Microsatellite analysis of Tio Taka, the first rice commercial cultivar released from the recurrent selection breeding method[1]

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Rice (*Oryza sativa*) has one of the largest germplasm collections in the world. The genus *Oryza* originated 130 million years ago in Gondwanaland and different species have since been distributed in different continents. Rice is cultivated between 55° north latitude and 36° south latitude, and grows under diverse conditions found in irrigated, rainfed lowland, rainfed upland and flood-prone ecosystems. Human selection and adaptation to diverse environments have created a large number of cultivars, and it is estimated that about 120 000 varieties of rice exist in the world (Khush, 1997).

Rice production doubled between 1966 and 1990, following the release of high-yielding cultivars. However, the use of elite germplasm in breeding programmes reduced the genetic variability available for selection. This reduction in genetic variability is thought to be the main reason for the yield plateau reached in many regions; in addition, it increases susceptibility to disease and insect epidemics.

With the increase in world population, it is projected that in 2025, there will be demand for an additional 290 million tonnes in relation to today's rice production of 560 million tonnes, as part of a scenario that includes the reduction of useful land area due to the degradation of natural resources and increase in urbanization (Tanksley and McCouch, 1997; Khush, 1997). It is, therefore, necessary to obtain rice cultivars with higher yield potential and yield stability. It is also extremely important to broaden the rice genetic basis so as to permit that new allelic combinations can be obtained and selected.

Thanks to knowledge of molecular markers, estimates of genetic diversity stored in gene banks can be made much more efficiently than with phenotypical analysis alone. Accessions with a DNA profile quite distinct from that of modern germplasm are likely to contain the greatest number of novel alleles (Tanksley and McCouch, 1997). Also, two breeding strategies can be efficiently used for the exploitation of genetic resources: backcross and recurrent selection methods.

The backcross method is generally used to breed a deficient trait in a cultivar (Fehr, 1987). When the backcross involves a cultivar as the recurrent parent and an exotic germplasm as the donor parent, two or three backcrosses are necessary in the direction of the cultivated parent, to produce a progeny without the undesirable traits incorporated by linkage drag (which reduces the proportion of donor fragments by 12.5 to 6.25 percent). In this case, the broadening of the genetic base is restricted to localized genomic regions close to the introgressed gene of interest.

Recurrent selection permits the accumulation of genetic gain in the successive cycles (Rangel, Zimmermann and Fagundes, 2000). Hull (1945) described for the first time recurrent selection: a method of reselecting, generation after generation, with the intercross of the selected families, to obtain genetic gains. It permits the generation of progenies obtained from recombination and selection; thanks to the broad genetic basis population, improved lines with a broad genetic basis can be produced. The methodology has been used successfully to improve rice in several countries in Latin America (Guimarães, 2005).

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Tio Taka, a rice commercial cultivar released in 2002 in Brazil with the cooperation of EMBRAPA (Brazilian Agricultural Research Corporation) and EPAGRI (Empresa de Pesquisa Agropecuária e Difusao de Tecnologia de Santa Catarina), was the first cultivar in the world originating from a rice recurrent selection programme. It was obtained from the recurrent selection population CNA-IRAT 4 (Rangel and Neves, 1997), which was in turn obtained in 1990 from the intercross of nine lines of the *indica* group: BG 90-2, CNA-7, CNA-3815, CNA-3848, CNA-3887, Colombia 1, Eloni, Nanicão and UPR 103 80 1 2, with IR36, the androsterility source. After 2 years of selection/recombination cycles, families were selected from CNA-IRAT 4 to be evaluated in multiple environments. After 5 years of evaluation and selection, the SC 169 family was selected in Santa Catarina State, showing high yield, rice blast resistance and tolerance of iron toxicity. For 3 years, the SC 169 line was evaluated in yielding experiments in Santa Catarina State, showing an average of 8 561 kg/ha (i.e. significantly more productive than both the other lines and the controls). The SC 169 line was named SCS BRS 113 (Tio Taka) and showed the following main characteristics: high yielding, low plant height (100 cm), lodging resistance, high tillering capacity, and high milling and cooking qualities.

The increasing availability of highly polymorphic genetic markers and the decreasing cost of typing provide high power for resolving the true biological relationship between individuals (Presciuttini *et al.*, 2002). Microsatellite markers (also known as simple sequence repeats [SSR]) were used to infer the genetic variability resulting from the higher informativity in relation to other classes of molecular markers (Rafalski *et al.*, 1996). Rice has a large collection of microsatellite markers, extensively produced by SSR-enriched libraries (Chen *et al.*, 1997; Brondani *et al.*, 2001).

This paper aims:

- to estimate the genetic variability of the genitors of the CNA-IRAT 4 population and the cultivar Tio Taka derived from this population; and
- to discuss this variability in relation to rice germplasm under cultivation in Brazil.

MATERIAL AND METHODS

Plant material

Seeds of Tio Taka, 10 accessions that originated the population CNA-IRAT 4, 43 rice cultivars, 14 rice breeding lines, 9 red rice accessions, 14 rice landraces and 4 *Oryza glumaepatula* accessions (Tables 1 and 2) were germinated in order to generate the plant material for DNA isolation. The DNA was extracted following the protocol described by Doyle and Doyle (1987). The DNA concentration was estimated using electrophoresis in 0.8 percent agarose gel and by comparison with the lambda DNA standard, and adjusted to 3 ng/µl.

Microsatellite analysis

Genetic analysis was done using previously published microsatellite markers (Akagi *et al.*, 1996; Chen *et al.*, 1997; Temnykh *et al.*, 2000; Brondani *et al.*, 2001). Sixteen microsatellite markers were chosen on the basis of their informative content and representative of all 12 rice chromosomes (Table 3).

The SSR reactions were performed in a final volume of 13 µl containing the following constituents: 0.3 µM of each primer, 1 U of *Taq* DNA polymerase, 0.2 mM of each dNTP, 1 mM TRIS-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1.3 µl of DMSO (50%) and 7.5 ng of template DNA.

The PCR (polymerase chain reaction) amplification reactions were performed in a PT-100 thermocycler (MJ Research) with the following programme: one pre-cycle at 96°C for 2 minutes; followed by 30 cycles at 94°C for 1 minute, 56°C for 1 minute and 72°C for 1 minute; and a final stage at 72°C for 7 minutes. The amplification was checked by horizontal electrophoresis in 3 percent agarose gel containing TBE 1x buffer (0.09M TRIS-Borate and 2 mM EDTA, pH 8.3) and 0.2 µg/ml ethydium bromide. The allelic polymorphism between rice genotypes was detected in 6 percent denaturing polyacrylamide gels containing 7M urea and 1x TBE

TABLE 1
List of genitors of CNA-IRAT 4 and their relative theoretical contribution in Tio Taka cultivar

Lines/varieties	Genitors
BG 90-2	IR262/Remadja
CNA 7	T 141/IR665-1-1-75-3
CNA 3815	Cica 4/BG 90-2//SML 1517
CNA 3848	IR36/Cica 7//5461
CNA 3887	BG 90-2/Tetep//4440
Colombia 1	Napal/Takao Iku 18
Eloni	IR454/SML Kapur//SML 66410
Nanicão	Landrace
UPR 103-80-1-2	IR24/Cauvery
IR36 (*msms*)	IR36 male sterile mutant

Source: Rangel and Neves, 1997.

buffer, and visualized by silver staining (Bassam, Caetano Anolles and Gresshoff, 1991).

STATISTICAL ANALYSIS

The allele frequencies were determined using the TFPGA program (Miller, 1997). The dendrogram was constructed from the genetic distance matrix obtained by the J&C genetic distance coefficient (Jukes and Cantor, 1969) and grouping by UPGMA (unweighted pair group method with arithmetic mean) clustering, using the NTSYS program (Rohlf, 1989). The private alleles and polymorphism information content (PIC) were estimated using the GDA program (Lewis and Zaykin, 2000).

RESULTS AND DISCUSSION

SSR analysis

The 16 SSR markers used produced high values of polymorphism information content (PIC), varying from 0.89 (OG17) to 0.45 (MRG4879), with an average of 0.70 (Table 4). These SSR markers identified 203 alleles,

TABLE 2
Ninety-five genotypes analysed in this study

	Genotype	Germplasm		Genotype	Germplasm
1	Tio Taka	Cultivar	49	CNA 8502	Breeding line
2	BG 90-2 (*)	Breeding line	50	CNAi 8859	Breeding line
3	CNA 3815 (*)	Breeding line	51	CNAi 8860	Breeding line
4	UPR 103 80 1 2 (*)	Breeding line	52	CNAi 8870	Breeding line
5	CNA 3848 (*)	Breeding line	53	CNAi 9025	Breeding line
6	CNA 3887 (*)	Breeding line	54	CNAi 9051	Breeding line
7	IR36 (*)	Cultivar	55	CNAi 9150	Breeding line
8	Eloni (*)	Cultivar	56	CNAi 9606	Breeding line
9	Colombia 1 (*)	Cultivar	57	CNAi 9687	Breeding line
10	Nanicão (*)	Landrace	58	CNAi 9705	Breeding line
11	CNA 7(*)	Breeding line	59	CNAi 9747	Breeding line
12	AS 3510	Breeding line	60	CNAi 9748	Breeding line
13	Basmati 370	Cultivar	61	CNAi 9834	Breeding line
14	Blue Belle	Cultivar	62	CNAi 9838	Breeding line
15	BR IRGA 409	Cultivar	63	Diamante	Cultivar
16	BR IRGA 410	Cultivar	64	EEA 406	Landrace
17	BR IRGA 411	Cultivar	65	Embrapa 6 Chuí	Cultivar
18	BR IRGA 412	Cultivar	66	Embrapa 7 Taim	Cultivar
19	BR IRGA 413	Cultivar	67	Epagri 109	Cultivar
20	BR IRGA 417	Cultivar	68	Gen 1	Red rice
21	BR IRGA 418	Cultivar	69	Gen 11	Red rice
22	BR IRGA 419	Cultivar	70	Gen 13	Red rice
23	BR IRGA 420	Cultivar	71	Gen 141	Red rice
24	BRS Agrisul	Cultivar	72	Gen 145	Red rice
25	BRS Atalanta	Cultivar	73	Gen 19	Red rice
26	BRS Biguá	Cultivar	74	Gen 2	Red rice
27	BRS Bojuru	Cultivar	75	Gen 752	Red rice
28	BRS Bonança	Cultivar	76	Gen 9	Red rice
29	BRS Firmeza	Cultivar	77	IR22	Cultivar
30	BRS Formoso	Cultivar	78	IR8	Cultivar
31	BRS Jaburu	Cultivar	79	IR841	Cultivar
32	BRS Ligeirinho	Cultivar	80	Javaé	Cultivar
33	BRS Ouro Minas	Cultivar	81	Jequitiba	Cultivar
34	BRS Pelota	Cultivar	82	Marajó	Cultivar
35	BRS Soberana	Upland cultivar	83	Maravilha	Upland cultivar
36	BRS Talento	Upland cultivar	84	Metica 1	Cultivar
37	Pacholinha (CA 780320)	Landrace	85	*O. glumaepatula* P	Wild species
38	Precoce (CA 780403)	Landrace	86	*O. glumaepatula* RN	Wild species
39	Matão (CA 800120)	Landrace	87	*O. glumaepatula* RS	Wild species
40	Saquarema (CA 840018)	Landrace	88	*O. glumaepatula* VG	Wild species
41	Lageado (CA 840075)	Landrace	89	Oryzica 1	Cultivar
42	Palha Murcha (CA 840165)	Landrace	90	Paga Dívida	Landrace
43	Japonês (CA 940002)	Landrace	91	Rio Grande	Cultivar
44	Farroupilha (CA 940007)	Landrace	92	São Francisco	Cultivar
45	Cateto (CA 950011)	Landrace	93	SCS 112	Cultivar
46	Cica 4	Cultivar	94	SCS BRS 111	Cultivar
47	Cica 8	Cultivar	95	Skrivimangoti	Landrace
48	Cica 9	Cultivar			

* Genitors who originated the population CNA-IRAT 4.

TABLE 3
Chromosomal assignment, primer reference and sequence information of 16 SSR loci for rice used

SSR marker	Chromosome	Source	Primer sequence (5′ – 3′)
OG17	2	Brondani *et al.*, 2001	Forward CATGCATCAACAACGATC Reverse GTGCTCAAGTTAGCTGCTC
RM207	2	Chen *et al.*, 1997	Forward CCATTCGTGAGAAGATCTGA Reverse CACCTCATCCTCGTAACGCC
OG44	3	Brondani *et al.*, 2001	Forward ACACCAGCTCAGCTCATC Reverse TGTCCAGGTAGTACAAGCTC
MRG4879	4	http://www.monsanto.com	Forward CAGAGATCGATTGGTAGC Reverse CCTTGTACTCAGCTCCAT
OG61	5	Brondani *et al.*, 2001	Forward GCATGCTGATGACTGAAGG Reverse GAAACGAACGGATGGACA
RM204	6	Chen *et al.*, 1997	Forward GTGACTGACTTGGTCATAGGG Reverse GCTAGCCATGCTCTCGTACC
RM11	7	Panaud *et al.*, 1996	Forward TCTCCTCTTCCCCCGATC Reverse ATAGCGGGCGAGGCTTAG
RM248	7	Chen *et al.*, 1997	Forward TCCTTGTGAAATCTGGTCCC Reverse GTAGCCTAGCATGGTGCATG
RM223	8	Chen *et al.*, 1997	Forward GAGTGAGCTTGGGCTGAAAC Reverse GAAGGCAAGTCTTGGCACTG
OG106	9	Brondani *et al.*, 2001	Forward GGCCGTGTCACCATCTTCTCTA Reverse GGGGATCTGACATGGCATATGA
RM304	10	Temnhyk *et al.*, 2000	Forward TCAAACCGGCACATATAAGAC Reverse GATAGGGAGCTGAAGGAGATG
OG7	11	Brondani *et al.*, 2001	Forward CAGGTTCTTGTGAAATGTGT Reverse ACACTGACCACCATCTCC
RM224	11	Chen *et al.*, 1997	Forward ATCGATCGATCTTCACGAGG Reverse TGCTATAAAAGGCATTCGGG
MRG4961	11	http://www.monsanto.com	Forward CCACTTGTCTCCTGTATGCT Reverse GTGATGTGAACGCCTCTACT
RM229	11	Chen *et al.*, 1997	Forward CACTCACACGAACGACTGAC Reverse CGCAGGTTCTTGTGAAATGT
RM247	12	Chen *et al.*, 1997	Forward TAGTGCCGATCGATGTAACG Reverse CATATGGTTTTGACAAAGCG

TABLE 4
Results of 16 SSR marker analyses

SSR	Allele number	Polymorphism information content (PIC)	Allele size in Tio Taka	Allele frequency in genitors	Allele frequency in all genotypes
RM207	18	0.82	142	0.4	0.36
OG61	17	0.82	108	0.0	0.07
RM247	15	0.56	148	0.5	0.65
RM304	15	0.58	182	0.8	0.56
OG17	15	0.89	126	0.1	0.12
RM204	14	0.76	116	0.2	0.24
OG106	14	0.76	218	0.4	0.39
OG44	12	0.50	158	0.1	0.01
RM224	12	0.83	158	0.3	0.15
OG7	12	0.73	154	0.2	0.46
RM223	11	0.60	164	0.7	0.52
RM229	11	0.72	134	0.2	0.48
RM248	11	0.78	94	0.1	0.21
RM11	10	0.73	148	0.6	0.43
MRG4879	10	0.45	108	0.9	0.7
MRG4961	6	0.75	140	0.4	0.38
Average	**12.7**	**0.70**	**-**	**0.31**	**0.36**

TABLE 5
Observed heterozygosity in rice germplasm based on 16 SSR markers

Genotype	Observed heterozygosity	Number of heterozygotes
CNA 3815	0.063	1
UPR 103 80 1 2	0.063	1
Blue Belle	0.063	1
BRS Biguá	0.063	1
BRS Formoso	0.125	2
BRS Pelota	0.063	1
BRS Atalanta	0.188	3
Cica 9	0.063	1
EEA 406	0.313	5
BR IRGA 419	0.125	2
Javaé	0.063	1
Metica 1	0.250	4
Rio Grande	0.188	3
IR8	0.188	3
Cica 4	0.063	1
IR22	0.188	3
CNAi 9025	0.188	3
Maravilha	0.313	5
BRS Bonança	0.063	1
BRS Talento	0.063	1
Gen 9	0.125	2
Gen 11	0.313	5
Gen 13	0.125	2
Gen 145	0.188	3
CNAi 9051	0.125	2
Skrivimangoti	0.125	2
O. glumaepatula RN	0.125	2
O. glumaepatula RS	0.063	1
O. glumaepatula VG	0.188	3
CA 940002	0.063	1
CA 840018	0.25	4
CA 840075	0.063	1
CA 840165	0.063	1
CA 780320	0.063	1

TABLE 6
Number of private alleles in 95 analysed germplasm

Genotype	Marker	Allele	Frequency
AS3510	RM204	130	1
Basmati 370	RM248	74	1
Blue Belle	RM304	170	1
BR IRGA 413	RM229	132	1
BRS Bojuru	RM204	190	1
BRS Bojuru	OG61	96	1
BRS Bojuru	OG106	228	1
BRS Formoso	RM207	114	0.5
BRS Talento	RM204	200	0.5
BRS Talento	RM204	180	0.5
CA780403	RM223	174	1
CA780403	RM247	150	1
CA780403	MRG4879	146	1
CA800120	RM207	146	1
CA840018	RM223	166	1
CA840018	OG106	212	0.5
CA940002	RM247	166	1
CA940002	MRG4879	116	0.5
CA940007	OG106	250	1
CA950011	RM207	118	1
CNA3815	RM207	158	1
CNA7	RM304	186	1
CNA8502	RM304	194	1
Gen11	RM304	176	1
Gen11	OG106	226	1
Gen11	MRG4879	150	0.5
Gen13	OG61	140	1
Gen141	RM247	190	1
Gen141	RM304	156	1
Gen145	RM224	154	0.5
Gen752	RM248	100	1
IR8	RM207	150	1
Metica1	OG61	124	0.5
O. glumaepatula P	RM223	154	1
O. glumaepatula P	RM229	114	1
O. glumaepatula P	RM247	140	1
O. glumaepatula P	OG44	186	1
O. glumaepatula P	RM224	132	1
O. glumaepatula P	OG07	136	1
O. glumaepatula RN	RM247	134	1
O. glumaepatula RN	OG17	146	0.5
O. glumaepatula RN	OG44	184	1
O. glumaepatula RN	OG07	144	0.5
O. glumaepatula RS	OG106	242	1
O. glumaepatula RS	RM247	130	1
O. glumaepatula RS	OG44	180	1
O. glumaepatula RS	RM224	130	1
O. glumaepatula RS	MRG4879	128	1
O. glumaepatula RS	OG07	124	0.5
O. glumaepatula RS	RM207	100	1
O. glumaepatula VG	RM247	136	0.5
O. glumaepatula VG	OG61	112	1
O. glumaepatula VG	OG17	140	1
O. glumaepatula VG	OG44	182	1
Paga Dívida	OG61	132	1
Paga Dívida	RM248	96	1
Skrivimangoti	RM304	158	1
UPR 103 80 1 2	RM11	140	1

with an average of 12.7 alleles per locus, similar to the 15.3 alleles per locus found in rice by Blair, Hedetale and McCouch (2002). Considering the cultivated rice genotypes alone, 137 alleles were identified, with an average of 8.6 alleles per locus. These reductions reflect the lesser variability of cultivated genotypes in relation to the complete pool of genotypes analysed. The average number of alleles found in rice was similar to that in two other cereal species: 7.4 alleles were found in wheat (Prasad *et al.*, 2000) and 8.6 alleles in barley (Struss and Plieske, 1998). In general, SSR markers with a high number of detected alleles produced the highest values of PIC (Table 4).

A total of 58 private (exclusive) alleles were observed, distributed in 31 of the 95 genotypes analysed. The highest number of private alleles (21) was found in the wild species *Oryza glumaepatula*: six alleles from the Pantanal (P) accession, four from Rio Negro (RN), seven from Rio Solimões (RS) and four from Veredas de Goiás (VG) (Table 6). From commercial cultivars, BRS Bojuru showed three private alleles – the same number as the weed red rice Gen 11. Private alleles in wild species populations are generally related to reproductive isolation.

In rice varieties, the private alleles result from genitors whose ancestors were developed under very specific environmental conditions, subjected to mutation, migration, selection and adaptation, and for this reason, generating more diverse germplasm with low frequency alleles. With the use of highly polymorphic SSR markers, the detection of private alleles was facilitated. Private alleles are important for defining the genetic identity of each germplasm and can be used for its genetic evaluation, the assessment of genetic relationships between them, and to follow the allele frequency during the recurrent selection programme. The existence of exclusive alleles in recurrent selection populations would facilitate the discovery of the real contribution of each parent in the released cultivars. No private alleles were detected in CNA-IRAT 4 genitors with the 16 SSR analysed. However, increasing the number of SSR markers to genotype the genitors, private alleles may be identified and haplotypes for each genitor may be determined. This information can be used as an alternative to private alleles to identify the genitor with the highest probability of generating the best performed lines.

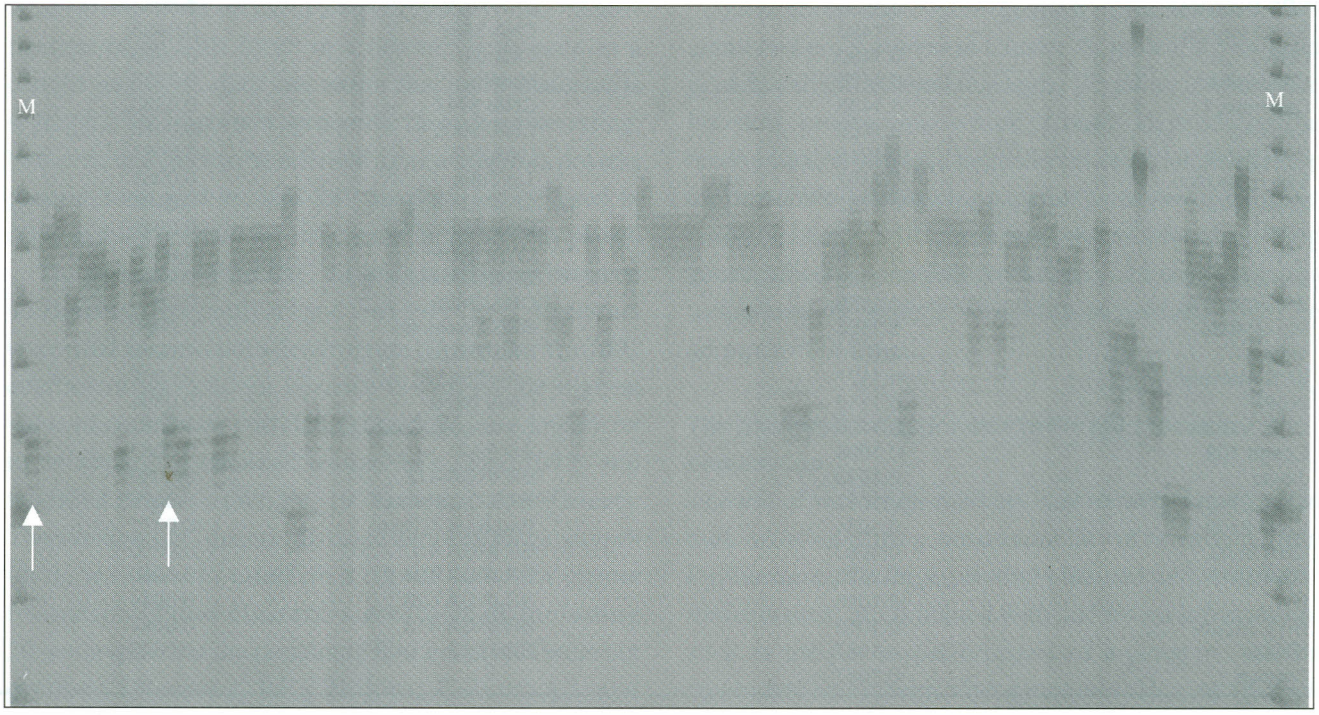
Analysis of CNA-IRAT 4 genitors

Based on the 16 SSR markers analysed, the detected alleles in Tio Taka cultivar were used to determine its frequencies in CNA-IRAT 4 genitors. The frequency varied from 0.9 (MRG4879) to zero (OG61), with an average of 0.31. Considering all 95 analysed genotypes, the frequency varied from 0.7 (MRG4879) to 0.01 (OG61), with an average of 0.36 (Table 4). In general, the most frequent alleles in genitors were also frequent in all accessions genotyped.

TABLE 7
Number of alleles in common and J&C distance coefficient between Tio Taka and the genitors of CNA-IRAT 4 population

Genitor	Shared alleles	J&C genitor × Tio Taka
BG 90-2	11	0.0510
UPR 103 80 1 2	9	0.0833
CNA 3815	8	0.0888
CNA 3848	6	0.1056
Nanicão	6	0.1056
CNA 3887	5	0.1171
IR36	5	0.1171
Eloni	4	0.1287
CNA 7	3	0.1404
Colombia 1	2	0.1524

FIGURE 1
Allelic polymorphism of OG61 SSR marker in 95 analysed genotypes. The allele 108 (indicated by an arrow) was present in Tio Taka (lanes 1 and 12) and absent in progenitors of Tio Taka. Between the lanes 1 and 12 (from left to right), are located the CNA-IRAT 4 genitors: BG 90-2, CNA 3815, UPR 103 80 1 2, CNA 3848, CNA 3887, IR36, Eloni, Colombia 1 and Nanicão. M = molecular mass marker: Ladder 10 bp (invitrogen)



The breeding line, BG 90-2, was the CNA-IRAT 4 genitor that showed the highest number (11) of alleles in common with Tio Taka and, consequently, the lowest J&C distance coefficient to Tio Taka (Table 7, Figure 2). The genitor Colombia 1 showed two alleles in common with Tio Taka and the higher J&C distance coefficient. The donor of the male sterility gene, the genitor IR36, shared five alleles with Tio Taka, meaning that the recombination and selection procedures prevented a higher contribution of IR36 in the Tio Taka genetic background, as observed by Ferreira *et al.* (2000). The high similarity between BG 90-2 and Tio Taka may be due to the good general combining ability of BG 90-2 in relation to other CNA-IRAT 4 genitors. In addition, BG 90-2 has good plant architecture and high yield (traits that can be easily identified and selected) and would have contributed to increasing the proportion of BG 90-2 alleles in the Tio Taka genome.

The Tio Taka allele 108, from SSR marker OG61, was not identified in the ten genitors that originated the population CNA-IRAT 4 (Figure 1, Table 4). This allele is common in the rice gene pool, since it was identified in the cultivars: BRS Formoso, BRS Ourominas, SCS 112 and BRS Talento, and in the breeding lines: CNAi 9834 and CNAi 9838. There are two possible reasons for the occurrence of this allele in Tio Taka: the seed mixture originating a rice plant that could pollinate any male sterile plants (*msms*); or the pollen migration from a rice variety cultivated in adjacent areas of the CNA-IRAT 4 field experiment, during recombination of the recurrent selection population. The male sterile gene is responsible for important cost reductions during the recombination step of the recurrent selection method. However, due to the risk of pollen contamination, it is recommended to maintain the population under recombination as far as possible to other rice genotypes, since

TABLE 8
The J&C distance coefficient average of 69 rice cultivars, breeding lines and traditional varieties

Genotype	J&C coefficient	Genotype	J&C coefficient
Maravilha	0.1884	IR22	0.1055
BRS Bojuru	0.1776	Diamante	0.1051
BRS Bonança	0.1729	CNAi 9150	0.1049
Blue Belle	0.1721	BRS Formoso	0.1033
AS 3510	0.1677	CNAi 9687	0.1029
BR IRGA 411	0.1618	Cica 4	0.1023
BRS Talento	0.1591	CNA 3848	0.0985
BRS Soberana	0.1539	BRS Agrisul	0.0983
BRS Firmeza	0.1490	CNA 3887	0.0983
CNA 7	0.1490	Cica 8	0.0983
CNAi 9051	0.1476	Marajo	0.0983
Colombia 1	0.1438	CNAi 9747	0.0980
Nanicão	0.1405	CNAi 9606	0.0976
BRS Bigua	0.1215	Cica 9	0.0961
Sao Francisco	0.1192	Tio Taka	0.0953
CNAi 9025	0.1192	BR IRGA 413	0.0950
Eloni	0.1186	SCS BRS 111	0.0946
CNAi 9834	0.1180	CNAi 8859	0.0942
CNAi 9838	0.1180	BR IRGA 417	0.0934
BR IRGA 419	0.1169	CNAi 8860	0.0920
Rio Grande	0.1164	BRS Ligeirinho	0.0920
Metica 1	0.1161	BRS Pelota	0.0915
BRS Atalanta	0.1127	Jequitiba	0.0902
IR36	0.1115	CNAi 8870	0.0899
UPR 103 80 1 2	0.1106	Embrapa 6 Chuí	0.0887
Epagri 109	0.1098	BG 90-2	0.0882
Javae	0.1093	BR IRGA 412	0.0882
CNAi 9748	0.1092	BRS Ouro Minas	0.0875
CNAi 9705	0.1084	Embrapa 7 Taim	0.0871
CNA 8502	0.1083	BR IRGA 410	0.0860
CNA 3815	0.1070	BR IRGA 409	0.0860
IR8	0.1068	BR IRGA 420	0.0844
BRS Jaburu	0.1068	BR IRGA 418	0.0842
IR841	0.1067	SCS 112	0.0839
Oryzica 1	0.1057		

the pollen has the potential to fecundate plants at least 10 m from the pollen source (Messeguer *et al.*, 2001).

Genetic variability of rice germplasm

The use of highly informative SSR markers permits the identification and monitoring of specific alleles during cultivar development in recurrent selection populations. Despite the preferentially autogamous reproductive habit of rice, it is possible to identify loci in a heterozygous state, even in commercial cultivars. When genetic identity was based on phenotypic evaluation at whole plant level, this assumption was often an acceptable approximation to reality. For registered modern varieties, genetic heterogeneity may be diagnostic of unwanted seed mixtures, outcrossing or, in rare cases, mutation (Olufowote *et al.*, 1997). The genotypes showing the highest number of heterozygous loci were: Maravilha (upland cultivar), EEA 406 (landrace) and Gen 11 (red rice) with five loci in heterozygosity; and Metica 1 (lowland cultivar) and Saquarema (landrace) with four loci in heterozygosity (Table 5). Considering only the commercial cultivars expected to have a well-defined genetic identity with very low frequency residual heterozygosity, at least one heterozygous locus was observed in Blue Belle, BR IRGA 419, BRS Atalanta, BRS Biguá, BRS Bonança, BRS Formoso, BRS Pelota, BRS Talento, Cica 4, Cica 9, IR22, IR8, Javaé, Maravilha, Metica 1 and Rio Grande, corresponding to 33 percent of analysed cultivars (Table 5). In relation to the breeding lines analysed, 21 percent showed at least one SSR locus in heterozygosity. The identification of an SSR locus in a heterozygous state in a certain genotype does not mean that such material is segregating to important agronomic traits. It is interesting, however, that lines to be released as new cultivars possess genetic homogeneity, and SSR markers can be used to achieve this goal with the selection of homozygous plants.

The estimates of the J&C distance coefficient between 44 Brazilian rice cultivars and ten CNA-IRAT 4 genitors were used to construct a dendrogram (Figure 2). The J&C coefficient was chosen because it showed the highest value of cophenetic correlation ($r = 0.92$) in comparison to dendrograms obtained using Nei's (Nei, 1972) and modified Rogers's (Wright, 1978) distance coefficients. The average J&C genetic distance between all genotypes (0.11) was used to establish a cut-off value for cluster formation. Four clusters were formed including 50 genotypes, and the remaining four genotypes were not grouped (Nanicão, BRS Bojuru, Colombia 1 and Maravilha) (Figure 2). Cluster A was formed with 43 lowland rice genotypes, including Tio Taka and seven of the genitors of CNA-IRAT 4 (BG 90-2, CNA 3815, CNA 3848, CNA 3887, UPR 103 80 1 2, IR36 and Eloni). Cluster B included CNA 7 (a genitor of CNA-IRAT 4) and BRS Soberana (an upland rice cultivar). Cluster C included BRS Firmeza and its two genitors, Blue Belle and BR IRGA 411. Cluster D included two upland rice cultivars, BRS Bonança and BRS Talento, which have two of its three genitors in common. The other two CNA-IRAT 4 genitors, Nanicão and Colombia 1, were not included in clusters.

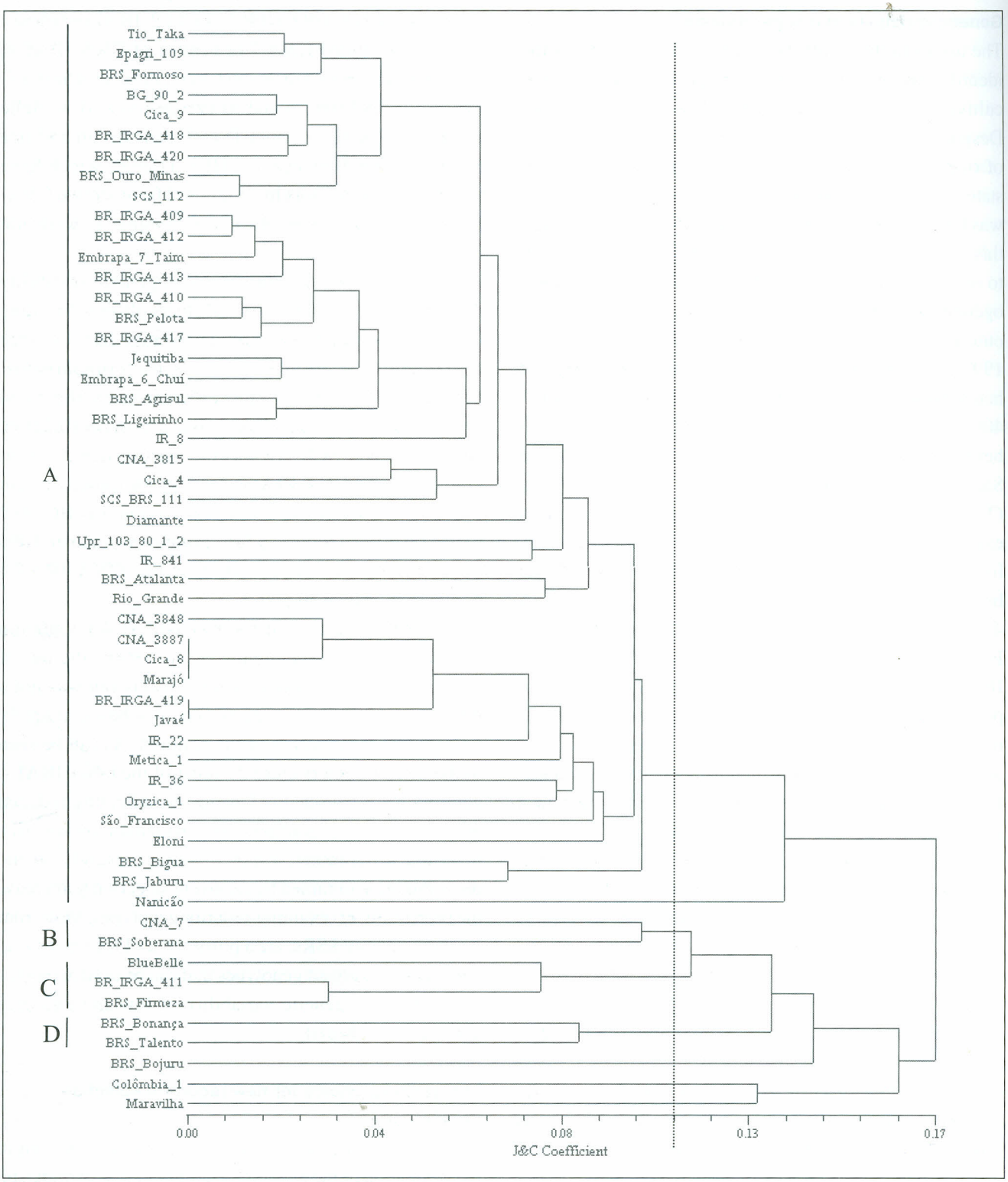
In order to determine the degree of genetic variability of each genotype in relation to all other cultivars, breeding lines and traditional varieties, the average J&C distance coefficient was used to proceed the comparisons. Maravilha, an upland rice cultivar, was the most genetically distant from all other rice genotypes (0.1884), and the cultivar SCS 112 was the least distant (0.0839) (Table 8). From CNA-IRAT 4 genitors, the most divergent was CNA 7 (0.15), and the least divergent was BG 90-2 (0.088). Tio Taka showed a J&C distance average of 0.095 – i.e. inferior to all genitors of the CNA-IRAT 4 population, except BG 90-2.

When the genitors of the CNA-IRAT 4 population were chosen at the beginning of the 1990s, the use of molecular markers in rice genetic evaluation was not a routine, and the selection performed was based uniquely on pedigree information of the genitors. As can be seen in the dendrogram (Figure 2), most of the CNA-IRAT 4 genitors were included in Cluster A, indicating reduced genetic variability available to the Brazilian lowland rice breeding programme, which strongly depends on the recombination obtained by recurrent selection to increase the probability of obtaining superior genotypes. Molecular markers, mainly SSRs, are a powerful tool for identifying genetically divergent genotypes that can be used in crosses to increase the genetic variability of lowland elite rice germplasm in Brazil.

The genitors' choice for new recurrent selection populations

The use of molecular markers is extremely important in the evaluation of the genetic variability of rice germplasm. Furthermore, the fingerprinting obtained with molecular markers must be combined with additional information, such as the *per se* performance of a genotype and its combining ability with other genotypes. This information

FIGURE 2
Dendrogram with 54 genotypes, including Tio Taka and cultivated germplasm, with distances obtained by J&C distance coefficient and UPGMA method (Dotted line corresponds to the cut-off value 0.11, obtained with the average J&C distance coefficient)



can aid genitor selection, revealing genetic divergence and good agronomic performance. Another advantage in fingerprinting the genitors of a recurrent selection population is the possibility to monitor the allele frequencies of SSR markers between the different cycles of recombination. In order to avoid the genetic drift, which could reduce the genetic variability and, consequently, the genetic gain, SSR markers can be used to monitor the allelic frequencies of all genitors of the population. If the frequency of a certain allele is reduced, or even lost, the genitors that have this allele can be included in the recombination step of this population.

CONCLUSION

The broadening of the genetic basis of rice cultivars can be accomplished by a recurrent selection programme. The choice of genitors in the development of recurrent selection populations is decisive for the generation of variable populations that will produce breeding lines with unique favourable allele combinations. SSR markers are a useful tool in the selection of genitors and for monitoring allele variation during the successive recurrent cycles in the recurrent selection programme.

Since there is low genetic variability in elite lowland rice germplasm adapted to Brazilian cultivation, two strategies are recommended to obtain better results:

- First, integrate more diverse germplasm, such as rice introductions from abroad, upland cultivars, landraces and lines derived from interspecific crosses, into the elite rice background, by the implementation of a pre-breeding programme based on backcrosses, which will generate breeding lines with broader genetic variability to be used as genitors of the Brazilian rice breeding programme.
- Second, use the recurrent selection method to maximize the opportunity of recombination of this genetically variable germplasm.
- The release of Tio Taka, a highly productive rice cultivar, developed from a population obtained with less divergent genitors, is an indication that the increase of genetic variability of elite genitors in new recurrent selection populations will produce even better lowland rice cultivars.

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