Journal of Plant Biology, October 2006, 49(5) : 339-347

Genetic Diversity and Evolutionary Relationships of *Oryza* Species with the B- and C-Genomes as Revealed by SSR Markers

Ying Bao[1,2], Hai Fei Zhou[3], De Yuan Hong[1,3], and Song Ge[3]*

[1]College of Life Sciences, Zhejiang University, Hangzhou 310029, China
[2]College of Life Science, Qufu Normal University, Qufu 273165, Shandong, China
[3]State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Genetic diversity and evolutionary relationships of 72 accessions representing six species with the B-, C-, and BC-genomes in the genus *Oryza* were investigated by seven microsatellite markers. Of four diploid species, *Oryza officinalis* maintained the highest diversity (*P*=71.4%, *He*=0.565), followed by *Oryza eichingeri* (*P*=57.1%, *He*=0.376), *Oryza punctata* (*P*=57.1%, *He*=0.272) and *Oryza rhizomatis* (*P*=42.9%, *He*=0.222). In comparison, a higher level of genetic diversity was revealed in the tetraploid (*P*=71.4%, *He*=0.461-0.637). UPGMA dendrograms based on genetic distance revealed an obvious genetic differentiation between Asian and African races of *O. eichingeri*. Three BBCC species clustered with different accessions of the diploid *O. punctata*, suggestive of their multiple origins. The results inferred from the dendrogram suggested that diploid species, *O. officinalis* and African *O. eichingeri* might be the C-genome donors for tetraploid species, *Oryza minuta* and *O. punctata*, respectively, while the C-genome ancestor of *Oryza malampuzhaensis* seemed to be either *O. rhizomatis* or the Sri Lankan *O. eichingeri* species. The genetic relationship among the CC and BBCC species further indicated that the tetraploid species with the BC-genome have originated independently, at least three times in history. In addition, we have demonstrated successful cross-species amplification of seven rice SSR loci across *Oryza* species with B-and C-genomes.

Keywords: B- and C-genome, evolutionary relationships, genetic diversity, microsatellite, *Oryza*

Rice is an economically important crop that is the staple food for more than one-half of the world's population. However, intensive selection in modern breeding practices has led to a severe loss of genetic diversity in rice and then limited its productivity (Lu, 1999). In contrast to the cultivated species, wild relatives of rice hold abundant useful genes and can be exploited both to broaden the narrow genetic base and enrich the existing varieties with desired agronomically important traits (Khush, 1997; Tanksley and McCouch, 1997; Ge et al., 2001). In the rice genus (*Oryza*), there are approx. 23 species represented cytogenetically by 10 genome groups (i.e., the A-, B-, C-, BC-, CD-, E-, F-, G-, HJ- and HK-genomes; Ge et al., 1999, 2001). The species group with the B-, C-, BC-, CD-, and E-genomes, also called the *O. officinalis* complex by Vaughan (1989), consists of about 10 species and is well known for its taxonomic complexity and particularly for the difficulty in taxonomic treatment of the polyploids (Tateoka, 1962; Nayar, 1973; Vaughan, 1989; Ge et al., 2001; Bao et al., 2005). Of three C-genome diploids, *O. officinalis* Wall. ex Watt is the most common species widely distributed in southern China and South and Southeast Asia while *O. eichingeri* A. Peter is disjunctively distributed in Sri Lanka and Africa. The third species, *O. rhizomatis* Vaughan, is endemic to Sri Lanka. The single B-genome diploid species is *O. punctata* Kotechy ex Steud., which occurs in Africa and is sympatric to its tetraploid form with the BC-genome. Other BC-genome tetraploids include *O. malampuzhaensis* Krish. et Chand. confined to India and *O. minuta* J.S. Presl. et C.B. Presl, in Southeast Asia and Papua New Guinea. Three CCDD species are distributed exclusively in Central and South America and their origin and phylogenetic relationships have been investigated recently (Bao and Ge, 2004). The earliest divergent lineage in this complex is *Oryza australiensis*, the single E-genome diploid endemic to northern Australia (Vaughan, 1994; Ge et al., 1999).

Because the B-, C-, and BC-genome species possess abundant valuable genes useful for rice improvement (Jena and Khush, 1990; Brar and Khush, 1997; Vaughan et al., 2003), the germplasm identification and evolutionary relationships among species in this complex have been undertaken using morphological, cytological and molecular data (see reviews in Nayar, 1973; Federici et al., 2002; Vaughan et al., 2003; Bao et al., 2005). Based on sequence data of the integrase coding domain of a *gypsy*-like retrotransposon, Shcherban et al. (2001) studied the diversity and phylogenetic relationships among species of the *O. officinalis* complex. Phylogenetic studies based on molecular sequences (Ge et al., 1999; Bao and Ge, 2003, 2004) have revealed the phylogenetic relationships of five diploid species (one BB, three CC, and one EE species) in this group and demonstrated that three CCDD species originated from a single hybridization event, while the BBCC species had different origins with their maternal parents as either the B- or C- genome. However, the polyploid speciation involving different diploids has long been in debate and what roles these diploids might have played in the formation of polyploid species are largely unclear (Ge et al., 2001).

Microsatellites or SSRs (simple sequence repeats) have been proven to be highly informative and distributed throughout genomes and, as co-dominant markers, provide powerful markers for studying genetic variation and phylogenetic relationships among species (Peakall et al., 1998; Alvarez et al., 2001; Garris et al., 2005). Although 2740 SSR markers have been developed for the cultivated rice (McCouch et al., 2002) and widely used as tools in fingerprinting and variety identification, genetic diversity and

*Corresponding author; fax +86-10-62590843
e-mail gesong@ibcas.ac.cn

genetic mapping (e.g., Wu et al., 1993; Yang et al., 1994; Panaud et al., 1996; Chen et al., 1997; McCouch et al., 1997; Temnykh et al., 2000; Garris et al., 2005), limited studies have been undertaken toward the wild species (Federici et al., 2002; Gao et al., 2002; Ren et al., 2003; Zhou et al., 2003; Nishikawa et al., 2005). In the present study, we investigated the genetic diversity and evolutionary relationships among species with the B-, C-, and BC-genomes in *Oryza* using SSR markers. Our objectives are: (1) to verify the utility of the microsatellite markers from cultivated rice in cross species (genome) amplification in the species with the B-, C-, and BC-genomes; (2) to detect the level of genetic diversity at both species and genome levels; (3) to reveal the evolutionary relationships among the species in this group. This information will facilitate the collection, conservation and effective use of the wild rice germplasm.

MATERIALS AND METHODS

Plant Materials

One hundred and twenty-three individuals from 72 accessions representing six species with the B-, C-, and BC-genomes in the genus *Oryza* were sampled (Table1). For *O. punctata* that has both the diploid and polyploid forms, the diploid and tetraploid accessions were treated separately. Two to four individuals from each of 33 accessions were selected to assess variation within accessions. One individual was randomly sampled from each of the remaining accessions. All accessions were kindly provided by the International Rice GenBank at the International Rice Research Institute (Philippines) (IRRI). Identity of most accessions used in this study has been confirmed by chromosome counting and PCR-RFLP method (Bao et al., 2005). Total genomic DNA was extracted from 2-week-old seedlings following the procedure described previously by Bao et al. (2005) and each seedling was treated individually.

Primer Screening and Amplification of Microsatellites

One hundred and eight SSR primer pairs which were developed in the cultivated rice were used for primer screening for their utility in *O. officinalis* complex, including 72 RM and 36 OSR primers. The RM primers were designed using rice sequences from genomic libraries and

Table 1. Plant materials used in the study.

Species (Genome) (Sample size)	Origin	Accession No. (No. of individuals sampled)
O. eichingeri (CC)	Sri Lanka	105407 (1), 105413 (1), 105415 (4)
6 (9)*	Uganda	101422 (1), 105159 (1), 105162 (1)
O. officinalis (CC)	Bangladesh	102460 (3)
29 (48)	Brunei	101152 (2), 105100 (1), 105103 (1)
	China	104972 (1), 105395 (2)
	India	100953 (3), 101412 (2)
	Indonesia	81796 (1), 104615 (1), 105680 (2)
	Malaysia	100180 (1), 105093 (1)
	Myanmar	80755 (2), 81943 (2), 105081 (2), 106368 (2), 106443 (1)
	Papua New Guinea	106519 (1), 106520 (1), 106522 (3), 106524 (2)
	Philippines	101115 (1), 105082 (1), 105085 (1), 105084 (3), 105120 (2)
	Thailand	105376 (1)
	Vietnam	105080 (2)
O. rhizomatis (CC)	Sri Lanka	103414 (1), 103421 (1), 105950 (3)
3 (5)		
O. punctata (BB)	Cameroon	105980 (3), 105984 (1)
13 (29)	Chad	104067 (1), 105607 (1), 106292 (3)
	Kenya	101417 (3), 104974 (3), 105690 (3)
	Nigeria	104064 (3), 106304 (3)
	Tanzania	103896 (1), 103903 (2), 105180 (2)
O. malampuzhaensis (BBCC)	India	80764 (1), 80765 (1), 105223 (1), 105224 (1)
8 (14)		80766 (3), 105322 (3), 80767 (2), 105328 (2)
O. minuta (BBCC)	Philippines	101081 (1), 103874 (1), 101141 (3)
3 (5)		
O. punctata (BBCC)	Ghana	100937 (1), 101408 (3)
10 (13)	Kenya	105158 (1)
	Nigeria	101389 (1), 104059 (1), 105154 (2)
	Zaire	105137 (1)
	Uganda	105160 (1), 105181 (1), 105182 (1)

*Numbers out and in parentheses are the numbers of accessions and individuals sampled, respectively. Capital letters in parentheses indicate genome type.

GenBank (Panaud et al., 1996; Chen et al., 1997; Temnykh et al., 2000), while the OSR primers were derived from the DDBJ database (Akagi et al., 1996). PCR amplification was conducted in 10 mM Tris-HCl (pH 8.3), 2.0 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 1 unit of *Taq* DNA polymerase (Takara, Japan) and 20 ng of template DNA per 25-μL reaction using a PTC-200 (PE) thermal cycler for 40 cycles. After initial pre-amplification for 2 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C and 1 min extension at 72°C, followed by 38 cycles of 30 s at 94°C, 30 s at 55°C, 1 min at 72°C, and 10 min at 72°C for final extension. Amplified products were electrophoresed on 6% polyacrylamide gels and the banding patterns were visualized using silver staining as described by Panaud et al. (1996).

Statistical Analyses

An amplification product was recorded as a genotype with two and four capital letters representing the genotype of diploids and tetraploids, respectively. Data were entered in the form of single-individual genotypes. Genetic polymorphism for each population was assessed by calculating the mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), mean expected heterozygosity under Hardy-Weinberg equilibrium (H_e). The above analyses were

Table 2. Genotype frequencies for each locus and genetic characteristics of the *Oryza* species with the B- and C-genomes based on seven microsatellite loci.

Locus	*O. eichingeri* (CC) 6 (9)*	*O. officinalis* (CC) 29 (48)	*O. rhizomatis* (CC) 3 (5)	*O. malampuzhaensis* (BBCC) 8 (14)	*O. minuta* (BBCC) 3 (5)	*O. punctata* (BBCC) 10 (13)	*O. punctata* (BB) 13 (29)
OSR16							
a	0.667	0.271					
b	0.333	0.375				0.423	
c		0.354			0.600		
d			1.000	0.500			
e				0.500	0.400	0.577	0.931
f							0.069
OSR32							
a		0.135		0.533	0.400	0.635	0.897
b		0.219		0.167	0.300		0.034
c							0.069
d	1.000	0.604	1.000	0.300	0.300	0.365	
e		0.042					
OSR34							
a						0.115	0.448
b		0.271				0.038	
c		0.115		0.167		0.019	
d	0.833	0.021	0.800	0.367		0.404	0.034
e	0.167	0.594	0.200		0.300		0.017
f				0.467	0.300	0.308	0.500
g					0.200		
h					0.200	0.115	
RM10							
a		0.125	0.100				0.103
b		0.594			0.100	0.154	0.690
c	0.333	0.188		0.733	0.600	0.538	0.207
d	0.667	0.094	0.900	0.267	0.300	0.308	
RM215							
a	0.167	0.698		0.867	0.800	0.385	1.000
b	0.333		0.500			0.192	
c						0.231	
d	0.500	0.302	0.500	0.133	0.200	0.192	
P	57.1%	71.4%	42.9%	71.4%	71.4%	71.4%	57.1%
He	0.376	0.565	0.222	0.461	0.609	0.637	0.272
A	1.71	2.71	1.43	2.00	2.00	2.71	2.14

*Numbers out and in parentheses are the numbers of accessions and individuals sampled, respectively. Capital letters in parentheses indicate genome types.

calculated with the BIOSYS-1 software (Swofford and Selander, 1981). The genetic distance measures of Tomiuk and Loeschcke (1995) for codominant alleles was implemented with software PPOPDIST, Version. 1.1.0 (Guldbrandtsen et al., 2000). Relationships among accessions and species were then inferred using the UPGMA clustering method on the genetic distance with the PHYLIP3.6 software (Felsenstein, 2004).

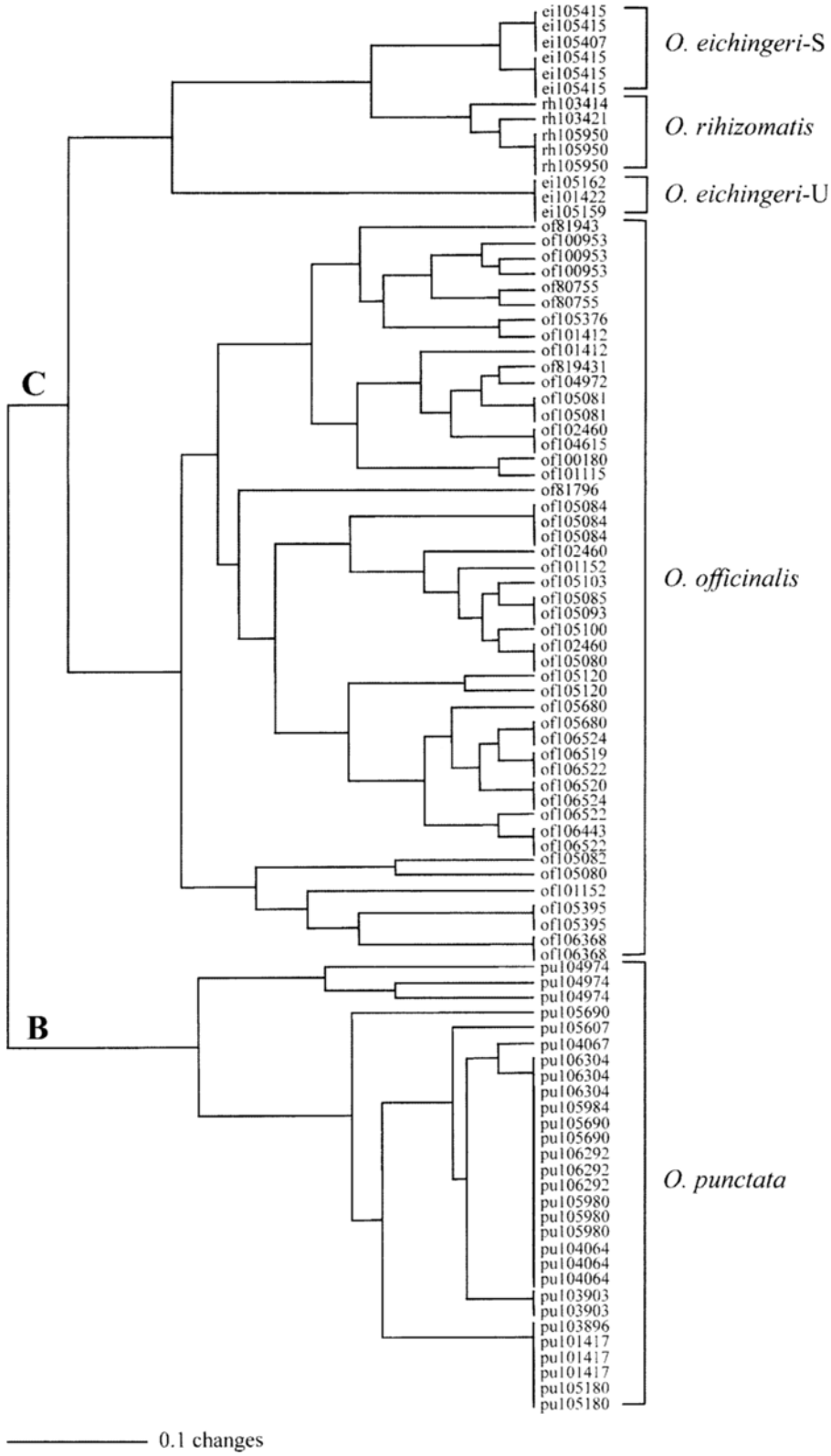

Figure 1. UPGMA dendrogram based on genetic distance showing the genetic relationships among the accessions of diploid *Oryza* species with the B- and C-genomes.

RESULTS

Microsatellite Polymorphisms and Genetic Diversity

After screening 108 primer pairs against 35 samples of 11 accessions representing all the six species with the B-, C-, and BC-genomes, seven primer pairs that produced clear bands of the predicted sizes were chosen for further accession screening. The seven loci were dispersed in rice chromosomes 3, 5, 6, 7, 9, and 12, respectively. The number of alleles varied widely among the seven loci with a total of 30 alleles identified (Table 2). The most variable locus was OSR34 that had 8 alleles, followed by OSR16 (6 alleles), OSR32 (5 alleles), RM215 (4 alleles), and RM10 (4 alleles). Two loci (OSR22 and RM238B) had only one allele across the 123 samples.

Five alleles were found unique to species. Two alleles (OSR16-f and OSR32-c) were specific for diploid *O. punctata*, one (OSR32-e) for *O. officinalis*, one (OSR34-g) for *O. minuta*, and one (RM215-c) for tetraploid *O. punctata* (Table 2). Two alleles (OSR34-g in *O. minuta* and RM215-c in tetraploid *O. punctata*) were found exclusively in tetraploids (Table 2) and might result from the limited samples for the diploid ancestors. Additionally, four alleles were specific for genome, with OSR16-e and OSR34-f being unique to B-genome, while OSR32-d and RM215-d were unique to C-genome (Table 2).

At species level, all the genetic parameters ranged greatly with *O. rhizomatis* being the lowest (P=42.9%, He=0.222, A=1.43) and the tetraploid *O. punctata* being the highest (P=71.4%, He=0.637, A=2.71). In general, a higher level of genetic diversity was revealed in tetraploid than in diploid species (P: 71.4% vs.42.9%-71.4%, He: 0.461-0.637 vs. 0.222-0.565, A: 2.0-2.71 vs.1.43-2.71) (Table 2), suggesting the heterozygous nature of the allotetraploid species. Of four diploid species, the widespread *O. officinalis* exhibited the highest level of polymorphism (P=71.4%, He=0.565, A=2.71), while the other three species maintained comparable levels of diversity (Table 2). Among three tetraploid species, *O. punctata* (He=0.637, A=2.71) and *O. minuta* (He=0.609, A=2.0) had significantly higher diversity than *O. malampuzhaensis* (He=0.461, A=2.0) that was confined to a small area of India.

Relationships among Accessions and Species

A cluster analysis with the UPGMA based on average genetic distance of diploid samples was constructed. All the diploid samples were clustered into two distinct groups corresponding to their genome constitutions, i.e., the B-genome group (*O. punctata*) and the C-genome group (*O. eichingeri*, *O. officinalis*, and *O. rhizomatis*). All the accessions from the same species were grouped together and formed distinct clusters except for the accessions of *O. rhizomatis*, which were also grouped together but included within the cluster of *O. eichingeri*. In this cluster, accessions of *O. eichingeri* from Sri Lanka were grouped with the accessions of *O. rhizomatis*, while those of from Uganda were grouped separately. Of three diploid species, *O. eichingeri* and *O. rhizomatis* seemed more closely related (Fig. 1).

In order to detect the origin of the tetraploids and the relationships among all the diploid and tetraploid species, an UPGMA dendrogram consisting of all the 123 individuals from 72 accessions was generated. It was evident that the relative positions or relationships among accessions of the diploid species were the same as those in the diploid dendrogram where two main groups were present. It was noteworthy that two types of samples were found for each of the three tetraploid species (*O. malampuzhaensis*, *O. minuta*, and *O. punctata*) with one type of samples forming a cluster with the B-genome group while the other type with the C-genome group. For example, two individuals (one accession) from the tetraploid *O. punctata* clustered with the B-genome group while the other 11 individuals representing nine accessions from the same species were nested in the C-genome group. Similarly, 11 individuals (seven accessions) and three individuals (one accession) of *O. malampuzhaensis* formed clusters with the B- and C- genome accessions, respectively. Although only five individuals from three accessions were screened for *O. minuta* in this study, both the B and the C types of accessions were also detected (Fig. 2).

Because there were three diploid species with the C-genome, Figure 2 provided additional insights into the origin of the tetraploid species. In the C-genome cluster, accessions of the tetraploid *O. punctata* clustered with the *O. eichingeri* accessions from Uganda, whereas accessions of *O. malampuzhaensis* clustered with the accessions of *O. rhizomatis* and *O. eichingeri* from Sri Lanka, suggesting that the two allotetraploid species originated independently with different C-genome diploid species as one of their ancestors. It was also evident that the *O. minuta* accessions nested into the cluster of *O. officinalis* accessions, indicating *O. officinalis* might be the C-genome donor in the formation of *O. minuta*. In the B-genome cluster, two allotetraploid species clustered with the different accessions of the diploid *O. punctata*, with all *O. malampuzhaensis* accessions clustering with three diploid *O. punctata* accessions but the tetraploid *O. punctata* accessions clustering with the other accessions of diploid *O. punctata* (Fig. 2). This suggested that *O. malampuzhaensis* and the tetraploid *O. punctata* might be formed independently with different populations as their B-genome donors.

DISCUSSION

Transferability of Microsatellite Markers in Wild Rice

Because of their hypervariability, codominant trait, ease of assay by PCR, and distribution throughout the whole genome, microsatellite markers have become a powerful tool for addressing a variety of genetic and evolutionary questions, including the gene mapping, DNA fingerprinting, population genetics and evolutionary relationships of the cultivated rice and its wild relative (Yang et al., 1994; Chen et al., 1997; McCouch et al., 1997; Temnykh et al., 2000; Ishii et al., 2001; Gao et al., 2002; Ren et al., 2003; Zhou et al., 2003; Garris et al., 2005). However, ascertaining microsatellite markers that are sufficiently polymorphic in a previously unexamined species presents a challenge because the development of novel microsatellite markers is a laborious and costly exercise, in particular if more than one species are the target of study. An alternative is cross-

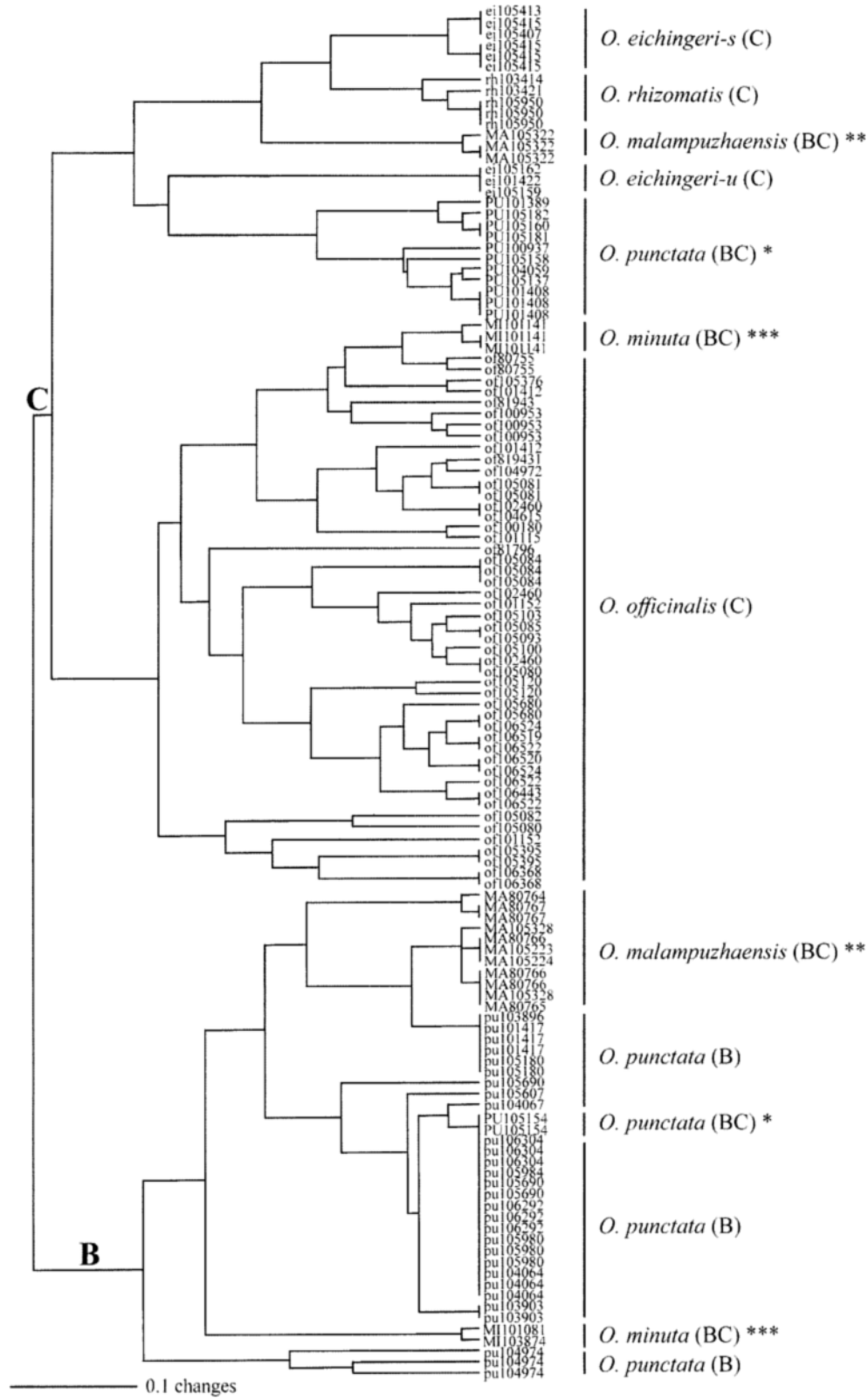

Figure 2. UPGMA dendrogram based on genetic distance showing the genetic relationships among all 123 accessions of the *Oryza* species with the B- and C-genomes. *, Indicates the tetraploid *O. punctata*; **, The tetraploid *O. malampuzhaensis*; ***, The tetraploid *O. minuta*.

species amplification, i.e., microsatellite primers developed for one species can be used to detect polymorphism at homologous sites in related species (Peakall et al., 1998; Rossetto, 2001). The availability of SSRs in certain groups of species has increased interests in primer transferability across closely related taxa. For example, Chambers et al. (2004) screened 47 human-derived markers to assess their utility in the white-handed gibbon. Alvarez et al. (2001)

tested 17 microsatellite loci from tomato on nine species of the genus *Lycopersicon*. Using soybean SSR flanking primers, Peakall et al. (1998) have successfully amplified microsatellite loci from several legume genera.

In rice, a total of 2740 confirmed microsatellite markers have been released (McCouch et al., 2002). However, few markers have been used for cross-amplification on wild rice. Ren et al. (2003) used 27 SSR primer pairs to estimate genetic relationships of the A-genome species. Using 21 and 10 polymorphic rice microsatellite loci, respectively, Gao et al. (2002) and Zhou et al. (2003) investigated the genetic structure of the Chinese wild populations of *Oryza rufipogon*, the most closely related wild species to the cultivated rice. However, fewer studies have been undertaken on more phylogenetically distinct species in *Oryza*.

In the present study, a total of 108 primer pairs have been examined and clear amplification has been obtained from 7 primer pairs on the B-, C- and BC-genome species. Therefore, the percentage of successful amplification (6.5%) is low when microsatellite primers developed for the Asian cultivated rice used for species with the B-, C- and BC-genomes. In our previous study on *O. rufipogon*, an A-genome species closely related to *O. sativa*, Zhou et al. (2003) found that 10 out of 134 primers produced clear bands. Recently, some authors (Bautista et al., 2006) screened 551 SSR primers derived from *O. sativa* on selected C-genome accessions and found 12 (2.2%) were useful for cross amplification. The low rate of successful cross-species amplification of microsatellite in these studies might reflect the genetic affinity between the target species and the species from which SSRs were developed. Panaud et al. (1996) found that amplification in wild rice was reliable in species most closely related to cultivated rice but became less successful as the genetic distance increased. This decline of amplification success with increase of genetic distance was also observed in other groups. Kutil and Williams (2001) found 100% microsatellite transfer rates from *Pinus taeda* to *Pinus palustris*, a close relative in the same subsection of *Pinus*, but only 47% successful rate to *Pinus halepensis* in a different subsection. In spite of low marker transferability among rice species with B-, C- and BC-genome in this study, cross-species amplification is an alternative and useful method for studies of population genetics and evolutionary relationships of wild rice species.

Genetic Diversity

Despite many SSR studies on the cultivated rice and its closely related wild species (McCouch et al., 1997; Ishii et al., 2000; Gao et al., 2002; Zhou et al., 2003; Garris et al., 2005), relatively few studies have been undertaken on the population genetics of the B- and C-genome species using SSRs (Gao and Zhang, 2005). Of the four diploid species in this study, *O. officinalis* maintained the highest diversity (P=71.4%, A=2.71, He=0.565), followed by *O. eichingeri* (P=57.1%, A=1.71, He=0.376), *O. punctata* (P=57.1%, A=2.14, He=0.272) and *O. rhizomatis* (P=42.9%, A=1.43, He=0.222) (Table 2). However, this level of diversity is relatively lower than that found in other wild rice species. For example, Zhou et al. (2003) revealed much higher level of genetic diversity in the Chinese populations of the A-genome *O. rufipogon* using 10 SSR primers (P=100%, A=10.6, He=0.787). Recently, Gao and Zhang (2005) applied six and seven SSR loci to investigate the genetic structure of six populations throughout the range of the Chinese *O. rufipogon* and *O. officinalis*, respectively. They found that *O. rufipogon* possessed higher levels of genetic diversity (P=100%, He=0.580) than *O. officinalis* (P=57.1%, He=0.283). Similar results have also been obtained by our previous allozyme studies although lower level of polymorphism associated with allozyme was expected. Gao et al. (2000, 2001) analyzed the genetic diversity and population genetic structure of the Chinese populations of the A-genome *O. rufipogon* and the C-genome *O. officinalis* using allozyme technique, and found that *O. rufipogon* maintained much higher diversity (P=22.7%, A=1.33, He=0.068) than *O. officinalis* (P=12.5%, A=1.13, He=0.029) at population level.

Significantly higher diversity of the tetraploid species (P=71.4%, A=2.0-2.71, He=0.461-0.637) is expected because all of them are allotetraploids and maintain genetic diversity from both parental lineages (Nayar, 1973; Ge et al., 1999). It is noteworthy that the genetic diversity of *O. officinalis* is about twice times higher than that of the other diploids and is comparable to those of the tetraploids (Table 2). The higher genetic diversity within *O. officinalis* may derive from its wider geographical distribution given the fact that *O. rhizomatis* is endemic to Sri Lanka and *O. eichingeri* is disjunctly distributed in small areas in Sri Lanka and Africa. However, many other life history characters, such as mating system, population size and gene flow may contribute to the difference in the levels of genetic diversity for the B- and C-genome species. Further population genetic study with more extensive sampling is needed to clarify the population pattern and dynamics of these species.

Relationships among Species and Speciation of the Allotetraploids

The genetic relationship of the diploid species revealed by SSR loci is consistent with the species genomic constitutions, with three C-genome species forming one cluster distinct to the cluster of the B-genome species (Fig. 1). All the accessions from the same species were generally formed a cluster except for *O. eichingeri*. In *O. eichingeri*, the accessions from Sri Lanka were not clustered with their African counterparts but instead formed a cluster with the accessions of *O. rhizomatis*, which was indigenous to Sri Lanka. In their phenogram developed based on sequences of a *gypsy*-like retrotransposon, Shcherban et al. (2001) found that *O. eichingeri* from two continents fell into different clusters, and suggested that *O. eichingeri* was more closely related to the ancestral species of the complex. RFLP analysis (Federici et al., 2002) and multigene phylogenetic study (Bao and Ge, 2003) also detected high degree of differentiation between the two geographical races. In fact, the taxonomic status of *O. eichingeri* confined to Sri Lanka has been a subject of controversy (Bor, 1960; Tateoka, 1962; Nayar, 1973; Vaughan, 1990). This species was originally described as *O. sativa* var. *collina* by Trimen (1889), subsequently was included in *O. officinalis* (Roschevicz, 1931), and Bor (1960) and Tateoka (1962) included it under the African species *O. eichingeri*. However, Sharma and Shastry (1965) proposed a specific

status for this race (*O. collina*). Our present result indicates that gene flow may exist between *O. eichingeri* and *O. rhizomaits* in Sri Lanka to some extent. The result consists with the study by Bautista et al. (2006). Using RAPD and SSR markers, Bautista et al. (2006) studied the genetic diversity of A- and C-genome species in southern South Asia and found that one *O. rhizomatis* accession clustered with the *O. eichingeri* accessions. Nevertheless, further investigations using multiple approaches are needed to clarify the genetic relationships between the two species and verify the taxonomic status of the two races of *O. eichingeri*.

As a codominant marker, microsatellites provide some lights on the origin of polyploid species. Bernardo et al. (2000) analyzed the parental contribution and coefficient of coancestry among maize inbreds by comparing RFLP and SSR data and confirmed that SSR markers were superior to RFLP markers for estimating genetic relationships. Moretzsohn et al. (2004) reported that SSR data was useful for identity of the ancestor progenitor species of cultivated peanut, an allotetraploid species. In the genus *Oryza*, the origin of the allotetraploids with BC-genome has long been in debate (Wang et al., 1992; Aggarwal et al., 1999; Ge et al., 1999), because the modern distribution patterns of the tetraploids have not always consisted with the distributions of their parental diploids. By generating and comparing two nuclear gene (*Adh*1 and *Adh*2) trees and a chloroplast gene (*mat*K) tree of all *Oryza* species, Ge et al. (1999) revealed that the BBCC species had different origins, with their maternal parents being either a B- or C-genome. Wang et al. (1992) indicated that the B-genome donor of three BBCC species was the diploid *O. punctata*, but their closest C-genome diploid accessions were found in different locations and close to the locations of the corresponding BBCC species, suggesting that there was a possibility of independent origins of the C-genome of BBCC species. In the present study, the closely affinities among diploid *O. punctata* and three BC-genome tetraploid (Fig. 2) also suggests that the diploid *O. punctata* is the B-genome donors of all BC-genome species. Importantly, three BC-genome species clustered with different accessions of the diploid *O. punctata*, implying that three tetraploids might originate independently. For example, *O. malampuzhaensis* clustered with accessions pu103896, pu101417 and pu105180 of the diploid *O. punctata*, whereas the tetraploid *O. punctata* clustered with other accessions of this diploid species (Fig. 2).

The genetic relationship between the C-genome diploids and the BC-genome species (clade C in Fig. 2) further suggests the independent origins of the BC-genome species. It is evident that the putative C-genome donors of *O. minuta* and the tetraploid *O. punctata* were *O. officinalis* and the African *O. eichingeri*, respectively, while the C-genome ancestor of *O. malampuzhaensis* was either *O. rhizomatis* or the Sri Lanka *O. eichingeri* (Fig. 2). Therefore, it is reasonable to assume that the tetraploid species with the BC-genome have originated independently, at least three times in history. The multiple and independent polyploidization events in the genus *Oryza* supports the previous argument that the present estimates of frequency of multiple origins were low and that multiple origins of polyploid might be a rule rather than an exception (Soltis and Soltis, 1993).

ACKNOWLEDGEMENTS

We thank the International Rice Research Institute (Philippines) for providing seed samples for this study. This research was supported by the National Natural Science Foundation of China (30430030, 30121003 and 30470124), Program for Key International S & T Cooperation project of P.R. China (20011CBand11103) and China Postdoctoral Science Foundation (20040350710).

Received May 23, 2006; accepted July 27, 2006.

LITERATURE CITED

Aggarwal RK, Brar DS, Nandi S, Huang N, Khush GS (1999) Phylogenetic relationships among *Oryza* species revealed by AFLP markers. Theor Appl Genet 98: 1320-1328

Akagi H, Yokozeki Y, Inaaki A, Fujimura T (1996) Microsatellite DNA marker for rice chromosomes. Theor Appl Genet 93: 1071-1077

Alvarez AE, van de Wiel CCM, Smulders MJM, Vosman B (2001) Use of microsatellites to evaluate genetic diversity and species relationships in the genus *Lycopersicon*. Theor Appl Genet 103: 1283-1292

Bao Y, Ge S (2003) Phylogenetic relationships among diploid species of *Oryza* officinalis complex revealed by multiple gene sequences. Acta Phytot Sinica 41: 497-508

Bao Y, Ge S (2004) Origin and phylogeny of *Oryza* species with the CD genome based on multiple-gene sequence data. Plant Syst Evol 249: 55-66

Bao Y, Lu BR, Ge S (2005) Identification of genomic constitutions of *Oryza* species with the B and C genomes by the PCR-RFLP method. Genet Res Crop Evol 52: 69-76

Bautista NS, Vaughan D, Jayasuriya AHM, Liyanage ASU, Kaga A, Tomooka N (2006) Genetic diversity in AA and CC genome *Oryza* species in southern South Asia. Genet Res Crop Evol 53: 631-640

Bernardo R, Romero-Severson J, Ziegle J, Hauser J, Joe L, Hookstra G, Doerge RW (2000) Parental contribution and coefficient of coancestry among maize inbreds: Pedigree, RFLP, and SSR data. Theor Appl Genet 100: 552–556

Bor NL (1960) The grasses of Burma, Ceylon, India and Pakistan. Pergamon, Oxford, pp 767

Brar DS, Khush GS (1997) Alien introgression in rice. Plant Mol Biol 35: 35-47

Chambers KE, Reichard UH, Möller A, Nowak K, Vigilant L (2004) Cross-species amplification of human microsatellite markers using noninvasive samples from white-handed gibbons (*Hylobates lar*). Am J Primatol 64: 19-27

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

Federici MT, Shcherban AB, Capdevielle F, Francis M, Vaughan D (2002) Analysis of genetic diversity in the *Oryza officinalis* complex. J Biotechnol 5: 173-181

Felsenstein J (2004) PHYLIP (Phylogeny Inference Package) version 3.6. University of Washington Press, Seattle

Gao LZ, Ge S, Hong DY (2000) Allozyme variation and population genetic structure of common wild rice, *Oryza rufipogon* Griff. in China. Theor Appl Genet 101: 494-502

Gao LZ, Ge S, Hong DY (2001) High levels of genetic differentiation of *Oryza officinalis* Wall. ex Watt. from China. Heredity

92: 511-516
Gao LZ, Schaal BA, Zhang CH, Jia JZ, Dong YS (2002) Assessment of population genetic structure in common wild rice *Oryza rufipogon* Griff. using microsatellite and allozyme markers. Theor Appl Genet 106: 173-180
Gao LZ, Zhang CH (2005) Comparisons of microsatellite variability and population genetic structure of two endangered wild rice species, *Oryza rufipogon* and *O. officinalis*, and their conservation implications. Biodivers Conserv 14: 1663-1679
Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch S (2005) Genetic structure and diversity in *Oryza sativa* L. Genetics 169: 1631-1638
Ge S, Sang T, Lu BR, Hong DY (1999) Phylogeny of rice genomes with emphasis on origins of allotetraploid species. Proc Natl Acad Sci USA 96: 14400-14405
Ge S, Sang T, Lu BR, Hong DY (2001) Phylogeny of the genus *Oryza* as revealed by molecular approaches, *In* GS Khush, DS Brar, B Hardy, eds, Rice Genetics IV. Proceedings of the Fourth International Rice Genetics Symposium. Los Banos, Philippines, IRRI, pp 89-105
Guldbrandtsen B, Tomiuk J, Loeschcke V (2000) POPDIST, Version 1.1.1: A Program to calculate population genetic distance and identity measures. J Hered 91: 178-179
Ishii T, Xu Y, McCouch SR (2001) Nuclear- and chloroplast-microsatellite variation in A genome species of rice. Genome 44: 658-666
Jena KK, Khush GS (1990) Introgression of gene from *O. officinalis* Well ex Watt. to cultivated rice, *O. sativa*. Theor Appl Genet 80: 737-745
Khush GS (1977) Disease and insect resistance in rice. Adv Agron 29: 265-361
Kutil B, Williams CG (2001) Triplet-repeat microsatellites shared among hard and soft pines. J Hered 92: 327-332
Lu BR (1999) Taxonomy of the genus *Oryza* (Poaceae): A historical perspective and current status. Int Rice Res Notes 24: 4-8
McCouch SR, Chen XL, Panaud O, Temnykh S, Xu Y, Cho YG, Takashige NH, Ishii T, Matthew B (1997) Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Mol Biol 35: 89-99
McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Zh, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9: 199-207
Moretzsohn MdeC, Hopkins MS, Mitchell SE, Kresovich S, Valls JFM, Ferreira ME (2004) Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Bio 4: 11
Nayar NM (1973) Origin and cytogenetics of rice. Adv Genet 17: 153-292
Nishikawa T, Vaughan DA, Kadowaki K-I (2005) Phylogenetic analysis of *Oryza* species, based on simple sequence repeats and their flanking nucleotide sequences from the mitochondrial and chloroplast genomes. Theor Appl Genet 110: 696-705
Panaud O, Chen X, McCouch SR (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol Gen Genet 252: 597-607
Peakall R, Gilmore S, Keys W, Morgante M, Rafalski A (1998) Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. Mol Biol Evol 15: 1275-1287
Ren F, Lu BR, Li S, Huang J, Zhu Y (2003) A comparative study of genetic relationships among the AA-genome *Oryza* species using RAPD and SSR markers. Theor Appl Genet 108: 113-120
Roschevicz RI (1931) A contribution to the study of rice. Turdy Prikl Bot Genet Selek 27: 3-133
Rossetto M (2001) Sourcing of SSR markers from related plant species, *In* RJ Henry, ed, Plant Genotyping: The DNA Fingerprinting of Plants. CAB International, New York, pp 211-224
Sharma SD, Shastry SVS (1965) Taxonomic studies in the genus *Oryza* L. IV. the Ceylonese *Oryza* spp. affin. *O. officinalis* Wall. ex Watt. India J Genet Plant Breed 25: 168-172
Shcherban AB, Vaughan DA, Tomooka N, Kaga A (2001) Diversity in the integrase coding domain of a gypsy-like retrotransposon among wild relatives of rice in the *Oryza officinalis* complex. Genetica 110: 43-53
Soltis PS, Soltis DE (1993) Molecular data and the dynamic nature of polyploidy. Crit Rev Plant Sci 12: 243-273
Swofford DL, Selander RB (1981) BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J Hered 72: 281-283
Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277: 1063-1066
Tateoka T (1962) Taxonomic studies of *Oryza* I. *O. latifolia* complex. Bot Mag Tokyo 75: 418-427
Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100: 697-712
Tomiuk J, Loeschcke V (1995) Genetic identity combining mutation and drift. J Hered 74: 607-615
Trimen H (1889) Additions to the flora of Ceylon. J Bot 27: 161-172
Vaughan DA (1989) The Genus *Oryza* L. Current Status of Taxonomy. International Rice Research Institute, Philippines, pp 7-9
Vaughan DA (1990) A new rhizomatous *Oryza* species (Poaceae) from Sri Lanka. Bot J Linn Soc 103: 159-163
Vaughan DA (1994) The Wild Relatives of Rice: A Genetic Resources Handbook. International Rice Research Institute, Philippines, pp1-137
Vaughan DA, Morishima H, Kadowaki K (2003) Diversity in the *Oryza* genus. Curr Opin Plant Biol 6: 139-146
Wang ZY, Second G, Tanksley SD (1992) Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor Appl Genet 83: 565-581
Wu K, Steven S, Tanksley D (1993) Abundance, polymorphism and genetic mapping of miscrosatellites in rice. Mol Gen Genet 241: 225-235
Yang GP, Saghai Maroof MA, Xu CG, Zhang Q, Biyashev RM (1994) Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. Mol Gen Genet 245: 187-194
Zhou HF, Xie ZW, Ge S (2003) Microsatellite analysis of genetic diversity and population genetic structure of a wild rice (*Oryza* rufipogon Griff.) in China. Theor Appl Genet 107: 332-339