ANALYSIS OF GENES CONTROLLING PEROXIDASE ISOZYMES IN *ORYZA SATIVA* AND *O. PERENNIS*¹⁾

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Isozyme variation has been observed for various enzymes of higher organisms and genetic analysis has been made, as reviewed by Whitt (1967), Shannon (1968), Latner and Skillen (1968), and by Shaw (1969). Variants so far examined were in most cases considered to be specified by "structural" genes, which were either allelic or non-allelic. The "null form" or "silent enzyme", lacking activity, was found to be controlled by a different gene behaving as a recessive allele (*e.g.*, Yoshitake and Akiyama 1964 in acid phosphatase of silk worm; Endo and Schwartz 1966 in catechol oxidase of maize; Johnson 1966 in alkaline phosphatase of *Drosophila melanogaster*; Endo 1967 in peroxidase of *Pharbitis nil*; Ohba and Sasaki 1968 in esterase of *Drosophila virilis*; etc.).

Enzyme synthesis in plants may presumably be controlled by a system of structural and regulatory genes, in the same manner as in microorganisms. However, no positive evidence for regulatory genes seems to have been presented. To investigate the genetic control of peroxidase variations, we have observed the zymograms of hybrids between strains of *Oryza sativa* L. and its wild progenitor, the Asian form of *O. perennis* Moench. The results suggested the presence of a regulatory gene, in addition to allelic and non-allelic genes specifying the isozyme bands.

MATERIALS AND METHODS

Four *sativa* and four Asian *perennis* strains (Table 1) were chosen for crosses from the material preserved in the National Institute of Genetics, Japan. The *sativa* strains were pure lines. A *perennis* strain, W107, was also a pure line obtained from a haploid plant by chromosome doubling. The other *perennis* strains were selfed progenies of plants raised from seeds collected in natural populations. Six crosses were made, and the parental, F₁ and F₂ plants were observed (Tables 2 and 3). In one cross-combination, B₁ plants were also observed. Further, diallel crosses were made of the plants randomly chosen from a population of *perennis* (W120, perennial type), which was raised from seeds collected in a pond at Cuttack, India. The F₁ plants and selfed progenies of the parents were observed in 1967, and the F₂ and corresponding selfed generations of the

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parents in 1968.

Seedlings raised in a greenhouse were transplanted in middle May into concrete beds with automatic short-day control (12-13 hrs.), dressed with 6-6-6 NPK (g/m^2). Before or at the heading time, the third leaf blades from the top were sampled to make the homogenates. Peroxidase isozymes were observed by starch-gel electrophoresis method described by Endo (1966) or Chu (1967). The isozyme bands migrating towards the anode and cathode were numbered 1A, 2A, ... and 1C, 2C, ..., respectively. Bands 1A, 1C and 2C were excluded from the records as they were often indistinct.

Fig. 1. Photographs showing peroxidase bands 4C, 2A, 3A and 4A.

RESULTS

1. *Crosses between sativa and perennis strains*

In *O. sativa*, the majority of Indica varieties show band 4C which is absent or very weak in most Japonica varieties (Chu 1967). Asian strains of *O. perennis* varied in the presence or absence of bands 4C, 2A, 3A and 4A (Fig. 1). The zymograms of the parental strains used for crosses are shown in Table 1.

Table 1. Strains used for crosses and their zymograms

Acc. no.	Species	Type	Origin	Zymogram
108	<i>sativa</i>	Indica	Taiwan	4C 2A —
T.65	"	Japonica	"	—* 2A —
219	"	"	Philippines	4C 2A —
325	"	"	Indonesia	— 2A —
W106	<i>perennis</i>	Annual	India	— 2A —
W107	"	"	"	— 2A —
W124	"	Interm. ¹⁾	"	4C 2A —
W1294	"	Perennial	Philippines	4C — 4A
W120 ²⁾	"	"	India	4 types (Table 4)

* Activity is very weak.

1) Intermediate annual-perennial type

2) 10 plants randomly sampled from a population

The data for hybrid plants are presented in Tables 2 and 3. The occurrence of band 4C in the F_1 plants indicates that the gene specifying it is active. Deviations from 3:1 ratios were found; they were generally large in Indica-Japonica crosses due to an excess of plants having 4C (Table 2). The B_1 ratio in $(108 \times T.65) \times T.65$ also showed deviation from a 1:1 ratio in the same manner. But, *sativa-perennis* crosses showed much smaller deviations. The F_1 pollen fertility of $108 \times T.65$ was 49% and that of $219 \times T.65$ was 72%, while other crosses showed high F_1 pollen fertilities. The significant deviation in $108 \times T.65$ may be due to gametic selection caused by F_1 sterility, as shown by Oka (1957; 1964) for various monogenic characters.

The 4C band in these crosses thus seems to be specified by an active structural gene. It is denoted by Pe^{4C} and its recessive allele by pe^{4C-} .

A *perennis* strain, W1294, had band 4A but no 2A band, while another *perennis* strain, W107, had 2A but no 4A. Their F_1 plants showed three bands, 2A, 3A and 4A (Fig. 1). The F_2 segregated into 2A, 2A-3A-4A and 4A plants in a 1:2:1 ratio (Table 3). This indicates that the 2A and 4A bands are specified by allelic genes Pe^{2A} and Pe^{4A} , respectively, and the two alleles produce a hybrid dimer band, 3A, in their heterozygotes.

The F_2 between 108 (*sativa*) and W124 (*perennis*), which had the same zymogram (4C-2A), showed no segregation among their F_2 plants, indicating that peroxidase isozymes in *sativa* and *perennis* may be controlled by the same genic system. The two loci, Pe^{4C}/pe^{4C-} and Pe^{2A}/Pe^{4A} , were found to be independent of each other.

Table 2. Segregation for peroxidase band 4C in *sativa* and *perennis* crosses

Plant	Observed no. 4C		Exp. ratio	Chi-square
<i>sativa</i> × <i>sativa</i>				
108	3			
T .65		3		
F ₁ 108×T .65	3			
F ₂	30	1	3 : 1	7.8**
B ₁ (F ₁ ×T .65)	20	6	1 : 1	7.5**
219	3			
F ₁ 219×T .65	3			
F ₂	21	5	3 : 1	0.5 ^{n.s.}
325		3		
F ₁ 108×325	3			
F ₂	35	5	3 : 1	3.3 ^{n.s.}
<i>sativa</i> × <i>perennis</i>				
108	3			
W106		3		
F ₁ 108×W106	3			
F ₂	21	11	3 : 1	1.5 ^{n.s.}
W107		3		
F ₁ 108×W107	3			
F ₂	23	9	3 : 1	0.2 ^{n.s.}

** Significant at 1% level, n.s.—non-significant

Table 3. Segregation for peroxidase bands 2A, 3A and 4A

Plant (Genotype)	Observed number 2A 2A-3A-4A 4A			Expected ratio	Chi-square
W107 (<i>Pe</i> ^{2A} / <i>Pe</i> ^{2A})	3				
W1294 (<i>Pe</i> ^{4A} / <i>Pe</i> ^{4A})			3		
F ₁ W107×W1294		3			
F ₂	13	29	12	1 : 2 : 1	0.33 ^{n.s.}
W120 (Diallel crosses)					
<i>Pe</i> ^{4A} / <i>Pe</i> ^{4A} × <i>Pe</i> ^{2A} / <i>Pe</i> ^{4A}		27	43	1 : 1	3.66 ^{n.s.}
<i>Pe</i> ^{2A} / <i>Pe</i> ^{4A} self (1967)	5	9	11	1 : 2 : 1	4.84 ^{n.s.}
" " (1968)	20	49	43	1 : 2 : 1	11.2**

** Significant at 1% level, n.s.—non-significant.

2. Diallel crosses in a *perennis* population

The zymograms of ten plants of W120 population used for diallel crosses are given in Table 4. Four of them had band 4C, while in the remaining six it was absent or very weak. Seven had 4A, while the remaining three had the hybrid zymogram, 2A-3A-4A. The segregation pattern observed in the F₁ plants and selfed progenies of the parents (in 1967) is given in Table 5.

Table 4. Peroxidase zymograms and estimated genotypes of ten plants of a *perennis* population (W120)

Plant no.	Zymogram				Genotype		
	4C	2A	3A	4A			
1	—	—	—	+	$Pe^{4C}/pe^{4C}-$	R^{4C}/r^{4C}	Pe^{4A}/Pe^{4A}
4	—	—	—	+	$Pe^{4C?}/Pe^{4C?}$	R^{4C}/R^{4C}	Pe^{4A}/Pe^{4A}
5	+	—	—	+	$Pe^{4C}/pe^{4C}-$	r^{4C}/r^{4C}	Pe^{4A}/Pe^{4A}
7	+	+	+	+	$Pe^{4C}/pe^{4C}-$	r^{4C}/r^{4C}	Pe^{2A}/Pe^{4A}
11	—	—	—	+	$Pe^{4C}/pe^{4C}-$	R^{4C}/r^{4C}	Pe^{4A}/Pe^{4A}
14	+	—	—	+	$Pe^{4C}/pe^{4C}-$	r^{4C}/r^{4C}	Pe^{4A}/Pe^{4A}
18	—	—	—	+	$Pe^{4C}/Pe^{4C?}$	R^{4C}/r^{4C}	Pe^{4A}/Pe^{4A}
19	+	+	+	+	$Pe^{4C}/pe^{4C}-$	r^{4C}/r^{4C}	Pe^{2A}/Pe^{4A}
21	—	+	+	+	$Pe^{4C}/Pe^{4C?}$	R^{4C}/r^{4C}	Pe^{2A}/Pe^{4A}
23	—	—	—	+	$Pe^{4C}/Pe^{4C?}$	R^{4C}/r^{4C}	Pe^{4A}/Pe^{4A}

Table 5. Segregation pattern in diallel crosses among ten plants taken from a *perennis* population (W120, in 1967)

Plant	no. 1	no. 4	no. 5	no. 7	no. 11	no. 14	no. 18	no. 19	no. 21	no. 23
no. 1 a)	2 : 10									
no. 1 b)	0 : 0 : 8									
no. 4 a)	0 : 4	1* : 12								
no. 4 b)	0 : 0 : 4	0 : 0 : 4								
no. 5 a)	2 : 2	0 : 4	2 : 4							
no. 5 b)	0 : 0 : 4	0 : 0 : 4	0 : 0 : 6							
no. 7 a)	2 : 1	0 : 5	3 : 0	5 : 3						
no. 7 b)	0 : 0 : 3	0 : 1 : 4	0 : 0 : 3	3 : 4 : 2						
no. 11 a)	0 : 4	0 : 1	2 : 4	0 : 4	0 : 5					
no. 11 b)	0 : 0 : 4	0 : 0 : 1	0 : 0 : 6	1* : 2 : 1	0 : 0 : 6					
no. 14 a)	2 : 2	0 : 7	3 : 0	2 : 2	1 : 7	3 : 5				
no. 14 b)	0 : 0 : 4	0 : 0 : 4	0 : 0 : 3	1* : 1 : 2	0 : 0 : 10	0 : 0 : 8				
no. 18 a)	0 : 2	0 : 2		3 : 1	4 : 1	1 : 1	3 : 2			
no. 18 b)	0 : 0 : 2	0 : 0 : 2		0 : 1 : 3	0 : 0 : 5	0 : 0 : 2	0 : 0 : 5			
no. 19 a)		0 : 2	1 : 3	1 : 3				7 : 1		
no. 19 b)		0 : 1 : 1	0 : 2 : 3	0 : 1 : 3				1 : 2 : 5		
no. 21 a)	0 : 7	0 : 5	0 : 4	0 : 4	1 : 3	4 : 0	0 : 3	2 : 3	3 : 5	
no. 21 b)	0 : 0 : 7	0 : 3 : 1	0 : 2 : 2	0 : 3 : 1	0 : 2 : 3	0 : 2 : 2	0 : 1 : 2	0 : 1 : 2	1 : 3 : 4	
no. 23 a)	0 : 2	0 : 2	1 : 0	3 : 1	0 : 4	1 : 3	2 : 2	1 : 1	2 : 4	1 : 3
no. 23 b)	0 : 0 : 2	0 : 0 : 2	0 : 0 : 1	0 : 3 : 1	0 : 0 : 4	0 : 0 : 4	0 : 0 : 4	0 : 1 : 1	2* : 4 : 0	0 : 0 : 4

* Contamination due to out-crossing

It may be recognized from Table 5 that not only the selfed but also all crossed progenies of plant no. 4 did not show band 4C (or its activity was very low). This suggests that the 4C band in W120 is controlled by two genes, one of which may be Pe^{4C} found from other crosses while the other may be a regulatory gene repressing 4C isozyme. Plant no. 4 seems to be homozygous for the regulatory gene. It is symbolized by R^{4C} and its recessive allele by r^{4C} .

The segregation pattern in the F_2 and corresponding selfed progenies of the parents (observed in 1968) is shown in Table 6, in comparison with the F_1 data. As the table shows, plants supposedly heterozygous for R^{4C} gave a 1:3 or a 3:13 ratio for band 4C. The data indicate that there must be a set of two loci (Pe^{4C} and R^{4C}), and plants with Pe^{4C} and r^{4C} produce the 4C band, but those either with pe^{4C-} or with R^{4C} do not show it. There was a tendency for pe^{4C-} to increase against Pe^{4C} , possibly due to gametic selection (Table 6). This segregation distortion made it difficult to distinguish between 1:3 and 3:13 ratios to determine whether a plant showing such a ratio was homozygous or heterozygous for Pe^{4C} . The genotype of such plants was expressed by $Pe^{4C}/Pe^{4C?}$ (Tables 4 and 6).

Table 6. Segregation ratios for peroxidase 4C band in diallel crosses

Parental genotype	Year	Plant	Observed no. 4C		Expected ratio	Chi-square
$Pe^{4C}/pe^{4C-} \quad r^{4C}/r^{4C}$	1967	5, 7, 14 & 19 self				
		& mutual cross	27	21	3:1	9.0**
	1968	5 (self) -2	2	4		
		7 (self) -1	6	4		
		1×5-1	22	9		
		7×1-1	28	12		
		7×23-1	21	9		
			79	38	3:1	3.49 ^{n.s.}
	$Pe^{4C}/Pe^{4C?} \quad R^{4C}/r^{4C}$	1, 11, 18, 21 & 23				
		self & mutual	18	57	{ 1:3 3:13	0.04 ^{n.s.}
		cross				1.36 ^{n.s.}
		1968				
		1 (self) -1	3	7		
		1×4-1	12	41		
		7×4-1	29	51		
		7×11-1	7	23		
		11×1-1	5	11		
		11×14-1	4	8		
		19×4-1	2	16		
			62	157	{ 1:3 3:13	1.28 ^{n.s.} 13.14**
$Pe^{4C}/Pe^{4C?} \quad R^{4C}/r^{4C}$	1967	16 crosses				
×		between two	25	38	{ 1:1 3:5	2.68 ^{n.s.}
$Pe^{4C}/pe^{4C-} \quad r^{4C}/r^{4C}$		plant groups given above				0.13 ^{n.s.}

** Significant at 1% level (possibly due to gametic selection)

The segregation pattern for 2A, 2A-3A-4A and 4A in the diallel crosses was reasonably explained by the assumption of two allelic genes, Pe^{2A} and Pe^{4A} , as already mentioned (Table 3). The genotypes of the ten parental plants were thus determined from the data, as shown in Table 4.

DISCUSSION

The genes controlling peroxidase variation found between *sativa* and *perennis* strains were at two independent loci. One of them had two alleles $Pe^{2A} : Pe^{4A}$ specifying bands 2A and 4A, respectively, and produced in heterozygotes a hybrid dimer band, 3A. The other locus had alleles $Pe^{4C} : pe^{4C-}$, the former specifying band 4C while the latter producing no active band. They produced no detectable hybrid band in heterozygotes. These may be considered to be "structural" genes.

Further, from diallel crosses in a *perennis* population, the presence of a gene repressing the active band, 4C, was found out. Within the scope of the present experiment, this may be considered to be a "regulatory" gene. Otherwise it may represent some change in an inducer system which behaves like a dominant gene. In the present work, we observed zymograms of mature leaf blades only. Isozymes are organ-specific, while the pattern may differ according to strains. Their activities may also differ according to aging and other physiological conditions. To distinguish whether the gene in question is really repressor-producing, or belongs to an inducer system, it seems necessary to look into the genetic control of organ-specificity patterns.

As Chu (1967) has pointed out, the variation in isozymes of cultivated rice varieties is relatively limited, while their wild progenitor, the Asian form of *perennis*, carries much wider variability. The wild population of perennial type we observed contained genic changes for peroxidase in at least three loci, one of which could be regulatory. Chu (1967) however showed that a *perennis* population of annual type was homogeneous. Populations of perennial wild rice may have a high capacity to store up genetic variations.

SUMMARY

Eight strains belonging to *Oryza sativa* and *O. perennis* (Asian form) were intercrossed, and the hybrid plants were observed for peroxidase isozymes of the leaf blades by starch-gel electrophoresis method. Band 4C was found to be specified by an active gene, Pe^{4C} . Bands 2A and 4A were specified by allelic genes, Pe^{2A} and Pe^{4A} , respectively. Their heterozygotes produced a hybrid dimer band, 3A, between 2A and 4A. Further, diallel crosses were made among ten plants randomly chosen from a population of *O. perennis*. The data suggested that there might be a regulatory gene, R^{4C} , repressing band 4C. The loci of Pe^{4C} , R^{4C} and Pe^{2A}/Pe^{4A} appeared to be independent of one another.

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