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Abnormal segregations in crosses between two cultivated rice species

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An F_1 hybrid between the two cultivated rice species *Oryza glaberrima* and *O. sativa* spp. *japonica* was pollinated by four different *O. sativa* genotypes to obtain four back-cross progenies with 9–36 plants each. These progenies were followed for genetic marker segregations. Segregation distortions were demonstrated at several loci. Preferential allele associations were detected. Some distortions could be explained by the sterility of the female gametes of the F_1 hybrid caused by a sporogametophytic interaction gene, while an early differential zygotic selection could be the origin of the other distortions.

Keywords: genetic resources, interspecific hybrids, *Oryza glaberrima, Oryza sativa*, reproductive barriers, segregation distortion.

Introduction

Rice offers a rare opportunity to study the relationships between two cultivated species belonging to the same genomic group (AA genome, Morinaga, 1964). Furthermore, since rice is a major crop, this is not only of theoretical interest but also important for the use of rice germplasm.

The analysis of isozyme polymorphism (Second, 1982) and those of chloroplastic RFLPs (Dally & Second, 1990) and nuclear RFLPs (Wang *et al.*, 1992) support the hypothesis of distinct domestication of the two cultivated rice species, in Asia for *Oryza sativa* and in West Africa for *Oryza glaberrima* (for review see Oka, 1988). Agronomical studies revealed useful characteristics in some *O. glaberrima* cultivars, such as early tillering (observations of plant breeders), tolerance to drought (Sano *et al.*, 1984), tolerance to Rice Yellow Mottle Virus (Attere & Fatokun, 1983; John *et al.*, 1985; John & Thottapilly, 1987) or some insects (Vercambre, 1982; Sauphanor, 1985). However, despite these characteristics, it is the distinct genetic origin of *O. glaberrima* that has been used to argue that the African species is a potential source of improvement for *O. sativa*. This idea is confirmed by the results of Silue & Notteghem (1991) who tested 99 *O. glaberrima* accessions for blast resistance and who observed for some accessions a resistance that was not higher than that of *O. sativa* but different.

The use of *O. glaberrima* for improvement of *O. sativa* is difficult because of strong reproductive barriers between the two cultivated species (Chu *et al.*, 1969; Sano, 1986; Yabuno, 1990). Sano *et al.* (1980) showed a tendency to the non-independent transmission of morphological and viability traits in the progenies of crosses between *O. glaberrima* and *O. sativa*. However, the influence of reproductive barriers on the variability of progenies obtained from such crosses remains poorly known.

In a previous paper (Bougerol & Pham, 1989), we studied the influence of the *O. sativa* genotypes on the characteristics of F_1 hybrids between *O. glaberrima* and *O. sativa*. This paper reports a part of a study of the influence of the *O. sativa* parent on back-cross progenies. We used an F_1 hybrid, *O. glaberrima* × *O. sativa* spp. *japonica*, as constant female parent and studied the back-cross progenies obtained after pollinating with four different *O. sativa* genotypes. We present here our results dealing with the segregations of genetic markers observed in the four progenies. Although the number of plants studied is low (from 9 to 36 depending on the progeny), it is, as far as we know, the first time that so many plants have been studied from back-crosses involving *O. glaberrima* and *O. sativa*.

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Materials and methods

Plant material, cultivation and experimental design

Four *O. sativa* genotypes were used and were classed into the *indica* and *japonica* subspecies based on isozyme studies (Second, 1982; Ghesquière & Second, 1983; de Kochko, 1987). These are ES70-6 (Tanzania, *japonica*), YS309 (Guinea, *japonica* showing presumed introgression from *O. longistaminata*), YS45-1 (Guinea, *japonica*) and SS404 (Senegal, *indica*). These traditional African cultivars were obtained from the ORSTOM† collection. The *O. glaberrima* genotype WO25 was provided several years ago by the National Institute of Genetics (Misima, Japan) and was used to study the relationships between *O. sativa* and *O. glaberrima* (Sano *et al.*, 1979; Sano, 1983). The purity of parental lines was ensured by successive generations of controlled self-fertilization.

Initially, the parents WO25 (female) and ES70-6 (male) were crossed to obtain F_1 hybrids. No difficulty was observed at this step. The F_1 plants showed a very low pollen fertility (< 1 per cent) which is usual in such crosses (Morishima *et al.*, 1962; Chu *et al.*, 1969; Yabuno, 1977; Bougerol & Pham, 1989) and a complete seed sterility was observed on the panicles that were not used in crossing. These F_1 hybrids were probably female-sterile (Chu *et al.*, 1969) since it was difficult to obtain seeds by pollinating the F_1s with each of the four *O. sativa* parents. The rate of successful back-cross pollination was about 4 per cent and did not differ significantly between back-crosses. This rate is similar to that observed by Sano (1983). Twelve to 46 seeds per progeny were obtained. As the female parent was constant, each of the four progeny will therefore be denoted according to the male parent: //ES70-6, //YS309, //SS404 and //YS45-1.

Seeds were dehulled, disinfected in a solution of sodium hypochlorite, rinsed in distilled water, and sown in Petri dishes with nutrient solution. After 10 days, seedlings were planted out in 2-l pots. Plants were cultivated in a glasshouse with constant irrigation. A randomized block design was used which included the four back-cross progenies, the F_1 hybrid WO25 × ES70-6, and parental lines.

Genetic markers

Six isozyme marker loci and one morphological marker locus were used. The electrophoresis methods on starch gel that were used are described by Trouslot & Second (1980) and de Kochko (1987). Four enzymatic systems were used: esterases (*Est*), phosphoglucose isomerase (*Pgi*), catalase (*Cat*) and shikimate dehydrogenase (*Sdh*). The *Pgi-1* locus is located on chromosome 3, the loci *Est-2, Cat-1* and *Sdh-1* are on chromosome 6, and the loci *Est-1* and *Est-9* are on chromosome 7 (using the unified chromosome nomenclature published in Rice Genetics Newsletter 1990).

The *O. glaberrima* parent WO25 and the *O. sativa* parent YS309 showed spikelets with a red coloured apiculus (locus *C*). Following Sano *et al.* (1979), these genotypes were considered as having the *C* allele, the other parents having the C^+ allele (non coloured apiculus). The *C* locus is located on chromosome 6 and is linked to the sporogametophytic interaction locus *S-1* (Sano, 1986). It is also linked to the isozyme locus *Est-2* (about 9 per cent recombination calculated from intra-*O. sativa* crosses, genetic map published in Rice Genetics Newsletter 1987).

Statistical methods

The conformity of mono-locus segregations to Mendelian proportions was tested following Sokal & Rohlf (1981) using the binomial test for progenies //ES70-6, //YS45-1 and //YS309 (frequencies < 25), and the Chi-square test with continuity correction for progeny //SS404 (frequencies between 25 and 200). The co-segregation of all the pairs of loci were tested (Chi-square test) in the progeny //SS404 only because of the small sample sizes of the other progenies.

Results

Development of plants

Plant losses were observed during the cultivation, varying from 37 to 60 per cent depending on the progeny. All the progenies showed a similar proportion of non-germinated seeds (< 20 per cent). This rate is similar to that observed for the F_1 hybrid WO25/ES70-6 and to the results of Chu *et al.* (1969). The second level of loss was at the seedling stage, during the 2 weeks following germination. Significant differences appeared at this stage between the progenies. The highest level of mortality was scored in the progeny //ES70-6 (53 per cent of germinated seeds). It was 29, 19 and 10 per cent in the progeny //YS245-1, //SS404 and //YS309, respectively.

Segregations of genetic markers

Table 1 shows the results of mono-locus segregations. Out of the 23 observed segregations, 16 agreed with Mendelian proportion 1:1, while 7 were significantly distorted at the 5 per cent level. At least one abnormal segregation was observed in each of the four progenies.

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Table 1 Segregations observed at marker loci for each of the four backcross progenies, and test of their conformity to Mendelian proportions 1:1

	Back-cross											
	//SS404			//ES70-6			//YS309			//YS45-1		
Locus	G	S	test (1)	G	S	test (2)	G	S	test (2)	G	S	test (2)
Cat-1	17	15		10	4		7	2		8	7	
	$\chi^2 = 0.03$		ns	$P = 0.09$		ns	$P = 0.09$		ns	$P = 0.50$		ns
Est-1	—	—		7	7		—	—		11	5	ns
				$P = 0.61$		ns				$P = 0.23$		
Est-2	27	5		12	2		—	—		—	—	
	$\chi^2 = 13.78$		***	$P = 0.01$		**						
Est-9	17	16		3	11		6	3		8	9	
	$\chi^2 = 0.00$		ns	$P = 0.03$		*	$P = 0.25$		ns	$P = 0.50$		ns
Pgi-1	20	14		6	8		5	4		7	10	
	$\chi^2 = 0.74$		ns	$P = 0.40$		ns	$P = 0.50$		ns	$P = 0.31$		ns
Sdh-1	18	16		7	7		9	0		11	6	
	$\chi^2 = 0.03$		ns	$P = 0.61$		ns	$P = 0.002$		**	$P = 0.23$		ns
C	22	8		13	1		—	—		13	4	
	$\chi^2 = 5.63$		*	$P = 0.001$		***				$P = 0.03$		*

(1) χ^2 test with continuity correction.
(2) Binomial test.
G: plants with the allele donated by the *O. glaberrima* parent of the F_1 hybrid.
S: plants with the allele donated by the *O. sativa* parent of the F_1 hybrid.
ns, non-significant; *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

The linked loci *Est-2* and *C* were subject to distortions in all of the progenies where they were scored: //SS404 and //ES70-6 for the locus *Est-2*; //SS404, //ES70-6 and //YS45-1 for the locus *C*. An abnormal segregation was observed for *Sdh-1* in the progeny //YS309. All these distortions showed an excess of the allele contributed by the *O. glaberrima* parent WO25. However, an excess of the allele donated by the *O. sativa* parent ES70-6 was observed for *Est-9* in the progeny //ES70-6. Thus, this progeny showed two opposite deviations.

Although not significant at the 5 per cent level, trends in deviation from Mendelian proportions were observed for *Cat-1* in the progenies //YS309 and //ES70-6, for *Est-1* and *Sdh-1* in the progeny //YS45-1. If one pooled the segregation for *Cat-1* of the progenies //YS309 and //ES70-6, a significant excess of the allele donated by WO25 was noted.

All the co-segregations of the pairs of loci studied in the progeny //SS404 were consistent with previous data on the independence of the involved loci (Pham *et al.* 1990). However, a multilocus analysis showed a tendency towards preferential association of some alleles. In this analysis five independent isozyme loci were considered. There are $2^5 = 32$ possible combinations for all the alleles. Only 19 of them were observed among the 29 observed plants. Moreover, if we only considered the four loci *Est-2*, *Est-9*, *Pgi-1* and *Cat-1*, seven plants out of 29 showed the same allele combination as the *O. glaberrima* parent WO25. This proportion differed significantly from the theoretical proportion 1:16 ($X^2 = 4.994$, 1. d.f., $P = 0.025$). However, it did not differ from the theoretical proportion calculated using the hypothesis of a random association of gametes and observed allele progenies.

Discussion

Analysis of segregation distortions

Wendel *et al.* (1987) give several examples of plant species where abnormal segregations were observed. In rice, distortions frequently occurred in crosses within *O. sativa* (Pham *et al.*, 1990) and in crosses between *O. sativa* and *O. longistaminata* (Causse & Ghesquière, 1991). The skewed segregations of marker loci are caused by the selection against chromosome segments containing these markers. This selection may act either at the haploid gametophyte stages during the prezygotic phase, or after the fertilization. Ottaviano &

Mulcahy (1986) defined the gametophytic selection, including fertilization events, and sporophytic selection.

Using isogenic lines Sano *et al.* (1979) proposed a one-locus sporogametophytic interaction model that explains part of the sterility of the hybrids between *O. sativa* and *O. glaberrima*. The model is the following: the genotypes of 108 (*O. sativa* spp. *indica*) and WO25 (*O. glaberrima*, also used here) are supposed to be *S-1*^{a} *S-1*^{a} and *S-1 S-1*, respectively. The genotype of F_1 hybrid is *S-1*^{a} *S-1*. The presence of the *S-1* allele in the maternal tissue leads to the sterility of male and female gametes with the *S-1*^{a} allele. These authors located this gene ('gamete killer') on chromosome 6 by linkage with the *C* locus. Sano (1983) then identified the gene *S-3* on chromosome 11, acting in the same way, but only on the male gametophytes ('pollen killer').

This model of gametic selection can explain the deviations observations for the *Est-2* and *C* loci. Let us suppose that WO25 and ES70-6 have alleles *S-i* and *S-i*^{a}, respectively, acting in the same way as *S-1* and *S-1*^{a}. The excess of *O. glaberrima* alleles at the *Est-2* and *C* loci in the progenies could be explained by a linkage between these loci and the gene *S-i*. Although we cannot demonstrate it, it is likely that *S-i* is the *S-1* gene studied by Sano *et al.* (1979) since the same *O. glaberrima* genotype WO25 was used, and since both are located on chromosome 6.

The observation of Mendelian and non-Mendelian segregations at *Est-9* and *Sdh-1* loci depending on the considered progeny demonstrates that the sporogametophytic interaction model is not sufficient to explain the distortions observed in this case. Using this model, the same deviation would be expected in all of the backcross progenies obtained from the F_1 hybrid, except if one imagines particular correction mechanisms.

It is therefore necessary to consider the genotype of the pollinating parent of the back-cross, i.e. ES70-6 for *Est-9*, YS309 for *Sdh-1*. It is difficult, in the case of gametophytic selection, to imagine a preferential selection of some ovules by the pollen. Since there is only one ovule per spikelet, it would suppose a non independent fertilization of the spikelets. The observation of hybrids between ES70-6 and all *O. sativa* parents argues against the hypothesis of incompatibility systems.

We may propose the hypothesis of sporophytic selection during the early development of the seeds, at the germination stage or just after this stage. These two last possibilities can be rejected because: (i) the losses observed at this stage are not high enough to explain high segregation deviations and, moreover, these losses are homogeneous between progenies; and (ii) it is unlikely that the mortality of seedlings is the source of distortions at locus *Sdh-1* for the progenies //SS404, //YS45-1 and //YS309. Whereas this mortality rate is roughly the same in these three progenies, they differ for segregations at locus *Sdh-1*. Moreover, the mortality rate in the progeny //YS309 cannot explain the magnitude of distortion at this locus. On the other hand, the progeny //ES70-6 shows an high mortality rate, about 50 per cent, that could explain the distortion noted at locus *Est-9*. Some examples of weakness or non viability were recorded in the *Sativa* group after intra- or interspecific hybridization (Chu *et al.*, 1969), but the genetic models proposed cannot be applied here.

These data suggest that the early abortion of the zygote is the more likely hypothesis to explain the distortion at locus *Sdh-1*, and perhaps at *Est-9*. The hypothesis of early differential zygotic abortion was chosen by Rick (1963) and Gadish & Zamir (1987) to explain abnormal segregations in crosses between *Lycopersicon esculentum* and *Lycopersicon chilense* and between *Lycopersicon esculentum* and *Lycopersicon pennellii*, respectively. An unfavourable interaction between the endosperm and embryo was suggested to explain such phenomena (Grant, 1975). At the moment, we do not know the genetic basis of the observed differential zygotic selection. A possible way to study this would be the model developed by Chu & Oka (1970) and Ghesquière (1988) to explain the failure of endosperm development in F_1 hybrids of crosses between *O. sativa* and the wild species *O. longistaminata*. This model is based on an unfavourable allelic dosage of two genes in the endosperm.

Consequences of abnormal segregations

The general existence of sporogametophytic interaction genes creates problems for the integration of *O. glaberrima* genes into *O. sativa*. Let us assume that we want to introduce an *O. glaberrima* trait into an *O. sativa* genotype, and that the genes governing this trait are independent of the sterility gene. The production of near-isogenic lines of *O. sativa* will be slowed down by this sterility gene, since the proportion of offspring having an undesirable allele G linked to the sterility gene is equal to $1 - p$, where p is the recombination rate between the locus G and the sterility gene.

The model of sporogametophytic interaction involved in the reproductive barriers between the two cultivated species was also used to explain hybrid sterilities in crosses between the *O. sativa* subspecies *indica* and *japonica* (Oka, 1988; Ikehashi & Araki, 1986). Varieties overcoming the sterility system, called Wide Compatibility Varieties, were found in *O. sativa*

(Ikehashi *et al.*, 1991). This property of wide compatibility is caused by a 'neutral' allele *Sn*. The question is whether it could exist in *O. sativa* or *O. glaberrima* varieties compatible with the other cultivated species. As far as we know, no such variety has been detected. Sano (1990) demonstrated the existence of a modifier gene, transforming the action of a pollen killer gene into that of a gamete killer. This mechanism suggests that it could be of interest to study strategies to inactive the pollen killer action.

Even when sterility genes are not involved, restrictions to recombination occur in interspecific crosses (Paterson *et al.*, 1990). The multilocus analysis of the progeny //SS404 clearly showed the excess of some allelic combinations of the WO25 parent. We observed that in all the progenies most of the distortions are in favour of alleles contributed by *O. glaberrima*. Further analyses will be made to study if the same tendency is observed at the morphological level.

Finally, this study shows that interspecific crosses can reveal novel genetic diversity of *O. sativa*, since different levels of zygotic selection were observed according to the *O. sativa* genotype. This underlines the idea that the evaluation of germplasm must include a study of crosssing barriers, in addition to surveys of morphological, biochemical and molecular polymorphisms.

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