GENETIC CONVERGENCE OF THE SPECIFIC ACID PHOSPHATASE ZYMGRAMS IN ORYZA SATIVA

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In our previous paper (Shahi et al. 1969a), the variations in acid phosphatase isozymes were compared among four groups of rice, i.e., 1) cultivars of Oryza sativa L., 2) semi-wild or intermediate perennis-sativa forms collected from Jeypore, India, 3) Asian and other wild forms belonging to O. perennis Moench, and 4) various other Oryza species. The wild forms, especially perennial type of perennis, showed various zymograms, i.e., in total 147 strains contained 31 different zymograms, while in 115 sativa cultivars and in 26 strains of the semi-wild forms only two were located. Moreover, it was noted among cultivars that the Indica and Japonica types generally differed in their zymograms.

Since cultivated rice, O. sativa, is believed to have originated from the Asian group of wild rice, O. perennis, it may be rather natural that the two zymograms of cultivars were observed in the wild forms. In the present paper, genes specifying the zymograms were analyzed in order to compare two cultivars with two wild strains having the same zymograms. A discussion follows about the convergence of genic systems specifying the zymograms during the course of rice domestication.

MATERIALS AND METHODS

Two perennis and two sativa strains preserved in the National Institute of Genetics, Misima, were mainly used for crossing. The former were W107 (P1, annual) and W1294 (P2, perennial), both mentioned in our previous papers (Shahi et al. 1969b; Endo 1971). The latter were 108 (P3, Indica type) and T65 (P4, Japonica type). These were pure lines, except for W1294 which was homozygous for the isozymic characters examined. Parental, F1, F2 and B1 plants were observed.

Zymographic examinations in O. perennis were carried out mainly of lemma and palea taken together and partly of leaf blade and leaf sheath, but the latter two were chiefly examined in O. sativa. Experimental details for the extraction of cell sap, starch

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RESULTS

The cell saps from lemma plus palea as well as mature leaf sheath of the two *Oryza* species gave fine acid phosphatase zymograms of *P*₁, *F*₁ and *P*₂ plants. The *P*₁ plants (*W*₁₀₇) had one major band, 3*A*, and, at least, four minor bands, 4*A'*, 2*A*, 1*A* and 1*C*. The *P*₂ plants (*W*₁₂₉₄) had one major band, 5*A*, and, at least, five minor bands, 6*A*, 4*A'*, 3*A'*, 2*A* and 1*A*. In the *F*₁ plants, three major bands, 5*A*, 4*A* and 3*A*, and, at least, four minor bands, 6*A*, 2*A*, 1*A* and 1*C*, were detected. The 4*A* band of *F*₁ plants seems to be a dimeric hybrid band composed of 3*A* and 5*A* monomer components from *P*₁ and *P*₂ strains, respectively. This band may also overlap the 4*A'* band from both parents. Band 3*A* in *P*₁ showed the same mobility as 3*A'* in *P*₂ and the two bands may overlap in *F*₁. Since there is no evidence for hybridization between 3*A'* and 5*A* bands in the plants, both bands must be specified by non-allelic genes.

It may be interesting that the zymograms of the two wild strains, *W*₁₀₇ (*P*₁) and *W*₁₂₉₄ (*P*₂), correspond to those of two cultivars, *₁₀₈* (*P*₃) and *₁₆₅* (*P*₄), respectively. This was examined in *F*₁ plants from *P*₁ × *P*₃ and from *P*₂ × *P*₄, and no difference was found in the zymograms among the *F*₁ and parents. The leaf sheath zymograms of *P*₃, *F*₁ (*P*₁ × *P*₂) and *P*₄ are shown in Fig. 2. There was no appreciable difference between the acid phosphatase zymograms from lemma plus palea and from mature leaf sheath or leaf blade of the same plants, though the intensity of each isozyme band was more or less changing due to the aging of organs during the summer season. It should be noted that a set of anodal bands was located near 6*A* band toward the anode in leaf blade of all four strains in the autumnal season, but there was no difference among them. We have confirmed that the zymograms of *P*₁, *F*₁ (*P*₁ × *P*₂) and *P*₂ closely resemble those of *P*₃, *F*₁ (*P*₃ × *P*₄) and *P*₄, respectively.

Among ⁶⁰ *F*₂ segregants from a cross between the two *perennis* strains, there were found no new zymograms other than those of *P*₁, *F*₁ and *P*₂ types, as they segregated into ⁸*P*₁, ³⁶*F*₁ and ¹⁶*P*₂ types. Some of them are represented in Fig. 3. Very similar results were obtained from the cross between the two *sativa* strains, ¹⁰₈ (*P*₃) and *₁₆₅* (*P*₄): Namely, ¹₆₃*F*₂ plants segregated into ⁵⁷*P*₃, ⁷⁰*F*₁ and ³⁶*P*₄ types. Their six zymograms are shown in Fig. 4, where the band ⁵*A* of immature leaf sheath (plant No. 3-2) was relatively weak. *F*₂ progenies also showed the three types, ²⁰*P*₃, ³⁸*F*₁ and ¹²*P*₄, obtained from four *F*₂ heterozygotes, while *B*₁ (*F*₁ × *P*₄) progenies showed the two types, ⁹*F*₁ and ¹⁷*P*₄ (Table 1). The ratios for the three types indicate single locus segregation, though they may be modified by gametic selection. We then designated the allelic genes which specify bands ³*A* and ⁵*A* by *A*ₚ₁³*A* and *A*ₚ₁⁵*A* at the *A*ₚ₁ locus, respectively. Also, the genes specifying band ¹*C* of *P*₁ (or *P*₃) and its null form of *P*₂ (or *P*₄) were designated by *A*ₚ₂¹*C* and *A*ₚ₂⁰ at the *A*ₚ₂ locus, respectively. Obviously, the two loci, *A*ₚ₁ and *A*ₚ₂, are closely linked.

The genes specifying ⁴*A'*, ²*A* and ¹*A* bands appear to be common to both parental
strains. The minor band 6A was always accompanied by 5A major band. The gene specifying the minor band may be linked with Acp1, unless it was due to in vivo modification of the major band.

Thus, the present experiments revealed that the isozymic differences between the two zymograms of both species were due to alleles at Acp1 and Acp2 loci. Namely, the Indica type zymogram (P1 and P3) was specified by Acp1^A and Acp2^C, while the Japonica type zymogram (P2 and P4) was specified by Acp1^M and Acp2^O. The inheritance of these isozyme bands is diagrammatically represented in Fig. 5.
Although *perennis* wild strains and *sativa* cultivars are cytologically closely related, both species are considerably different. Morphologically and zymographically the former is polymorphic, and shows more grain shedding as well as many other wild characters. The species has a series of intergrades varying from annual to perennial forms. The latter species, including Indica, Japonica and intermediate types, shows less grain shedding and is potentially perennial, though cultivated as an annual. For cultivated

<table>
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<th>Generation</th>
<th>P₁ (or P₃) type</th>
<th>F₁ type</th>
<th>P₂ (or P₄) type</th>
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<th>χ²-test</th>
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1) Indica type. 2) Japonica type.

![Diagram](image.png)

**Fig. 5.** A diagram for the inheritance of acid phosphatase isozymes between two strains of *O. sativa* (or *O. perennis*).

**DISCUSSION**

Although *perennis* wild strains and *sativa* cultivars are cytologically closely related, both species are considerably different. Morphologically and zymographically the former is polymorphic, and shows more grain shedding as well as many other wild characters. The species has a series of intergrades varying from annual to perennial forms. The latter species, including Indica, Japonica and intermediate types, shows less grain shedding and is potentially perennial, though cultivated as an annual. For cultivated
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rice, the conditions of cultivation differ from locality to locality. Since any genetic system as a whole is presumably moulded by cultivation, this may cause a convergence of the genetic systems responsible for morphological and physiological characters, resulting in the formation of local varieties. Thus, local varieties must be, more or less, different from each other. A series of intervariental morphological variation (e.g. Oka 1958) and of hybrid sterility has been observed (e.g. Hinata and Oka 1962). However, the species showed to have only two kinds in acid phosphatase zymograms, when we neglect mutant zymograms expected in any cultivar.

The present experiments revealed that the genic systems specifying the two acid phosphatase zymograms of two sativa cultivars almost completely corresponded to those of two perennis strains. Then, it appears that among cultivars the two kinds of genic systems are to the exclusion of others distributed, in contrast to the intervarietal differential genic systems controlling morphological and physiological characters.

A similar fact was already observed in leaf peroxidase zymograms of both species. In wild perennial populations the zymograms were much more polymorphic than in the annual populations. Furthermore like the case of acid phosphatase, sativa cultivars (Chu 1967) and semi-wild perennis-sativa strains (Chu and Oka 1967) were found to have two kinds of peroxidase zymograms representing the Indica and Japonica types, respectively, which differed in the presence or absence (or very weak intensity) of 4C band, i.e., most strains of the Indica type have this band but those of the Japonica type generally lack it.

From these results may be drawn a tentative hypothesis that even at a primitive cultivation as observed in Jeypore, India, some particular zymograms were rapidly selected and others were lost. In other words, when the genetic systems of primitive rice forms are moulded by cultivation, convergence of the genetic systems may occur at a certain isozyme level. Actually the cultivation experiments with O. perennis and its hybrid with O. sativa demonstrated that the frequency of alleles expressing cultivated characters quickly increased and that selection for the cultivated characters resulted in homozygosity, probably due to increased selfing rate (Oka and Morishima 1971). However, the correlation between the cultivated characters and specific zymograms remains to be further examined.

**SUMMARY**

In a number of rice cultivars, Oryza sativa, acid phosphatase zymograms were found to be of two kinds. Genetic experiments revealed that the isozymic difference between the two was due to alleles at Acpl and Acp2 loci which are closely linked. Those two zymograms were detected in wild rice strains of O. perennis which is believed to be the wild progenitor of O. sativa. The genetic behavior of the isozymes was very similar to that of the isozymes of O. sativa. Although O. perennis showed many different acid phosphatase zymograms, semi-wild perennis-sativa strains were found like O. sativa to have the same two kinds. Probably, domestication, even in a primitive stage, may lead to genetic convergence for the genes specifying the acid phosphatase zymograms of rice.
ACKNOWLEDGMENTS

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LITERATURE CITED


