イネPx-1遺伝子座支配のパーオキシダーゼ・アイソザイムの差別的調節

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Differential regulation of peroxidase isozymes coded by *Px-1* locus in rice[1)]

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ABSTRACT

Alleles of Px^{2A} and Px^{4A} at the *Px-1* locus in wild and cultivated rice strains code dimeric peroxidase isozymes, which were detected zymographically with lemmata and paleae at higher intensity as well as with leaf blade and leaf sheath at lower intensity. In a heterozygote, $Px^{2A}Px^{4A}$, three isozyme bands 2A, 3A and 4A appear. Distinct differences in the intensity ratio of the band-morphs were observed, and they were classified into types I to V. A cross between Japonica *sativa* and Asian *perennis* showed type I only. Another cross between two strains of Asian *perennis* showed type II only, but a cross between Japonica *sativa* and African *perennis* showed types I to III. A receptor mutant gene adjacent to the *Px-1* locus and two regulator gene loci independent from the *Px-1* locus were assumed to account for the inheritance.

1. INTRODUCTION

Asynchronous or preferential allelic expression in isozyme loci has been widely known in interspecific or intergeneric hybrids in plants and animals, though genetic analysis is usually difficult due to the lack of offspring after the F_1 generation. Some genetic analysis for a similar phenomenon is done in intraspecific or intervarietal hybrids in organs of early developmental stages (Efron 1970, 1971; Schwartz 1971; Chao and Scandalios 1971) as well as in advanced ones (Endo 1971, 1981) in plants. Cause of this phenomenon has been explained by a regulator mutant (Efron 1971) or a promotor mutant (Schwartz 1976) in the *Adh-1* locus and by the assignment of a modulator gene in the *Amy-1* locus (Chao and Scandalios 1975) in maize.

In the previous paper (Endo 1971), a preferential allelic expression was reported in the *Px-1* locus specifying isozymes of peroxidase (EC 1.11.1.7) in rice. In the present report, further experimental results are presented in addition to the previous one.

2. MATERIALS AND METHODS

Two cultivated strains of *Oryza sativa* and three wild strains of *O. perennis* were used (Table 1). All the strains were homozygous at the *Px-1* locus,

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Table 1. *Genotypes, origins and traits of the strains crossed*

Strain	*Px-1* locus	Species, Origins and Traits
T65	$Px^{2A}Px^{2A}$	*O. sativa*, Japonica type (Taiwan)
563	$Px^{2A}Px^{2A}$	*O. sativa*, Japonica type (Japan), glutinous
W107	$Px^{2A}Px^{2A}$	*O. perennis*, annual (India), diploid by nuclear restitution
W1294	$Px^{4A}Px^{4A}$	*O. perennis*, perennial (Philippines)
W648	$Px^{4A}Px^{4A}$	*O. perennis barthii**, perennial (Sierra Leone)

* African *perennis* or *O. longistaminata* in synonym.

either $Px^{2A}Px^{2A}$ or $Px^{4A}Px^{4A}$, where the former specifies a slow-moving band, 2A, and the latter specifies a fast-moving band, 4A, to the anode. The crosses between the two genotypes were done by Dr. H.I. Oka of our Institute. It should be noted that to cross *O. sativa* with African *perennis* was very difficult and the rate of success was around 0.1%. Perennial strains, F_1 hybrids and their offspring were grown in a concrete bed with automatic short-day control (12 h) during the summer season.

Zymographic experiments were carried out mainly with lemmata and paleae at the flowering stage, and partly with mature leaves in order to examine the heterozygosity. The procedures for starch gel electrophoresis and zymographic development were described elsewhere (Endo 1971, 1972; Pai *et al.* 1973). Densitometer scan was done with Tôyô Densitorol DMU-2 fitted with a filter of 624 nm.

3. RESULTS

From the densitometer scanning of the peroxidase zymograms, as shown in Fig. 1, the activities of both Px^{2A} and Px^{4A} alleles of the structure gene locus *Px-1* appeared similar in lemma and palea of the strains T65 and W1294 around the flowering stage. Although the intensities on zymograms of the crude extracts from each of the homozygotes were not completely the same, their F_1 heterozygote, $Px^{2A}Px^{4A}$, showed an intensity ratio (%) of 28.4 : 44.1 : 27.5 for the bandmorph of 2A : 3A : 4A, which was not significantly different from the ratio of 25.0 : 50.0 : 25.0. This result suggests that both alleles have the same activity.

Among 51 F_2 segregants, 20 were heterozygotes, and six heterozygotes were derived from one of the F_2 heterozygotes as the F_3 segregants. These F_2 and F_3 heterozygous segregants showed the bandmorphs for the intensity ratio of 2A : 3A : 4A similar to that of the F_1 hybrid. The bandmorph of these heterozygotes was designated as type I or normal intensity ratio.

The second type of heterozygote bandmorph was found in the F_1 hybrid between W107 and W1294. As shown in Fig. 2, band 2A was low, 4A was

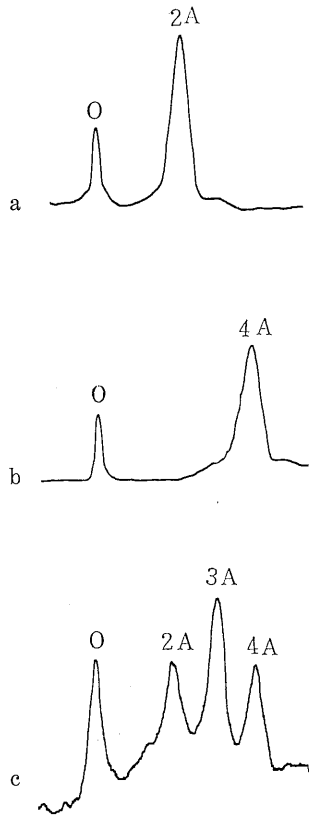

Fig. 1. Densitometer scan of peroxidase bandmorphs from lemma and palea. a: 2A band from T65, b: 4A band from W1294, c: type I bandmorph from T65×W1294. Types I and IV are the same in appearance.

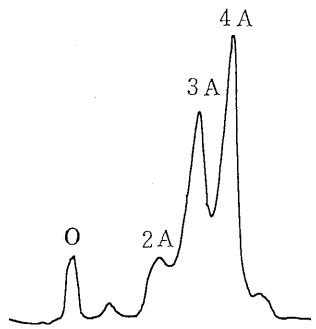

Fig. 2. Densitometer scan of type II peroxidase bandmorph from W107×W1294.

high and the hybrid band 3A was intermediate in intensity. This bandmorph was designated as type II. Among the 25 F_2 heterozygous segregants, 24 showed type II as the F_1 did, and the remaining one showed type I. This

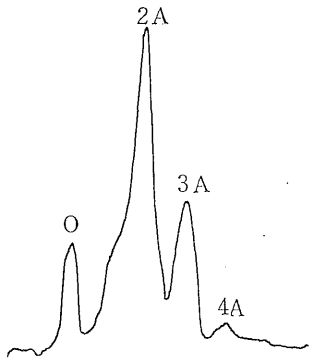

Fig. 3. Densitometer scan of type III peroxidase bandmorph of an F_3 segregant from 563×W648.

heretical segregant has already been cited previously (Endo 1971). At present, this abnormal individual was explained as the result of pollen contamination due to the partial self-sterility of the F_1 plants. It was also noted that three F_2 heterozygotes produced only type II in their F_3 offspring without any other bandmorphs.

The bandmorph, type III, was found in the F_2 and the F_3 generations of the cross between a Japonica cultivar 563 and an African *perennis* W648. As shown in Fig. 3, band 2A was high, 4A was low and 3A was intermediate in this type. It should be noted that the bandmorph of the F_1 hybrids was apparently the same as type I. Nevertheless, this F_1 hybrid produced three bandmorph types, types I, II and III in the F_2 generations. The bandmorph of this F_1 was designated as type IV.

The characteristics of parental combinations and their offspring were summarized in Fig. 4 where the homozygous segregants of F_2 and F_3 were omitted. Although only the three heterozygous types, types I to III, were depicted, type IV was included in type I in the F_2 segregants of the cross, 563×W648. It was noted in the cross that the offspring of the F_1 having type IV bandmorph were 12 type I : 5 type II : 3 type III; this ratio may agree with 10 : 3 : 3. The F_2 showing type II segregated into 6 type I : 13 type II; this ratio is not significantly different from 1 : 3. Also, the F_2 showing type III segregated into 7 type I : 19 type III; this ratio is not significantly different from 1 : 3. Further explanation will be added later.

A curious bandmorph appeared in a F_3 segregant, No. 7-13, from type II of the F_2 of the cross, 563×W648. Lemmata and paleae of the plant were repeatedly sampled and examined during the same season, and the same bandmorph was obtained; one of which is shown in Fig. 5a. This curious bandmorph consisted of 2A and 4A bands but lacks the hybrid band 3A. This plant was highly sterile and showed perennial form. Three clonal stocks were

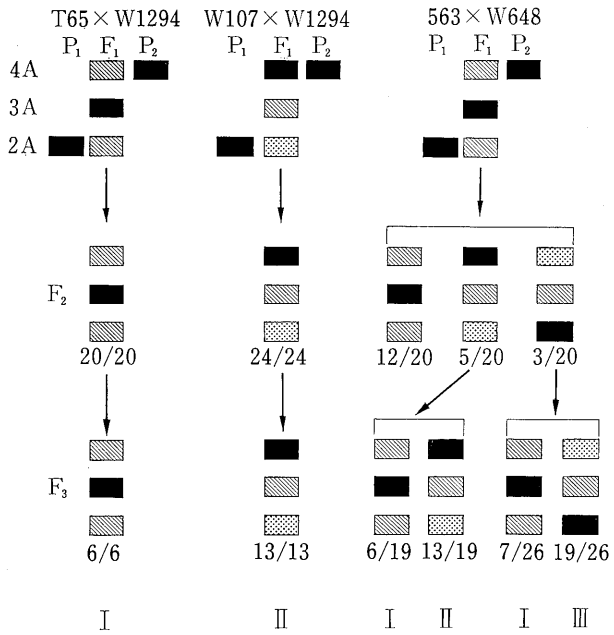

Fig. 4. Combinations of parental strains and their segregation into types I to III. Homozygous segregants are not included.

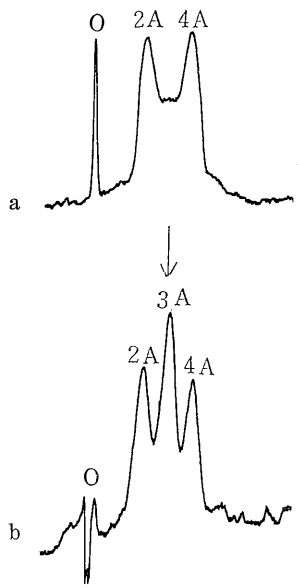

Fig. 5. Densitometer scan of non-reproducible bandmorph of type V which appeared in plant No. 7-13, an F_3 segregant from 563×W648. a: No. 7-13, b: clonal offspring from No. 7-13.

Table 2. *Relative intensities of three peroxidase bands coded by $Px^{2A}Px^{4A}$ and those calculated by m values in five heterozygous bandmorphs, types I to V*

Type of bandmorphs	Relative intensity ratio (%)		
	2A	3A	4A
I observed	28.4	44.1	27.5
calculated (m=0.98)	25.5	50.0	24.5
II observed	10.9	37.7	51.4
calculated (m=2.17)	9.9	43.2	46.9
III observed	67.2	29.5	3.3
calculated (m=0.22)	67.0	29.7	3.3
IV observed	27.1	46.8	26.1
calculated (m=0.98)	25.5	50.0	24.5
V observed	49.3	0.0	50.7
calculated (m=1.01)	24.8	50.0	25.2

taken from this plant and grown in pots in a greenhouse. Surprisingly, the bandmorphs of these stocks had changed to type I (or type IV) in the next year, as shown in Fig. 5b. The orignal bandmorph of this plant was tentatively designated as type V.

By densitometer scan, relative intensity of the bandmorphs from types I to V was examined in several plants, and the allelic activities specifying the separated bands in each type can be estimated using the following formula

$$SS : SF : FF = 1 : m(1+a) : m^2a.$$

Here, S and F are isozyme subunits or monomers, m is the ratio in amount of the two monomers (F/S), and a is the molecular specific activity ratio of the two homodimers (FF/SS). When the three dimers SS, SF and FF are assigned to bands 2A, 3A and 4A, respectively, and a is allocated to be 1 in the equation, the observed and the calculated intensity ratios represented by m in the heterozygotes from types I to V are as shown in Table 2. It appears that there are no significant differences between the observed and the calculated relative intensities of the hybrid band 3A except type V. This means that the hybridization between homodimers SS and FF or 2A and 4A is almost completely random. Alternatively, under the assumption that both m and a are 1 in the equation, the intensity of the hybrid band 3A should always be the highest of the bandmorph. Thus the assumption is ruled out.

As shown in the Table, m values of types I, IV and V are similar, but those of types I, II and III are much different. Such distinctive differences in m values may be explained by the differences in the coding rates, as will be discussed later.

4. DISCUSSION

With regard to the segregation ratios of heterozygous bandmorphs in the F_2 and F_3 generations cited in Fig. 4, the participation of variant gene other than the structure genes of the *Px-1* locus is considered probable. When the segregation ratio of 1 : 2 : 1 in the cross T65 × W1294 is normal and the standard, then, the segregation ratios from the cross W107 × W1294 suggest the participation of a regulator or a receptor gene mutation in addition. As described in RESULT only type II was produced at the F_1 of the latter cross and not segregated at the generations of F_2 and F_3. This difference from the standard can be explained by the participation of a receptor or a regulator gene which located close to the *Px-1* locus.

According to the definition by Britten and Davidson (1969), receptor genes consist of multiple promotor regions adjacent to the corresponding producer or structure genes. If so, it is supposed that a recessive receptor gene, rcp^{2A}, takes position adjacent to Px^{2A} in W107 strain, and a dominant receptor gene, Rcp^{4A}, takes position adjacent to Px^{4A} in W1294 strain. In the heterozygotes, $rcp^{2A}Px^{2A}/Rcp^{4A}Px^{4A}$, predominant amount of unidentified activating substance (or activator RNA in Britten-Davidson model) acts on a dominant receptor Rcp^{4A}, and the remaining part acts on a recessive receptor rcp^{2A}. With this way, isozyme 4A would be formed predominatly over isozyme 2A. In addition to this, if recombination between the *Rcp* and *Px-1* regions is considered negligible due to the close linkage, the bandmorph type II shown by the F_1 will be maintained throughout the successive generations without segregation. One other explanation is that the presence of a regulator variant gene linked with Px^{2A}, but this possibility becomes remote if the results of segregation from the third cross is taken in consideration.

The segregation ratios from the third cross, 563 × W648, require two unlinked gene loci, $Reg\text{-}1^{2A}$, and $Reg\text{-}2^{4A}$, both of which are unlinked also with *Px-1* locus. As mentioned in RESULTS, the F_2 segregation ratio of the heterozygous genotype, $Px^{2A}Px^{4A}$, fits with the theoretical ratio of 10 : 3 : 3 for ($Reg\text{-}1^{2A}Reg\text{-}2^{4A}$ + $reg\text{-}1^{2A}reg\text{-}2^{4A}$): $Reg\text{-}1^{2A}$ $reg\text{-}2^{4A}$: $reg\text{-}1^{2A}$ $Reg\text{-}2^{4A}$. This means that both the regulator genotypes, $Reg\text{-}1^{2A}Reg\text{-}2^{4A}$ and $reg\text{-}1^{2A}reg\text{-}2^{4A}$, produce similar bandmorphs because the same $Px^{2A}Px^{4A}$ is activated. This is expected since the ultimate amounts of 2A, 3A and 4A isozymes should be different but the relative amount ratios of 2A, 3A and 4A may stay the same between $Reg\text{-}1^{2A}Reg\text{-}2^{4A}$ and $reg\text{-}1^{2A}reg\text{-}2^{4A}$. In other words, it is assumed that there was no competition between $reg\text{-}1^{2A}reg\text{-}2^{4A}$ and $reg\text{-}2^{4A}reg\text{-}2^{4A}$ as well as between $Reg\text{-}1^{2A}Reg\text{-}1^{2A}$ and $Reg\text{-}2^{4A}Reg\text{-}2^{4A}$ on the allelic activation of Px^{2A} and Px^{4A}.

The genetic independency of *Reg-1* and *Reg-2* loci was inferred from the data of F_3 segregations. When the type II segregants of F_2 were selfed, type I and II were segregated at the F_3 generation. The type III segregants of F_2 segregated into type I and type III; i.e., monohybrid segregation. Since the

Table 3. *Regulator and receptor genotypes assumed for the Px-1 locus in rice strains used*

Strain	Regulator genotype	Receptor genotype
T65	$reg\text{-}1^{2A}reg\text{-}1^{2A}reg\text{-}2^{4A}reg\text{-}2^{4A}$	$Rcp^{2A}Rcp^{2A}$
563	$reg\text{-}1^{2A}reg\text{-}1^{2A}reg\text{-}2^{4A}reg\text{-}2^{4A}$	$Rcp^{2A}Rcp^{2A}$
W1294	$reg\text{-}1^{2A}reg\text{-}1^{2A}reg\text{-}2^{4A}reg\text{-}2^{4A}$	$Rcp^{4A}Rcp^{4A}$
W107	$reg\text{-}1^{2A}reg\text{-}1^{2A}reg\text{-}2^{4A}reg\text{-}2^{4A}$	$rcp^{2A}rcp^{2A}$
W648	$Reg\text{-}1^{2A}Reg\text{-}1^{2A}Reg\text{-}2^{4A}Reg\text{-}2^{4A}$	$Rcp^{4A}Rcp^{4A}$

strains 563 and T65 are Japonica type cultivars and closely related genetically, presumably, both dominant regulator genes are present in W648 (Table 3).

Returning once more to the fixation of type II in the second cross, W107 × W1294, it is difficult to assume that $Reg\text{-}1^{2A}$ is linked with the *Px-1* locus in W107, because three loci of *Reg-1*, *Reg-2* and *Px-1* are unlinked each other. But it may be possible that the third regulator gene locus, $Reg\text{-}3^{2A}$, is closely linked with the *Px-1* in this strain. However, it is too complicated to analyze if two regulator loci are considered for the control of a single structural allelic gene.

The mechanism for the deletion of hybrid band in type V remains unknown. Although the enzymic system responsible for dimerization or multimerization of polypeptide subunits is not identified yet, such systems must be existed in intracellular systems, and are expected to be sensitive to environmental conditions of external biophysical as well as of internal biochemical. What environmental conditions are involved in the behavior of type V is under examination.

In the present experiment, noteworthy interactions were observed between the alleles of a structure gene locus and the alleles of a regulator gene locus or loci. In maize, a normal regulatory allele, $Adh\text{-}R^N$ (originally $Adh_r{}^N$) activates structure alleles of $Adh\text{-}1^F$ and $Adh\text{-}1^S$ to a similar extent in the scutellum, and produces almost the same amount of FF dimer of ADH coded by $Adh\text{-}1^F$ as that of SS dimer of ADH by $Adh\text{-}1^S$ (Efron 1970). This was clearly proved immunoelectrophoretically by Schwartz (1973). Another regulatory allele, $Adh\text{-}R^L(Adh_r{}^L)$, however, activates $Adh\text{-}1^S$ less but $Adh\text{-}1^F$ as similarly as $Adh\text{-}R^N$ (Efron 1970). It means that some regulator allele activates only specific structure allele or alleles in a locus but not the entire locus. It looks curious, but the phenomenon observed in the present experiment resembles with this. It is assumed that a regulator allele of $Reg\text{-}1^{2A}$ and of $Reg\text{-}2^{4A}$ activates only incompletely a specific structure allele of Px^{2A} and Px^{4A}, respectively, in return, $Reg\text{-}1^{2A}$ completly activates Px^{4A} and $Reg\text{-}2^{4A}$ completely activates Px^{2A}. Although such an assumption can be made, no actual knowledge has yet been obtained on the relationships between the actions of regulator genes and the products of structure genes in higher organisms.

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