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Ribosomal gene spacer length variability in cultivated and wild rice species

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Summary. Restriction fragment length polymorphism of the rDNA spacer was studied in the genus *Oryza* using a cloned rice rDNA probe. One-hundred-five accessions, including 58 cultivated rice and 47 wild species with various genome types, were analysed. Seven size classes differing from one another by an "increment" of ca. 300 bp were observed amongst the Asiatic cultivated rice of the species *O. sativa*. A general tendency from a smaller spacer in the *Japonica* subtypes to longer ones in *Indica* is observed. Classification as *Japonica* or *Indica* on the basis of rDNA pattern generally agrees with classification based on isozyme patterns. In contrast, African rice of the species *O. glaberrima* does not display any rDNA size variation. When wild species are considered, extensive variation is observed, but the fragment sizes do not fall into regularly increasing size classes except for *O. rufipogon* and *O. longistaminata*. The variation is greater in these species than in the cultivated ones.

Key words: Cultivated rice – Wild rice – RFLP – Ribosomal RNA genes – Hybridisation

Introduction

Higher eucaryote ribosomal genes are organised in tandem repeats. In plants their copy number can vary from a few hundred, as in *Arabidopsis*, to several thousands in most species (Rogers and Bendich 1987).

Each repeat contains sequences coding for mature rRNAs and an intergenic region, the external large spacer, which contains the signals for transcription initiation and termination. The precursor rRNA undergoes successive cleavages to give rise to the mature 18S, 5.8S and 25S RNAs. The study of restriction patterns of plant genomic or cloned fragments of ribosomal genes has shown a size heterogeneity of the non-transcribed intergenic region in certain species (for a review on plant rDNA, see Flavell 1986; Rogers and Bendich 1987; Hemleben et al. 1988). In several plants, including wheat, maize, broad bean and cucumber, this length heterogeneity of the intergenic region is accounted for by variation in the copy number of small subrepeats. The length and number of these subrepeats vary considerably from one species to another. Similar repeats also exist in plants in which no spacer length variation has yet been reported (Tremousaygue et al. 1988; Delcasso-Tremousaygue et al. 1988).

Preliminary experiments indicated that rice rDNA is heterogeneous (Oono and Sugiura 1980; Olmedilla et al. 1984; Pental and Barnes 1985), but another group (Reddy and Padayatty 1987) reported a single type of unit in variety IR 20. The coding sequence of a cloned rice rDNA unit has been determined so that detailed structural information on this part of the gene is available (Takaiwa et al. 1984, 1985a, b). However, no extensive survey of rice species has been made to estimate the extent of variability in either cultivated or wild-type species and to determine if it could be related to a variable number of small subrepeats within the rDNA intergenic region.

Cytogenetic studies have distinguished several genomic types among the *Oryza* genus (Tsunoda and Takahashi 1984), while two species complexes, *O. sativa* and *O. glaberrima*, represent all the cultivated species and their direct ancestors, *O. rufipogon* and *O. breviligulata* (=*O. barthii*), respectively. Both *O. sativa* and *O. glaberrima* have an AA-type genome. The Asiatic cultivated rice belong to the *sativa* complex and are subdivided on

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the basis of morphophysiological characteristics into the *Japonica* and *Indica* types (Oka 1988; Tsunoda and Takahashi 1984). However, many genetic exchanges occurred between these subgroups, so that many cultivated rices occupy an intermediate position in this classification. The African cultivated rice belong to the *glaberrima* complex.

The relationships between these different species and subspecies have already been investigated by using isozyme polymorphism (Second 1982, 1985; Glaszmann 1987, 1988; De Kochko 1987) as well as chloroplast DNA RFLP (Ichikawa et al. 1986; Ishii et al. 1986, 1988; Dally 1988), and hypothetic scenarios for evolution and exchange between these genomes have been deduced. In this report we have analysed the extent of variability at the level of the length of the intergenic rDNA region, and we demonstrate that similar grouping can be recognised using rDNA as a probe. This observation indicates that a correlation can be established between rDNA spacer organization and different taxonomic units.

Materials and methods

Material

The plant material consisted of:

– 52 accessions of cultivated rice (*O. sativa*) which are part of the collection studied by Second (1982). They have previously been characterized for their isozyme patterns as *Indica*, intermediate *Indica, Japonica* or intermediate *Japonica*. Some of them were also studied by Glaszmann (1987, 1988 and unpublished results). Fifteen of these accessions were considered as ancestors of the *Japonica* and *Indica* types on the basis of the high pollen sterility which is observed when they are crossed (Oka 1958). These plants were used as references for typical *Japonica* and *Indica*, because their isozyme patterns were consistent with this conclusion (Second 1982).

– 6 accessions of the African cultivated rice, *O. glaberrima.*

– 47 accessions of wild-type species representative of the following genome types: AA – *O. rufipogon* (20), *O. breviligulata* (8) and *O. longistaminata* (2), BB – *O. punctata* (2), BBCC – *O. punctata* (1), *O. minuta* (2), *O. malampuzahensis* (1), CC – *O. officinalis* (4), CCDD – *O. latifolia* (2) and *O. alta* (1). Three species formerly classified as *Oryza* (Tateoka 1963) – *Porteseria coarctata* (1), *Rynchoryza subulata* (1), *Chickusichloa brachyanthera* (1), as well as the closely related species *Zizania latifolia* (1) – were also analysed.

All plants were grown in pots partly immersed in water in a greenhouse maintained at 25 °C with a 13-h day/11-h night photoperiod during winter.

DNA extraction

Total DNA was isolated from leaves of one individual according to a published method which has been slightly modified (Dellaporta et al. 1983). Extracts were centrifuged at 4,000 g instead of 20,000 g: an RNase digestion step (Merck, 1.3 units, 1 h at 37 °C) was introduced between the two isopropanol precipitations, followed by two phenol-chloroform (v/v) extractions and one chloroform extraction. A few samples were also prepared using a CsCl-ethidium bromide purification step (Delcasso-Tremousaygue et al. 1988).

Gel electrophoresis and hybridisation

Approximately 1 µg of total DNA was digested with 10 units of BamHI for 1 h at 37 °C. Restriction fragments were separated by electrophoresis on 0.8% agarose gels in 40 m*M* TRIS-acetate (pH 8), 2 m*M* EDTA, 5 m*M* sodium acetate. Their size was estimated with reference to marker lambda DNA fragments. DNA was transferred to a nylon membrane (Gene Screen Plus). Hybridisation was carried out with a probe consisting of a rice ribosomal gene repeat unit (plasmid RR 217) isolated and partially sequenced by Takaiwa et al. (1984, 1985a, b). A non-radioactive probe was used for the visualisation of restriction fragments in most of this work. The probe DNA was modified by sulphonation of cytosine residues using a kit from Orgenics. A hybridised probe was detected using antibodies against sulphonated cytosine and an enzyme-linked antibody reaction, according to the protocol given by the manufacturer. Alternatively, a classical nick-translation with radioactive ^{32}P-dCTP was used (Olmedilla et al. 1984). When several fragments corresponding to the spacer region were present, their relative proportion was estimated by densitometric scanning using a Bio Rad model 1650 densitometer.

Results

Use of sulphonated probes to detect rDNA RFLP

As the transfer of this technology to Third World country laboratories is anticipated, non-radioactive probes were used in this study. The chemical modification procedure developed by Orgenics was chosen because it avoids the enzymatic incorporation of modified nucleotides. The other major advantage of this non-radioactive method is that results can be obtained within a working day instead of one or several full days. Whenever the same samples were analysed by using either the non-radioactive or the radioactive probe, qualitatively similar results were obtained. However, quantitative differences were noticed because saturation of the coloured reaction occurs at a much lower probe concentration than saturation of an autoradiographic X-ray film. Therefore, the relative ratio of two fragments detected with the non-radioactive probe might be biased if the concentration of the probe which hybridises to one or both of them is higher than that giving a saturated response.

From previous results (Olmedilla et al. 1984; Takaiwa et al. 1984, 1985a, b), it was decided to use a BamHI digest, because all samples investigated so far contained two conserved sites delimiting a constant 3.8-kb fragment containing part of the coding sequences and a variable larger fragment containing the complete intergenic region and bordering coding sequences. Most of these experiments were also carried out with EcoRI which cuts only once per unit, but no more information was obtained from these experiments. Larger BamHI fragments were reproducibly observed. They correspond to the size of the repeat unit or to its multimers. These longer fragments were not considered in the following analysis because they may have arisen from incomplete digestion or

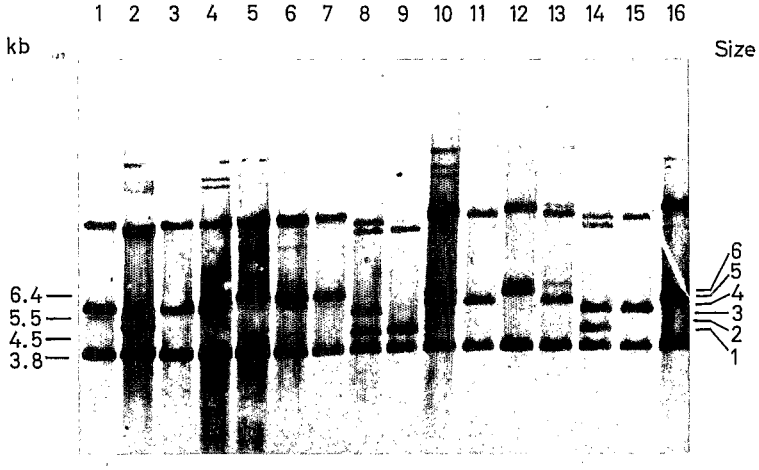

Fig. 1. Example of rDNA patterns in Asiatic cultivated rice. One microgram of DNA was digested with BamHI as described. After gel electrophoresis and Southern transfer, the DNA was hybridised with RR217, which contains the complete ribosomal repeat unit from *Oryza sativa*. The probe was sulphonated and hybrids detected as described. *Lane 1*=S18, *2*=S19, *3*=S20, *4*=S21, *5*=S22, *6*=S23, *7*=S24, *8*=S26, *9*=S27, *10*=S28, *11*=S30, *12*=S31, *13*=S34, *14*=S35, *15*=S36, *16*=S38. Other names and origins are given in Table 1

from site unavailability due to methylation. Alternatively, they may represent additional variant units which lack one or both BamHI sites. Similar problems arose from DNA purified through a CsCl cycle and, therefore, this pattern does not result from poor purification of the DNA.

rDNA polymorphism within AA genomes

The sativa complex: cultivated varieties. Among the investigated accessions corresponding to this complex, seven size classes were observed for the rDNA spacer. Each class differs from the next by an increment of 300 bp except for the last, which differs from the preceding one by twice this increment. A typical experiment is presented in Fig. 1 and the information which has been derived from numerous similar gels and hybridisations is summarised in Table 1. In this table the samples analysed have been ordered according to increasing spacer size. Many samples have more than one size class, thus confirming previous reports of length heterogeneity (Oono and Sugiura 1980; Olmedilla et al. 1984). Only two, S25 and 446, have three size classes and one, S19, has four. When several size classes are present their relative abundance ratio, estimated by densitometry, varies from 25% to 75%. Most frequently, fragments from samples with two size classes fall into adjacent classes.

The most interesting observations arise from the comparison of our results with those based on isozyme studies obtained on the same accessions. Second (1982, 1985) has reported four major groups: *Japonica, Indica,* intermediate *Japonica* and intermediate *Indica.* Glaszmann (1987) has reported six: group 1 corresponds to *Indica* and group 6 to *Japonica.* Groups 2–5 correspond to intermediates between *Japonica* and *Indica.* Differences between the two classifications result from differences in the number of loci and accessions analysed by these authors. In addition, Dally (1988) determined the chloroplast genome type on the same plant collection using several restriction enzymes. In this way he could also recognise typical *Indica* and *Japonica* patterns. From Table 1, it appears clearly that most typical *Japonica* samples have spacer fragments falling preferentially into classes 1–3 and that typical *Indica* have generally longer spacers, ranging from classes 4–8. In fact, classes 1 and 2 are represented only in *Japonica* or intermediate *Japonica,* while classes 5 and 6 are present only in typical *Indica* and intermediate *Indica.* The only exception is sample 619, which is considered to be a typical *Indica* on the basis of morphological characteristics and isozyme patterns, but which has rDNA spacer fragments of the *Japonica* type.

The sativa complex: wild species. This complex is formed by two major species: *O. rufipogon,* which presents numerous, world-wide spread forms, and *O. longistaminata,* which is a perennial species restricted to Africa.

Special attention was paid to *O. rufipogon* because it is considered to be the direct ancestor of *O. sativa.* This species shows a greater variability than the cultivated *O. sativa,* with as many as 13 different sizes for the external spacer (Table 2). All studied accessions except two (DN13 and W1654), have spacer fragments of the same size or deriving from each other by the same 300-bp increment as *O. sativa* accessions. Some *O. rufipogon* lines show intra-individual heterogeneity with up to four different spacer fragments. At least one accession (DN41; Fig. 3, lane 1) has an additional BamHI site in the spacer. In contrast to the situation in *O. sativa,* spacers with considerably different sizes can be present within the same plant (e.g. W1655). The comparison of these results with those at the isozyme level for *O. rufipogon* (Second 1985) does not show a clear congruence as found in cultivated rice: the strains originating from China do not have a closer relationship with the *Japonica* type which is supposed to derive from them. Further, the

Table 1. Classification of cultivated varieties of *sativa* complex according to the length of the rDNA spacer. (a) The results of Second (1982, 1985) and Glaszman (1987) are based on isozyme studies. The samples are classified according to these authors. Dally (1988) used several restriction enzymes to determine the chloroplast genome type, which is indicated by the symbol □ for *Japonica* and ○ for *Indica*. Sample S-24 has a slighty different chloroplast genome, close to the *Indica* type represented by △. Ancestor samples studied by Oka (1958) are underlined; (b) the rDNA spacers are classified by their size, their relative proportion was estimated by densitometric scanning and is indicated as a percentage

Samples	Names	Origins	Classification (a)			Size classes (b)							
			Second	Glaszmann	Dally	1	2	3	4	5	6	7	8
S27	Cigalon	France	J	6	□	100							
S09	Nan ching Shiang Tao	China	J		□	100							
446	Poung nyen	Thailand	J			40	35	25					
S19	M2-2 (T2-325)	Philippines	int. J	6	□	20	30	30	20				
S08	Jaguary	Brazil	J		□	50		50					
S26	Iguape Cateto	Brazil	int. J	6	□	50		50					
S35	IRAT 13	Ivory Coast	int. J	6	□	50		50					
S12	Canabong bong	Philippines	J		□	50		50					
S41	Taichung 65	Taiwan	J	6	□		100						
S10	Fujisaka 5	Japan	J	6	□		100						
563	Kinoshita moshi	Japan	J	6			100						
504	Taichung 65	Taiwan	J				100						
571	Mansaku	Japan	J	6			100						
701	Ta-tung-tsallal	China	J	6			100						
S13	Texas Fortuna	USA	J	6	□		55	45					
564	Nakamura	Japan	J	6			60	40					
S25	Santa cruz	South America	int. J	1			20	35	45				
619	Padi hotjong	Indonesia	I	1			50		50				
S16	Siampang	Sumatry	J	6	□			100					
S15	Vista	USA	J	6	□			100					
S04	Sunbonnet	USA	J	6	□			100					
S36	Moroberekan	Ivory Coast	int. J	6	□			100					
S17	Page miniak	Sumatra	J	6				75	25				
S07	Basilanon	Philippines	int. I	6–1	□			35	65				
S40	Taichung native 1	Taiwan	int. I	1	○			35	65				
S18	Hu nan hsien	China	int. I	1	○			30	70				
130	Tuan-kuang-huoa-le	Taiwan	I					50	50				
S38	Peh kuh	Taiwan	I	I	○				100				
108	Peh kuh	Taiwan	I		○				100				
S37	D 52-37	Niger	I		○				100				
S11	Khao Nok	Laos	J		□				100				
S03	Mack kova	Laos	int. J	6					100				
S20	Mack Fay Deng.	Laos	int. J		□				100				
S21	500 A (M 21)	Madagascar	int. J	2	○				100				
S30	H. 105 (T 6-802)	Sri-lanka	int. I	1	○				100				
S06	Tan Reug Chan	China	int. I		○				100				
S05	Linzhoubayoya zao	China	I	I	○				100				
S02	Hong Xie nuo	China	I	I	○				100				
S39	Chuh tu	Taiwan	I	I	○				100				
S14	IR 28	Philippines	I	I	○				100				
716	Pe-yang-ts20	China	I						100				
237	Apostol	Philippines	I	I					100				
610	Padi ravekang	Indonesia	I						100				
S01	Chinsurah boro II	India	int. I	2	□				45	55			
S32	Vary vato	Madagascar	I		□				45	55			
S23	3 LS 102-4	Madagascar	int. I	2	○				40	60			
S22	2 LS 102	Madagascar	int. I	2	○				40	60			
S24	L 78-9148	Zaire	I		△				40	60			
512	Padi-bali	Indonesia	I						50		50		
S34	Sixa (T 5-337)	South America	int. I	I	○				60		40		
160	Hong-ka-chiu	China	I						50				50
S31	Segadis	Indonesia	int. I	I	○					60	40		

Table 2. Classification of wild species of *Oryza sativa* complex according to the length of the rDNA spacer. (a) Dally (1988) used several restriction enzymes to determine the chloroplast genome type, which is indicated by the symbols □ for *Japonica*, and ○ for *Indica*, + for American and * for Australian accessions of *O. rufipogon;* (b) the rDNA spacer fragments are classified according to increasing size classes with their relative proportion as in Table 1. Two samples, DN 13 and W 1654, have size classes intermediate between the usual ones

Samples	Species	Origins	a	b												
			Chloro.	1	2	3	4	5	6	7	8	9	10	11	12	13
DN 9	O. rufi.	India	□	100												
DN 12	O. rufi.	India		100												
DN 21	O. rufi.	India		100												
W 1191	O. rufi.	America	+	100												
DN 13	O. rufi.	India	□		100											
W 1627	O. rufi.	Australia	*		100											
W 1629	O. rufi.	Australia	*		100											
W 1654	O. rufi.	China	□			25	25						50			
W 1534	O. rufi.	India	○			100										
DN 40	O. rufi.	India				50	50									
W 162	O. rufi.	Thailand				15		30	25	30						
W 1681	O. rufi.	India	○			30				70						
DN 33	O. rufi.	India				20		80								
W 1655	O. rufi.	China	□				33							33	33	
DN 41	O. rufi.	India					25								25	50
W 1187	O. rufi.	America					15	30	15						40	
W 133	O. rufi.	India	□					100								
W 555	O. rufi.	Ceylan	○					100								
W 612	O. rufi.	America	□					40					15	30	15	
W 135	O. rufi.	India	○								100					
EL 15, 17	O. long.	Tanzania					50		15	35						
EL 34	O. long.	Tanzania						50	50							

strains originating from western India which were found to have a high similarity with the *O. glaberrima* complex at the isozyme level (accessions DN9, DN12, DN13; Lolo and Second 1988) share an rDNA spacer length similar to that of the *Japonica* type.

The two accessions of the other AA-genome species, *O. longistaminata,* have spacer fragments within the same size classes as *O. rufipogon.*

The glaberrima complex. This complex contains the cultivated African rice, *O. glaberrima,* and their presumed ancestor, *O. breviligulata.* When DNA from different accessions of these species was analysed, a clearly different situation was observed, as shown in Fig. 2. Indeed, all the samples analysed so far have the same simple pattern with a single class of spacer fragment. It is smaller than the smallest *O. sativa* spacer fragment by more than 300 bp. Therefore, the difference cannot readily be explained by the loss of a single subrepeat. The accessions of *O. breviligulata* originate from the whole distribution area from Senegal to Tchad and Mali.

rDNA polymorphism within other genomes

Figure 3 is representative of the rDNA patterns of several accessions of wild species belonging to different genome types. The range of variation observed is much larger than for cultivated rice, with spacer BamHI fragments up to 7 kbp, and most of the accessions can be distinguished from one another. When the species are ordered by increasing spacer size, it is not possible to determine a regular size increment, as was observed for cultivated species or for *O. rufipogon.* This indicates that variation in the spacer length among all these species cannot be accounted for simply by variation in the number of small subrepeats. In addition, other rearrangements, such as deletions or insertions, have certainly taken place. This justifies careful analysis of spacer regions in rDNA from wild-type species.

It may be noted that allotetraploid species do not show greater polymorphism than their diploid ancestors. Indeed some have a single spacer length within an individual plant more frequently than diploids. Such an example is shown in Fig. 3 (lanes 10: *O. minuta* and 13: *O. malampuzahensis*) for two BBCC species. On the other hand, analysis of isozymes clearly shows additivity of the parental patterns (Second 1984). Although the number of individuals studied is small, it is clear that a homogenisation process or a deletion of part of the parental rDNA probably occurred rather frequently.

Species belonging to four genera closely related to *Oryza, Rynchoryza subulata, Zizania latifolia, Chiku-*

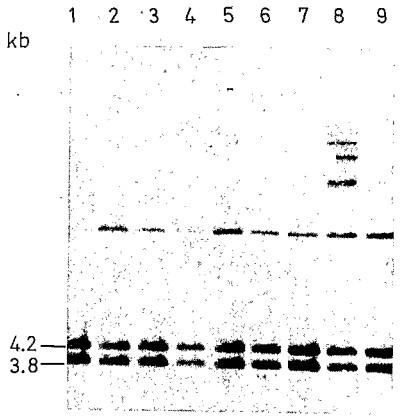

Fig. 2. Example of rDNA patterns in African rice. Samples were prepared and hybridised with the RR 217 probe as described in the legend to Fig. 1. *Lane 1: O. breviligulata* (IL B 05), *2: O. breviligulata* (SB 321), *3: O. beviligulata* (SB 304), *4: O. breviligulata* (NB 02), *5: O. breviligulata* (1B 03), *6: O. brevigulata* (UB 35), *7: O. glaberrima* (glutinous), *8: O. glaberrima* (4 LG 32), *9: O. glaberrima* (CG-161-5)

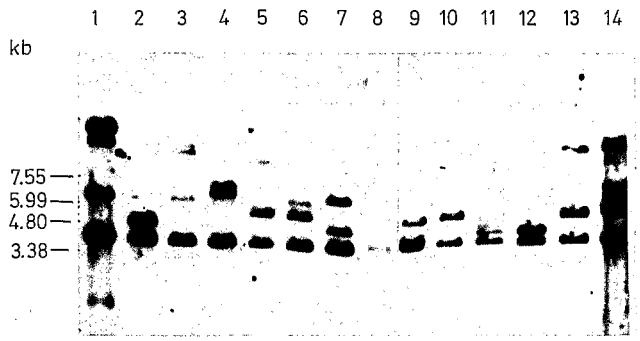

Fig. 3. Example of rDNA patterns in wild rice. Samples were prepared as decribed in the legend to Fig. 1, but were hybridised with RR 217 labelled by nick-translation with ^{32}P dCTP. *Lane 1:* DN 41 (*O. rufipogon,* India), *2:* 100963 (*O. latifolia,* IRRI), *3:* W 1655 (*O. rufipogon,* Thailand), *4:* EL 34 (*O. longistaminata,* Tanzania), *5:* W 65 (*O. officinalis,* India), *6:* D04 (*O. officinalis,* India), *7:* W 17 (*O. alta,* South America), *8:* W 551 (*Porteresia coarctata,* India), *9:* 104314 (*O. officinalis,* China), *10:* W 1344 (*O. minuta,* The Philippines), *11:* W 1306 (*O. officinalis,* India), *12:* UB 35 (*O. breviligulata,* Cameroon), *13:* W 1159 (*O. malampuzahensis,* India), *14:* EL 15–17 (*O. longistaminata,* Tanzania)

sichloa brachyanthera and *Porteresia coarctata* have been studied; they all have patterns similar in size and complexity to those of wild rice except that the spacer fragments poorly hybridise (Fig. 3, lane 8).

Discussion

Although size variation in the rDNA spacer has been reported for many plant species (Rogers and Bendich 1987), including rice (Oono and Sugiura 1984, 1985a, b; Olmedilla et al. 1984; Pental and Barnes 1985), no extensive survey within the *Oryza* genus has yet been reported. The regular increase in rDNA spacer size by units of ca. 300 bp strongly indicates that subrepeats similar to those described in other species exist in rice and are responsible for size variation. This was recently confirmed by restriction mapping and sequence analysis of subclones of RR217 (F. Cordesse, unpublished results; Sano and Sano 1988). The general significance of size variation in spacer length is not obvious. They are supposed to play a role as binding sites for pre-initiation complexes or as enhancers in rDNA transcription (Flavell 1986). This suggests that a correlation should be observed between a given ecotype and the length of the rDNA spacer. The extensive survey of *Japonica, Indica* and various intermediates between these two types supports this notion, since a general trend from a short spacer length in *Japonica* to longer ones in *Indica* clearly emerges from our study. The situation is much less clear for the wild species, because many fewer samples were examined for each species and because these species cover many different ecological conditions.

Another interesting point outlined by our study is the relatively good agreement of our results with data previously obtained independently by analysing isozymatic patterns or chloroplast DNA restriction patterns (Second 1982, 1985; Glaszmann 1987; Dally 1988). This indicates that rDNA spacer length polymorphism can be used as a reliable tool in analysing the evolution of rice species. Indeed, during our study few discrepancies with previous studies were noticed. When the isozyme pattern analysis was repeated on these accessions it became clear that tagging errors accounted for the discrepancy. The only one which is not yet resolved concerns sample 619, which has rDNA genes of the *Japonica* type but clearly has isozyme patterns of the *Indica* type. A possible explanation is that this situation results from limited introgression of rDNA genes from a *Japonica* into an *Indica* type. Many examples of genetic exchanges between both genomes are obvious from studies on isozyme patterns and reflect either natural crossing or the activity of early rice breeders (Second 1985). The same hypothesis holds for the *O. rufipogon* strains from Western India (DN9, DN12, DN13), with close similarity to the *O. glaberrima* complex at the isozyme level but to the *O. sativa* complex at the rDNA spacer length level. The simple Mendelian genetic determinism of rDNA (only one locus was detected in one *O. sativa* cross, McCouch et al. 1988) is in agreement with this hypothesis. It is thus clear that variation in rDNA spacer length can reflect both the phylogenetic relationships and a limited ecological introgression which has been fixed because it fitted well to conditions. In any case, reorganisation of this variation in allotetraploid species seems to occur relatively rapidly.

The fact that the same size variation was observed between the *O. rufipogon* and the Asian cultivated rice confirms the assumption that this species might be the ancestor of *O. sativa*. Similarly for African cultivated rice, our results support the idea that *O. breviligulata* is a likely ancestor of *O. glaberrima*, but that *O. longistaminata* is not. Indeed, *O. longistaminata* seems to be closer to *O. rufipogon* and might result from the spreading in Africa of a common ancestor of present day *O. rufipogon* and *O. longistaminata* during geological times. On the other hand, *O. breviligulata* and the domesticated species *O. glaberrima* have certainly evolved independently from another more ancient ancestor. All these data are consistent with the evolutionary model proposed by Second (1984).

It was surprising that no size variation was observed between the accessions of *O. glaberrima* and *O. breviligulata*. This contrasts with the diversity noticed at the isozyme level (Second 1985). This observation suggests that the mechanisms which generate size variation in the rDNA spacer have been lost in this species during evolution. Variation in length of the spacer is assumed to arise through unequal crossing-over between the subrepeat arrays of different rDNA genes. A possibility is that the subrepeat array has been altered in such a way that unequal crossing-over is no longer possible or is considerably reduced. It is remarkable in this respect that the spacer fragment of *O. glaberrima* is the shortest of the series.

Another interesting point, already noted for other plant species, is that the extent of variability is smaller in cultivated species than in wild types. Therefore, domestication is associated with a reduction in the variability of the rDNA spacer.

Finally, our results indicate that rDNA spacer size has probably played a significant role during the evolution and domestication of Asian cultivated rice, but has been rather neutral during evolution of the African rice.

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