Theoretical and Applied Genetics 42, 351–356 (1972)

Intra and inter Subspecific Variation in Soluble Proteins of *Oryza sativa* L.

E. A. Siddiq, Y. S. Nerkar and **S. L. Mehta**

Division of Genetics, Indian Agricultural Research Institute, New Delhi (India)

Summary. Types representing three subspecies of *Oryza sativa*, namely, *indica, japonica* and *javanica*, and a group of intermediate types collected from North East India, were studied for variation in soluble proteins using acrylamide gel electrophoresis. The study revealed that there was a marked variation within and between varietal groups. Variability for number and intensity of protein bands in *indica* was wider than in *japonica* and *javanica*. Protein pattern in the group comprising N.E. Indian types transgressed that of all three established groups by displaying a wide spectrum. Relative homology, as measured from percentage similarities of N.E. Indian types to the three subspecies, suggested the existence of six different groups. Comparison of varietal groups for the protein mobility pattern showed that *japonica* and *javanica* varieties tended to show higher percentages of slow mobility proteins than *indica*. It appears, from the narrow variability and relatively low percentage of slow mobility proteins, that the *japonica* and *javanica* races are of later origin compared to *indica*. However, the study with a limited number of types suggested the monophyletic origin of varietal groups from an *indica*-like base predominantly found in the secondary centres of origin of *O. sativa*.

Introduction

Protein and enzyme variations are now known to provide an additional clue to our understanding of phylogenetic relationships in higher plants, including the genus *Oryza* (c. f. Fox *et al.*, 1964; Johnson and Hall, 1965; Vaughan *et al.*, 1966; Garber, 1965; Desborough and Peloquin, 1966; Loeschcke and Stegemann, 1966; Hart and Bhatia, 1967; Chu, 1967; Chu and Oka, 1967 and Shahi *et al.* 1969 a, b). Of the twenty-four species of the genus *Oryza*, *O. sativa* and *O. glaberrima* are the only cultivated species. Evolution of the cultivated forms, and further differentiation into geographical groups of *O. sativa* in particular, have been the subject of controversy for a long time (c. f. Chatterjee, 1948; Sampath and Govindaswami, 1958; Morishima *et al.*, 1961; Kato, 1930; Mizushima, 1950 and Oka, 1958). The present study was undertaken with the object of gaining further insight into the intervarietal relationships of *O. sativa* through the electrophoretic patterns of soluble protein fractions.

Material and Methods

Material used in the present study comprised representative cultivars belonging to three subspecies of *O. sativa*, namely, *indica*, *japonica* and *javanica*, and a set of intermediate forms collected from North East India which is considered to fall on the periphery of the centre of origin of cultivated rice.

Employing the polyacrylamide gel electrophoresis technique outlined by Davis (1964) and Ornstein (1964), the soluble protein pattern was studied. Extraction was done by soaking the endosperm overnight at 4 °C in 0.05 M Tris-HCl buffer (pH 7.6) containing 6 mM B-mercaptoethanol and subsequently grinding and homogenizing in a pestle and mortar. The cell paste suspension was centrifuged at 20,000 × g for 20 min. Protein content in the supernatant was estimated by the method described by Lowry *et al.* (1951). Samples containing 200 µg of protein were applied to each gel column over spacer gel and electrophoresis was carried out using Trisglycine buffer (pH 8.3) for 90 min. at 4 m A current per gel column. After electrophoresis the gels were removed and stained with 0.1% amido-black in 7% acetic acid for 30 min. and excess stain was removed by 7% acetic acid. The patterns of soluble proteins in the gels were photographed using a polaroid camera and phoretophot assembly of the Canalco, U.S.A. The densitographs of the spectrum of soluble protein were prepared by scanning the gels on chromoscan (Joyce Loeble, England). Rf values were calculated for each band as

$$R_f = \frac{\text{distance travelled by the band from top of the running gel}}{\text{distance travelled by tracking dye}}$$

The R_f value for each band was computed from the mean of observations obtained from four independent electrophoretic runs on two separate extractions.

Results

The electrophoretic patterns of soluble proteins in the representative varieties of each group and the diagrammatic representation of the corresponding band patterns are presented in Figs. 1 and 2. Fig. 3 gives a comparative spectrum of protein of all the varieties studied.

The results in general showed that variation in the number and intensity of protein bands within the subspecific groups *japonica* and *javanica* was relatively narrow compared with that in the *indica* and intermediate types. For instance, the number of protein bands ranged between 14 and 15, and 15 and 17, for *japonica* and *javanica* respectively, while it varied from 12 to 16, and 9 to 16, in the *indica* and North East Indian intermediate types, respectively.

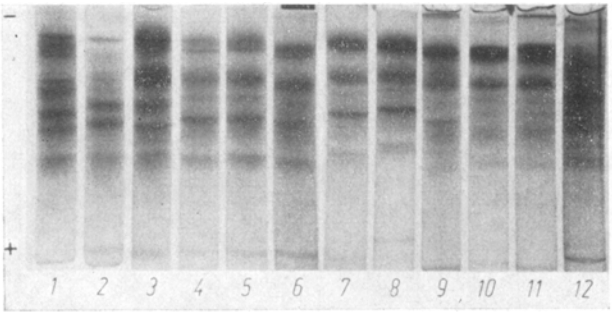

Fig. 1. Photograph showing the electrophoretic pattern displayed by soluble endosperm proteins of varieties representing *indica, japonica, javanica* and N.E. Indian types (1—3 *indica*; 4—6 *japonica*; 7—8 *javanica* and 9—12 N.E. Indian types)

The three subspecies were also compared for the presence or absence of bands at different R_{f_s}. The study revealed that band 6 at R_f 0.12 was present in the *indica* and *javanica* varieties and absent in the *japonica* varieties. Band 9 at R_f 0.17 was present in *javanica* and N. P. 130, an *indica* variety. Similarly, the *javanica* types and variety Asd-4 of the *indica* group had band 17 at R_f 0.35, while band 10 at R_f 0.18 was absent in these varieties. Bands 18 and 19, at R_f 0.38 and 0.39 respectively, which were characteristic of *japonica* and *javanica* types, were interestingly absent in the varieties of the *indica* group. Band 13 (R_f 0.26) was exclusively found in

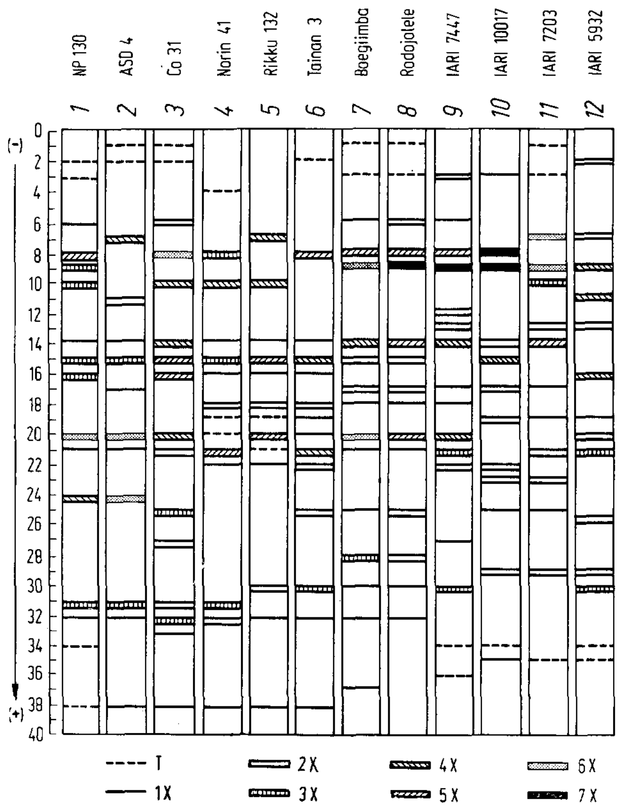

Fig. 2. Diagrammatic representation of Fig. 1 showing the number and intensity of protein bands in *indica, japonica, javanica* and N.E. Indian types

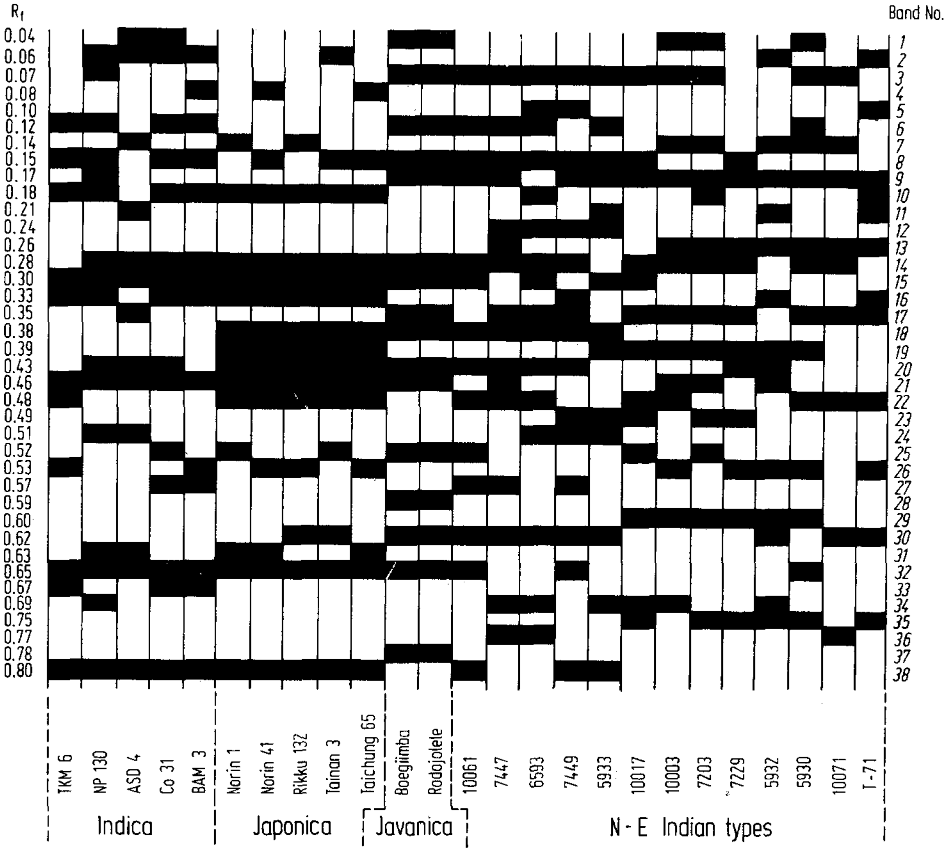

Fig. 3. Diagram showing the comparative spectrum of soluble protein in representative varieties of *indica, japonica, javanica* and N.E. Indian collection

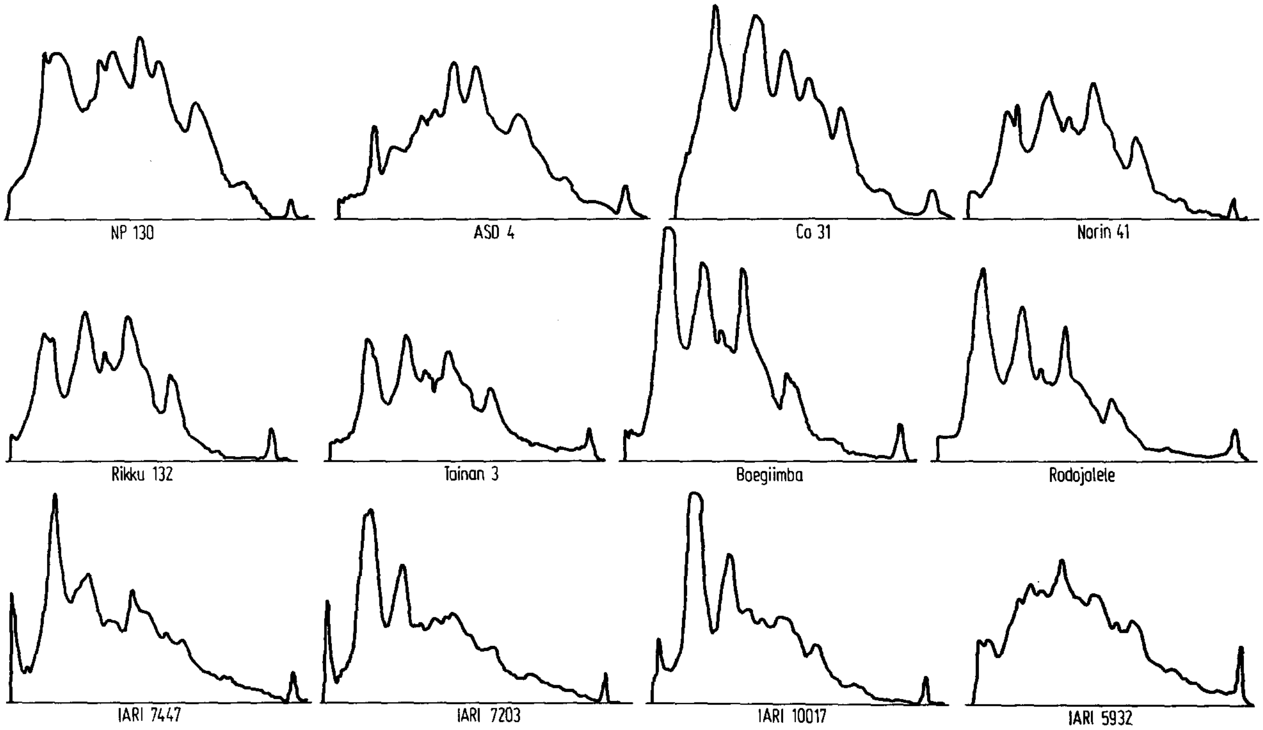

Fig. 4. Densitographs of soluble protein fractions in the (a—d) representative varieties of a) *indica*, b) *japonica*, c) *javanica* and d) N.E. Indian collection

javanica. In contrast to the established varietal groups, the protein patterns of the North East Indian types were found to transgress those of the three subspecies. Some of the varieties of this group exhibited extra bands, such as 12, 23, 29, 35 and 36, respectively at R_f 0.24, 0.49, 0.60, 0,75 and 0.77.

The varietal groups were also found to differ in the intensity of corresponding bands. Band 16 at R_f 0.33, for instance, was very intense and sharp in the *indica* types while it was faint and diffused in *japonica* types and altogether absent in *javanica* types. Similarly, the three groups showed quantitative differences for bands 14, 20 and 21 (R_f 0.28, 0.43 and 0.46 respectively).

Based on the densitograph readings (Fig. 4), the distribution of the protein fractions expressed as percentages were broadly grouped into three mobility groups, namely, slow (0.0—0.2), intermediate (0.2 to 0.4 and 0.4—0.6) and fast (0.6—0.8) (Table 1). The data, in general, suggested that the *javanica* types and some of the *japonica* types displayed higher percentages of slow mobility proteins than the *indica* type, while the reverse was true for fast mobility proteins. Although the proportion of the intermediate mobility fraction was similar in all three, *javanica* differed slightly in showing a relatively low proportion, compared with *indica* and *japonica* varieties, in the R_f range of 0.4—0.6. The North East Indian types, however, displayed a wide range overlapping all the three subspecies.

Percentage similarities between all possible pairs of varieties, based on major protein bands summari-

Table 1. *Distribution of soluble proteins in O. sativa on the basis of electrophoretic mobility (in percentage)*

Varietal group	Variety	Slow mobility (0—0.2) R_f	Intermediate mobility (0.2—0.4) R_f	(0.4 · 0.6) R_f	Fast mobility (0.6—0.8) R_f
indica					
1.	TKM-6	23.77	33.12	25.60	17.51
2.	NP-130	25.90	30.32	30.17	13.62
3.	ASD-4	9.68	30.46	37.93	21.93
4.	Co.-31	23.80	33.10	22.62	13.48
5.	BAM-3	23.70	33.68	28.90	13.72
japonica					
6.	Norin-1	21.57	30.35	35.22	12.84
7.	Norin-41	22.95	34.97	30.64	11.44
8.	Rikku-132	28.81	36.40	36.40	5.61
19.	Tainan-3	27.11	38.23	29.01	5.65
0.	Taichung-65	22.00	35.75	31.64	10.61
javanica					
11.	Boegiimba	29.08	35.88	26.25	8.79
12.	Rodojolele	30.91	36.52	25.56	7.01
N. E. Indian types					
13.	IARI 10061	27.52	38.81	25.17	8.50
14.	IARI 7447	27.17	33.14	27.79	11.91
15.	IARI 6593	22.36	28.84	32.66	16.15
16.	IARI 7449	21.67	34.50	27.80	16.03
17.	IARI 5933	19.74	34.88	32.33	13.25
18.	IARI 10017	33.72	32.32	35.14	8.82
19.	IARI 10003	35.39	31.55	23.90	9.16
20.	IARI 7203	31.98	30.32	27.58	10.12
21.	IARI 7229	33.45	33.65	25.25	7.65
22.	IARI 5932	24.48	35.90	27.54	12.08
23.	IARI 5930	25.72	33.53	31.34	9.41
24.	IARI 10071	28.48	37.13	27.00	7.39
25.	T (71)	25.87	37.63	28.81	7.69

sed in Table 2, were calculated as follows:

$$\text{Percentage similarity} = \frac{\text{No. of pairs of similar bands}}{\text{No. of different bands} + \text{No. of similar bands}} \times 100$$

The data showed that within the *indica* group homology varied between 50.0 and 79.9% (excluding the variety Asd. 4), while it was 75.0 to 100% within *japonica*. The homology between the *indica* and *japonica* types varied from 33.3 to 62.5%, while it was 26.3 to 42.1% between *indica* and *javanica* and 30.4 to 45.0% between the *japonica* and *javanica* types.

Percentage similarities among varieties of the *indica* group and the N. E. Indian intermediate forms ranged from 5.5 to 53.3%. Similarly, homology between the *japonica* and intermediate types and the *javanica* and intermediate types ranged from 15.0 to 58.8 and 20.0 to 55.5%, respectively.

Thus the study revealed the existence of variability for number and intensity of soluble protein bands between groups as well as within groups, and the occurrence of types resembling and also differing from all three established varietal groups in the N. E. Indian rice collection, in the pattern of protein fractions.

Discussion

O. sativa, the only widely cultivated species of the genus *Oryza*, has been extensively studied with respect to its origin, geographical distribution and subspecific differentiation. On the basis of a set of morphological differences and sexual affinity, early workers, like Kato *et al.* (1928), proposed to divide

Table 2. *Percentage similarities between the varieties based on soluble protein component homologies*

indica					*japonica*					*javanica*		North East Indian Types												
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25*
1	50.0	22.2	53.3	69.2	46.7	53.3	46.7	43.7	53.3	29.4	29.4	25.0	23.5	35.7	25.0	21.0	20.0	16.6	12.5	16.6	22.2	22.2	05.9	25.0
	2	50.0	66.6	60.0	53.0	58.8	40.0	50.0	56.3	40.0	40.0	40.0	42.1	36.8	42.1	31.5	21.0	15.0	20.0	21.0	21.0	21.0	11.0	16.6
		3	35.3	29.4	43.7	38.9	38.9	33.3	38.9	26.3	26.3	26.3	20.0	27.7	33.3	21.0	16.6	23.5	22.2	16.6	22.2	22.2	17.7	11.0
			4	76.9	56.3	50.0	44.4	62.5	53.0	42.1	42.1	53.3	31.5	33.3	36.8	20.0	20.0	10.0	21.0	15.8	16.6	05.9	05.9	10.6
				5	38.9	53.0	47.1	47.1	56.3	30.0	30.0	41.1	26.3	31.5	31.5	21.0	23.5	16.6	15.7	16.6	15.8	22.2	05.9	05.5
					6	75.0	76.5	75.0	75.0	31.8	31.8	42.1	22.7	30.0	27.2	18.1	26.3	26.6	31.5	15.8	25.0	31.6	18.7	15.0
						7	75.0	75.0	100.0	30.4	30.4	42.1	28.5	36.8	33.3	20.0	26.3	26.6	19.0	26.6	25.0	26.3	10.5	21.1
							8	75.0	75.0	31.8	31.8	38.8	28.5	38.8	33.3	23.8	20.0	33.3	25.0	20.0	35.3	31.6	18.6	35.0
								9	75.0	45.0	45.0	58.8	35.0	44.4	40.0	30.0	33.3	20.0	25.0	20.0	21.0	20.0	16.6	21.1
									10	31.8	31.8	38.8	28.5	36.8	33.3	26.3	26.2	22.2	19.0	26.6	25.0	25.0	10.6	21.1
										11	100.0	55.5	55.5	42.1	31.5	30.0	31.5	25.0	30.0	31.6	23.8	25.0	29.4	20.0
											12	55.5	55.5	42.1	31.5	30.0	32.5	25.0	30.0	31.6	23.8	25.0	29.0	20.0
												13	52.9	47.0	47.0	35.2	33.3	15.0	14.3	21.0	14.3	26.3	25.0	20.0
													14	56.2	50.0	31.5	27.7	31.2	26.6	35.3	23.5	37.5	42.8	29.4
														15	44.4	41.1	23.5	15.7	15.0	22.2	10.0	21.0	26.7	23.6
															16	47.0	26.3	14.2	19.0	33.3	19.0	15.8	23.6	10.6
																17	29.4	10.0	15.0	22.2	21.0	14.2	11.8	11.8
																	18	46.6	50.0	53.8	16.6	40.0	30.8	18.7
																		19	61.5	53.8	50.0	75.0	54.5	35.7
																			20	50.0	37.5	46.7	38.5	20.0
																				21	40.0	21.7	30.8	26.7
																					22	37.5	28.6	38.5
																						23	50.0	28.6
																							24	41.7
																								25

* Serial numbers represent varieties in order as in Table 1.

O. sativa into two varietal groups. Later Terao and Mizushima (1944) introduced an intermediate group, *javanica*, based on the degree of crossability. The occurrence of groups within the subspecies *japonica*, namely 'tropical' and 'temperate' forms, was shown by Oka (1958). Further studies using many more criteria, including the application of biometrics, reported the existence of more than three groups (c. f. Mizushima, 1948, 1950; Oka, 1958; Jawahar Ram and Panwar, 1970 and Vairavan, 1971).

As in many crop plants, the degree of homology in protein fractions has been used recently as a dependable tool for measuring intra and inter-specific relationships in rice (c. f. Chu and Oka, 1967; Shahi *et al.*, 1969 a, b).

In the present study, soluble proteins of the three subspecies of *O. sativa* and intermediate forms collected from N. E. India were fractionated on acrylamide gel electrophoresis and the extent of homology in number and intensity of protein bands was studied. Comparative study of the extent of intra-variations in protein fractions in the subspecies revealed that the group of intermediate types showed the maximum variability followed, in order, by *indica, japonica* and *javanica*. This agrees with earlier reports on variability for morphological traits, intervarietal hybrid sterility, etc., thus showing *indica* to be more primitive than other subspecies (c. f. Richharia *et al.*, 1962; Jennings, 1964 and Oka, 1964b). Shahi *et al.* (1969), surveying a few selected enzymes in different species and subspecies of *Oryza*, concluded that widely distributed wild types showed more variability than localised types. The present study shows that not only the primitive groups like *indica*, but also the population undergoing differentiation and approaching towards definite groups, might show maximum variability. This was obvious from the percentage of similarity computed for the pairs of varieties, which indicated a high percentage of homology among varieties within the *japonica* and *javanica* types but much less in the *indica* and intermediate types.

Duke and Glassman (1968), working on 29 species of *Drosophila*, reported that the electrophoretic mobility of isozymes tended to be reduced as the species became advanced. Although the present study was not based on any structurally or functionally definite protein bands, the gross fraction suggests that the varietal groups differ considerably in their mobility pattern. Much as expected, the *japonica* and *javanica* groups tended to show a higher percentage of slow mobility proteins than the *indica* group, thus further strengthening the view that the former two groups are of later origin than the latter.

The relative homology of each N. E. Indian type to the three subspecies, based on percentage similarities, may be worked out as given in Table 3. The study of even a very limited number of types suggested the existence of more than the established number of groups. This is, to a great extent, in agreement with earlier reports. For instance, using multivariate analysis of a representative collection of 190 cultivars of N. E. India, Vairavan (1971) reported the existence of nine divergent clusters. The occurrence of *japonica* types and several intermediate forms bridging the races has also been reported by many workers from their study of similar collections made in Nepal, Sikkim, N. E. India, Jeypore tract etc. (c. f. Nakao, 1957; Kihara and Katayama, 1963; Sharma *et al.*, 1971; Govindaswami and Krishnamurthy, 1968; Oka *et al.*, 1959). Thus the results provide additional evidence that the N. E. Indian types represent a continuous array of intermediate types bridging the different subspecies of *O. sativa*.

Table 3. *Grouping of the N. E. Indian types based on their relative homology to the three subspecies of O. sativa*

Culture	Relative homology			Group
IARI 10061	javanica >	japonica >	indica	F
IARI 7447	javanica >	indica >	japonica	E
IARI 6593	japonica >	javanica >	indica	D
IARI 7449	indica >	japonica >	javanica	A
IARI 5933	indica >	japonica >	javanica	
IARI 10017	japonica >	javanica >	indica	D
IARI 10003	japonica >	javanica >	indica	
IARI 7203	japonica >	javanica >	indica	
IARI 7229	javanica >	japonica >	indica	F
IARI 5932	japonica >	javanica >	indica	D
IARI 5930	japonica >	javanica >	indica	
IARI 10071	javanica >	japonica >	indica	F
T-71	japonica >	indica >	javanica	C

There are two different views on the mode of origin. According to Oka (1964a), the subspecies might have evolved independently (monophyletic origin) from the ecotypes of the putative species, *O. perennis*, through introgression. Richharia *et al.* (1962) hold the view that the '*aus*' types of *indica* might have given rise to *japonica* on the one hand and *javanica* on the other. From the present limited study on the variability for protein fractions in the established geographical groups and the primitive cultivars of N. E. India, it seems that *indica* could be one of the stabilised forms like *japonica* and *javanica* rather than being the progenitor of the latter two. From the continuous nature of the variability observed in the varietal collection of N. E. India, which is supposed to be on the periphery of the centre of origin of cultivated rice, it appears that, depending upon the degree of adaptation developed to specific environments, a few groups like *indica, japonica* etc. might have emerged, possibly as a result of disruptive selection, and subsequently have been preserved by man. Intermediate types, as evident from various reports, appear to show a tendency to evolve into many more cultivated groups.

Acknowledgement

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

Literature

1. Chatterjee, D.: A modified key and enumeration of the species of *Oryza sativa* L. Indian J. Agric. Sci. **18**, 185—192 (1948). — 2. Chu, Y. E.: Variation on peroxidase isozymes of *Oryza perennis* and *O. sativa*. Japan. J. Genetics **42**, 233—244 (1967). — 3. Chu, Y. E., Oka, H. I.: Comparison of variation in peroxidase isozymes between *perennis-sativa* and *breviligulata-glaberrima* series of *Oryza*. Bot. Bull. Acad. Sinica **8** (Special No.), 261—270 (1967). — 4. Davis, R. J.: Disc electrophoresis II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. **121**, 404—427 (1964). — 5. Desborough, S. L., Peloquin, S. J.: Disc electrophoresis of tuber proteins from *Solanum* species and interspecific hybrids. Phytochemistry **5**, 727—733 (1966). — 6. Duke, E. J., Glassman, E.: Evolution of Xanthine dehydrogenase in *Drosophila*. Genetics **58**, 101—112 (1968). — 7. Fox, D. J., Thurman, D. A., Butler, O.: Studies of the proteins of seeds of the *Leguminosae* I. Albumins. Phytochemistry **3**, 417—419 (1964). — 8. Garber, E. D.: The genus *Collinsia* XXVIII: A paper chromatographic and disc electrophoresis study of leaf extracts from 14 species and progeny from 5 interspecific hybrids. Can. J. Genet. Cytol. **7**, 551—558 (1965). — 9. Govindaswamy, S., Krishnamurty, A.: On the occurrence of *japonica* type of grains in Jeypore tract. Rice News Teller **6**(4) 22—24 (1958). — 10. Hart, G. E., Bhatia, C. R.: Acrylamide gel electrophoresis of soluble leaf proteins and enzymes from *Nicotiana* species. Can. J. Genet. Cytol. **9**, 367—374 (1967). — 11. Jawahar Ram, Panwar, D. V. S.: Interspecific divergence in rice. Indian J. Genet. **30**(1), 1—10 (1970). — 12. Jennings, P. R.: Evaluation of partial sterility in *indica* × *japonica* rice hybrids. Technical Bull. **5**, IRRI, Los Baños, Philippines, 63 (1964). — 13. Johnson, B. L., Hall, O.: Analysis of phylogenetic affinities in the *Triticinae* by protein electrophoresis. Am. J. Bot. **52**, 506—513 (1965). — 14. Kato, S. C.: On the affinity of the cultivated varieties of rice plants, *Oryza sativa*, L. Kyushu Imp. Univ. Dept. Agr. J. **2**(9), 241—276 (1930). — 15. Kato, S., Kosaka, H., Hara, S.: On the affinity of rice varieties as shown by the fertility of hybrid plants. Kyusu Imp. Univ. Fakult. Terkult, Bull. Sci. Report **3**, 132 (1928). — 16. Kihara, H., Katayama, C.: Occurrence of *indica* and *japonica* types among Sikkemese rice varieties. National Inst. Genet. Annual Report No. **14**, 67 (1963). — 17. Loeschcke, V., Stegemann, H.: Proteine der Kartoffelknollen in Abhängigkeit von Sorte und Virosen (Polyacrylamid-Elektrophorese). Phytochemistry **5**, 985—991 (1966). — 18. Lowry, O. M., Rosebrough, N. J., Farr, A. L., Randall, R. J.: Protein measurement with folin phenol reagent. J. Biol. Chem. **193**, 265—275 (1951). — 19. Mizushima, U.: Studies on sexual affinity among rice varieties, *Oryza sativa* L. I. Analysis of affinity of Japanese, American and Javanese varieties. Biology (Asakura-Shoten, Tokyo) 3(2), 41—52 (1948). — 20. Mizushima, U. C.: Studies on sexual affinity among rice varieties, *Oryza sativa* L. II. Analysis of affinity of other Asiatic and Hawaiian varieties. Tohoku J. Agr. Res., Japan 1(2), 151—160 (1950). — 21. Morishima, H., Oka, H. I., Chang, W. T.: Directions of differentiations in populations of wild rice, *Oryza perennis* and *O. sativa* fatua *spontanea*. Evolution XV(3), 326—339 (1961). — 22. Nakao, S.: Rice — In: Peoples of Nepal, Himalaya Scientific results of the Japanese expedition to Nepal Himalaya. Fauna Flora Res. Soc. Kyoto Univ. **3**, 398—400 (1957). — 23. Oka, H. I.: Intervarietal variation and classification of cultivated rice. Indian J. Genet. 18(2), 80—89 (1958). — 24. Oka, H. I.: Pattern of interspecific relationships and evolutionary dynamics in *Oryza*. In: Rice Genetics and Cytogenetics, ed. R. F. Chandler, pp. 71 — 90. Amsterdam: Elsevier 1964a. — 25. Oka, H. I.: Considerations on the genetic basis of intervarietal sterility in *Oryza sativa*. Ibid. pp. 158—174 (1964b). — 26. Oka, H. I., Chang, W. T., Nanse, T.: A preliminary note on investigations of wild and cultivated rice strains collected from the mountain region of Orissa state, India. Ann. Rept. Natl. Inst. Genet. (Japan) 1958, **18**, 34—42 (1959). — 27. Ornstein, L.: Disc Electrophoresis. I. Background and theory. Ann. N.Y. Acad. Sci. **121**, 321—349 (1964). — 28. Richharia, R. H., Misro, B., Rao, R. K.: Sterility in the rice hybrids and its significance. Euphytica **11**, 137—142 (1962). — 29. Sampath, S., Govindaswami, S.: Wild rices of Orissa and their relationship to cultivated rices. Rice News Teller, July, 1958 (1958). — 30. Shahi, B. B., Chu, Y. E., Oka, H. I.: Analysis of genes controlling peroxidase isozymes in *Oryza sativa* and *O. perennis*. Japan. J. Genet. **44**(5), 321—328 (1969b). — 31. Shahi, B. B., Morishima, H., Oka, H. I.: A survey of variations in peroxidase, acid phosphatase and esterase isozymes of wild and cultivated *Oryza* species. Japan. J. Genet. **44**(5), 303—319 (1969a). — 32. Sharma, S. D., Vellanki, J. M. R., Hakim, K. L., Singh, R. K.: Primitive and current cultivars of rice in Assam — A rich source of valuable genes. Curr. Sci. 15(6), 126—128 (1971). — 33. Terao, H., Mizushima, V.: On the affinity of rice varieties cultivated in East Asia and America. B. Agr. Expt. Sta. Ministry Agr. and Commerce (Japan) **55**, 1—7 (1944). — 34. Vairavan, S.: Studies on the nature of genetic divergence in some rice strains from Assam and North East Himalayas. M. Sc. Thesis, P. G. School, I.A.R.I., India (1971). — 35. Vaughan, J. G., Waite, A., Boulter, D., Waiters, S.: Comparative studies of the seed proteins of *Brassica campestris*, *B. oleracea* and *B. nigra*. J. Exptl. Bot. **17**, 332—349 (1966).

Received February 3, 1972

Communicated by M. S. Swaminathan

E. A. Siddiq
Y. S. Nerkar
S. L. Mehta
Division of Genetics
Indian Agricultural Research Institute
New Delhi-12 (India)