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Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers

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Abbreviations: AFLP: amplified fragment length polymorphism
H: genic diversity
NA: number of alleles
SSR: simple sequence repeat

In Venezuela, pedigree analyses indicate that the rice varieties currently under cultivation are closely related. Effective breeding programs, based on knowledge of the genetic diversity of cultivars, are needed to broaden the genetic bases of rice germplasm in the country. In this study, we used a set of 48 simple-sequence-repeat (SSR) markers to assess the genetic diversity of 11 Venezuelan rice cultivars, released by the National Rice Breeding Program between 1978 and 2007. A total of 203 alleles were detected, the number of alleles (*NA*) per marker ranged from 2 to 9, with an average of 4.23. The average genic diversity (*H*) over all SSR loci for the 18 genotypes was 0.524, ranging from 0.105 to 0.815. Positive correlations were found between *H* at each locus, *NA*, the allele size range and the maximum number of repeats. Venezuelan cultivars showed lower *H* (mean = 0.37) and *NA* (total = 124, mean = 2.58) than the whole sample. UPGMA-cluster-analysis based on genetic distance coefficients clearly separated all the genotypes, and showed that the Venezuelan rice varieties are closely related. Molecular identification of 7 Venezuelan cultivars could be done with 9 primers pairs which produced 10 genotype-specific-alleles. Although the genetic diversity was low, SSRs proved to be an efficient tool in assessing the genetic diversity of rice genotypes. Implications of the low genetic diversity detected and relatedness of Venezuelan cultivars are discussed.

The commercial exploitation of a reduced genetic base and the prevalence of a small set of landraces in the breeding process had been the general approach for several crops species (Souza and Sorrells, 1989; Dilday, 1990; Cuevas-Perez et al. 1992). Rice (*Oryza sativa* L.) is one of the most important staple food crops supporting the world population. Compared with other crop species, the genetic diversity in the world rice germplasm is quite large. Three subspecies, *i.e.*, *indica*, *japonica*, and *javanica*, compose a large reservoir of rice germplasm including a variety of local landraces and cultivars (Khush, 1997; Lu et al. 2005; Garris et al. 2005). In addition, there are a number of wild relatives that provide potentially valuable resources for the improvement of cultivated rice (Khush, 1997; Ren et al. 2003).

Despite the richness of genetic resources, only a small proportion of the world rice germplasm collections have been used in breeding programs. As a consequence a high genetic similarity is found within several commercial rice germplasms around the world. The limited use made of the rice genetic diversity available worldwide has been a concern in Latin America since the late 1980s. Cuevas-Perez et al. (1992) analyzed a total of 143 commercial varieties released in the region from 1971 to 1989, it was found that 101 different landraces were involved in the crosses that produced the varieties, however only 14 ancient cultivars contributed 70 percent of the genes. A similar situation has been reported for the upland varieties cultivated in the region. Six native varieties make up the base for the upland varieties released up to 1992. Forty ancestors were involved in crossing to develop varieties, but only 11 of them accounted for 81 percent of the genes for the varieties released between 1971 and 1993 (Guimaraes et al. 1995). Although differences in genetic diversity and relatedness are observed within rice germplasms of different countries, the general feature is a very close relationship among cultivars (Cuevas-Perez et al. 1992; Guimaraes et al. 1995; Rangel et al. 1996; Fuentes et al. 1999). In Venezuela, for example, irrigated rice varieties currently under cultivation are closely related among them and with cultivars from Colombia, Brazil and Ecuador (Cuevas-Perez et al. 1992).

Knowledge regarding the amount of genetic variation in germplasm accessions and genetic relationships between genotypes are important considerations for designing effective breeding programs. In the past, the characterization of germplasm diversity was carried out by means of morphological and biochemical markers which, in many cases, did not have the resolution power for revealing polymorphisms in genetic analyses and/or for differentiating between closely related genotypes. Advances in plant genetics and molecular biology have led to the development of many types of molecular markers which can be used to characterize germplasm. Different types of DNA markers are available nowadays, each method differing in principle, application, type and amount of polymorphism detected, and cost and requirement. These include random amplification of polymorphic DNA (RAPDs), restriction fragment length polymorphisms (RFLPs) amplified fragment length polymorphisms (AFLP) and simple sequence repeats (SSRs). SSRs are codominant, abundant and highly reproducible and exhibit a high degree of allelic variation (Panaud et al. 1996; Temnykh et al. 2000). SSRs are an excellent molecular marker system for many types of genetic analyses, including linkage mapping, germplasm surveys, and phylogenetic studies. They have been used for characterizing genetic diversity in several crop species including sorghum (Dean et al. 1999; Smith et

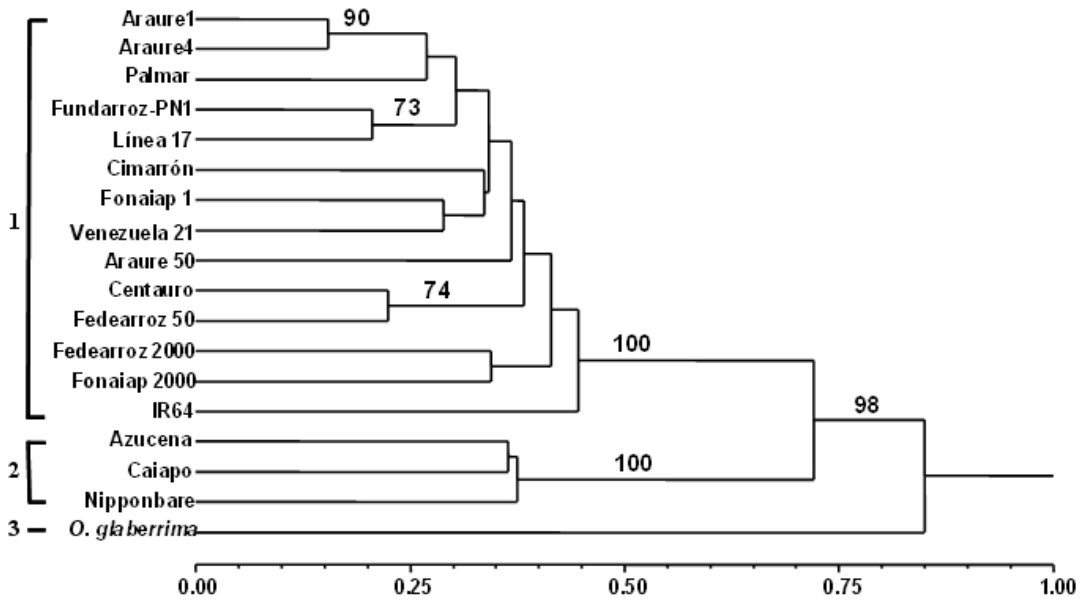

Figure 1. Dendrogram of 18 rice genotypes clustered based on Rogers' genetic distance calculated from 48 SSR markers (203 alleles) by UPGMA analysis. The strength of dendrogram nodes was estimated with a bootstrap analysis using 1000 permutations.

al. 2000), maize (Senior et al. 1998), cotton (Liu et al. 2000) and wheat (Prasad et al. 2000). In rice, SSRs have been used to assess the genetic diversity of both wild and cultivated species (Yang et al. 1994; Gealy et al. 2002; Ni et al. 2002; Ren et al. 2003; Sarla et al. 2003; Yu et al. 2003; Siwach et al. 2004; Xu et al. 2004; Brondani et al. 2005; Jeung et al. 2005; Neeraja et al. 2005). These studies showed that SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes.

The objectives of this study were to use SSR markers to estimate the genetic diversity within a core of 11 Venezuelan rice cultivars released by the National Rice Breeding Program between 1978 and 2006, to reveal genetic relationships among them, and to distinguish different accessions by means of specific SSR alleles.

MATERIALS AND METHODS

Rice germplasm

Venezuelan rice germplasm is represented by 11 irrigated cultivars/breeding lines ('Araure1', 'Araure 4', 'Araure 50', 'Centauro', 'Cimarrón', 'Fonaiap1', 'Fonaiap 2000', 'Fundarroz PN-1', 'Línea 17', 'Palmar' and 'Venezuela 21',) from the Venezuelan Rice Breeding Program at the National Institute for Agricultural Research (INIA) (Table 1). These *indica* varieties represent commercial rice cultivars released by INIA between 1970 and 2006 (Álvarez et al. 2004). Other seven rice cultivars representing different species and subspecies were analyzed in order to compare the genetic diversity of Venezuelan germplasm and evaluate the discrimination power of selected SSRs. These extra accessions included three *indica* genotypes ('Fedearroz 50' and 'Fedearroz 2000', two irrigated cultivars from Colombia, and 'IR64', a lowland variety developed at IRRI, Philippines), three *japonica* genotypes ('Nipponbare' a *temperate japonica* variety from Japan; 'Azucena' and 'Caiapó', two *tropical japonica* varieties from Philippines and Brazil, respectively) and the African wild species, *Oryza glaberrima* (IRGC 103544). The rice varieties, 'IR64', 'Nipponbare' and 'Azucena' also served as control for determining allele molecular weight because they had been previously assayed at the same SSR loci (Chen et al. 1997; Temnykh et al. 2000). Venezuelan accessions were obtained from INIA in Venezuela, the rest of accessions were supplied by the Centro Internacional de Agricultura Tropical (CIAT) located in Colombia.

SSR assay

DNA was extracted from fresh seedling leaves of each accession following the method of Dellaporta et al. (1983) with slight modifications (Dellaporta et al. 1983; Posso-Duque and Ghneim-Herrera, 2008). Forty-eight SSR markers covering all the twelve chromosomes at about a 30 cM intervals were selected for the genetic diversity analysis based on the published rice SSR framework maps (Temnykh et al. 2000; Coburn et al. 2002). The loci, chromosome position, repeat motifs and primer sequences for these markers are presented in Table 2. SSR primers were obtained from Integrated DNA Technologies, Inc. (Skokie, IL, USA).

PCR amplification was carried out in 20 ml reaction mixtures, each containing 20 ng of template DNA, 0.13 mM of each primer, 250 mM of each dNTPs, 1X PCR buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl, 2 mM

Table 1. List of Venezuelan rice cultivars analyzed in this study.

Accession	Pedigree	Progenitors	Year of release
Araure 1	P 849-45-1M-40-4-3-1M	IR930-147-13/Colombia 1	1978
Araure 4	P2217-F4-30-4-1B	CICA 7//CICA 8/Remadja	1984
Cimarrón	Chianung Sen Yu 23	Hebi G11330//Chianung Sen Yu/IR1561	1988
Palmar	P2231-F4-138-6-2-1	CICA7//CICA8/PELITA I-1	1988
Fonaiap 1	P4070 F3-RH3-7-1- BA	P1386-6-8M-1-3M-1/P 3767	1993
Fonaiap 2000	CT 8240-1-3-9P-M	P5446-6-3-2/CT5690-3-19-2//P3059-F4-79-1-1B	2000
Fundarroz-PN1	CT10310-15-3-2P-4-3	P3084-F4-56-2-2/ITA 306//CT8154-1-9-2	2000
Venezuela 21	FL00147-8P-6-15P-M	CT8008-16-31-3P-M//CT9682-2-M-14-1-M-1/CT10310-15-3-2P-4-3	2003
Araure 50	FL00459-21P-11-2P-M	CT9682-2-M-14-1-M-1/CT 10310-15-3-2P-4-3//CT8222-7-6-2P-1X	2005
Centauro	FL00984-8P-11-2P-M-M	ECIA 38-2-4-2-5-6/CT8222-7-6-2P-1X//FB007-3-1-6-1-M	2005
Línea 17	FL00470-23P-3-2P-M	CT10825-1-2-1-3-M/CT10310-15-3-2P-4-3//CT8455-1-23-7P-1X	-

$MgCl_2$, 1% Triton X-100, 1% BSA) and 1 U of *Taq* DNA polymerase. An MJ Research (PTC-100 TM 96V) thermo-cycler was used along with the following PCR profile: an initial denaturation step of 3 min at 94ºC, followed by 30 cycles of 30 sec at 94ºC, 45 sec at 48-65ºC, 1 min at 72ºC, and a final extension at 72ºC for 5 min.

The PCR products were resolved on a 6% sequencing gel followed by silver staining following the standard protocol (Temnykh et al. 2000). Silver-stained gels were scanned to capture digital images of the gels after air drying. The allele size of the amplified band for each SSR locus was determined based on its migration relative to the 10 bp DNA ladder (Invitrogen Corp., CA, USA) and three check varieties ('Azucena', 'IR64' and 'Nipponbare') that were loaded in each gel as references.

For genetic distance estimates and cluster analysis, the allele molecular sizes were used as codes for the different alleles detected in each locus.

Data analyses

The allelic diversity of the SSR was calculated according to the diversity index, *H*, described by Nei (1987), in the following formula:

$$H_i = 1 - \sum_{j=1}^{n} p_{ij}^2,$$

Where p_{ij} is the frequency of *j*th allele for the marker *i* and summation extends over *n* alleles. For each marker locus, the total numbers of alleles and allele sizes were calculated using Powermarker (version 3.25). The most frequent alleles and genotype-specific alleles were found using MICROSAT (version 1.5d) (Minch et al. 1997). The "maximum repeat count" for each SSR locus was calculated as described by Cho et al. (2000) using the following formula, Max repeat count = [(max allele MW - reference allele MW) / x] + reference repeat count (x = 2 or 3 for dinucleotide and trinucleotide repeats, respectively). The reference repeat count was taken directly from the known sequence of 'Nipponbare'. Pearson correlation coefficients were used to evaluate the relationships between the genetic diversity, the number of alleles, the maximum repeat count or the size range; these calculations were made using SPSS (version 10.0.1).

Data from all polymorphic SSR markers were used for phylogenetic analysis to determine genetic relationships. Genetic distances (Rogers, 1972) between two entries were computed as $RD_{ij} = ½ [\sum (X_{ai} - X_{aj})^2]^{½}$, where X_{ai} is the frequency of the allele a for individual *i,* and X_{aj} is the frequency of the allele a for the individual *j*. A dendrogram showing genetic relationships of the 18 accessions was constructed based on these distances using the unweighted pair-group method with the arithmetic mean (UPGMA). The strength of the dendrogram nodes was estimated with a bootstrap analysis using 1000 permutations with Phylip software (version 3.65) (Felsenstein, 2004).

Table 2. List of the 48 simple sequence repeat (SSR) markers used in this study.

Locus	Chr.	Repeat Motif	PRIMER F	PRIMER R
RM110	2	(GA)15	TCGAAGCCATCCACCAACGAAG	TCCGTACGCCGACGAGGTCGAG
RM129	1	(CGG)8	TCTCTCCGGAGCCAAGGCGAGG	CGAGCCACGACGCGATGTACCC
RM134	7	(CCA)7	ACAAGGCCGCGAGAGGATTCCG	GCTCTCCGGTGGCTCCGATTGG
RM144	11	(ATT)11	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG
RM148	3	(TG)12	ATACAACATTAGGGATGAGGCTGG	TCCTTAAAGGTGGTGCAATGCGAG
RM165	1	(CT)13	CCGAACGCCTAGAAGCGCGTCC	CGGCGAGGTTTGCTAATGGCGG
RM169	5	GA)12	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC
RM174	2	(AGG)7(GA)10	AGCGACGCCAAGACAAGTCGGG	TCCACGTCGATCGACACGACGG
RM179	12	(TG)7	CCCCATTAGTCCACTCCACCACC	CCAATCAGCCTCATGCCTCCCC
RM186	3	(CGG)5	TCCTCCATCTCCTCCGCTCCCG	GGGCGTGGTGGCCTTCTTCGTC
RM188	5	(CA)8	TCCGCCTCTCCTCTCGCTTCCC	GCAACGCACAACCGAACCGAGC
RM206	11	(CT)21	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
RM212	1	(CT)24	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
RM216	10	(CT)18	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
RM239	10	(AG)5TG(AG)2	TACAAAATGCTGGGTACCCC	ACATATGGGACCCACCTGTC
RM262	2	(CT)16	CATTCCGTCTCGGCTCAACT	CAGAGCAAGGTGGCTTGC
RM263	2	(CT)34	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
RM272	1	(GA)9	AATTGGTAGAGAGGGGAGAG	ACATGCCATTAGAGTCAGGC
RM286	11	(GA)16	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC
RM296	9	(GA)10	CACATGGCACCAACCTCC	GCCAAGTCATTCACTACTCTGG
RM30	6	(AG)9A(GA)12	GGTTAGGCATCGTCACGG	TCACCTCACCACACGACACG
RM300	2	(GTT)14	GCTTAAGGACTTCTGCGAACC	CAACAGCGATCCACATCATC
RM338	3	(CTT)6	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC
RM402	6	(ATA)7	GAGCCATGGAAAGATGCATG	TCAGCTGGCCTATGACAATG
RM408	8	(CT)13	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
RM411	3	(GTT)7	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG
RM414	1	(TGCA)6	ATTGCAGTCATGCAGCAGTC	ATATCTCCAATGTGGCAGGG
RM436	7	(TAA)6	ATTCCTGCAGTAAAGCACGG	CTTCGTGTACCTCCCCAAAC
RM441	11	(AG)13	ACACCAGAGAGAGAGAGAGAGAG	TCTGCAACGGCTGATAGATG
RM453	12	(TC)10	CGCATCTCTCTCCCTTATCG	CTCTCCTCCTCGTTGTCGTC
RM471	4	(GA)12	ACGCACAAGCAGATGATGAG	GGGAGAAGACGAATGTTTGC
RM477	8	(AATT)5	TCTCGCGGTATAGTTTGTGC	ACCACTACCAGCAGCCTCTG
RM494	6	(AGA)16	GGGAGGGGATCGAGATAGAC	TTTAACCTTCCTTCCGCTCC
RM495	1	(CTG)7	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC
RM507	5	(AAGA)7	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC
RM508	6	(AG)17	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC
RM517	3	(CT)15	GGCTTACTGGCTTCGATTTG	CGTCTCCTTTGGTTAGTGCC
RM519	12	(AAG)8	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC
RM542	7	(CT)22	TGAATCAAGCCCCTCACTAC	CTGCAACGAGTAAGGCAGAG
RM547	8	(ATT)19	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG
RM551	4	(AG)18	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG
RM556	8	(CCAG)6	ACTCCAAACCTCACTGCACC	TAGCACACTGAACAGCTGGC
RM559	4	(AACA)6	ACGTACACTTGGCCCTATGC	ATGGGTGTCAGTTTGCTTCC
RM560	7	(CT)12	GCAGGAGGAACAGAATCAGC	AGCCCGTGATACGGTGATAG
RM590	10	(TCT)10	CATCTCCGCTCTCCATGC	GGAGTTGGGGTCTTGTTCG
RM598	5	(GCA)9	GAATCGCACACGTGATGAAC	ATGCGACTGATCGGTACTCC
RM6	2	(AG)16	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC
RM60	3	(AATT)5AATCT(AATT)	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC

RESULTS

SSR diversity

All 48 SSR markers were found to be polymorphic among the 18 rice genotypes. These markers revealed a total of 203 alleles (Table 3); the average number of alleles per locus was 4.23, ranging from 2 to 9. The overall size of

Table 3. Data on the number of alleles, allele size range, highest allele frequency and genic diversity (*H*) at each 48 loci found among 18 rice genotypes. Allele number and genic diversity for each SSR marker were also calculated considering only the Venezuelan accessions (in brackets).

Marker	Chromosome location	No. of alleles	Size range (bp)	Difference (bp)	Highest frequency allele		*H*
					Size (bp)	Frequency (%)	
RM110	2	7 (5)	142-162	20	142	36.1	0.792 (0.731)
RM129	1	3 (2)	188-208	20	208	72.2	0.426 (0.355)
RM134	7	3 (2)	85-99	14	95	66.7	0.475 (0.426)
RM144	11	8 (5)	220-257	37	220	38.9	0.779 (0.689)
RM148	3	3 (2)	131-138	7	131	58.8	0.526 (0.473)
RM165	1	4 (1)	180-190	10	190	77.8	0.377 (0.000)
RM169	5	5 (2)	166-180	14	166	77.8	0.383 (0.142)
RM174	2	6 (4)	204-228	24	212	33.3	0.765 (0.710)
RM179	12	5 (4)	188-195	7	192	36.1	0.752 (0.743)
RM186	3	2 (2)	115-132	47	132	63.9	0.461 (0.355)
RM188	5	3 (1)	212-225	13	212	88.9	0.204 (0.000)
RM206	11	6 (4)	130-152	22	135	77.8	0.384 (0.275)
RM212	1	4 (3)	113-135	22	117	52.8	0.628 (0.606)
RM216	10	4 (3)	131-146	15	135	36.1	0.707 (0.624)
RM239	10	5 (3)	143-152	9	146	44.4	0.710 (0.544)
RM262	2	5 (4)	138-158	20	156	36.1	0.742 (0.660)
RM263	2	6 (4)	158-187	29	160	33.3	0.753 (0.698)
RM272	1	2 (1)	120-123	3	120	94.4	0.105 (0.000)
RM286	11	5 (3)	98-123	25	98	55.6	0.636 (0.379)
RM296	9	4 (3)	117-128	11	121	66.7	0.512 (0.544)
RM30	6	5 (4)	77-87	10	82	72.2	0.455 (0.388)
RM300	2	6 (3)	116-172	56	125	50.0	0.679 (0.521)
RM338	3	2 (1)	183-186	3	186	88.9	0.198 (0.000)
RM402	6	3 (2)	130-140	10	135	61.1	0.512 (0.473)
RM408	8	5 (2)	120-130	10	123	52.8	0.640 (0.453)
RM411	3	3 (2)	107-115	8	107	66.7	0.475 (0.260)
RM414	1	2 (1)	220-225	5	220	83.3	0.278 (0.000)
RM436	7	3 (1)	83-134	51	85	77.8	0.364 (0.000)
RM441	11	5 (3)	186-195	9	189	50.0	0.685 (0.544)
RM453	12	7 (4)	165-190	25	173	47.2	0.640 (0.568)
RM471	4	3 (2)	200-210	10	210	50.0	0.526 (0.500)
RM477	8	3 (1)	218-225	7	220	77.8	0.364 (0.000)
RM494	6	3 (1)	178-207	29	178	83.3	0.290 (0.000)
RM495	1	2 (2)	149-161	12	149	61.1	0.475 (0.426)
RM507	5	2 (1)	250-255	5	250	77.8	0.346 (0.000)
RM508	6	4 (3)	221-255	34	221	44.1	0.642 (0.517)
RM517	3	4 (2)	260-287	27	260	66.7	0.512 (0.260)
RM519	12	3 (1)	119-138	19	138	77.8	0.364 (0.000)
RM542	7	7 (3)	91-113	22	94	66.7	0.537 (0.272)
RM547	8	9 (4)	200-295	95	200-295	27.8	0.815 (0.675)
RM551	4	5 (4)	180-198	18	192	32.4	0.758 (0.686)
RM556	8	2 (2)	88-95	7	88	72.2	0.401 (0.260)
RM559	4	2 (1)	158-161	3	158	77.8	0.346 (0.000)
RM560	7	6 (4)	237-268	31	239	50.0	0.687 (0.565)
RM590	10	4 (3)	130-146	16	146	69.4	0.480 (0.210)
RM598	5	5 (4)	149-167	18	160	66.7	0.525 (0.568)
RM6	2	5 (4)	145-163	18	163	38.9	0.724 (0.660)
RM60	3	3 (1)	160-167	7	164	83.3	0.290 (0.000)
Total		203 (124*, 112**)					
Mean		4.23 (2.58*, 3.11**)				60.9	0.524 (0.370*, 0.490**)

PCR products amplified using 48 primer pairs ranged from 77 to 295 bp. The molecular size difference between the smallest and the largest allele for a given locus varied from 3 to 95 bp (Table 3). SSR markers with ATT, GA, TAA and GTT motifs showed the maximum variation in allele size. There was a considerable range in allele frequency (27.8-94.4%), on average 60.2% of the varieties shared a common allele (Table 3).

The average genic diversity over all SSR loci for the 18 genotypes was 0.524, ranging from 0.105 (RM272 on chromosome 1) to 0.815 (RM547 on chromosome 8) (Table 3). The genic diversity at each SSR locus was significantly correlated with the number of alleles detected ($r = 0.739$, $P < 0.01$) and with the allele size range ($r = 0.402$ $P < 0.01$) (Table 4). Loci with two-nucleotide repeat motifs (mean number of alleles 4.8, n = 24) and complex repeat motifs (mean number of alleles 4.8, n = 4) were more polymorphic than those with tri-nucleotide repeat motifs (mean number of alleles 3.9, n = 15) or tetra-nucleotide motifs (mean number of alleles 2.2, n = 5). The maximum number of repeats within the SSRs was significantly correlated with the genic diversity ($r = 0.362$, $P < 0.05$) and with the number of alleles at a locus ($r = 0.505$, $P < 0.01$) (Table 4).

Analyses of genetic diversity and allele number were also performed considering only the Venezuelan accessions (Table 3). For these accessions, only thirty-six SSR markers showed polymorphism. Analysis based only on polymorphic markers showed a total of 112 alleles (Table 3). The average number of alleles decreased to 3.11, ranging from 2 to 5, while the average genic diversity was reduced to 0.49, ranging from 0.14 (RM169 on chromosome 5) to 0.73 (RM110 on chromosome 2). As for the whole set of accessions, the genic diversity observed at each locus for the Venezuelan accessions was significantly correlated with the number of alleles detected ($r = 0.861$, $P < 0.01$), and the maximum number of repeats ($r = 0.323$, $P < 0.05$).

Genetic relationships among rice cultivars

A dendrogram was constructed based on the Rogers genetic distance calculated from the 203 SSR alleles generated from the 18 rice accessions. All 18 rice cultivars could be easily distinguished. The UPGMA cluster tree analysis led to the grouping of the 18 varieties in three major groups (Figure 1). Group I is comprised of *indica* varieties, which includes the Venezuelan and Colombian cultivars and 'IR64'. Group II is constituted by the *japonica* varieties 'Nipponbare', 'Azucena' and 'Caiapó'. The African wild species *Oryza glaberrima* is clearly differentiated from all other accessions in Group III. The bootstrap analysis supported the consistence of these groups; *japonica* and *indica* cultivars were clearly separated 100% of the times, while *O. glaberrima* was out-grouped 98% of the times (Figure 1).

Several additional sub-clusters were observed within the *indica* group, although these were weakly supported by bootstrap analysis. Only three sub-clusters, 'Araure 1' and 'Araure 4', 'Fundarroz-PN1' and 'Línea 17', 'Centauro' and 'Fedearroz 50' showed associations strongly supported by bootstrap analysis (they appeared 90%, 73% and 74% of the times, respectively) (Figure 1). Mean genetic distance for Venezuelan accessions was 0.31 (ranging from 0.15 to 0.57) indicating a close relationship among these cultivars (Table 5).

Genotype-specific alleles

Seventy of the 203 alleles detected are specific for a given genotype (Table 6): 35 alleles are specific for *O. glaberrima*, 7 for 'Azucena', 5 for 'Caiapó', 5 for 'Nipponbare', 7 for 'IR64', 3 for 'Fedearroz', 2 for each 'Araure 50', 'Cimarrón', 'Fonaiap 2000' and 'Palmar', and 1 for each 'Araure 1', 'Fonaiap 1' and 'Línea 17'. Nine of the 14 *indica* cultivars, 7 of them Venezuelan, can be differentiated from the other *indica* accessions using a set of 15 primers pairs while differentiation among *japonica* cultivars can be done with 11 primer pairs.

DISCUSSION

The assessment of genetic diversity is an essential component in germplasm characterization and

Table 4. **Correlations (Pearson's coefficient) calculated for genic diversity (*H*), the number of alleles, the number of repeats and the allele size range.** Correlations were calculated based on data derived from the analysis of 48 SSRs on the 18 genotypes.

	H	Allele number	Max. repeat number	Size range
H	1			
Allele number	0,739**	1		
Max. repeat number	0,362*	0,505**	1	
Size range	0,402**	0,541**	0,689**	1

*, ** Correlation significant at the 0.05 and 0.01 probability levels, respectively.

Table 5. Genetic distances among pairs of Venezuelan rice cultivars. Genetic distances were calculated using Rogers' coefficient (Rogers, 1972). Two Colombian cultivars, 'Fedearroz 2000' and 'Fedearroz 50', are included to show their relationship with Venezuelan cultivars.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1.	'Araure 1'	0,00												
2.	'Araure 4'	0,15	0,00											
3.	'Araure 50'	0,42	0,39	0,00										
4.	'Centauro'	0,33	0,32	0,36	0,00									
5.	'Cimarrón'	0,38	0,33	0,39	0,36	0,00								
6.	'Fedearroz 2000'	0,39	0,33	**0,52**	**0,50**	0,46	0,00							
7.	'Fedearroz 50'	0,35	0,28	**0,51**	0,22	0,39	0,37	0,00						
8.	'Fonaiap 1'	0,36	0,32	0,45	0,41	0,35	0,35	0,39	0,00					
9.	'Fonaiap 2000'	0,43	0,42	**0,57**	0,47	0,36	0,34	0,31	0,34	0,00				
10.	'Fundarroz-PN1'	0,29	0,31	0,29	0,39	0,38	0,39	0,45	0,40	**0,50**	0,00			
11.	'Línea 17'	0,32	0,28	0,33	0,34	0,34	0,36	0,37	0,35	**0,50**	0,21	0,00		
12.	'Palmar'	0,28	0,26	0,36	0,43	0,34	0,38	0,38	0,31	0,38	0,28	0,34	0,00	
13.	'Venezuela 21'	0,39	0,34	0,31	0,41	0,32	0,36	0,39	0,29	0,43	0,30	0,29	0,30	0,00

conservation. The results derived from analyses of genetic diversity at the DNA level could be used for designing effective breeding programs aiming to broaden the genetic bases of commercially grown varieties. A narrow genetic base has been reported for Latin American commercial rice cultivars, mainly based on pedigree and/or biochemical analyses (Cuevas-Perez et al. 1992; Guimaraes et al. 1995; Rangel et al. 1996; Fuentes et al. 1999; Aguirre et al. 2005). To our knowledge this work represents the first study of genetic diversity and molecular characterization of Venezuelan rice germplasm using SSR markers. Furthermore, there are few published reports of DNA fingerprinting of Latin American commercial rice cultivars (Fuentes et al. 1999; Aguirre et al. 2005; Giarrocco et al. 2007).

SSRs were chosen for the analysis of genetic diversity of Venezuelan rice cultivars because several works have showed these markers are very powerful for differentiating individual germplasm accessions, particularly when they are closely related (Bligh et al. 1999; Xu et al. 2004; Jeung et al. 2005). Additionally, SSRs show a series of advantages when compared with other DNA-based markers, such as abundance in the genome, high level of polymorphism, repeatability, co-dominance and cost-effectiveness (Ni et al. 2002).

In this study we evaluated 48 SSR markers in 18 rice cultivars, eleven of these genotypes represent *indica* varieties commercially cultivated in Venezuela. The other seven cultivars represent 3 *indica* and 3 *japonica* cultivars and the African wild species *Oryza glaberrima*. All 48 SSRs were polymorphic across the 18 genotypes. A total of 203 alleles were detected with an average number of alleles of 4.23 per locus (range 2 to 9 per locus). This value is quite low compared with those reported for the worldwide collection (range 2 -11, mean = 6.3) and other large scale studies (range = 3-17, mean = 7.4) (Olufowote et al. 1997; Yu et al. 2003) but quite comparable to values reported for studies performed on smaller germplasm sets (Cho et al. 2000; Hashimoto et al. 2004; Siwach et al. 2004). A set of 12 SSR markers (25%) resulted monomorphic when evaluated exclusively in the set of Venezuelan accessions, the average number of alleles for the 36 SSRs that showed polymorphism was 3.11 (range 2 to 5), a value significantly lower than previously reported for SSRs.

H value was 0.52 for the 18 genotypes evaluated and was reduced to 0.37 when the analysis was performed only with

Table 6. **Genotype-specific alleles identified for 13 rice cultivars analyzed in this study.**

Genotype	Locus	Allele (bp)	Genotype	Marker	Allele (bp)
O. glaberrima	RM110	151	'Araure 1'	RM206	139
	RM129	200	'Araure 50'	RM300	130
	RM134	99	'Cimarrón'	RM144	249
	RM144	240		RM542	91
	RM165	180	'Fedearroz 2000'	RM206	148
	RM169	170		RM263	158
	RM174	204		RM590	143
	RM179	191	'Fonaiap 1'	RM453	190
	RM188	225	'Fonaiap 2000'	RM169	180
	RM206	130		RM598	165
	RM212	128	'IR64'	RM188	215
	RM239	152		RM206	150
	RM262	138		RM263	182
	RM263	180		RM272	123
	RM286	107		RM408	124
	RM296	117		RM547	233
	RM30	87		RM551	189
	RM300	116	'Línea 17'	RM560	238
	RM402	130	'Palmar'	RM206	152
	RM408	120		RM547	222
	RM436	134			
	RM453	165	'Azucena'	RM110	154
	RM453	172		RM165	189
	RM471	200		RM169	171
	RM477	218		RM300	172
	RM494	180		RM411	109
	RM508	233		RM542	108
	RM517	287		RM547	208
	RM519	119	'Nipponbare'	RM144	230
	RM542	100		RM148	132
	RM547	205		RM169	169
	RM560	252		RM542	113
	RM598	149		RM547	235
	RM6	150	'Caiapó'	RM144	250
	RM60	160		RM174	217
				RM453	178
				RM542	105
				RM547	260

the 11 Venezuelan accessions. Both values are lower than the estimates for the world rice accessions, which included 83 landraces, 15 breeding lines and 95 improved varieties from eight major rice-growing regions of the world (Yu et al. 2003). In the case of Venezuelan cultivars, *H* value is about half the world estimate (*H* = 0.68). Data of genetic distances between Venezuelan cultivar pairs (mean = 0.31) also indicated a high degree of relatedness and low genetic diversity within the Venezuelan accessions. Our results support the reports of narrow genetic variation in Latin American cultivars (Cuevas-Perez et al. 1992; Guimaraes et al. 1995; Fuentes et al. 1999). Similar low *H* values have been reported for Japanese (Hashimoto et al. 2004) and Korean cultivars (Song et al. 2002) for which a narrow genetic base has been documented by means of SSR markers.

The structure and length of simple sequence repeats are considered to be the major factors affecting microsatellite variability (Brinkmann et al. 1998). In general, SSR loci with more repeats tend to be more polymorphic and have larger amplitude of variation (Panaud et al. 1996; Innan et al. 1997; Schug et al. 1998; Cho et al. 2000). The results obtained in this study are in concordance with this general pattern, SSR markers with many repeat units (RM110, RM144, RM262, and RM547) exhibited higher *H* values, higher number of alleles and larger size differences among alleles, thus high correlation coefficients were found between these parameters.

In spite of the low variability, we identified SSR markers that produce unique alleles for the genotypes studied. Several SSR markers were identified that could readily distinguish Venezuelan cultivars from the rest of accessions. More specifically, nine SSR markers (RM144, RM169, RM206, RM300, RM453, RM542, RM547, RM560 and RM598) produced ten genotype-specific alleles that distinguished seven Venezuelan rice cultivars (‘Araure 1’, ‘Araure 50’, ‘Cimarrón’, ‘Fonaiap 1’, ‘Fonaiap 2000’, ‘Línea 17’ and ‘Palmar’); these markers can be used for the molecular identification/characterization of the Venezuelan germplasm.

The UPGMA cluster analysis showed that all 18 rice cultivars could be easily distinguished based on the information generated by the 48 SSR markers. As expected, cultivars were separated in three clear groups corresponding to the *indica* and *japonica* subspecies and *O. glaberrima*. The 48 SSR markers also allowed the distinction among *indica* accessions; all 14 *indica* cultivars were clearly distinguished even though a high relatedness or similarity was measured between cultivar pairs. Our results support the contention that SSR marker systems can distinguish genetically close breeding lines and cultivars, and validate their use in the characterization of rice germplasm accessions.

Venezuelan cultivars used in the present study have not been examined previously in terms of genetic relatedness using molecular markers. The low genetic diversity found among the Venezuelan accessions evidence the narrow genetic bases used in our breeding programmes. References to the narrow genetic base of cultivated rice varieties are available from several regions, including Latin America (Cuevas-Pérez et al. 1992; Guimaraes et al. 1995; Fuentes et al. 1999; Aguirre et al. 2005), Japan (Hashimoto, 2004), USA (Dilday, 1990; Xu et al. 2004), Korea (Song et al. 2002) and Taiwan (Lin, 1991). In the case of Latin American rice germplasm, an extensive study based on pedigree analysis carried out in 1992 indicated that a group of only 14 landraces accounted for nearly 70 irrigated rice cultivars released in the region during the period 1971-1988 (Cuevas-Perez et al. 1992). The commercial rice varieties analyzed in this study represent 79% of the rice cultivars liberated by the Venezuelan Rice Breeding Program since 1978. Our results indicate that, despite breeding efforts, the varieties under current cultivation (‘Fundarroz PN-1’, ‘Venezuela 21’, ‘Centauro’, and ‘Cimarrón’) are closely related among them and even with cultivars released more than 20 years ago (‘Araure 1’ and ‘Araure 4’). A recent study by Arnao et al. (2008) using AFLP markers showed similar results, even when cultivars and experimental lines from other national breeding programs were included in the study. A pedigree analysis included in this work showed a strong contribution of only few progenitors to main cultivars and breeding lines (Arnao et al. 2008)

Our results indicate that it is essential to broaden the genetic base of the rice varieties cultivated in the country to reduce its vulnerability to diseases and insect pests. Recent studies carried out by the International Rice Research Institute showed there is still a tremendous amount of unexploited genetic diversity in the primary gene pool of rice that can be used for enhancing the diversity in local germplasms and their performance under diverse agroecological conditions (Guimaraes, 2000; Ali et al. 2006; Lafitte et al. 2006). Wild species of *Oryza* also represent a potential source of new alleles for improving yield, quality, and stress resistance in rice cultivars (Xiao et al. 1998; Moncada et al. 2001; Ahn et al. 2002; Thomson et al. 2003; McCouch, 2004; Kovach and McCouch, 2008). Several studies report improvements in performance because the introgression of valuable genes from wild germplasm into elite rice cultivar. Lines derived from crossing the wild species *Oryza rufipogon* with *Oryza sativa* cultivars showed higher yields than their progenitors and are tolerant to several abiotic stresses (Moncada et al. 2001; Nguyen et al. 2003; Tian et al. 2006; Xie et al. 2006; McCouch et al. 2007). Yield and grain quality enhancing alleles have also been identified from *O. glaberrima* (Aluko et al. 2004; Li et al. 2004; Sarla and Swamy, 2005) and *O. glumaepatula* (Brondani et al. 2002; Rangel et al. 2005). Utilisation of these “novel” gene sources is underway in rice breeding programs of several Latin American countries including Venezuela.

REFERENCES

AGUIRRE, Carlos; ALVARADO, Roberto and HINRICHSEN, Patricio. Identificación de cultivares y líneas de mejoramiento de arroz de Chile mediante amplificación de fragmentos polimórficos (AFLP). *Agricultura Técnica*, December 2005, vol. 65, no. 4, p. 356-369.

AHN, S.N.; SUN, J.P.; OH, C.H.; LEE, S.J. and SUH, H.S. Development of introgression lines of weedy rice in the background of Tongil-type rice. *Rice Genetic Newsletter*, December 2002, vol. 19, p. 14.

ALI, A.J.; XU, J.L.; ISMAIL, A.M.; FU, B.Y.; VIJAYKUMAR, C.H.M.; GAO, Y.M., DOMINGO, J.M.; MAGHIRANG, R.; YU, S.B.; GREGORIO, G.; YANAGHIHARA, S.; COHEN, M.; CARMEN, B.; MACKILL, D. and LI, Z.K. Hidden diversity for abiotic and biotic stress tolerances in the primary gene pool of rice revealed by a large backcross breeding program. *Field Crop Research*, May 2006, vol. 97, no. 1, p. 66-76.

ALUKO, G.; MARTINEZ, C.; TOHME, J.; CASTANO, C.; BERGMAN, C. and OARD, J. QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* x *O. glaberrima*. *Theoretical and Applied Genetics*, August 2004, vol. 109, no. 3, p. 630-639.

ÁLVAREZ, R.M.; MORENO, O.; DELGADO, N.; REYES, E.; ACEVEDO, M. and TORREALBA, G. Mejoramiento Genético. *El cultivo de arroz en Venezuela*. Serie Manuales de Cultivo INIA No. 1. Instituto Nacional de Investigaciones Agrícolas. Maracay, Venezuela. 2004, 202 p.

ARNAO, Erika; JAYARO, Yorman; HINRICHSEN, Patricio; RAMIS, Catalina; MARIN, Carlos and PEREZ-ALMEIDA, Iris. Marcadores AFLP en la evaluación de la diversidad genética de variedades de arroz y líneas élites de arroz en Venezuela. *Interciencia*, Mayo 2008, vol. 33, no. 5, p. 359-364.

BLIGH, H.F.J.; BLACKHALL, N.W.; EDWARDS, K.J. and McCLUNG A.M. Using amplified length polymorphisms and simple sequence length polymorphisms to identify cultivars of brown and white milled rice. *Crop Science*, November 1999, vol. 39, no. 6, p. 1715-1721.

BRINKMANN, Bernd; KLINTSCHAR, Michael; NEUHUBER, Franz; HUHNE, Julia and ROLF, Burhard. Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. *American Journal of Human Genetics*, June 1998, vol. 62, no. 6, p. 1408-1415.

BRONDANI, C.; RANGEL, N.; BRONDANI, V. and FERREIRA, E. QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. *Theoretical and Applied Genetics*, May 2002, vol. 104, no. 6-7, p. 1192-1203.

BRONDANI, R.P.V.; ZUCCHI, M.I.; BRONDANI, C.; RANGEL, P.H.N.; BORBA, T.C.D.O.; RANGEL, P.N.; MAGALHAES M.R. and VENCOVSKY, R. Genetic structure of wild rice *Oryza glumaepatula* populations in three Brazilian biomes using microsatellite markers. *Genetica*, November 2005, vol. 125, no. 2-3, p. 115-123.

CHEN, X.S.; TEMNYKH, S.; XU, Y.; CHO, Y.G. and McCOUCH, S.R. Development of microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, September 1997, vol. 95, no. 4, p. 553-567.

CHO, Y.G.; ISHII, T.; TEMNYKH, S.V.; CHEN, X.; LIPOVICH, L.; McCOUCH, S.R.; PARK W.D.; AYRES, N. and CARTINHOUR, S. Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, March 2000, vol. 100, no. 5, p. 713-722.

COBURN, J.R.; TEMNYKH, S.V.; PAUL, E.M. and McCOUCH, S.R. Design and application of microsatellite marker panels for semi-automated genotyping of rice (*Oryza sativa* L.). *Crop Science*, November 2002, vol. 42, no. 6, p. 2092-2099.

CUEVAS-PEREZ, Federico E.; GUIMARAES, Elcio P.; BERRIO, Luis E. and GONZALES, Daniel I. Genetic base of the irrigated rice in Latin America and the Caribbean. *Crop Science*, July 1992, vol. 32, no. 4, p. 1054-1059.

DEAN, R.E.; DAHLBERG, J.A.; HOPKINS, M.S.; MITCHELL, S.E. and KRESOVICH, S. Genetic redundancy and diversity among 'Orange' accessions in the U.S. national Sorghum collection as assessed with simple sequence repeat (SSR) markers. *Crop Science*, July-August 1999, vol. 39, no. 4, p. 1215-1221.

DELLAPORTA, Stephen L.; WOOD, Jonathan and HICK, James B. A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter*, September 1983, vol. 1, no. 4, p. 19-21.

DILDAY, R.H. Contribution of ancestral lines in the development of new cultivars of rice. *Crop Science*, July-August 1990, vol. 30, no. 4, p. 905-911.

FELSENSTEIN, J. PHYLIP (Phylogeny Inference Package), version 3.65c. Department of Genetics, University of Washington, Seattle. 2004.

FUENTES, Juan Luis; ESCOBAR, Fabio; ALVAREZ, Alba; GALLEGO, Gerardo; DUQUE, Miriam Cristina; FERRER, Mirle; DEUS, Juan Enrique and TOHME, Joe. Analyses of genetic diversity in Cuban rice varieties using isozyme, RAPD and AFLP markers. *Euphytica*, September 1999, vol. 109, no. 2, p. 107-115.

GARRIS, A.J.; TAI, T.H.; COBURN, J.R.; KRESOVICH, S. and McCOUCH, S.R. Genetic structure and diversity in *Oryza sativa* L. *Genetics*, March 2005, vol. 169, no. 3, p. 1631-1638.

GEALY, D.R.; TAI, T.H. and SNELLER, C.H. Identification of red rice, rice, and hybrid populations using microsatellite markers. *Weed Science*, May-June 2002, vol. 50, no. 3, p. 333-339.

GIARROCCO, L.E.; MARASSI, M.A. and SALERNO, G.L. Assessment of the genetic diversity in Argentine rice cultivars with SSR Markers. *Crop Science*, March 2007, vol. 47, no. 2, p. 853-860.

GUIMARAES, E.P. Mejoramiento poblacional del arroz en América Latina: dónde estamos y para dónde vamos. *Avances en el mejoramiento poblacional en arroz*. Santo Antônio de Goiás, Brazil, 2000, p. 299-311.

GUIMARAES, E.P.; BORRERO, J. and OSPINA-REY, Y. Genetic diversity of upland rice germplasm distributed in Latin America. *Pesquisa Agropecuaria Brasileira*, 1995, vol. 31, no. 3, p. 187-194.

HASHIMOTO, Z.; MORI, N.; KAWAMURA, M.; ISHII, T.; YOSHIDA, S.; IKEGAMI, M.; TAKUMI, S. and NAKAMURA, C. Genetic diversity and phylogeny of Japanese sake-brewing rice as revealed by AFLP and nuclear and chloroplast SSR markers. *Theoretical and Applied Genetics*, November 2004, vol. 109, no. 8, p. 1586-1596.

INNAN, H.; TERAUCHI, R. and MIYASHITA, T. Microsatellite polymorphism in natural populations of the wild plant *Arabidopsis thaliana*. *Genetics*, August 1997, vol. 146, no. 4, p. 1441-1452.

JEUNG, J.U.; HWANG, H.G.; MOON, H.P. and JENA, K.K. Fingerprinting temperate *japonica* and tropical *indica* rice genotypes by comparative analysis of DNA markers. *Euphytica*, December 2005, vol. 146, no. 3, p. 239-251.

KHUSH, Gurdev S. Origin, dispersal cultivation and variation of rice. *Plant Molecular Biology*, September 1997, vol. 35, no. 1-2, p. 25-34.

KOVACH, M.J. and McCOUCH, S.R. Leveraging natural diversity: back through the bottleneck. *Current Opinion in Plant Biology*, February 2008, vol. 11, no. 2, p. 193-200.

LAFITTE, H.R.; LI, Z.K.; VIJAYAKUMAR, C.H.M.; GAO, Y.M.; SHI, Y.; XU, J.L.; FU, B.Y.; YU, S.B.; ALI, A.J.; DOMINGO, J.; MAGHIRANG, R.; TORRES, R. and MACKILL, D. Improvement of rice drought tolerante through backcross breeding: Evaluation of donors and selection in drought nurseries. *Field Crops Research*, May 2006, vol. 97, no. 1, p. 77-86.

LI, J.; JINHUA, X.; GRANADILLO, S.; JIANG, L.; WAN, Y.; DENG, Q.; YUANG L. and McCOUCH, S. QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice. *Genome*, August 2004, vol. 47, no. 4, p. 697-704.

LIN, Maw Sun. Genetic base of *Japonica* rice varieties released in Taiwan. *Euphytica*, July 1991, vol. 56, no. 1, p. 43-46.

LIU, S.; CANTRELL, R.G.; McCARTY, J.C. and STEWART, J.M.D. Simple sequence repeat-based assessment of genetic diversity in cotton race stock accessions. *Crop Science*, September-October 2000, vol. 40, no. 5, p. 1459-1469.

LU, H.; REDUS, M.A.; COBURN, J.R.; RUTGER, J.N.; McCOUCH, S.R and TAI, T.H. Population structure and breeding patterns of 145 US rice cultivars base don SSR marker analysis. *Crop Science*, January 2005, vol. 45, no. 1, p. 66-76.

McCOUCH, S.; SWEENEY, M.; LI, J.; JIANG, H.; THOMSON, M.; SEPTININGSIH, E.; EDWARDS, J.; MONCADA, P.; XIAO, J. and GARRIS, A. Through the genetic bottleneck: *O. rufipogon* as a source of trait-enhancing alleles for *O. sativa*. *Euphytica*, March 2007, vol. 154, no. 3, p. 317-339.

McCOUCH, Susan. Diversifying selection in plant breeding. *PLoS Biology*, October 2004, vol. 2, no. 10, p. 1507-1512.

MINCH, E.; RUIZ-LINARES, A.; GOLDSTEIN, D.; FELDMAN, M. and CAVALLI-SFORZA, L. MICROSAT (ver. 1.5d): A computer program for calculating various statistics on microsatellite allele data. Updated 1997. Available from Internet: http://human.standford.edu/microsat/ microsat.html.

MONCADA, P.; MARTÍNEZ, C.P.; BORRERO, J.; CHATEL, M.; GAUCH, H. JR.; GUIMARAES, E.; TOHME, J. and McCOUCH, S.R. Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment. *Theoretical and Applied Genetics*, January 2001, vol. 102, no. 1, p. 41-52.

NEERAJA, C.N.; HARIPRASAD, A.S.; MALATHI, S. and SIDDIQ, E.A. Characterization of tall landraces of rice (*Oryza sativa* L.) using gene derived simple sequence repeats. *Current Science*, January 2005, vol. 88, no. 1, p. 149-152.

NEI, M. Molecular evolutionary genetics. New York; Columbia University Press, 1987. 512 p. ISBN 0231063210.

NGUYEN, B.D.; BRAR, D.S.; BUI, B.C.; NGUYEN, T.V.; PHAM, L.N. and NGUYEN, H.T. Identification and mapping of the QTL for aluminium tolerante introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, February 2003, vol. 106, no. 4, p. 583-593.

NI, Junjian; COLOWIT, Peter M. and MACKILL, David J. Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science,* March-April 2002, vol. 42, no. 2, p. 601-607.

OLUFOWOTE, Johnson O.; XU, Yunbi; CHEN, Xiuli; GOTO, Mak; MCCOUCH, Susan R.; PARK, WIlliam D.; BEACHELL, Henry M. and DILDAY, Robert H. Comparative evaluation of within-cultivar variation of rice (*Oryza sativa* L.) using microsatellite and RFLP markers. *Genome*, June 1997, vol. 40, no. 3, p. 370-378.

PANAUD, O.; CHENAYD, X. and McCOUCH, S.R. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Molecular and General Genetics*, October 1996, vol. 252, no. 5, p. 597-607.

POSSO-DUQUE, Duina and GHNEIM-HERRERA, Thaura. Uso de marcadores moleculares para la estimación de la diversidad genética en plantas. Manual de Laboratorio. *Ediciones IVIC*. 2008. p. 75.

PRASAD, M.; VARSHNEY, R.K.; ROY, J.K.; BALYAN, H.S. and GUPTA, P.K. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. *Theoretical and Applied Genetics,* February 2000, vol. 100, no. 3-4, p. 584-592.

RANGEL, P.H.N.; BRONDANI, C.; RANGEL, P.N.; BRONDANI, R.P.V. and ZIMMERMANN, F.J.P. Development of rice lines with gene introgression from wild *Oryza glumaepatula* by the AB-QTL methodology. *Crop Breeding and Applied Biotechnology*, 2005, vol. 5, no. 1, p. 10-19.

RANGEL, P.H.N.; GUIMARAES, E.P. and NEVES P.C.F. The genetic base of Brazilian irrigated rice (*Oryza sativa* L) cultivars. *Pesquisa Agropecuaria Brasileira*, 1996, vol. 31, p. 349-357.

REN, F.; LU, B.R.; LI, S.; HUANG, J. and ZHU, Y. A comparative study of genetic relationships among the AA-genome *Oryza* species using RAPD and SSR markers. *Theoretical and Applied Genetics*, December 2003, vol. 108, no. 1, p. 113-120.

ROGERS, J.S. Measures of genetic similarity and genetic distance. In: *Studies in Genetics VII*. University of Texas Publication 7213, Austin, Texas, 1972, p. 145-153.

SARLA, N. and SWAMY, B.P. *Oryza glaberrima*: a source for the improvement of *Oryza sativa*. *Current Science*, September 2005, vol. 89, no. 6, p. 955-963.

SARLA, N.; BOBBA, S. and SIDDIQ, E.A. ISSR and SSR markers based on AG and GA repeats delineate geographically diverse *Oryza nivara* accessions and reveal rare alleles. *Current Science*, March 2003, vol. 84, no. 5, p. 683-690.

SCHUG, M.D.; HUTTER, C.M.; WETTERSTRAND, K.A.; GAUDETTE, M.S.; MACKAY, T.F.C. and AQUADRO, C.F. The mutation rates of di-, tri- and tetranucleotide repeats in *Drosophila melanogaster*. *Molecular Biology and Evolution*, December 1998, vol. 15, no. 12, p. 1751-1760.

SENIOR, M.L.; MURPHY, J.P.; GOODMAN, M.M. and STUBER, C.W. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Science*, July-August 1998, vol. 38, no. 4, p. 1088-1098.

SIWACH, P.; JAIN, S.; SAINI, N.; CHOWDHURY, V.K. and JAIN, R.K. Allelic diversity among Basmati and non-Basmati long-grain indica rice varieties using microsatellite markers. *Journal of Plant Biochemistry and Biotechnology*, January 2004, vol. 13, p. 25-32.

SMITH, J.S.C.; KRESOVICH, S.; HOPKINS, M.S.; MITCHELL, S.; DEAN R.E.; WOODMAN, W.L.; LEE, M. and PORTER, K. Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. *Crop Science*, January-February 2000, vol. 40, no. 1, p. 226-232.

SONG, M.T.; LEE, J.H.; CHO, Y.S.; JEON, Y.H.; LEE, S.B.; KU, J.H.; CHOI, S.H. and HWANG, H.G. Narrow genetic background of Korean rice germplasm as revealed by DNA fingerprinting with SSR markers and their pedigree information. *Korean Journal of Genetics*, 2002, vol. 24, p. 397-403.

SOUZA, E. and SORRELLS, M.E. Pedigree analysis of North American oat cultivars released from 1941 to 1985. *Crop Science*, May-June 1989, vol. 29, no. 3, p. 595-601.

TEMNYKH, S.; PARK, W.D.; AYES, N.; CARTINHOUR, S.; HAUCK, N.; LIPOVICH, L.; CHO, Y.G., ISHII, T. and McCOUCH, S.R. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, March 2000, vol. 100, no. 5, p. 697-712.

THOMSON, M.J.; TAI, T.H.; McCLUNG, A.M.; LAI, X.H. and HINGA, M.E. Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza* cultivar Jefferson. *Theoretical and Applied Genetics*, February 2003, vol. 107, no. 3, p. 479-493.

TIAN, F.; LI, D.; FU, Q.; ZHU, Z.; FU, Y.; WANG, X. and SUN, C. Construction of introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*Oryza sativa* L.) background and characterization of introgressed segments associated with yield related traits. *Theoretical and Applied Genetics*, February 2006, vol. 112, no. 3, p. 570-580.

XIAO, J.; LI, J.; GRANDILLO, S.; AHN, S.N. and YUAN, L. Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. *Genetics*, June 1998, vol. 150, no. 2, p. 899-909.

XIE, Xiaobo; SONG, Mi-Hee; JIN, Fengxue; AHN, Sang-Nag; SUH, Jung-Pil; HWANG Hung-Goo and MCCOUCH, Susan. Fine mapping of a grain weight quantitative trait locus on rice chromosome 8 using near-isogenic lines derived from a cross between *Oryza sativa* and *Oryza rufipogon*. *Theoretical and Applied Genetics*, September 2006, vol. 113, no. 5, p. 885-894.

XU, Yunbi; BEACHELL, Henry and McCOUCH, Susan R. A marker-based approach to broadening the genetic base of rice in USA. *Crop Science,* November-December 2004, vol. 44, p. 1947-1959.

YANG, G.P.; SAGHAI, M.A.; XU, C.G.; ZHANG, Q. and BIYASHEV, R.M. Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Molecular and General Genetic*, March 1994, vol. 245, no. 2, p. 187-194.

YU, S.B.; XU, W.J.; VIJAYAKUMAR, C.H.M.; ALI, J.; FU, B.Y.; XU, J.L.; JIANG, Y.Z.; MARGHIRANG, R.; DOMINGO, J.; AQUINO, C.; VIRMANI, S.S. and LI, Z.K. Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. *Theoretical and Applied Genetics,* December 2003, vol. 108, no. 1, p. 131-140.