

Results of Genetic Diversity Assessment of Local Rice Genetic Resources

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ABSTRACT: According to some research results, rice genetic resources in the Northern Mountainous Region of Vietnam are the most diverse in the world. Research on genetic diversity and classification of genetic resources is the first work to be carried out, which is not only meaningful in the conservation of indigenous rice varieties but also in the exploitation, development and breeding of specialty rice genetic resources. Nowadays, molecular markers have been widely used as a useful tool in genetic research, and allow evaluation of a large number of loci throughout the rice genome. Information on genetic diversity at the DNA level can detect the smallest differences between varieties, thereby helping to quickly and accurately identify rare and precious varieties in the populations of indigenous rice varieties.

KEYWORDS: Rice, Nitrogen fertilizer, rice embryo, larger embryo, lipids content, γ -oryzanol content

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I. INTRODUCTION

According to some research results, rice genetic resources in the Northern Mountainous Region of Vietnam are the most diverse in the world. Research on genetic diversity and classification of genetic resources is the first work to be carried out, which is not only meaningful in the conservation of indigenous rice varieties but also in the exploitation, development and breeding of specialty rice genetic resources. Nowadays, molecular markers have been widely used as a useful tool in genetic research, and allow evaluation of a large number of loci throughout the rice genome. Information on genetic diversity at the DNA level can detect the smallest differences between varieties, thereby helping to quickly and accurately identify rare and precious varieties in the populations of indigenous rice varieties.

DNA markers are widely and effectively used in studying genetic structure and evolutionary process of rice, clarifying the purity of breeding materials, etc., because these are co-dominant markers for high and stable polymorphisms. Currently, more than 15,000 DNA markers have been established, and covered on the genetic association map of rice (Giarrocco et al., 2007). Many studies on genetic diversity using SSR and DNA fingerprinting to identify rice varieties have been published in recent years. In this research paper, DNA markers are used to study genetic diversity of 25 rice genetic resources collected in 3 districts of Son La province, located in the Son La hydropower reservoir bed, helping to identify varieties, detect possibility of duplication, and conserve indigenous rice genetic resources.

II. RESEARCH METHODOLOGY AND CONTENT

2.1. Experimental objects and materials

Objective: Analyze genetic diversity of 25 rice varieties using DNA markers

2.2. Research materials and methods

2.2.1. Materials: 25 rice DNA samples with symbol: DNA01-DNA25

2.2.2. Research methods

2.2.2.1. Sampling: Seeds are soaked to germinate so that fresh leaf samples are collected in paper bags and stored at 4°C for 1 week for DNA extraction.

2.2.2.2. DNA extraction: DNA of rice leaf samples is extracted in accordance with the DNA extraction method of Dellaporta et al., 1983

Cut the vacuum-dried leaf samples with forceps into 0.5-4 cm pieces into a 1.5 mL tube which the name of varieties has already been marked on; Grind the samples with a bead shaker at 1800 rpm, 60s, rest for 10s each time, 2 repetitions; Add 600 μ l extraction buffer (10 mM Tris, pH 8.0; 1 mM EDTA, pH 8.0) (incubated at 65°C)

and mix well. Then incubate the samples at 65°C for 30 minutes; Add 5M potassium acetate equal to 1/3 of the volume of extraction buffer. Incubate the samples in the refrigerator for 30 minutes; Centrifuge at 12000 rpm for 15 minutes at 4°C; Carefully aspirate the supernatant (approximately 400 µl) into a new eppendorf tube; Add an equal amount of Isopropanol, and mix well; Centrifuge at 14000 rpm for 30 minutes, at 4°C; Carefully discard the supernatant, avoid pouring the solution into another well and not dropping the precipitate; Wash the precipitate with 200µl of 70% ethanol, do not shake, just gently drip; Centrifuge at 14000 rpm, for 10 minutes at 4°C, discard the supernatant; Remove all ethanol at room temperature or at 37°C; Add 100µl of deionized water to dissolve the DNA precipitate. Check the total DNA obtained using electrophoresis for 20 min at 100 V, use 1% agarose gel premixed with ethidium bromide 1 µg/mL. Observe the results of electrophoresis under UV light and take pictures.

2.2.2.3. PCR: PCR is performed with denaturation step at 95°C for 5 minutes, followed by 35 cycles, the conditions for each subsequent cycle are as follows: denaturing at 95°C for 30 seconds, binding primers at 55°C depending on each primer for 30 seconds, extending the strand at 72°C for 1 minute, and ending the extension at 72°C for 7 minutes. Keep the PCR sample at a temperature below 15°C. PCR product is separated by electrophoresis in 2% agarose gels, using the loading dye with available ABT Gelred DNA dye at 150V for 40 minutes. Observe the results of electrophoresis under UV light and take pictures.

Table 2. List of 35 SSR markers

Marker	Chr.	Position cM	Anneal temp	PCR Cycles
RM495*	1	2.8	55	30
RM283*	1	31.4	61	30
RM237*	1	115.2	55	30
RM431*	1	178.3	55	30
RM154*	2	4.8	61	30
RM452*	2	58.4	61	30
OSR13*	3	53.1	53	40
RM338*	3	108.4	55	40
RM514*	3	216.4	55	30
RM124*	4	150.1	67	30
RM507*	5	0	55	30
RM413*	5	26.7	53	30
RM161*	5	96.9	61	30
RM133*	6	0	63	30
RM162*	6	108.3	61	30
RM125*	7	24.8	63	30
RM455*	7	65.7	57	30
RM118*	7	96.9	67	30
RM408*	8	0	55	30
RM152*	8	9.4	53	40
RM44*	8	60.9	53	30
RM284*	8	83.7	55	30
RM433*	8	116	53	40
RM447*	8	124.6	55	30
RM316*	9	1.8	55	30
RM215*	9	99.4	55	30
RM271*	10	59.4	55	30
RM484*	10	97.3	55	30
RM536*	11	55.1	55	30
RM277*	12	57.2	55	30

RM307	4	0	55	30
RM552	11	40,6	55	30
RM19	12	40,9	55	30
RM105	9	32,1	63	30
RM474	10	0	55	30

2.2.2.4. Genetic diversity analysis

The genetic diversity tree is constructed using the unweighted pair-group method with arithmetic mean (UPGMA) in which the DNA bands obtained by electrophoresis of PCR product using each marker are considered as alleles, and are denoted by 1 in the variety sample with DNA band and 0 in the variety sample without the corresponding DNA band. All allele types of all varieties are entered directly using NTedit software. The digitized data is then analyzed for similarity using NTSYS-pc version 2.10, thereby the pairs of variety samples, branches of the genetic diversity tree represent the similarity of the group.

The Polymorphic Information Content (PIC) coefficient of each SSR locus is calculated under the formula: $PIC(i) = 1 - \sum P_{ij}^2$ (Weir, 1996). Where: P_{ij} is the frequency of j^{th} allele with i^{th} SSR locus.

2.3. Results

2.3.1. DNA extraction results

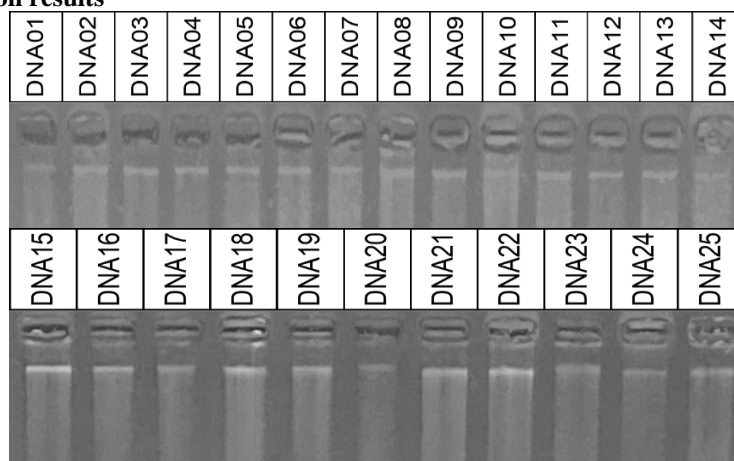


Figure 1. Test results of total DNA of 25 samples

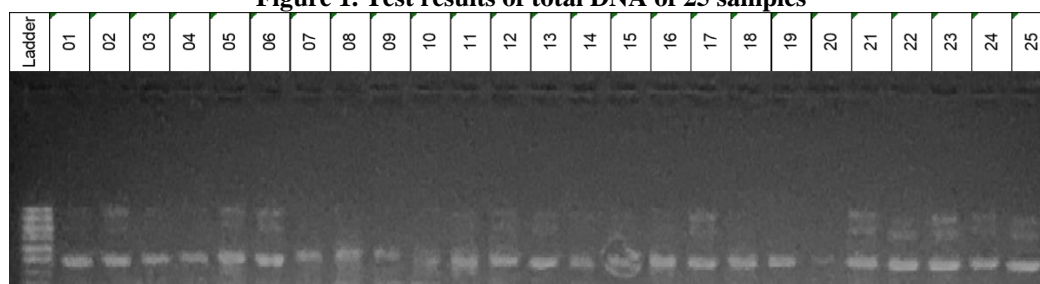


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM495 marker

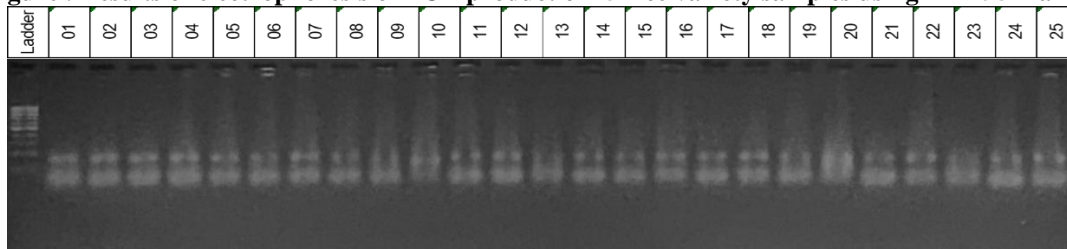


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM283 marker

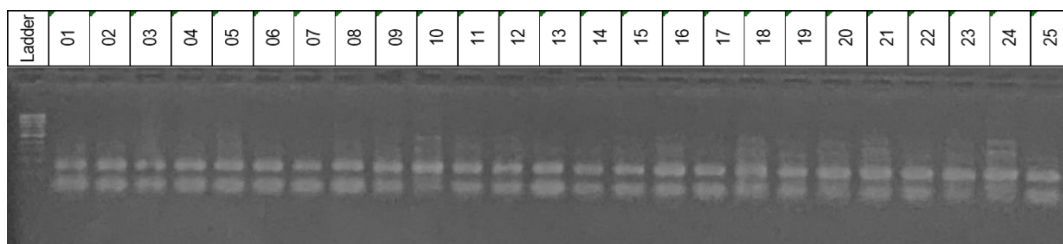


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM237 marker

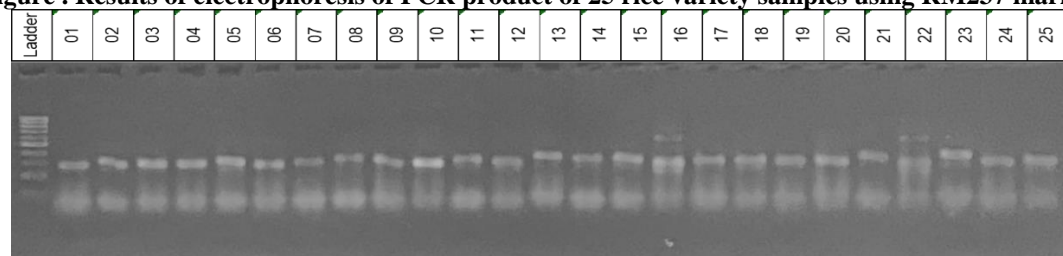


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM431 marker

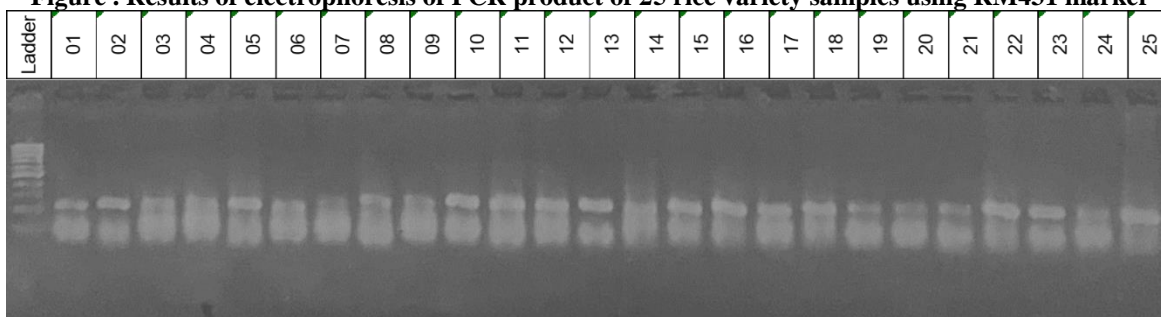


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM452 marker



Figure . Results of electrophoresis of PCR product of 25 rice variety samples using OSR13 marker

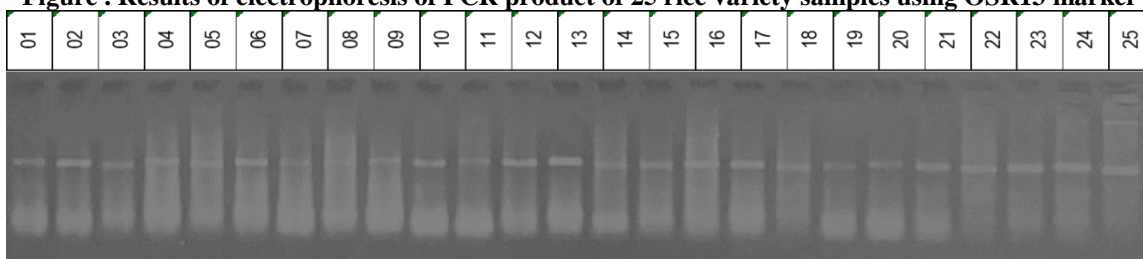


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM338 marker



Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM514 marker

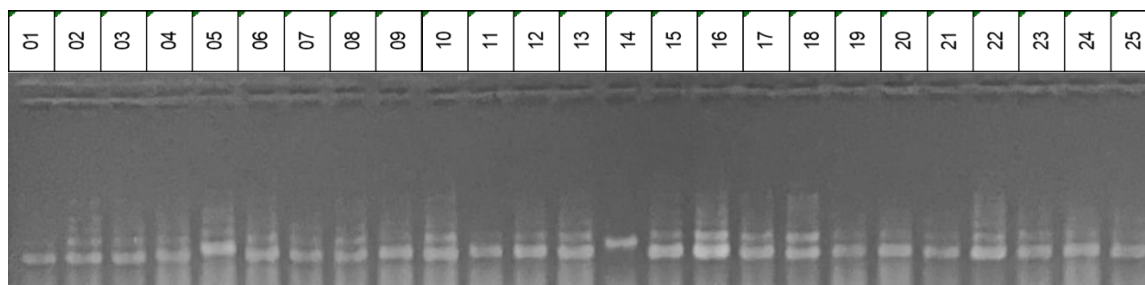


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM507 marker

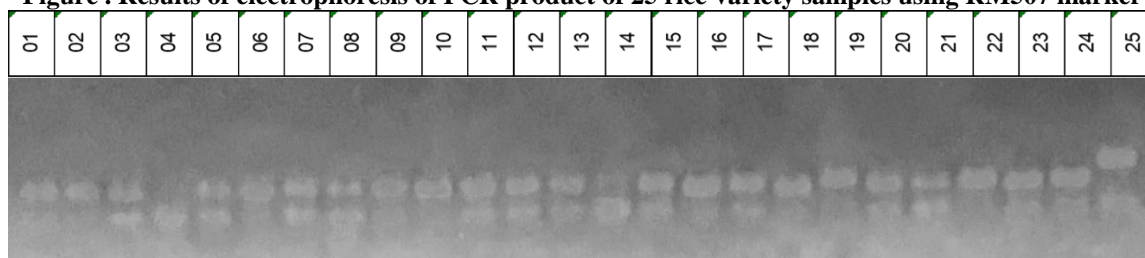


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM413 marker

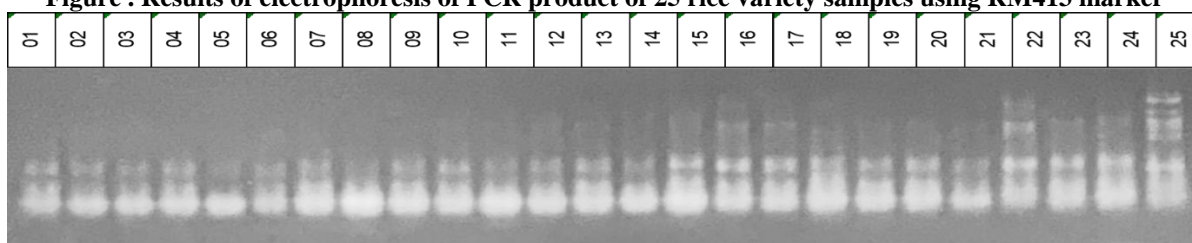


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM161 marker

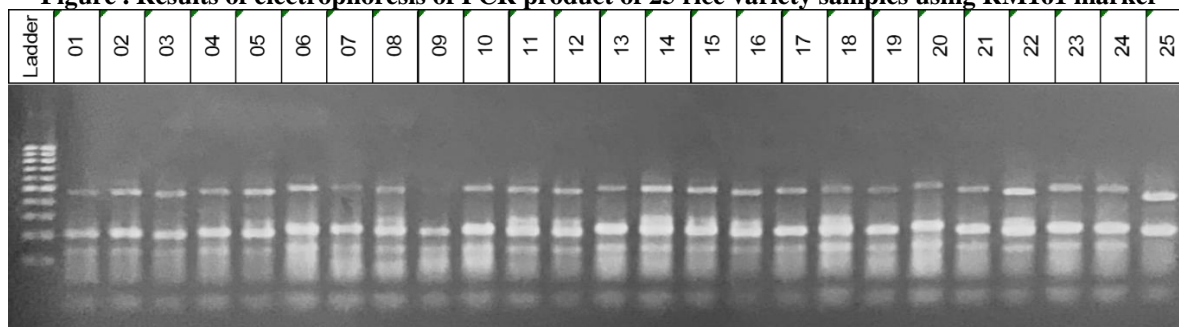


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM133 marker



Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM162 marker

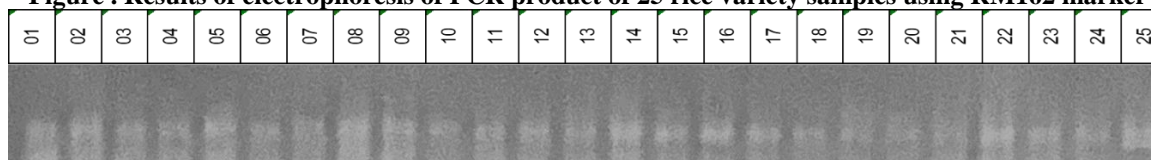


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM125 marker

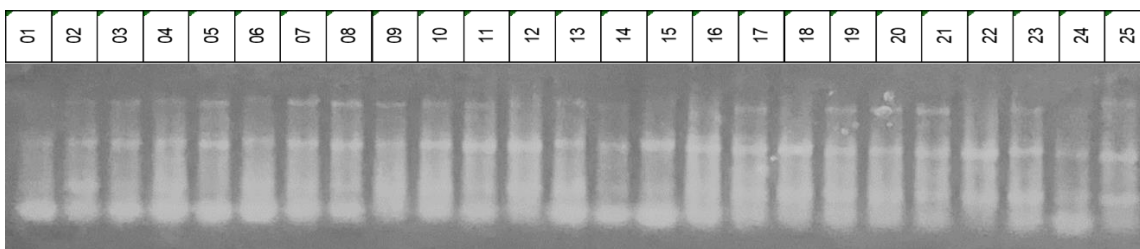


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM455 marker

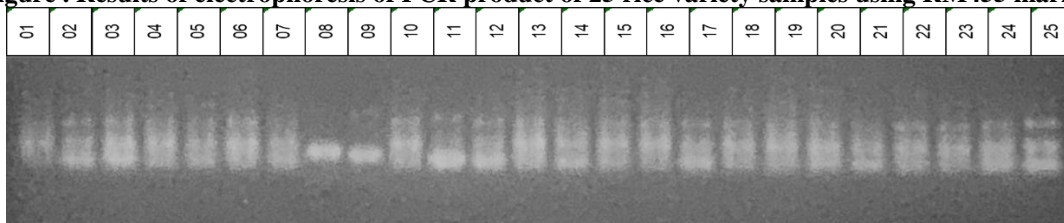


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM118 marker

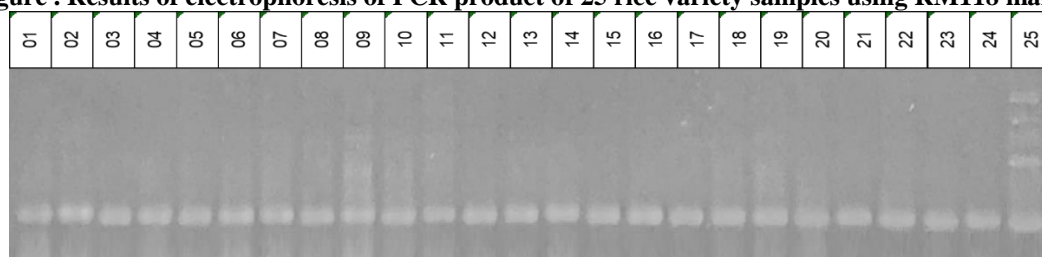


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM408 marker

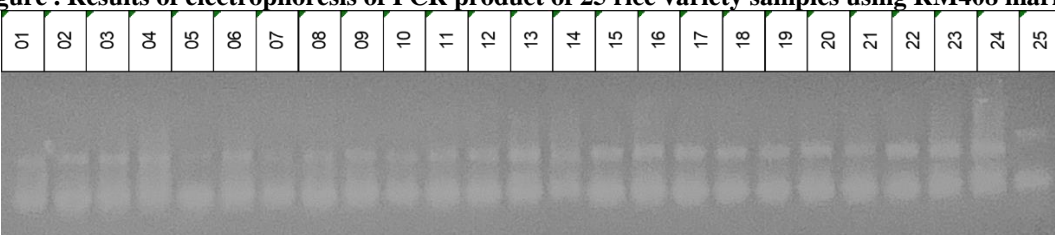


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM152 marker

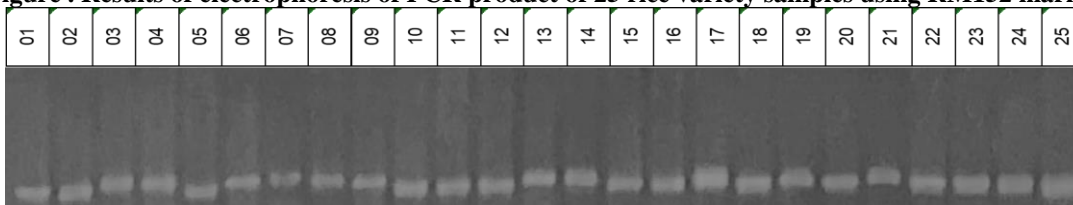


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM284 marker

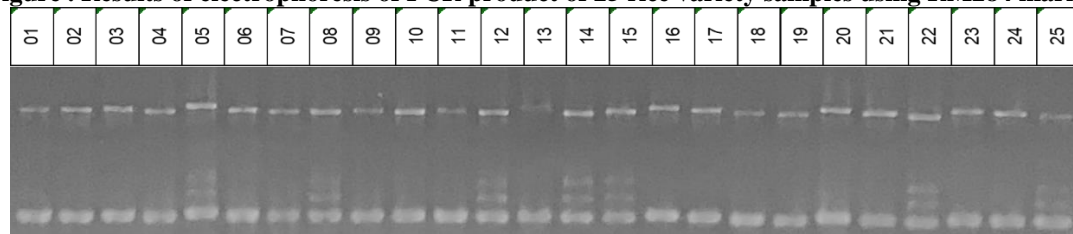


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM277 marker

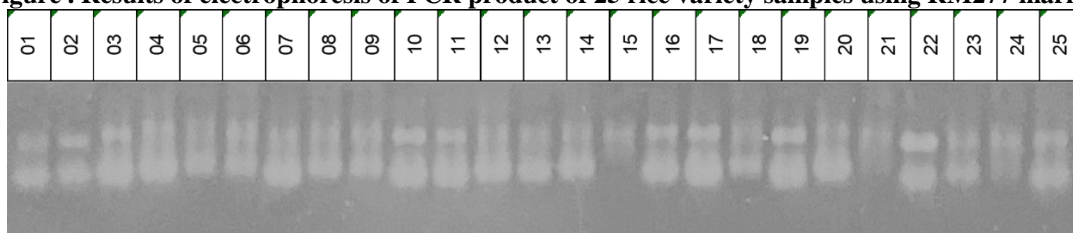


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM307 marker



Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM552 marker

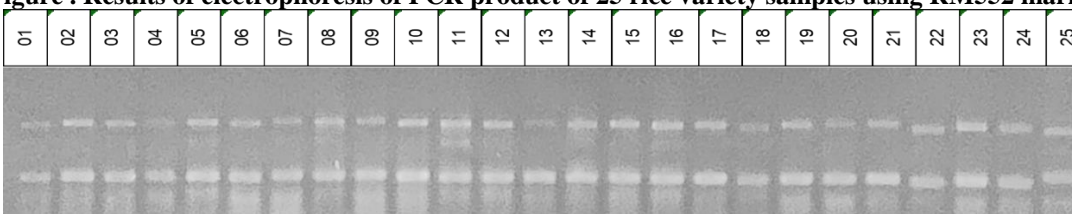


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM19 marker

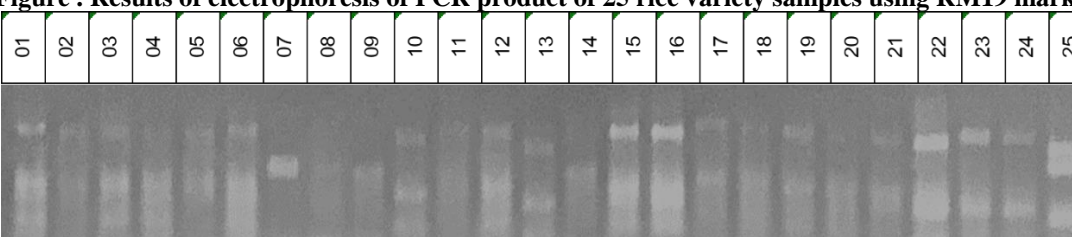


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM105 marker

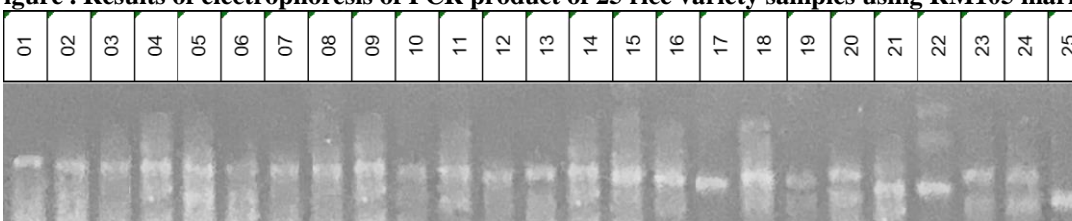


Figure . Results of electrophoresis of PCR product of 25 rice variety samples using RM474 marker

The results of determining the degree of genetic similarity

	DNA1	DNA2	DNA3	DNA4	DNA5	DNA6	DNA7	DNA8	DNA9	DNA10	DNA11	DNA12	DNA13	DNA14	DNA15	DNA16	DNA17	DNA18	DNA19	DNA20	DNA21	DNA22	DNA23	DNA24	DNA25
DNA1	1,00																								
DNA2	0,89	1,00																							
DNA3	0,88	0,89	1,00																						
DNA4	0,87	0,83	0,89	1,00																					
DNA5	0,76	0,77	0,81	0,75	1,00																				
DNA6	0,89	0,88	0,84	0,83	0,80	1,00																			
DNA7	0,84	0,83	0,84	0,88	0,82	0,86	1,00																		
DNA8	0,73	0,75	0,81	0,75	0,86	0,70	0,84	1,00																	
DNA9	0,80	0,78	0,80	0,76	0,67	0,86	0,81	0,75	1,00																
DNA10	0,82	0,83	0,84	0,81	0,72	0,81	0,78	0,75	0,78	1,00															
DNA11	0,73	0,72	0,78	0,67	0,76	0,77	0,72	0,83	0,84	0,75	1,00														
DNA12	0,77	0,81	0,82	0,73	0,84	0,78	0,76	0,84	0,69	0,73	0,82	1,00													
DNA13	0,83	0,80	0,86	0,82	0,73	0,84	0,82	0,73	0,82	0,84	0,76	0,77	1,00												
DNA14	0,71	0,70	0,78	0,75	0,83	0,72	0,77	0,86	0,72	0,70	0,81	0,87	0,71	1,00											
DNA15	0,80	0,78	0,82	0,78	0,84	0,78	0,78	0,82	0,76	0,81	0,80	0,86	0,80	0,82	1,00										
DNA16	0,71	0,72	0,73	0,67	0,78	0,70	0,70	0,73	0,67	0,75	0,78	0,75	0,69	0,73	0,84	1,00									
DNA17	0,83	0,89	0,88	0,80	0,78	0,82	0,80	0,76	0,77	0,82	0,76	0,82	0,83	0,73	0,84	0,78	1,00								
DNA18	0,80	0,81	0,77	0,76	0,75	0,81	0,78	0,75	0,78	0,86	0,80	0,81	0,77	0,77	0,86	0,82	0,82	1,00							
DNA19	0,87	0,81	0,82	0,83	0,75	0,90	0,86	0,75	0,93	0,83	0,80	0,71	0,84	0,75	0,81	0,72	0,80	0,83	1,00						
DNA20	0,83	0,80	0,83	0,82	0,71	0,84	0,84	0,78	0,87	0,82	0,81	0,72	0,81	0,76	0,80	0,73	0,78	0,77	0,89	1,00					
DNA21	0,82	0,78	0,82	0,73	0,80	0,83	0,78	0,80	0,81	0,81	0,84	0,78	0,80	0,77	0,76	0,75	0,80	0,73	0,83	0,87	1,00				
DNA22	0,69	0,72	0,73	0,63	0,73	0,67	0,60	0,71	0,63	0,70	0,73	0,80	0,69	0,71	0,80	0,78	0,76	0,72	0,65	0,64	0,70	1,00			
DNA23	0,80	0,76	0,82	0,76	0,75	0,83	0,76	0,72	0,81	0,78	0,80	0,76	0,87	0,70	0,81	0,75	0,84	0,73	0,86	0,82	0,86	0,75	1,00		
DNA24	0,80	0,76	0,80	0,76	0,70	0,81	0,71	0,70	0,78	0,78	0,80	0,73	0,80	0,67	0,78	0,75	0,80	0,76	0,81	0,84	0,88	0,72	0,90	1,00	
DNA25	0,57	0,60	0,61	0,58	0,61	0,63	0,58	0,59	0,55	0,51	0,61	0,70	0,59	0,64	0,60	0,52	0,64	0,53	0,53	0,57	0,63	0,66	0,63	0,65	1,00

The results of building the genetic diversity tree

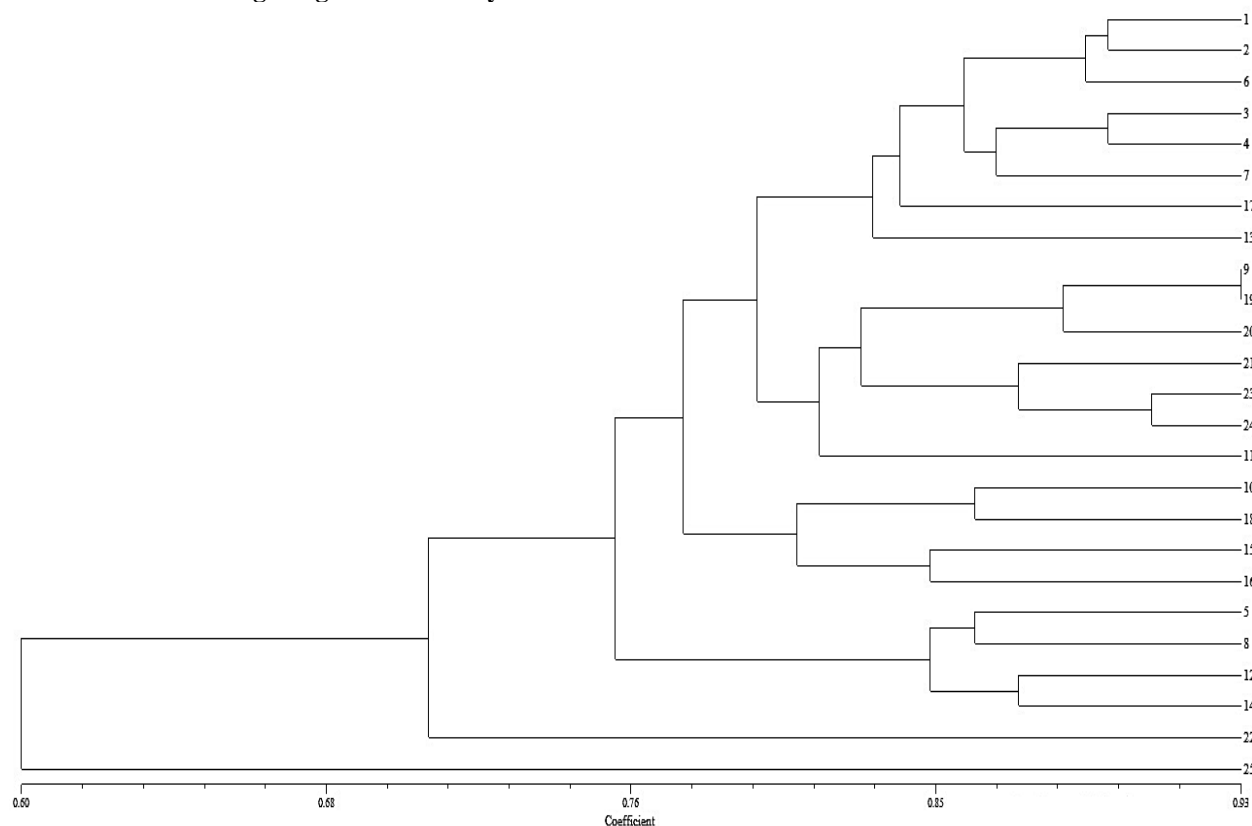


Figure . The genetic diversity tree of 25 rice variety samples

2.3.3. Polymorphic Information Content (PIC) values of 25 markers used to assess genetic diversity

No.	Primer	Locus	Polymorphic locus	Rate (%)	PIC
1	RM495	5	3	60	0.30
2	RM283	2	2	100	0.21
3	RM237	4	2	50	0.22
4	RM431	2	1	50	0.07
5	RM452	1	0	0	0.00
6	OSR13	6	5	83	0.38
7	RM338	2	1	50	0.04
8	RM514	2	2	100	0.21
9	RM507	5	5	100	0.25
10	RM413	3	3	100	0.22
11	RM161	5	4	80	0.14
12	RM133	4	3	75	0.15
13	RM162	6	4	67	0.30
14	RM125	2	1	50	0.04
15	RM455	4	3	75	0.20
16	RM118	3	3	100	0.39
17	RM408	3	2	67	0.05
18	RM152	1	0	0	0.00
19	RM284	3	2	67	0.17
20	RM277	4	2	50	0.20
21	RM307	1	0	0	0.00
22	RM552	4	3	75	0.28

23	RM19	4	2	50	0.13
24	RM105	5	5	100	0.37
25	RM474	2	2	100	0.32

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