

Origin of Cultivated Rice as detected by Isoenzyme Variations

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Cultivated rice (*Oryza sativa* L.) distributes in worldwide tropical and temperate areas and is composed of various groups of varieties that show wide variations among them. Cross hybridization between the groups very often causes gametic selection and hybrid sterility^{1,2)}. These reproductive barriers indicate the substantial differentiation that has occurred in cultivated rice. Rice breeding in Japan has an increasing demand for introduction of exotic useful genes, since the domestic genes are limited. Examples are noted in the introduction of resistant genes to pests and diseases, and more recently a trial is being continued for high ability of leaf photosynthesis³⁾. Successful introduction of these desirable traits can be facilitated by clear understandings of affinity among the groups so that adverse effects of reproductive barriers or undesirable linkage are minimized. Where can we expect the most useful materials for future rice breeding in Japan—this is our main purpose to pursue genetic variations and origin of cultivated rice.

Approach to the origin of species could be based on Vavilov's "gene center theory"⁴⁾ that the genetic variability in populations is greatest in the place where the species arose and from which it subsequently spread elsewhere. The authors used esterase isoenzymes in rice leaves as the clue to genetic variability, since their varietal specificity in kinds and activities is maintained regardless of growth stages and environmental conditions. Enzyme extraction was done by a newly developed homogenizer⁵⁾, and electrophoresis for zymogram analysis by horizontal agar gel thin

layer method⁶⁾.

Genic analysis for esterase isoenzymes

Activities of esterase isoenzymes in 1,095 native varieties collected from various rice growing countries of the world were found in 14 all anodic bands (Fig. 1). Genic analysis for each band was investigated with F₁ and F₂ plants from crosses between various varieties. The results for five most conspicuous bands, 1A, 6A, 7A, 12A and 13A, showed that they were controlled by seven alleles at three loci⁷⁾. Activity of band 1A is controlled by a dominant allele symbolized *Est*₁, its null allele is *Est*₁⁰. Band 6A and 7A are controlled by codominant alleles at the same locus, which are symbolized *Est*₂^S and *Est*₂^F. The null allele is symbolized *Est*₂⁰. Band 12A and 13A are controlled by codominant alleles, which are symbolized *Est*₃^S and *Est*₃^F.

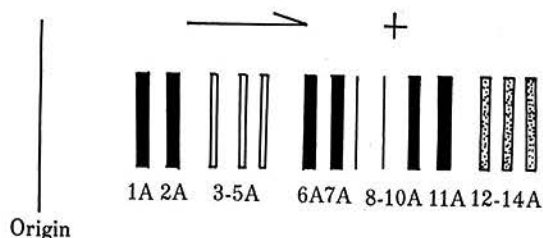


Fig. 1. Esterase isoenzymes detected in leaves of native rice varieties

Combinations of the isoenzymic alleles, $2(Est_1, Est_1^0) \times 3(Est_2^S, Est_2^F, Est_2^0) \times 2(Est_3^S, Est_3^F)$, give rise to twelve gametic genotypes as follows:

- 1: $Est_1^0 Est_2^S Est_3^F$ (1A-6A-13A)
- 2: $Est_1^0 Est_2^S Est_3^S$ (1A-6A-12A)
- 3: $Est_1^0 Est_2^F Est_3^S$ (1A-7A-13A)
- 4: $Est_1^0 Est_2^F Est_3^F$ (1A-7A-12A)
- 5: $Est_1^0 Est_2^0 Est_3^F$ (1A-13A)
- 6: $Est_1^0 Est_2^0 Est_3^S$ (1A-12A)
- 7: $Est_1^0 Est_2^S Est_3^F$ (6A-13A)
- 8: $Est_1^0 Est_2^S Est_3^S$ (6A-12A)
- 9: $Est_1^0 Est_2^F Est_3^F$ (7A-13A)
- 10: $Est_1^0 Est_2^F Est_3^S$ (7A-12A)
- 11: $Est_1^0 Est_2^0 Est_3^F$ (13A)
- 12: $Est_1^0 Est_2^0 Est_3^S$ (12A)

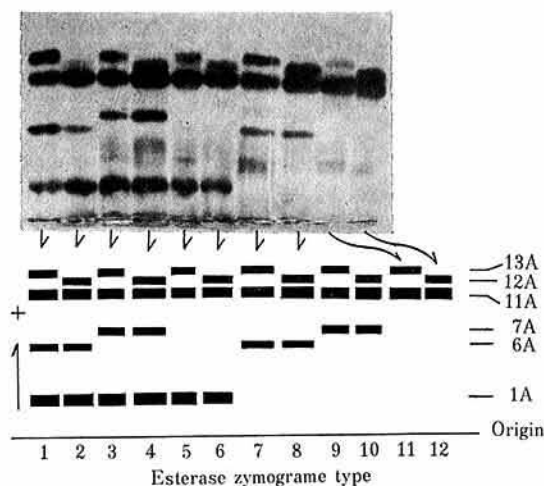


Fig. 2. Twelve zymograms comprising three esterase loci, Est_1 , Est_2 , Est_3 , in native rice varieties

Their zymograms are demonstrated in Fig. 2. Distributions of twelve genotypes were ex-

amined with 1,095 native varieties. The results showed that 217 varieties (19.8%) belonged to Type 1, 33(3.0%) to Type 2, 156(14.2%) to Type 3, 9(0.8%) to Type 4, 81(7.4%) to Type 5, 546(49.9%) to Type 6, 9(0.8%) to Type 7, 16(1.5%) to Type 8, 2(0.2%) to Type 9, 8(0.7%) to Type 11, and 18(1.6%) to Type 12. All expected genotypes except Type 10 actually existed, and no other types than these twelve were found.

Geographic cline in distribution of isoenzymes

Geographic distribution in percent occurrence 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 with 776 out of 1,095 varieties collected from the known sites (Table 1). The sites are grouped into eight areas: A (Sri Lanka), B (India, Bangladesh), C (Nepal, Bhutan, Assam), D (Burma, Thailand, Malaysia), E (Cambodia, Vietnam), F (southern China including Yangtze Valley, Taiwan), G (northern China, Korea) and H (Japan). The results showed that 1A occurred almost commonly in all areas. Band 6A dominated in area A and B, decreased northwardly and virtually disappeared in G and H. Band 7A increased northwardly upto E and F, but suddenly disappeared in further north areas. Band 12A and 13A showed more remarkable geographic cline; 12A dominated

Table 1. Geographic distribution in percent occurrence for five major bands of esterase isoenzymes in native rice varieties

Collecting area	Number of varieties	Est_1 1A	Est_2^S 6A	Est_2^F 7A	$Est_2^S + Est_2^F$ 6A + 7A	Est_3^S 12A	Est_3^F 13A	$Est_3^S + Est_3^F$ 12A + 13A
A	105	97.1	92.4	6.7	99.3	0.0	86.7	86.7
B	41	100.0	73.2	7.3	80.5	14.6	85.4	100.0
C	103	95.1	44.7	21.4	66.0	22.3	77.7	100.0
D	49	93.9	51.0	28.6	79.6	12.2	87.8	100.0
E	39	97.4	30.8	48.7	79.5	15.4	84.6	100.0
F	107	98.1	9.3	49.5	58.9	28.0	70.1	98.1
G	51	100.0	0.0	0.0	0.0	90.2	3.9	94.1
H	281	98.2	3.9	1.4	5.3	98.9	1.1	100.0

in the northern areas and gradually decreased in the southern areas, whereas 13A behaved just oppositely to 12A.

Center of genetic diversity

Genetic variation for each area was represented by frequency distribution of genotypes comprising two loci, *Est*₂ and *Est*₃, that involved the pronounced geographic cline. The expected genotypes include I: *Est*₂^S *Est*₃^S (6A-12A), II: *Est*₂^S *Est*₃^F (6A-13A), III: *Est*₂^F

*Est*₃^S (7A-12A), IV: *Est*₂^F *Est*₃^F (7A-13A), V: *Est*₂^O *Est*₃^S (12A only), and VI: *Est*₂^O *Est*₃^F (13A only).

As shown in Fig. 3 (genotype III was omitted due to its very low presence through the areas), the northern areas (G, H) were dominated by type V and the southern areas (A, B) by type II, indicating that these areas involve only simple variations. On the contrary, area C and D involved wide variations of genotypes without being dominated by any particular type. This area with wide variations

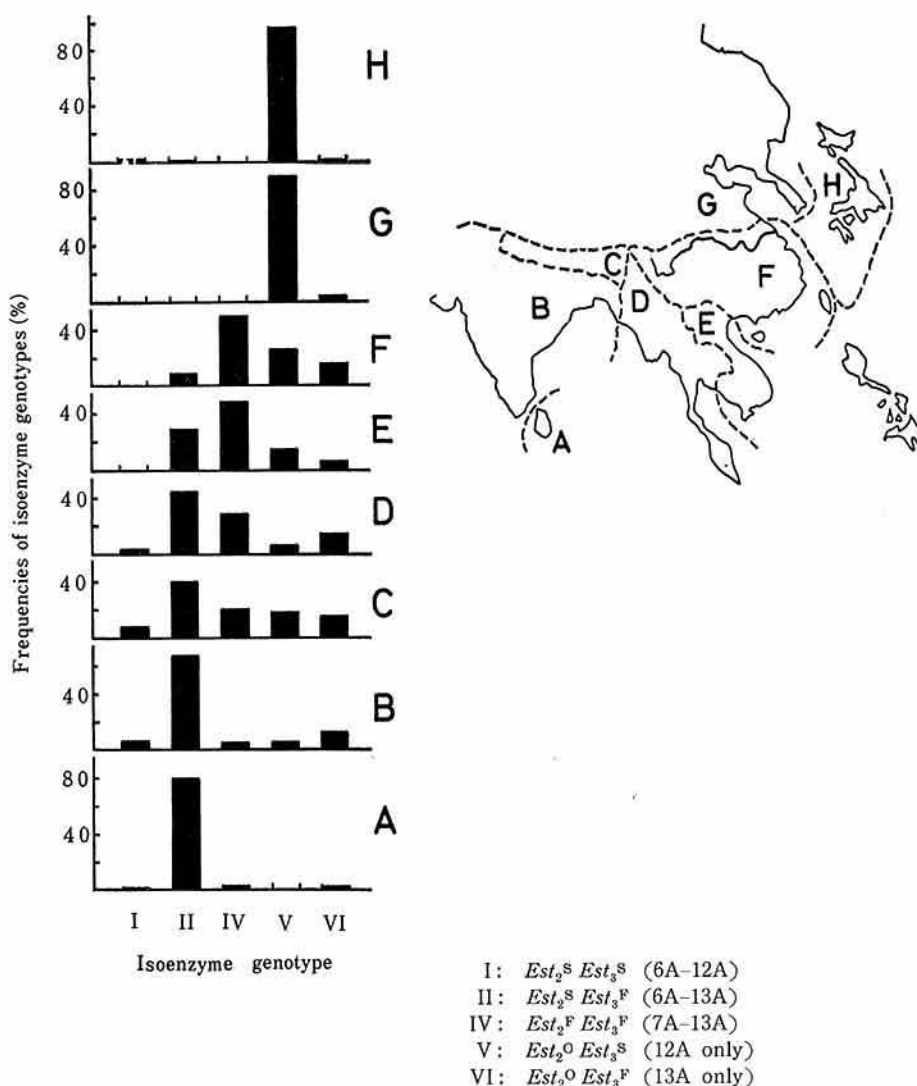


Fig. 3. Geographic cline in esterase zymograms in native rice varieties

actually includes east Nepal, Bhutan, Assam, Burma, Indo-China and Yunnan of China. It then follows that the center of genetic variations for esterase isoenzymes genes exists in this area and the variations become simpler in areas remote from the center.

Conclusion

It has hitherto generally been said that origin of cultivated rice exists in tropical lowland of India⁸⁾. From the results of F_1 fertility in crosses between varieties of various groups, Morinaga⁹⁾ concluded that origin of Japanese rice existed in southeast Nepal. In studies using morphological traits including old rice grains recovered from ruins, Watabe¹⁰⁾ postulated Assam and Yunnan areas as the origin of cultivated rice. Our analysis on variations in isoenzymic genotypes in native rice varieties has shown that the center of genetic diversity exists in the areas including east Nepal, Bhutan, Assam, Burma, Indo-China and Yunnan. These areas are very close to the areas postulated by Morinaga and Watabe. Chang¹¹⁾ also recently postulated, based on his personal observation and other information, that the same area could provide the

richest spectrum of varietal diversity. To make our postulation valid, more studies are needed on variation in other genetic characters.

Fig. 4 shows schematically the center of genetic variations of esterase isoenzymic genotypes in rice varieties and also the trend in which the variations become gradually simpler as they locate apart from the center. The arrows in the Fig. 4 are assumed to indicate also migrating route of cultivated rice. It follows that differentiation of various groups of the present varieties would be the results of adaptation or genetic drift to various areas through migration of original population.

Introduction of useful exotic genes is essential for future progress of Japanese rice. This can most effectively be done by choosing breeding materials from areas where varieties affinitive to Japanese rice but still diverse in genetic variation exist. Accordingly, the varieties growing in the areas posulated as the center of genetic diversity by the present study would be useful for future progress of rice breeding in Japan. The author's method reported here would be applicable to other crop plants and would encourage international co-operation for the exploitation of useful plant germ plasm.

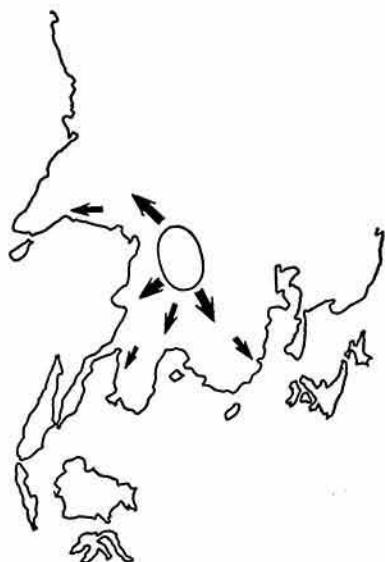


Fig. 4. Center of genetic diversity of cultivated rice

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