THE DIFFERENTIATION, CLASSIFICATION AND CENTER OF GENETIC DIVERSITY OF CULTIVATED RICE (*Oryza sativa* L.) BY ISOZYME ANALYSIS

Masahiro NAKAGAHRA *

Introduction

Rice cultivated in Asia (*Oryza sativa* L.) also grows in tropical and temperate areas of the world and consists of several varietal groups with wide genetic variations. Cross experiments between the groups often revealed gametic selection and hybrid sterility which are the main causes of reproductive isolation and non-random recombination (Nakagahra 1972, Nakagahra *et al.* 1972, Oka 1963, 1974). The remarkable varietal differentiation is due to these reproductive barriers. Rice breeding in Japan increasingly requires the introduction of useful exotic genes, since the domestic genetic sources are limited. For example, genes resistant to pests and diseases, and some physiological traits such as high capacity of leaf photosynthesis (Hayashi *et al.* 1977) have been introduced. Successful introduction of these desirable traits can be facilitated by a clear understanding of affinities among the groups so as to minimize adverse effects of reproductive barriers or undesirable linkage. Where can we expect to obtain the most useful materials for the future rice breeding in Japan? Our main purpose is to pursue genetic variations and investigate the center of genetic diversity of cultivated rice.

"Isozyme analysis" is one of the most effective means to estimate the extent of genetic variations in native plant populations, because most enzymes can be divided into some polymorphic forms, called isozymes which can be separated by electrophoresis. The author used esterase isozymes in rice leaves as a clue to find out genetic variability, since their varietal specificity in kinds and activities are maintained regardless of growth stages and environmental conditions. Enzyme extraction was performed by a newly developed homogenizer (Nakagahra *et al.* 1976), and electrophoresis for zymogram analysis by using the horizontal agar gel thin layer method (Nakagahra *et al.* 1975).

Genetics for esterase isozymes

Activities of esterase isozymes in leaves of 1,317 varieties collected from various countries were found in all 14 anodic bands. Genic analysis for each band was carried out with F_1 and F_2 plants stemming from crosses between various varieties. The results indicated the presence of five major bands, 1A, 6A, 7A, 12A and 13A, which were specified by seven alleles at three loci (Nakagahra 1977). Presence or absence of band 1A was specified by a dominant allele symbolized as Est_1, its null form being $Est_1{}^O$. Band 6A and 7A were specified by codominant alleles at the same locus, which were symbolized as $Est_2{}^S$ and $Est_2{}^F$, the null form being $Est_2{}^O$. Band 12A and 13A were specified by codominant alleles, which were symbolized as $Est_3{}^S$ and $Est_3{}^F$, respectively. No significant linkage relationship was found between Est_1 and Est_2, or between Est_2 and Est_3.

Combinations of the isozymic alleles would be 2 (Est_1, $Est_1{}^O$) x 3 ($Est_2{}^S$, $Est_2{}^F$ and $Est_2{}^O$) x 2 ($Est_3{}^S$, $Est_3{}^F$), and then give rise to twelve gametic genotypes as follows:

---

* Plant Geneticist, National Institute of Agricultural Sciences, Ohara, Hiratsuka, Kanagawa 254, Japan

Type	Genotype	Zymogram	Type	Genotype	Zymogram
1.	Est_1 $Est_2{}^S$ $Est_3{}^F$	(1A-6A-13A)	7.	$Est_1{}^O$ $Est_2{}^S$ $Est_3{}^F$	(6A-13A)
2.	Est_1 $Est_2{}^S$ $Est_3{}^S$	(1A-6A-12A)	8.	$Est_1{}^O$ $Est_2{}^S$ $Est_3{}^S$	(6A-12A)
3.	Est_1 $Est_2{}^F$ $Est_3{}^F$	(1A-7A-13A)	9.	$Est_1{}^O$ $Est_2{}^F$ $Est_3{}^F$	(7A-13A)
4	Est_1 $Est_2{}^F$ $Est_3{}^S$	(1A-7A-12A)	10.	$Est_1{}^O$ $Est_2{}^F$ $Est_3{}^S$	(7A-12A)
5.	Est_1 $Est_2{}^O$ $Est_3{}^F$	(1A-13A)	11.	$Est_1{}^O$ $Est_2{}^O$ $Est_3{}^F$	(13A only)
6.	Est_1 $Est_2{}^O$ $Est_3{}^S$	(1A-12A)	12.	$Est_1{}^O$ $Est_2{}^O$ $Est_3{}^S$	(12A only)

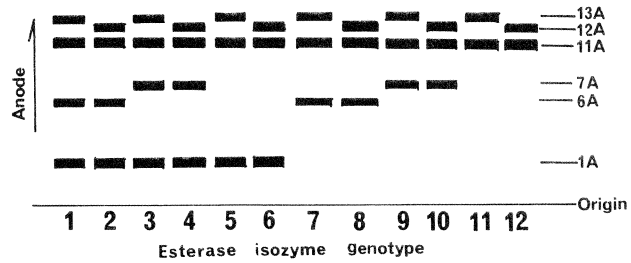

Fig. 1. Twelve zymogram genotypes comprising three esterase loci, Est_1, Est_2 and Est_3, in rice varieties.

Their zymograms are demonstrated in Fig. 1, also zymograms of 1,317 varieties were examined. The results showed that 266 varieties (20.2%) belonged to Type 1, 48 (3.6%) to Type 2, 205 (15.6%) to Type 3, 16 (1.2%) to Type 4, 81 (6.2%) to Type 5, 563 (42.7%) to Type 6, 18 (1.4%) to Type 7, 43 (3.3%) to Type 8, 5 (0.3%) to Type 9, 11 (0.8%) to Type 11, and 61 (4.6%) to Type 12. All expected genotypes except type 10 were found in the varieties tested, and no other type than these twelve was observed. These findings suggest that the esterases in rice varieties are controlled by seven alleles at three loci, as far as the five major bands are concerned.

Geographic cline in frequency of isozyme alleles

Geographic distribution for five major bands, 1A (Est_1), 6A ($Est_2{}^S$), 7A ($Est_2{}^F$), 12A ($Est_3{}^S$) and 13A ($Est_3{}^F$), was examined in 1,190 out of 1,317 varieties collected from the

Table 1. Percentage occurrence of five major esterase bands in each area of Asia.

Area	1A Est_1	6A $Est_2{}^S$	7A $Est_2{}^F$	6A+7A Est_2	12A $Est_3{}^S$	13A $Est_3{}^F$	No. of variety
A	98.2	80.4	6.1	86.5	11.7	88.3	163
B	96.9	50.9	20.8	71.7	20.8	79.2	159
C	80.3	59.0	21.3	80.3	27.9	72.1	61
D	59.5	39.2	26.6	65.8	27.8	72.2	79
E	80.6	46.8	35.5	82.3	37.1	62.9	62
F	65.1	24.5	27.4	51.9	63.2	36.8	106
G	98.7	10.4	58.3	68.7	23.8	76.2	151
H	100.0	0.0	0.0	0.0	96.6	3.4	59
I	98.9	3.1	1.1	4.2	99.4	0.6	350

known sites (Table 1). The sites were grouped into nine areas as follows: A (India, Sri Lanka), B (Nepal, Bhutan, Assam district of India), C (Thailand, Malaysia), D (Burma), E (Laos, Cambodia, Vietnam), F (Indonesia), G (southern China including Yangtze valley, Taiwan) H (northern China, Korea), and I (Japan).

The results showed that 1A was commonly noted in the varieties from southern and northernmost areas (A,B,G,H,I), but its frequency decreased in Southeast Asia (C,D,E,F). Particularly, the area D (Burma) showed a band 1A in only 59.5 percent. Band 6A ($Est_2{}^S$) dominated in area A, but gradually decreased northwards and virtually disappeared in areas H and I. Band 7A ($Est_2{}^F$) increased northwards up to areas E and G, but suddenly disappeared in farther northern areas. In other words, the null allele (Est_2O) gradually increased from southern to northern areas. Band 12A and 13A showed more remarkable cline; 12A dominated in the northern areas, whereas 13A behaved oppositely.

Center of genetic diversity

Genetic variation for each area is represented by the frequency distribution of the genotypes consisting of three loci, Est_1, *and* Est_2 and Est_3, which involved a clear geographic cline. The expected genotypes are twelve as described in the previous section. The percentage

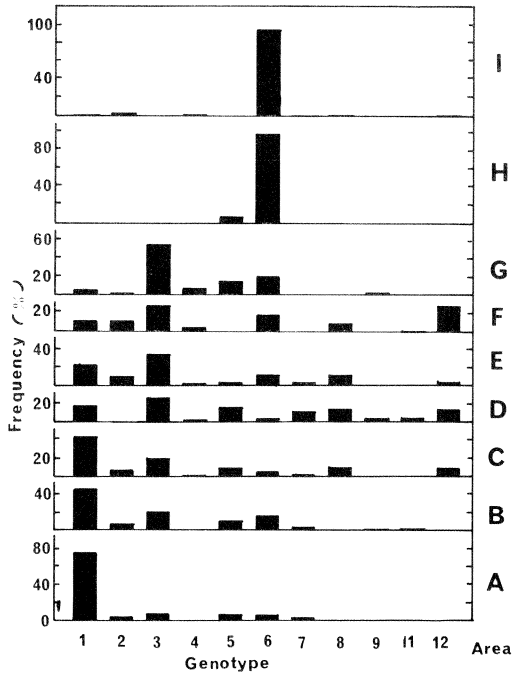

Fig. 2. Geographic cline in esterase zymograms in native rice varieties in Asia.

of occurrence of the genotypes in each area is shown in Fig. 2. The northern areas (H, I) were dominated by genotype 5, the southern areas (A, B) by genotype 1, and area G (southern China) by genotype 3. Hence, these areas involve simple variations as far as the esterase genotypes are concerned. On the contrary, areas D and E involve wide variations of genotypes without being dominated by any particular type. The area with the widest variations actually included Assam district of India, northern Burma, Laos and probably Yunnan province of China. It then follows that the center of genetic diversity for esterase isozyme genotypes exists in this area and the variations become simpler in the areas distant from the center.

On the basis of the results of F_1 semi-sterility in various crosses between varietal groups, Morinaga (1968) concluded that the Japanese rice originated from southeast Nepal. In studies using ecological traits and grain shape of old rice excavated from ruins in Southeast Asia, Watabe (1976) postulated Assam in India and Yunnan in China as being at the origin of cultivated rice. Our analysis on variations in esterase isozyme polymorphism in native rice varieties has shown that the center of genetic diversity existed in the areas including Assam in India, the northern part of Burma, Laos and Yunnan in China. These areas are very close to the area proposed by Morinaga and Watabe. Chang (1976) also recently postulated, on the basis of personal observations and other information, that the above mentioned area could provide the richest spectrum of varietal diversity.

The validity of our results in esterase isozymes has been confirmed also by our recent studies on the distribution of other polymorphic genes such as certation genes (Nakagahra 1976), phenol reaction and glutinous endosperm characters (Iizuka *et al.*). Fig. 3 shows the richest area in genetic variations of esterase genotypes in rice varieties.

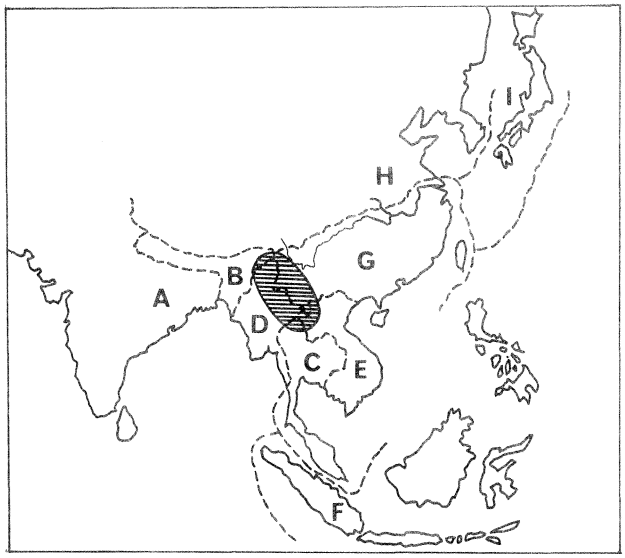

Fig. 3. Center of genetic diversity as demonstrated by esterase isozyme genotypes.
A - I show grouped areas for sampling native cultivars, respectively.

Classification of Asian cultivated rice

Classification of rice varieties has long been an important research subject, but no definite criteria have yet been established. The isozyme genotypes reported here can be an effective tool for that purpose.

As shown in Fig. 2, the esterase genotype 1 (Est_1 $Est_2{}^S$ $Est_3{}^F$; 1A-6A-13A) corresponded well to the majority of Indian varieties, Type 3 (Est_1 $Est_2{}^F$ $Est_3{}^F$; 1A-7A-13A) to that of southern China, Type 6 (Est_1 $Est_2{}^O$ $Est_3{}^S$; 1A-12A) to that of the northernmost areas. Type 8 ($Est_1{}^O$ $Est_2{}^S$ $Est_3{}^S$; 6A-12A) and Type 12 ($Est_1{}^O Est_2{}^O$ $Est_3{}^S$; 12A only) are representatives of hill and mountain rice in Southeast Asia.

Table 2 shows a new classification of rice varieties by using isozyme analysis as compared with conventional methods. The isozymic genotypes corresponded well to the varietal groups that were classified on the basis of morphological characteristics, distribution of genetic markers, photoperiodic response, F_1 pollen and seed fertility, etc. (Kato 1930, Matsuo 1952, Oka 1958, Morinaga and Kuriyama 1955, 1958, Kudo 1968, Chang 1976, Nakagahra 1977). No particular

Table 2. Classification of rice varieties based on zymogram analysis and its correspondence to other conventional classifications.

Item	Varietal group and geographical distribution				
New classification					
Isozyme genotype	1	3	6	8	12
Groupe name	Indica	Sinica	Japonica	Javanica 1	Javanica 2
Distribution	India	Southern China, Vietnam	Northern area	Hilly area in Southeast Asia	
Correspondence to conventional classification					
Kato *et al.* (1928)	Indica	Indica	Japonica	Indica or Japonica	Indica or Japonica
Matsuo (1952)	C	C	A and B	B	B
Oka (1958)	Continental	Continental	Temperate insular	Tropical insular	Tropical insular
Morinaga (1968)	*aman, aus*		*japonica*	*bulu*	*bulu*
Chang (1976)	Indica	Indica	Japonica or Sinica	Javanica	Javanica
Agroecotype	aman, aus, boro, tjereh	hsien	keng, (japonica)	bulu, hill rice	bulu, hill rice

esterase genotype was found to be dominant in the low lands of Southeast Asia. This suggests that the native varieties in these areas have not yet been differentiated in the zymogram variation and probably in many genetic characters. To confirm the present hypothesis, more studies are needed on the relationship between isozyme genotypes and conventional varietal groups.

References

1. CHANG, T. T. (1976). The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. *Euphytica* **25**, 425–441.
2. IIZUKA, K., NAKAGAHRA, M., HAYASHI, K., MIYAZAKI, S. & KAWAKAMI, J. Geographic distribution for phenol reaction genes and glutinous endosperm character in Asian cultivated rice. *Japan. J. Breed. Suppl.* **2** (in press).
3. HAYASHI, K., YAMAMOTO, T. & NAKAGAHRA, M. (1977). Genetic control for leaf photosynthesis in rice, Oryza sativa L. *Japan. J. Breed.* **27**, 49–56.
4. MORINAGA, T. (1968). Origin and geographical distribution of Japanese rice. *JARQ* **3**, 1–5.
5. NAKAGAHRA, M. (1972). Genetic mechanism of the distorted segregation of marker genes belonging to the eleventh linkage group in cultivated rice. *Japan. J. Breed.* **22**, 232–238.
6. ———— (1976). The origin of cultivated rice traced back by geographic distribution of marker genes. *Advances in Breeding* **17**, 35–44 (In Japanese).
7. ———— (1977). Genic analysis for esterase isoenzymes in rice cultivars. *Japan. J. Breed.* **27**, 141–148.
8. NAKAGAHRA, M., OMURA, T. & IWATA, N. (1972). Gametophyte genes and their loci on the eleventh linkage group of cultivated rice. *Ibid.* **22**, 305–312.

9. NAKAGAHRA, M., AKIHAMA, T. & HAYASHI, K. (1975). Genetic variation and geographic cline of esterase isozymes in native rice varieties. *Japan. J. Genet.* **50**, 373–382.
10. NAKAGAHRA, M., HAYASHI, K., YAMAMOTO, T. & OZAWA, T. (1976). A new apparatus for homogenizing plant tissues. *Japan. J. Breed.* **26**, 76–78 (In Japanese).
11. OKA, H. I. (1953). The mechanism of sterility in the intervarietal hybrid. Phylogenetic differentiation of the cultivated rice plant V. *Japan. J. Breed.* **2**, 217–224.
12. ———— Analysis of gene controlling F_1 sterility in rice by the use of isogenic lines. *Genetics* **77**, 521–534.
13 WATABE, T. (1975). Migration of Asian cultivated rice. *Dolmen* **2**, 14–27 (In Japanese).

Discussion

K. Kawano, Colombia: Your method seems to be highly relevant to field work. For instance, in what part of the world should we try to collect more genetic material to enrich our collection? Which among the wild species within the same genera should we try to collect?

Answer: Isozyme techniques can be applied to many crops and wild plants and also represent one of the most effective means to investigate genetic diversity in native cultivars and wild plant populations, particularly when there are no differences in morphological characteristics among populations.

S. Iyama, Japan: Do you have any idea as to the relation between the origin of cultivated rice and isozyme variations in wild rice?

Answer: Wild rice populations (annual and perennial AA genome) usually show wider variations in the enzyme pattern as compared with cultivars, including some identical isozyme bands between the two. However there is a great difference in zymogram patterns between AA genome rice and the other wild relatives. The next step will be to investigate the relationship between wild species with AA genome and the cultivars, as regards enzyme variations.

A. N. Lampang, Thailand: Have you done any work on other enzymes, in addition to esterase?

Answer: Yes we have. However, some of the enzymes were not stable during the growing stages while others showed great variations in their zymogram patterns. On the other hand similar results have been obtained in polymorphic marker genes, supporting our present data.

I. Tarumoto, Japan:

1. What kind of genotypes were used in your study? Were they leading varieties or wild types?

2. If your materials were leading varieties your classification could depend on the expression of genotype-environment interaction.

Answer: All our materials were native and indigenous and did not consist of improved varieties. Such studies can only be conducted by using indigenous strains whose genotypes are not modified by artificial crossing or other methods. Thus, improved varieties in southern areas, such as those developed at IRRI have new genotypes in respect to isozyme loci as a result of recombination between both parental lines.

Y. Yamada, Japan: Is it possible to use this technique for classifying environments?

Answer: Our results are based upon the combinations of three esterase loci as sole marker genes. Although your suggestion is interesting, I believe that we should be careful in using biochemical characteristics because we do not have information about their functions in plant tissue nor about the relationship existing between isozyme and morphological traits.