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ANALYSIS OF GENETIC DIVERSITY OF 26 CASSAVA VARIETIES (*Manihot esculenta* CRANTZ) WITH DIFFERENT RESPONSES TO WATERLOGGING CONDITIONS BY USING RAPD MARKERS

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Abstract. RAPD (Randomly Amplified Polymorphic DNA) is an indicator for high and stable polymorphism, widely used in the study of the diversity of cassava. In this paper, the results of using 20 polymorphic primers OPK combined with the establishment of the phylogenetic tree to analyze the genetic diversity of 26 cassava varieties with different responses to waterlogging conditions by using the RAPD-PCR technique were presented. The purpose of this experiment was to show the genetic relevance of the studied cassava varieties. The results showed that the flood tolerance of cassava was not related to the polymorphism and branching characteristics of the stem. This information may be use as a basis for selecting flood-tolerant cassava varieties for cassava production, as well as the basis for selecting genetically different parents for breeding.

Keywords: cassava, RAPD marker, genetic diversity, primer.

1. Introduction

Cassava is an important food crop in the world with characteristics such as simple cultivation and good growth in nutrient-poor soil. In Vietnam, the role of cassava has changed from a food crop to an industrial crop, so the cassava growing area is being developed and expanded to include new areas. High-efficiency cassava production areas have large fluctuations in annual yield due to climate change, causing cassava growing areas to be flooded causing heavy losses to cassava yield [1]. Today, molecular markers are widely applied in genetic research and allow the assessment of a number of loci on the genome of many plant species. Information about genetic diversity at the DNA level can detect the smallest differences between breeds, this helps researchers to quickly and accurately distinguish cultivars with the required characteristics in crop groups [2].

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The Random Amplified Polymorphic DNA (RAPD) is a simple dominant marker system widely used in Biology. The effectiveness of RAPD can be improved by selecting and redesigning primers whose priming sites occur in the target sequence(s) of gene or organism at an optimum distance. RAPD molecular marker for its high and stable polymorphism is widely used in the study of genetic structure, evolution, clarification of the purity of breeding materials and analysis of genetic diversity of cassava varieties have different phenotypes [3-5]. RAPD primers are of decamer (10 base pairs) size and are randomly generated. The success of Polymerase Chain Reaction (PCR) is highly dependent on these short arbitrary oligonucleotides that hybridize onto the complementary DNA fragments [6]. Therefore, the study of the genetic diversity of cassava varieties that responded differently to waterlogging conditions had the purpose to confirm the relevance of the experimental cassava varieties with good tolerance to waterlogging conditions to serve as a basis for analyzing the differences in genetic variation and breeding cassava varieties with good flood tolerance.

2. Content

2.1. Materials and methods

2.1.1. Plant materials

In this study, 26 varieties of cassava from the Institute of Agricultural Genetics were used as materials. These materials were categorized into three groups, including poor waterlogging tolerance (21, 22, 24, Hanoi3, C10, C13, C34, C42, C66, C71, C84, C86, and 50C49), medium waterlogging tolerance (C15, C23, C25, C31, C53, C58, BK, HL-S12, and Hanoi5) and good waterlogging tolerance (26, 31, C60 and KU50).

Table 1. General characteristics of 26 cassava varieties

No.	Varieties	ID	Pedigree		Type of response to waterlogging	Characte-ristics of stem
1	BK	BK			Medium	
2	KU50 mutation	50C49			Poor	Branching
3	KU50	KM94	Rayong 1 * Rayong 90		Good	Branching
4	HL-S12	HL-S12			Medium	
5	CM 9912-167	21			Poor	
6	GM 214-62	22			Poor	
7	GM 1263-6	24			Poor	
8	GM 1406-13	26			Good	
9	SM 1521-10	31			Good	

10	IITA-TMS-IBA980505	Hanoi3			Poor	
11	IITA-TMS-IBA920057	Hanoi5			Medium	
12	AR 12-11	C10	C-33 * CW234-2		Poor	Branching
13	AR 36-9	C13	C-127 * CW259-10		Poor	
14	AR 37-32	C15	C-33 * CW259-42		Medium	Branching
15	CR 52A-2	C23	C-243 * SM1219-9		Medium	
16	AR 1-152	C25	C-127 * CW257-12		Medium	
17	AR 9-34	C31	C-243 * CW257-12		Medium	
18	CR 15B-1	C34	C-33 * CM523-7		Poor	Branching
19	AR 35-1	C42	C-243 * CW257-10		Poor	
20	AR 12-37	C53	C-33 * CW234-2		Medium	Branching
21	AR 14-3	C58	C-33 * CW257-10		Medium	
22	AR 15-6	C60	C-33 * CW257-12		Good	Branching
23	AR 17-23	C66	C-33 * CW258-17		Poor	Branching
24	AR 42-3	C71	C-413 * CW259-42		Poor	
25	AR 1-1	C84	C-127 * CW257-12		Poor	
26	AR 7- 5	C86	C-127 * CW234-2		Poor	Branching

Note: branching characteristics of cassava stems as described by Ha et al., 2015 [7].

2.1.2. DNA extraction

The DNA extraction method was carried out as previously described by Osena et al., 2017 [8]. Particularly, 100 mg of young cassava leaves (the 3rd leaf from the top) were

used to ground into powder, then added 500 µL of Cetyltrimethylammonium Bromide (CTAB) solution and 150 µL of Sodium Dodecyl Sulphate (SDS) solution. Incubate the sample in a temperature bath of 55 ºC for 20 minutes with inversions of the microtubes 3 - 5 times after every 5 minutes. The homogenate was centrifuged at 13,800 rpm at room temperature for 5 minutes, transfer the supernatant to a new microtube and add chloroform-isoamyl alcohol (24:1) to equal the volume and mixed by inversion for 5 min (repeat 3 times). Then the sample was centrifuged at 13,800 rpm at room temperature for 5 minutes and added 50 µL of 7.4 M ammonium acetate and 2 volume of ice-cold absolute ethanol, and incubate at -20 ºC for 20 min. Centrifuge at 10,000 rpm for 5 min at room temperature. The pellet was washed 2 times with 500 µL of a wash solution (75% ethanol and 15 mM ammonium acetate), pour out the wash solution and the pellet was dried by inverting the microtube onto the paper for 10 minutes or until the alcohol has evaporated. For DNA purification, add 70 µL of TE buffer and 3 µL of 10 mg/mL ribonuclease A to digest RNA. Then incubate the DNA at 37 ºC for 30 min in a water bath and store it at -20 ºC for further use. Purity was checked by a nanodrop machine combined with electrophoresis on 1% agarose gel.

2.1.3. PCR procedure

Conducted polymorphism analysis with the number of RAPD reactions was 26 DNA samples of cassava lines/varieties + 1 negative control sample × 20 polymorphic primers OPK (random primer). PCR technique was conducted with a total volume of 10 µL/sample, the mixture included the following components: 1 µL DNA 100 ng; 1 µL primer; 5 µL Dream Taq Master Mix 2X and 3 µL water. PCR reaction cycle includes 6 steps, specifically as follows: step 1: 94 ºC - 4 minutes; step 2: 94 ºC - 1 min; step 3: 35 ºC - 1 min; step 4: 72 ºC - 2 min; repeat 45 cycles from step 2 to step 4; step 5: 72 ºC - 6 min; kept at 10 ºC.

Table 2. List of polymorphic primers used in genetic diversity assessment

Primer	Sequence 5'-3'	Primer	Sequence 5'-3'
OPK1	CATTCGAGCC	OPK11	AATGCCCCAG
OPK2	GTCTCCGCAA	OPK12	TGGCCCTCAC
OPK3	CCAGCTTAGG	OPK13	GGTTGTACCC
OPK4	CCGCCCAAAC	OPK14	CCCGCTACCC
OPK5	TCTGTCGAGG	OPK15	CTCCTGCCAA
OPK6	CACCTTTCCC	OPK16	GAGCGTCGAA
OPK7	AGCGAGCAAG	OPK17	CCCAGCTGTG
OPK8	GAACACTGGG	OPK18	CCTAGTCGAG
OPK9	CCCTACCGAC	OPK19	CACAGGCGGA
OPK10	GTGCAACGTG	OPK20	GTGTCGCGAG

The primers on this list are based on the research of Marmey et al., 1993 [3].

2.1.4. Electrophoresis

PCR products with primer OPK were denatured and electrophoresed on 1% agarose gel using a Mupid-2plus-Optima machine. The gel was fixed with TE + 5 µL nucleic acid staining solution and stained with Fastgene 100 bp DNA ladder RTU 50 µg/500 µL, then examined under UV light and photographed.

2.1.5. Statistical analysis

The data was analyzed by using PyElph 1.3 software to set up a binary matrix. The bands on the gel were data coded according to binary encoding with the divisor not numbered band 0, all samples have band number 1. Using NTSYS pc 2.1 software: determine heritability and build a phylogenetic tree based on similarity coefficients.

2.2. Results and discussions

The results of DNA extraction and DNA content used in PCR reaction were shown in Table 3 as follows:

Table 3. Results of DNA extraction and DNA content for use in PCR reactions

No.	Sample ID	Nucleic Acid Conc.	Unit	260/280	260/230	PCR 50 (µL)	
						H_20 (µL)	DNA (µL)
1	21	1278.2	ng/µL	1.9	1.61	46	4
2	22	812.9	ng/µL	1.88	1.4	44	6
3	24	898.9	ng/µL	1.84	1.28	45	5
4	26	960.2	ng/µL	1.95	1.56	45	5
5	31	309.1	ng/µL	1.91	1.41	34	16
6	Hanoi3	640.4	ng/µL	2.16	1.75	42	8
7	Hanoi5	553.9	ng/µL	1.77	1.32	41	9
8	C10	1397.2	ng/µL	1.96	1.82	46.5	3.5
9	C13	3300.1	ng/µL	1.79	1.46	48.5	1.5
10	C15	1062.1	ng/µL	1.94	1.75	45	5
11	C23	1709.5	ng/µL	1.96	1.93	47	3
12	C25	595.3	ng/µL	1.93	1.55	42	8
13	C31	1786.2	ng/µL	1.87	1.61	47	3
14	C34	674	ng/µL	1.91	1.41	43	7
15	C42	1079.6	ng/µL	1.91	1.8	45.5	4.5
16	C53	699.4	ng/µL	1.88	1.44	43	7
17	C58	1631.3	ng/µL	1.94	1.75	47	3
18	C60	278.5	ng/µL	1.91	1.06	32	18

19	C66	804.2	ng/µL	1.75	1.39	44	6
20	C71	909.5	ng/µL	1.89	1.58	44.5	5.5
21	C84	762	ng/µL	1.85	1.28	43.5	6.5
22	C86	1182.7	ng/µL	2.04	1.98	46	4
23	BK	687	ng/µL	1.78	1.23	43	7
24	KU50	219.9	ng/µL	2.01	1.39	28	22
25	HLS-12	296.7	ng/µL	1.72	0.84	33	17
26	50C49	452.7	ng/µL	1.76	1.02	39	11

Note: nucleic acid conc. = nucleic acid concentration

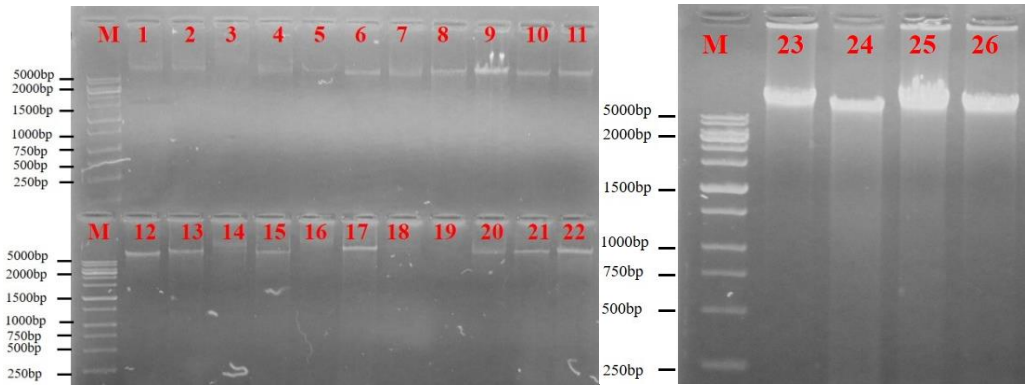

Figure 1. Electrophoresis of total DNA extracted from leave of 26 cassava lines/varieties in order 1-26: 21, 22, 24, 26, 31, Hanoi3, Hanoi5, C10, C13, C15, C23, C25, C31, C34, C42, C53, C58, C60, C66, C71, C84, C86, BK, KU50, HL-S12 and 50C49. M = DNA marker 1kb intron

In this experiment, 20 primers were used to analyze the DNA polymorphisms of 26 cassava lines/varieties with different responses to waterlogging. As a result of 20 Primer OPKs used, only 1 primer did not appear, polymorphic DNA band, which was OPK5 primer, and 18 primers appeared polymorphic DNA band, of which 2 primers that OPK9 and OPK15 primers, appeared DNA band but exhibits a monomorphic DNA. The remaining 16 primers showed polymorphic DNA of cassava varieties. This result proves that Primer OPK was capable of analyzing polymorphisms of cassava varieties with different responses to waterlogging conditions.

From the results of electrophoresis of PCR products, the software PyElph 1.3 was used to set up the binary matrix, the bands on the gel were coded according to the binary code table with the divisor fund no numbered band 0, any sample has tape number 1. NTSYS pc 2.1 software: was used to determine heritability and analyze genetic relationships that were similar to each other, thereby building a phylogenetic tree based on close the genetic similarity coefficients of each other (Figure 2) by the UPGMA program. From the phylogenetic tree, the similarities and relatedness of cassava varieties with different responses to waterlogging can be seen.

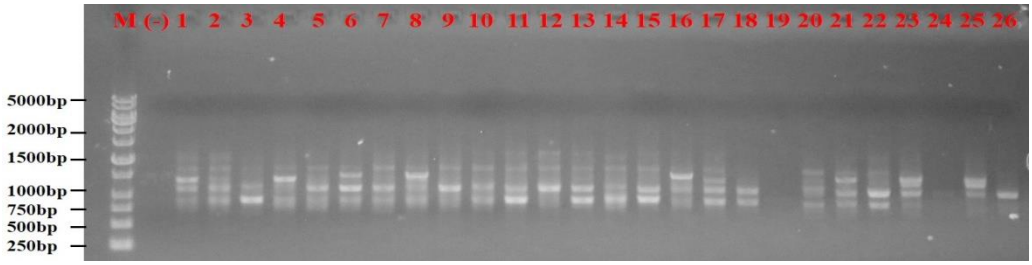

Figure 2. Electrophoresis results of PCR products using OPK4 primer representing 26 cassava lines/varieties in order 1-26: 21, 22, 24, 26, 31, Hanoi3, Hanoi5, C10, C13, C15, C23, C25, C31, C34, C42, C53, C58, C60, C66, C71, C84, C86, BK, KU50, HL-S12 and 50C49. M = DNA marker 1kb intron, (-) negative control

Table 4. The genetic similarity coefficients of 26 cassava varieties

ID	21	22	24	26	31	Ha noi3	Ha noi5	C10	C13	C15	C23	C25	C31
21	1												
22	0.78	1											
24	0.79	0.79	1										
26	0.75	0.73	0.78	1									
31	0.76	0.76	0.75	0.73	1								
Hanoi3	0.68	0.68	0.72	0.7	0.77	1							
Hanoi5	0.64	0.71	0.63	0.67	0.79	0.78	1						
C10	0.7	0.67	0.73	0.79	0.78	0.73	0.75	1					
C13	0.58	0.62	0.59	0.57	0.64	0.63	0.7	0.66	1				
C15	0.6	0.64	0.59	0.61	0.66	0.65	0.72	0.68	0.78	1			
C23	0.59	0.54	0.6	0.64	0.72	0.64	0.71	0.72	0.64	0.71	1		
C25	0.59	0.54	0.53	0.6	0.65	0.62	0.62	0.67	0.68	0.71	0.76	1	
C31	0.58	0.53	0.59	0.57	0.68	0.54	0.59	0.64	0.59	0.63	0.77	0.75	1
C34	0.58	0.66	0.63	0.63	0.64	0.61	0.61	0.66	0.67	0.72	0.71	0.73	0.76
C42	0.61	0.61	0.62	0.58	0.65	0.6	0.6	0.63	0.68	0.68	0.67	0.72	0.82
C53	0.55	0.62	0.63	0.63	0.58	0.61	0.57	0.66	0.63	0.63	0.64	0.64	0.67
C58	0.53	0.55	0.59	0.61	0.55	0.63	0.61	0.64	0.63	0.61	0.64	0.58	0.61
C60	0.58	0.6	0.61	0.57	0.62	0.57	0.5	0.55	0.5	0.52	0.6	0.55	0.57
C66	0.63	0.61	0.66	0.62	0.61	0.6	0.51	0.59	0.55	0.53	0.61	0.59	0.6
C71	0.6	0.58	0.63	0.61	0.55	0.52	0.5	0.64	0.57	0.57	0.62	0.53	0.65
C84	0.58	0.55	0.59	0.65	0.58	0.57	0.59	0.66	0.7	0.63	0.66	0.55	0.63
C86	0.61	0.59	0.6	0.64	0.7	0.62	0.66	0.7	0.68	0.62	0.65	0.65	0.64
BK	0.58	0.6	0.61	0.65	0.62	0.57	0.59	0.71	0.57	0.61	0.6	0.55	0.57
KU50	0.63	0.63	0.66	0.66	0.59	0.58	0.53	0.63	0.55	0.53	0.61	0.59	0.55
HL-S12	0.64	0.68	0.65	0.63	0.66	0.57	0.61	0.64	0.63	0.65	0.62	0.53	0.57
50C49	0.6	0.58	0.61	0.65	0.55	0.54	0.5	0.62	0.57	0.52	0.58	0.6	0.54

Table 5. The genetic similarity coefficients of 26 cassava varieties (continues)

ID	C34	C42	53	58	C60	C66	C71	C84	C86	BK	KU50	HL-S12	50C49
C34	1												
C42	0.77	1											
53	0.8	0.73	1										
58	0.7	0.66	0.78	1									
C60	0.7	0.62	0.72	0.61	1								
C66	0.68	0.63	0.71	0.58	0.88	1							
C71	0.67	0.66	0.78	0.72	0.7	0.66	1						
C84	0.7	0.75	0.78	0.74	0.65	0.64	0.78	1					
C86	0.6	0.72	0.62	0.62	0.64	0.61	0.62	0.73	1				
BK	0.61	0.64	0.63	0.61	0.63	0.58	0.7	0.7	0.68	1			
KU50	0.68	0.63	0.71	0.58	0.86	0.93	0.64	0.66	0.63	0.62	1		
HL-S12	0.67	0.62	0.65	0.61	0.7	0.66	0.67	0.67	0.68	0.74	0.68	1	
50C49	0.67	0.62	0.7	0.54	0.76	0.84	0.61	0.65	0.62	0.65	0.88	0.67	1

To analyze the genetic relationship of 26 cassava lines/varieties with good, medium, and poor tolerance to waterlogging, we used the analysis method by UPGMA program based on the genetic similarity coefficients created a phylogenetic tree of 26 cassava lines/varieties that responded differently to waterlogging conditions (Figure 3).

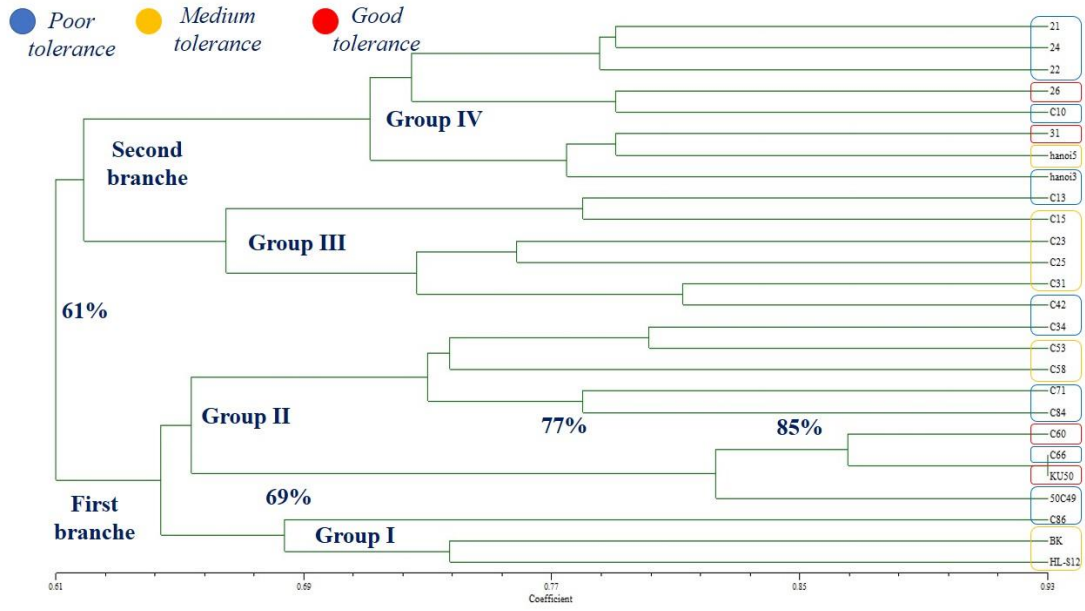

Figure 3. Phylogenetic tree of 26 cassava lines/varieties with different responses to waterlogging as determined by RAPD with primer OPK

The phylogenetic tree showed the relationship between the experimental cassava varieties. It was divided into two main branches with a genetic distance was 39%. The first branch consisted of 2 groups. Group I with genetic similarity coefficients (GSC) was 68%, there were 3 varieties, in which there were 2 varieties with medium tolerance (BK and HL-S12) with GSC were 74% and one poor tolerance variety (C86). Group II with GSC was 67%, including 9 varieties, of which there were 5 varieties with poor tolerance (50C49, C66, C84, C71, and C34) with different GSC, 2 varieties with medium tolerance (C53 and C58) with GSC closeness of 78% and 2 good tolerance varieties (C60 and KU50) with GSC of 86%. Group III with GSC was 63%, there were 6 varieties of which there were 2 poor tolerance varieties (C42 and C13) with 68% GSC and 4 medium tolerance varieties (C31, C25, C23, and C15) with GSC of 63%. Group IV had 8 varieties with GSC of 64%, of which there were 5 varieties with poor tolerance (Hanoi3, C10, 22, 24, and 21) with GSC of 68%, one variety with medium tolerance (Hanoi5) with 78% GSC and 2 good tolerance varieties (31 and 26) with 73% GSC. Theoretically, the higher the GSC between the varieties, the more closely related the cassava varieties, but the results showed that the distribution of some varieties on different tolerant groups had more GSC in the same group, as in group II KU50 (good tolerance) with C66 (poor tolerance) had a GSC of up to 93% while KU50 with C60 of the same good tolerance had GSC lower (86%) or in group IV 26 (good tolerance) and C10 (poor tolerance), in the same group 31 (good tolerance) with Hanoi5 (medium tolerance), they had GSC was 79% while 26 and 31 had GSC of 73%. When looking at the groups according to genetic similarity coefficients, it was found that most of the varieties also had the same distribution according to the response group, especially the poor and medium tolerance varieties but only the good tolerance group were quite distinctive, which showed that the nature of cassava was poor flood tolerance and the good tolerance of cassava had a specials genes that is not related to polymorphism. Therefore, there was no clear relationship between the subgroups according to the RAPD markers and the response of cassava to waterlogging conditions. RAPD markers are widely used for finding genetic diversity and genetic relationships in plants and animals [6]. The research genetic diversity of cassava majority assessing between cassava varieties at different locations, with wild types or comparing genetic diversity with morphological characteristics [9, 10, 11, 12] but there were no results on the assessment of physiological responses to adverse conditions of cassava as well as waterlogging conditions. According to the above results, we found that 20 OPK primers used in this study assessed quite accurately the genetic relationship between the cassava varieties.

3. Conclusions

The study of polymorphisms of 26 cassava lines/varieties that responded differently to waterlogging conditions by using OPK primer and technique RAPD-PCR analysis did not relate with the polymorphisms and branching characteristics of the stem of the experimental cassava varieties. This information may contribute to the basis for the selection of cassava varieties that are resistant to flooding for cassava production, as well as the basis for the selection of genetically different parents for breeding.

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