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Research Article

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Genetic Diversity Analysis of High-Quality Mutant Rice (*Oryza sativa* L.) Lines with by Using SSR Markers

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Abstract The objective of this study was to evaluate the genetic diversity of 36 mutant high-quality rice lines including 01 non-mutant rice variety by using 30 SSR markers. A total of 1075 DNA bands involving in 51 different alleles were documented. The average PIC coefficient of the primers was 0.182 ranged from 0.0 to 0.68, respectively. It also revealed that the mutant quality rice lines have had high genetic purity by 1.01% on the average of the heterozygote. At the genetic similarity of all lines ranged from 66% - 94% were divided into 5 different groups. Mutant lines of the different groups have significant differences in some agronomic traits including the growth duration, the shape of seeds and amylose content. The obtained results in this study may provide useful information to further use the mutant rice materials for the breeding program.

Keywords Mutant rice, allele, SSR markers, genetic diversity

Introduction

Rice (*Oryza sativa* L.) is one of the most principle crops in Vietnam and is providing daily meals for nearly 100 million persons in this country. Genetics of rice materials and its genetic diversityis important source and information to launch rice breeding program which helps to generate new rice varieties with high quality and yield as well as tolerant to abiotic and biotic stresses to cope with climate change.

The recent advanced molecular markers are a powerful tool for evaluation of genetic variation and characterization of genetic relationships within and among species and varieties [1-2]. Currently, there are numerous difference of multilocus molecular markers are obtainable for assessing the genetic diversity of rice and other species such as including amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), random amplification of polymorphic DNAs (RAPD), single nucleotide polymorphism (SNP), simple sequence repeats (SSRs), inter simple sequence repeat (ISSRs), and expressed sequence tag- simple sequence repeat (EST-SSRs), which play a key role in plant breeding. Among them, SSR markers are considered the most capable markers for genetic diversity assessment due to their highly informative, mostly monolocus, co-dominant, multi-allelic nature, relative abundance, conveniently analysed and cost-effective as well as able to detect a high level of allelic diversity [3, 4, 5].

In our previous studies, we have used 40 SSR markers to evaluate genetic diversity of local-colored rice landraces, of which 11 markers including M250, RM302, RM10926, RM208, RM227, RM17231, RM23251, RM5647, RM1376, RM339 and RM228 which showed the unique allele [6]. Moreover, Vu et al [1] studied on the genetic diversity of 40 Vietnamese lowland rice varieties using 30 SSR markers covering in all rice chromosomes and showed their relationship to the seedling vigour under submergence.

In rice, as a principle, worldwide food crops, numerous reports over the decades have worked on their genetic diversities via whole rice genome analyses and focused on the specific traits such as high quality and yield as well as their abiotic and biotic tolerances stresses by using approximately 20.000 SSR markers. The generally obtained results showed highly significant allelic variation or polymorphism among the rice accessions/varieties or the polymorphism information content (PIC) value by applying SSR markers [7]. In this study, we attempted to analyse the genetic diversity of 36 rice lines by using 30 SSR markers. The obtained results in this study may provide useful information to further use the mutant rice materials for a breeding program.

Materials and Methods

Plant Materials

In this study, a total of 35 mutant rice lines were selected from M6 generations. The dried seeds of ST19 rice variety was mutated by the gamma radiation (Co60) with the application of the different dose (150 Gray, 200 Gray, 300 Gray and 350 Gray). The selected rice lines were shown some specific agronomic traits of interests as shown in Table 2

A total of 30 SSR markers used to analyse the different loci which were provided by Invitrogen (Thermo Fisher Scientific corporation). The information on SSR markers was listed in Table 1.

Methods

Total DNA extraction from the young leaves of two weeks rice seedlings were made following the CTAB method [8]. The amplification component of chloroplast ITS region includes 2.5µl dNTP Mix (concentration of 0.2 mM); 2.5 µl 10X Buffer; 0.625 DreamTaqTM DNA polymerase; 0.5 µl of primer (concentration of 0.175 µM) 0.5 µl of primer (concentration of 0.175 µM); 5 µl of DNA sample (concentration of 50 ng / ml), distilled water was 25µl. The primers of ITSF (GTTTCTTTTCCTCCT) and ITSR (AGGAGAAGTCGTAACAAG) are mounted in 23S and 18S areas, used for amplification and sequence reading.

PCR reactions were performed by Veriti 96-well Thermal cycler. Total volume was 15 µl,included: 5µl DNA; 0.15µM primer; 0.2 mM dNTPs; 1X Buffer PCR; 2.5mM MgCl2 and 0.25 Taq polymerase as following the method of Diep et al [9]. PCR program was set up as follows: initial denaturation at 95°C for 5 min; 35 DNA replication cycles (denatured at 94°C for 2 min, primers at 58°C for 1 min, extended at 72°C for 2 min), respectively. The final extension stage at 72 ^{o}C for 10 min. PCR products were performed on electrophoresis on 6% gel polyacrylamide for further analysis.The gels werestained in 0.5 mg/ml ethidium bromide and were documented using Alpha Imager 1220 (Alpha Innotech, CA,USA). Then, automatic PCR sequencing products were made by Macrogen company, Seoul, Korea.

Statistical Analyses

All data were observed based on the occurrence or absence of DNA bands (alleles) and statistically analysed by NTSYsp 2.1 and Excel version 2017. PIC coefficient (Polymorphic Information Content value) was calculated according to the formula:

$$PIC = 1 - \Sigma P_i^2 \text{ (where Pi is the frequency of the allele i).}$$

Results and Discussion

The PIC coefficients, the number of alleles and the total number of DNA bands per SSR primer pair

PIC coefficients are considered to be the polymorphism of alleles at each SSR locus. High PIC coefficients reflect a high polymorphism in the subjects and vice versa. In this study, we have analysed 30 SSR markers on a total of 35 mutant rice lines. The results showed that there were a total of 1076 DNA bands of the 51 different alleles. Also, there were 15 markers showing monomorphism including *xa5add35*, RM 122, RM 247, srwd5 and p3, RM 1, RM 310, salt, RM 13, pita, RM 337, RM 323, drep1a, RM 160 and RM 341, it means only observed 1 allele was available. Whereas, a total of 15 primer pairs revealed polymorphic locus. Among them, 12 markers showing 2 alleles, 1 primer pair observed 3 alleles, and another obtained 4 alleles as shown in Table 1.

In this study, we have found that the aromatic rice group has had less diversity of alleles based on the SSR loci. The PIC coefficients of 30 SSR markers were ranged from 0.0 (in primer pairs, only monoband appears to 0.68

(in primer pairs that appear 4 types of alleles - pikp). The average PIC coefficient of 30 primer pairswas low at 0.182.

The results have revealed that the mutant lines and compared with original ST19 variety, the coefficient of the diversity of primers is lower than other previous reports on aromatic rice groups. Specifically, Raj et al [10] used 12 SSR markers to evaluate genetic diversity of fragrant Indian rice varieties. The PIC coefficients of this study ranged from 0 to 0.830 [10]. Also, Jayamani&Lambodar [11] assessed the genetic diversity of 179 local rice varieties in the different 19 cultivated areas in Portugal by SSR markers. The results showed that PIC coefficients werefrom 0.179 to 0.894. Similarly, McCouch et al [12] worked on the genetic relationship between 52 aromatic Basmati rice varieties and 17 other Indian rice varieties by using 30 SSR markers and reported that the number of detected alleles ranged from 3 to 22 and the PIC coefficient ranged from 0.2 to 0.9. Adegbaju et al. [13] analyzed polymorphisms in 6 rice varieties by 129 SSR primers and detected 492 alleles, the average was 3.8 alleles/locus, PIC coefficients varied from 0.0-0.375. Masuduzzanman et al [14] studied on the genetic diversity of 160 collected rice varieties collected from some countries including India, Vietnam, Indonesia, Bangladesh, Sri Lanka and 4 varieties from IRRI by applying 30 SSR markers and discovered 337 alleles, the average was 11 alleles/locus, PIC values range from 0.44-0.91.

Table 1: Number of alleles present and PIC coefficients of 30 pairs of SSR primers

Order No	Name of marker	Chr. No	Number of alleles	PIC	No	Name of marker	Chr.No	Number of alleles	PIC
1	mp1-2	5	2	0.38	18	RM 431	8	2	0.5
2	xa5add35	6	1	0.00	19	RM 341	2	1	0
3	wxy	6	1	0.38	20	RM 160	12	1	0
4	bad2	8	3	0.50	21	RM 225	11	2	0.15
5	RM 122	4	1	0.00	22	drep1a	8	1	0
6	RM 225	3	2	0.24	23	pikp	2	4	0.68
7	RM 234	7	2	0.28	24	RM 323	1	1	0
8	RM 246	5	2	0.4	25	RM 337	8	1	0
9	qac7	6	2	0.22	26	RM 452	2	2	0.5
10	RM 302	10	2	0.41	27	pi ta	8	1	0
11	RM 247	7	1	0	28	RM 13	5	1	0
12	RM 224	2	2	0.47	29	salt	11	1	0
13	wx	11	2	0.41	30	RM 310	2	1	0
14	srwd5	9	1	0					
15	p3	9	1	0					
16	pta248	8	2	0.45		**Total**		**51**	**5.47**
17	RM 1	8	1	0		**Average**		**1.7**	**0.182**

3.2. The rate of heterozygotes alleles (H%) and missing alleles (M%) among the studied rice lines

Microsatellite or Simple Sequence Repeat (SSR) isa short stretch of DNA of which one to six bases can repeat over five to hundred times at each locus [15]. Hence, SSRs are dominant markers and often show highly polymorphic which can be used to distinguish the lines carrying heterogeneous genotypes. In this study, the missing alleles (M%) and (M) and heterozygotes alleles (H%) among the 35 mutant rice lines were compared based on the analysis of 30 SSR as presented in Table 2.

Table 2: Heterozygotes alleles (H%) and missing alleles (M%) among the rice lines

No	Lines name	M%	H%	No	Lines name	M%	H%
1	Line 1	0	0	19	Line 19	0	3.33
2	Line 2	0	0	20	Line 20	0	0
3	Line 3	0	0	21	Line 21	0	0
4	Line 4	0	0	22	Line 22	0	0
5	Line 5	3.33	0	23	Line 23	0	0
6	Line 6	0	3.33	24	Line 24	0	0

7	Line 7	0	6.67	25	Line 25	0	0
8	Line 8	0	0	26	Line 26	0	0
9	Line 9	0	0	27	Line 27	0	0
10	Line 10	0	0	28	Line 28	0	0
11	Line 11	0	0	29	Line 29	3.23	0
12	Line 12	0	0	30	Line 30	0	0
13	Line 13	0	3.33	31	Line 31	0	0
14	Line 14	0	0	32	Line 32	0	3.33
15	Line 15	0	0	33	Line 33	0	6.67
16	Line 16	0	0	34	Line 34	0	0
17	Line 17	0	0	35	Line 35	0	0
18	Line18	0	0	36	Line 36	3.23	0
		Total average				**0.31**	**1.01**

Note: Line 1: ST19 (original rice variety), 2-36 lines were the mutated lines in M6 generations

As shown in Table 2, it demonstrated that all the rice lines have a relatively high genetic purity. Of the 35 mutant lines, 28 lines had a heterozygous rate at 0% (equal to the heterozygous level of the original ST19 rice variety), which means that the lines were homogeneous in all 30 SSR primers (only one unique allele/ locus). We found that 7 lines showing heterozygotes were 3.33% - 6.67%. Lines 7 and 33 have had the highest heterozygous rate (6.67%). The average heterozygosity of all lines is 1.01%. The obtained results were lower than the report of Cong et al [16] who studied on the genetic diversity and relationships of 24 hybrid lines, the average heterozygous rate was 2.13, while, Trung et al [17] used 31 SSR to evaluate the genetic diversity of some native rice accessions and showed the heterozygosity was 0.00-14.29%.

The missing allele (M%) found in the studied rice lines is rather low, of which 3 lines have had 3.23% as shown in Table 2. Thus, the data obtained from all 35 rice lines are very high reliability for evaluating the genetic relationships and further used them as useful materials for rice breeding program.

Polymorphic analysis and genetic relationships in the rice lines

PCR products with SSR were presented in Figure 1 and Figure 2. The results of PCR product analysis of 35 mutant lines derived from ST19 original varieties have been statistically analyzed by NTSYSpc version 2.1 software, and then, factors of genetic similarity and diagram tree were made based on the polymorphic SSR data (Figure 3).

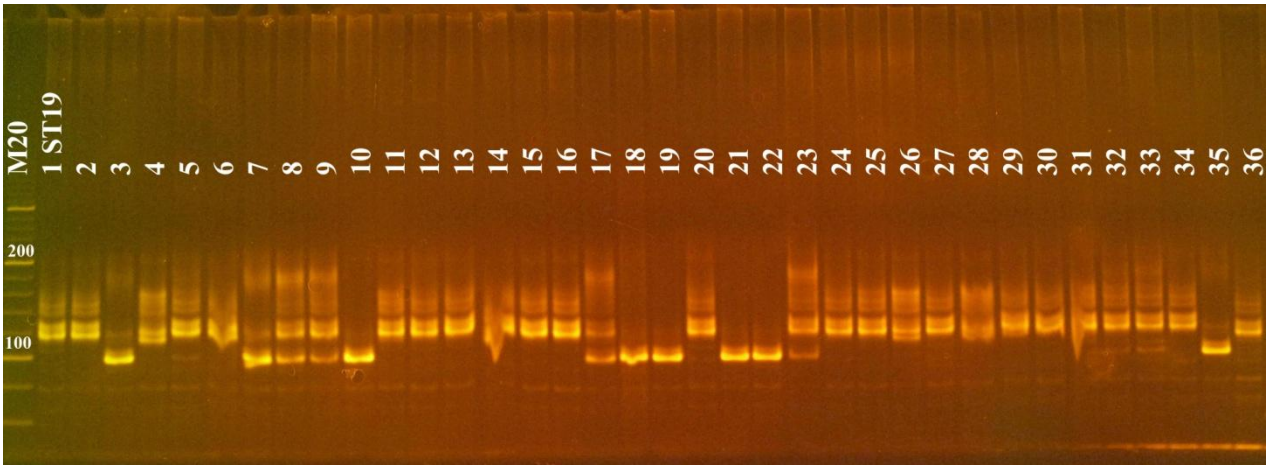

Figure 1: PCR and electrophoresis using RM246 markers

(M120: Ladder marker; lane 1: ST19 (original variety); 2-36 lanes: the mutant lines in M6 generation)

The results showed the variation of the genetic similarity coefficient of 35 rice lines was ranged from 0.85 (as shown in lines 1, 2) to 0.94 (line 17, 21). The 35 lines were divided into 5 groups as follows:

*Group I: line 1 and 2 (line 1 is original ST19 variety as the control). The lowest genetic similarity coefficient in this group is 0.85. Based on the morphological characteristics and SSR data, it showed having a high similarity in this group.

*Group II: lines 4, 29, 11 and 27 have a genetic similarity coefficient of about 0.78

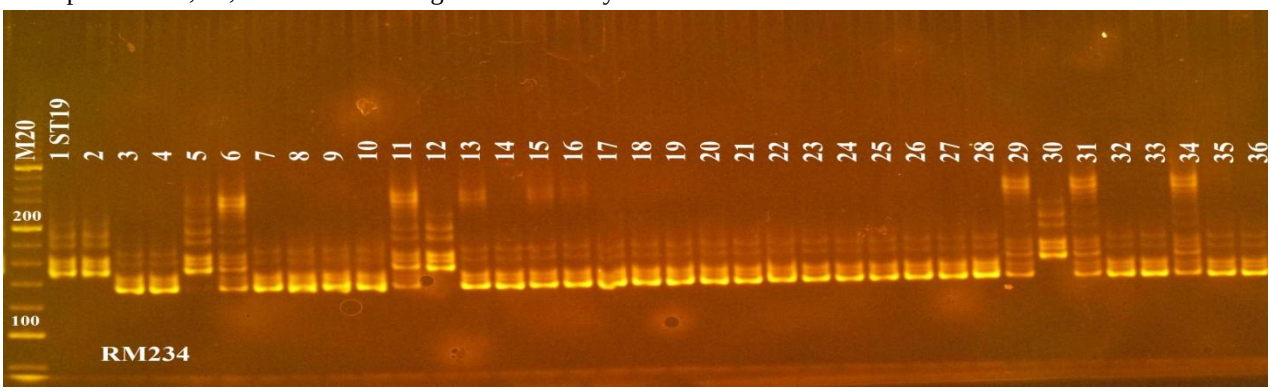

Figure 2: PCR and electrophoresis of using RM234 marker

M120: Ladder marker; lane 1: ST19 (original variety); 2-36 lanes: the mutant lines in M6 generation)

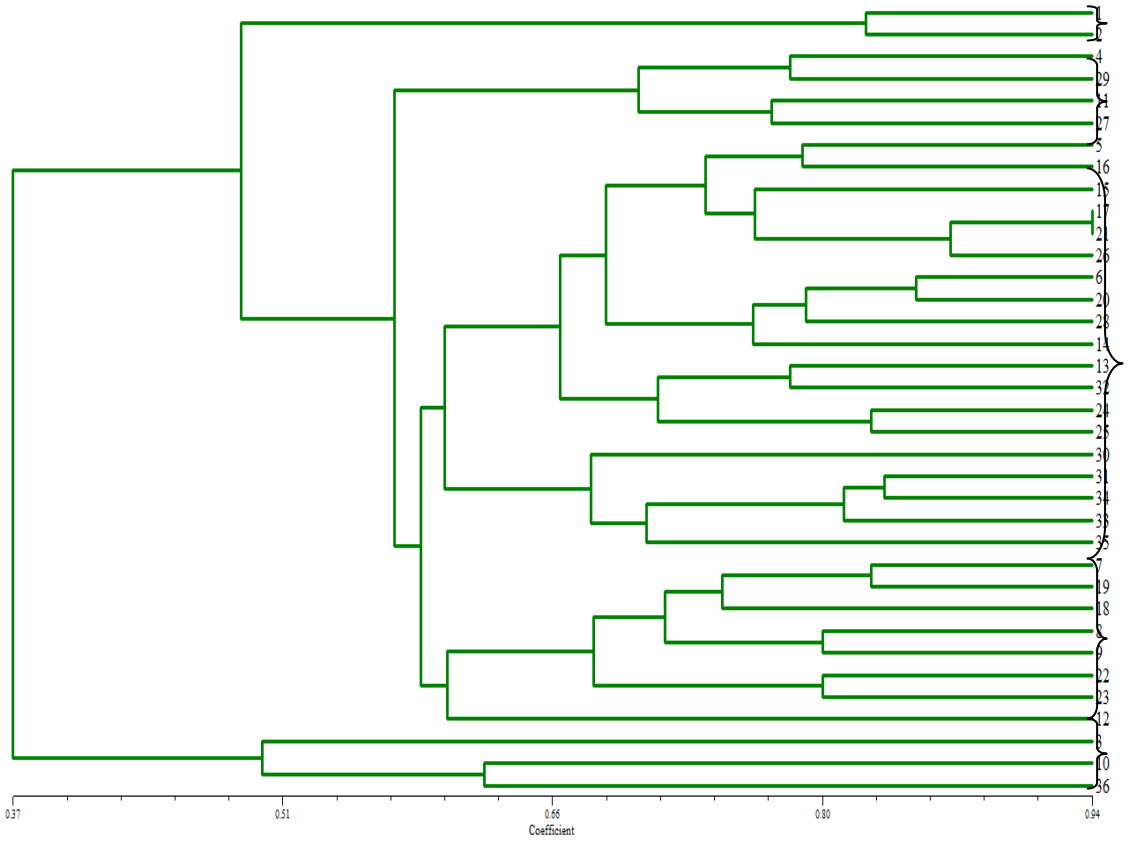

Figure 3: Tree diagram of the genetic relationship of 36 rice lines studied

*Group III: consists of 19 lines, in which two lines 17 and 21 have genetic similarity coefficient of 0.94 (as of observation, these lines have almost similar genotypes in all 30 loci). The remaining lines with genetic similarities were from 0.7-0.9. With such genetic similarity coefficient, it is shown that the obtained lines have a very close genetic relationship as following:

*Group IV: includes 8 lines: 7, 8, 9, 12, 18, 19, 22 and 23

*Group V: consists of 3 lines: 3 10, 36 with genetic similarity coefficients that werefluctuated around 0.6 and shown the lowest in the study.

Genetic homology coefficients of 36 rice lines including the original varieties were ranged from 66% - 94%. The two lines with the highest ratios of homology are lines 17 and 21, accounting for 94%. Therefore, it found that similar agronomic traits like the growth duration, the shape of seeds and amylose content have ranged in the same group.

Conclusions

In conclusion, by using 30 SSR markers to evaluate the genetic relationship of 36 mutant rice lines. A total of 1075 DNA bands involving in 51 different alleles. PIC coefficients of 30 primers vary from 0.0 to 0.68 (average 0.2).The mutant rice lines studied had relatively high genetic purity. At the genetic similarity of 66% - 94%, 36 lines were divided into 5 different groups with a specific difference in important agronomic traits as the growth duration, the shape of seeds and amylose content. The obtained results in this study may provide useful information to further use the mutant rice materials for the breeding program.

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