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Transferability of microsatellite and sequence tagged site markers in *Oryza* species

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The genus *Oryza* comprises 22 species which are potentially useful as a source of genetic variability that can be introgressed into the worldwide cultivated rice, *Oryza sativa*. Molecular markers are useful tools for monitoring gene introgressions and for detecting polymorphism among species. In this study, cross-amplification was estimated among 28 accessions of 16 *Oryza* species, representing the genomes **AA**, **BB**, **CC**, **BBCC** and **CCDD**, using 59 microsatellite (OG, OS and RM series) and 15 STS (Sequence Tagged Sites) markers. All markers amplified at least one *Oryza* species, indicating different levels of transferability across species. Markers based on microsatellite sequences amplified 37 % of the accessions, with an average of 6.58 alleles per locus and an average polymorphism information content (PIC) of 70 %. For STS markers, the amplification level was 53.3 %, and the average number of alleles and PIC values were 1.6 and 10 %, respectively. These results showed that although the STS markers detected a reduced level of genetic diversity, the transferability was higher, indicating that they can be used for genetic analysis when evaluating less genetically related species of *Oryza*. Among the microsatellite markers, an analysis of species with an **AA** genome showed that the OG markers produced the highest level of polymorphic loci (54.6 %), followed by RM markers (48 %). Highly polymorphic and transferable molecular markers in *Oryza* can be useful for exploiting the genetic resources of this genus, for detecting allelic variants in loci associated with important agronomic traits, and for monitoring alleles introgressed from wild relatives to cultivated rice.

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The genus *Oryza* consists of two cultivated and 21 wild species. Of these, the most important species is rice (*O. sativa*), which is cultivated worldwide. The current classification of *Oryza* species based on the ease of gene transfer to rice by interspecific hybridization is of special interest to rice breeders who desire the introgression of exotic genes. The *Oryza sativa* species complex (primary gene pool) contains the **AA** genome, as in cultivated rice, and gene transfer to rice is relatively easy. The *Oryza officinalis* complex includes species with **BB**, **CC**, **BBCC**, **CCDD**, **EE** and **FF** genomes (secondary gene pool), and there is limited gene transfer to rice which generally requires embryo rescue approaches in order to succeed. The tertiary gene pool includes representatives of the *O. meyeriana*, *O. ridleyi* and *O. schlechteri* complexes, and it is extremely difficult or even impossible to obtain fertile hybrids with rice (KHUSH 1997).

BRAR and KHUSH (1997) list a series of gene introgressions, basically genes associated with disease resistance and cytoplasmic male sterility, obtained from crosses with wild rice species. Interspecific crosses are useful for increasing the genetic variability of cultivated rice, and have produced a favorable general effect in important agronomic traits. Favorable genes that are apparently not based on the phenotype of the wild species have been identified (XIAO et al. 1998; BRONDANI et al. 2002a). Advanced backcross quantitative trait loci (AB-QTL) analysis (TANKSLEY and NELSON 1996) has been used to identify these genes and consists of an interspecific cross followed by two to three backcrosses in the direction towards rice and then several generations of selfing. This strategy, which results in rice lines with different introgressed fragments, is the basis of the QTL analysis done using molecular markers combined with phenotypic analysis in replicated experiments. The use of molecular markers can help in estimating the overall genetic variability, visualize the proportion of the genome introgressed from the donor, identify the genes related to the increase in the phenotypic value of analyzed traits, and then allow marker assisted selection in subsequent generations of these introgression lines.

Several types of PCR-based markers are available for genetic analysis. Of these, microsatellites or simple sequence repeats (SSR) (WEBER and MAY 1989) are very attractive since they are co-dominant, detect

a high number of alleles per locus, and are abundant and uniformly dispersed throughout the genome. In addition, the amplification of SSR across species, which depends on the sequence conservation of the flanking primer regions, was reported for closely related species in *Oryza* (Wu and Tanksley 1993). These characteristics are particularly interesting for rice researchers since a huge number of SSR markers are available for rice, and based on its high information content, they can give good estimates of genetic variability or can be used for extensive genomic analysis among broad interspecific crosses.

In this work, the extent of genome conservation and genetic similarity among 28 accessions from 16 *Oryza* species (**AA**, **BB**, **CC**, **BBCC** and **CCDD** genomes) chosen as potentially useful for the transfer of fragments containing genes of interest to rice breeding programs were examined. For these analyses, SSR markers from genomic enriched libraries (OG and RM markers), expressed sequences associated to SSR motifs (OS markers) and STS sequences from databank (STSG markers) were used, and the genetic information content among them was compared.

MATERIAL AND METHODS

Plant material

Twenty eight accessions of 16 *Oryza* species, 15 of which were from the *O. sativa* complex and 13 were from the *O. officinalis* complex and obtained from the rice germplasm bank were used in this study (Table 1). Genomic DNA was extracted from leaves as described by Brondani et al. (1998).

Marker analysis

Seventy four PCR-based markers, representatives of all rice chromosomes, were used for the genetic analysis. Two new SSR markers, OG 56 (5′ CGCAGGTAATGAACAGCTTG 3′ and 5′ GTAGCAGCAAAAGGGAAAGG 3′, 21 AG repeat motif) and OG 58 (5′ CTGAGAGCGTGCTGACATGTG 3′ and 5′ TGCTTCCATCTGCGGCTT 3′, 20 AG repeat motif), both with 56°C of annealing temperature, were produced. An additional set of 72 previously published marker sequences were used. From the SSR markers available in the rice literature, 18 were selected from the RM series (Panaud et al. 1996; Chen et al. 1997): RM 1, RM 5, RM 6, RM 7,

Table 1. *Accessions of cultivated and wild Oryza species analyzed (*).*

Species	Abbreviation	Accession	Genome/Region	Complex
Oryza sativa	SAT-01	IR-36	AA	*O. sativa*
Oryza sativa	SAT-02	BG-90-2	AA	*O. sativa*
Oryza glumaepatula	GLU-03	RS20	AA/Brazil	*O. sativa*
Oryza glumaepatula	GLU-04	PPA4	AA/Brazil	*O. sativa*
Oryza longistaminata	LON-05	IRRI101202	AA/Africa	*O. sativa*
Oryza longistaminata	LON-06	IRRI104075	AA/Africa	*O. sativa*
Oryza barthii	BAR-07	IRRI100223	AA/Africa	*O. sativa*
Oryza barthii	BAR-08	IRRI100122	AA/Africa	*O. sativa*
Oryza glaberrima	GLA-09	IRRI102202	AA/Africa	*O. sativa*
Oryza glaberrima	GLA-10	IRRI102254	AA/Africa	*O. sativa*
Oryza breviligulata	BRE-11	EB04	AA/Africa	*O. sativa*
Oryza breviligulata	BRE-12	EB22	AA/Africa	*O. sativa*
Oryza meridionalis	MER-13	IRRI101147	AA/Australia	*O. sativa*
Oryza latifolia	LAT-14	IRRI105133	CCDD/Brazil	*O. officinalis*
Oryza alta	ALT-15	IRRI100161	CCDD/Brazil	*O. officinalis*
Oryza grandiglumis	GRA-16	IRRI101405	CCDD/Brazil	*O. officinalis*
Oryza punctata	PUN-17	IRRI103889	BB/Africa	*O. officinalis*
Oryza punctata	PUN-18	IRRI103917	BB/Africa	*O. officinalis*
Oryza punctata	PUN-19	IRRI101408	BBCC/Africa	*O. officinalis*
Oryza punctata	PUN-20	IRRI105137	BBCC/Africa	*O. officinalis*
Oryza minuta	MIN-21	IRRI101083	BBCC/Asia	*O. officinalis*
Oryza minuta	MIN-22	IRRI101101	BBCC/Asia	*O. officinalis*
Oryza eichingeri	EIC-23	IRRI101426	CC/Africa	*O. officinalis*
Oryza eichingeri	EIC-24	IRRI105162	CC/Africa	*O. officinalis*
Oryza officinalis	OFF-25	IRRI105961	CC/Africa	*O. officinalis*
Oryza officinalis	OFF-26	IRRI105962	CC/Africa	*O. officinalis*
Oryza rhizomatis	RHI-27	IRRI103410	CC/Africa	*O. officinalis*
Oryza rhizomatis	RHI-28	IRRI105449	CC/Africa	*O. officinalis*

*The *Oryza* classification is according to Khush (1997).

RM 8, RM 11, RM 16, RM 17, RM 18, RM 22, RM 26, RM 30, RM 31, RM 34, RM 38, RM 55, RM 201 and RM 202; 15 from the OS series (AKAGI et al. 1996): OS 1, OS 4, OS 9a, OS 12, OS 15, OS 16, OS 17, OS 22, OS 23, OS 26, OS 27, OS 28, OS 29, OS 30 and OS 34; and 26 from the OG series (BRONDANI et al. 2001): OG 5, OG 7, OG 8, OG 10, OG 16, OG 17, OG 19, OG 22, OG 25, OG 26, OG 29, OG 31, OG 32, OG 35, OG 44, OG 45, OG 56, OG 57, OG 58, OG 60, OG 61, OG 63, OG 64, OG 65, OG 73 and OG 99. Of the sequences obtained from NCBI (http://www.ncbi.nlm.gov), 15 STS markers previously developed by BRONDANI et al. (2001) were used: STSG 6, STSG 8, STSG 20, STSG 22, STSG 25, STSG 26, STSG 33, STSG 39, STSG 40, STSG 41, STSG 42, STSG 43, STSG 44, STSG 47 and STSG 71.

PCR and electrophoresis of microsatellites

The PCR reactions were done in a final volume of 13 µl, containing 15 ng of DNA, 0.3 µM of each primer, 0.25 mM of each dNTP, 1.5 mM of $MgCl_2$, 1 mM TRIS–HCl (pH 8.3), 50 mM KCl, 5 % of DMSO and 1 unit of Taq DNA polymerase. The reactions were done in a 96-well PT-100 thermocycler (MJ Research), with an initial 4 min incubation at 94°C, followed by 30 cycles at 94°C for 1 min, then 1 min at 50, 52 or 56°C, 1 min at 72°C, and a final extension at 72°C for 7 min. The amplified products were electrophoresed on denaturing 4 % acrylamide gels that were silver stained according to BASSAM et al. (1991).

Allele scoring and data analysis

After the silver staining, the **AA** genome alleles were scored to determine the polymorphism information content (PIC) as described by ANDERSON et al. (1993) for self-pollinated species, calculated as:

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

where P_{ij} is the frequency of the jth allele for marker i, and n is the number of alleles. To dendrogram construction, all individuals were scored for presence (1) and absence (0) of alleles for each marker. The Jaccard similarity coefficient was used to generate the similarity matrix, which was utilized to group the individuals using the unweighted pair-group method (UPGMA) available in NTSYS-pc software (ROHLF 1998). The bootstrap analysis was conducted using the Bood software version 3.01, kindly provided by Dr. Alexandre Coelho (Federal University of Goiás, Brazil).

RESULTS AND DISCUSSION

The genetic diversity of 13 accessions of *Oryza* species with an **AA** genome was analyzed using 74 PCR-based markers. The OG and RM microsatellite markers, derived from SSR-enriched genomic libraries, showed a number of alleles per locus ranging from 1 (RM 34) to 11 (OG 10 and OG 60), with an average of 6.8 alleles, and PIC values ranging from 0 to 0.89, with an average of 0.74 (Table 2). The number of alleles was similar to that observed by NI et al. (2002) in a study with subspecies of rice using microsatellite markers in which an average of 6.8 alleles per locus was found. For OS markers derived from expressed sequences containing microsatellite fragments, an average of 5.87 alleles per locus and a PIC value of 0.58 were obtained. Among the STS markers, which are based on unique sequences found anywhere in the genome, the level of genetic diversity was relatively low, with an average of 1.57 alleles per locus and a PIC of 0.10 per locus. Markers obtained from genomic microsatellite-enriched libraries (OG and RM markers) showed greater genetic diversity than microsatellite markers obtained from NCBI sequences (OS markers), as also previously found by CHO et al. (2000). The reduced level of polymorphism among OS microsatellite markers was congruent with the conservative nature of the expressed sequences, with lower mutation rates compared to markers located in non-translated regions. However, this class of microsatellite markers is very attractive since it could help in establishing a relationship between candidate genes and specific QTLs.

Based on the number of repetitive sequences of OG and RM markers, there was no positive relationship between the number of tandem repeats and the level of polymorphism for the 44 loci evaluated. Several SSRs, ranging from 12 to 29 repeats in length, were highly polymorphic, with PIC values greater than 80 %. In contrast, the longest SSR, OG 5 (30 AG/TC repeats), showed a lower PIC (0.73). Similar results, in which longer repeats did not necessarily show the highest level of polymorphism, have been reported for *Brassica* (SZEWC-MCFADDEN et al. 1996) and *Eucalyptus* (BRONDANI et al. 1998, 2002b) species, in contrast to the positive relationship noted by NI et al. (2002) for rice.

All markers were amplified in at least one *Oryza* species. However, different levels of transferability across species were observed. The marker series with the highest capacity to generate amplified products (53.3 %) in all *Oryza* species analyzed were the STS and OS markers obtained from expressed sequences. The greater capacity of the STS and OS markers to generate amplified products compensated for the re-

Table 2. *PCR-based markers analyzed and its Oryza genome specificity. The value between parenthesis is the percentage of amplified markers in relation to all markers of the same series. P = differentiation of **BB** and **CC** genomes in O. punctata; M = differentiation of **BB** and **CC** genomes in O. meridionalis.*

Marker series	All genomes	**AA** specific	All genomes except **BB**	All genomes except **CC**	**BB** and **CC** genome differentiation in **BBCC**	Average PIC value
OG (26 markers)	5,17,25,31, 56,61,45, 64,65,99, 44,73 (41.15 %)	8,10,16,22, 29,32,35,58, 60 (34.62 %)	26,57,63 (11.54 %)	7 (3.84 %)	17 (P), 45(P), 61 (P/M), 64 (P/M), 65 (P/M),44(P), 99(P/M) (26.92 %)	0.7760
OS (15 markers)	4,9a,12,15,16,22,26, 34 (53.33 %)	1,17,23,28,29 (33.33 %)	30 (6.67 %)	–	12(M), 22(P/M), 3(P/M) (20 %)	0.5845
RM (18 markers)	18,30,202 (16.67 %)	1,6,7,8,11,13,16,17,22,31, 34,38,55 (72.22 %)	–	5 (5.56 %)	202(P/M), 30(P) (11.11 %)	0.6904
STSG (15 markers)	25,33,41, 42,43,44, 47,71 (53.33 %)	8,26 (13.33 %)	20,22,39,40 (26.67 %)	–	–	0.1368

duced level of allelic variability detected. The least transferable markers were in the RM series, with just 16.7 % of the markers amplifying all genomes. For OG markers, 41.2 % were useful for amplifying all *Oryza* species tested (Table 2). The reduced level of PCR amplification for microsatellite markers in some *Oryza* species suggested that conservation of the DNA sequences at the primer annealing sites may not extend beyond specific genomes, indicating a distant phylogenetic relationship among them. In order to preliminarily infer the genetic relationship among *Oryza* species, we constructed a dendrogram based on the presence and absence of alleles. We used the Jaccard similarity coefficient, which produced the highest value of the cophenetic correlation ($r = 0.96$) in comparison to Dice and Simple Matching similarity coefficients. The use of molecular markers to infer phylogeny is controversial, since the co-migrated alleles can be resultant from paralogy (identical in state) or orthology (identical by descent). However, we observed that the 74 PCR-based markers produced a dendrogram with two clusters, one grouping all **AA** genome species and another with **BB**, **CC**, **BBCC** and **CCDD** genome species (Fig. 1). This result indicates that the use of a high number of molecular markers can diminish the problem of paralogy, as also described by Ishii et al. (2001).

Among the 13 accessions of the *Oryza sativa* complex analyzed, and considering all markers evaluated, *O. breviligulata* and *O. barthii* showed the highest (71 %) and lowest (24 %) levels of polymorphic markers, respectively. As also observed by Cho et al. (2000) *O. longistaminata*, which is native to Africa, failed to be amplified by 14 % of the molecular markers. This situation probably reflects the lack of a primer annealing site as a result of DNA deletions, insertions or rearrangements in these genomic regions. All remaining **AA** genome species were amplified by all microsatellite and STS markers. Of the *O. officinalis* complex, *O. punctata* (**BB** genome) was amplified by only 46 % of the markers tested whereas values greater than 60 % were obtained for the remaining species. In addition, the markers used were useful to identify the genomic origin of alleles from **BB** and **CC** genomes of the tetraploid **BBCC** species *O. punctata* and *O. minuta* (Table 2 and Fig. 2).

The availability of molecular markers that are transferable among species and which allow the identification of allelic variability is very important for genetic analysis in rice, as one moves from QTL mapping to an effective search for QTL-allele tagging, comparative QTL mapping, gene-flow studies and a direct search for new allelic variations within germplasm collections (Brondani et al. 1998). In addition, there is no need to develop SSR markers for

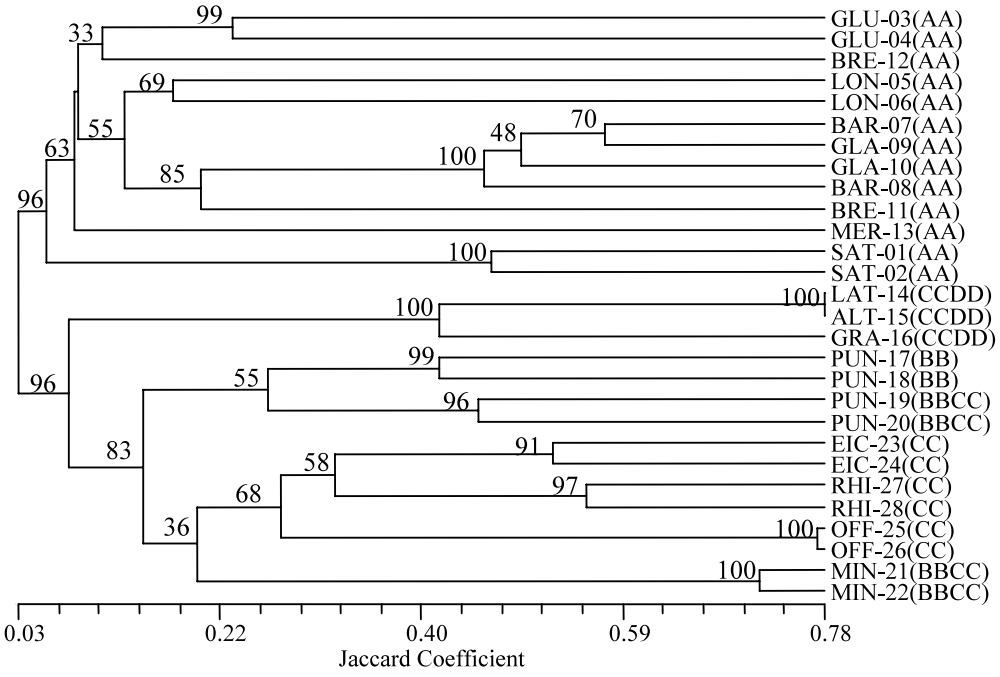

Fig. 1. Dendrogram based on genetic similarity coefficient of Jaccard calculated from 74 PCR-based marker alleles of 28 *Oryza* species. The species names are according to the abbreviation indicated on Table 1. The values on the dendrogram nodes correspond to the percentage of times that the grouping occurred by bootstrap re-sampling analysis.

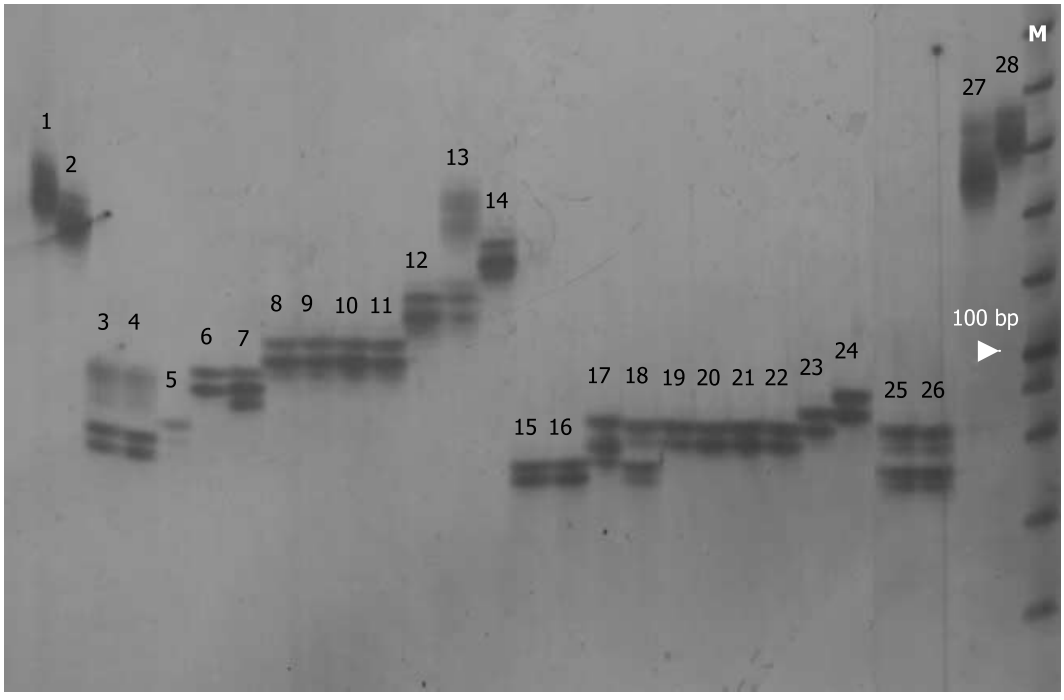

Fig. 2. SSR pattern of OG 61, which can differentiate the **BB** and **CC** genome amplification in tetraploid **BBCC** (individuals 17, 18, 25 and 26). The species are: *O. glumaepatula* **AA** (1,2); *O. latifolia* **CCDD** (3); *O. alta* **CCDD** (4); *O. grandiglumis* **CCDD** (5); *O. longistaminata* **AA** (6,7); *O. barthii* **AA** (8,9); *O. glaberrima* **AA** (10,11); *O. breviligulata* **AA** (12,13); *O. meridionalis* **AA** (14); *O. punctata* **BB** (15,16); *O. punctata* **BBCC** (17,18); *O. eichingeri* **CC** (19,20); *O. officinalis* **CC** (21,22); *O. rhizomatis* **CC** (23,24); *O. minuta* **BBCC** (25,26) and *O. sativa* **AA** (27,28). M = 10 bp Ladder.

species of the *O. sativa* complex (**AA** genome). Based on a previous *O. sativa* × *O. glumaepatula* QTL analysis (BRONDANI et al. 2002a), some markers related to the grain yield trait were also tested in the present work, such as RM 1 (% phenotypical variance explained, PV = 39.1), OS 15 (PV = 38.3), RM 16 (PV = 19.7) and RM 11 (PV = 13.9). The PIC values for these markers (0.85, 0.82, 0.63 and 0.78 for RM 1, OS 15, RM 16 and RM 11, respectively) showed that they could be useful for finding alleles related to the grain yield trait among **AA** genome species, and can also be used to monitor the gene introgression of such alleles in interspecific crosses with cultivated rice.

REFERENCES

Akagi, H., Yokozeki, Y., Inagaki, A. and Fujimura, T. 1996. Microsatellite DNA markers for rice chromosomes. – Theor. Appl. Genet. 93: 1071–1077.

Anderson, J. A., Churchill, G. A., Autrique, J. E. et al. 1993. Optimizing parental selection for genetic linkage maps. – Genome 36: 181–186.

Bassam, B. J., Caetano-Anolles, G. and Gresshoff, P. M. 1991. Fast and sensitive silver staining of DNA in polyacrilamide gels. – Anal. Biochem. 196: 80–83.

Brar, D. S. and Khush, G. S. 1997. Alien introgression in rice. – Plant Mol. Biol. 35: 35–47.

Brondani, R. P. V., Brondani, C., Tarchini, R. and Grattapaglia, D. 1998. Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and *E. urophylla*. – Theor. Appl. Genet. 97: 816–827.

Brondani, C., Brondani, R. P. V., Rangel, P. H. N. and Ferreira, M. E. 2001. Development and mapping of *Oryza glumaepatula*-derived microsatellite markers in the interspecific cross *O. glumaepatula* × *O. sativa*. – Hereditas 134: 59–71.

Brondani, C., Rangel, P. H. N., Brondani, R. P. V. and Ferreira, M. E. 2002a. QTL mapping and introgression of yield related traits from *Oryza glumaepatula* to cultivated rice (*O. sativa*) using microsatellite markers. – Theor. Appl. Genet. 104: 1192–1203.

Brondani, R. P. V., Brondani, C. and Grattapaglia, D. 2002b. Towards a genus-wide reference linkage map for *Eucalyptus* based exclusively on highly informative microsatellite markers. – Mol. Genet. Genomics 267: 338–347.

Chen, X., Temnykh, S., Xu, Y. et al. 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L). – Theor. Appl. Genet. 95: 553–567.

Cho, Y. G, Ishii, T., Temnykh, S. et al. 2000. Diversity of microsatellite derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). – Theor. Appl. Genet. 100: 713–722.

Ishii, T., Xu, Y. and McCouch, S. R. 2001. Nuclear- and chloroplast-microsatellite variation in A-genome species of rice. – Genome 44: 658–666.

Khush, G. S. 1997. Origin, dispersal, cultivation and variation of rice. – Plant Mol. Biol. 35: 25–34.

Ni, J., Colowit, P. M. and Mackill, D. 2002. Evaluation of genetic diversity in rice subspecies using microsatellite markers. – Crop Sci. 42: 601–607.

Panaud, O., Chen, X. and McCouch, S. R. 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). – Mol. Gen. Genet. 252: 597–607.

Rohlf, F. J. 1998. Numerical taxonomy and multivariate analysis system, version 2.02. User guide.

Szewc-McFadden, A. K., Kresovich, S., Bliek, S. M. et al. 1996. Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated *Brassica* species. – Theor. Appl. Genet. 93: 534–538.

Tanksley, S. D. and Nelson, J. C. 1996. Advanced backross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. – Theor. Appl. Genet. 92: 191–203.

Weber, J. L. and May, P. E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. – Am. J. Hum. Genet. 44: 388–396.

Wu, K. S. and Tanksley, S. D. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. – Mol. Gen. Genet. 241: 225–235.

Xiao, J., Li, J., Grandillo, S. et al. 1998. Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. – Genetics 150: 899–909.