ISSN 2300-9675

International Letters of Natural Sciences

SciPress

Volume 67, 2018

International Letters of Natural Sciences

Volume 67

SciPress

2018

International Letters of Natural Sciences

ISSN: 2300-9675

Journal is published and owned by SciPress Ltd

About:
International Letters of Natural Sciences (ILNS) is an open-access peer-reviewed international journal, which is focused on the publication of advanced theoretical and practical achievements in the fields of the agricultural science, zoology, plant science, biodiversity and evolution, environment and ecology, forestry, microbiology and biotechnology, geology and aquatic research. The ILNS is indexed in Emerging Sources Citation Index (Web of Science).

Journal Scope: Agricultural science, zoology, plant science, biodiversity and evolution, environment and ecology, forestry, microbiology and biotechnology, geology, aquatic research

Full text available online at http://www.scipress.com.

If you have any questions please contact us at ilns@scipress.com

SciPress Ltd
Seestrasse 24c
CH-8806 Bach
Switzerland
http://www.scipress.com

Table of Contents

International Letters of Natural Sciences
ISSN: 2300-9675, Vol. 67, pp 24-34
doi:10.18052/www.scipress.com/ILNS.67.24

Submitted: 2017-08-18
Revised: 2018-01-04
Accepted: 2018-01-31

Evaluation of Genetic Diversity of Local-Colored Rice Landraces Using SSR Markers

Hoang Thi Hue[1], La Tuan Nghia[1], Hoang Tuyet Minh[2], La Hoang Anh[3], Le Thi Thu Trang[1], Tran Dang Khanh[3*]

[1]Plant Resources Center (PRC), Hoaiduc district, Hanoi, Vietnam,

[2]Vietnam Seed Association (VSA), Hanoi, Vietnam,

[3]Agricultural Genetics Institute, Hanoi, Vietnam.

t.d.khanh@scipress.com

Keywords: Local-colored rice, genetic diversity, rice landraces, SSR marker.

Abstract. Analysis of genetic diversity of 90 Vietnamese local-colored rice accessions was carried out using 40 SSR markers. The numbers of polymorphic alleles ranged from 3 to 12 alleles per locus and average of 7.1 alleles per locus. The similarity coefficients of the rice landraces fluctuated from 0.76 to 0.93; at a genetic correlation level of 0.78. Ninety accessions of rice landraces were divided into five groups based on analysis of genetic relationships. The results have indicated that 11 markers included M250, RM302, RM10926, RM208, RM227, RM17231, RM23251, RM5647, RM1376, RM339 and RM228 which gave the unique allele. These markers can be used effectively for genetic diversity of colored rice and provided a specific database and useful materials for landraces identification, local germplasm conservation for further colored rice improvement on rice quality via rice breeding programs in Vietnam.

Introduction

Colored rice (*Oryza sativa* L.) is a rich sources of fat-soluble bioactive components which has high concentrations of protein, total essential amino acids, antioxidant compounds, vitamin B1 and other minerals to compare with the common rice [1-3]. Vietnam is known to be the center of rice diversity germplasm. Rice landraces are much diversified and played a key role in rice breeding for rice quality improvement in this country [4]. Among rice germplasm, local-colored rice landraces are abundant in this country, therefore, it should be exploited to develop commercial rice of high quality and rich medicinal value via rice breeding program.

The recent advances in terms of molecular biology such as PCR, DNA sequencing and data analysis technologies have significantly contributed to analysis and evaluation of rice genome. Among these mentioned technologies, the PCR method has emerged as a useful and popular technique that can be used for analysis of rice genome, particularly in the estimation of rice genetic diversity [5]. SSR markers are able to estimate genetic diversity between cultivars e.g. between parents of genepool or between plants extracted from a population or between populations. Microsatellites are more powerful for the identification of within cultivar variation.

Based on the characterization and evaluation of rice genetic diversity, plant breeders have been perceived the necessary information to identify initial materials for rice breeding via mutation, hybridization to produce landraces with high yield and good quality as well as resistance to biotic and abiotic stresses. Parallel to breeding, information gained from analysis of rice genetic diversity are also very useful for sustainable conservation of plant genetic resources for food and sustainable agriculture.

Recent research on the genetic diversity of colored rice mostly focused on anthocyanin content [6], Fe and Ze content [7, 8], or genetic diversity using both SSR markers and morphological characters [9]. The objective of this study was to evaluate the genetic diversity of 90 local - colored rice landraces in Vietnam using SSR markers. This study will provide useful information on genetic diversity among the colored rice landraces for further rice breeding.

Materials and Methods

Plant Materials

The plant materials included 90 diverse rice accessions were kindly provided by the Plant Resources Center. The rice landraces were collected from some different districts and provinces in the Central and the North of Vietnam as shown in Table 1. In order to evaluate the genetic diversity of rice landraces, 40 SSR primers were selected and used as listed in Table 2. These primers were selected to cover the rice genome with a representation for the 12 chromosome and based on the published paper [10].

Methods

- *DNA extraction*:

Total genomic DNA extraction from leaves of three weeks old seedlings were carried out following the CTAB method [11].

- *PCR assay*:

Polymerase chain reaction was implemented in the Veriti 96 well Thermal cycler. The total reaction solution was 20 µl including 2 µl PCR buffer 10x; 1.6 µl dNTP 2.5mM; 1.4 µl primer (forward and reverse primer) 25ng/µl; 0,1 µl green Taq (5U/ µl) and 5 µl DNA (5ng/µl). A programmable thermal controller set for 35 cycles in which each cycle is for 1 min at 94ºC, 1 min at 55ºC, and 1 min at 72ºC. PCR products were electrophoresed on 8% polyacrylamide gel, and analyzed under the UV trans-illuminator for DNA detection.

Band scoring: The gels were scored for computer analysis on the basis of the presence and absence of the amplified products. If a product was present in a genotype, it was designated as '1' and if absent, it was designated as '0'.

- *Data analysis*

The genetic similarity was analyzed by using Jaccard similarity coefficient in NTSYSpc 2.11X software using the method of Rohlf [12]. The genetic distance was calculated using the method of Nei [13]. Polymorphic Information Content (PIC) was calculated applying the methodology of Mohammadi [14].

Table 1. Accession number and origin of rice genotypes in current study

No	Name of rice landrace	Accession number	Origin	No	Name of rice landrace	Accession number	Origin
1	Beo cu	GBVN001912	Trang Dinh, Lang Son	46	Khau ho he	GBVN008678	Tuong Duong, Nghe An
2	Pe le chua	GBVN002013	Than Uyen, Lao Cai	47	Khau cam co	GBVN008693	Tuong Duong, Nghe An
3	Plei ha	GBVN002019	Than Uyen, Lao Cai	48	Ple lau sang	GBVN008713	Bac Yen, Son La
4	Nep cam Nuong	GBVN002022	Than Uyen, Lao Cai	49	Ple sang loi	GBVN008717	Bac Yen, Son La
5	Bao dam	GBVN002024	Than Uyen, Lao Cai	50	Khau cam ky	GBVN008755	Phu Yen, Son La
6	Nep cam den	GBVN002056	Bao Thang, Lao Cai	51	Lo cam	GBVN008770	Phu Yen, Sơn La
7	Khau pet Lanh	GBVN002093	Yen Chau, Son La	52	Plao co cam	GBVN009446	Da Bac, Hoa Binh
8	Ne diem	GBVN002102	Yen Chau, Son La	53	Khau lech	GBVN009914	Song Ma, Son La
9	Khau pe	GBVN002468	Mai Son, Son La	54	Ngo hieng	GBVN012319	Ky Son, Nghe An
10	Khau lech 1	GBVN002509	Dien Bien, Lai Chau	55	Khau lech	GBVN012352	Dien Bien
11	Khau lech 2, dang 2	GBVN002515	Dien Bien, Lai Chau	56	Khau tang san cha	GBVN012565	Da Bac, Hoa Binh
12	Na ple la	GBVN003562	Mu Cang Chai, Yen Bai	57	Khau cao lan danh	GBVN012593	Da Bac, Hoa Binh
13	Khau lo	GBVN003918	Quynh Nhai, Son La	58	Blau ca dayk	GBVN012983	Bac Yen, Son La
14	Pe lanh	GBVN003921	Thuan Chau, Son La	59	Ble chong la	GBVN013001	Bac Yen, Son La

15	Khau lec	GBVN003926	Thuan Chau, Son La	60	Ble trong la	GBVN013005	Bac Yen, Son La
16	Khau lec	GBVN003929	Dien Bien, Lai Chau	61	Ble mua chua	GBVN013010	Bac Yen, Son La
17	Ble sa	GBVN003969	Tuan Giao, Lai Chau	62	Ple sang	GBVN013282	Muong La, Son La
18	Ta cu dang 2	GBVN004003	Bat Sat, Lao Cai	63	Khau lech	GBVN013293	Muong La, Son La
19	Ble chua te dang 1	GBVN004019	Bac Ha, Lao Cai	64	Beo cu	GBVN013321	Quynh Nhai, Son La
20	Ble chua te dang 2	GBVN004020	Bac Ha, Lao Cai	65	Ple blau sang	GBVN013354	Quynh Nhai, Son La
21	Khau xien Pan	GBVN004083	Trang Dinh, Lang Son	66	Bieu cu	GBVN013392	Tua Chua, Dien Bien
22	Mo ta dang 2	GBVN004153	Bach Thong, Bac Thai	67	Ble blau xa	GBVN014210	Quynh Nhai, Son La
23	Nep cam co Rau	GBVN004199	Na Hang, Tuyen Quang	68	Khau ma cha	GBVN014220	Quynh Nhai, Son La
24	Ble to	GBVN004688	Tuan Giao, Lai Chau	69	Ple la	GBVN014259	Mai Son, Son la
25	Te mun	GBVN004732	Ba Thuoc, Thanh Hoa	70	Khau pe lanh	GBVN014269	Mai Son, Son la
26	Khau gioi Ho He	GBVN005017	Tuong Duong, Nghe An	71	Ple la gia	GBVN014271	Mai Son, Son la
27	Po le po lau Xa	GBVN005034	Ky Son, Nghe An	72	Blau đu	GBVN014276	Mai Son, Son la
28	Lua bat	GBVN005078	Thach Ha, Ha Tinh	73	Ble sang	GBVN014283	Mai Son, Son la
29	Nep than	GBVN005101	Huong Ha, Quang Tri	74	Plau xa	GBVN014360	Than Uyen, Lai Chau
30	Cu nho cu San	GBVN005102	Huong Hoa, Quang Tri	75	Ple ma mu	GBVN014413	Tram Tau, Yen Bai
31	Dep cu hom	GBVN005175	A Luoi, Thua thien Hue	76	Ple ma mu	GBVN014414	Tram Tau, Yen Bai
32	Pau cam	GBVN006402	Binh Gia, Lang Son	77	Ple chua	GBVN014418	Tram Tau, Yen Bai
33	Nep be lanh	GBVN007146	Ba Thuoc, Thanh Hoa	78	Ple ma mu	GBVN014419	Tram Tau, Yen Bai
34	Pe lanh	GBVN007151	Quan Hoa, Thanh Hoa	79	Ple chua	GBVN014471	Tram Tau, Yen Bai
35	Vong đo doi	GBVN007209	Ba Thuoc, Thanh Hoa	80	Ple mang chinh	GBVN014482	Tram Tau, Yen Bai
36	A tut dang 2	GBVN007282	Huong Hoa,Quang Tri	81	Plau song	GBVN014617	Mu Cang Chai, Yen Bai
37	Khau doi dang 2	GBVN007715	Muong Lat, Thanh Hoa	82	Plau sang	GBVN014644	Mu Cang Chai, Yen Bai
38	Khau lech Lon	GBVN008212	Mai Son, Son La	83	Ble blau sang	GBVN014650	Mu Cang Chai, Yen Bai
39	Khau cam ky	GBVN008231	Moc Chau, Son La	84	Ple ban cang	GBVN014654	Mu Cang Chai, Yen Bai

Table 2. List of 40 SSR primers used in this study

No.	Primer	Chr	Annealing temp (oC)	PCR products (bp)	Forward Sequence	Reverse Sequence
1	RM 250	2	55	153	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG
2	RM 270	12	55	108	GGCCGTTGGTTCTAAAATC	TGCGCAGTATCATCGGCGAG
3	RM 302	1	55	156	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
4	RM 3825	1	55	147	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG
5	RM 201	9	55	158	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
6	RM 1359	4	55	170	AACGAATTCTATTTTGCGTC	TTCTTCTCATTTCAATTCGC
7	RM 23251	8	55	259	TCCGATACTCCCATAGTTAGACC	ATGTGGGTTGGCTATAGTCTAGG
8	RM 27027	11	55	182	GTTGTCGCTGCACTCCACAATGG	GATCCGGCCTCGTGATTACCG
9	RM 6836	6	55	240	TGTTGCATATGGTGCTATTTGA	GATACGGCTTCTAGGCCAAA
10	RM 6314	4	50	169	GATTCGTGTCGGTTGTCAAG	GGTTCAGGGACGAATTTCAG
11	RM 227	3	55	106	ACCTTTCGTCATAAAGACGAG	GATTGGAGAGAAAAGAAGCC
12	RM 21969	7	55	99	AGTTTCCTCCTCTTCTCCTTTAGTGC	ACACAGAACTACAGAAGCACTCTGC
13	RM 20589	6	55	263	CATGTATTTGTGTGCACGTACCG	ACCTTTCTTGGGCCTTTCTTGG
14	RM 20590	6	55	243	TTCGATGAGCACCTTTCCTTGTCC	GCCTCGCCGATTCACTTATGC
15	RM 347	3	55	207	CACCTCAAACTTTTAACCGCAC	TCCGGCAAGGGATACGGCGG
16	RM 122	5	55	277	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC

17	RM 144	11	55	237	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG
18	RM 224	11	55	157	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
19	RM 611	5	55	213	CAACAAGATGGCCTCTTACC	TACAAACAAACAGCTTGTGC
20	RM 27274	11	55	389	GGTTCAATGCTTACCTGCGTAGC	AGCAAAGGTGCACAACAATGAGC
21	RM10926	1	55	147	CCCACTCTTGCAGCAGTTCTCG	GTCAGACTCGTCGCCACTTCACC
22	RM 20177	7	55	187	GCTGTCTGCCTAGCATTATTGG	AACACTATGCTTGAGACCACTTGC
23	RM 138	2	55	233	AGCGCAACAACCAATCCATCCG	AAGAAGCTGCCTTTGACGCTATGG
24	RM 14226	2	55	284	AAACCTCCACGACGATGACG	GGGTTACATCACAATCATCCTTCC
25	RM 1233	11	55	175	TTCGTTTTCCTTGGTTAGTG	ATTGGCTCCTGAAGAAGG
26	RM 208	2	58	173	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC
27	RM 247	12	55	131	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG
28	RM 541	6	55	158	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG
29	RM 5536	1	55	150	GAATCCTGCAGGGATGAAAC	ATACTAATCCCGTCATCCGG
30	RM 5948	5	55	141	AGTTCCCCAACTTGTTCCAC	GCCTGCGCTTAGCTTAAGTG
31	RM 3866	4	55	161	AGTTGGTCATCTACCAGAGC	GATCTTCTTGCCTCAGAAAG
32	RM1376	8	55	199	CATGTGTGATGACTGACAGG	GGTGCTGTGATGATTCTTTC
33	RM 5647	8	55	119	ACTCCGACTGCAGTTTTTGC	AACTTGGTCGTGGACAGTGC
34	RM 5473	4	55	105	ACACGGAGATAAGACACGAG	CGAGATTAACGTCGTCCTC
35	RM 5364	12	50	148	GTATTACGCTCGATAGCGGC	GTATCCTTTCTCGCAATCGC
36	RM 316	9	55	192	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC
37	RM 339	8	55	148	GTAATCGATGCTGTGGGAAG	GAGTCATGTGATAGCCGATATG
38	RM 228	10	55	154	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC
39	RM346	7	55	175	CGAGAGAGCCCATAACTACG	ACAAGACGACGAGGAGGGAC
40	RM 17231	4	55	95	AATGGCAACAACGAGACTAGG	GTCTCTGAATCCTAACCCTAACC

AT: Annealing temperature; bp: PCR product (bp)

Results and Discussion

Variation of rice gain color among 90 accessions

From database of Plant Resources Center, Vietnam national gene bank (http://en.prc.org.vn/), all of 90 rice gain color accessions were selected with description based on Biodiversity International standard (Table 3). Of those the dominant number of accessions was variable purple and the smallest number (5 accessions) was purple color. Thus, this rice gain color population reflected local-colored rice landraces in North central of Vietnam.

Table 3. Number of accession with different seed coat color

No	Seed coat color	Number of accession	Percentage (%)
1	Brown	13	14.4
2	Red	30	33.3
3	Variable purple	42	46.7
4	Purple	5	5.6

DNA extraction

The DNA concentration ranged from 150-300 ng/µl as shown in Fig. 1. A total DNA of 90 colored rice landraces were extracted following to the method of Zheng et al. [11]. PCR products were electrophoresed in the 1% agarose gel with 50ng/µl of Lamda DNA; and DNA concentration was measured by the spectrum nanodrop machine at the wavelength OD_{260}/OD_{280}.

The DNA products were shown to be high yield, good quality and concentration at 150-300 ng/µl which are good enough to implement further PCR assay (Fig. 1).

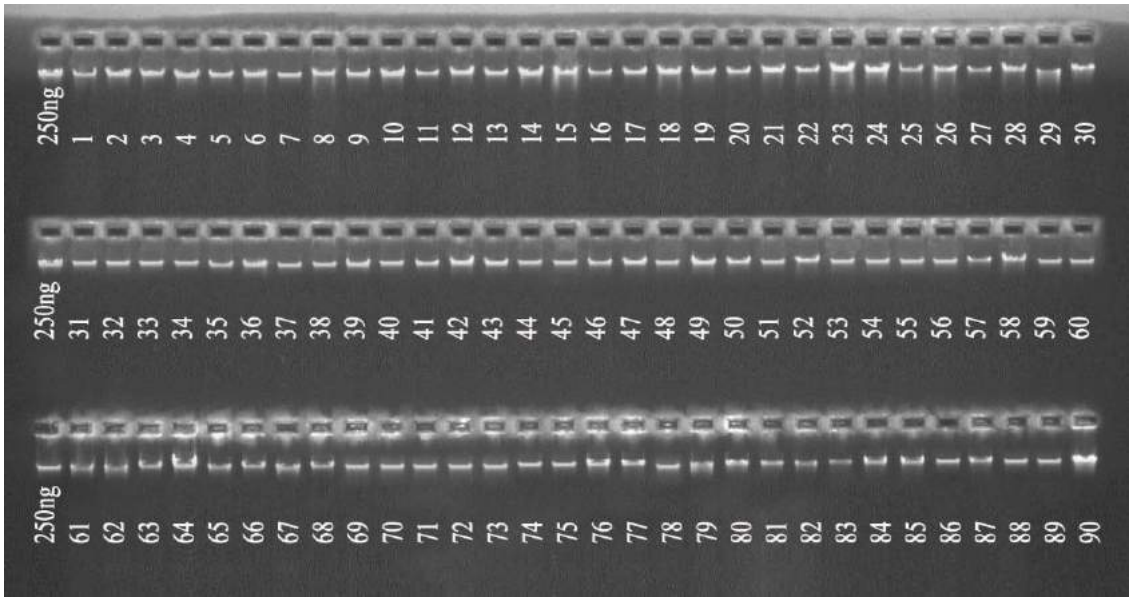

Figure 1. Total DNA in 1% agarose gel

Analysis of genetic diversity of colored rice landraces using SSR markers

The results of the PCR products were yielded DNA bands ranging 80-425 bp. At each locus, the size of alleles varied from 7 bp (RM 144) to 125bp (RM20590) (Fig. 2).

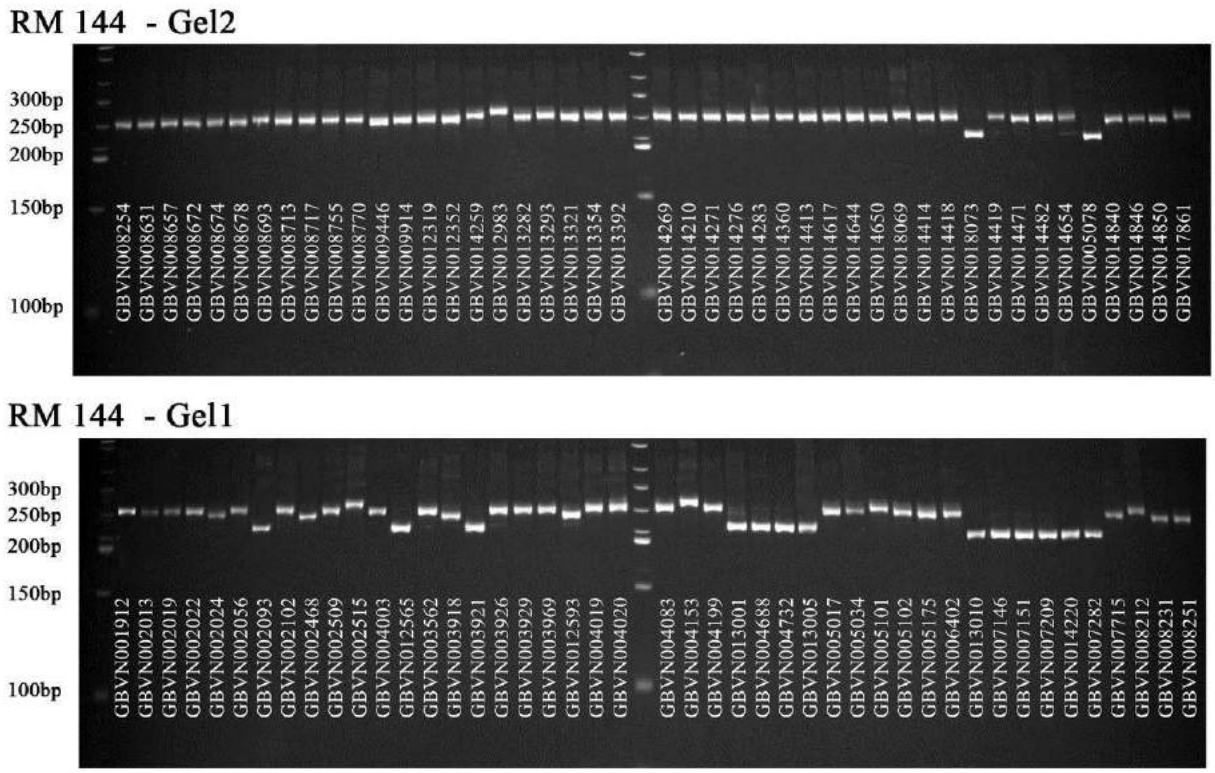

Figure 2. PCR products of colored rice landraces amplified by using the RM144 primer

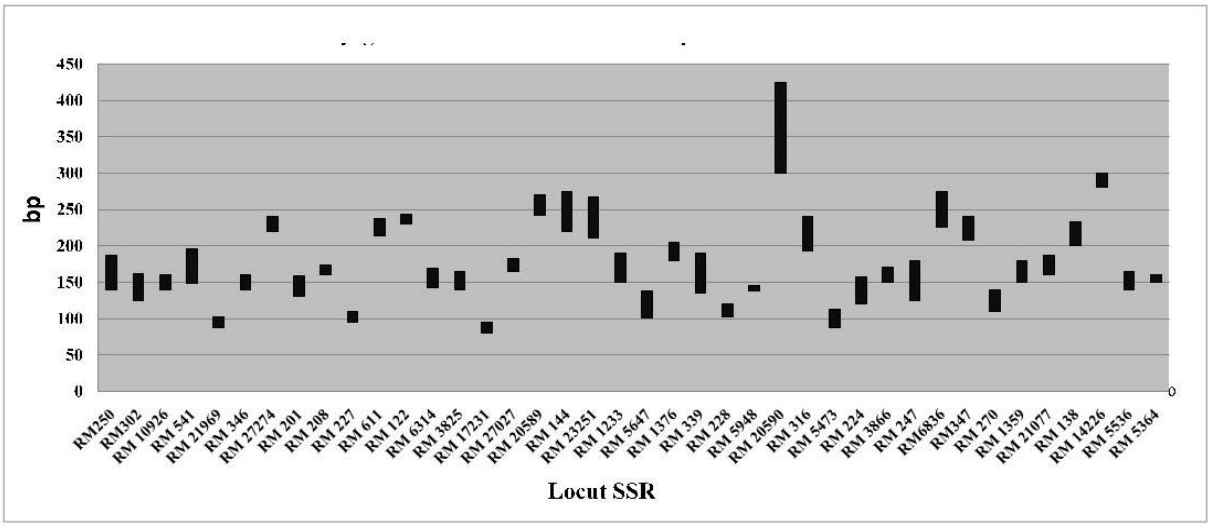

Figure 3. The diagram showed variation of DNA band sizes at each locus

In total, 184 alleles were scored at 40 loci, the number of allele per locus ranged from 2 to 12, and average of 5.7 alleles per locus. The number of polymorphic alleles per locus were from 3 (RM5364) to 12 (RM5647), and average of 7.1 alleles per locus. There were 5 marker pairs recorded for 4 alleles (RM27274, RM201, RM5948, RM347, RM138); 6 primer pairs recorded for 5 alleles (RM346, RM208, RM17231,RM3866, RM280, RM14226); 4 marker pairs recorded for 10 alleles (RM541 RM6314, RM23251, RM339) and only 2 marker pairs RM250, RM228 recorded for 11 alleles (Fig. 3).

Table 4 showed that 11 markers were found giving unique allele, including RM250, RM302, RM10926, RM208, RM227, RM17231, RM23251, RM5647, RM1376, RM339 and RM228; these unique alleles can be used to identify the colored rice landraces collection. For example, Khau cam xang variety (GBVN018073), originated from Con Cuong, Nghe An, can be identified using 3 markers RM10926, RM208 and RM5647. Similar to Plemamu landrace (GBVN014419) also recognized by using markers RM227, RM23251 and RM339; and Khau phach variety (GBVN008251), originated from Moc Chau, Son La, can be also identified by using two markers RM5647 and RM228.

Table 4. Polymorphic data using SSR markers for local colored rice landraces

No	Locus	Chr.	No. observed alleles	Minimum allele size (bp)	Maximum allele size (bp)	No. unique allele	Acc. Number of landraces having unique allele	PIC value
1	RM250	2	11	140	187	1	GBVN00201 3 (140bp)	0.84
2	RM302	1	8	125	162	1	GBVN00178 61 (125bp)	0.72
3	RM 10926	1	9	140	160	2	GBVN00823 1 (140bp) GBVN01807 3 (160bp)	0.78
4	RM 541	6	10	148	195	-	-	0.80
5	RM 21969	7	9	88	102	-	-	0.85
6	RM 346	7	5	140	160	-	-	0.68
7	RM 27274	11	4	220	240	-	-	0.61
8	RM 201	9	4	130	158	-	-	0.60
9	RM 208	2	5	160	173	1	GBVN01807 3 (173bp)	0.41
10	RM 227	3	6	95	110	1	GBVN014419 (110bp)	0.49

11	RM 611	5	9	213	238	-	-	0.81
12	RM 122	5	8	230	243	-	-	0.84
13	RM 6314	4	10	142	169	-	-	0.87
14	RM 3825	1	9	140	165	-	-	0.84
15	RM 17231	4	5	80	95	1	GBVN00728 2 (80bp)	0.73
16	RM 27027	11	8	165	182	-	-	0.81
17	RM 20589	6	6	242	270	-	-	0.73
18	RM 144	11	6	220	275	-	-	0.76
19	RM 23251	8	10	210	267	1	GBVN00419 9 (267bp)	0.83
20	RM 1233	11	9	150	190	-	-	0.76
21	RM 5647	8	12	98	138	2	GBVN00825 1(98bp), GBVN01807 3 (102bp)	0.89
22	RM 1376	8	7	180	205	1	GBVN00944 6 (205bp)	0.76
23	RM 339	8	10	135	190	2	GBVN00501 7(138bp), GBVN01441 9 (135bp)	0.79
24	RM 228	10	11	102	122	1	GBVN00825 1 (118bp)	0.82
25	RM 5948	5	4	138	145	-	-	0.49
26	RM 20590	6	8	300	425	-	-	0.83
27	RM 316	9	6	192	240	-	-	0.78
28	RM 5473	4	8	87	112	-	-	0.84
29	RM 224	11	7	120	157	-	-	0.68
30	RM 3866	4	5	150	170	-	-	0.72
31	RM 247	12	6	125	180	-	-	0.7
32	RM6836	6	7	225	275	-	-	0.81
33	RM347	3	4	207	240	-	-	0.48
34	RM 270	12	5	110	140	-	-	0.77
35	RM 1359	4	7	150	180	-	-	0.82
36	RM 21077	7	6	160	187	-	-	0.82
37	RM 138	2	4	200	233	-	-	0.75
38	RM 14226	2	5	280	300	-	-	0.76
39	RM 5536	1	8	140	165	-	-	0.85
40	RM 5364	12	3	150	160	-	-	0.66
	Minimum		**3**	**80**	**95**	**1**		**0.41**
	Average		**7.1**					**0.74**
	Maximum		**12**	**300**	**425**	**2**		**0.89**
	Total		**284**			**14**		

After examining the SSR loci, the PIC values ranged from 0.41 (RM208) to 0.89 (RM5647), the average PIC value was 0.74. This result was lower than PIC value reported by Gowda et al. [7] where an average PIC was 0.84 in the analysis of the 45 Indian cultivated landraces. But this study's PIC value was higher than the studies of Freeg et al. [15] with an average PIC of 0.52 or higher than the results published by Soe et al. [16] with an average number of alleles of 6.83 and the average PIC reached 0.55.

Analysis of genetic relationships among the local-colored rice landraces.

Cluster analysis showed significant genetic variation among the landrace rice varieties studied, with genetic distance ranging from 0.76 to 0.93 (Fig. 4). With a genetic distance of 0.78, the cluster revealed 5 major groups.

Group I consisted of 59 landraces, in which the majority of collection's seed coat color as variable purple (33 out of 59) and the Khau tang san cha (GBVN012565) and Naple la (GBVN003562) landraces were separated from the others; at the genetic correlation level of ~0.73, the rest of group I was divided into three subgroups:

Sub-group I-a consisted of 7 landraces: GBVN001912, GBVN002013, GBVN002019, GBVN002020, GBVN002056, GBVN002102, GBVN002509, in which 2 rice landraces as GBVN001912 and GBVN002013 having the highest similarity coefficient 0.88.

Sub-group I-b had 50 landraces, divided into 2 subgroups as I-b.1 and I-b.2. The sub-group I-b.1 consisted of 15 landraces in which 8 accessions collected from Northwest (GBVN002515, GBVN003926, GBVN003929, GBVN003969, GBVN004019, GBVN004020, GBVN004199, GBVN01454), 02 accessions from Northeast (GBVN004082, GBVN014654) and 5 accessions from North Central (GBVN005017, GBVN005034, GBVN005101,GBVN005102, GBVN005175). The similarity coefficient among these landraces ranged from 0.81 to 0.90

Sub-group I-b.2 consisted of 35 landraces with similarity coefficient ranging from 0.84 to 0.93. The pair of landraces (GBVN008674) and Khau ho he (GBVN008678) had the greatest similarity coefficient of 0.93and interestingly, these two samples originated from North Central. Sub-group I-c had 2 landraces GBVN012565 andGBVN003562 with similarity coefficient of 0.83, both of them having red seed coat.

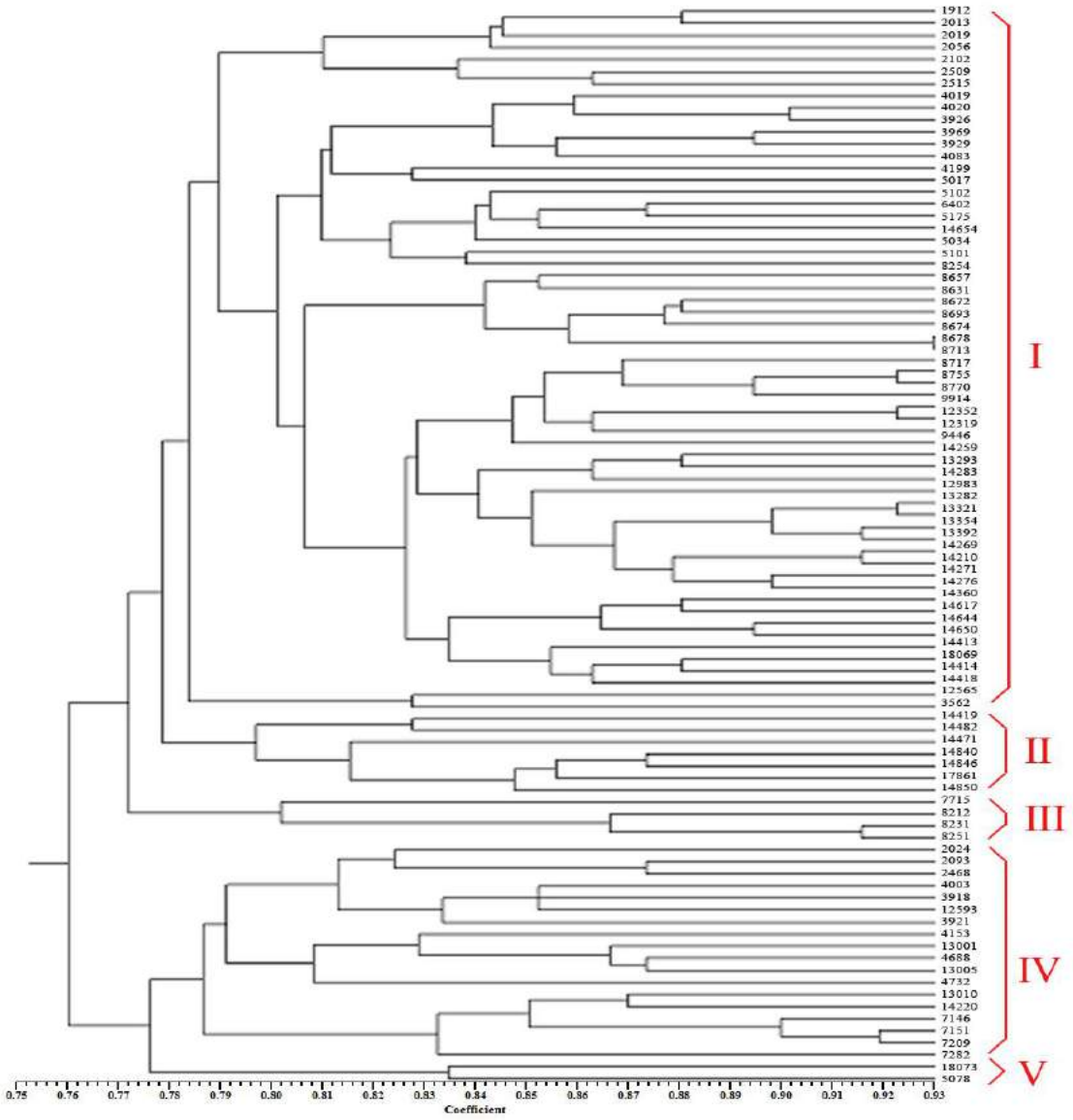

Figure 4. Dendrogram of genetic relationship among 90 colored rice genotypes based on 40 SSR markers

Group II had 7 landraces GBVN014419, GBVN 014482, GBVN014471, GBVN014840, GBVN014846 and GBVN014850 obtained from Northwest and only GBVN017861 collected from North Central.The similarity coefficient of this group was from 0.80 to 0.88.

Group III: consisted of 4 landraces GBVN007715, GBVN008212, GBVN008231 and GBVN008251 with similarity coefficient ranging from 0.80 to 0.92. In this group, most of the genotypes originated from Son La province, only the Khau doi dang 2 (GBVN007715) originated from Thanh Hoa province.

Group IV: 18 landraces and divided into three sub-groups:

Group IV-a: comprising of 7 landraces GBVN002024, GBVN002093, GBVN002468, GBVN004003, GBVN003918, GBVN012593 and GBVN003921; all of these were collected from Northwest, only accession GBVN004003 had seed coat brown and the remaining were red seed coat. The similarity coefficient among this group ranged from 0.82 to 0.87.

Subgroup IV-b: contained 03 landraces: GBVN004153, GBVN013001, GBVN004688 with brown seed coat and only GBVN013005 was red seed coat. The similarity coefficient among this group ranged from 0.81 to 0.87.

Sub-group IV-c: consisted of 6 genotypes with red seed coat (GBVN013010, GBVN014220, GBVN007146, GBVN007151 and GBVN007282) and similarity coefficient of 0.83 to 0.92. Regarding to origin, this sub-group had 4 varieties collected from Thanh Hoa province (Central of Vietnam) and 2 varieties obtained from Son La provice (Northwest).

Group V had two landraces Khau cam xang (GBVN018073 and GBVN005078) derived from North Central of Vietnam.with the similarity coefficient of 0.83.

Conclusions

The genetic diversity of 90 local-colored rice accessions in Vietnam were evaluated using 40 SSR markers which were distributed through the 12 rice chromosomes. The results indicated that the numbers of polymorphic alleles ranged from 3 to 12 alleles per locus and average of 7.1 alleles per locus; 11 markers such as RM250, RM302, RM10926, RM208, RM227, RM17231, RM23251, RM5647, RM1376, RM339 and RM228 gave the unique allele for 10 colored rice landraces. Polymorphic Information Content (PIC) at analyzed locus was high with an average of 0.74. The similarity coefficients of the 90 landraces studied ranged from 0.76 to 0.93; at a level of 0.78; 90 colored rice landraces were divided into five groups. The majority of varieties with variable purple were in group I, accounting for 78.9%; interestingly, cluster IV and V consisted of only varieties with brown and red seed coat color. The results of this study has found 11 markers that can be used effectively for genetic diversity of colored rice and also provide useful materials and information for genetic resources conservation as well as breeding of colored rice in Vietnam.

References

[1] R.S. Chaudhary, Speciality rices of the world: Effect of WTO and IPR on its production trend and marketing, Food Agric. Environ. 1 (2003) 34-41.

[2] M. Suzuki et al., Comparison of mineral contents in 8 cultivars of pigmented brown rice, J. Jpn. Soc. Food Sci. Technol. 51 (2004) 424-427.

[3] H.Y. Yoshida, Y. Tomiyama, Y. Mizushina, Lipid components, fatty acids and triacylglycerol molecular species of black and red rice, Food. Chem. 123(2) (2010) 210-215.

[4] L.H. Anh et al., Effect of salt on growth of rice landraces in Vietnam, Int. Let. Nat. Sci. 59 (2016) 72-81.

[5] V.C. Lapitan et al., Assessment of genetic diversity of Philippine r ice cultivars carrying good quality traits using SSR markers, Breeding Sci. 57(4) (2007) 263-270.

[6] K.T. Le, Y. Yang, Morphological characterization and photochemical analysis of pigmented rice cultivars from diverse regions, Breeding, Genetic and Physiology. (2005) 77-86.

[7] S.J.M Gowda et al., Morpho-agronomic and simple sequence repeat-based diversity in colored rice (*Oryza sativa* L.) germplasm from peninsular India, Genetic Resources and Crop Evolution. 59(2) (2012) 179-189.

[8] Brara et al., Correlation of molecular marker allele size with physio-morphological and micronutrient (Zn, Fe) traits among rice genotypes, Int. J. Curr. Sci. 15 (2015) 42-50.

[9] F. Ahmad et al., Genetic divergence and heritability of 42 coloured upland rice genotypes (*Oryza sativa*) as revealed by microsatellites marker and agro-morphological traits, PloS ONE. 10(9) (2015) e0138246.

[10] H.T. Hue et al., Evaluation of genetic diversity, yield and grain quality of local rice (*Oryza sativa* L.) accessions collected from the North of Vietnam, Annual scientific conference at Plant Resources Center, 2015.

[11] K. Zheng, Rapid DNA isolation for marker assisted selection in rice breeding, Rice Genet. Newsl. 12 (1995) 255-258.

[12] F.J. Rohlf, NTSYS-pc: Numerical taxonomy and multivariate analysis System, Exeter Publishing Ltd, ver. 2.1, 2002.

[13] M. Nei, Analysis of gene diversity in subdivided populations, Proceedings of the National Academy of Sciences. 70(12) (1973) 3321-3323.

[14] S.A. Mohammadi, B.M. Prasanna, Analysis of genetic diversity in crop plants salient statistical tools and considerations, Crop Sci. 43(4) (2003) 1235-1248.

[15] H.A. Freeg et al., Genetic diversity among some rice genotypes with different drought tolerance based on SSR Markers, Cercetari Agronomice in Moldova. 49(3) (2016) 39-50.

[16] I. Soe et al., Genetic diversity of rice *(Oryza sativa*) germplasm from six countries using simple sequence repeat markers, African Journal of Biotechnology. 15(37) (2016) 2038-2044.

Keyword Index

Author Index