九州大学学術情報リポジトリ
Kyushu University Institutional Repository

Genetic Diversity in Vietnamese Upland Rice Germplasm Revealed by SSR Markers

Nguyen, Thuy T. T.
Department of Molecular Biology, Agricultural Genetics Institute

Nguyen, Nguyet M. T.
Department of Molecular Biology, Agricultural Genetics Institute

Hoang, Long H.
Department of Molecular Biology, Agricultural Genetics Institute

Furuya, Naruto
Faculty of Agriculture, Kyushu University

他

https://doi.org/10.5109/25195

出版情報：九州大学大学院農学研究院紀要. 57 (2), pp.383-391, 2012-09-20. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

KYUSHU UNIVERSITY

J. Fac. Agr., Kyushu Univ., **57** (2), 383–391 (2012)

Genetic Diversity in Vietnamese Upland Rice Germplasm Revealed by SSR Markers

Thuy T. T. NGUYEN[1]*, Nguyet M. T. NGUYEN[1], Long H. HOANG[1], Naruto FURUYA[2] and Kenichi TSUCHIYA[2]

Laboratory of Plant Pathology, Division of Agricultural Bioresource Science,
Department of Bioresource Science, Faculty of Agriculture,
Kyushu University, Fukuoka 812–8581, Japan
(*Received April 5, 2012 and accepted May 10, 2012*)

Tolerance to water deficit condition is an important prerequisite for optimal performance of rice in drought prone environments. The purpose of this study was to evaluate the genetic diversity within upland rice accessions, collected from Northern part of Vietnam. Genetic diversity of the rice varieties was evaluated at the DNA level. Forty–one accessions were surveyed with 30 simple sequence repeat (SSR) markers revealing the genetic relationship among the varieties. A total of 192 polymorphic bands were detected. The number of alleles per locus ranged from 3 to 12, with an average of 6.4. Cluster analysis based on genetic similarities grouped the rice accessions into two major groups. These groups were divided into five subgroups. These clusters agree with origin information available on the accessions. The results suggested that a relatively small number of SSR markers could be used for analysis of genetic diversity in rice germplasm. The upland rice germplasm presents a valuable gene source and sufficient genetic background for future breeding and mapping works on drought tolerance rice in Vietnam.

Keywords: allele, drought tolerance, genetic diversity, rice

INTRODUCTION

Drought is an important factor which affected the world food security can cause 70% of yield loss (Bray *et al*., 2000). Forty six thousand ha of rice land in Asia cultivated under rainfed condition every year. In Vietnam, the rice planting area is approx. 6 million ha. Of which, 1.5 million ha are rainfed and highland area. Twenty percent of the poor ethnic minority people that account for 20% of total population, live on rice production and have important responsiblity for the forest and watershed protection. Making them selfsufficient in food shall be defined as very important task in the development strategy of the country. It is suggested that with losses of arable land in delta areas due to the raised water level, rice production in these areas should be shifted to highland regions. In addition, insufficient water for rice production frequently happens even though in lowland irrigated rice fields bring measurable yield damage, affected rice farmer life. The development of drought tolerant cultivars is an effective approach for the contribution to the secure of stable rice production, increase rice farmer income and reduce poverty in Asia. However, attempts of the development of the cultivars with improved yield production were not much succeeded.

Rice has one of the largest ex situ germplasm collection in the world (Jackson and Juggan, 1993). This germplasm has made great contributions to rice breeding (Junjia, *et al*., 2002). Much success has been made in rice breeding in Vietnam with an average yield of 3.69 tones per ha in 1995 to 5.23 tones per ha in 2009 (General Statistics Office of Vietnam, 2010, http://www.gso.gov.vn). However, this value is equivalent to rice yield in China of 3.35 tones per ha in the 1950s and 6.23 tones per ha in the 1990s (Yongwen *et al*., 2006). Since 1996, rice cultivars from different Vietnam ecosystems throughout the country have been collected by National Plant Resource Center and maintained ex situ, including over 3,000 accessions in the National Genebank. However, the evaluation of germplasm has been performed only for morphological traits. The molecular tools have not been used systematically to evaluate large Vietnamese rice germplasm. Despite the richness of genetic resources, only a small proportion of this rice germplasm collection has been used in national rice breeding program. A few preliminary studies indicated that the gene sources for drought tolerance are quite abundant in Vietnamese local rice cultivars (Lang *et al*., 2009). However, the exploitation and efficient application of the gene sources are still limited.

Molecular markers have been used extensively to determine the genetic structure and diversity pattern of rice cultivar of interest (Herrera *et al*., 2008). Compared to morphological analysis, molecular markers can reveal differences among accessions at DNA level and thus provide a more direct reliable and efficient tool for germplasm conservation and management. The knowledge regarding the amount of genetic variation in germplasm accession and genetic relationships among genotypes is an important consideration for designing effective breeding programs (Herrera *et al*., 2008). Different types of molecular markers are available for evaluation of genetic variation in rice: restriction fragment length polymorphism (RFLP) (Botstein *et al*., 1980), random amplified

[1] Department of Molecular Biology, Agricultural Genetics Institute, Pham Van Dong, Hanoi, Vietnam
[2] Faculty of Agriculture, Kyushu University, Hakozaki, Fukuoka 812, Japan
* Corresponding author (E–mail: thuynt.khcn@mard.gov.vn)

polymorphism DNA (RAPD) (William *et al*., 1990), amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995), and simple sequence repeat (SSR) (Tautz, 1989). The AFLP marker has proven to be useful for characterization of the closely related rice cultivars (Spada *et al*., 2004). SSR approach is more suitable for differentiation of the closely related genomes, to study family structure, and to allow allelic changes due to their ability to detect higher polymorphism and codominance (Akagi *et al*., 1997).

Numerous studies on rice genetic diversity have been performed using molecular markers to analyze wild population or landraces in different countries (Jayamani *et al*., 2007). More concern is often expressed at cultivars because the domestication and modern plant breeding have led to a reduction in the genetic diversity of crops and loss of genes, which could result in dramatic consequences in crop production, such as genetic vulnerability to novel pests, diseases and climatic changes (Roussel *et al*., 2004). Genetic diversity of Vietnamese local rice landraces have been performed with 200 salt tolerance assessions. However, the study was assessed using agro–morphological characters (Lang *et al*., 2009). The same research was conducted with traditional upland rice varieties in the Philippines using morphometric markers (Florence *et al*., 2010).

In this context, to ensure food security and stable development of agriculture rice land areas will be

Table 1. Distribution of the upland and improved Vietnamese rice germplasm used in present study

Code	Name	Location	Cultivation
Upland varieties			
842	Nhong Haiduong	Red river delta	Summer season
1832	Khau nuot cung	Northeastern	Summer season, terraced field, rained field
1837	Lua mo trang	Northeastern	Summer season, terraced field, rained field
2125	Ble' tolo	Northwest	Summer season, upland variety
2127	Ble blu	Northwest	Summer season, upland variety
2131	Ble la tong	Northwest	Summer season, upland variety
2135	Ble lenh xi	Northwest	Summer season, upland variety
2367	Nep cuc	Red river delta	Spring season, field in hollows land, rained field
2642	B'le la	Northeastern	Summer season, irrigated field, cultivated rice
3550	Chanh trui	North Central	Spring season, field in hollows land, cultivated rice
3935	Mong lu	Northwest	Summer season, upland variety
3970	Ble ch– cau	Northwest	Summer season, upland variety
4123	Khau lay khao	Northeastern	Summer season, upland variety
4723	Cham soong	North Central	Summer season, upland variety
4726	Nep cai can	North Central	Summer season, upland variety
4748	Hang ngua	North Central	Summer season, upland variety
4762	Lo cang	North Central	Summer season, upland variety
4792	Khau ma giang	North Central	Summer season, upland variety
4793	Khau non	North Central	Summer season, upland variety
4794	Khau hin	North Central	Summer season, upland variety
4806	Blao sinh sai	Northwest	Summer season, upland variety
4840	Blao dong	Northwest	Summer season, upland variety
4843	Blao co nem	Northwest	Summer season, upland variety
5011	Khau noong mo	North Central	Summer season, upland variety
5015	Chao luu	North Central	Summer season, upland variety
5018	Khau san	North Central	Summer season, upland variety
5020	Khau do don	North Central	Summer season, upland variety
5057	Khau cu	North Central	Summer season, upland variety
6111	Te tep	Red river delta	Lowland variety
6188	Cuom dang 1	Red river delta	Spring season, irrigated field, cultivated rice
6203	Ngoi tia	Red river delta	Spring season, field in hollows land, cultivated rice
6430	Khau ken	Northeastern	Summer season, upland variety
6432	Bieo hong sui	Northeastern	Summer season, upland variety
7349	Manh gie	North Central	Summer season, upland variety
Commercial varieties			
4666	IR64	Introduced from IRRl	Lowland variety
N/A	Jasmine 85	Thailand origin	Lowland variety
N/A	SL12	Northern cultivated	Irrigated field, cultivated rice
N/A	DT54	Northern cultivated	Irrigated field, cultivated rice
8177	Khang Dan	China origin	Irrigated field, cultivated rice
8179	Q5	China origin	Irrigated field, cultivated rice
N/A	LT25	Northern cultivated	Irrigated field, cultivated rice

expanded to the high land area, where the irrigation is depended on the rainfall. Therefore, the deployment of the basic research on drought tolerance in rice plays an important role in the application and development research. The objectives of this study were: (1) to evaluate the genetic diversity among selected local Vietnamese rice gene pool, paying particular attention to variation among potential drought tolerant varieties; and (2) to determine rice accessions with high genetic diversity that will be used to develop mapping population for construction of rice drought quantitative trait locus (QTL) maps and marker–assisted molecular breeding of high yield rice variety with drought tolerance.

MATERIALS AND METHODS

Rice germplasm

Rice germplasm (Table 1) were kindly provided by National Gene Bank of Plant Resource Center of Vietnam, represented the different unique upland cultivars of Northern part, which have been evaluated for drought tolerance and showed high level of resistance to water deficit condition (data not shown). A total of 41 accessions including 34 local drought tolerant varieties distributed in twelve provinces and 7 varieties originated from others in order to compare the genetic diversity of Vietnam unique upland rice germplasm and identify gene source for breeding program of drought tolerance in rice.

SSR analysis

Fresh leaf tissues were harvested at the seedling stage from plants grown in the greenhouse. Total genomic DNA was extracted from parental lines leaf tissues using NaOH extraction method improved from the simple method of preparing plant samples for PCR (Wang *et al*., 1993). Thirty SSR markers covering all the twelve chromosomes on rice genome were selected for the genetic diversity analysis based on the Gramene Markers Database (http://www.gramene.org/markers/). These markers were chosen based on ability to be high polymorphic among rice varieties in the preliminary screening performed in previous studies. PCR was performed in 15 μl reaction mixture containing 30 ng of template DNA, 0.4 μM of each primer, 200 μM for each dNTPs, 1x PCR buffer (10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$) and 1.0 units *Taq* DNA polymerase (Takara) in a 96 wells PCR plate. An Verity 96 Well Thermal Cycler (Applied Biosystems Co. Ltd.,) was used along with the following PCR profile: an initial denaturing step of 5 min at 94˚C, followed by 35 cycles of 30 sec at 94˚C, 30 sec at 55˚C, 1 min at 72˚C, and a final extension at 72˚C for 7 min. Amplified products were resolved on 3% agarose gels with ethidium bromide staining. Gels were visualized under ultraviolet light using Herolab, Germany. The size in nucleotide base pairs (bp) of allele band with highest intensity for each SSR marker was scored as main allele band and determined based on its migration relative to 50 bp DNA ladder (Fermentas, Lithuania).

Data analysis

The allelic diversity of the SSR was calculated according to the Polymorphism Information Content, PIC, described by Anderson *et al*. (1993), in the following formula:

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

Where p_{ij} is the frequency of jth allele at the locus i and summation extends over n alleles. Heterogeneity (HG) by accession and by marker was calculated as percentage of heterogeneous loci per accession across all accessions and loci, respectively. For the diversity representation, markers were scored based on the band pattern generated from the gel imaging system for the presence or absence of the corresponding band among the genotypes and then converted to a genetic similarity (GS) matrix using Dice coefficients (Sneath and Sokal, 1973). Using the binary coding system, '1' indicates the presence of clear and unambiguous bands and '0' indicates the absence of bands. A genetic relationship matrix was used to produce a dendrogram in a sequential agglomerative hierarchical nested cluster analysis (SAHN), based on the unweighted pair–group method with arithmetical average (UPGMA) clustering of the GS matrix.

RESULTS

SSR diversity

The 41 rice accessions were analyzed with 30 SSR markers and all of them were shown polymorphic with 192 bands. The size of PCR products ranging from 65 bp to 280 bp across 41 rice varieties for each of the 30 markers (Fig. 1 and Table 2).

There was no correlation detected between the number of alleles and the number of repeats in SSR loci (Table 2). The number of alleles per locus ranged from 3 alleles (RM3431) to 12 alleles (RM1364), with an average of 6.4 alleles across the 30 loci. The PIC values ranged from 0.447 (RM3431) to 0.888 (RM3515), with an average of 0.735. Unique allele, an allele that was observed in only one of the 41 rice varieties, were identified at 16 loci in total of 30 loci, with the maximum of 3 unique alleles in RM1364 locus and RM3476 locus. The frequency of the most common allele at each locus ranged from 13.95% (RM3515) to 71.43% (RM3431). On average, 37.76% of the 41 rice accessions shared a common major allele at any given locus (Table 2).

The average HG percentage of the 30 SSR markers over all 41 accessions (Table 2) was accounted for 5.28%. Fifteen of the 30 (50%) SSR markers detected no heterogeneous accessions, while the remaining markers showed one or more heterogeneous accessions. Remarkably, RM5599 detected cultivar heterogeneity in 21 accessions (HG=51.22), and RM1364 detected cultivar heterogeneity in 17 accessions (HG=41.46) while the remaining 13 SSR markers identified one to four heterogeneous accessions (HG=2.44 to HG=9.76). Determination of the HG% of 41 accessions across all SSR markers (Table 3) revealed that 24 accessions were homogeneous at all loci and 17

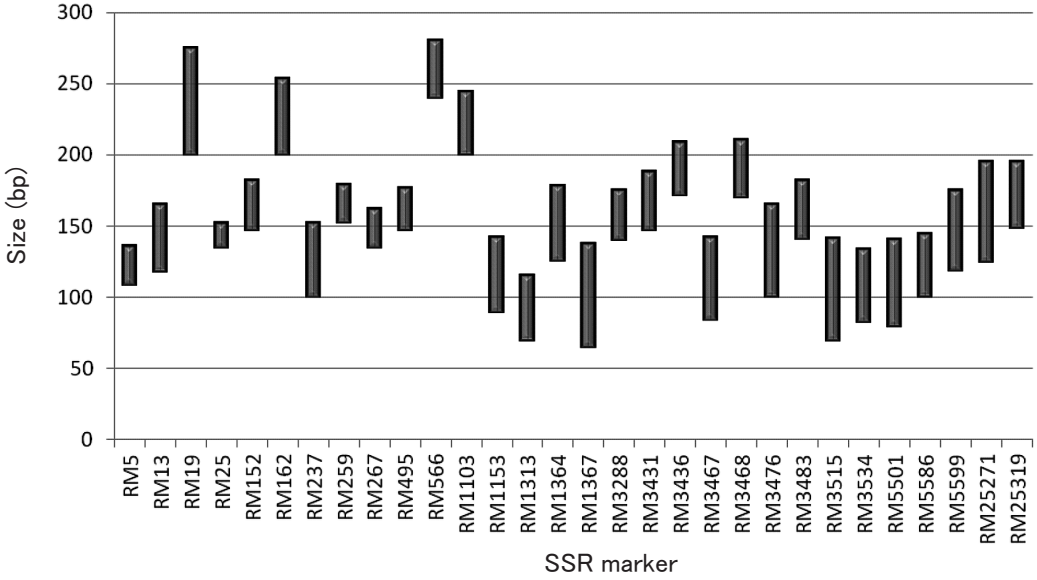

Fig. 1. Allele size variation of the surveyed SSR loci.

Table 2. Allele variation, Polymorphism Information Content (PIC) and heterogeneity (HG%) for SSR loci identified in 41 rice varieties

Marker	Chromosome location	No. of allele	PCR product size (bp)	Frequency of the most common allele	Unique allele	PIC	HG (%)
RM5	1	7	109–136	24.39	0	0.844	0
RM13	5	5	118–165	41.463	0	0.709	0
RM19	12	5	200–275	36.585	0	0.746	0
RM25	8	4	135–152	51.22	1	0.563	0
RM152	8	5	147–182	51.22	0	0.657	0
RM162	6	6	200–253	26.829	0	0.813	0
RM237	1	4	100–152	51.22	0	0.651	0
RM259	1	6	153–179	20.93	0	0.829	4.88
RM267	5	6	135–162	51.163	2	0.654	7.32
RM495	1	6	147–176	42.857	1	0.709	4.88
RM566	9	7	240–280	26.19	1	0.808	2.44
RM1103	12	4	200–244	35.897	0	0.707	0
RM1153	4	7	90–142	39.535	2	0.677	4.88
RM1313	2	7	70–115	26.829	2	0.795	0
RM1364	7	12	126–178	24.561	3	0.855	41.46
RM1367	2	7	65–137	39.024	1	0.74	0
RM3288	4	5	140–175	26.19	0	0.787	2.44
RM3431	6	3	147–188	71.429	0	0.447	0
RM3436	3	5	172–209	48.718	1	0.68	0
RM3467	3	7	84–142	37.209	0	0.778	4.88
RM3468	1	9	170–210	19.048	1	0.864	2.44
RM3476	5	7	102–165	54.545	3	0.642	7.32
RM3483	12	3	141–182	41.026	0	0.657	0
RM3515	2	10	70–141	13.953	0	0.888	9.76
RM3534	4	7	83–133	41.463	1	0.751	0
RM5501	1	7	80–140	51.163	0	0.698	7.32
RM5586	4	7	100–144	32.432	2	0.783	2.44
RM5599	11	5	119–175	56.667	1	0.62	51.22
RM25271	10	9	125–195	28.571	1	0.844	4.88
RM25319	10	10	149–195	20.513	2	0.86	0
Total		192			25		
Mean		6.4		37.76	0.83	0.735	5.28
Min		3	65	13.95	0	0.447	0
Max		12	280	71.43	3	0.888	51.22

Table 3. Unique allele and heterogeneity (HG%) identified in 41 rice varieties

Varieties Name	Code	Unique allele			HG%
		Total	SSR marker	Size (bp)	
Nhong Haiduong	842	2	RM25, RM1364	152, 126	6.67
Khau nuot cung	1832	2	RM1364, RM3534	160, 96	6.67
Lua mo trang	1837	3	RM267, RM495, RM5599	144, 147	10
B'le tolo	2125	0			0
Ble blu	2127	1	RM3476	165	3.33
Ble la tong	2131	2	RM1153, RM25319	135, 184	6.67
Ble lenh xi	2135	1	RM3476	117	3.33
Nep cuc	2367	1	RM1367	100	3.33
Ble' la	2642	1	RM3468	170	3.33
Chanh trui	3550	1	RM566	261	3.33
Mong lu	3935	0			0
Ble ch– cau	3970	0			0
Khau lay khao	4123	0			0
Cham soong	4723	0			0
Nep cai can	4726	0			0
Hang ngua	4748	1	RM1313	89	3.33
Lo cang	4762	0			0
Khau ma giang	4792	0			0
Khau non	4793	0			0
Khau hin	4794	0			0
Blao sinh sai	4806	1	RM267	162	3.33
Blao dong	4840	1	RM25271	185	3.33
Blao co nem	4843	1	RM3476	136	3.33
Khau noong mo	5011	0			0
Chao luu	5015	0			0
Khau san	5018	0			0
Khau do don	5020	0			0
Khau cu	5057	1	RM25319	175	3.33
Te tep	6111	0			0
Cuom dang 1	6188	1	RM5586	100	3.33
Ngoi tia	6203	3	RM1153, RM1313	90, 76, 144	10
Khau ken	6430	2	RM1364, RM3436	169, 209	6.67
Bieo hong sui	6432	0			0
Manh gie	7349	0			0
IR64	4666	0			0
Jasmine 85		0			0
SL12		0			0
DT54		0			0
Khang Dan	8177	0			0
Q5	8179	0			0
LT25		0			0
Total		25			
Mean		0.61			2.03
Max		25			10

accessions had at least two alleles at one (single) locus. Eleven accessions had the minimum HG percentage indicating a single heterogeneous locus (3.33%).

Twenty five unique alleles detected are specific for 17 rice accessions (Table 3). All of these alleles detected in traditional rice varieties, and two varieties, Lua mo trang and Ngoi tia, harbored three unique alleles in each (Table 3).

Cluster analysis

The genetic similarities obtained from SSR data were used to create a cluster diagram. Cluster analysis based on Dice coefficients using UPGMA grouped 41 rice accessions into 2 main clusters I and Ⅱ (Fig. 2). The cluster analysis represented a significant genetic variation among the rice accessions. The genetic similarity ranged from null for ten accessions pairs to 0.81 with a mean of 0.32 indicated a significant genetic variation among rice accessions. The highest value (0.81) corresponded to 'Khang Dan'–'DT54' pair (Table 4). The dendrogram revealed 2 distinct groups (I and Ⅱ) at the Dice coefficient of 0.12 (Fig. 2). The first group (Group I) consists of 16 rice vari-

Table 4. Genetic relationships among 41 rice accessions

		NhongHaiduong	Khaunuotcung	Luamotrang	B'letolo	Bleblu	Blelatong	Blelenhxi	Nepcuc	Ble'la	Chanhtrui	Monglu	Blechucau	KhaulaykhAo	Chamsoong	Nepcaican	Hangngua	Locang	Khaumagiang	Khaunon	Khauhin	Blaosinhsai	Blaodong	Blaoconem	Khaunoongmo	Chaoluu	Khaucu	Khaudodon	Khaucu	Tetep	Cuomdang1	Ngoitia	Khauken	Bieohongsui	Manhgie	Manhgie	IR64	Jasmine85	SL12	DT54	KD	Q5	LT25
		842	1832	1837	2125	2127	2131	2135	2367	2642	3550	3935	3970	4123	4723	4726	4748	4762	4792	4793	4794	4806	4840	4843	5011	5015	5018	5020	5057	6111	6188	6203	6430	6432	7349	4666	Jas85	SL12	DT54	KD	Q5	LT25
NhongHaiduong	842	***																																								
Khaunuotcung	1832	0.14	***																																							
Luamotrang	1837	0.62	0.18	***																																						
B'letolo	2125	0.16	0.40	0.10	***																																					
Bleblu	2127	0.07	0.51	0.03	0.41	***																																			
Blelatong	2131	0.13	0.33	0.07	0.53	0.54	***																																		
Blelenhxi	2135	0.53	0.07	0.52	0.10	0.00	0.00	***																																	
Nepcuc	2367	0.22	0.45	0.23	0.39	0.30	0.39	0.09	***																																
Ble'la	2642	0.43	0.34	0.45	0.23	0.13	0.16	0.47	0.25	***																															
Chanhtrui	3550	0.16	0.37	0.14	0.44	0.44	0.50	0.10	0.51	0.16	***																														
Monglu	3935	0.22	0.41	0.08	0.46	0.44	0.50	0.04	0.41	0.30	0.39	***																													
Blechucau	3970	0.49	0.18	0.47	0.17	0.07	0.10	0.56	0.16	0.49	0.17	0.29	***																												
KhaulaykhAo	4123	0.52	0.17	0.67	0.07	0.07	0.07	0.58	0.16	0.47	0.10	0.22	0.75	***																											
Chamsoong	4723	0.20	0.39	0.14	0.46	0.43	0.30	0.10	0.34	0.17	0.30	0.49	0.07	0.10	***																										
Nepcaican	4726	0.19	0.37	0.10	0.41	0.41	0.34	0.13	0.27	0.19	0.25	0.43	0.07	0.07	0.72	***																									
Hangngua	4748	0.19	0.30	0.17	0.47	0.32	0.34	0.13	0.30	0.16	0.34	0.46	0.14	0.13	0.52	0.50	***																								
Locang	4762	0.14	0.31	0.07	0.52	0.27	0.30	0.10	0.22	0.14	0.30	0.34	0.11	0.10	0.55	0.59	0.52	***																							
Khaumagiang	4792	0.16	0.37	0.07	0.44	0.41	0.44	0.03	0.18	0.10	0.28	0.39	0.10	0.10	0.52	0.56	0.47	0.56	***																						
Khaunon	4793	0.13	0.30	0.07	0.31	0.51	0.44	0.03	0.21	0.10	0.28	0.43	0.14	0.13	0.52	0.53	0.44	0.39	0.75	***																					
Khauhin	4794	0.17	0.36	0.11	0.43	0.44	0.43	0.07	0.29	0.14	0.37	0.46	0.11	0.14	0.53	0.47	0.47	0.46	0.58	0.53	***																				
Blaosinhsai	4806	0.16	0.35	0.07	0.39	0.43	0.33	0.03	0.26	0.13	0.27	0.45	0.10	0.06	0.51	0.55	0.42	0.48	0.67	0.52	0.68	***																			
Blaodong	4840	0.16	0.46	0.10	0.46	0.47	0.40	0.03	0.29	0.16	0.43	0.46	0.10	0.10	0.61	0.49	0.43	0.58	0.62	0.55	0.59	0.66	***																		
Blaoconem	4843	0.16	0.37	0.07	0.32	0.39	0.25	0.10	0.24	0.13	0.35	0.25	0.07	0.10	0.47	0.44	0.41	0.40	0.51	0.41	0.37	0.52	0.59	***																	
Khaunoongmo	5011	0.18	0.41	0.10	0.38	0.39	0.29	0.10	0.18	0.07	0.29	0.40	0.10	0.10	0.50	0.48	0.41	0.50	0.57	0.48	0.54	0.55	0.53	0.52	***																
Chaoluu	5015	0.12	0.35	0.06	0.39	0.33	0.27	0.06	0.28	0.09	0.21	0.27	0.00	0.03	0.56	0.51	0.45	0.50	0.54	0.39	0.38	0.49	0.44	0.58	0.48	***															
Khaucu	5018	0.07	0.20	0.00	0.41	0.26	0.19	0.13	0.12	0.07	0.25	0.18	0.03	0.03	0.40	0.44	0.41	0.63	0.38	0.29	0.31	0.28	0.31	0.29	0.42	0.58	***														
Khaudodon	5020	0.13	0.17	0.07	0.32	0.23	0.22	0.10	0.06	0.07	0.19	0.18	0.00	0.03	0.37	0.41	0.32	0.57	0.44	0.38	0.34	0.28	0.31	0.32	0.42	0.55	0.77	***												
Khaucu	5057	0.13	0.23	0.07	0.28	0.32	0.28	0.06	0.18	0.10	0.25	0.32	0.14	0.10	0.36	0.34	0.47	0.36	0.44	0.44	0.40	0.39	0.43	0.48	0.41	0.51	0.44	0.48	***											
Tetep	6111	0.31	0.17	0.32	0.13	0.10	0.10	0.34	0.26	0.21	0.27	0.07	0.39	0.31	0.11	0.07	0.10	0.07	0.10	0.07	0.14	0.06	0.10	0.14	0.17	0.16	0.14	0.14	0.10	***										
Cuomdang1	6188	0.32	0.23	0.33	0.13	0.10	0.09	0.39	0.24	0.26	0.25	0.11	0.41	0.39	0.10	0.06	0.09	0.03	0.09	0.09	0.10	0.06	0.09	0.16	0.16	0.12	0.06	0.10	0.09	0.80	***									
Ngoitia	6203	0.10	0.21	0.03	0.23	0.13	0.16	0.20	0.18	0.10	0.19	0.19	0.14	0.14	0.31	0.29	0.25	0.37	0.32	0.23	0.24	0.22	0.22	0.30	0.30	0.34	0.30	0.26	0.26	0.24	0.19	***								
Khauken	6430	0.16	0.23	0.10	0.22	0.19	0.18	0.10	0.24	0.10	0.25	0.28	0.17	0.16	0.32	0.31	0.40	0.35	0.34	0.31	0.43	0.39	0.33	0.34	0.34	0.29	0.22	0.22	0.37	0.13	0.13	0.44	***							
Bieohongsui	6432	0.16	0.24	0.14	0.22	0.23	0.25	0.13	0.21	0.10	0.29	0.29	0.14	0.13	0.27	0.29	0.48	0.27	0.35	0.30	0.44	0.37	0.34	0.29	0.32	0.30	0.26	0.29	0.38	0.20	0.19	0.39	0.69	***						
Manhgie	7349	0.40	0.17	0.27	0.06	0.06	0.10	0.39	0.15	0.20	0.19	0.07	0.38	0.43	0.07	0.06	0.13	0.07	0.03	0.06	0.13	0.07	0.03	0.06	0.13	0.10	0.06	0.10	0.10	0.13	0.55	0.58	0.20	0.13	0.13	***					
IR64	4666	0.32	0.13	0.27	0.06	0.06	0.03	0.39	0.18	0.26	0.13	0.07	0.34	0.43	0.13	0.16	0.09	0.07	0.09	0.09	0.13	0.09	0.06	0.13	0.16	0.09	0.16	0.13	0.13	0.43	0.47	0.23	0.12	0.19	0.48	***					
Jasmine85	Jas85	0.37	0.21	0.24	0.10	0.10	0.13	0.37	0.18	0.23	0.23	0.14	0.37	0.44	0.14	0.13	0.16	0.10	0.06	0.10	0.14	0.06	0.16	0.13	0.10	0.09	0.10	0.10	0.19	0.47	0.48	0.23	0.13	0.20	0.77	0.68	***				
SL12	SL12	0.32	0.22	0.19	0.17	0.21	0.17	0.32	0.23	0.32	0.28	0.23	0.33	0.33	0.15	0.14	0.17	0.11	0.07	0.10	0.11	0.07	0.10	0.11	0.07	0.13	0.18	0.11	0.28	0.39	0.42	0.18	0.10	0.14	0.64	0.55	0.76	***			
DT54	DT54	0.33	0.24	0.28	0.06	0.13	0.06	0.40	0.22	0.23	0.19	0.07	0.28	0.34	0.10	0.16	0.10	0.10	0.03	0.06	0.14	0.06	0.13	0.07	0.10	0.03	0.10	0.07	0.10	0.48	0.52	0.17	0.13	0.16	0.52	0.52	0.60	0.61	***		
KhangDan	KD	0.25	0.22	0.25	0.07	0.07	0.03	0.32	0.26	0.21	0.21	0.04	0.33	0.32	0.07	0.10	0.10	0.11	0.00	0.00	0.07	0.03	0.10	0.07	0.07	0.07	0.14	0.03	0.14	0.44	0.45	0.14	0.10	0.11	0.47	0.59	0.54	0.60	**0.81**	***	
Q5	Q5	0.30	0.23	0.27	0.16	0.13	0.16	0.33	0.27	0.20	0.25	0.07	0.28	0.33	0.10	0.16	0.13	0.07	0.13	0.13	0.17	0.06	0.09	0.16	0.13	0.15	0.16	0.19	0.16	0.47	0.51	0.20	0.19	0.26	0.43	0.54	0.52	0.49	0.75	0.74	***
LT25	LT25	0.29	0.13	0.22	0.08	0.04	0.00	0.33	0.17	0.13	0.12	0.08	0.30	0.35	0.09	0.08	0.04	0.09	0.00	0.00	0.04	0.04	0.08	0.04	0.13	0.08	0.16	0.12	0.08	0.40	0.40	0.17	0.08	0.16	0.51	0.68	0.60	0.48	0.67	0.73	0.69	***
Min		**0.00**	0.07	0.07	0.00	0.06	0.00	0.00	0.03	0.06	0.07	0.10	0.04	0.00	0.03	0.07	0.06	0.04	0.03	0.00	0.00	0.04	0.03	0.06	0.04	0.07	0.03	0.06	0.03	0.08	0.13	0.13	0.14	0.08	0.11	0.43	0.52	0.52	0.48	0.67	0.73	0.69	
Max		**0.81**	0.62	0.51	0.67	0.53	0.54	0.50	0.58	0.51	0.49	0.43	0.49	0.75	0.44	0.72	0.59	0.52	0.63	0.75	0.55	0.68	0.66	0.59	0.58	0.48	0.58	0.77	0.48	0.38	0.80	0.58	0.44	0.69	0.26	0.77	0.68	0.76	0.61	**0.81**	0.74	0.69	

Genetic similarity were calculated using Dice coefficient (Dice, 1945; Nei and Li, 1979).

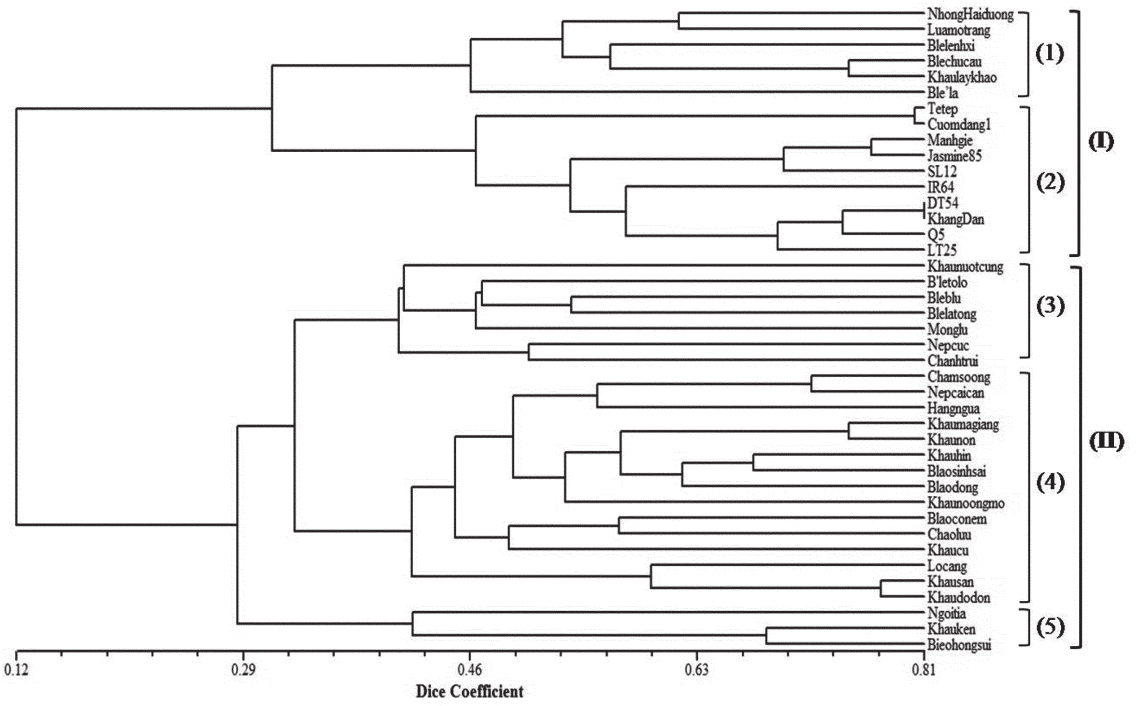

Fig. 2. Dice's similarity coefficient based on 30 SSR makers showing the relationships among 41 rice accessions. Dendrogram derived from UPGMA cluster analysis. (1) – (5): sub group; (I), (II): group.

eties with the Dice coefficients ranged between ~0.32 and 0.81. The second group (Group II) consists of 25 remaining accessions, with the genetic coefficients ranged between ~0.29 and 0.76. Group I was further sub–divided into several sub–groups. All of 7 commercial varieties (Jasmine85, SL12, IR64, DT54, Khang Dan, Q5, and LT25) were clustered in one sub–group with three other varieties (Tetep, Cuom dang1, and Manh gie). The second group (Group II) was also further sub–divided into three sub–groups, consisted of 7, 15, and 3 varieties, respectively (Fig. 2). Cluster analysis had grouped most of the traditional upland varieties together showing a high level of genetic relatedness.

DISCUSSION

The number of studies have been performed for genetic diversity in different rice germplasm using molecular markers (Nagaraju *et al*., 2002; Yu *et al*., 2003; Gao *et al*., 2005; Jeung *et al*., 2005; Herrera *et al*., 2007; Wong *et al*., 2009). In this study, we present a genetic diversity analysis of upland drought tolerant and improved Vietnamese rice varieties based on microsatellite marker data. We evaluated 30 SSR markers in 41 rice varieties, seven of these genotypes represent *indica* varieties commercially cultivated in Vietnam, originated from China, Thailand and IRRI. The other 34 genotypes rep-

Table 5. Genetic diversity of different rice germplasms

Author	No of accessions	No of marker	No of allele	Average			
				Allele number	PIC	Number of unique alleles (rare allele*)	HG (%)
Giarrocco *et al*., 2007	69	26	219	8.4	0.69	1.7	3.4
Wong *et al*., 2009	8	12	31	2.6	0.52	–	–
Nagaraju *et al*., 2002	24	19	70	3.8	–	–	–
Alvarezet *et al*., 2007	50	10	66	6.6	0.74	1.4	–
Thomson *et al*., 2007	330	30	394	13.1	0.66	9*	–
Herrera *et al*., 2008	18	48	203	4.2	0.52	–	–
Yu *et al*., 2003	193	101	628	6.2	0.68	–	–
Chakravarthi *et al*., 2006	15	30	462	–	–	–	–
This study	41	30	192	6.4	0.73	0.6	5.3

resent Vietnam traditional and upland varieties (Table 1). A total of 192 alleles were detected with an average number of alleles of 6.4 per locus (ranged from 3 to 12 per locus). This value is higher than those reported for the studies performed on smaller germplasm sets (Nagaraju *et al*., 2002; Herrera *et al*., 2007; Wong *et al*., 2009) and comparable to values reported in Yu *et al*. (2003), Alvarez *et al*. (2007), but quite lower than those reported for other large scale collection (Giarrocco *et al*., 2007; Thomson *et al*., 2007) (Table 5). The results demonstrated that the varieties selected possess a high level of microsatellite variation (PIC=0.73). The overall genetic diversity (PIC=0.73) of the 41 rice germplasm accessions included in this study was similar to the value reported in previous studies (PIC=0.74) (Gao *et al*., 2003; Alvarez *et al*., 2007; Ghneim *et al*., 2008), but higher than those reported in the studies: Yu *et al*. (2003) reported a PIC value of 0.68 using 193 accessions, Xu *et al*. (2004) reported 0.66 using 236 accessions, Garris *et al*. (2005) reported 0.67 using 334 accessions, and Giarrocco *et al*. (2007) reported 0.69 using 69 accessions. The results presented in this study suggest the genetic basis of the local upland and traditional Vietnamese rice varieties is desirably broad.

Based on phenotypic characteristics (data not shown) and information supplied by Gene Bank on the origin of the varieties, we hypothesize that traditional upland rice represents alternative genetic pools to those presented in the improved cultivars. The same observation was found for Cuban rice varieties (Alvarez *et al*., 2007). The hypothesis was also corroborated by cluster analysis based on genetic relationship estimates (Fig. 2). As expected, all the improved varieties grouped in the same genetic group (group I), confirming the close relationship. The cluster of some traditional upland varieties in group I suggested their close genetic relationship with improved varieties. The Gene Bank information also indicated the *indica* relationship among these varieties. Out of 16 accessions of Group I, four improved cultivars LT25, Q5, Khangdan and DT54 were clustered together in one subgroup, which cultivated in lowland conditions. The cultivar IR64 introduced from IRRI was placed alone in a subgroup. The varieties Cuomdang1, a traditional adapted to salinity condition was placed together with Tetep in one subgroup. The other six traditional varieties clustered in one subgroup, which originated either from Red river Delta or Northwest region (Fig. 2). This indicated the varieties clustered into one subgroup came from the same origin, representing the alternative genetic pools.

In the dendrogram assembled for the 41 rice accessions (Fig. 2), there was a relatively positive correlation between cluster groupings and geographic distance. For example, most of the rice accessions from the North Central and Northwest provinces of Vietnam were clustered into group Ⅱ comprised 25 varieties and consisted of three subgroups. The similarity coefficient of this group ranged from 0.29 and 0.76. The phenotypic evaluation on drought tolerance exhibited high level of tolerance to water deficit of these traditional upland varieties (data not shown).

There are few studies which have been performed in genetic diversity survey for Vietnamese rice germplasm (Fukuoka *et al*., 2006; Lang *et al*., 2009) using molecular markers (RAPD) and phenotypic profiles. Vietnamese upland varieties used in the present study have not been examined previously in term of genetic relatedness using molecular markers. The high genetic diversity was found among the Vietnamese traditional accessions, and evidences of the broad genetic bases can be used in our breeding program. Comparison between results of this study and previous similar works of different rice germplasms showed high level of genetic diversity for Vietnamese rice germplasm (Table 5). Based on phenotypic evaluation data provided by National Gene Bank, the traditional rice varieties showed resistance to different diseases and adapted to abiotic stress conditions. This germplasm can provide potential gene sources for breeding program. Our results indicated that it is essential to broaden the genetic base of the cultivated rice to reduce its vulnerability to diseases and insect resistance, to enhance its ability to stress conditions. Recent studies

showed that 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 germplasm and their performance under diverse agro- ecological conditions (Yu, 2003; Pfeiffer *et al*., 2005; Lafitte *et al*., 2006; Thomson, 2007; Herrera, 2008; Wong, 2009).

The correct identification of genetic diversity of varieties in the local germplasm is important for rice breeding programs, allowing selection of the desired rice accessions for crossing. Thus, the determination of the genetic distance among the accessions will be important to maximize their use in breeding program. Furthermore, the assessment of genetic diversity of rice germplasm present in the collection will help breeder to formulate crosses by choosing varieties with different genetic backgrounds and will assist in the development of mapping populations with high level of marker polymorphism (Alvarez *et al*., 2007; Jayamani *et al*., 2007).

In conclusion, this is the first study on characterization of the molecular diversity in traditional drought tolerant upland rice originated from Northern part of Vietnam. The phenotyping and SSR fingerprinting data showed high level of genetic diversity in this germplasm. The data will be useful to Vietnam rice breeders by improving the selection of parental lines for cross and enhancing plasticity or resistance of the cultivars.

ACKNOWLEDGEMENTS

This work was conducted at Agricultural Genetics Institute and financially supported by National Foundation for Science and Technology (Nafosted), Vietnam. We also acknowledge Plant Resource Center for kindly providing the rice materials from National Gene Bank, Hanoi, Vietnam. The authors of this paper would like to acknowledge the kind advice of Jeff Stein from Donald Danforth Plant Science Center, USA.

REFERENCES

Akagi, H., Y. Yokozeki, A. Inagaki and T. Fujimura 1997 Highly polymorphic microsatellites of rice consist of TA repeats, and a classification of closely related cultivars with these microsatellite loci. *Theor. Appl. Genet*., **94**: 61–67

Alvarez, A., J. L. Fuentes, V. Puldón, P. J. Gómez, L. Mora, M. C. Duque, G. Gallego and J. M. Tohme 2007 Genetic diversity analysis of Cuban traditional rice (*Oryza sativa* L.) varieties based on microsatellite markers. *Genet. Mol. Biol*., **30**(4): 1109–1117

Anderson J. A., G. A. Churchill, J. E. Autrique, S. D. Tanksley and M. E. Sorrells 1993 Optimizing parental selection for genetic linkage maps. *Genome*, **36**: 181–186

Botstein, D., R. L. White, M. Skolnick and R. W. Davis 1980 Construction of a genetic linkage map in man using restriction fragment polymorphisms. *Am. J. Hum. Genet*., **32**: 314–331

Bray, E. A., J. Bailey–Serres and E. Weretilnyk 2000 Responses to abiotic stresses. *In* W Gruissem, B Buchannan, R Jones, eds, Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp. 1158–1249

Chakravarthi, K. B. and R. Naravaneni 2006 SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L). *Afr. J. Biotech*., **5**(9): 684–688

Dice, L. R. 1945 Measures of the amount of ecologic association between species. *Ecology*, **26**: 297–302

Florence, C., Lasalita–Zapico, J. A. Namocatcat and J. L. Carino–Turner 2010 Genetic diversity analysis of traditional upland rice cultuvars in Kihan, Malapatan, Sarangani province, Philippines using morphometric markers. *Philipp. J. Sci*., **139**(2): 177–180

Fukuoka S., D. S. Tran, K. Ebana, N.T. Luu, T. Nagamine and K. Okuno 2006 Diversity in phenotypic profiles in landrace populations of Vietnamese rice: A case study of agronomic characters for conserving crop genetic diversity on farm. *Gen. Res. and Crop Evolution*, **53**(4): 753–761

Gao, L., B. A. Schaal, I. Jia and Y. Dong 2003 Assessment of population genetic structure in common wild rice *Oryza rufipogon* Griff. using microsatellite and allozyme markers. *Theor. Appl. Genet*., **106**: 1631–1638

Gao, L. Z. 2005 Microsatellite diversity and population genetic structure of an endangered wild rice, *Oryza officinalis* (Poaceae) from China. *Mol. Ecol*., **14**: 4287–4297

Garris, A. J., T. H. Tai, J. Coubur, S. Kresovich and S. McCouch 2005 Genetic structure and diversity in *Oryza sativa* L. *Genetics*, **169**: 1631–1638

Ghneim, T., D. P. Duque, I. Petez, G. Torrealba, A. J. Pieters, C. P. Martinez and J. M. Tohme 2008 Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. *Electron. J. Biotechnol*., **11**: 1–13

Giarrocco, L. E., M. A. Marassi and L. Salerno 2007 Assessment of the genetic diversity in Argentina rice cultivars with SSR markers. *Crop Sci*., **47**: 853–860

Herrera, T. G., D. P. Duque, I. P. Almeida, G. T. Núñez, A. J. Pieters, C. P. Martinez and J. M. Tohme 2008 Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. *Electron. J. Biotechnol*., **11**(5): 14

Jackson, M. T. and R. Juggan 1993 Sharing the diversity of rice to feed the world. *Diversity*, **9**: 22–25

Jayamani, P., S. Negrao, B. Martins, B. Macas and M. Oliveria 2007 Genetic relatedness of Portuguese rice accession from diverse origin as assessed by microsatellite marker. *Crop Sci*., **47**: 879–886

Jeung, J. U., H. G. Hwang, H. P. Moon and K. K. Jena 2005 Fingerprinting temperate *janponica* and tropical *indica* rice genotypes by comparative analysis of DNA markers. *Euphytica*, **146**(3): 239–251

Junjiam, N., M. C. Peter and J. Mackill 2002 Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Sci*., **42**: 601–607

Lafitte, H. R., Z. K. Li, C. H. M. Vijayakumar, Y. M. Gao, Y. Shi, J. L. Xu, B. Y. Fu, S. B. Yu, A. J. Ali, J. Domingo, R. Maghirang, R. Torres, D. Mackill 2006 Improvement of rice drought tolerance through backcross breeding: evaluation of donors and selection in drought nurseries. *Field Crop Res*., **97**: 77–86

Lang, N. T., P. T. B. Tu, N. C. Thanh, B. C. Buu and A. Ismail 2009 Genetic diversity of salt tolerance rice landraces in Vietnam. *Plant Breeding Crop Sci*., **1**(5): 230–243

Nagaraju, J., M. Kathirvel, K. R. Ramesh, E. A. Siddiq and E. H. Seyed 2002 Genetic analysis of traditional and evolved Basmati and non–Basmati rice varieties by using fluorescence–based ISSR–PCR and SSR markers. *PNAS*, **99**(9): 5836–5841

Nei, M. and W. H. Li 1979 Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci*. **76**: 5269–5273

Roussel, V., J. Koenig, M. Beckert and F. Balfourier 2004 Molecular biodiversity in French bread wheat accession related to temporal trends and breeding program. *Theor. Appl. Genet*., **108**: 920–930

Sneath, P. H. A. and R. R. Sokal 1973 *Numerical taxonomy: The principles and practice of numerical classification*. W. H. Free–man, San Francisco, CA.

Spada, A., R. Mantegazza, M. Biloni, E. Caporali and F. Sala 2004 Italian rice varieties: Historical data, molecular markers, and pedigree to reveal their genetic relationships. *Plant Breed*.,

123: 1005–1011

Tautz, D. 1989 Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res*., **17**: 6463–6471

Thomson, M. J., E. M. Septiningsih, F. Suwardjo, T. J. Santoso, T. S. Silitonga and S. R. McCouch 2007 Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. *Theor. Appl. Genet*., **114**: 559–568

Vos, P., R. Hogers, M. Bleeker and Reijans 1995 AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res*., **23**: 4407–4414

William, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey 1990 DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., **18**: 6531–6535

Wang H. M. Qi and A. J. Cutler 1993 A simple method of preparing plant samples for PCR. *Nucleic Acids Res*., **21**(17): 4153–4154

Wong, S. C., P. H. Yiu, S. T. W. Bong, H. H. Lee, P. N. P. Neoh and A. Rajan 2009 Analysis of Sarawak Bario Rice Diversity Using Microsatellite Markers. *Am. J. Agr. Biol. Sci*., **4**(4): 298–304

Xu, Y., H. Beachell and S. R. McCouch, 2004 Marker–based approach to broadening the genetic base of rice in the USA. *Crop Sci*., **44**: 1947–1959

Yongwen, Q. I., D. Zhang, H. Zhang, M. Wang, J. Sun, X. Wei, Z. Qiu, S. Tang, Y. Cao, X. Wang and Z. Li 2006 Genetic diversity of rice cultivars (*Oryza sativa* L.) in China and the temporal trends in recent fifty years. *Chinese Sci. Bull*., **51**: 681–688

Yu, S. B., W. J. Xu, C. H. M. Vijayakumar, J. Ali, B. Y. Fu, J. L. Xu, Y. Z. Jiang, R. Marghirang, J. Domingo, C. Aquino, S. S. Virmani and Z. K. Li 2003 Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. *Theor. Appl. Genet*., **108**: 131–140