

Isozyme markers in rice: genetic analysis and linkage relationships¹

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Segregations of rice isozymes made it possible to ascertain the genetic control of 13 enzymes by 30 loci. In addition to numerous cases of independence, several linkages were observed. *Got-2* and *Enp-1* were localized on chromosome 3 and a linkage group composed of *Pox-3*, *Pox-4*, and *Est-1* was identified. Inconsistencies of recombination rates between linked loci among different crosses were noted. Several cases of distorted segregations and pseudolinkages were recorded. The relationships between these results and the study of genome organization in cultivated rice are discussed.

Key words: *Oryza*, isozyme, genetic analysis, segregation distortion, linkage.

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L'étude de ségrégations enzymatiques chez le riz a permis de vérifier le déterminisme génétique de 13 systèmes enzymatiques. Trente locus sont concernés. Outre de nombreux cas d'indépendance, plusieurs liaisons entre locus ont été observées. Les locus *Got-2* et *Enp-1* ont été localisés sur le chromosome 3, et un groupe de linkage comportant *Pox-3*, *Pox-4* et *Est-1* a été identifié. Des contradictions sur les taux de recombinaison entre locus liés ont été notées entre descendance. Plusieurs cas de distortions de ségrégations et de pseudo-liaisons ont été observés. Les relations entre ces résultats et l'étude de l'organisation du génome des riz cultivés sont discutées.

Mots clés : *Oryza*, isozyme, analyse génétique, distorsion de ségrégation, liaison.

Introduction

Isozyme variation for more than 40 presumed loci has been used in studies on the genetic structure of cultivated rice and its closest wild relatives in the genus *Oryza* (Second 1982, 1985; Glaszmann 1987; de Kochko 1987). In contrast, there are few reports on the inheritance of isozymes and their linkage relationships in rice (for a review see Morishima and

Sano, 1984; Sano and Barbier 1985). The interpretation of the zymograms was initially based on segregations observed in inbreeding (species) versus outbreeding species of the genus *Oryza*. Species of the former group, in particular the two cultivated species *O. sativa* (Asian origin) and *O. glaberrima* (African origin), and their respective wild relatives *O. rufipogon* (annual form) and *O. breviligulata*, generally present a pattern of bands that can be attributed to homozygous loci. On the other hand, the closely related wild allogamous species *O. longistaminata* and the perennial form of *O. rufipogon* often show heterozygous patterns. These findings permitted the proposal of genetic models to explain the variation and the formulation of loci nomenclature (Second and Trouslot 1980). Recently, the use of trisomics permitted assigning several loci to their respective chromosomes (Ishikawa et al. 1986; Ranjhan et al. 1988; Wu et al. 1988). The present paper describes isozyme segregations in F₂ progeny obtained mostly from crosses between varieties of the common cultivated rice (*O. sativa*) and also from crosses involving *O. rufipogon* and *O. breviligulata*. Results obtained in four different laboratories are presented together. The experimental procedures differed slightly from one laboratory to the other, but there is value in bringing the overall information together. These joint results confirmed the genetic basis proposed previously for 30 genes

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TABLE 1. Parental accessions

Accession	Collection ^a	Origin	Subspecific classification ^b
<i>O. breviligulata</i>			
101196	IRRI	Cameroon	
NB2	ORSTOM	Niger	
IB 3.5	ORSTOM	Ivory Coast	
2LB 104	ORSTOM	Mali	
SB 309	ORSTOM	Senegal	
WB 01	ORSTOM	Botswana	
<i>O. rufipogon</i>			
W106	NIG	India	
<i>O. sativa</i>			
Bansi	IRAT	India	<i>indica</i>
Bete 3	IRAT	Ivory Coast	<i>japonica</i>
BG 90-2	IRRI	Sri Lanka	<i>indica</i>
BS 20	ORSTOM	Guinea-Bissau	<i>indica</i>
Carreon	IRAT	Philippines	<i>indica</i>
Century Patna 231	IRRI	United States	<i>japonica</i>
Chianan 8	IRAT	Taiwan	<i>japonica</i>
C8669	NIG	India	<i>indica</i>
ES 70-6	ORSTOM	Tanzania	<i>japonica</i>
ES 79	ORSTOM	Tanzania	<i>indica</i>
Gaebyo	IRRI	Korea	<i>japonica</i>
H 105	IRAT	Sri Lanka	<i>indica</i>
IR 2298-PLPB	IRRI		<i>indica</i>
IR 50	IRRI		<i>indica</i>
IRAT 13	IRAT		<i>japonica</i>
Lung Sheng 1	IRAT	Taiwan	<i>japonica</i>
Moroberekan	IRAT	Ivory Coast	<i>japonica</i>
PJ 110	IRAT		Intermediate
Pursigui	IRAT	Philippines	<i>indica</i>
RT 1031-69	IRAT	Zaire	<i>japonica</i>
Simtharo	IRRI	Nepal	<i>indica</i>
SS 404	ORSTOM	Senegal	<i>indica</i>
Taichung Native 1	IRAT	Taiwan	<i>indica</i>
Tatsuminochi	IRAT	Japan	<i>japonica</i>
Tjempo Welut	IRAT	Indonesia	<i>japonica</i>
YS 309	ORSTOM	Guinea	<i>japonica</i>
YS 45-1	ORSTOM	Guinea	<i>japonica</i>
108	NIG	Taiwan	<i>indica</i>
143	NIG	Taiwan	<i>indica</i>
221	NIG	Philippines	<i>japonica</i>
414	NIG	India	<i>indica</i>
504 ^c	NIG	Taiwan	<i>japonica</i>
761	NIG		
868	NIG	China	<i>indica</i>

^aIRAT, Institut de recherches agronomiques tropicales et des cultures vivrières (Montpellier, France); IRRI, International Rice Research Institute (Manila, Philippines); NIG, National Institute of Genetics (Miyama, Japan); ORSTOM, Institut français de recherche scientifique pour le développement en coopération (Paris, France).

^bClassification of *O. sativa* accessions is based on their enzymatic profiles (Second 1982; Glaszmann 1987).

^cThree isogenic lines of 504 were used: 504 *Rc wx dl* (noted 504Rc), 504 *g l l g la* (noted 504g), and 504 *wx* (noted 504wx).

encoding 13 enzymes. Cosegregation analyses identified several linkages, which could be compared and integrated with the linkage map established on the basis of trisomic analysis.

progeny studied are listed in Table 2. Of these, 28 are from *O. sativa* × *O. sativa* crosses, 3 are from *O. breviligulata* × *O. breviligulata* crosses, and 1 is from a cross *O. sativa* × *O. rufipogon*. The open-pollinated and self-pollinated (bagging of

TABLE 2. Origin of F₂ and BC progeny: parents, F₁ fertility, control of self-pollination

Cross		F ₁ fertility (%)	F ₁ hybrid pollination
SB 309	× WB 01		Controlled
IB 3.5	× 2LB 104		Controlled
101196	× NB2		Controlled
BS20	× YS 45-1	76	Controlled
108	× BS 20	98	Controlled
ES 70-6	× YS 309	27	Controlled
ES 70-6	× SS 404	25	Controlled
ES 70-6	× ES 79		Controlled
YS 45-1	× ES79		Controlled
108	× YS 309	80	Controlled
BS 117	× YS 138-3	20	Controlled
IR 50	× IR 2298		Open
CP 231	× Gaebyo		Open
IR 50	× Simtharo		Open
868	× 504	48	Controlled
143	× 221	26	Controlled
108	× W106	91	Controlled
C8669	× 221	51	Controlled
414	× 504	48	Controlled
761	× 504		Controlled
(761 × 504)	× 504		
Pooled F ₂			
Carreon	× Chianan 8	30	Open
Carreon	× IRAT 13		Open
Carreon	× Bete 3		Open
Bansi	× Tjempo Welut		Open
Bansi	× IRAT 13		Open
Bete 3	× Bansi	77	Open
H 105	× RT 1031-69		Open
Pursigui	× Lung Sheng	27	Open
Pursigui	× Chianan 8	35	Open
PJ 110	× Taichung Native 1		Open
Moroberekan	× PJ 110		Open

monly observed in F₁ (Pai et al. 1975; Pai and Fu 1977; Second 1982; Glaszmann et al. 1984; Glaszmann 1987; de Kochko 1987).

Isozyme electrophoresis

Isozyme electrophoresis was performed in starch gels. Descriptions of most electrophoretic buffer systems used in the various laboratories involved are listed in references cited in Table 3. The systems used at the National Institute of Genetics (Misima) (references 5 and 6 in footnote *a* of Table 3) are as follows.

System 1—Electrode buffer 0.19 M boric acid, adjusted to pH 8.0; gel buffer 9 parts 0.032 M Tris (adjusted to pH 8.0 with citric acid) : 1 part electrode buffer.

System 2—Electrode buffer 0.4 M Tris, adjusted to pH 8.0 with citric acid; gel buffer 0.015 M histidine-HCl, adjusted to pH 8.0 with Tris.

Thirteen enzymes were studied: alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), isocitrate dehydrogenase (ICD), 6-phosphogluconate dehydrogenase (PGD), shikimate dehydrogenase (SDH), phosphoglucose isomerase (PGI), catalase (CAT), peroxidase (POX), glutamate oxaloacetate transaminase (aspartate aminotransferase) (GOT), esterase (EST), acid phosphatase (ACP), endopeptidase (ENP), and aminopeptidase (AMP).

For loci nomenclature, the recommendations of the Committee on Gene Symbolisation, Nomenclature and Linkage Groups of the Rice Genetics Cooperative were followed (Morishima and Glaszmann 1986). The correspondence with previously used nomenclatures is given in Table 3.

Nonisozymic markers

Seven nonisozyme loci, whose chromosomal locations are known (Kinoshita 1984, numbering system by Khush et al. 1984), segregated in some crosses; *wx* (glutinous endosperm, chromosome 3), *Rc* (brown pericarp and seed coat, chromosome 7), *g* (long sterile lemmas, chromosome 7), *lg* (liguleless, chromosome 12), *dl* ("daikoku dwarf," chromosome 5), *la* ("lazy" growth habit, chromosome 11), and *Ph* (phenol staining of the hulls, chromosome 12). Relationships between various systems of numbering of chromosomes and linkage groups are given by Khush et al. (1984).

Statistical analysis

Most of the F₂ progeny were considered individually. However, a special population, called "pooled F₂," was constituted by grouping 11 small F₂ populations derived from crosses between different varietal types (Table 2); the three genotypic classes were obtained by pooling, respectively, female parent, F₁, and male parent genotypes.

For each locus under study, the observed segregations were tested against the expected Mendelian ratio (1:2:1 or 3:1, according to whether identification of heterozygous genotypes was possible or not). In case of non-Mendelian segregations, the equiprobability of alleles (*p*, *q*) and the distribution of genotype frequencies according to $p^2:2pq:q^2$ (random assortment) were tested to check the occurrence of gametic or zygotic selection. The frequency of null alleles was estimated on the basis of the frequency of the double-recessive homozygote. Independence of allelic associations at all

TABLE 3. Relationships between various nomenclatures for isozyme loci

This paper	Previous nomenclature ^a						
	1	2	3	4	5	6	7
<i>Acp-1</i>	PAC.AMC			ACP-1			
<i>Acp-2</i>	PAC.FaSa	PAC-2		ACP-2			
<i>Acp-4</i>		PAC-1					
<i>Adh-1</i>	ADH-A						Adh-1
<i>Amp-1</i>	LAP-E	LAP					
<i>Amp-2</i>		AAP				Amp-2	
<i>Amp-3</i>	LAP-F					Amp-1	
<i>Cat-1</i>	CAT-A	CAT			CAT-1		
<i>Enp-1</i>	EP-A						
<i>Est-1</i>	EST-D	EST-3	EST-1				
<i>Est-2</i>	EST-E	EST-4	EST-2		EST-2		
<i>Est-3</i>	EST-J		EST-3				
<i>Est-5</i>	EST-B						
<i>Est-6</i>	EST-C	EST-2					
<i>Est-7</i>	EST-I	EST-5					
<i>Est-8</i>		EST-6					
<i>Est-9</i>	EST-Ca	EST-1				Est-C1	Est-8
<i>Got-1</i>	GOT-A						
<i>Got-2</i>	GOT-B						
<i>Got-3</i>	GOT-C						
<i>Icd-1</i>	ICD-A						
<i>Mdh-1</i>	MDH-A						
<i>Pgd-1</i>	PGD-A					Pgd-1	
<i>Pgi-1</i>	PGI-A	PGI-1			PGI-1		Pgi-1
<i>Pgi-2</i>	PGI-B	PGI-2			PGI-2		
<i>Pgi-3</i>		PGI-3					
<i>Pox-2</i>				Px2			
<i>Pox-3</i>	POX-B	POX					
<i>Pox-4</i>	POX-C						
<i>Sdh-1</i>	SDH-A					Sdh-1	Sdh-1

^a1, Trouslot and Second 1980; Second 1982; de Kochko 1987; 2, Glaszmann et al. 1984; 3, Nakagahra et al. 1975; Nakagahra 1977; 4, Pai et al. 1973; Pai et al. 1975; 5, Morishima and Sano 1984; 6, Sano and Barbier 1986; 7, Ranjhan et al. 1988.

pairs of loci was tested. The goodness of fit (χ^2) of the two

ular interest because they show traces of introgression from

TABLE 4. Monolocus F₂ segregations at 30 presumed loci

Locus	Cross P ₁ × P ₂	Species or subspecies ^a	No. of F ₂ genotypes			χ ²
			P ₁	F ₁	P ₂	
<i>Acp-1</i>	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	48	187	159	63.56***
	BS 20 × YS 45-1	<i>i</i> × <i>j</i>	59	133	75	1.92
	108 × BS 20	<i>i</i> × <i>i</i>	18	22	8	4.50
	143 × 221	<i>i</i> × <i>j</i>	32	55	23	1.47
	C8669 × 221	<i>i</i> × <i>j</i>	73	124	55	2.63
	761 × 504R	<i>i</i> × <i>j</i>	47	69	30	4.40
	761 × 504g	<i>i</i> × <i>j</i>	34	64	21	3.52
<i>Acp-2</i>	ES 70-6 × YS 309	<i>j</i> × <i>j</i>	85	—247—		0.06
	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	47	—351—		36.90***
	BS 20 × YS 45-1	<i>i</i> × <i>j</i>	—193—		74	1.05
<i>Acp-4</i>	Pooled F ₂		63	—199—		0.13
	Pooled F ₂		62	100	61	2.38
<i>Amp-1</i>	ES 70-6 × YS 309	<i>j</i> × <i>j</i>	76	175	73	2.14
	108 × YS 309	<i>i</i> × <i>j</i>	24	49	11	6.36*
	IR 50 × Simtharo	<i>i</i> × <i>i</i>	48	67	31	4.95
	868 × 504	<i>i</i> × <i>j</i>	25	48	18	1.35
	C8669 × 221	<i>i</i> × <i>j</i>	17	42	23	0.93
<i>Amp-2</i>	868 × 504R	<i>i</i> × <i>j</i>	16	46	29	3.73
	414 × 504R	<i>i</i> × <i>j</i>	18	44	27	1.83
	C8669 × 221	<i>i</i> × <i>j</i>	24	43	15	2.17
	Pooled F ₂		68	132	79	1.67
<i>Amp-3</i>	IR 50 × Simtharo	<i>i</i> × <i>i</i>	37	170	104	1.39
	CP 231 × Gaebyo	<i>j</i> × <i>j</i>	3	27	56	80.23***
	IR 50 × IR 2298	<i>i</i> × <i>i</i>	53	93	49	0.58
	868 × 504R	<i>i</i> × <i>j</i>	25	48	18	1.35
	C8669 × 221	<i>i</i> × <i>j</i>	17	42	23	0.93
<i>Adh-1</i>	BS 20 × YS 45-1	<i>i</i> × <i>j</i>	69	130	62	0.38
	108 × BS 20	<i>i</i> × <i>i</i>	54	90	29	7.51*
<i>Cat-1</i>	ES 70-6 × YS 309	<i>j</i> × <i>j</i>	76	149	90	2.16
	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	105	178	108	3.18
	414 × 504	<i>i</i> × <i>j</i>	36	45	29	4.53
	868 × 504	<i>i</i> × <i>j</i>	18	45	18	1.00
	C8669 × 221	<i>i</i> × <i>j</i>	83	85	37	26.62***
<i>Enp-1</i>	Pooled F ₂		32	63	28	0.33
	ES 70-6 × ES 79	<i>j</i> × <i>i</i>	—76—		39	4.87*
<i>Est-1</i>	YS 45-1 × ES 79	<i>j</i> × <i>i</i>	—65—		15	1.67
	ES 70-6 × YS 309	<i>j</i> × <i>j</i>	90	—238—		1.04
<i>Est-2</i>	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	94	—295—		0.15
	BS 20 × YS 45-1	<i>i</i> × <i>j</i>	—145—		57	1.12
	BS 117 × YS 138-3	<i>i</i> × <i>j</i>	—34—		14	0.44
	IR 50 × Simtharo	<i>i</i> × <i>i</i>	24	—122—		5.70*
	IR 50 × IR 2298	<i>i</i> × <i>i</i>	39	—156—		2.60
	Pooled F ₂		23	—56—		0.71
	ES 70-6 × YS 309	<i>j</i> × <i>j</i>	64	—264—		2.98
	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	23	—375—		78.42***
<i>Est-3</i>	108 × BS 20	<i>i</i> × <i>i</i>	40	80	53	2.93
	108 × YS 309	<i>i</i> × <i>j</i>	20	45	30	2.37
	IR 50 × Simtharo	<i>i</i> × <i>i</i>	39	66	41	1.40
	IR 50 × IR 2298	<i>i</i> × <i>i</i>	53	92	50	0.71
	CP 231 × Gaebyo	<i>j</i> × <i>j</i>	30	—56—		73.81***
	Pooled F ₂		14	28	8	2.16
	868 × 504R	<i>i</i> × <i>j</i>	—75—		16	2.67
	143 × 221	<i>i</i> × <i>j</i>	22	57	31	1.62
	C8669 × 221	<i>i</i> × <i>j</i>	—187—		67	0.25
	IR 50 × Simtharo	<i>i</i> × <i>i</i>	9	28	9	2.17
<i>Est-5</i>	BS 117 × YS 138-3	<i>i</i> × <i>j</i>	8	—40—		1.78
<i>Est-6</i>	BS 117 × YS 138-3	<i>i</i> × <i>j</i>	—40—		8	1.78
	Pooled F ₂		41	—97—		1.63
<i>Est-7</i>	BO4 × NB2	<i>B</i> × <i>B</i>	24	—77—		0.08
<i>Est-8</i>	Pooled F ₂		23	—59—		0.44
	Pooled F ₂		59	—167—		0.90

TABLE 4 (concluded)

Locus	Cross P ₁ × P ₂	Species or subspecies ^a	No. of F ₂ genotypes			χ ²	
			P ₁	F ₁	P ₂		
<i>Est-9</i>	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	19	41	26	1.32	
	IR 50 × IR 2298	<i>i</i> × <i>i</i>	36	102	57	4.94	
	414 × 504	<i>i</i> × <i>j</i>	34	45	31	3.80	
	868 × 504R	<i>i</i> × <i>i</i>	21	43	27	1.07	
	C8669 × 221	<i>i</i> × <i>j</i>	19	31	32	9.00*	
	Pooled F ₂		66	89	54	5.98	
<i>Got-1</i>	BS 20 × YS 45-1	<i>i</i> × <i>j</i>	76	135	47	7.08*	
	108 × BS 20	<i>i</i> × <i>i</i>	22	48	21	0.30	
<i>Got-2</i>	SB 309 × WB 01	<i>B</i> × <i>B</i>	39	86	41	0.27	
<i>Got-3</i>	ES 70-6 × YS 309	<i>j</i> × <i>j</i>	80	168	70	1.33	
	108 × YS 309	<i>i</i> × <i>j</i>	16	51	30	4.30	
<i>Icd-1</i>	IR 50 × IR 2298	<i>i</i> × <i>i</i>	37	113	45	5.58	
	CP 231 × Gaebyo	<i>j</i> × <i>j</i>	15	43	28	3.93	
<i>Mdh-1</i>	BO4 × NB2	<i>B</i> × <i>B</i>	27	44	30	1.85	
<i>Pgd-1</i>	ES 70-6 × YS 309	<i>j</i> × <i>j</i>	121	143	45	39.60***	
	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	99	186	112	2.43	
	108 × BS 20	<i>i</i> × <i>i</i>	31	50	11	9.39**	
	108 × YS 309	<i>i</i> × <i>j</i>	13	31	5	6.06*	
	C8669 × 221	<i>i</i> × <i>i</i>	26	40	16	2.49	
	414 × 504	<i>i</i> × <i>j</i>	42	36	8	29.16***	
	868 × 504	<i>i</i> × <i>j</i>	21	43	27	1.07	
	<i>Pgi-1</i>	SB 309 × WB 01	<i>B</i> × <i>B</i>	42	86	38	0.41
		108 × BS 20	<i>i</i> × <i>i</i>	20	49	19	1.16
		108 × YS 309	<i>i</i> × <i>j</i>	24	44	29	1.35
BS 117 × YS 138-3		<i>i</i> × <i>j</i>	13	22	13	0.33	
IR 50 × Simtharo		<i>i</i> × <i>i</i>	36	80	30	1.84	
Morober. × PJ 110		<i>j</i> × <i>i</i>	11	32	11	1.85	
868 × 504		<i>i</i> × <i>j</i>	28	48	15	3.99	
C8669 × 221		<i>i</i> × <i>j</i>	74	111	68	4.08	
143 × 221		<i>j</i> × <i>i</i>	23	60	27	1.20	
108 × W106		<i>i</i> × <i>R</i>	22	39	23	0.45	
<i>Pgi-2</i>	868 × 504R	<i>i</i> × <i>j</i>	28	48	15	4.99	
	761 × 504R	<i>i</i> × <i>j</i>	55	56	35	13.40**	
	761 × 504g	<i>i</i> × <i>j</i>	21	64	34	3.52	
	SB 309 × WB 01	<i>B</i> × <i>B</i>	34	86	46	1.95	
	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	56	206	136	32.65***	
	BS 20 × YS 45-1	<i>i</i> × <i>j</i>	68	123	65	0.46	
	108 × YS 309	<i>i</i> × <i>j</i>	22	43	32	3.31	
	IR 50 × IR 2298	<i>i</i> × <i>i</i>	51	92	51	0.52	
	868 × 504R	<i>i</i> × <i>j</i>	24	44	23	0.12	
	C8669 × 221	<i>i</i> × <i>j</i>	71	104	78	8.39*	
<i>Pgi-3</i>	143 × 221	<i>i</i> × <i>j</i>	26	54	30	0.33	
	108 × W106	<i>i</i> × <i>R</i>	16	45	20	1.40	
	Pooled F ₂		28	33	29	6.42*	
	761 × 504R	<i>i</i> × <i>j</i>	56	64	26	14.55***	
			65	187	—	0.08	
	<i>Pox-2</i>	143 × 221	<i>i</i> × <i>j</i>	—83—	—	27	0.01
		761 × 504R	<i>i</i> × <i>j</i>	—124—	—	22	7.68**
		761 × 504g	<i>i</i> × <i>j</i>	—96—	—	26	0.63
	<i>Pox-3</i>	IB 3.5 × 2LB 104	<i>B</i> × <i>B</i>	15	29	11	0.75
		108 × BS 20	<i>i</i> × <i>i</i>	10	45	13	7.38*
108 × YS 309		<i>i</i> × <i>j</i>	8	26	22	7.29*	
<i>Pox-4</i>	IB 3.5 × 2LB 104	<i>B</i> × <i>B</i>	14	—41—	—	0.01	
	ES 70-6 × YS 309	<i>j</i> × <i>j</i>	65	102	76	7.26*	
	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	71	133	84	2.85	
<i>Sdh-1</i>	ES 70-6 × SS 404	<i>j</i> × <i>i</i>	45	185	157	65.57***	
	BS 20 × YS 45-1	<i>i</i> × <i>i</i>	72	133	59	1.30	
	108 × BS 20	<i>i</i> × <i>i</i>	19	50	19	1.64	
	C8669 × 221	<i>i</i> × <i>j</i>	16	47	19	1.98	
	414 × 504	<i>i</i> × <i>j</i>	26	40	23	1.11	

^a*B*, *O. breviligulata*; *R*, *O. rufipogon*; *i*, *indica*; *j*, *japonica*.NOTE: *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$.

TABLE 5. Distorted F₂ segregations

Chromosome	Locus	Cross (P ₁ × P ₂)	Allele frequency		χ ²	
			P ₁	P ₂	Allele frequency homogeneity	F ₂ distribution (p ² :2pq:q ²)
1	<i>Got-1</i>	BS 20 × YS 45-1	0.556	0.444	6.52*	0.93
3	<i>Pgi-2</i>	ES 70-6 × SS 404	0.399	0.601	32.16**	2.47
	<i>Amp-3</i>	CP231 × Gaebyo	0.192	0.808	65.3 ***	0.01
	<i>Est-2</i>	ES 70-6 × SS 404	0.240	0.760		
		CP231 × Gaebyo	0.193	0.807		
	<i>Cat-1</i>	C8669 × 221	0.612	0.388	20.64***	3.29
		ES 70-6 × ES 79	0.404	0.596	8.42**	0.10
	<i>Enp-1</i>	ES 70-6 × ES 79	0.418	0.582	8.42**	0.10
4	<i>Pgi-1</i>	761 × 504Rc	0.568	0.432	5.48**	6.97**
6	<i>Acp-1</i>	ES 70-6 × SS 404	0.359	0.641	62.54***	0.38
	<i>Pox-2</i>	761 × 504Rc	0.388	0.612		
	<i>Sdh-1</i>	ES 70-6 × SS 404	0.355	0.645	64.83***	0.73
7	<i>Est-9</i>	C8669 × 221	0.579	0.421	4.12*	4.13*
8	<i>Amp-2</i>	108 × YS 309	0.577	0.423	4.02*	3.20
11	<i>Adh-1</i>	108 × BS 20	0.572	0.428	7.23*	0.68
	<i>Pgd-1</i>	ES 70-6 × YS 309	0.623	0.377	37.39***	0.07
		108 × BS 20	0.609	0.391	8.70*	1.83
		108 × YS 309	0.582	0.418	2.61	4.41*
?	<i>Est-1</i>	IR50 × Simtharo	0.405	0.595		
	<i>Pox-3</i>	108 × BS 20	0.478	0.522	0.26	7.38*
		108 × YS 309	0.375	0.625	7.00*	0.01
	<i>Pox-4</i>	ES 70-6 × YS 309	0.477	0.523	1.00	6.13*

NOTE: *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$.

Loose linkage (over 35% recombination) was found, in one progeny for each pair of loci, between *Amp-2* and *Pgi-3*, *Pgi-3* and *Acp-2*, *Pox-3* and *Pgi-2*, *Pgi-1* and *Est-3*, *Acp-1* and *Est-6*, and *lg* and *Pox-2* (results not shown). For the reasons that are discussed below, such results have to be considered with care, and further analyses are required for confirmation.

Discussion

The results presented here provide new data on the inheritance of enzymatic systems and linkage of isozyme loci. They also point out cases of distorted monolocus and bilocus segregations.

Genetic control and linkage relationships newly found

For each of the 30 investigated loci, in particular for loci *Acp-4*, *Enp-1*, *Est-6*, *Est-7*, *Est-8*, *Got-2*, *Got-3*, *Mdh-1*, *Pgi-3*, and *Pox-4*, for which no segregation data had been presented before, at least one progeny supported the hypothesis of a monogenic segregation. The inheritance models previously proposed are therefore confirmed, in spite of the occurrence of some deviant segregations.

Numerous cases of independence between loci were demonstrated. None of them is in contradiction with the information previously obtained either from classical linkage studies or from trisomic analyses (Ishikawa et al. 1986; Ran-

Various progeny yielded contradictory results for the pairs of loci *Acp-1/Pox-2*, *Acp-1/Sdh-1*, and *Cat-1/Est-2*. Ranjhan et al. (1988) and Wu et al. (1988) localized *Sdh-1* and *Acp-1* on chromosome 6 by dosage effect in heterozygous trisomics. Furthermore, Wu et al. (1988) reported the existence of a tight linkage between *Pox-2* and *Sdh-1* and a moderate linkage between *Pox-2* and *Acp-1*, which suggested that *Pox-2* resides between *Sdh-1* and *Acp-1*. Thus, the associations we observed probably indicate a genuine linkage. Other inconsistencies of linkage values are noteworthy: the linkage between *Est-2* and *Cat-1* observed in 1 progeny of 3 is in contradiction with the independence reported by Second and Morishima (1981). Thus, this might be another instance of inconsistent recombination value on a chromosome, since Wu et al. (1988) localized *Cat-1* on chromosome 3 by trisomic analysis, where *Est-2* is also located.

Another contradiction involving *Est-2* was observed in the only F₂ ES70-6 × SS404, where a significant result of linkage test was calculated for couples *Est-2/Acp-1* and *Est-2/Sdh-1*. These results are in contradiction with those obtained with trisomics, which show the location of *Acp-1* and *Sdh-1* on chromosome 6 (Ranjhan et al. 1988; Wu et al. 1988).

Our observations combined with the information presented by Wu et al. (1988) are summarized in Fig. 2. *Got-2*

TABLE 6 (concluded)

Loci		Cross (parental genotypes)		Segregation ^a						χ^2			% recombination (mean \pm SD)		
				Locus A	Locus B					Locus A	Locus B	Linkage ^b			
A	B	AB	ab		AA	BB	B-	Bb	b-				bb		
<i>Est-2</i>	<i>Sdh-1</i>	IR 50 \times IR 2298		AA	47		7		0	0.90	0.52		7.5 \pm 1.4		
				Aa	4		78		10						
				aa	0		8		42						
		868 \times 504		A-	49		74		17	3.75	2.05	38.88***	19.7 \pm 3.3		
				aa	2		8		22						
		143 \times 221		AA	16		6		0	1.62	0.33	95.72***	13.2 \pm 2.5		
				Aa	9		43		5						
				aa	1		5		25						
		108 \times W06		AA	18		6		0	0.57	1.59		10.7 \pm 2.5		
				Aa	5		34		1						
				aa	0		5		15						
		<i>Est-2</i>	<i>Sdh-1</i>	C8669 \times 221		A-	74		91		20	0.34	7.97*	122.76***	16.0 \pm 2.5
A-	3						13		51						
ES 70-6 \times SS 404				AA	3		8		10	76.4 ***	64.8 ***	11.9 ***			
				a-	45		176		146						
				AA	6		18		4	1.91	1.62	4.69			
108 \times BS20				Aa	11		20		9						
				aa	2		13		7						
				AA	47		4		0	0.63	0.58		7.2 \pm 1.4		
<i>Pgi-2</i>	<i>Amp-3</i>			IR 50 \times IR 2298		Aa	6		79		7				
						aa	0		10		42				
						AA	18		4		2	1.35	0.12		15.6 \pm 3.0
				868 \times 504		Aa	7		35		2				
		aa	0				9		14						
		AA	14				6		3	3.34	0.93		16.6 \pm 3.2		
		C8669 \times 221		Aa	2		29		2						
				aa	1		7		18						
				AA	17		13		4	1.95	0.26	26.02***	30.8 \pm 3.2		
		SB 309 \times WB 01		Aa	15		55		16						
				aa	7		18		21						
				A-	2		28		11	0.01	0.77		5.3 \pm 3.1		
<i>Pox-4</i>	<i>Pox-3</i>	IB 3.5 \times 2 LB 104		aa	13		1		0						
				AA	24		34		14	1.30	2.45	36.00***	34.4 \pm 2.7		
				Aa	27		79		27						
		C8669 \times 221		aa	6		19		34						
				AA	5		12		1	0.41	1.71	5.07	38.7 \pm 5.2		
				Aa	7		24		10						
		414 \times 504		aa	4		10		8						
				AA	7		15		5	1.36	1.11	2.15	47.3 \pm 5.3		
				Aa	13		17		11						
		<i>wx</i>	<i>Est-2</i>	868 \times 504		aa	6		8		7				
						A-			67		8	2.67	2.67	10.11***	24.0 \pm 3.6
						aa			8		8				
143 \times 221				A-	21		50		12	0.01	1.62	33.56***	19.6 \pm 4.2		
				aa	1		7		19						
				AA	61				133	0.53	0.34	10.19***	24.5 \pm 2.2		
C8669 \times 221				aa	6				52						

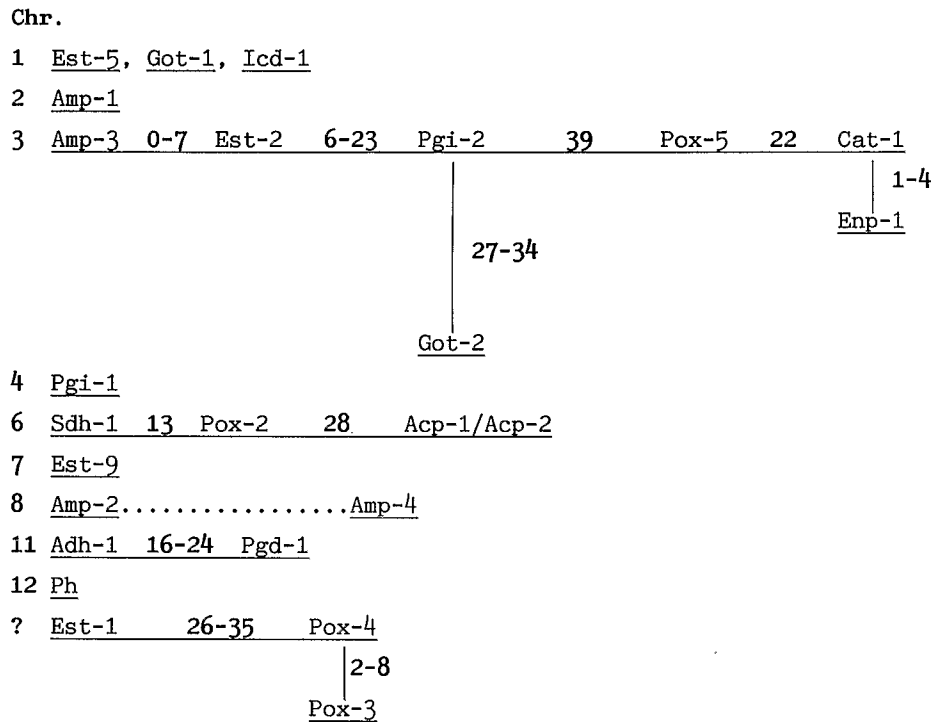


FIG. 2. Chromosomal location of isozyme loci in rice (from our results and those of Wu et al. 1988). The distances given are the extreme calculated values. independent; ———, linked. Orthogonal positions indicate linkages that are not orientated in relation to the others. Chr., chromosome.

tion of isozyme markers throughout the genome is heterogeneous since seven of them are found on chromosome 3. However, at least eight chromosomes are marked, which makes isozyme loci potentially useful genetic markers in breeding programs.

This is also of some value for the study of rice genetic resources. Several of the diagnostic loci for the *indica-japonica* differentiation, such as *Acp-1* and *Ph* (Oka 1958; Shahi et al. 1969; Pai and Fu 1977; Inouye and Hagiwara 1980; Ghesquière and Second 1983), *Pgi-1*, *Pgi-2/Est-2*, *Cat-1* (Second 1982), or *Pgi-1*, *Acp-2*, and *Amp-2* (Glaszmann et al. 1984), are not chromosomally linked. Therefore, gametic disequilibrium should be attributed to another cause (drift-selection).

Abnormal segregations

A number of well-known loci have been reported to occasionally display aberrant segregations. This was recently observed for restriction fragment length polymorphism markers by McCouch et al. (1988), not only in *ssp. indica* × *ssp. japonica* progeny but also in *ssp. japonica* × *ssp. japonica* crosses. Various models have been proposed to explain aberrant segregations in rice, involving both gametophytic and sporophytic selection mechanisms

mal segregations are more likely in crosses between subspecies than within subspecies, since only 2 of the 11 crosses where distortions were observed are, *sensu stricto*, within the same subspecies. (ii) Eighteen loci are affected, which are distributed on at least seven chromosomes (Table 5); thus, a large portion of the genome is involved. (iii) The deviations from Mendelian ratios result from gametic selection rather than from zygotic selection and the alteration of allelic frequencies in the pool of F_1 gametes is the major source of segregation distortions.

Cosegregations were observed between *Est-2* and both locus *Acp-1* and locus *Sdh-1*, located on chromosomes 3 and 6, respectively. This was observed in the cross between subspecies (*japonica* × *indica*) ES70-6 × SS404 and displaying strongly distorted monolocus segregations. Such pseudolinkage between independent genes was observed by Oka (1956, 1983), who explained it by their linkage with duplicate gametic development genes, whose recombination produces disadvantaged gametes. In particular, this was noted between the loci *Rc* (red pericarp) and *wx*. The latter locus is linked to *Est-2*. This suggests that the same causal mechanism could be involved.

Occurrence of such restrictions to recombination leads one to suspect the validity of the weak linkages noted above.

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- ALLARD, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. *Hilgardia*, **24**: 235-278.
- DE KOCHKO, A. 1987. Isozymic variability of traditional rice (*Oryza sativa* L.) in Africa. *Theor. Appl. Genet.* **73**: 675-682.
- GHSQUIÈRE, A., and SECOND, G. 1983. Polymorphisme enzymatique et évolution d'*O. sativa* en Afrique. In *Colloque électrophorèse et taxonomie*. Société Zoologique de France, Paris. pp. 263-272.
- GLASZMANN, J.C. 1987. Isozymes and classification of Asian rice varieties. *Theor. Appl. Genet.* **74**: 21-30.
- GLASZMANN, J.C., BENOIT, H., and ARNAUD, M. 1984. Classification des riz cultivés (*Oryza sativa* L.). Utilisation de la variabilité isoenzymatique. *Agron. Trop.* **39**: 51-66.
- IKEHASHI, H. 1982. Prospects for overcoming barriers in the utilisation of *indica-japonica* crosses in rice breeding. *Oryza*, **19**: 69-77.
- INOUE, J., and HAGIWARA, T. 1980. Classification of floating rice varieties by acid phosphatases and peroxidase zymograms. *Jpn. J. Trop. Agric.* **24**: 159-164.
- ISHIKAWA, R., KINOSHITA, T., and MORISHIMA, H. 1986. Trisomic analysis of genes for isozymes: location of *Cat-1*, *Acp-1* and *Pox-2* on chromosomes. *Rice Genet. Newsl.* **4**: 75-76.
- IWATA, N., NAGAMATSU, T., and OMURA, T. 1964. Abnormal segregation of waxy and apiculus coloration by a gametophyte gene belonging to the first linkage group in rice. *Jpn. J. Breed.* **14**: 33-39. (In Japanese with English summary.)
- KHUSH, G.S., SINGH, R.J., SUR, S.C., and LIBROJO, A.L. 1984. Primary trisomics of rice: origin, morphology, cytology and use in linkage mapping. *Genetics*, **107**: 141-163.
- KINOSHITA, T. 1984. Gene analysis and linkage map. In *Biology of rice*. Edited by S. Tsunoda and N. Takeda. Japan Science and Technology Agency, Tokyo. pp. 117-118.
- OKA, H.I. 1953. Phylogenetic differentiation of the cultivated rice plant. IX. Influence of intervarietal hybrid sterility on segregation ratios in rice. *Jpn. J. Breed.* **3**: 31-39. (In Japanese with English summary.)
- _____. 1956. Phylogenetic differentiation of the cultivated rice plant. XIII. Restriction on gene recombination in hybrid populations of rice. *Jpn. J. Breed.* **6**: 185-191.
- _____. 1958. Intervarietal variation and classification of cultivated rice. *Indian J. Genet. Plant Breed.* **18**: 79-89.
- _____. 1983. The *indica-japonica* differentiation of rice cultivars. A review. In *Crop improvement research*. Edited by T.C. Yap, K.M. Graham, and J. Sukaimi. Proceedings of the 4th International SABRAO Congress, SABRAO. pp. 117-128.
- PAI, C., and FU, P.Y. 1977. Genetic analysis for peroxidase and acid phosphatase isozymes in cultivated rice. *Agronomy Bulletin*, National Chungshing University, Taichung, **2**: 75-85. (In Chinese with English summary.)
- PAI, C., ENDO, T., and OKA, H.I. 1973. Genic analysis for peroxidase isozymes and their organ specificity in *Oryza perennis* and *O. sativa*. *Can. J. Genet. Cytol.* **15**: 845-853.
- _____. 1975. Genic analysis for acid phosphatase isozymes in *Oryza perennis* and *O. sativa*. *Can. J. Genet. Cytol.* **17**: 637-650.
- RANJHAN, S., GLASZMANN, J.C., RAMIREZ, D., and KHUSH, G.S. 1988. Chromosomal location of four isozyme loci by trisomic analysis in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **75**: 541-545.
- SANO, R., and BARBIER, P. 1985. Analysis of five isozyme genes and chromosomal location for *Amp-1*. *Rice Genet. Newsl.* **2**: 60-62.
- SECOND, G. 1982. Origin of the genetic diversity of cultivated rice (*Oryza* spp.): study of the polymorphism scored at 40 isozyme loci. *Jpn. J. Genet.* **57**: 25-57.
- _____. 1985. Evolutionary relationships in the *Sativa* group of *Oryza* based on isozyme polymorphism. *Genet. Sel. Evol.* **17**: 89-114.
- SECOND, G., and MORISHIMA, H. 1981. Mendelian segregation analysis at 3 isozyme loci in rice cultivars. Annual report, 1980. National Institute of Genetics, Misima. pp. 117-118.