Isozyme markers in rice: genetic analysis and linkage relationships[1]

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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 descendances. 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 F_2 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]
O. breviligulata			
101196	IRRI	Cameroon	
NB2	ORSTOM	Niger	
IB 3.5	ORSTOM	Ivory Coast	
2LB 104	ORSTOM	Mali	
SB 309	ORSTOM	Senegal	
WB 01	ORSTOM	Botswana	
O. rufipogon			
W106	NIG	India	
O. sativa			
Bansi	IRAT	India	*indica*
Bete 3	IRAT	Ivory Coast	*japonica*
BG 90-2	IRRI	Sri Lanka	*indica*
BS 20	ORSTOM	Guinea-Bissau	*indica*
Carreon	IRAT	Philippines	*indica*
Century Patna 231	IRRI	United States	*japonica*
Chianan 8	IRAT	Taiwan	*japonica*
C8669	NIG	India	*indica*
ES 70-6	ORSTOM	Tanzania	*japonica*
ES 79	ORSTOM	Tanzania	*indica*
Gaebyo	IRRI	Korea	*japonica*
H 105	IRAT	Sri Lanka	*indica*
IR 2298-PLPB	IRRI		*indica*
IR 50	IRRI		*indica*
IRAT 13	IRAT		*japonica*
Lung Sheng 1	IRAT	Taiwan	*japonica*
Moroberekan	IRAT	Ivory Coast	*japonica*
PJ 110	IRAT		Intermediate
Pursigui	IRAT	Philippines	*indica*
RT 1031-69	IRAT	Zaire	*japonica*
Simtharo	IRRI	Nepal	*indica*
SS 404	ORSTOM	Senegal	*indica*
Taichung Native 1	IRAT	Taiwan	*indica*
Tatsuminochi	IRAT	Japan	*japonica*
Tjempo Welut	IRAT	Indonesia	*japonica*
YS 309	ORSTOM	Guinea	*japonica*
YS 45-1	ORSTOM	Guinea	*japonica*
108	NIG	Taiwan	*indica*
143	NIG	Taiwan	*indica*
221	NIG	Philippines	*japonica*
414	NIG	India	*indica*
504[c]	NIG	Taiwan	*japonica*
761	NIG		
868	NIG	China	*indica*

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

[b]Classification of *O. sativa* accessions is based on their enzymatic profiles (Second 1982; Glaszmann 1987).

[c]Three isogenic lines of 504 were used: 504 *Rc wx dl* (noted 504Rc), 504 *g gl lg 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.

Material and methods

Material

The institutions storing collections and the country of origin of parental accessions are presented in Table 1. For *O. sativa* samples, the subspecific classification as determined by isozymes (Second 1982; Glaszmann 1987) is indicated. The 32 F_2 and 1 backcross 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 immature panicles) F_1 plants produced F_2 progeny (Table 2). Fertility measured in terms of seed set was determined for some of the F_1 plants (Table 2). This is important information because high sterility may result in a segregation bias in the following generation (Oka 1953). It would obviously be better to use only progeny of highly fertile hybrids, but lack of isozyme polymorphisms in related accessions often requires hybridization of varieties belonging to distinct varietal groups, in which semisterility is com-

TABLE 2. Origin of F_2 and BC progeny: parents, F_1 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 F_1 (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), *d1* ("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 F_2 progeny were considered individually. However, a special population, called "pooled F_2," was constituted by grouping 11 small F_2 populations derived from crosses between different varietal types (Table 2); the three genotypic classes were obtained by pooling, respectively, female parent, F_1, 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 equifrequency 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
Acp-1	PAc.AMC			ACP-1			
Acp-2	PAc.FaSa	PAC-2		ACP-2			
Acp-4		PAC-1					
Adh-1	ADH-A						Adh-1
Amp-1	LAP-E	LAP					
Amp-2		AAP				Amp-2	
Amp-3	LAP-F					Amp-1	
Cat-1	CAT-A	CAT			CAT-1		
Enp-1	EP-A						
Est-1	EST-D	EST-3	EST-1				
Est-2	EST-E	EST-4	EST-2		EST-2		
Est-3	EST-J		EST-3				
Est-5	EST-B						
Est-6	EST-C	EST-2					
Est-7	EST-I	EST-5					
Est-8		EST-6					
Est-9	EST-Ca	EST-1				Est-C1	Est-8
Got-1	GOT-A						
Got-2	GOT-B						
Got-3	GOT-C						
Icd-1	ICD-A						
Mdh-1	MDH-A						
Pgd-1	PGD-A					Pgd-1	
Pgi-1	PGI-A	PGI-1			PGI-1		Pgi-1
Pgi-2	PGI-B	PGI-2			PGI-2		
Pgi-3		PGI-3					
Pox-2				Px2			
Pox-3	POX-B	POX					
Pox-4	POX-C						
Sdh-1	SDH-A					Sdh-1	Sdh-1

[a]1, 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 marginal segregations to a Mendelian segregation and the linkage between these loci were calculated. Progeny showing strong one-locus distortions were not used for linkage calculations. Recombination values and standard errors were estimated by the maximum-likehood method (Allard 1956).

Results

Monogenic segregations

Segregations were analysed for a total of 30 loci among 121 populations (Table 4).

Ninety-five segregations fitted expected monogenic Mendelian segregation, whereas 26 significantly deviated from it. However, each locus displayed at least one Mendelian segregation among the several F_2s tested.

Of the 30 loci investigated, 18 showed deviations from expected ratios in 12 different progeny (including "pooled F_2"). Eight of these progeny were derived from controlled self-pollination. Thus, cross-pollination, if it occurred, is not a sufficient explanation for the deviations observed in free-pollinated progeny.

Seven of the deviant progeny were obtained from crosses between *O. sativa* subspecies *indica* and *japonica*, and four from crosses within the same subspecies. Among the latter, two involved parents BS20 and YS309, which are of particular interest because they show traces of introgression from the wild species *O. longistaminata*.

The distorted segregations were analysed to describe the parameters of distortion (Table 5). Allelic frequencies significantly differed from the expected (1:1) ratio in almost all cases, mostly in favor of *O. sativa* ssp *indica* alleles. However, a random reassortment of gametes was observed in most F_2s.

Linkage

Linkage tests for 155 pairs of isozyme loci and other markers are summarized in Fig. 1. Detailed cosegregations for the loci among which linkage was detected are presented in Table 6.

Evidence was found for linkage between *Acp-1* and *Acp-2*, *Adh-1* and *Pgd-1*, *Pgi-2* and *Got-2*, and *Enp-1* and *Cat-1* and for the existence of linkage groups *Pgi-1/Est-2/Amp-3/wx* and *Pox-3/Pox-4/Est-1*.

Contradictory results, i.e., either independence or linkage, depending on the progeny, were found in the combinations *Acp-1/Pox-2*, *Sdh-1/Acp-1*, *Est-2/Cat-1*, *Est-2/Sdh-1*, and *Est-2/Acp-1*. The recombination frequency between *Pgi-2* and *wx* also showed some variation. However, enough evidence can be obtained to show that these two loci belong to the same linkage group.

TABLE 4. Monolocus F_2 segregations at 30 presumed loci

Locus	Cross $P_1 \times P_2$	Species or subspecies[a]	No. of F_2 genotypes P_1	F_1	P_2	χ^2
Acp-1	ES 70-6 × SS 404	*j* × *i*	48	187	159	63.56***
	BS 20 × YS 45-1	*i* × *j*	59	133	75	1.92
	108 × BS 20	*i* × *i*	18	22	8	4.50
	143 × 221	*i* × *j*	32	55	23	1.47
	C8669 × 221	*i* × *j*	73	124	55	2.63
	761 × 504R	*i* × *j*	47	69	30	4.40
	761 × 504g	*i* × *j*	34	64	21	3.52
Acp-2	ES 70-6 × YS 309	*j* × *j*	85	⊢—247—⊣		0.06
	ES 70-6 × SS 404	*j* × *i*	47	⊢—351—⊣		36.90***
	BS 20 × YS 45-1	*i* × *j*	⊢—193—⊣		74	1.05
	Pooled F_2		63	⊢—199—⊣		0.13
Acp-4	Pooled F_2		62	100	61	2.38
Amp-1	ES 70-6 × YS 309	*j* × *j*	76	175	73	2.14
	108 × YS 309	*i* × *j*	24	49	11	6.36*
	IR 50 × Simtharo	*i* × *i*	48	67	31	4.95
	868 × 504	*i* × *j*	25	48	18	1.35
	C8669 × 221	*i* × *j*	17	42	23	0.93
Amp-2	868 × 504R	*i* × *j*	16	46	29	3.73
	414 × 504R	*i* × *j*	18	44	27	1.83
	C8669 × 221	*i* × *j*	24	43	15	2.17
	Pooled F_2		68	132	79	1.67
Amp-3	IR 50 × Simtharo	*i* × *i*	37	170	104	1.39
	CP 231 × Gaebyo	*j* × *j*	3	27	56	80.23***
	IR 50 × IR 2298	*i* × *i*	53	93	49	0.58
	868 × 504R	*i* × *j*	25	48	18	1.35
	C8669 × 221	*i* × *j*	17	42	23	0.93
Adh-1	BS 20 × YS 45-1	*i* × *j*	69	130	62	0.38
	108 × BS 20	*i* × *i*	54	90	29	7.51*
Cat-1	ES 70-6 × YS 309	*j* × *j*	76	149	90	2.16
	ES 70-6 × SS 404	*j* × *i*	105	178	108	3.18
	414 × 504	*i* × *j*	36	45	29	4.53
	868 × 504	*i* × *j*	18	45	18	1.00
	C8669 × 221	*i* × *j*	83	85	37	26.62***
	Pooled F_2		32	63	28	0.33
Enp-1	ES 70-6 × ES 79	*j* × *i*	⊢—76—⊣		39	4.87*
	YS 45-1 × ES 79	*j* × *i*	⊢—65—⊣		15	1.67
Est-1	ES 70-6 × YS 309	*j* × *j*	90	⊢—238—⊣		1.04
	ES 70-6 × SS 404	*j* × *i*	94	⊢—295—⊣		0.15
	BS 20 × YS 45-1	*i* × *j*	⊢—145—⊣		57	1.12
	BS 117 × YS 138-3	*i* × *j*	⊢—34—⊣		14	0.44
	IR 50 × Simtharo	*i* × *i*	24	⊢—122—⊣		5.70*
	IR 50 × IR 2298	*i* × *i*	39	⊢—156—⊣		2.60
	Pooled F_2		23	⊢—56—⊣		0.71
Est-2	ES 70-6 × YS 309	*j* × *j*	64	⊢—264—⊣		2.98
	ES 70-6 × SS 404	*j* × *i*	23	⊢—375—⊣		78.42***
	108 × BS 20	*i* × *i*	40	80	53	2.93
	108 × YS 309	*i* × *j*	20	45	30	2.37
	IR 50 × Simtharo	*i* × *i*	39	66	41	1.40
	IR 50 × IR 2298	*i* × *i*	53	92	50	0.71
	CP 231 × Gaebyo	*j* × *j*	30	⊢—56—⊣		73.81***
	Pooled F_2		14	28	8	2.16
	868 × 504R	*i* × *j*	⊢—75—⊣		16	2.67
	143 × 221	*i* × *j*	22	57	31	1.62
	C8669 × 221	*i* × *j*	⊢—187—⊣		67	0.25
Est-3	IR 50 × Simtharo	*i* × *i*	9	28	9	2.17
Est-5	BS 117 × YS 138-3	*i* × *j*	8	⊢—40—⊣		1.78
Est-6	BS 117 × YS 138-3	*i* × *j*	⊢—40—⊣		8	1.78
	Pooled F_2		41	⊢—97—⊣		1.63
Est-7	BO4 × NB2	*B* × *B*	24	⊢—77—⊣		0.08
	Pooled F_2		23	⊢—59—⊣		0.44
Est-8	Pooled F_2		59	⊢—167—⊣		0.90

TABLE 4 (*concluded*)

Locus	Cross $P_1 \times P_2$	Species or subspecies[a]	No. of F_2 genotypes P_1	F_1	P_2	χ^2
Est-9	ES 70-6 × SS 404	*j* × *i*	19	41	26	1.32
	IR 50 × IR 2298	*i* × *i*	36	102	57	4.94
	414 × 504	*i* × *j*	34	45	31	3.80
	868 × 504R	*i* × *i*	21	43	27	1.07
	C8669 × 221	*i* × *j*	19	31	32	9.00*
	Pooled F_2		66	89	54	5.98
Got-1	BS 20 × YS 45-1	*i* × *j*	76	135	47	7.08*
	108 × BS 20	*i* × *i*	22	48	21	0.30
Got-2	SB 309 × WB 01	*B* × *B*	39	86	41	0.27
Got-3	ES 70-6 × YS 309	*j* × *j*	80	168	70	1.33
	108 × YS 309	*i* × *j*	16	51	30	4.30
Icd-1	IR 50 × IR 2298	*i* × *i*	37	113	45	5.58
	CP 231 × Gaebyo	*j* × *j*	15	43	28	3.93
Mdh-1	BO4 × NB2	*B* × *B*	27	44	30	1.85
Pgd-1	ES 70-6 × YS 309	*j* × *j*	121	143	45	39.60***
	ES 70-6 × SS 404	*j* × *i*	99	186	112	2.43
	108 × BS 20	*i* × *i*	31	50	11	9.39**
	108 × YS 309	*i* × *j*	13	31	5	6.06*
	C8669 × 221	*i* × *i*	26	40	16	2.49
	414 × 504	*i* × *j*	42	36	8	29.16***
	868 × 504	*i* × *j*	21	43	27	1.07
Pgi-1	SB 309 × WB 01	*B* × *B*	42	86	38	0.41
	108 × BS 20	*i* × *i*	20	49	19	1.16
	108 × YS 309	*i* × *j*	24	44	29	1.35
	BS 117 × YS 138-3	*i* × *j*	13	22	13	0.33
	IR 50 × Simtharo	*i* × *i*	36	80	30	1.84
	Morober. × PJ 110	*j* × *i*	11	32	11	1.85
	868 × 504	*i* × *j*	28	48	15	3.99
	C8669 × 221	*i* × *j*	74	111	68	4.08
	143 × 221	*j* × *i*	23	60	27	1.20
	108 × W106	*i* × *R*	22	39	23	0.45
	868 × 504R	*i* × *j*	28	48	15	4.99
	761 × 504R	*i* × *j*	55	56	35	13.40**
	761 × 504g	*i* × *j*	21	64	34	3.52
Pgi-2	SB 309 × WB 01	*B* × *B*	34	86	46	1.95
	ES 70-6 × SS 404	*j* × *i*	56	206	136	32.65***
	BS 20 × YS 45-1	*i* × *j*	68	123	65	0.46
	108 × YS 309	*i* × *j*	22	43	32	3.31
	IR 50 × IR 2298	*i* × *i*	51	92	51	0.52
	868 × 504R	*i* × *j*	24	44	23	0.12
	C8669 × 221	*i* × *j*	71	104	78	8.39*
	143 × 221	*i* × *j*	26	54	30	0.33
	108 × W106	*i* × *R*	16	45	20	1.40
	Pooled F_2		28	33	29	6.42*
	761 × 504R	*i* × *j*	56	64	26	14.55***
Pgi-3	Pooled F_2		65	⊢—187—⊣		0.08
Pox-2	143 × 221	*i* × *j*	⊢—83—⊣		27	0.01
	761 × 504R	*i* × *j*	⊢—124—⊣		22	7.68**
	761 × 504g	*i* × *j*	⊢—96—⊣		26	0.63
Pox-3	IB 3.5 × 2LB 104	*B* × *B*	15	29	11	0.75
	108 × BS 20	*i* × *i*	10	45	13	7.38*
	108 × YS 309	*i* × *j*	8	26	22	7.29*
Pox-4	IB 3.5 × 2LB 104	*B* × *B*	14	⊢—41—⊣		0.01
	ES 70-6 × YS 309	*j* × *j*	65	102	76	7.26*
	ES 70-6 × SS 404	*j* × *i*	71	133	84	2.85
Sdh-1	ES 70-6 × SS 404	*j* × *i*	45	185	157	65.57***
	BS 20 × YS 45-1	*i* × *i*	72	133	59	1.30
	108 × BS 20	*i* × *i*	19	50	19	1.64
	C8669 × 221	*i* × *j*	16	47	19	1.98
	414 × 504	*i* × *j*	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 F_2 segregations

Chromosome	Locus	Cross ($P_1 \times P_2$)	Allele frequency P_1	Allele frequency P_2	χ^2 Allele frequency homogeneity	χ^2 F_2 distribution (p^2:2pq:q^2)
1	*Got-1*	BS 20 × YS 45-1	0.556	0.444	6.52*	0.93
3	*Pgi-2*	ES 70-6 × SS 404	0.399	0.601	32.16**	2.47
	Amp-3	CP231 × Gaebyo	0.192	0.808	65.3 ***	0.01
	Est-2	ES 70-6 × SS 404	0.240	0.760		
		CP231 × Gaebyo	0.193	0.807		
	Cat-1	C8669 × 221	0.612	0.388	20.64***	3.29
		ES 70-6 × ES 79	0.404	0.596	8.42**	0.10
	Enp-1	ES 70-6 × ES 79	0.418	0.582	8.42**	0.10
4	*Pgi-1*	761 × 504Rc	0.568	0.432	5.48**	6.97**
6	*Acp-1*	ES 70-6 × SS 404	0.359	0.641	62.54***	0.38
	Pox-2	761 × 504Rc	0.388	0.612		
	Sdh-1	ES 70-6 × SS 404	0.355	0.645	64.83***	0.73
7	*Est-9*	C8669 × 221	0.579	0.421	4.12*	4.13*
8	*Amp-2*	108 × YS 309	0.577	0.423	4.02*	3.20
11	*Adh-1*	108 × BS 20	0.572	0.428	7.23*	0.68
	Pgd-1	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*
?	*Est-1*	IR50 × Simtharo	0.405	0.595		
	Pox-3	108 × BS 20	0.478	0.522	0.26	7.38*
		108 × YS 309	0.375	0.625	7.00*	0.01
	Pox-4	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; Ranjhan et al. 1988; Wu et al. 1988). Our results also confirmed the linkage of *Acp-1* and *Acp-2* (Pai et al. 1973), of *Adh-1* and *Pgd-1* (Wu et al. 1988), of *Pgi-2* and *Est-2* (Morishima and Sano 1984), and of *wx*, *Est-2* and *Amp-3* (Nakagahra and Hayashi 1976; Sano and Barbier 1985). New linkages were demonstrated, namely, between *Pgi-2* and *Got-2*, *Enp-1* and *Cat-1*, *Est-1* and *Pox-4*, and *Pox-3* and *Pox-4*.

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 F_2 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* and *Enp-1* are newly localized on chromosome 3. Their precise position on that chromosome remains to be determined. A new isozyme linkage group is identified with *Est-1*, *Pox-3*, and *Pox-4* but is not assigned as yet to any given chromosome.

Of the 30 loci under study, 18 could be assigned to one of the 12 chromosomes. As shown in Fig. 2, the distribu-

TABLE 6. Cosegregation data for 19 pairs of loci

Loci		Cross (parental genotypes)		Segregation[a]						χ^2			
					Locus *B*								
A	*B*	*AB*	*ab*	Locus *A*	*BB*	*B-*	*Bb*	*b-*	*bb*	Locus *A*	Locus *B*	Linkage[b]	% recombination (mean ± SD)
Acp-1	*Pox-2*	868 × 504		*AA*		19			6	0.00	2.01	1.59	44.1 ± 6.7
				Aa		32			8				
				aa		10			6				
		143 × 221		*AA*		28			4	1.47	0.01	6.65*	35.0 ± 5.4
				Aa		42			13				
				aa		13			10				
		761 × 504		*AA*		45			2	7.68*	4.40*	8.26*	33.5 ± 4.6
				Aa		57			12				
				aa		28			8				
		761 × 504		*AA*		37			1	7.48*	4.71*	22.16***	17.3 ± 3.6
				Aa		64			6				
				aa		10			12				
		(868 × 504) × 504 Backcross		*Aa*			20		4	1.60	0.01		12.5 ± 5.2
				aa			1		15				
Adh-1	*Pgd-1*	108 × BS 20		*AA*	13		5		2	3.72	2.04		20.3 ± 4.0
				Aa	7		26		4				
				aa	0		4		6				
Amp-2	*Pgi-3*	Pool F_2		*AA*		31			22	3.11	0.10	12.09*	37.0 ± 4.0
				Aa		70			23				
				aa		53			9				
Amp-3	*Est-2*	IR 50 × Simtharo		*AA*	87		0		0	2.82	2.82		0
				Aa	0		170		0				
				aa	0		0		104				
		IR 50 × IR 2298		*AA*	53		0		0	0.58	0.71		0.3 ± 0.3
				Aa	0		92		1				
				aa	0		0		49				
		868 × 504		*AA*		25			0	1.35	2.67		4.9 ± 2.3
				Aa		47			1				
				aa		3			15				
		C8669 × 221		*AA*	17			0		0.73	0.10		2.6 ± 1.8
				Aa	2			40					
				aa	0			22					
		CP 231 × Gaebyo		*AA*		3			0	73.83***	73.83***		0
				Aa		27			0				
				aa		0			56				
Amp-2	*Pgi-3*	Pooled F_2		*AA*		31			22	3.11	0.10	12.09**	37.0 ± 4.0
				Aa		70			23				
				aa		53			9				
Cat-1	*Est-2*	868 × 504Rc		*AA*		17			1	1.00	1.19	7.64*	27.5 ± 5.7
				Aa		38			7				
				aa		10			8				
		C8669 × 221		*AA*	25			58		26.62***	0.37	2.20	
				Aa	23			62					
				aa	7			30					
		ES70-6 × YS309		*AA*	14			62		2.28	5.20*	0.59	
				Aa	31			117					
				aa	16			74					
Enp-1	*Cat-1*	ES 70-6 × ES 79		*A-*	18		56		2	4.87*	10.43**		2.2 ± 1.4
				aa	0		1		38				
Est-1	*Pox-4*	Es 70-6 × YS 309		*AA*	38		21		11	1.88	7.26*	46.16***	29.5 ± 3.4
				a-	27		81		65				
		ES 70-6 × SS 404		*A-*	32		28		8	0.08	3.62	28.90***	31.9 ± 3.3
				aa	38		99		75				
Est-2	*Acp-1*	Es 70-6 × SS 404		*AA*	3		12		8	76.85***	63.82***	14.33***	
				a-	45		174		151				
Est-2	*Acp-2*	ES 70-6 × YS309		*A-*		193			66	5.53	0.01	0.04	
				aa		47			15				
Est-2	*Pgi-2*	108 × YS 309		*AA*	16		3		1	2.39	4.81		13.5 ± 2.7
				Aa	4		33		8				
				aa	1		5		24				
		Bete 3 × Bansi		*A-*	5		16		15	0.24	3.92	25.84***	18.1 ± 5.9
				aa	11		2		1				

TABLE 6 (*concluded*)

Loci		Cross (parental genotypes)		Segregation[a]						χ^2			
					Locus *B*								
A	*B*	*AB*	*ab*	Locus *A*	*BB*	*B-*	*Bb*	*b-*	*bb*	Locus *A*	Locus *B*	Linkage[b]	% recombination (mean ± SD)
		IR 50 × IR 2298		*AA*	47		7		0	0.90	0.52		7.5 ± 1.4
				Aa	4		78		10				
				aa	0		8		42				
		868 × 504		*A-*	49		74		17	3.75	2.05	38.88***	19.7 ± 3.3
				aa	2		8		22				
		143 × 221		*AA*	16		6		0	1.62	0.33	95.72***	13.2 ± 2.5
				Aa	9		43		5				
				aa	1		5		25				
		108 × W06		*AA*	18		6		0	0.57	1.59		10.7 ± 2.5
				Aa	5		34		1				
				aa	0		5		15				
		C8669 × 221		*A-*	74		91		20	0.34	7.97*	122.76***	16.0 ± 2.5
				A-	3		13		51				
Est-2	*Sdh-1*	ES 70-6 × SS 404		*AA*	3		8		10	76.4 ***	64.8 ***	11.9 ***	
				a-	45		176		146				
		108 × BS20		*AA*	6		18		4	1.91	1.62	4.69	
				Aa	11		20		9				
				aa	2		13		7				
Pgi-2	*Amp-3*	IR 50 × IR 2298		*AA*	47		4		0	0.63	0.58		7.2 ± 1.4
				Aa	6		79		7				
				aa	0		10		42				
		868 × 504		*AA*	18		4		2	1.35	0.12		15.6 ± 3.0
				Aa	7		35		2				
				aa	0		9		14				
		C8669 × 221		*AA*	14		6		3	3.34	0.93		16.6 ± 3.2
				Aa	2		29		2				
				aa	1		7		18				
Pgi-2	*Got-2*	SB 309 × WB 01		*AA*	17		13		4	1.95	0.26	26.02***	30.8 ± 3.2
				Aa	15		55		16				
				aa	7		18		21				
Pox-4	*Pox-3*	IB 3.5 × 2 LB 104		*A-*	2		28		11	0.01	0.77		5.3 ± 3.1
				aa	13		1		0				
Sdh-1	*Acp-1*	BS 20 × YS 45-1		*AA*	24		34		14	1.30	2.45	36.00***	34.4 ± 2.7
				Aa	27		79		27				
				aa	6		19		34				
		C8669 × 221		*AA*	5		12		1	0.41	1.71	5.07	38.7 ± 5.2
				Aa	7		24		10				
				aa	4		10		8				
		414 × 504		*AA*	7		15		5	1.36	1.11	2.15	47.3 ± 5.3
				Aa	13		17		11				
				aa	6		8		7				
wx	*Est-2*	868 × 504		*A-*		67			8	2.67	2.67	10.11***	24.0 ± 3.6
				aa		8			8				
		143 × 221		*A-*	21		50		12	0.01	1.62	33.56***	19.6 ± 4.2
				aa	1		7		19				
		C8669 × 221		*A-*	61			133		0.53	0.34	10.19***	24.5 ± 2.2
				aa	6			52					
wx	*Amp-3*	868 × 504		*A-*	25		40		10	2.67	1.35		23.9 ± 5.0
				aa	0		8		8				
		C8669 × 221		*A-*	15		32		13	0.15	0.93	5.90	34.6 ± 6.2
				aa	2		10		10				
wx	*Pgi-2*	143 × 221		*A-*	22		45		16	0.01	0.33	11.36***	32.8 ± 5.3
				aa	4		9		14				
		868 × 504		*A-*	22		37		16	2.67	0.12	3.32	36.3 ± 6.0
				aa	2		7		7				
		C8669 × 221		*A-*	61		76		57	0.15	3.34	0.66	45.6 ± 6.7
				aa	10		28		21				

NOTE: *Acp-1* and *Acp-2* are very tightly linked (Fig. 1); thus, they do not both appear in the table and are discussed together in the text. *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$.

[a]Symbols *A*-, B-, and *b*- are used when heterozygous genotypes cannot be identified because of segregation of a null allele.

[b]Test was not performed when some classes were too small. This only occurred in clear cases of linkage.

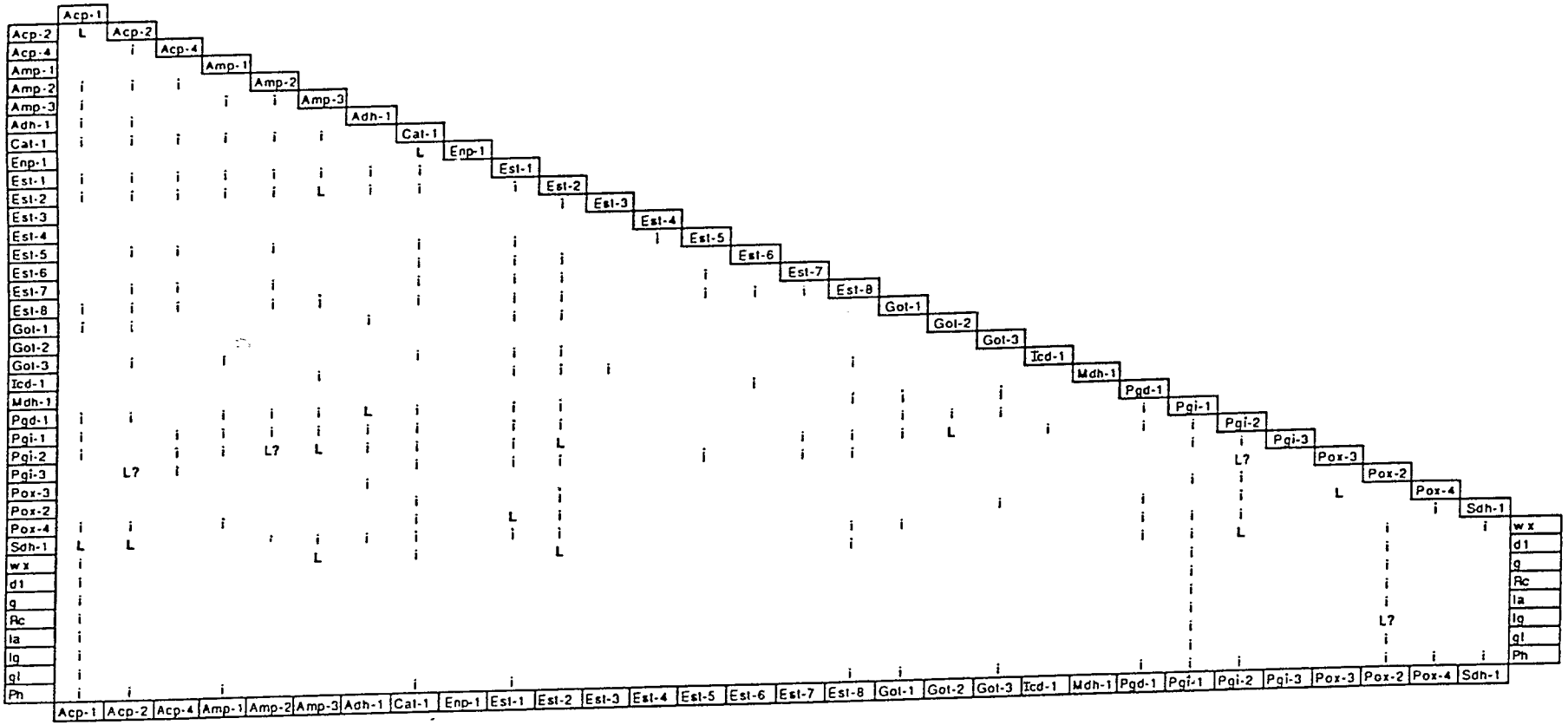

FIG. 1. Relationships between loci. *i*, independence; L, linkage; L?, uncertain linkage (see text).

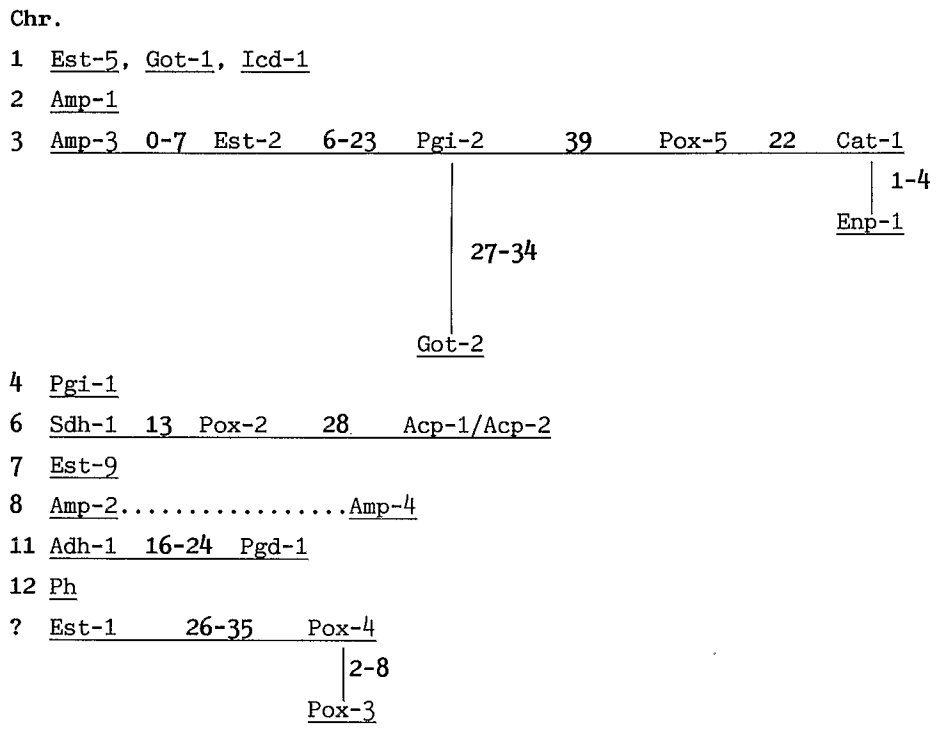

FIG. 2. Chromosomal location of isozyme loci in rice (from our results and those of Wu et al. 1988). The distances given are the extreme calculated values. ····, independent; ———, linked. Orthogonal positions indicate linkages that are not orientated in relation to the others. Chr., chromosome.

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 (Ikehashi 1982; Oka 1983). In particular, the *wx* locus is frequently subject to segregation distortion as a result of its linkage with certation genes (Iwata et al. 1964; Nakagahra et al. 1974) on chromosome 3, where *Pgi-2*, *Est-2*, and *Amp-3* are also located. F_2 or BC_1 data are not sufficient to determine the cause of the distortions reported here (Table 6). However, several comments can be made from our results, keeping in mind that the number of plants studied and, therefore, the power of the test for detection of distortion were not the same in every progeny. (*i*) Abnormal 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. These linkages are all the more doubtful since they were estimated from crosses between different varietal groups and concern loci strongly involved in the discrimination between these groups.

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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.

GHESQUIÈRE, 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.

INOUYE, 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. Takahashi. Japan Scientific Societies Press, Tokyo; Elsevier, Amsterdam. pp. 187–274.

MCCOUCH, S.R., KOCHERT, G., YU, Z.H., WANG, Z.Y., KHUSH, G.S., COFFMAN, W.R., and TANKSLEY, S.D. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. **76**: 815–829.

MORISHIMA, H., and GLASZMANN, J.C. 1986. Gene symbols for isozymes. Rice Genet. Newsl. **3**: 15–17.

MORISHIMA, H., and SANO, Y. 1984. Genetic analysis for isozymes in rice. Rice Genet. Newsl. **1**: 117–118.

NAKAGAHRA, M., and HAYASHI, K. 1976. Detection of esterase isozyme loci of *O. sativa* L. Jpn. J. Breed. **26**: (Suppl. 1): 114–115. (In Japanese.)

NAKAGAHRA, M., OMURA, T., and IWATA, N. 1974. New certation gene on the first linkage group found by inter-subspecific hybridization of cultivated rice. J. Fac. Agric. Kyushu Univ. **18**: 157–167.

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 peroxydase and acid phosphatase isozymes in cultivated rice. Agronomy Bulletin, National Chunghsing University, Taichung, **2**: 75–85. (In Chinese with English summary.)

PAI, C., ENDO, T., and OKA, H.I. 1973. Genic analysis for peroxydase 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.

SECOND, G., and TROUSLOT, P. 1980. Polymorphisme de 13 zymogrammes observés parmi diverses espèces sauvages et cultivées du genre *Oryza*. *In* Electrophorèse d'enzymes de riz. Trav. Doc. ORSTOM, 120.

SHAHI, B.B., MORISHIMA, H., and OKA, H.I. 1969. A survey of variations in peroxydase, acid phosphatase and esterase isozymes of wild and cultivated *Oryza* species. Jpn. J. Genet. **44**: 303–319.

TROUSLOT, P., and SECOND, G. 1980. Technique d'électrophorèse en gel d'amidon appliquée à l'étude du polymorphisme de 14 enzymes de riz. *In* Electrophorèse d'enzymes de riz. Trav. Doc. ORSTOM, 120.

WU, K.S., GLASZMANN, J.C., and KHUSH, G.S. 1988. Chromosomal location of ten isozyme loci in rice (*Oryza sativa* L.) through trisomic analysis. Biochem. Genet. **26**: 303–320.