COMPARISON OF VARIATIONS IN PEROXIDASE ISOZYMES BETWEEN *PERENNIS-SATIVA* AND *BREVILIGULATA-GLABERRIMA* SERIES OF *ORYZA*[1]

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(Received July 10 1967)

A survey of variations in peroxidase isozymes within and between populations of *Oryza perennis* Moench and *O. sativa* L. was reported by the senior writer in a previous paper (Chu 1967). He pointed out that the leaf blade as well as the leaf sheath, a few days after attaining the final size, give a high repeatability in the zymogram, and therefore are suitable for the observation of differences between plants. Populations of perennial habit from India (*O. perennis* subsp. *balunga* or *perennis* type) and from Africa (subsp. *barthii*) were appreciably polymorphic, while those of annual habit appeared to be homogeneous. More than ten different zymograms of leaf blade were found among *O. perennis* strains of different origin. Of six different zymograms found among the strains of tropical Asia, two, peculiar to the Asian strain-group, were found to characterize *sativa* varieties.

For a comparison with these, we have observed peroxidase-isozyme variations among strains of another cultivated rice species, *O. glaberrima* Steud., and its wild progenitor, *O. breviligulata* Chev. et Roehr, both endemic to West Africa. The present paper deals with a comparison between two parallel series of evolution of cultivated rice, from *perennis* to *sativa* and from *breviligulata* to *glaberrima*. Also, F_1 hybrids and back-cross progenies between *sativa* and *glaberrima* strains were observed. The writers are indebted to Dr. T. Endo of the National Institute of Genetics, Japan, for his technical advices.

Materials and Methods

Materials used are in total 28 *breviligulata* (including 13 semi-wild forms) and 27 *glaberrima* strains. They were mostly collected by Oka and Chang

(1) Contribution from National Institute of Genetics, Japan, No. 649
The writers wish to express their sincere thanks to The International Rice Research Institute for the generous financial support.
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(1964) from West African countries (Guinee to Tchad), and are preserved in the National Institute of Genetics, Japan. Each strain was represented by three plants raised from the seeds of the original population. For crossing experiments, a *sativa* strain, Pei-ku (Acc. no. 108, Indica) and a *glaberrima* strain, W025, were used as the parents. The plants were grown in concrete beds with automatic short-day equipments. Leaf blades, 3 to 5 days after reaching the final size, were sampled from the plants in the vegetative stage in July. Each plant was observed twice on different dates.

The procedures of electrophoresis were the same as those described by Chu (1967). A piece of a leaf blade was ground in a mortar, and a filter paper strip (Toyo no. 50, 6 mm × 18 mm), absorbing the homogenate, was inserted in starch gel blocks which were prepared in 0.03 M borate buffer at pH 8.5 (set in plastic molds, 20 cm × 20 mm × 6 mm in internal dimension), at 8 cm distance from the cathode. After a constant current of 10 V/cm (ca. 1.7 mA) was applied for 3 hours at 10°C, the gel blocks, sliced into two halves, were stained with the reacting mixture containing 0.03% hydrogen peroxide, 0.1% benzidine acetate and 0.01 M Tris-acetic acid buffer adjusted at pH 4.0 (cf. Endo 1966).

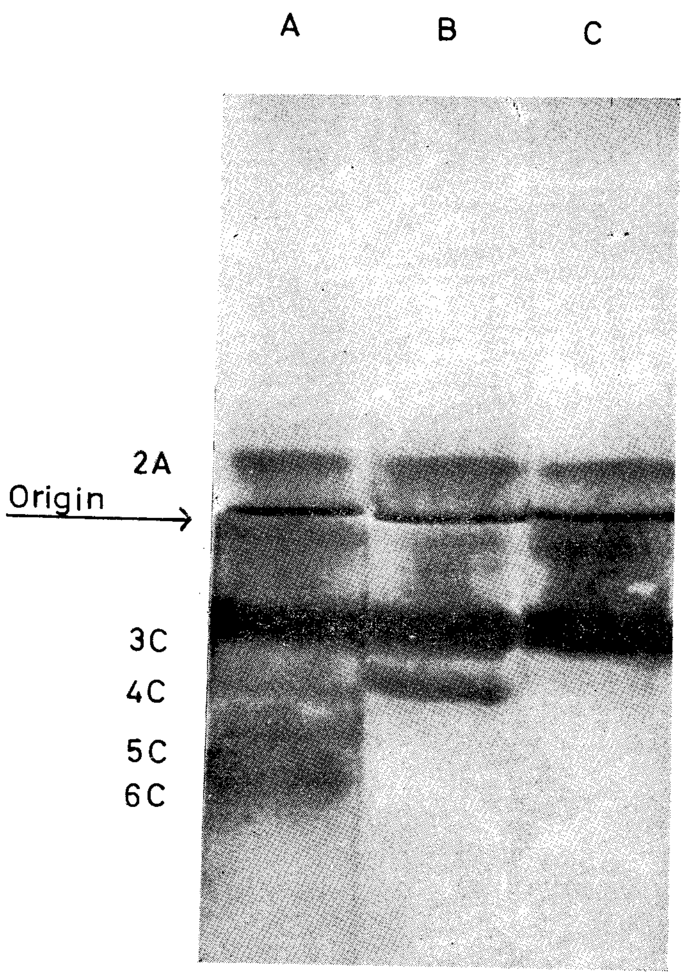
Results

1. *Zymogram variation among strains of* **O. glaberrima** *and* **O. breviligulata**

In each strain, three plants derived from the original population were compared. No zymogram differences between plants were found, except for two *glaberrima* and one *breviligulata* strains. This indicates that the populations would be generally homogeneous, in contrast to the polymorphic populations of *O. perennis*. In these species, variability in peroxidase isozymes might be for a greater part maintained as between-populational variations.

The isozyme bands of leaf blade found among the observed plants were, according to the senior writer's designation, 2A, 5A, 6A (running toward the anode), 1C, 2C, 3C, 4C, 5C and 6C (running toward the cathode). Of them, 2A, 5A, 6A, 3C and 4C invariably occurred in all strains (with one exception given below), and 1C and 2C were faint and indistinct; they were excluded from strain-comparison. The bands whose presence or absence were used for comparing the strains were 5C and 6C. The occurrence of 6C, absent in *perennis-sativa* series, characterized this plant group. (Fig. 1)

The variations found among the tested strains are given in Table 1. As the table shows, three different zymogram types were found, namely, 4C, 4C–6C, and 4C–5C–6C. 4C–5C was not found. Among them, 4C occurred only in four *glaberrima* strains (SL22, GN-4-9, ML32 and CA6), which were collected from Sierra Leone, Guinee, Mali and northern Cameroon, respectively. All



A : *O. glaberrima* (W025)
B : *O. sativa,* Indica (108)
C : *O. sativa,* Japonica (521)

Fig. 1. Comparison of the peroxidase zymogram of leaf blade between *sativa* and *glaberrima* strains.

other *glaberrima* strains showed 4C–5C–6C. Exceptionally, a *glaberrima* strain of floating habit from Tombouctou, Mali (ML32) gave the zymogram 4A–4C, band 2A being absent. As shown by Chu (1967), the presence of 4A and absence of 2A characterize *O. perennis* subsp. *barthii.* In the deep-water paddy where this *glaberrima* strain was grown, *barthii* plants were growing as a weed. It may be suspected that the *glaberrima* population might have had some introgression of genes from the *barthii* plants.

Strains of *O. breviligulata* showed either 4C–6C or 4C–5C–6C. So far as peroxidase isozymes of the leaf blade are concerned, *O. breviligulata* seems to be much less variable than *O. perennis.*

Table 1. *Peroxidase-zymogram variations among strains from different countries of* ***O. glaberrima*** *and* ***O. breviligulata***

Country	*glaberrima*			semi-wild			*breviligulata*		
	4C	4C-6C	4C-5C-6C	4C	4C-6C	4C-5C-6C	4C	4C-6C	4C-5C-6C
Gambia			2					1	
Guinee	1		5			1			
Sierra Leone	1		5		1			2	
Mali	1*		7			7		4	4
Niger			2					1	
Nigeria			3			1			
Cameroon	1		1			4			
Tchad								1	1
Total no. of strains	4		25		1	13		9	5

Classification into *glaberrima*, semi-wild and *breviligulata* groups was made by the discriminant scores shown in Page 5.
* This strain (ML32) showed zymogram 4A-4C.

2. Comparison between ***perennis-sativa*** *and* ***glaberrima-breviligulata*** *series*

O. perennis is distributed throughout tropical countries of the world and comprises many different forms which are largely classified into Asian, Oceanean, American and African (*barthii*) groups. Among 111 strains of this species so far observed, eleven zymogram types in total were found for leaf blade, by which the geographical groups could be to some extent distinguished (Chu 1967). The perennial (*perennis* or *balunga*) and annual (*spontanea* or *fatua*) types, into which the Asian forms tend to differentiate, showed no particular difference in zymogram. Some of the Asian perennial forms might be the progenitor of *O. sativa*, and the Indica-Japonica differentiation might have proceeded as the plants approached cultivated forms (Oka and Chang 1962; Oka 1964). The pattern of variations in peroxidase zymogram found among the Asian forms of *O. perennis* and *sativa* varieties is diagrammatically shown in Fig. 2. As the figure shows, the Asian *perennis* strains had six different zymogram types, and two of them, which were peculiar to the Asian group (not occurring in other geographical groups), characterized *sativa* varieties and intermediate wild-cultivated forms from the Jeypore Tract, India. Most Indica strains showed zymogram 2A-4C, while most Japonica strains had 2A.

Fig. 2 shows further that in the *perennis-sativa* series, the variation in peroxidase isozymes is reduced as the plants approach cultivated forms. It may be suggested that wild plants with certain peroxidase isozymes are the progenitors of cultivated forms so far as the genes controlling the isozymes are concerned.

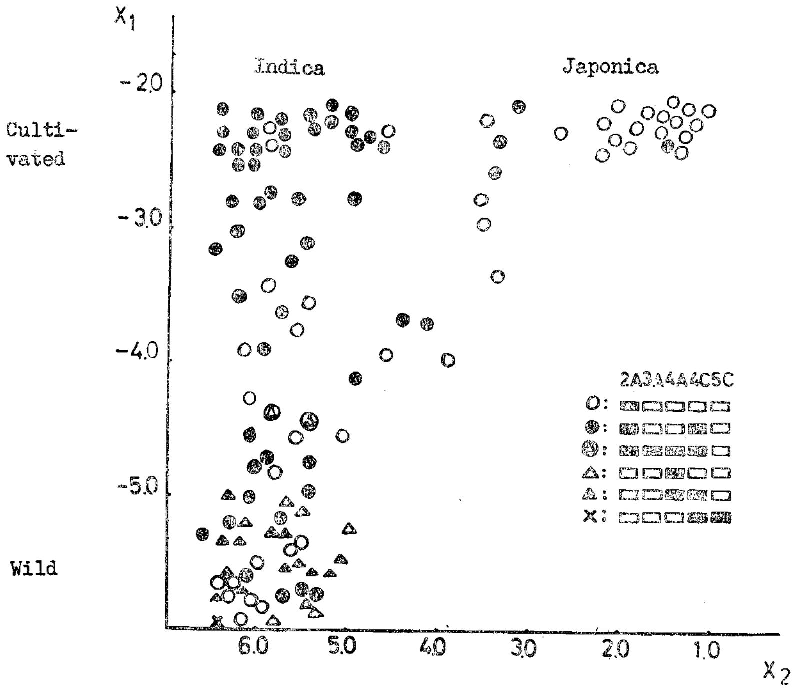


Fig. 2. Peroxidase zymograms of *perennis-sativa* strains scattered according to the scores given by two discriminant formulas, one (ordinate; X_1) for classifying wild and cultivated forms, and the other (abscissa; X_2) for Indica and Japonica types. (cf. Oka and Chang 1962)

A similar diagrammatic comparison of wild and cultivated forms in the *breviligulata-glaberrima* series is shown in Fig. 3. The strains vary between wild (*breviligulata*) and cultivated (*glaberrima*) forms showing many intermediates, which were mostly growing wild in habitats disturbed by man. This variation was measured by a discriminant formula combining an index for awn development (A), anther length (B), percentage of grain shedding (C), and an index for seed dormancy (D), as $A + 0.45B + C + 0.18D$ (cf. Oka and Morishima 1967). In this plant group, such a differentiation trend as the Indica-Japonica differentiation of *sativa* varieties is not found (Morishima *et al.* 1962). A remarkable variation trend in this plant group might be that some of them are of deep water habit, while others are not. Though the floating abilities of the strains were not measured, our data (unpublished) suggest that the number of elongated internodes (longer than 1 cm) could be taken as an estimate of deep-water habit. The strains were then scattered, in Fig. 3, according to the discriminant score classifying wild and cultivated forms (ordinate) and the number of elongated internodes (abscissa).

The figure shows that the strains varied in a wide range as to deep-water habit. Of the two zymogram types found among *breviligulata* strains, one,

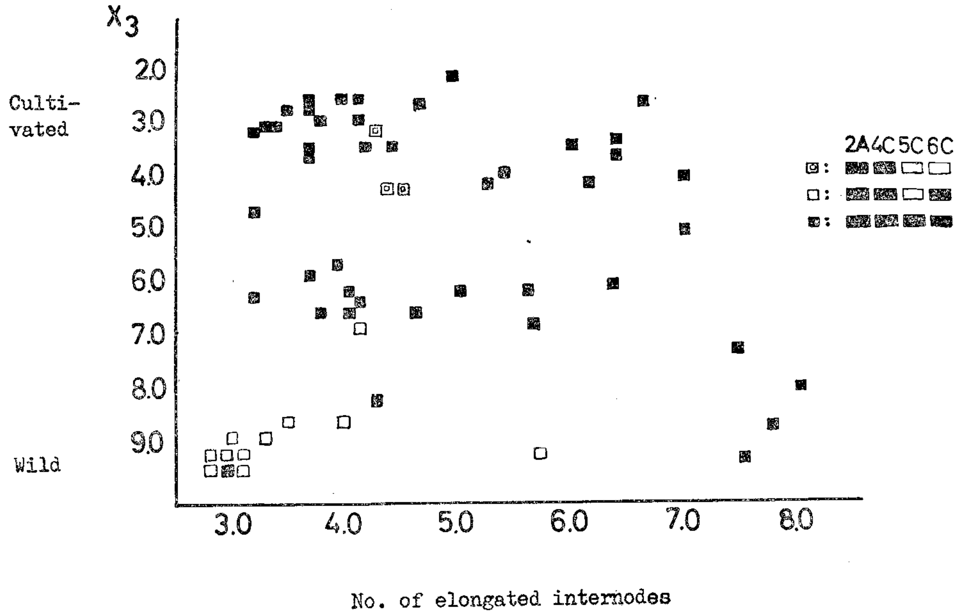


Fig. 3. Peroxidase zymograms of *breviligulata-glaberrima* stirans scattered by the score of a discriminant formula classifying wild and cultivated forms (ordinate), and by the number of elongated internodes.

4C–5C, was limited to strains with a few elongated internodes, possibly not adapted to deep-water habitats, and the other, 4C–5C–6C, was distributed among strains with many elongated internodes and also in intermediate wild-cultivated strains. The latter zymogram is also commonly found in cultivated varieties of *O. glaberrima*. Thus, *breviligulata* has two peroxidase zymogram types one of which characterizes *glaberrima*. So far as these isozyme variations are concerned, it may be assumed that deep-water forms of *breviligulata* with 4C–5C–6C might be the progenitor of cultivated forms, though a few *glaberrima* varieties with 4C only could have descended from *breviligulata* strains with 4C–6C.

3. *Hybrids between **O. sativa** and **O. glaberrima***

To estimate whether or not, the isozyme bands occurring in the two species series are controlled by the same set of genes F_1 hybrids between a *sativa* and a *glaberrima* strain, and their B_1 and B_2 progenies, were observed regarding leaf-blade zymograms. The results are given in Table 2. The F_1 hybrids were highly pollen sterile, but had about 35% normal embryosacs so that back-crosses could be made. The *sativa* parent had zymogram 2A–4C, while the *glaberrima* parent had zymogram 2A–4C–5C–6C. All F_1 plants showed 2A–4C–5C–6C, and no new bands.

The B_1 plants of *sativa* × *glaberrima* × *sativa* (s-g-s) and *glaberrima* × *sativa* × *sativa* (g-s-s) segregated into 4C, 4C–6C and 4C–5C–6C, all equally having

Table 2. *Peroxidase zymograms of F_1 hybride between **O. sativa** and **O. glaberrima** and their back-cross progenies*

Generation	Cross combination	Leaf blade zymogram		
		4C	4C-6C	4C-5C-6C
P_1	*sativa*(108)	5		
P_2	*glaberrima*(W025)			5
F_1	s-g			4
F_1	g-s			4
B_1	{s-g-s {g-s-s	1(a)	3(b, c, d)	1(e)
B_2	{s-g-s-s {g-s-s-s	{3(b), {2(e), {6(a, c)	1(b)	2(e)
B_2	{s-g-s-g {g-s-s-g			15(a, b, c, e)
B_1	g-s-g			4(f, g, h, i)
B_2	g-s-g-s	2(i)	{2(g), {2(i)	{2(g), {8(f, h)
B_2	g-s-g-g			17(f, g, h, i)

s-g shows *sativa* × *glaberrima*. The rest to follow this example.
a, b, c,...show individual B_1 plants and their B_2 progenies.

2A. No segregants showed 4C–5C, in the same manner as it was not found among various *glaberrima* and *breviligulata* strains. When a B_1 plant showing 4C–6C was back-crossed with the *sativa* parent (4C), the B_2 progeny segregated into 4C and 4C–6C plants. Also, back-crossing of another B_1 plant with the *sativa* parent produced B_2 plants showing 4C and 4C–5C–6C. All back-crosses with the *glaberrima* parent gave 4C–5C–6C plants.

These experimental results suggest that the genes controlling bands 5C and 6C, carried by the *glaberrima* parent, are non-allelic and dominant. Possibly, the 5C gene works only when the 6C gene is present. The *sativa* and *glaberrima* parents may have the same genes for bands 2A and 4C. Inter-crossing experiments between back-cross segregants may demonstrate allelic relations of genes more clearly.

Discussion

The two parallel evolutionary series, *perennis-sativa* and *breviligulata-glaberrima*, were compared by Morishima *et al.* (1963) regarding the mode of evolution of cultivated forms. The similarities found between the two series were: 1) In both series, the cultivated and the wild species could be distinguished by similar character differences. 2) In both series, the wild species showed in their populations a latent tendency to vary toward cultivated forms. 3) Examining materials collected from certain regions, in both series was found a continuous array of intergrades between wild and cultivated forms, which may be indicative of the evolutionary pathways.

Major differences found between the two series were: 1) *O. perennis* is distributed world-widely comprising various geographical forms, and the Asian perennial forms may be the progenitor of *O. sativa*, while *O. breviligulata* and *O. glaberrima* are endemic to West Africa. *O. sativa* might have originated in tropical Asia, and *O. glaberrima* in Africa independently from the former. 2) The Asian forms of *O. perennis* vary in such a way that both extremes are represented by perennial and annual types. *O. breviligulata* and *O. glaberrima* are typically annual grasses. 3) Populations of *O. perennis* were found to contain more genetic variability than those of *O. breviligulata*. Variations in the latter species appeared for a greater part as differences among populations. 4) Varieties of *O. sativa* are divided into two major groups, Indica and Japonica types. Such varietal differentiation is not found in *O. glaberrima*. 5) In *O. sativa*, F_1 hybrids between distantly related varieties show different degrees of sterility, though their hybrids with their putative progenitors, Asian forms of *O. perennis*, are generally fertile. F_1 hybrids between strains of *O. glaberrima* and *O. breviligulata* are generally fertile, but some hybrid combinations show F_1 weakness.

It may be suggested that a certain difference in the genetic system of the ancestral wild species gives rise to different modes of evolution of cultivated forms.

We observed in the present study that *breviligulata-glaberrima* series had less variability in peroxidase isozymes of leaf blade than *perennis-sativa* series. The same tendency was also found in some other characters (Morishima *et al.* 1963). This may be at least partly attributed to the annual habit of the former series. Their small within-populational variability may also be attributed to their low outcrossing rate (Oka 1964). The evolutionary potentiality of a species may be a function of genetic variability accumulated by the species. Hinata and Oka (1962) postulated that Asian *perennis* populations would have a high enough capacity for storing up genetic variations so that they could evolve *O. sativa*. However, carrying a smaller amount of genetic variations, *O. breviligulata* gave rise to *O. glaberrima*. Presumably, there might be some additional genetic adjustment that enables a wild species to respond to cultivation by man.

In general, the occurrence of an isozyme band is controlled by a gene (Kikkawa 1964; Schwartz and Endo 1966, etc.), though its intensity may be polygenic (McCune 1961). Some isozyme genes are known to be allelic (Schwartz 1960; Yoshitake and Akiyama 1960), but others are non-allelic (Yoshitake 1963; Lewontin and Hubby 1966). An isozyme is thought to perform a particular physiological action at a particular phase of development (Beckman and Johnson 1964; Endo 1966). Naturally, different species may have different isozymes of an enzyme. As to this point, our experimental results seem to indicate that *perennis-sativa* and *breviligulata-glaberrima* series might have basically the same peroxidase-isozyme genes. Not only the running distances of certain bands were the same, but also the hybrids showed no new bands lacking in the parents. The allelic relations of genes controlling the isozyme bands are under observation.

Summary

The two cultivated rice species, *O. sativa* and *O. glaberrima*, are thought to have independently evolved from Asian forms of *O. perennis* and African *O. breviligulata*, respectively. The isozymatic variations in leaf-blade peroxidase were investigated in strains of *O. breviligulata* and *O. glaberrima*, and were compared with those previously observed in *O. perennis* and *O. sativa*. The *breviligulata* and *glaberrima* strains showed only three zymogram types, and appeared to be much less variable than the *perennis-sativa* series that showed eleven different zymogram types. Also the within-populational variability in isozymes was quite small in *O. breviligulata*, in contrast to the polymorphic populations of *O. perennis*. The evolutionary paths as shown by the zymogram variations were discussed. Observations of *sativa-glaberrima* F_1 hybrids and back-cross progenies suggested that the two species might have basically the same genes for peroxidase isozymes, showing differences in the presence or absence of certain bands.

Peroxidase isozyme 在稻屬 *perennis-sativa* 和 *breviligulata-glaberrima* 之比較

朱 耀 源　　岡 彥 一

歷來 *Oryza sativa* 和 *O. glaberrima* 被認爲各別由 *O. perennis* 和 *O. breviligulata* 進化而來。在這個實驗裏，我們觀察 *glaberrima* 和 *breviligulata* 各系統葉身所含的 peroxidase isozyme 之變異性，同時與以前所觀察的 *perennis* 與 *sativa* 的資料作一個比較。在 *perennis-sativa* series 裏發現有11種不同的 isozyme 型，但在 *breviligulata-glaberrima* series 裏只能發現有 3 種的型式。而且集團內的變異性也較之 *perennis* 要少得多。由此種結果可能想像到兩種進化的型式是各有特徵。再則從分析 *sativa* × *glaberrima* 雜交和回交後代的結果，得知 *sativa* 和 *glaberrima* 由特定 isozyme band 之有無而能區別。因此兩種之間的 peroxidase isozyme 可說具有相同的遺傳基礎。

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