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Genetic Diversity Analysis of Hybrid Rice Parental Lines and Genetic Purity Assessment of Hybrid Seeds of China

Haiya Cai[1,†], Yuxia Lu[1,2,†], Gang Liu[1], Shuo Zhang[1], Haitao Jia[1], Aiqing You[1] & Chunhai Jiao[1]

[1] Food Crops Institute, Hubei Academy of Agricultural Sciences, Wuhan, China

[2] College of Life Science, Yangtze University, Jingzhou, China

Correspondence: Chunhai Jiao, Food Crops Institute, Hubei Academy of Agricultural Sciences, Wuhan, 430064, China. Tel: 86-1398-6152-279. E-mail: jiaoch@hotmail.com

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† *These authors contributed equally to this work.*

Abstract

Thirty-five pairs of SSR primers were used for genetic diversity analysis and DNA fingerprinting of 31 hybrid rice core parental lines developed in central- and southern-China using one *japonica* rice line and three inbred rice lines as the check varieties. The average number of alleles (Na) per SSR locus was 4.02, with a range of two to eight, the effective number of alleles (Ne) was 83.16 with a mean of 2.38, ranging from 1.19 to 4.66. The polymorphic information content (PIC) ranged from 0.16 to 0.79, with an average number of 0.52. The results of the cluster analysis indicated that the check varieties viz., one *japonica* rice and three inbred rice, were clustered into two groups with similarity coefficients of 0.62 and 0.71 respectively indicating their relatedness. Thirty-one hybrid rice parental lines were clustered into 6 groups according to their different types, pedigrees and regions of development with similarity coefficients of approximately 0.76. The highest genetic similarity coefficient (0.94) was observed between Y58S and C815S, and the lowest (0.63) was observed between Quan9311A and Peiai64S. The purity of one hybrid rice cultivar was tested using characteristic marker and the field test, and it was demonstrated that the purities obtained using the two methods were similar. This research will be helpful for rice breeding, new cultivar registration and seed production.

Keywords: SSR marker, hybrid rice, DNA fingerprinting, genetic diversity

1. Introduction

Rice is the consumed staple food by more than half of the people in the world (Zhang et al., 2007). Approximately 70 percent in China is dependent on rice, that necessitated more attention to its varietal improvement. The development of new rice varieties in China has been continuously increased in recent years. For example, 565 varieties were developed in 2008, and 943 varieties were developed in 2018, among which *indica* rice accounted for 77 percent, and hybrid rice accounted for 72 percent (http://www.ricedata.cn/variety/index.htm). Due to rapid increase in quantity of developed hybrids, a better method for variety identification and genetic diversity analysis is required.

Four basic requirements should be met in crop variety identification: stability of the environment, ability to identify existing variation among varieties, minimum variation within varieties and reliability of the results. The DNA fingerprinting, defined as the electrophoretic profiles reveal differences among individual organisms, not only has the characteristics mentioned above but also has the advantages of saving time and labor with reliability in results obtained. Therefore, this method is currently playing a more important role in rice breeding (Joshi et al., 2001). The simple sequence repeat (SSR) markers are widely distributed in the genome and exhibit the advantages of codominance, better repeatability, higher polymorphism, stable amplification, ease of handling (McCouch et al., 1997; Cook et al., 2002). Thus, they are widely used in rice fingerprinting and genetic diversity analysis (Zhang et al., 2015; Meng et al., 2018; Mnasri et al., 2018; Satturu et al., 2018; Bhattacharjee et al., 2019).

The DNA fingerprinting has also been gradually used in establishing distinctness, uniformity, stability (DUS) of plant varieties for the purpose of registration of new plant varieties due to the advantages mentioned above. The

International Union for the Protection of New Varieties of Plants (UPOV) evaluated different DNA marker parameters for use in the DUS test to examine plant varieties (Bredemeijer et al., 2002; Röder et al., 2002; UPOV-BMT, 2002), and a similar policy was also released by the Ministry of Agriculture in China.

The DNA fingerprinting can also be effectively used for the identification of seed authenticity, especially for those that are difficult to distinguish morphologically. Few elite parental lines are used frequently in modern breeding practices. As a result, the genetic background and morphological traits become increasingly narrow and small, but it is easier to distinguish these varieties based on genomic differences using molecular markers (Weising et al., 1991; Akkaya et al., 1992; Beyermann et al., 1992) and this method is widely used in rice now (Ramakishana et al., 1995; Nandakumar et al., 2004; Moorthy et al., 2011). The DNA fingerprinting can also be used in seed purity assessment. Purity should be guaranteed across seed production and marketing. When compared with the field test, the molecular marker test is more suitable for seed purity assessment (Akagi et al., 1997).

With the aid of fingerprinting using molecular markers, genetic similarity analysis and cluster analysis can be performed on parental lines used in breeding programs to determine varietal genetic diversity, relatedness, and genetic differences to help predict heterosis, choose appropriate parental lines to make crosses, and improve breeding efficiency.

In this paper, 35 pairs of SSR primers were used to fingerprint 4 check varieties and 31 hybrid rice core parental lines. Based on the benefits stated above, genetic diversity was analyzed using cluster analysis, and in the end, the feasibility was evaluated by assessing seed purity using characteristic markers. This research will provide guidance for rice breeding, variety identification and seed production at the molecular level.

2. Method

2.1 Plant Materials

There were two types of hybrid rice with similar cultivated area in China, three-line system and two-line system. For the former, the maintainer lines were needed to propagate cytoplasmic male sterile (CMS) lines, and then hybrid seeds were produced by crossing CMS lines with restorer lines which contain restorer genes. And for the letter, the fertility transformation of the male sterile (MS) lines depended on photoperiod and temperature, so no maintainer line was needed, and nearly all varieties be able to restore them. Thirty-five rice varieties were used in this study, including 31 hybrid rice core parental lines and 4 check varieties (Table 1). The parental lines of hybrid rice consisted of 4 three-line CMS lines, 7 two-line MS lines and 20 restorer lines. One japonica rice and 3 inbred lines of rice were used as the check varieties. Seed purity assessment was performed using the hybrid rice Guangliangyou476, with Guangzhan63S as the female parent and R476 as the male parent. All the varieties of seeds and hybrid seeds were provided by the Food Crops Institute, Hubei Academy of Agricultural Sciences, and all the seeds were planted in Wuhan, Hubei province.

Table 1. List of rice varieties used for DNA fingerprinting

No.	Variety	Year of release	Province of release	No.	Variety	Year of release	Province of release
Three-line sterile line				18	Huazhan	2008	Zhejiang
1	Quan9311A	2012	Anhui	19	R1468	2017	Hunan
2	Zhenshan97A	1984	Jiangxi	20	Huarun2	2014	Hubei
3	229A	2015	Hubei	21	Huanghuazhan	2005	Guangdong
4	JufengA	2008	Hubei	22	Fengxianghui1	2013	Anhui
Two-line sterile line				23	YR343	2017	Anhui
5	Y58S	2005	Hunan	24	Xiang5	2016	Hubei
6	C815S	2004	Hunan	25	93-11	1997	Jiangshu
7	Peiai64S	2000	Hunan	26	R476	2010	Hubei
8	Guangzhan63S	2003	Jiangshu	27	Feng3592	2010	Anhui
9	Enong1S	2016	Hubei	28	Minghui63	1984	Fujian
10	Longke638S	2014	Hunan	29	Shuhui527	2001	sichuan
11	Jing4155S	2014	Hunan	30	R1128	2014	Guangdong
Restorer line				31	R60	2018	Hubei
12	R534	2009	Guangdong	*Inbred rice*			
13	Huahui1308	2017	Hunan	32	Yuzhenxiang	2009	Hunan
14	R1377	2012	Guangdong	33	Yuewangsimiao	2013	Guangdong
15	Yuejingsimiao2	2006	Guangdong	34	Guiyu9	2014	Guangxi
16	Yuehesimiao	2014	Guangdong	*Japonica rice*			
17	Efengsimiao1	2018	Hubei	35	Nipponbare	1957	Japan

2.2 DNA Extraction, PCR Amplification and PCR Products Detection

For each variety, the same quantity of leaves from five sample plants was mixed, and then the CTAB method was employed to extract DNA (Doyle et al., 1978). The DNA concentration was adjusted to 30 ng/μL for PCR amplification using a NanoDrop. The DNA amplification was carried out in a 20 μL mixture containing 2.0 μL template DNA, 2.0 μL 25 mmol/L buffer, 1.0 μL primers (10 mmol/L for both forward and reverse primers), 200 μM each dNTP, 0.5 units of Taq DNA polymerase, and 13.8 μL ddH_2O. The PCR consisted of an initial denaturation of the template DNA at 94 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 1 min; annealing at 55 °C for 1 min; and extension at 72 °C for 1 min; The last cycle consisted of extension at 72 °C for 10 min.

Each reaction was mixed with 4 μL loading buffer, and then, 2 μL of the mixture was analyzed on a 6 percent polyacrylamide gel. A modified silver-staining procedure was employed after electrophoresis as follows: the gel was separated from the plate, placed into a staining solution containing 2 percent (w/v) silver nitrate, and shaken gently for 8 min. The gel was then immersed in distilled water for 10 s and transferred to a developing solution containing 15 percent (w/v) sodium hydroxide and 3 percent formaldehyde until the band was visible. Finally, the gel was imaged using a lamp box with light emitting diode (LED) light source.

2.3 SSR Markers

The SSR markers used in this paper were selected from a protocol for the identification of rice varieties using the SSR marker method (NY/T1433-2014), which was released by the Ministry of Agriculture of China. Together, there are four sets of makers with 12 markers per set. Thirty-five markers with good polymorphism and clear electrophoresis bands were used for rice fingerprinting and genetic analysis (Table 2).

Table 2. Details of the 35 markers used for molecular characterization of all rice varieties

No.	Markers	Primer sequences (5'→3')	Chromosome	Na	Ne	Pic
1	RM583	F: AGATCCATCCCTGTGGAGAG R: GCGAACTCGCGTTGTAATC	1	4	3.49	0.71
2	RM71	F: CTAGAGGCGAAAACGAGATG R: GGGTGGGCGAGGTAATAATG	2	5	2.52	0.6
3	RM85	F: CCAAAGATGAAACCTGGATTG R: GCACAAGGTGAGCAGTCC	3	2	1.25	0.2
4	RM471	F: ACGCACAAGCAGATGATGAG R: GGGAGAAGACGAATGTTTGC	4	3	1.85	0.46
5	RM190	F: CTTTGTCTATCTCAAGACAC R: TTGCAGATGTTCTTCCTGATG	6	5	2.65	0.62
6	RM336	F: CTTACAGAGAAACGGCATCG R: GCTGGTTTGTTTCAGGTTCG	7	8	4.24	0.76
7	RM72	F: CCGGCGATAAAACAATGAG R: GCATCGGTCCTAACTAAGGG	8	5	2.44	0.59
8	RM311	F: GGTAGTATAGGTACTAAACAT R: TCCTATACACATACAAACATAC	10	4	2.16	0.54
9	RM209	F: ATATGAGTTGCTGTCGTGCG R: CAACTTGCATCCTCCCCTCC	11	5	3.55	0.72
10	RM1195	F: ATGGACCACAAACGACCTTC R: CGACTCCCTTGTTCTTCTGG	1	4	1.63	0.39
11	RM208	F: TCTGCAAGCCTTGTCTGATG R: AAGTCGATCATTGTGTGGACC	2	4	3.39	0.71
12	RM232	F: CCGGTATCCTTCGATATTGC R: CCGACTTTTCCTCCTGACG	3	4	2.75	0.64
13	RM119	F: ATCCCCCTGCTGCTGCTGCTG R: GCCGGATGTGTGGGACTAGCG	4	3	2.24	0.55
14	RM253	F: TCCTTCAAGAGTGCAAAACC R: GCATTGTCATGTCGAAGCC	6	3	2.05	0.51
15	RM481	F: TAGCTAGCCGATTGAATGGC R: CTCCACCTCCTATGTTGTTG	7	8	3.66	0.73
16	RM258	F: TGCTGTATGTAGCTCGCACC R: TGGCCTTTAAAGCTGTCGC	10	6	4.66	0.79
17	RM224	F: ATCGATCGATCTTCACGAGG R: TGCTATAAAAGGCATTCGGG	11	5	2.1	0.52
18	RM17	F: TGCCCTGTTATTTTCTTCTCTC R: GGTGATCCTTTCCCATTTCA	12	4	2.72	0.63
19	RM493	F: TAGCTCCAACAGGATCGACC R: GTACGTAAACGCGGAAGGTG	1	4	2.74	0.64
20	RM8277	F: AGCACAAGTAGGTGCATTTC R: ATTTGCCTGTGATGTAATAGC	3	5	2.79	0.64
21	RM598	F: GAATCGCACACGTGATGAAC R: ATGCGACTGATCGGTACTCC	5	3	1.34	0.25
22	RM176	F: CGGCTCCCGCTACGACGTCTCC R: AGCGATGCGCTGGAAGAGGTGC	6	3	1.19	0.16
23	RM432	F: TTCTGTCTCACGCTGGATTG R: AGCTGCGTACGTGATGAATG	7	3	2.22	0.55
24	OSR28	F: AGCAGCTATAGCTTAGCTGG R: ACTGCACATGAGCAGAGACA	9	5	2.1	0.52
25	RM590	F: CATCTCCGCTCTCCATGC R: GGAGTTGGGGTCTTGTTCG	10	3	1.65	0.4
26	RM21	F: ACAGTATTCCGTAGGCACGG R: GCTCCATGAGGGTGGTAGAG	11	4	2.74	0.64
27	RM490	F: ATCTGCACACTGCAAACACC R: AGCAAGCAGTGCTTTCAGAG	1	3	2.18	0.54

28	RM424	F: TTTGTGGCTCACCAGTTGAG R: TGGCGCATTCATGTCATC	2	3	1.59	0.37
29	RM423	F: AGCACCCATGCCTTATGTTG R: CCTTTTTCAGTAGCCCTCCC	2	3	1.19	0.16
30	RM571	F: GGAGGTGAAAGCGAATCATG R: CCTGCTGCTCTTTCATCAGC	3	3	2.41	0.58
31	RM567	F: ATCAGGGAAATCCTGAAGGG R: GGAAGGAGCAATCACCACTG	4	2	1.32	0.24
32	RM289	F: TTCCATGGCACACAAGCC R: CTGTGCACGAACTTCCAAAG	5	4	2.7	0.63
33	RM542	F: TGAATCAAGCCCCTCACTAC R: CTGCAACGAGTAAGGCAGAG	7	5	1.99	0.5
34	RM316	F: CTAGTTGGGCATACGATGGC R: ACGCTTATATGTTACGTCAAC	9	3	1.34	0.25
35	RM7102	F: TAGGAGTGTTTAGAGTGCCA R: TCGGTTTGCTTATACATCAG	12	3	2.32	0.57
	Mean			4.03	2.38	0.52

Note. The paramters in columns 5, 6, 7 labelled as Na, Ne and Pic indicate the number of alleles, effective number of alleles and polymorphism index content.

2.4 Variety Fingerprinting Identity Numbers

The SSR markers used in rice fingerprinting were ranked according to their group and chromosome number, as shown in Table 2. For the electrophoretic profile of each marker, each type of band was given a number according to its mobility; for example, the band with the lowest mobility scored one, and so on for the other bands. Each variety received a number from each marker, and all 35 numbers from the 35 SSR markers were used as the variety fingerprinting identity numbers.

2.5 Data analysis

The number of alleles (Na) is the sum of all the multiple loci. The formula for the effective number of alleles (Ne) is $Ne = 1/\Sigma(Pi)^2$, the formula for the polymorphism index content (PIC) is $PIC = 1 - \Sigma(Pi)^2$, and Pi is the gene frequency for the SSR marker at the i locus. The pairwise similarity coefficient (F) was calculated by $F = 2Nxy/(Nx + Ny)$, where Nxy is the number of polymorphic bands shared by rice varieties X and Y, and Nx and Ny are the number of polymorphic bands scored for rice varieties X and Y, respectively. The SSR products were numbered as "0" or "1" according to the electrophoretic profile. At the same mobility loci for a variety, if there is a band, the product was assigned "1"; otherwise, the product was assigned "0". Then, the data matrix was obtained. Using the statistical program NTSYS-pc (Rohlf, 2000), cluster analysis was performed with the data matrix using an unweighted pair group method with arithmetic mean (UPGMA) method.

3. Results

3.1 Polymorphisms of the SSR Markers

Thirty-five SSR markers with stable amplification, good polymorphism and even distribution in the genome were used in the genetic diversity analysis. Altogether, there were 141 alleles in 35 varieties with an average of 4.03 for each marker, ranging from two to eight. The effective number of alleles was 83.16 with a mean of 2.38, ranging from 1.19 to 4.66. The PIC values ranged from 0.16 to 0.79, with an average of 0.52 (Table 2).

3.2 SSR Fingerprinting Identity Numbers and Variety Characteristic Markers

Thirty-five SSR markers were used for PCR analysis of 35 rice varieties. The electrophoretic bands of the PCR products were numbered from small to large according to their mobility, and each variety was given a number according to the PCR product for each marker. All 35 SSR markers consisted of a variety fingerprinting identity number (Table 3). Fourteen varieties had unique electrophoresis bands among all the varieties with 10 SSR markers, so these markers could be used as characteristic markers for the corresponding variety. By combining two markers, the other 21 varieties also obtained their characteristic markers (Table 3). With the aid of these characteristic markers, the corresponding variety could be distinguished from the others.

Table 3. SSR fingerprinting identity numbers and characteristic markers of 35 rice varieties

No.	Variety	DNA fingerprints identity number	Characteristic marker
1	Quan9311A	241137312 322213424 35223321 213324421	RM224
2	Zhenshan97A	251246312 342223214 34223242 213323422	RM21
3	229A	451114425 144123211 12223423 213323422	RM336, RM258
4	JufengA	351244425 144223111 12223423 213323422	RM336, RM258
5	Y58S	131226321 242323411 43222444 113322423	RM190
6	C815S	131236311 242323411 23222444 113322433	RM590, RM21
7	Peiai64S	131236414 232322412 44321444 112222123	RM423
8	Guangzhan63S	131211321 223211311 23122424 113112432	RM481
9	Enong1S	141231324 221223341 24122424 113112432	RM224, RM8277
10	Longke638S	131116324 242313414 42222414 111322422	RM590
11	Jing4155S	231136212 242213114 22222424 123323432	RM72, RM224
12	R534	231113324 221213112 13223421 113221422	RM481, RM336
13	Huahui1308	331213324 221216142 33223523 113321421	RM481, RM336
14	R1377	231136313 221216411 12223421 113222422	RM493, RM336
15	Yuejingsimiao2	231236313 221215412 32223424 113223421	RM481, RM336
16	Yuehesimiao	221216313 222216414 33223421 113222421	RM71
17	Efengsimiao1	131216313 231213311 33223421 113122421	RM208, RM481
18	Huazhan	231232313 221216114 13223421 113221421	RM336
19	R1468	231115321 211326111 33223421 113212421	RM208, OSR28
20	Huarun2	131145314 211226111 33223521 113213521	RM542
21	Huanghuazhan	231135311 212216112 33223521 113222421	OSR28, RM209
22	Fengxianghui1	241231314 242123634 22223424 213222422	RM258
23	YR343	431235314 222317534 42113424 113222321	RM481
24	Xiang5	431231312 223124314 34223423 233223421	RM481, RM336
25	93-11	441216313 243113334 32222424 213222321	RM224, RM336
26	R476	451216334 243216314 32222421 213322421	RM71, RM481
27	Feng3592	351231214 222328342 32222244 223223322	RM481, RM336
28	Minghui63	341231214 412326542 32222544 213321422	OSR28, RM336
29	Shuhui527	311215244 412223142 32222523 213221421	RM71
30	R1128	131316224 222228542 42213434 233223322	RM590
31	R60	341211224 432225532 32222423 213222321	RM481, RM336
32	Yuzhenxiang	132217313 313126514 33222524 331322221	RM336, OSR28
33	Yuewangsimiao	142237313 233216511 33222221 233222221	OSR28, RM481
34	Guiyu9	132237311 212224514 33322224 333323222	RM481, RM336
35	Nipponbare	152346134 222332553 41231144 133114113	RM224

3.3 Cluster Analysis

Based on the genetic similarity coefficient, cluster analysis was performed, and a dendrogram was generated (Figure 1). The check variety Nipponbare (subgroup VIII) was clustered into one group with a genetic similarity of 62 percent with the other varieties in remaining clusters. With a similarity coefficient of 0.71, 3 inbred rice varieties were clustered into subgroup VII. With a similarity coefficient of approximately 0.76, all the other varieties were clustered into 6 different subgroups according to the variation existed among these lines, pedigrees and released regions, including three-line CMS line subgroups I and V, two-line sterile line subgroup IV, restorer line subgroups II, III and VI.

The pairwise genetic similarity coefficient indicated that among 31 hybrid rice parental lines, the highest genetic similarity coefficient (0.94) was observed between Y58S and C815S, and the lowest (0.63) was observed between Quan9311A and Peiai64S, with an average of 0.74 for all the plant materials (Table 4).

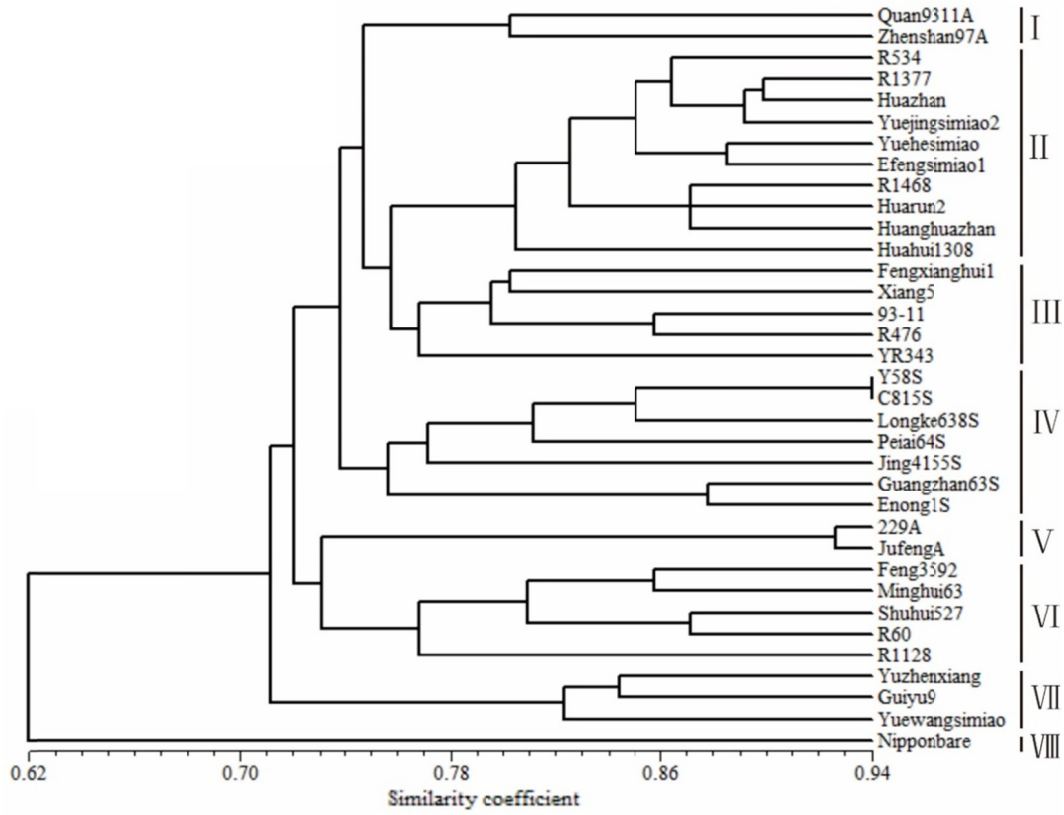

Figure 1. Dendrogram of genetic relationships among 35 rice varieties by UPGMA cluster analysis. Scale at the bottom is Jaccard's coefficient of similarity

3.4 Genetic Purity Assessment of Hybrid Rice Seeds

A hybrid seed genetic test was performed using two-line hybrid rice Guangliangyou476, with female parent Guangzhan63S and male parent R476, using RM481, which is the characteristic marker of Guangzhan63S. A random sample of 70 seeds was assessed, and three samples produced the same electrophoretic profile bands as the female parent Guangzhan63S (Figure 2). Therefore, the purity of the seed lot was approximately 95.7 percent. Genetic purity of the seed plot was also analyzed using the field test with randomly sampled seeds, and the purity was 96.2 percent for 500 plants. This result was similar with the result of the SSR marker test.

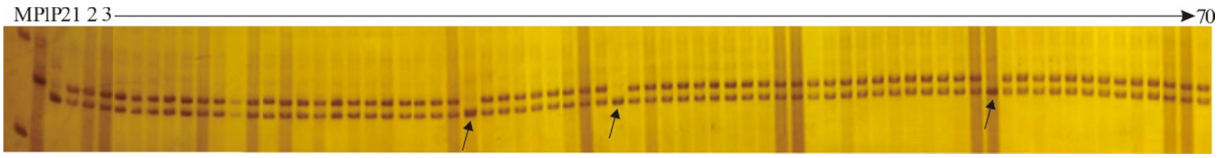

Figure 2. Amplification profile of RM481 in hybrid rice Guangliangyou 476; M: DNA ladder; P1: R476; P2: Guangzhan63S. Lane 1 to lane 70 were random samples of 70 seeds

Table 4. Similarity coefficient between pairs of varieties

Variety	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17
G2	0.80																
G3	0.70	0.77															
G4	0.70	0.79	0.93														
G5	0.69	0.76	0.73	0.75													
G6	0.70	0.76	0.70	0.72	0.94												
G7	0.63	0.69	0.65	0.66	0.83	0.83											
G8	0.65	0.65	0.68	0.69	0.79	0.80	0.69										
G9	0.68	0.68	0.68	0.71	0.77	0.80	0.73	0.88									
G10	0.72	0.73	0.75	0.72	0.87	0.83	0.77	0.76	0.74								
G11	0.76	0.77	0.73	0.75	0.76	0.82	0.69	0.75	0.74	0.82							
G12	0.76	0.72	0.77	0.77	0.76	0.73	0.69	0.79	0.77	0.79	0.77						
G13	0.73	0.69	0.72	0.76	0.73	0.70	0.66	0.75	0.74	0.72	0.70	0.86					
G14	0.79	0.73	0.73	0.73	0.75	0.76	0.73	0.75	0.74	0.79	0.80	0.87	0.76				
G15	0.80	0.77	0.71	0.74	0.78	0.80	0.77	0.77	0.76	0.75	0.80	0.84	0.81	0.89			
G16	0.80	0.79	0.70	0.72	0.79	0.79	0.75	0.75	0.73	0.76	0.75	0.82	0.79	0.87	0.88		
G17	0.75	0.76	0.72	0.73	0.80	0.80	0.75	0.79	0.78	0.76	0.73	0.82	0.79	0.85	0.85	0.89	
G18	0.79	0.73	0.69	0.73	0.73	0.75	0.70	0.75	0.73	0.72	0.77	0.89	0.83	0.90	0.89	0.89	0.85
G19	0.70	0.68	0.72	0.70	0.76	0.73	0.69	0.75	0.71	0.73	0.70	0.83	0.80	0.82	0.78	0.83	0.83
G20	0.72	0.70	0.68	0.70	0.70	0.70	0.69	0.70	0.71	0.69	0.70	0.80	0.79	0.80	0.80	0.80	0.83
G21	0.80	0.73	0.69	0.70	0.76	0.77	0.72	0.75	0.70	0.73	0.77	0.85	0.82	0.86	0.85	0.86	0.82
G22	0.77	0.79	0.76	0.76	0.76	0.79	0.73	0.72	0.78	0.77	0.79	0.76	0.69	0.79	0.78	0.80	0.76
G23	0.70	0.65	0.65	0.65	0.72	0.72	0.76	0.70	0.71	0.75	0.72	0.72	0.73	0.76	0.78	0.76	0.72
G24	0.76	0.77	0.75	0.73	0.70	0.72	0.70	0.72	0.71	0.68	0.73	0.73	0.73	0.76	0.82	0.80	0.79
G25	0.75	0.73	0.73	0.70	0.75	0.75	0.69	0.73	0.73	0.77	0.75	0.72	0.70	0.76	0.80	0.80	0.80
G26	0.75	0.76	0.76	0.76	0.75	0.73	0.68	0.75	0.71	0.79	0.76	0.75	0.79	0.77	0.77	0.82	0.80
G27	0.68	0.75	0.69	0.72	0.72	0.73	0.73	0.68	0.73	0.69	0.73	0.68	0.70	0.69	0.74	0.70	0.68
G28	0.72	0.75	0.70	0.73	0.75	0.76	0.72	0.66	0.73	0.72	0.72	0.68	0.76	0.69	0.71	0.70	0.68
G29	0.73	0.73	0.74	0.78	0.71	0.70	0.67	0.67	0.72	0.70	0.71	0.75	0.82	0.68	0.73	0.75	0.74
G30	0.65	0.70	0.73	0.73	0.74	0.70	0.73	0.70	0.72	0.75	0.73	0.75	0.73	0.73	0.75	0.73	0.71
G31	0.71	0.70	0.73	0.75	0.71	0.68	0.70	0.70	0.72	0.70	0.67	0.71	0.77	0.68	0.73	0.75	0.74
G32	0.68	0.69	0.65	0.63	0.72	0.72	0.66	0.69	0.64	0.72	0.66	0.65	0.70	0.68	0.71	0.76	0.76
G33	0.73	0.69	0.62	0.65	0.69	0.70	0.68	0.70	0.68	0.66	0.68	0.70	0.70	0.77	0.77	0.79	0.80
G34	0.69	0.75	0.65	0.68	0.75	0.76	0.70	0.72	0.70	0.70	0.75	0.68	0.68	0.69	0.74	0.73	0.73
G35	0.58	0.60	0.56	0.57	0.67	0.65	0.70	0.63	0.65	0.65	0.61	0.60	0.60	0.60	0.62	0.61	0.61

Table 4. Continued

Variety	G18	G19	G20	G21	G22	G23	G24	G25	G26	G27	G28	G29	G30	G31	G32	G33	G34
G19	0.82																
G20	0.82	0.87															
G21	0.87	0.87	0.87														
G22	0.77	0.72	0.70	0.76													
G23	0.77	0.73	0.70	0.76	0.79												
G24	0.80	0.73	0.75	0.76	0.80	0.75											
G25	0.76	0.70	0.69	0.73	0.85	0.79	0.80										
G26	0.77	0.76	0.72	0.76	0.76	0.75	0.77	0.86									
G27	0.68	0.65	0.68	0.69	0.77	0.73	0.76	0.75	0.72								
G28	0.69	0.68	0.69	0.73	0.79	0.70	0.7	0.72	0.73	0.86							
G29	0.73	0.73	0.74	0.77	0.75	0.70	0.73	0.75	0.77	0.78	0.84						
G30	0.68	0.68	0.70	0.68	0.74	0.77	0.73	0.71	0.70	0.81	0.74	0.75					
G31	0.68	0.71	0.70	0.73	0.78	0.74	0.73	0.80	0.77	0.80	0.82	0.87	0.77				
G32	0.72	0.70	0.73	0.73	0.69	0.66	0.75	0.76	0.73	0.65	0.72	0.68	0.65	0.70			
G33	0.79	0.72	0.76	0.79	0.72	0.69	0.76	0.79	0.77	0.70	0.70	0.70	0.67	0.75	0.83		
G34	0.72	0.68	0.73	0.75	0.72	0.69	0.76	0.69	0.69	0.73	0.73	0.67	0.71	0.68	0.85	0.82	
G35	0.58	0.60	0.63	0.58	0.60	0.65	0.58	0.58	0.61	0.65	0.63	0.58	0.70	0.59	0.60	0.61	0.62

Note. G1, Quan9311A; G2, Zhenshan97A; G3, 229A; G4, JufengA; G5, Y58S; G6, C815S; G7, Peiai64S; G8, Guangzhan63S; G9, Enong1S; G10, Longke638S; G11, Jing4155S; G12, R534; G13, Huahui1308; G14, R1377; G15, Yuejingsimiao2; G16, Yuehesimiao; G17, Efengsimiao1; G18, Huazhan; G19, R1468; G20, Huarun2; G21, Huanghuazhan; G22, Fengxianghui1; G23, YR343; G24, Xiang5; G25, 93-11; G26, R476; G27, Feng3592; G28, Minghui63; G29, Shuhui527; G30, R1128; G31, R60; G32, Yuzhenxiang; G33, Yuewangsimiao; G34, Guiyu9; G35, Nipponbare.

4. Discussion

In this paper, DNA fingerprinting and varietal characteristic markers for sterile lines and restorer lines that are widely used in China were reported. The cluster analysis and genetic diversity analysis were conducted based on DNA fingerprinting. Finally, the genetic purity of the seeds of a hybrid rice variety was determined using parental characteristic markers.

The DUS test has been indispensable in the process of rice variety registration to identify varietal characteristics that could distinguish new varieties from released varieties. Traditionally, the DUS tests of varieties depended on the field morphological descriptions observed during entire crop growth duration, which is time-consuming. In addition, the results of the DUS test are not exact because some descriptors rely on the experience, and the interaction between genotype and environment that may result in an unstable phenotype (Satturu et al., 2018). Furthermore, with the rapid development of the breeding program, some elite main parents are frequently used in breeding practice, which results in high genetic similarity among the developed varieties, so it is not suitable to distinguish them based on morphological traits. In addition, variety identification based on morphological traits cannot meet the requirements of crop breeding and crop improvement.

There are great advantages for variety identification at the genome level using DNA fingerprinting. For example, the use of SSR markers for fingerprinting has the advantages of codominance, high polymorphism, good genetic stability and so on, so they could be used in DUS tests and variety identification, especially for varieties with high morphological similarity (Akkaya et al., 1992; Beyermann et al., 1992; Nandakumar et al., 2004; Moorthy et al., 2011). The use of DNA fingerprinting in the DUS test has been extensively considered by the Biochemical and Molecular Techniques (BMT) Working Group of UPOV and the Ministry of Agriculture in China (UPOV-BMT, 2002). In this research, using SSR fingerprinting, 10 characteristic SSR markers were identified that could distinguish 14 varieties from other varieties. In addition, by combining two SSR markers, the other 21 varieties could also be distinguished. Therefore, SSR markers have great advantages in variety identification.

As the number of developed varieties in the market increases, especially varieties with similar phenotypes, some disputes about variety rights arise. For example, the very popular restorer line Huazhan, 148 developed hybrid rice cultivars using it as a male parent in 2008-2018, and some of these varieties also had female parents with high genetic similarity, so these varieties behaved similarly in the field. For some popular varieties, there will be imitation varieties with similar phenotypes for sale. Therefore, with the aid of varietal characteristic markers, these varieties can be easily distinguished to protect the rights of the variety owner.

The genetic purity of hybrid seeds is an essential requirement across their production and marketing because seed contamination would lead to yield loss and poor grain quality, resulting in disputes between farmers and seed sellers (Nandakumar et al., 2004). There are many means of hybrid seed contamination in production plots, such as pollen shedders, fertility restoration of two-line fertile lines, mechanical mixing during harvest and the following post-harvest handling procedures (Bora et al., 2016; Nethra et al., 2016). Currently, in China, approximately half of the hybrid rice varieties are two-line hybrid rice varieties, and fertility conversion is the main factor leading to seed contamination of these varieties. Both low temperature during the booting stage and genetic shift of the sterile line during its reproduction would lead to unstable fertility, and fertility restoration would then require to occur. Therefore, more attention should be paid to the genetic purity of two-line hybrid rice.

The results of the seed genetic purity assessment of a two-line hybrid rice in this study indicated that the purity was almost the same as that obtained by the field test and the varietal characteristic marker method, and all the mixed seeds were sterile lines. Therefore, the characteristic markers in this research could be effectively used in the genetic purity assessment of hybrid seeds.

Crop variety can be divided into different genetic pools according to their relatedness using cluster analysis based on the genetic similarity coefficient matrix, which will provide great help to breeders in choosing the appropriate parental lines to be crossed. Melchinger et al. (1990a) reported that with the aid of genetic distance based on the DNA markers, the yield of crosses between lines could be predicted. Genetic diversity and heterosis analysis of hybrid rice parental lines based on PCR markers indicated that there was a significantly positive correlation between yield, potential heterosis and genetic distance within *japonica* rice or *indica* rice (Xiao et al., 1996), which was also proven in other crops (Corbellini et al., 2002).

The dendrogram (Figure 1) of the hybrid rice parental lines in this research also indicated that the two-line sterile line, three-line sterile line and restorer line were all clustered into a single subgroup, and the similarity coefficient among the subgroups was approximately 0.76. However, the average similarity coefficient was higher within subgroups, such as 0.84 in the restorer line (III) and 0.79 in the two-line sterile line (subgroup IV), which

are the two largest subgroups in the dendrogram. Thus, a wide genetic distance between parental lines is a pre-requisite for heterosis of hybrid rice.

Although the genetic distance between the inbred rice subgroup and sterile line subgroup was wider (with a lower similarity of approximately 0.71), no hybrid rice variety was developed using either of these lines as the male parent. It was likely that all these inbred rice belong to high-grade good quality varieties with long slender grains though their yield was generally lower compared with that of the restorer lines. Zhang et al. (1996) reported that yield-related quantitative trait loci (QTLs) linked with markers determined the specific combining ability between the parental lines to be crossed, so the lack of yield-related QTLs may be the reason why inbred rice could not be used as male parents for hybrid rice.

5. Conclusion

In this paper, 35 pairs of SSR primers were used for genetic diversity analysis and DNA fingerprinting of 31 hybrid rice core parental lines developed in central- and southern-China using one *japonica* rice line and three inbred rice lines as the check varieties.

The average number of alleles (Na) per SSR locus was 4.02, with a range of two to eight, the effective number of alleles (Ne) was 83.16 with a mean of 2.38, ranging from 1.19 to 4.66. The polymorphic information content (PIC) ranged from 0.16 to 0.79, with an average number of 0.52. The results of the cluster analysis indicated that 31 hybrid rice parental lines were clustered into 6 groups with similarity coefficients of approximately 0.76. The highest genetic similarity coefficient (0.94) was observed between Y58S and C815S, and the lowest (0.63) was observed between Quan9311A and Peiai64S. The purity of one hybrid rice variety was tested and the results were reconfirmed with similarity in observations using characteristic markers and the field test methods. This research will be helpful for rice breeding, new cultivar registration and seed production.

References

Akagi, H., Yokozeki, Y., Inagaki, A., & Fujimura, T. (1997). Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci. *Theor. Appl. Genet., 94*, 61-67. https://doi.org/10.1007/s001220050382

Akkaya, M. S., Bhagwat, A. A., & Cregan, P. B. (1992). Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics, 132*(4), 1131-1139. https://doi.org/1131-1139.10.1101/gad.6.12b.2655

Beyermann, B., Nürnberg, P., Weihe, A., Meixner, M., Epplen, J. T., & Börner, T. (1992). Fingerprinting plant genomes with oligonucleotide probes specific for simple repetitive DNA sequences. *Theor. Appl. Genet., 83*, 691-694. https://doi.org/10.1007/BF00226686

Bhattacharjee, M., Prakash, S. H., Roy, S., Soumen, S., Begum, T., & Dasgupta, T. (2019). SSR-based DNA fingerprinting of 18 elite Indian varieties of sesame (*Sesamum indicum* L.). *The Nucleus, 63*, 67-73. https://doi.org/10.1007/s13237-019-00290-3

Bora, A., Choudhury, P. R., Pande, V., & Mandal, A. B. (2016). Assessment of genetic purity in rice (*Oryza sativa* L.) hybrids using microsatellite markers. *3 Biotech., 6*(1), 50.

Cook, R. J., Ganal, M. W., Peeters, R., Issac, P., Noordijk, Y., Rendell, S., … Vosman, B. (2002). Construction and testing of microsatellite database containing more than 500 tomato varieties. *Theor. Appl. Genet., 105*(6-7), 1019-1026. https://doi.org/10.1007/s00122-002-1038-6

Corbellini, M., Perenzin, M., Accerbi, M., Vaccino, P., & Borghi, B. (2002). Genetic diversity in bread wheat, as revealed by coefficient of parentage and molecular markers, and its relationship to hybrid performance. *Euphytica, 123*(2), 273-285. https://doi.org/10.1023/A:1014946018765

Doyle, J. J., & Doyle, J. L. (1978). Isolation of plant DNA from fresh tissue. *Focus, 12*, 13-14.

Joshi, S. P., Bhave, S. G., Chowdari, K. V., Apte, G. S., Dhonukshe, B. L., Lalitha, K., … Gupta, V. S. (2001). Use of DNA markers in prediction of hybrid performance and heterosis for a three-line hybrid system in rice. *Biochem. Genet., 39*, 179-200. https://doi.org/10.1023/A:1010293325482

McCouch, S. R., Chen, X. L., Panaud, O., Temnykh, S., Xu, Y., Cho, Y. G., … Blair, M. (1997). Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Mol. Biol., 35*, 89-99. https://doi.org/10.1023/A:1005711431474

Melchinger, A. E., Lee, M., Lamkey, K. R., & Woodman, W. W. (1990a). Genetic diversity for restriction fragment length polymorphisms: Relation to estimated genetic effects in maize inbreds. *Crop Sci., 30*(5), 1033–1040. https://doi.org/10.2135/cropsci1990.0011183X003000050016x

Meng, Y. S., Zhao, N., Li, H., Zhai, H., He, S., & Liu, Q. (2018). SSR fingerprinting of 203 sweetpotato (*Ipomoea batatas* (L.) Lam.) varieties. *J. Intergr. Agr., 17*(1), 86-93. https://doi.org/10.1016/S2095-3119 (17)61687-3

Mnasri, R. S., Saddoud, D. O., & Ferchichi, A. (2018). *Efficiency of SSR markers to analyze the molecular fingerprinting of some Tunisian olive cultivars*. Cham, Springer.

Moorthy, K., Babu, P., Sreedhar, M., Sama, A., Nataraj Kumar, P., Balachandran, S. M., & Sundaram, R. M. (2011). Identification of informative EST-SSR markers capable of distinguishing popular Indian rice varieties and their utilization in seed genetic purity assessments. *Seed Sci. and Technol., 39*(2), 282-292. https://doi.org/10.15258/sst.2011.39.2.02

Nandakumar, N., Singh, A. K., Sharma, R. K., Mohapatra, T., Prabhu, K. V., & Zaman, F. U. (2004). Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica, 136*, 257-264. https://doi.org/10.1023/B:EUPH.0000032706.92360.c6

Nethra, N., Pushpa, C., Rani, K. U., Gowda, R., & Rajendra Prasad, S. (2016). Fingerprinting and genetic purity assessment of rice varieties and hybrids through microsatellite markers. *Seed Science & Technology, 44*(3), 585-594. https://doi.org/10.15258/sst.2016.44.3.01

Ramakrishna, W., Chowdari, K. V., Lagu, M. D., Gupta, V. S., & Ranjekar, P. K. (1995). DNA fingerprinting to detect genetic variation in rice using hypervariable DNA sequences. *Theor. Appl. Genet., 90*, 1000-1006. https://doi.org/10.1007/BF00222913

Röder, M. S., Wendehake, K., Korzun, V., Bredemeijer, G. M. M., Laborie, D., Bertrand, L., … Ganal, M. (2002). Construction and analysis of a microsatellite-based database of European wheat varieties. *Theor. Appl. Genet., 106*, 67-73. https://doi.org/10.1007/s00122-002-1061-7

Rohlf, F. J. (2000) *NTSYS-pc: Numerical taxonomy and multivariate analysis system* (Version 2.1). Exeter Software, Setauket, NY.

Satturu, V., Rani, D., Gattu, S., Md, J., Mulinti, S., Kumar Nagireddy, R., … Yanda, R. (2018). DNA fingerprinting for identification of rice varieties and seed genetic purity assessment. *Agric. Res., 7*, 379-390. https://doi.org/10.1007/s40003-018-0324-8

UPOV-BMT. (2002). *BMT/36/10 Progress Report of the 36th Session of the Technical Committee, the technical working parties and working group on biochemical and molecular techniques and DNA-profiling in particular, Geneva*.

Weising, K., Beyermann, B., Ramser, J., & Kahl, G. (1991). Plant DNA fingerprinting with radioactive and digoxigenated oligonucleotide probes complementary to simple repetitive DNA sequences. *Electrophoresis, 12*(2-3), 159-169. https://doi.org/10.1002/elps.1150120211

Xiao, J., Li, J., Yuan, L., McCouch, S. R., & Tanksley, S. D. (1996). Genetic diversity and its relationships to hybrid performance and heterosis in rice as revealed by PCR-based markers. *Theor. Appl. Genet., 92*, 637-643. https://doi.org/10.1007/BF00226083

Zhang, L. W., Cai, R, R., Yuan, M. H., Tao, A. F., Xu, J., Lin, L., … Qi, J. (2015). Genetic diversity and DNA fingerprinting in jute (*Corchorus* spp.) based on SSR markers. *The Crop J., 5*, 416-422. https://doi.org/ 10.1016/j.cj.2015.05.005

Zhang, Q. F. (2007). Strategies for developing Green Super Rice. *Proc. Natl. Acad. Sci., 104*(42), 16402-16409. https://doi.org/10.1073/pnas.0708013104

Zhang, Q. F., Zhou, Z. Q., Yang, G. P., Xu, C. G., Liu, K. D., & Saghai Maroof, M. A. (1996). Molecular marker heterozygosity and hybrid performance in Indica and Japonica rice. *Theor. Appl. Genet., 93*, 1218-1224. https://doi.org/10.1007/bf00223453

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