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Génétique/*Genetics*

Identification of genetic markers for quantitative traits in rice (*Oryza sativa* L.)

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Abstract — 14 genetic markers, mostly isozyme loci located on at least 6 different chromosomes, were identified for several vegetative and reproductive traits in *Oryza sativa*.

Identification de marqueurs génétiques pour des caractères quantitatifs chez le riz (*Oryza sativa* L.)

Résumé — *14 marqueurs génétiques, en majorité des loci isozymiques, situés sur au moins 6 chromosomes différents, ont été identifiés pour plusieurs caractères végétatifs et reproductifs chez* Oryza sativa.

Version française abrégée — INTRODUCTION. — Les progrès sur la carte génétique du riz ([7], [8]) rendent possible chez cette plante la recherche de gènes contribuant aux caractères quantitatifs (QTL : quantitative trait loci) à l'exemple de la tomate ([1] à [4]) ou du maïs ([5], [6]).

Cette Note présente les résultats, issus de l'étude de 3 descendances F2 de croisements intervariétaux chez *Oryza sativa* L., sur l'identification de marqueurs génétiques pour des caractères végétatifs et reproductifs.

MATÉRIELS ET MÉTHODES. — 5 variétés d'*O. sativa* (collection ORSTOM) ont été utilisées : 3 variétés de la sous-espèce *japonica* ES70-6, YS309 et YS45-1, et 2 variétés *indica* SS404 et BS20. Les 3 descendances F2 ES70-6 × SS404 (effectif : 321), ES70-6 × YS309 (284) et BS20 × YS45-1 (175) ont été étudiées.

Les plantes F2 ont été suivies par électrophorèse d'enzymes ([9] à [12]), réaction au phénol des glumelles et mesurées pour de nombreux caractères agromorphologiques dont la fertilité paniculaire. 14 marqueurs, dont 13 loci isozymiques, ont été utilisés au total (tableau I), situés sur au moins 6 chromosomes différents [8].

Une différence significative entre classes génotypiques au locus L (analyse de variance ou éventuellement comparaison des distributions par test chi-2) est interprétée ([1], [3]) comme l'existence d'une liaison entre le locus L et un au moins des QTL du caractère étudié.

RÉSULTATS. — La recherche de QTL (tableau II) s'avère fructueuse : au moins un effet significatif est noté pour chaque caractère. Réciproquement, des effets significatifs sont observés pour chacun des marqueurs.

L'ensemble des QTL détectés se répartit (*fig.* 1) sur tous les chromosomes marqués. Certains secteurs chromosomiques apparaissent cependant plus denses que d'autres en effets détectés, à l'exemple de *Pgi*-2/*Est*-2 comparé à *Cat*-1.

DISCUSSION. — L'interprétation des résultats doit être tempérée pour des raisons méthodologiques : existence des risques de première et deuxième espèce, validité des résultats théoriquement restreinte aux variétés utilisées.

L'élaboration des caractères quantitatifs paraît largement répartie dans le génome. Ainsi la structure paniculaire semble relever de 5 chromosomes différents. Cette organisation pour un

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des caractères sélectionnés lors du processus de domestication [13] peut être opposée à celle des gènes déterminant les caractéristiques de l'épillet chez le mil ([14], [15]).

Certains des loci marqueurs ont été utilisés dans la classification des riz cultivés : *Ph* [16], *Acp*-1/2 ([17], [18]), *Est*-1 ([10], [19]), *Est*-2 et *Pgi*-2 [9]. Ce pouvoir discriminant pourrait être dû à la liaison de ces loci avec des QTL influant sur de nombreux caractères eux-mêmes discriminants.

La mise en évidence de marqueurs communs entre la hauteur et la fertilité apporte un support génétique à des observations de Oka [20] montrant une corrélation entre ces caractères.

INTRODUCTION. — The search for genes determining quantitative traits (QTL : Quantitative Trait Loci) was possible for those plants for which genetic maps of isozyme and/or molecular (RFLP) markers were dense enough: tomato ([1] and [4]) and maize ([5], [6]). Indeed, the search for QTL is so far indirect and is based on the detection of linkage between the QTL and marker loci. Recent progress in mapping of isozyme loci in rice ([7], [8]) make now possible such an approach in this cereal. This paper presents results on the identification of marker loci, mostly isozyme loci, for several vegetative and reproductive traits in *Oryza sativa*. These results were obtained from the observation of three F2 progenies of within-*O. sativa* crosses.

MATERIAL AND METHODS. — *Vegetal material and experimental design.* — Five *O. sativa* varieties were used (ORSTOM collection). When classed from their isozymic genotypes ([9], [10]), ES70-6, YS309 and YS45-1 belong to the *japonica* subspecies, SS404 and BS20 to the *indica* subspecies. All these varieties are homozygous for marker loci. Three crosses were made: ES70-6 × SS404, ES70-6 × YS309 and BS20 × YS45-1. The panicles of F1 hybrids were bagged to avoid cross-pollination. The F2 seeds were sown in glasshouse. The seedlings were planted out in a field divided in 3 parts, each of these in randomized design including one F2 progeny and parental lines. 321 (ES70-6 × SS404), 184 (ES70-6 × YS309) and 175 (BS20 × YS45-1) F2 plants were studied for both quantitative traits and marker segregations.

Quantitative traits. — The number of tillers was counted at 7 weeks (code: T7) and at maturity (TAM). The heading time (HEAD) was the number of days from sowing to heading of the first panicle. Other vegetative traits were scored at heading date: plant height (HGT), length (LGF) and width (WIDF) of the flag leaf. The panicle traits were estimated from the mean of the 3 first panicles: length (LGP), number of primary (BR1) and secondary (BR2) branches, number of grains (NGR) and number of spikelet insertions (NSI). Length (LGG) and width (WIDG) of grain were given by the mean of 10 grains. Four variables were calculated: LGF/WIDF, BR2/BR1, LGG/WIDG and NGR/NSI. This last one (seed fertility) is related to reproductive barriers.

Genetic markers. — Isozyme electrophoresis was done following [9], [11] and [12]. Ten enzymes were studied: alcohol dehydrogenase (*Adh*), 6-phosphogluconate dehydrogenase (*Pgd*), shikimate dehydrogenase (*Sdh*), phosphoglucose isomerase (*Pgi*), catalase (*Cat*), acid phosphatase (*Acp*), peroxydase (*Pox*), glutamate oxaloacetate transaminase (*Got*), esterase (*Est*) and aminopeptidase (*Amp*). The grains harvested on F2 plants were tested by phenol reaction. When soaked in a 2% phenol solution, the hulls of grains present two possible reactions depending of the maternal genotype at locus *Ph*: blackening

("+") or no coloration ("−"). An amount of 14 marker loci [8] were used (Table I), located on at least 6 of the 12 chromosomes.

Statistical analysis. − Each quantitative trait variable, in each F2 progeny, for each segregating locus L, was submitted to an analysis of variance for the factor "genotype at locus L" ([1], [3]), after Bartlett's test for homogeneity of variances and transformation where necessary. When no simple transformation was found, the distributions were compared between genotypic classes by chi-square test. A first analysis included all the genotypes, and a second one, in case of segregation with codominant alleles, included only homozygous genotypes. A significant effect observed in one of the 2 analyses may be interpreted as the existence of a linkage between the locus L and at least one of the QTL of the studied trait. It should be noted that no assumption was made on the biological nature of the QTL action.

RESULTS. − Table II presents the marker loci-quantitative trait associations. The search for QTL was successful, in that, at least one significant effect was detected for each trait. Reciprocally, all marker loci were useful since significant effects were calculated for each of them.

The QTL were distributed on all the marked chromosomes (*Fig.* 1). There was no evidence for specialization of the chromosomes toward a particular kind of traits (*Fig.* 1). However, although exact comparisons were not possible, some chromosomal areas seemed to be less dense than others for significant effects. For instance (Table II), *Cat*-1 was related to only two traits, while on the same chromosome, the segment *Est*-2/*Pgi*-2 marked all kinds of traits. The loci *Got*-1, *Ph*, *Sdh*-1 and *Acp*-1/2, *Est*-1 were other very involved chromosomal parts.

DISCUSSION. − Three preliminary remarks have to be made. (i) Many analyses of variance were necessary to study the data. It is therefore likely that some of the significant tests do not reflect a marker locus effect but a random fact (α-error). Nevertheless, numerous tests are significant at a level largely inferior to 5%, while some others are confirmed by similar results in another F2. (ii) A non-significant test may be interpreted by the independence between marker and trait, by an allelic identity of the parents at the QTL linked to the marker, or by a lack of power of the test to detect an effect. (iii) The conclusions of tests are true for the tested material only.

The genes contributing to each of the quantitative trait appear largely distributed throughout the genome. For instance, at least 5 chromosomes seem to be involved in

TABLE I

Observed alleles at segregating loci and chromosomal location.
Acp-1 and *Acp*-2 being tightly linked, they will be noted *Acp*-1/2 in text and Table II.
Allèles observés aux loci en ségrégation et localisation chromosomique.

Got-1	Amp-1	Est-2	Pgi-2	Cat-1	Sdh-1	Acp-1	Acp-2	Adh-1	Pgd-1	Ph	Got-3	Est-1	Pox-4
−	−									−	−		
chr. 1	2		3			6			11	12	?	?	
					Cross: ES70-6 × YS309								
	1/2	0/1		2/1			0/+		1/3	−/+	1/2	0/1	2/1
					Cross: ES70-6 × SS404								
			1/2	2/1	3/4	9/4	0/+		1/3	−/+		0/1	2/1
					Cross: BS20 × YS45-1								
2/1			3/1		2/3	4/9	+/0	2/1				1/0	

TABLE II

Marker locus-quantitative traits associations. *a* : F2 ES70-6 × YS309; *b*: ES70-6 × SS404; *c* : BS20 × YS45-1.

Associations entre marqueurs et caractères quantitatifs. a: F2 ES70-6 × YS309; b: ES70-6 × SS404; c: BS20 × YS45-1.

Character		Got-1			Amp-1			Est-2			Pgi-2			Cat-1			Sdh-1			Acp-1/2		
		chr. 1			chr. 2			chr. 3									chr. 5					
		d.f	F	p	d.f	F	p	d.f	F	p	d.f	F	p	d.f	F	p	d.f	F	p	d.f	F	p
T7	*a*		–			ns			ns			–			ns			–			ns	
	b		–			–			–		1,146	11.4	.001		ns		1,167	8.65	.004		ns	
	c	2,172	3.50	.032		–			–			ns			–			ns			ns	
TAM	*a*		–			ns			ns			–			ns			–		1,282	5.00	.026
	b		–			–			–			ns			ns			ns			ns	
	c	2,172	7.98	.001		–			–			ns			–			ns			ns	
HEAD	*a*		–		1,133	4.19	.034		ns			–			ns			–			ns	
	b		–			–			–		2,318	19.0	.000		ns		1,167	4.26	.040		ns	
	c	2,172	5.62	.004		–			–		2,172	8.09	.001		–			ns			ns	
HGT	*a*		–			ns		1,282	10.8	.001		–			ns			–			ns	
	b		–			–			–		2,318	6.67	.002		ns			ns		2,318	4.75	.009
	c		ns			–			–		1,90	6.48	.013		–			ns			ns	
LGF	*a*		–		1,133	5,76	.018		ns			–			ns			–			ns	
	b		–						–			ns			ns			ns			ns	
	c	2,172	4.08	.019		–			–			ns			–		1,88	6.73	.011		ns	
WDF	*a*		–			ns		X2:9	19.6	.024		–			ns			–			ns	
	b		–			–			–			ns		2,318	7.16	.001		ns			ns	
	c		ns			–			–			ns			–			ns			ns	
LGF/WIDF	*a*		–			ns			ns			–			ns			–			ns	
	b		–			–			–			ns			ns			ns		1,170	5.53	.002
	c	2,172	4.14	.018		–			–			ns			–		1,88	7.54	.007		ns	
LGP	*a*		–			ns			ns			–			ns			–			ns	
	b		–			–			–		1,146	5.56	.020		ns			ns			ns	
	c		ns			–			–			ns			–			ns			ns	
BR1	*a*		–			ns			ns			–			ns			–			ns	
	b		–			–			–		2,318	3.11	.046	1,167	4.99	.027		ns		2,318	3.24	.040
	c		ns			–			–		2,172	4.05	.019		–			ns		2,172	7.05	.001
BR2	*a*		–			ns		1,282	5.79	.017		–			ns			–			ns	
	b		–			–			–			ns			ns			ns			ns	
	c		ns			–			–			ns			–			ns		1,88	7.90	.006
BR1/BR2	*a*		–			ns			ns			–			ns			–			ns	
	b		–			–			–			ns			ns			ns			ns	
	c	1,83	4.96	.029		–			–			ns			–			ns			ns	
NSI	*a*		–			ns		1,282	6.10	.014		–			ns			–			ns	
	b		–			–			–			ns			ns			ns			ns	
	c		ns			–			–			ns			–			ns		1,88	4.96	.029
LGG	*a*		–		2,281	4,11	.017	1,282	4.29	.039		–			ns			–			ns	
	b		–			–			–			ns			ns			ns			ns	
	c		ns			–			–			ns			–			ns			ns	
WIDG	*a*		–		1,133	4.52	.035		ns			–			ns			–		1,282	7.69	.006
	b		–			–			–			ns			ns		1,167	8.23	.005	2,318	7.43	.001
	c		ns			–			–			ns			–		1,88	7.92	.006		ns	
LGG/WIDG	*a*		–		X2:6	13,4	.037		ns			–			ns			–		X2:9	18.4	.031
	b		–						–			ns			ns			ns		1,170	8.68	.004
	c		ns			–			–			ns			–			ns			ns	
NGR/NSI	*a*		–		1,133	5.12	.025		ns			–			ns			–			ns	
	b		–			–			–		2,318	8.37	.000		ns			ns			ns	
	c	2,172	3.06	.050		–			–		1,90	8.78	.004		–		1,88	5.61	.020		ns	

Character		Adh-1 / chr. 11			Pgd-1			Ph – chr. 12			Got-3 – ?			Est-1 / ?			Pox-4 / ?		
		d.f	F	p	d.f	F	p	d.f	F	p	d.f	F	p	d.f	F	p	d.f	F	p
	a		–			ns			ns			ns			ns			ns	
T7	*b*		–			ns			ns			–			ns			ns	
	c	1.86	4.11	.046		–			–			–			ns			–	
	a		–			ns			ns			ns			ns			ns	
TAM	*b*		–			ns			ns			–			ns			ns	
	c		ns			–			–			–			ns			–	
	a		–		1,149	8.00	.005		ns			ns			ns			ns	
HEAD	*b*		–			ns			ns			–			ns		1,132	5.43	.021
	c		ns			–			–			–			ns			–	
	a		–			ns			ns			ns			ns			ns	
HGT	*b*		–			ns			ns			–		1,319	9.34	.002		ns	
	c		ns			–			–			–			ns			–	
	a		–			ns		1,282	7.23	.008		ns			ns			ns	
LGF	*b*		–			ns		1,319	8.51	.004		–			ns		2,234	3.70	.026
	c	1,86	4.31	.041		–			–			–		1,173	8.23	.001		–	
	a		–			ns			ns		X2:14	26.2	.025	1,282	15.7	.000	1,125	9.31	.003
WDF	*b*		–			ns		1,319	39.6	.000		–		1,319	5.11	.025		ns	
	c		ns			–			–			–			ns			–	
	a		–			ns		1,282	5.44	.020		ns			ns		1,125	4.09	.045
LGF/WIDF	*b*		–		1,162	3.97	.048		ns			–			ns		2,234	4.03	.019
	c	1.86	4.19	.044		–			–			–			ns			–	
	a		–			ns			ns			ns		1,282	4.76	.030	1,125	4.19	.043
LGP	*b*		–		1,162	6.46	.012	1,319	3.96	.048		–			ns			ns	
	c		ns			–			–			–		1,173	8.23	.005		–	
	a		–			ns			ns			ns		1,282	4.80	.029		ns	
BR1	*b*		–			ns		1,319	30.3	.000		–		1,319	6.97	.009		ns	
	c		ns			–			–			–			ns			–	
	a		–			ns			ns			ns		1,282	6.29	.013		ns	
BR2	*b*		–			ns		1,319	13.8	.000		–			ns			ns	
	c		ns			–			–			–		1,173	5.86	.017		ns	
	a		–			ns			ns			ns			ns			ns	
BR2/BR1	*b*		–			ns			ns			–		1,319	7.03	.008		ns	
	c		ns			–			–			–		1,173	5.88	.016		–	
	a		–			ns			ns			ns		1,282	7.75	.006	1,125	4.84	.030
NSI	*b*		–			ns		1,319	14.1	.000		–			ns			ns	
	c		ns			–			–			–			ns			–	
	a		–			ns			ns			ns		1,282	7.72	.006		ns	
LGG	*b*		–			ns			ns			–			ns			ns	
	c		ns			–			–			–			ns			–	
	a		–			ns		1,282	4.10	.044		ns			ns			ns	
WIDG	*b*		–			ns			ns			–			ns			ns	
	c		ns			–			–			–		1,173	5.19	.024		–	
	a		–			ns		X2:9	18,6	.028		ns			ns			ns	
LGG/WIDG	*b*		–			ns			ns			–			ns			ns	
	c		ns			–			–			–			ns			–	
	a		–		1,149	6.10	.003		ns			ns			ns			ns	
NGR/NSI	*b*		–			ns			ns			–		1,319	9.03	.003		ns	
	c		ns			–			–			–			ns			–	

panicle traits. This organization for one of the traits selected during the domestication process of cereals [13] may be compared to that of the genes determining the spikelet morphology in pearl millet. They are linked ([14], [15]), a prerequisite to the domestication of allogamous plants, since it is necessary to maintain the integrity of "domestication syndrom" in front of the wild genetic pool. Our results would agree with the idea that such a linkage would not be necessary in autogamous plants.

The involvement of some loci as QTL markers is noteworthy because most of them were previously used to classify *O. sativa* varieties. Phenol reaction [16] is the best simple trait to recover the *indica-japonica* classification based on morphophysiological traits. This could be explained by the linkage of the locus *Ph* with QTL influencing discriminant traits. It could also be true for locus *Acp*-1/2 ([17], [18]), *Est*-1 [19] and *Est*-2 and *Pgi*-2 [9]. Further, some of these loci are found to be linked to seed fertility. Sterility relationships in intervarietal crosses in *O. sativa* were at the origin of *indica-japonica* distinction [16]. Although we did not identify the sterility genes, the association with fertility of the segment *Est*-2/*Pgi*-2 may be related to the genetic map, which locates these loci on chromosome 3 near several sterility genes. It is also of interest to note the occurence of common markers between traits for which correlations were noted, like plant height and fertility [20].

Isozyme markers were useful to detect some of the genes underlying quantitative traits. Further analyses are now needed to evaluate the effect of these QTL.

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REFERENCES

[1] S. D. TANKSLEY, H. MEDINA-FILHO and C. M. RICK, *Heredity*, 49, 1982, pp. 11-25.
[2] J. I. WELLER, *Heredity*, 59, 1987, pp. 413-421.
[3] J. I. WELLER, M. SOLLER and T. BRODY, *Genetics*, 118, 1988, pp. 329-339.
[4] A. H. PATERSON, E. S. LANDER, J. D. HEWITT, S. PETERSON, S. E. LINCOLN and S. D. TANKSLEY, *Nature*, 335, 1988, pp. 721-726.
[5] M. D. EDWARDS, C. W. STUBER and J. F. WENDEL, *Genetics*, 116, 1987, pp. 113-125.
[6] C. W. STUBER, M. D. EDWARDS and J. F. WENDEL, *Crop Sci.*, 27, 1987, pp. 639-648.
[7] K. S. WU, J. C. GLASZMANN and G. S. KHUSH, *Biochem. Genet.*, 26, 1988, pp. 303-320.
[8] J. L. PHAM, J. C. GLASZMANN, R. SANO, P. BARBIER, A. GHESQUIÈRE and G. SECOND, *Genome*, 1990 (in press).
[9] G. SECOND, *Jpn. J. Genet.*, 57, 1982, pp. 25-57.
[10] A. DE KOCHKO, *Evol. Trends Plants*, 1, 1988, pp. 105-110.
[11] P. TROUSLOT and G. SECOND, Travaux et Documents de l'ORSTOM, No. 120, 1980.
[12] A. DE KOCHKO, *Theor. Appl. Genet.*, 73, 1987, pp. 675-682,
[13] J. H. HARLAN, 1975, *Crops and man*, American Society of Agronomy, Crop Science Society of America Eds., 295 p.
[14] J. PERNES, *C. R. Acad. Sci. Paris*, série générale, 2, 1985, pp. 429-447.
[15] J. PERNES, *La Recherche*, 14, 1983, pp. 910-919.
[16] H. I. OKA, *Ind. J. Genet. Plant. Breed.*, 18, 1958, pp. 79-89.
[17] J. INOUYE and T. HAGIWARA, *Jpn. J. Trop. Agric.*, 24, 1980, pp. 321-328.
[18] B. B. SHAHI, H. MORISHIMA and H. I. OKA, *Jpn. J. Trop. Agric.*, 24, 1980, pp. 159-164.
[19] A. GHESQUIÈRE and G. SECOND, In *Electrophorèse et Taxonomie*, mémoire n° 42 de la Société Zoologique de France, 1986, pp. 263-272.
[20] H. I. OKA, *Jpn. J. Genet*, 53, 1978, pp. 397-410.

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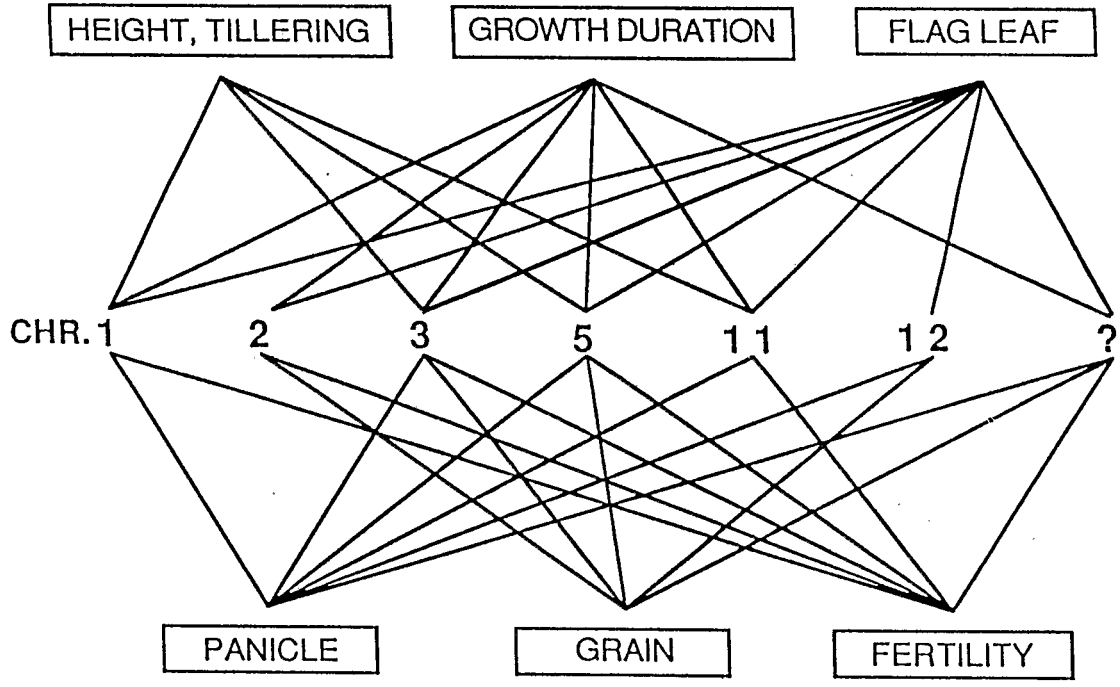

Fig. 1. – Associations between marked chromosomes and various traits.

Fig. 1. – Associations entre les chromosomes marqués et les différents caractères.