang, T.T.: Morphotaxonomic studies of *Oryza glaberrima* Steud. and its related wild taxa, *O.breviligulata* A. Chev. et Rochr. and *O.stapfii* Roscher. Bot. Mag. 79, 791-798 (1966)

Bhatia, C.R.: Electrophoresis of analogous enzymes in *Triticinae* In: Proc. Intern. Wheat Genet. Symp. 3rd, pp. 111-115. Canberra 1968

Bouharmont, J.: Researches cytogenetiques chez quelques hybrides inter specifiques d' *Oryza*. Cellule 63, 53-132 (1962)

Chevalier, A.: Nouvelle contribution a L' ehide systematique des *Oryza*. Rev. Int. Bot. Appl. Agric. Trop. 12, 1014-1032 (1932)

Chu, Y.E.: Variations in peroxidase isoenzymes of *Oryza perennis* and *O.sativa*. Japan, J. Genet. 42, 233-244 (1967)

Davis, B.J.: Disc electrophoresis. II. Method and application to serum proteins. Ann. N.Y. Acad. Sci. 121, 404-427 (1964)

Hunter, R.L.; Markert, C.L.: Histochemical demonstration of enzymes separated by Zone electrophoresis in starch gels. Science 125, 1294 (1957)

Internationsl Rice Research Institute: Rice Genetics and Cytogenetics. Proc. Symp. Los. Banos, Philippines, 274pp. Amsterdam: Elsevir 1963

Johnson, B.L.: Tetraploid wheat - seed protein electrophoretic pattern of Emmer and Timophevi groups. Science 158, 131-132 (1967)

Johnson, B.L.; Barnhar, O.; Hall, O.: Analysis of genome and species relationship in the polyploid wheat by protein electrophoresis. Amer. J. Bot. 54, 1078-1089 (1967)

Johnson, B.L.; Hall, O.: Electrophoretic studies of species relationship in *Triticum*. Acta Agric. Scand. Suppl. 16 (1966)

Lowry, O.M.; Posebrough, N.I.; Farr, A.L.; Randall, R.J.: Protein measurement with Folin Phenol reagent. J. Biol. Chem. 193, 265-275 (1951)

Morinaga, T.: The genealogy of Japanese rice. Agric. Hort. (Japan) 30, 1275-1277 (1955)

Morinaga, T.: Rice of China. Agric. Hort. (Japan) 31, 513-516 (1956)

Morinaga, T.; Kuriyama, H.: *Japonica* type rice in the subcontinent of India and Java. Japan. J. Breed. 7, 253-259 (1955)

Morinaga, T.; Kuriyama, H.: Interspecific hybrids and genomic consitution of various species in the genus *Oryza*. Agric. Hort. (Japan) 35, 936-938, 1091-1094, 1245-1247 (1960)

Nakao, S.: Rice. In: Peoples of Nepal Himalaya (ed. Kihara, H., Fauna Flora Res. Soc. Kyoto 4, 398-400 (1957)

Oka, H.I.; Chang, W.T.: Rice varieties intermediate between wild and cultivated forms and the origin of the *japonica* type. Bot. Ball. Acad. Sin. 3, 109-131 (1962)

Ornstein, L.: Disc electrophoresis. I. Background and theory. Ann. N.Y. Acad. Sci. 121, 312-349 (1964)

Porteres, R.: Taxonomic agrobotanique des riz. cultives *O.sativa* Linne. etc. *O.glaberrima* stendel. J. Agric. Trop. Bot. Appl. 3, 341-384, 541-580, 627-700, 821-856 (1956)

Ramiah, K.; Ghose, R.L.M.: Origin and distribution of cultivated plants of South Asia rice. Indian. J. Genet. 11, 7-13 (1951)

Richharia, R.N.: The origins of cultivated rices. Indian. J. Genet. 20, 1-14 (1960)

Sampath, S.: The genus *Oryza* its taxonomy and species interrelationships. Oryza 1, 1-29 (1962)

Sampath, S.; Jachuck, P.T.: The uses of wild rices in mutation breeding. In: Proc. Symp. Radiations and Radiomimetric Substances in Mutation Breeding, pp. 263-270. Trombay Bombay: BARC 1969

Sampath, S.; Rao, N.: Interrelationships between species in the genus *Oryza*. Indian. J. Genet. 11, 14-17 (1951)

Sampath, S.; Seetharaman, R.: The formation of geographical races in the cultivated rice, *Oryza sativa*. Rice News Teller 10, 17-19 (1962)

Shahi, B.B.; Chu, Y.E.; Oka, H.I.: A survey of variations in peroxidase, acid phosphatase and esterase isoenzymes of wild and cultivated *Oryza* species. Japan. J. Genet. 44, 321-328 (1969)

Sheen, S.J.: Peroxidase in the genus *Nicotiana*. Theor. Appl. Genet. 40, 18-25 (1970)

Sheen, S.J.: Isozymic evidence bearing on the origin of *Nicotiana tabaccum* L. Evolution 26, 143-154 (1972)

Siddiq, E.A.; Nerkar, Y.S.; Mehta, S.L.: Intra and inter subspecific variation in soluble protein of *O.sativa* L. Theor. Appl. Genet. 42, 351-356 (1972)

Sokal, R.R.; Sneath, P.H.: Principles of numerical taxonomy. San Fransisco: W.H. Freeman & Co. 1963

Wall, J.R.; Whitaker, T.W.: Genetic control of leucine amino peptidase and esterase isozymes in the interspecific cross *Cucurbita ecuadorensis* × *C. maxima*. Biochem. Genet. 5, 223-229 (1971)

West, N.B.; Garber, E.D.: An electrophoretic survey of esterases in the genus *Phaseolus*. Can. J. Genet. Cytol. 9, 640-645 (1967)

Yeh, B.; Henderson, M.T.: Cytogenetic relationship between cultivated rice *Oryza sativa* L. and five wild diploid forms of *Oryza*. Crop. Sci. 1, 445-450 (1961)

Yeh, B.; Henderson, M.T.: Cytogenetic relationship between African annual diploid species of *Oryza* and cultivated rice *O.sativa* L. Crop. Sci. 2, 463-467 (1962)

Received March 2, 1977
Communicated by B.R. Murty

K. Palanichamy
E.A. Siddiq
Division of Genetics
Indian Agricultural Research Institute
New Delhi-110012 (India)