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Genetic Diversity of Deep Water Rice, *Oryza sativa* L., Originated in Asian Countries on the Basis of Esterase Isozyme Loci

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Esterase zymograms of deep water rice varieties which originated in seven Asian countries were examined. Nine of 12 genotypes which are expected from the combinations of the five major bands (*Est-1*, *Est-2*^{S}, *Est-2*^{F}, *Est-3*^{S}, *Est-3*^{F}) were found in the present study. The gametic genotypes, *Est-1 Est-2*^{S} *Est-3*^{F} and *Est-1*^{nul} *Est-2*^{S} *Est-3*^{F}, which corresponded well to Indica type with a broad mean, were found in every country and the frequency was ca. 50% or less except in China. The genotype *Est-1 Est-2*^{n} *Est-3*^{S} which corresponded well to Japonica type was found in Bangladesh, Burma and India in a low frequency. With the exception of Thai deep water rice, no clear association between esterase genotype and elongation ability was found.

INTRODUCTION

Asian cultivated rice, *Oryza sativa* L., was classified by Kato (1930) into two groups, Indica and Japonica types, on the basis of morphological characteristics, F_1 pollen formation and seed fertility in the crosses between the different varietal groups. At present, however, many intermediate rice varieties in between the two have been recognized (Terao and Midusima 1939, Matsuo 1952, Oka 1958, Morinaga and Kuriyama 1958, Kudo 1968, Chang 1976).

Isozymic analysis has been a useful technique to investigate the phylogenetic relationships and genetic variability of rice varieties. Zymogram analysis can give useful information in classifying rice varieties into varietal groups.

Chu (1967) and Pai et ***al.*** (1973) suggested that rice varieties could be distinguished into Indica and Japonica types by the presence or absence of band 4C in peroxidase zymograms. Also, Pai et ***al.*** (1975) distinguished Indica and Japonica types according to the migration distance of band C in acid phosphatase zymograms.

Nakagahra (1978) assumed 12 esterase genotypes from association of five major bands. According to him, isozyme genotype 1 corresponded well to the majority of Indian varieties (aman, aus and boro), and genotype 6 corresponded well to the majority of Japanese varieties and Keng rice in China.

Glaszmann (1987) surveyed 15 polymorphic loci coding 8 enzymes, and classified Asian rices into six varietal groups. He showed that two major groups, group I and group VI, corresponded well to the anterior classifications' Indica and Japonica types, respectively. He also examined 27 varieties of deep water rice in Bangladesh, and found a wide variation distributing into group I , II, III and IV.

Deep water rice in Asia is generally considered to belong to Indica type. Recently, Inouye and Hagiwara (1980) reported some exceptions that some of the deep water varieties which originated in Bangladesh and India had the zymograms specific to the Japonica type varieties in both peroxidase and acid phosphatase. Grain shape of these varieties was rounder than the others. The majority of them are listed as adapted to high flood levels (Zaman 1978).

In the present study, deep water rice varieties which originated in Asian countries were investigated and classified on the basis of esterase zymograms.

MATERIALS AND METHODS

Materials used were 736 accessions of deep water rice (*Oryza sativa* L.) grown in Asia. Of them, 485 were from Bangladesh, 16 from Burma, 3 from China, 19 from India, 42 from Kampuchea, 90 from Thailand and 81 from Vietnam.

Plants were grown in a seedling box (42 × 32 × 10 cm) filled with paddy soil containing 0.6 g each of N, P_2O_5 and K_2O. Three plants of each variety were grown, and about fifty varieties were grown in one seedling box. Fully expanded leaf blades at the 8th or 9th leaf stages were used for zymogram analysis.

The apparatus and electrophoretic procedures were almost the same as those described by Nakagahra et al. (1975). The gel medium contained 0.9% agar and 2% of polyvinylpyrrolidone in the beronal buffer solution which was adjusted to pH 6.8. The gel was dissolved by using an autoclave, and about 23 ml dissolved gel was spread in a thin layer onto a clean glass plate (17 × 24 cm).

Immediately after sampling, each leaf blade was cut into small pieces and ground in a chilled mortar. The homogenate was absorbed onto defatted cotton threads (1 cm long), which were embedded in the gel at 3 cm distance from the cathode.

Constant current of 40 mA/24 cm was applied for 70 minutes at 3°C, then the plate was sprayed with distilled water and subsequently with an acetone solution containing 1% α- and 1% β-naphthyl acetate. Following incubation for 15 minutes at 37°C, the plate was sprayed with ca. 1% solution of diazo blue B salt.

The anodal isozyme bands were numbered from origin, 1A, 2A, ……, 13A, respectively, in the same manner as described by Nakagahra (1977). Each isozyme band was identified by comparison with those of the standard rice varieties for esterase isozyme genotypes. The migration distances from origin dealt with in this paper were as follows: 1A (*Est-1*) = 2.0 cm, 6A ($Est\text{-}2^S$) = 5.5 cm, 7A ($Est\text{-}2^F$) = 6.5 cm, 12A ($Est\text{-}3^S$) = 9.5 cm and 13A ($Est\text{-}3^F$) = 10.0 cm.

Elongation ability of each deep water rice variety was determined by the position of the lowest elongated internode (LEI). One plant was grown in each 420 ml pot. When plants reached the 8th leaf stage, each potted plant was submerged in a water tank about 25 cm in depth. The water depth was increased every other day up to around the highest lamina joint in most of the plants. Position of the LEI was examined from one to ten weeks after the beginning of submergence.

Table 1. Frequencies of alleles at *Est- 1, Est-2* and *Est-* 3 in deep water rice varieties originated in seven Asian countries.

Origin	No. of var.	Frequencies (%)						
		1 A (*Est-1*, *Est-I*"")		6 A (*Est-2*^{S},	7 A *Est-2*^{F},	*Est-2*^{nul})	12A (*Est-3*^{S},	13A *Est-3*^{F})
China	3	100	0	100	0	0	0	100
Kampuchea	42	78.6	21.4	50.0	50.0	0	0	100
Thailand	90	90.0	10.0	53.3	46.7	0	0	100
Vietnam	81	64.2	35.8	51.9	43.2	4.9	0	100
Burma	16	87.5	12.5	25.0	43.8	31.2	25.0	75.0
India	19	100	0	31.6	42.1	26.3	10.5	89.5
Bangladesh	485	99.0	1.0	51.6	16.9	31.5	11.5	88.5

Table 2. Distribution of deep water rice varieties originated in seven Asian countries in esterase genotypes.

Esterase genotype (*Est-1 Est-2 Est-3*)*	Frequencies (%)						
	China	Kampuchea	Thailand	Vietnam	Burma	India	Bangladesh
1 (+ S F)	100	35.7	44.4	28.4	18.8	31.6	51.2
2 (+ s S)							0.2
3 (+ F F)		42.9	45.6	34.6	37.6	42.1	16.1
4 (+ F S)							0.2
5 (+ nul F)				1.2	6.2	15.8	20.2
6 (+ nul S)					25.0	10.5	11.1
7 (nul S F)		14.3	8.9	23.5	6.2		0.2
9 (nul F F)		7.1	1.1	8.6	6.2		0.6
11 (nul nul F)				3.7			0.2

*symbols in the table refer to alleles at the loci (superscript).

RESULTS

Frequency of alleles at three esterase loci

Frequency of alleles at *Est-1,* Est-2 and Est-3 in deep water rice varieties of seven Asian countries are shown in Table 1. Among the seven alleles, three alleles, ***Est -1,*** *Est-2*^{S} and *Est-3*^{F}, were commonly found in every country. *Est-1*^{nul} was found in Kampuchea, Thailand, Vietnam, Burma and Bangladesh, though the frequency was very low in Bangladesh. *Est-2*^{F} was found in all countries except China, showing 50% or less frequency in every country. Inactive allele of ***Est-2,*** *Est-2*^{nul}, was found in Vietnam, Burma, India and Bangladesh, though the frequency was very low in Vietnam. *Est-3*^{F} was found in all countries, while the counterpart, *Est-3*^{S}, was found in three countries, Burma, Bangladesh and India in low frequency. And Est-3"" was not found in the present investigation.

Frequency of esterase genotype

As far as the five major bands are concerned, 1'2 esterase genotypes are assumed in Asian rice varieties (Nakagahra 1977). In the present study, nine of the 12 genotypes

were found (Table 2). Among those, genotype 1 (*Est-1 Est-2*^{S} *Est-3*^{F}) was found in all countries. The frequency of genotype 1 was highest in China, followed in descending order by Bangladesh, Thailand, Kampuchea, India, Vietnam and Burma.

Genotype 3 (*Est-1 Est-2*^{F} *Est-3*^{F}) was found in six countries, all except China. The frequency of genotype 3 was similar in Kampuchea, Thailand, Vietnam, Burma and India. In Bangladesh, however, the frequency was lower than a half of other countries.

Genotype 5 (*Est-1 Est-2*^{nul} *Est-3*^{F}) was found in four countries, and the frequency was high in India and Bangladesh. Genotype 6 (*Est-1 Est-2*^{nul} *Est-3*^{S}) was found in three countries, Burma, Bangladesh and India. It's frequency was higher in Burma than in Bangladesh and India.

Genotypes 7 (*Est-1*^{nul} *Est-2*^{S} *Est-3*^{F}) and 9 (*Est-1*^{nul} *Est-2*^{F} *Est-3*^{F}) were found in five countries. And the both were almost the same in geographical distribution but the frequency of the former was higher than the latter in Kampuchea, Thailand and Vietnam.

Genotype 11 (*Est-1*^{nul} *Est-2*^{nul} *Est-3*^{F}) was found in Vietnam and Bangladesh in low frequency. Genotypes 2 (*Est-1 Est-2*^{S} *Est-3*^{S}) and 4 (*Est-1 Est-2*^{F} *Est-3*^{S}) were found only in Bangladesh, in very low frequency. Genotypes 8 (*Est-1*^{nul} *Est-2*^{S} *Est-3*^{S}), 10 (*Est-1*^{nul} *Est-2*^{F} *Est-3*^{S}) and 12 (*Est-1*^{nul} *Est-2*^{nul} *Est-3*^{S}) were not found in the present investigation in any of the countries.

Relation between esterase genotype and the LEI position

Table 3 shows relation between average value for the position of the lowest elongated internode (LEI) and esterase genotypes in deep water rice varieties in different countries. Between genotypes 1 and 3, which were commonly observed in all countries, no marked difference was observed in the LEI position except Thailand. In Thai varieties, the average value for the LEI position of genotype 1 was about three nodes lower than that of genotype 3. Among other genotypes, no systematic relation with respect to the LEI position was observed.

Among the varieties from the seven different countries, the average value for the

Table 3. Relation between the esterase genotype and elongation ability (average value of LEI).

Esterase genotype	Average value of the LEI position						
	China	Kampuchea	Thailand	Vietnam	Burma	India	Bangladesh
1	11.0	11.2	9.7	10.3	13.0	9.0	9.5
2							14.0
3		11.4	13.2	11.4	11.0	10.2	9.6
4							15.0
5				9.0	14.0	8.3	8.7
6					15.5	7.0	8.5
7		8.3	9.6	10.4	17.0		7.0
9		13.5	11.0	10.5			7.0
11				9.5			7.0
Total	11.0	11.0	11.3	10.6	13.8	9.0	9.2

position of the LEI was smaller in Bangladesh and Indian varieties, and larger in Burmese varieties. Especially, of varieties with genotype 6 (Es-l *Est-2*^{nul} *Est-3*^{S}), the LEI position was much higher in Burmese varieties than in Bangladesh and Indian varieties.

DISCUSSION

Presence or absence of five major bands (1A, 6A, 7A, 12A and 13A) are specified by seven alleles at three loci, *Est-1, Est-2* and Est-3, in native rice varieties in Asia. There were no significant linkage relationships among the three loci (Nakagahra 1977).

In the present study, the number of deep water rice varieties used from China, India and Burma was not sufficient to realize the regional features compared with the other four countries. Within the scope of this examination, varieties from Bangladesh, India and Burma resembled each other, and Thai varieties resembled those from Kampuchea in the frequencies of the alleles at three loci.

Using 1,317 native rice varieties collected from various countries in Asia, Nakagahra (1978) examined the geographic distribution of the five major bands. In his examination, the materials included all kinds of rices, then the results of them seemed to represent ordinary rice varieties. Therefore, comparing the present results with those of Nakagahra, some differences between deep water rice and ordinary rice varieties may be observed.

A clear difference between deep water rice and ordinary rice was observed in the frequency of *Est-3*^{S} in China, Thailand, Vietnam and Kampuchea, i. e. the frequency of *Est-3*^{S} was nil in deep water rice but 24-37% in ordinary rice. A difference was also observed in the frequency of *Est-2*^{S} in China and India. *Est-2*^{S} was found in all three varieties of Chinese deep water rice, while it was about 10% in ordinary rice from the same country. And, in India, the frequency of *Est-2*^{S} was about 80% and that of *Est-2*^{F} was only about 6% in ordinary rice, while both of these alleles showed similar frequencies in deep water rice.

Nakagahra (1978) suggested that the isozymic genotypes corresponded well to the varietal groups that were classified on the basis of hybrid sterility and character variations (Kato 1930, Matsuo 1952, Oka 1958, Morinaga and Kuriyama 1958, Kudo 1968, Chang 1976). According to him, esterase genotype 1 (*Est-1 Est-2*^{S} *Est-3*^{F}) corresponded well to the Indica (Indian Indica), genotype 3 (*Est-1 Est-2*^{F} *Est-3*^{F}) to Sinica, Chinese Indica (Hsien), genotype 6 (*Est-1 Est-2*^{nul} *Est-3*^{S}) to the Japonica, and genotypes 8 (*Est-1*^{nul} *Est-2*^{F} *Est-3*^{S}) and 12 (*Est-1*^{nul} *Est-2*^{nul} *Est-3*^{S}) to Javanica (Hill rices).

Frequency distribution of the genotypes was grater in ordinary rice (Nakagahra 1984) than in deep water rice (Table 2). Especially, genotypes 2, 8 and 12 were often found in the former but scarcely found in the latter. Comparing the frequency of each genotype in deep water rice and ordinary rice, genotype 1, 3 and 7 were equally found in Vietnamese deep water rice, while genotype 3 was dominant in ordinary rice in the same country. In other countries, no obvious difference was observed in the frequency of any genotype. Especially, the frequency pattern of the genotypes in Bangladesh deep water rice was similar to that of ordinary rice from Assam district of India.

Endo and Morishima (1983) reported that Indica type varieties frequently showed

Est-2^{S} and *Est-3*^{F}, and Japonica type varieties *Est-2*^{nul} and *Est-3*^{S}. The former corresponds to Nakagahra's isozyme genotypes 1 (*Est-1 Est-2*^{S} *Est-3*^{F}) and 7 (*Est-1*^{nul} *Est-2*^{S} *Est-3*^{F}), and the latter to genotypes 6 (*Est-1 Est-2*^{nul} *Est-3*^{S}) and 12 (*Est-1*^{nul} *Est-2*^{nul} *Est-3*^{S}).

According to the division of Endo and Morishima (1983), in the present study, the frequency of Indica type varieties was 100% in China, and about 50% in Kampuchea, Thailand, Vietnam and Bangladesh, while it was less than 32% in Burma and India. On the other hand, frequency of Japonica type varieties found in Burma, India and Bangladesh was ca. 25%, 11% and 11%, respectively.

It is known that Japonica type varieties are frequently found in the hilly areas of southeast Asian countries (Morishima *et al.* 1980). Recently, Sato *et al.* (1986) suggested that Japonica type was adapted to upland conditions. Though it is uncertain whether the Japonica type mentioned in their studies are similar with those obtained in the present study from the viewpoint of isozyme analysis or not, it could be interesting that Japonica type was also found among the deep water rice varieties.

Concerning the relation between isozyme genotype and elongation ability, Inouye and Hagiwara (1980) reported that most varieties adapted to high flood levels in Bangladesh had the Japonica type zymograms in both acid phosphatase and peroxidase (*Acp-1*^{a}, *Pox-2*^{nul}). In some of those Japonica type varieties, *Est-2*^{nul} was detected (Morishima, personal communication). In our examination, too, most of the Bangladesh deep water rice varieties with *Acp-1*^{a} and *Pox-2*^{nul} had *Est-2*^{nul} and *Est-3*^{S}, and also had good elongation ability (unpublished).

It has been reported that most varieties of Thai deep water rice with good elongation ability belonged to Nakagahra's genotype 1 and those with less elongation ability belonged to genotype 3 (Inouye *et al.* 1984). This fact was reaffirmed in the present examination. With the exception of these results, no systematic relation between esterase genotype and elongation ability was found.

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