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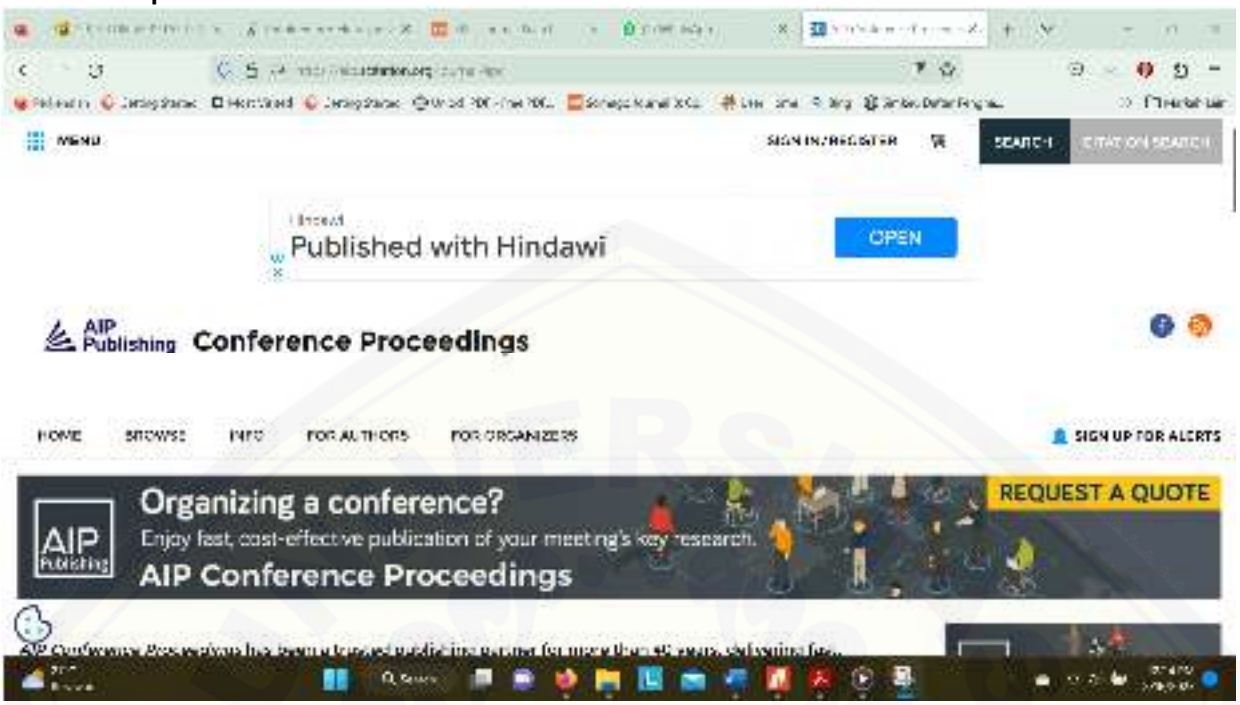

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Cite as: AIP Conference Proceedings **2583**, 020017 (2023); https://doi.org/10.1063/5.0117487
Published Online: 13 January 2023

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An Analysis on the Relationship and Genetic Diversity of Indonesian Cassava Accessions (*Manihot esculenta*)

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Abstract. The genetic improvement of Indonesian cassava is limited by the lack of knowledge about genetic diversity in circulating cassava clones. Good breeding results require information on the Relationship between the clones used. This study aims to study the genetic relationship between several Jember cassava clones through genotype and phenotype approaches. The genetic relationship between 22 cassava accessions was analyzed using RAPD and phenotypically using the key of determination. The genetic Relationship dendrogram results were obtained from 87 DNA bands and 18 phenotypic characters. The genetic relationships were analyzed using similarity coefficients and phenotypic genetic distances. Relationship analysis of 22 cassava accessions based on phenotypic characters yielded similarity coefficients ranging from 0.39 to 0.83. Relationship analysis of 22 cassava accessions based on RAPD markers, resulted in a similarity coefficient of 0.5-1.0, or 55 to 100%.

INTRODUCTION

Food diversification aims to support the sustainability of agriculture and national food security by reducing community dependence on rice and increasing consumption of corn, sago, and tubers such as potatoes, sweet potatoes, and cassava[1]. Cassava (Manihot esculenta Crantz) originates from Latin America, which can grow well in tropical and sub-tropical regions and is one of the rice substitutes that plays an important role in supporting food security in a region[2]. Cassava can grow in drought-prone environments, on soils that have low pH and even on soils with low nutrient content[3]. Farmers often use local cultivars and superior cultivars on the same land to maintain production stability in marginal areas[4].

Cassava has genetic variations that can be inherited. Several studies with morphological and agronomic characteristics reveal high diversity in cassava cultivars planted by farmers[5]. Plant breeding is one solution to increase

The 5th International Conference on Agriculture and Life Science 2021 (ICALS 2021)
AIP Conf. Proc. 2583, 020017-1–020017-12; https://doi.org/10.1063/5.0117487
Published by AIP Publishing. 978-0-7354-4216-0/$30.00

cassava productivity with various methods to improve the genetic character of plant. Research with molecular marker methods to determine population diversity is increasingly being conducted by researchers in the current-era of.DNA markers that are widely used to reveal genetic differentiation and relationships are markers of RAPD. The RAPD method is a method for identifying large amounts of DNA polymorphisms in the genome quickly and efficiently.

The diversity of the RAPD alleles is demonstrated by differences in migration of DNA bands in the gels of each sample. Based on the presence or absence of a band, the band profile is translated into binary data[6]. This binary data is processed to produce information on the genetic diversity of cassava in Jember districts, while the morphological data obtained from the results of qualitative variable scoring combined with quantitative variable values. Then it is converted into categorical data according to the similarity of each accession notation in DMRT advanced test for clustering through the NTSYSpc ver program. 2.02. The genetic enhancement of cassava in Indonesia, especially in Jember districts, is limited by a lack of knowledge about genetic diversity in cassava accession. This genetic information is expected to be a reference source for cassava breeding research in Jember districts.

MATERIALS AND METHODS

Study Area

The planting material consisted of 11 yellow cassava accessions and 11 cassava accessions from 11 sub-districts in Jember (Figure 1). Sampling was carried out by determining several sub-districts that could represent accessions of cassava planted in the southern, northern, eastern, and western part of Jember, East Java, Indonesia.

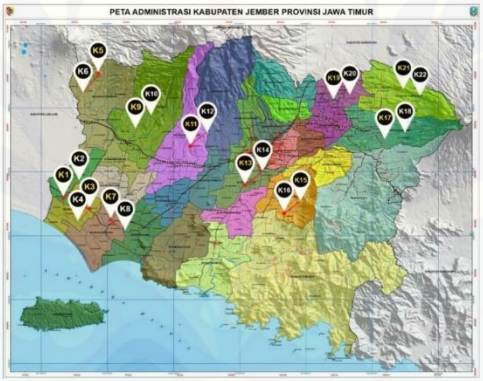

FIGURE 1. Sampling location map 22 accessions of cassava in Jember regency.

Procedures

DNA Extraction was carried out using the CTAB-PVP method to remove polyphenols [100 mM Tris-Cl (pH 8), 500 mM NaCl, 50 mM EDTA, 60 mL of 20% CTAB, 0.01 gr of 4% PVP, and 6 µl 2% β-Mercaptoethanol. 150 mg leaf samples were crushed using liquid nitrogen and put into a 1.5 ml tube centrifuge containing extraction buffer. The sample was incubated for 30 minutes at an incubator at 65oC. Add PCI (Phenol; Chloroform; IsoAmyl-Alcohol 25:24:1; v:v:v) as much as 200 µl and centrifugate at a speed of 12.000 rpm for 10 minutes at room temperature. Add PCI again to the supernatant and be centrifuged at 10.000 rpm for 10 minutes. The supernatant was then added with absolute EtOH. The sample was incubated at -20oC for 1 hour. The sample was re centrifuged at 10.000 rpm for 10 minutes. The pellets were dried at room temperature and add TE buffer of 50 µl and 3 µl ribonuclease. The concentration of DNA samples was measured by spectrophotometry Thermo Scientific Nano Drop™.

PCR Analysis. The DNA analysis was performed by PCR amplification using 10 RAPD primers namely OPK-04, OPK-08, OPK-11, OPK-12, OPK-20, OPA-19, OPE-02, OPC-19, 0PF-04, OPN-11 from Integrated DNA Technologies, Inc.US. The first step in the PCR process is to prepare a PCR tube containing 1 µl DNA template and 1 µl Primary, 5 µl MyTaq ™ Red Mix-Bioline and 5 µl ddH2O. The PCR-RAPD process starts with pre denaturation

at 94^0C for 5 minutes, denaturation at 94^0C for 30 seconds, amplification at 45^0C for 1 minute, extension at 72^0C for 1 minute 30 seconds, and a final extension at 72^0C for 5 minutes. PCR amplification was carried out for 40 cycles. The 3 µl PCR product will be separated by agarose gel with a 75-volt electrophoresis voltage within 60 minutes. The agarose gel is then visualized using Gel-Doc.

Data Analysis

Genotypic Relationship data analysis was performed based on the presence (1) or absence (0) the bands held together on each plant accession compared to the phenotype Relationship was analyzed using the key determinations of Fukuda [7]. Genetic similarity coefficient based on morphological data and RAPD data were analyzed using the SIMQUAL procedure in version 2.02 of the NTSYSpc program based on simple matching coefficient. The alignment between genotypic markers and phenotypic markers was reviewed by comparing two matrices with Pearson's product-moment correlation test MXCOMP function in the NTSYSpc program. The correlation between the two matrix pairs is tested with Z Coat statistics [7].

RESULTS AND DISCUSSION

So far, in practice, it is suspected that farmers obtain cassava seeds from other farmers who are near their location. So, it is thought that the cassava clone Relationship between nearby locations is very close. This study aims to test these hypotheses and examine the genetic relationship between several Jember cassava accessions through genotype and phenotype characters, and to find correlations between the two.

Phenotype Characteristics

Quantitative character analysis on Jember cassava plants included plant height, number of leaves, stem diameter, lobe length, lobe width, petiole length, the ratio of the length and width of the earlobe. Based on the results, all accessions have a very significant influence on all quantitative characters observed, except for the number of leaves which have a significant effect on the level of 5% (Table 1). Estimation on this character has variations between accessions so that further testing is done to see the variation using DMRT test.

TABLE 1. Average values and notations on quantitative observation variables.

Accessions	Parameters						
	TT (cm)	JD	DB (cm)	PL (cm)	LB (cm)	PP (cm)	RPL
K1	310.84 ^{efg}	29.5^{bcde}	1.58^{h}	14.00^{cdef}	4.18^{cdef}	16.45^{ef}	3.35^{b}
K2	300.98 ^{efg}	27.0^{bcde}	1.73^{gh}	15.25^{bcdef}	4.38^{bcdef}	17.73^{def}	3.49^{b}
K3	352.78 ^{cdefg}	37.0^{bcde}	2.54^{bcdefgh}	16.13^{bcdef}	5.15^{bcde}	21.93^{cdef}	3.12^{b}
K4	389.79 ^{bcdefg}	26.5^{bcde}	2.63^{bcdefg}	18.68^{bc}	4.88^{bcdef}	24.05^{bcdef}	3.83^{b}
K5	333.05 ^{defg}	44.0^{abc}	2.03^{cdefgh}	16.48^{bcde}	4.65^{bcdef}	16.80^{def}	3.55^{b}
K6	436.66 ^{bcdef}	29.0^{bcde}	2.81^{bcd}	17.30^{bcde}	5.38^{bc}	26.38^{abcd}	3.23^{b}
K7	298.51 ^{fg}	32.5^{bcde}	1.73^{gh}	14.85^{bcdef}	4.48^{bcdef}	16.63^{ef}	3.31^{b}
K8	251.64 ^{fg}	21.0^{de}	1.93^{defgh}	12.83^{ef}	3.68^{f}	17.13^{def}	3.43^{b}
K9	337.98 ^{defg}	25.0^{cde}	2.29^{bcdefgh}	14.58^{bcdef}	4.45^{bcdef}	19.20^{def}	3.27^{b}
K10	357.72^{bcdefg}	38.0^{bcde}	1.81^{efgh}	13.38^{ef}	4.15^{cdef}	17.00^{def}	3.23^{b}
K11	513.14 ^{bcd}	42.5^{bcd}	2.70^{bcdef}	16.20^{bcdef}	5.20^{bcde}	20.88^{cdef}	3.16^{b}
K12	315.78 ^{efg}	25.0^{cde}	2.34^{bcdefgh}	17.00^{bcde}	4.53^{bcdef}	18.93^{def}	3.76^{b}
K13	495.87 ^{bcde}	63.5 ^{a}	2.75^{bcde}	16.90^{bcde}	5.25^{bcd}	22.75^{bcdef}	3.22^{b}
K14	515.61 ^{bcd}	37.0^{bcde}	3.21^{ab}	18.35^{bcd}	5.63^{b}	33.50^{a}	3.26^{b}
K15	424.33 ^{bcdef}	30.5^{bcde}	1.86^{defgh}	13.78^{def}	3.93^{def}	18.83^{def}	3.51^{b}
K16	431.73 ^{bcdef}	37.0^{bcde}	2.48^{bcdefgh}	16.13^{bcdef}	4.05^{def}	24.30^{bcdef}	3.98^{b}
K17	550.14 ^{b}	33.0^{bcde}	2.70^{bcdef}	23.50^{a}	2.03^{g}	25.95^{abcde}	11.61^{a}
K18	224.50 ^{g}	17.0^{e}	1.70^{gh}	11.63^{f}	3.83^{f}	15.45^{f}	3.07^{b}
K19	328.11 ^{defg}	48.0^{ab}	1.75^{fgh}	13.80^{def}	4.15^{cdef}	17.25^{def}	3.32^{b}
K20	537.81 ^{bc}	27.5^{bcde}	2.90 ab	18.38^{bcd}	5.38^{bc}	28.88^{abc}	3.42^{b}
K21	352.78 ^{cdefg}	27.0^{bcde}	2.20^{cdefgh}	15.30^{bcdef}	4.68^{bcdef}	17.55^{def}	2.19^{c}
K22	722.84 ^{a}	27.5^{bcde}	3.74^{a}	18.88^{b}	5.75^{a}	31.38^{ab}	1.03^{d}

Note: TT, plant height; JD, number of leaves; DB, stem diameter; LL, width of lobe; PP, lobe length; PP, petiole length; RPL, the ratio of the length of the lobe. Numbers followed by different letters in the same column show results that are significantly different according to the DMRT test at the level of $\alpha = 5\%$.

The qualitative characters (Table 2), such as leaf color, lobe periphery, leaf vein color, first branch height, branching level, branch shape, and diversity of branching angle do not have diversity. Other qualitative characters have diversity with different percentages. Some plant organs such as leaves were able to provide much character information on 22 accessions of Jember cassava from lobe length, lobe width, width ratio, leaf color, lobe shape, leaf number, lobe edge surface, and leaf vein color. In the leaf color variable, there is no color difference in all cassava accessions [all accessions have a bright green color with scoring three[8]] were used the characterization and clustering of *Paspalum scrobiculatum* L. genotypes based on qualitative characters. Elliptic-lanceolate lobe is owned by K2, K3, K9, K10, K12, K16, K17, and K22 accessions. The form of lanceolate lobe is owned by K1, K4, K5, K6, K7, K11, K13, K14, K15, K19, and K21 accessions. While the straight or linear lobe is owned by K18accession. The variable number of lobes shows three types of variation on 22 cassava accessions. Accessions that have five lobes are K1, K2, K3, K5, K10, K16, K19, and K20 accessions. Accessions that have a total of seven lobes are K4, K6, K7, K9, K11, K12, K13, K15, K17, K18, K21, and K22. Whereas the accessions that have six lobes are K8 and K14. For the leaf edge surface variable, all accessions have smooth surfaces with a uniform vein color variable, namely Green.

In general, morphological features such as leaf stalk color, leaf color, stem color, and the shape of the fingers have higher heritability when compared to root length and yield which is more influenced by environmental conditions [9]. Unlike the leaf color variable, which categorically does not show diversity because all accessions have a bright green color. Petiole color show high color diversity at each accession (Table 2). Variable color of petiole has 4 color variations, yellowish-green, reddish-green, red, and purple. As the agro-morphological, the 96 elite cassava cultivars were grouped as 58 morphotypes thus indicating the presence of synonyms/homonyms/duplicates [10]

TABLE 2. Scoring of qualitative observation variables on 22 cassava accessions.

Accessions	Scoring										
	WD	**WP**	**BL**	**JL**	**PPL**	**WVD**	**TCP**	**TP**	**BC**	**SP**	**BT**
K1	Light Green	Red	Lanceolate	Five lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K2	Light Green	Yellow Green	Elliptic-lanceolate	Five lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K3	Light Green	Red	Elliptic-lanceolate	Five lobes	Smooth	Green	no branching	no branching	Erect	0°	Umbrella
K4	Light Green	Yellow Green	Lanceolate	Seven Lobes	Smooth	Green	no branching	no branching	Erect	0°	Umbrella
K5	Light Green	Yellow Green	Lanceolate	Five lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K6	Light Green	Yellow Green	Lanceolate	Seven Lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K7	Light Green	Red	Lanceolate	Seven Lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K8	Light Green	Red	Ovoid	Six Lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K9	Light Green	Red	Elliptic-lanceolate	Seven Lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K10	Light Green	Red	Elliptic-lanceolate	Five lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K11	Light Green	Reddish Green	Lanceolate	Seven Lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K12	Light Green	Yellow Green	Elliptic-lanceolate	Seven Lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K13	Light Green	Red	Lanceolate	Seven Lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K14	Light Green	Red	Lanceolate	Six Lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K15	Light Green	Yellow Green	Lanceolate	Seven lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K16	Light Green	Purple	Elliptic-lanceolate	Five lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K17	Light Green	Red	Elliptic-lanceolate	Seven lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K18	Light Green	Reddish Green	Straight or linear	Seven lobes	Winding	Green	no branching	no branching	Erect	0°	Cylindrical
K19	Light Green	Red	Lanceolate	Five lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K20	Light Green	Reddish Green	Ovoid	Five lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K21	Light Green	Reddish Green	Lanceolate	Seven lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical
K22	Light Green	Red	Elliptic-lanceolate	Seven lobes	Smooth	Green	no branching	no branching	Erect	0°	Cylindrical

Note: WD, leaf color; WP, petiole color; BL, the shape of the lobe; JL, number of leaves; PPL, the surface of the periphery of the lobe; WVD, leaf vein color; TCP, first branch height; TP, branching level; BC, branch form; SP, branching angle; and BT, plant form.

The quantitative data are converted into categorical data according to the similarity of each accession's notation in the DMRT advanced test. Data that have been converted into categorical data are then combined with qualitative variable data to be classified through the NTSYSpc version 2.02. This software also used by Yadav [11] to evaluate the genetic diversity analysis of different wheat (*Triticum aestivum* L.). NTSYSpc is one of the most popular software being used in molecular genetic qualitative data cluster analysis [12]

Based on observations, all accessions have a very significant influence on all quantitative characters observed, except for the number of leaves which has a significant effect on the level of 5%. Estimation on this character has variations between accessions so that further testing is carried out to see variations using DMRT advanced testing. The results of genetic similarity analysis based on phenotypic characteristics indicate that 22 accessions show varying distance coefficients (Table 3).

TABLE 3. The estimated matrix for genetic similarity of Jember cassava accession based on phenotypic characters with the STAND procedure and SIMMINT function processed with the NTSYS version 2.02 program.

	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18	K19	K20	K21	K22
K1	1.00																					
K2	0.67	1.00																				
K3	0.61	0.67	1.00																			
K4	0.50	0.56	0.50	1.00																		
K5	0.61	0.72	0.50	0.56	1.00																	
K6	0.61	0.61	0.50	0.61	0.67	1.00																
K7	0.72	0.72	0.61	0.61	0.61	0.67	1.00															
K8	0.56	0.56	0.50	0.39	0.56	0.50	0.61	1.00														
K9	0.56	0.72	0.67	0.50	0.67	0.56	0.72	0.61	1.00													
K10	0.72	0.72	0.67	0.50	0.61	0.56	0.61	0.67	0.67	1.00												
K11	0.56	0.56	0.61	0.50	0.56	0.61	0.67	0.50	0.61	0.50	1.00											
K12	0.56	0.78	0.56	0.56	0.72	0.67	0.61	0.56	0.83	0.61	0.56	1.00										
K13	0.61	0.50	0.50	0.56	0.61	0.67	0.67	0.56	0.61	0.56	0.61	0.61	1.00									
K14	0.67	0.56	0.56	0.50	0.56	0.61	0.67	0.61	0.56	0.61	0.61	0.50	0.61	1.00								
K15	0.61	0.67	0.50	0.61	0.67	0.78	0.67	0.61	0.61	0.61	0.61	0.67	0.61	0.61	1.00							
K16	0.61	0.72	0.72	0.50	0.56	0.61	0.61	0.50	0.67	0.67	0.56	0.61	0.56	0.56	0.67	1.00						
K17	0.56	0.56	0.56	0.44	0.44	0.56	0.61	0.50	0.61	0.61	0.56	0.56	0.56	0.56	0.56	0.56	1.00					
K18	0.44	0.50	0.39	0.39	0.44	0.50	0.56	0.50	0.50	0.44	0.56	0.50	0.50	0.44	0.50	0.44	0.44	1.00				
K19	0.72	0.61	0.56	0.44	0.72	0.56	0.61	0.61	0.67	0.72	0.56	0.56	0.61	0.61	0.67	0.56	0.50	0.44	1.00			
K20	0.61	0.61	0.56	0.44	0.56	0.61	0.56	0.56	0.50	0.61	0.56	0.50	0.50	0.67	0.56	0.61	0.50	0.50	0.56	1.00		
K21	0.56	0.67	0.56	0.56	0.67	0.61	0.72	0.50	0.67	0.56	0.67	0.61	0.56	0.56	0.67	0.56	0.56	0.50	0.56	0.56	1.00	
K22	0.56	0.56	0.56	0.44	0.44	0.56	0.61	0.50	0.61	0.61	0.50	0.56	0.56	0.56	0.56	0.56	0.67	0.44	0.50	0.50	0.56	1.00

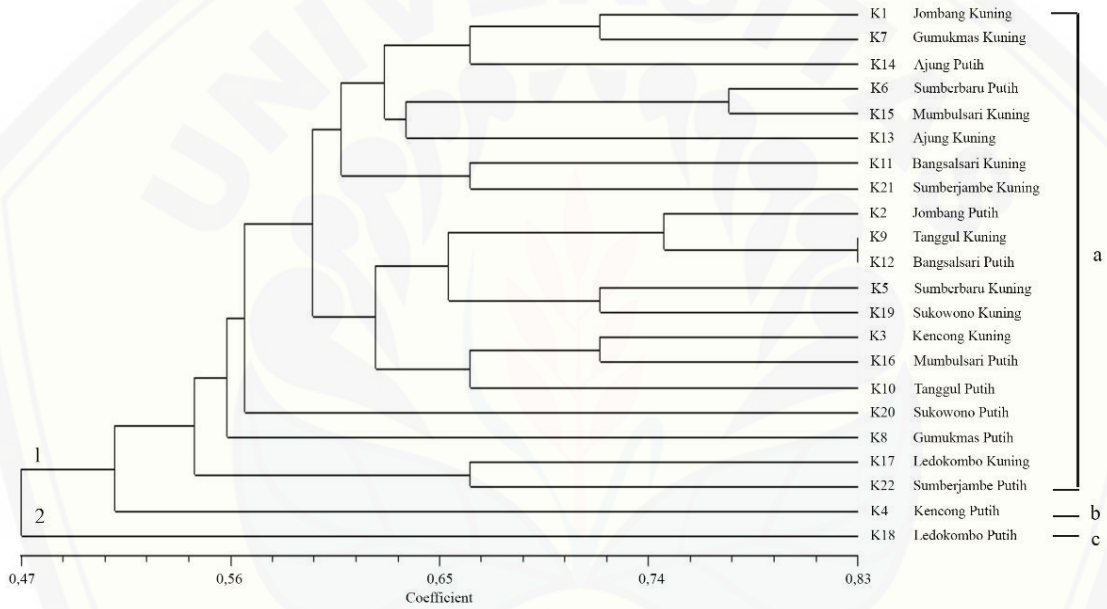

FIGURE 2. The Dendrogram 22 accession of Jember cassava is based on the phenotypic character with the STAND procedure and the SIMQUAL function is processed with the NTSYS version 2.02 program.

Result-2 (Phenotype Characteristics). The dendrogram formed based on the character of the phenotype forms 2 main groups (Figure 2). The dendrogram formed from NTSYSpc ver.2.02 is divided into two groups and three clusters (Table 4). A genetic similarity that distinguishes the coefficient of each accession is one of the parameters that can provide an indication of Relationship relations between populations. The grouping of accession similarities has a very high similarity coefficient if r> 0.9; high category if 0.8 <r <0.9; the category is quite high if 0.7 <r <0.8 and low category if r <0.7 22 [13]. All cassava accessions analyzed produced genetic similarity coefficient value based on phenotypic characters showing a correlation of 0.39 to 0.83 or a percentage of genetic similarity from 39% to 83%. The number of similarity coefficients which are below 0.7 (low category) is owned by 94% of accession pairs, namely 218 pairs of the total 231 accession pairs, while 13 other accessions have coefficients ranging from 0.72 to 0.83 tab. These results indicate that the majority of accessions have a low genetic similarity and a small proportion have fairly high similarity. Adeyemo [14] used the dendrogram to show the classification of smallholder cassava-based households. In dendrogram analysis can be defined distances farther than the distance to the right hand, the more diverse the similarity character of accession [15].

TABLE 4. The grouping of accession pairs based on the percentage of genetic similarity based on the character of the phenotype.

Category	Percentage of genetic similarity	Accession pairs
Very high	r > 0.9	-
High	0.8 < r < 0.9	K9-K12
Quite high	0.7 < r < 0.8	K2-K5. K2-K7. K2-K9. K2-K10. K2-K12. K2-K16. K3-K16. K5-K12. K6-K16. K7-K9. K7-K21. K10-K19
Low	r < 0.7	K1-K2. K1-K3. K1-K4. K1-K5. K1-K6. K1-K7. K1-K8. K1-K9. K1-K10. K1-K11. K1-K12. K1-K13. K1-K14. K1-K15. K1-K16. K1-K17. K1-K18. K1-K19. K1-K20. K1-K21. K1-K22. K2-K3. K2-K4. K2-K6. K2-K8. K2-K11. K2-K13. K2-K14. K2-K15. K2-K17. K2-K18. K2-K19. K2-K20. K2-K21. K2-K22. K3-K4.K3-K5.K3-K6.K3-K7.K3-K8.K3-K9.K3-K10.K3-K11.K3-K12.K3-K13. K3-K14.K3-K15.K3-K17.K3-K18.K3-K19.K3-K20. K4-K5. K4-K6. K4-K7. K4-K8. K4-K9. K4-K10. K4-K11. K4-K12. K4-K13. K4-K14. K4-K15. K4-K16. K4-K17. K4-K18. K4-K19. K4-K20. K4-K21. K4-K22. K5-K6. K5-K7. K5-K8. K5-K9. K5-K10. K5-K11. K5-K13. K5-K14. K5-K15. K5-K16. K5-K17. K5-K18. K5-K19. K5-K20. K5-K21. K5-K22. K6-K7. K6-K8. K6-K9. K6-K10. K6-K11. K6-K12. K6-K13. K6-K14. K6-K15. K6-K17. K6-K18. K6-K19. K6-K20. K6-K21. K6-K22. K7-K8. K7-K10. K7-K11. K7-K12. K7-K13. K7-K14. K7-K15. K7-K16. K7-K17. K7-K18. K7-K19. K7-K20. K7-K22. K8-K9. K8-K10. K8-K11. K8-K12. K8-K13. K8-K14. K8-K15. K8-K16. K8-K17. K8-K18. K8-K19. K8-K20. K8-K21. K8-K22. K9-K10. K9-K11. K9-K13. K9-K14. K9-K15. K9-K16. K9-K17. K9-K18. K9-K19. K9-K20. K9-K21. K9-K22. K10-K11. K10-K12. K10-K13. K10-K14. K10-K15. K10-K16. K10-K17. K10-K18. K10-K20. K10-K21. K10-K22. K11-K12. K11-K13. K11-K14. K11-K15. K11-K16. K11-K17. K11-K18. K11-K19. K11-K20. K11-K21. K11-K22. K12-K13. K12-K14. K12-K15. K12-K16. K12-K17. K12-K18. K12-K19. K12-K20. K12-K21. K12-K22. K13-K14. K13-K15. K13-K16. K13-K17. K13-K18. K13-K19. K13-K20. K13-K21. K13-K22. K14-K15. K14-K16. K14-K17. K14-K18. K14-K19. K14-K20. K14-K21. K14-K22. K15-K16. K15-K17. K15-K18. K15-K19. K15-K20. K15-K21. K15-K22. K16-K17. K16-K18. K16-K19. K16-K20. K16-K21. K16-K22. 17-K18. K17-K19. K17-K20. K17-K21. K17-K22. K18-K19. K18-K20. K18-K21. K18-K22. K19-K20. K19-K21. K19-K22. K20-K21. K20-K22. K21-K22

The ten primers used in the amplification of 22 accessions of Jember cassava produced 4-15 bands or an average yield of 5.9 bands per primer, with a total band produced by 657 bands with fragment sizes ranging from 250 bp-3000 bp (Figure 3).

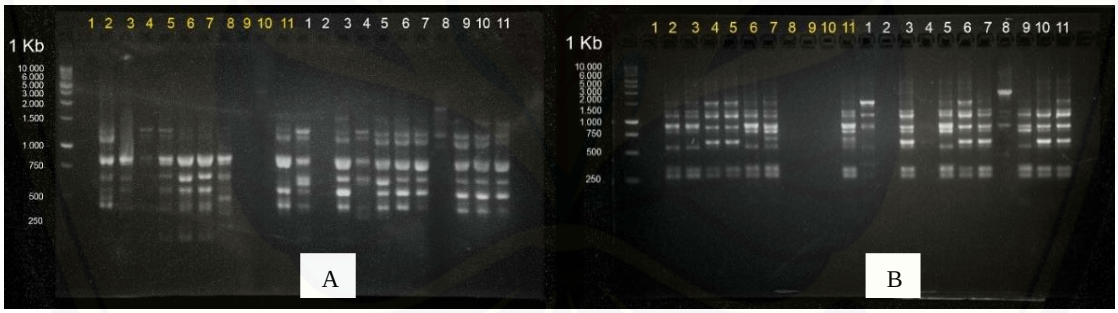

FIGURE 3. The results of visualization of RAPD-PCR from 22 accessions of Jember cassava with primary (A) OPC 19 and (B). OPK 12. Marker Promega BenchTop 1 kb DNA ladder at 2% Agarose.

The band polymorphism produced in this study was 54.23% (48 bands) out of 87 total cassava DNA bands obtained (Table 5). The results of this polymorphic band scoring were used to analyze the level of genotype Relationship of the 22 cassava accessions based on the genetic similarity coefficient value. Mahakosee et al. [16] were investigate the effect of seasonal variations in canopy size and yield of rayong 9 cassava genotype under rainfed and irrigated conditions. Khatemenla et al. [17] reported that the insights into the relative genetic diversity using morphological markers would be useful in making a core collection.

TABLE 5. Primary type, arrangement of nucleotide bases, percentage of G/C content and the number of amplified bands of 22 Jember cassava.

Primer	Base 5'- 3'	Total DNA bands	Total polymorphic bands	Polymorphisme (%)	DNA size (bp)
OPK-04	CCG-CCC-AAA-C	10	9	90.00	250-2000
OPK-08	GAA-CAC-TGG-G	7	2	28.57	500-4000
OPK-11	AAT-GCC-CCA-G	9	4	44.44	250-3000
OPK-12	TGG-CCC-TCA-C	12	10	83.33	250-5500
OPK-20	GTG-TCG-CGA-G	9	4	44.44	250-5000
OPA-19	CAA-ACG-TCG-G	7	4	57.14	750-1750
OPE-02	GGT-GCG-GGA-A	4	2	50.00	600-4000
OPC-19	GTT-GCC-AGC-C	15	6	40.00	250-2000
OPF-04	GGT-GAT-CAG-G	9	4	44.44	250-3000
OPN-11	TCG-CCG-CAA-A	5	3	60.00	500-400
	Total	87	48	542.38	
	Average	8.7	54.8	54.23 %	

The results of genetic similarity analysis showed that 22 cassava accessions were grouped with similarities between 0.5 - 1.0 or 55 to 100% (Table 6). The lowest genetic similarity (55%) was possessed by 105 cassava accession pairs, while the highest genetic similarity value (100%) was owned by the Tanggul Kuning (K9) and Ajung Kuning (K13) accession pairs (Table 7).

TABLE 6. The genetic similarity matrix of 22 Jember cassava accessions based on the character of the RAPD data with the SM function is processed with the NTSYS version 2.02 program.

	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18	K19	K20	K21	K22
K1	1.00																					
K2	0.55	1.00																				
K3	0.55	0.65	1.00																			
K4	0.55	0.65	0.75	1.00																		
K5	0.55	0.65	0.75	0.86	1.00																	
K6	0.55	0.65	0.68	0.68	0.68	1.00																
K7	0.55	0.65	0.68	0.68	0.68	0.91	1.00															
K8	0.55	0.65	0.68	0.68	0.68	0.69	0.69	1.00														
K9	0.90	0.55	0.55	0.55	0.55	0.55	0.55	0.55	1.00													
K10	0.90	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.95	1.00												
K11	0.55	0.65	0.68	0.68	0.68	0.77	0.77	0.69	0.55	0.55	1.00											
K12	0.83	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.83	0.83	0.55	1.00										
K13	0.90	0.55	0.55	0.55	0.55	0.55	0.55	0.55	1.00	0.95	0.55	0.83	1.00									
K14	0.55	0.65	0.71	0.71	0.71	0.68	0.68	0.68	0.55	0.55	0.68	0.55	0.55	1.00								
K15	0.90	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.92	0.92	0.55	0.83	0.92	0.55	1.00							
K16	0.55	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.55	0.55	0.64	0.55	0.55	0.64	0.55	1.00						
K17	0.55	0.65	0.75	0.82	0.82	0.68	0.68	0.68	0.55	0.55	0.68	0.55	0.55	0.71	0.55	0.64	1.00					
K18	0.55	0.65	0.71	0.71	0.71	0.68	0.68	0.68	0.55	0.55	0.68	0.55	0.55	0.74	0.55	0.64	0.71	1.00				
K19	0.78	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.78	0.78	0.55	0.78	0.78	0.55	0.78	0.55	0.55	0.55	1.00			
K20	0.55	0.65	0.71	0.71	0.71	0.68	0.68	0.68	0.55	0.55	0.68	0.55	0.55	0.74	0.55	0.64	0.71	0.79	0.55	1.00		
K21	0.55	0.65	0.71	0.71	0.71	0.68	0.68	0.68	0.55	0.55	0.68	0.55	0.55	0.74	0.55	0.64	0.71	0.83	0.55	0.79	1.00	
K22	0.55	0.65	0.71	0.71	0.71	0.68	0.68	0.68	0.55	0.55	0.68	0.55	0.55	0.74	0.55	0.64	0.71	0.83	0.55	0.79	0.92	1.00

TABLE 7. The action group formed based on the phenotypic dendogram analyzed by the STAND procedure and the SIMQUAL function is processed with the NTSYS version 2.02 program.

Group	Cluster	Accessions
1	A	K1, K7, K14, K6, K15 K13, K11, K21, K2, K9, K12, K5, K19, K3, K16, K10, K20, K8, K17, K22
	B	K4
2	C	K18

Dendrogram are formed into two groups and four clusters (Figure 3). Cluster A is occupied by K1, K9, K13, K10, K15, and K12. Cluster B is occupied by K19. The second group is occupied by two clusters; Cluster C and Cluster D. Cluster C is occupied by K2, K3, K4, K5, K17, K14, K18, K21, K22, K20, K6, K7, K11, and K8. Cluster D is occupied by K16 (Table 8).

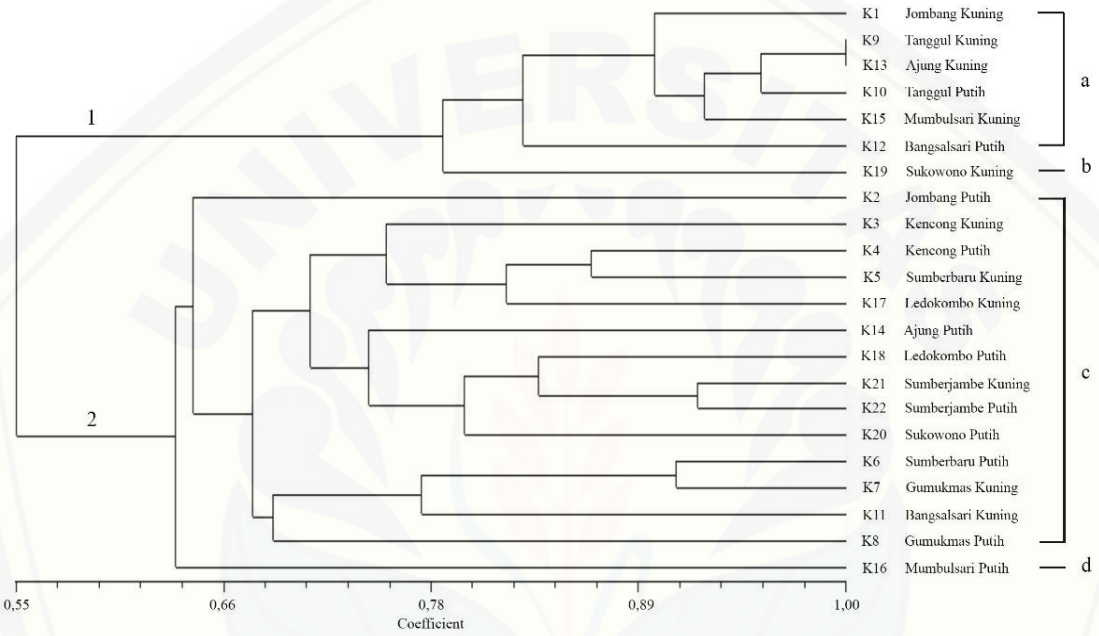

FIGURE 3. The genetic similarity of 22 dendrogram of Jember cassava accessions based on the character of the RAPD data with the SM function is processed with the NTSYS version 2.02 program.

TABLE 8. The accession group formed based on the genetic similarity dendrogram of 22 Jember cassava accessions analyzed by the SM function was processed using the NTSYS version 2.02 program.

Group	Cluster	Accessions
1	A	K1. K9. K13. K10. K15.12
	B	K19
2	C	K2. K3. K4. K5. K17. K14. K18. K21. K22. K20. K6. K7. K11. K8
	D	K16

All cassava accessions analyzed produced similarity coefficient values ranging from 55-100%, with varying degrees of similarity. 8 accession couples had genetic similarities> 0.9 (very high), 14 accession couples had 0.8 <r <0.9 (high) genetic similarity, 38 accession couples had 0.7 <r <0.8 (quite high) genetic similarity, and 171 accession pairs had similarities genetic <0.7 (low) (Table 9). These results indicate that 74% or the majority of accessions have low genetic similarity. Low genetic similarity shows that the same DNA sequence in the cassava genome is only small, whereas high genetic similarity shows many of the same DNA sequences in the accession genomes.

TABLE 9. The grouping of accession pairs based on the percentage of genetic similarity based ongenotype characters.

Category	Percentage of genetic similarity	Accession pairs
Very high	r > 0.9	K6-K7. K9-K10. K9-K13. K9-K15. K10-K13. K10-K15. K13-K15. K21-K22
High	0.8 < r < 0.9	K1-K9. K1-K10. K1-K12. K1-K13. K1-K15. K4-K5. K4-K17. K5-K17. K9-K12. K10-K12. K12-K13. K12-K15. K18-K21. K18-K22.
Quite high	0.7 < r < 0.8	K1-K19. K3-K4.K3-K5. K3-K14. K3-K17.K3-K18. K3-K20. K3-K21. K3-K22. K4-K12. K4-K18. K4-K20. K4-K21. K4-K22. K5-K14. K5-K18. K5-K20. K5-K21. K5-K22. K6-K11. K7-K11. K9-K19. K10-K19. K12-K19. K13-K19. K14-K17. K14-K18. K14-K20. K14-K21. K14-K22. K15-K19. K17-K18. K17-K20. K17-K21. K17-K22. K18-K20. K20-K21. K20-K22
Low	r < 0.7	K1-K2. K1-K3. K1-K4. K1-K5. K1-K6. K1-K7. K1-K8. K1-K11. K1-K14. K1-K16. K1-K17. K1-K18. K1-K20. K1-K21. K1-K22. K2-K3. K2-K4. K2-K5. K2-K6. K2-K7. K2-K8. K2-K9. K2-K10. K2-K11. K2-K12. K2-K13. K2-K14. K2-K15. K2-K16. K2-K17. K2-K18. K2-K19. K2-K20. K2-K21. K2-K22. K3-K6.K3-K7.K3-K8.K3-K9.K3-K10.K3-K11.K3-K12.K3-K13. K3-K15.K3-K16. K3-K19. K4-K6. K4-K7. K4-K8. K4-K9. K4-K10. K4-K11. K4-K13. K4-K14. K4-K16. K4-K19. K5-K6. K5-K7. K5-K8. K5-K9. K5-K10. K5-K11. K5-K12. K5-K13. K5-K15. K5-K16. K5-K19. K6-K8. K6-K9. K6-K10. K6-K12. K6-K13. K6-K14. K6-K15. K6-K16. K6-K17. K6-K18. K6-K19. K6-K20. K6-K21. K6-K22. K7-K8. K7-K9. K7-K10. K7-K12. K7-K13. K7-K14. K7-K15. K7-K16. K7-K17. K7-K18. K7-K19. K7-K20. K7-K21. K7-K22. K8-K9. K8-K10. K8-K11. K8-K12. K8-K13. K8-K14. K8-K15. K8-K16. K8-K17. K8-K18. K8-K19. K8-K20. K8-K21. K8-K22. K9-K11. K9-K14. K9-K16. K9-K17. K9-K18. K9-K20. K9-K21. K9-K22. K10-K11. K10-K14. K10-K16. K10-K17. K10-K18. K10-K20. K10-K21. K10-K22. K11-K12. K11-K13. K11-K14. K11-K15. K11-K16. K11-K17. K11-K18. K11-K19. K11-K20. K11-K21. K11-K22. K12-K14. K12-K16. K12-K17. K12-K18. K12-K20. K12-K21. K12-K22. K13-K14. K13-K16. K13-K17. K13-K18. K13-K20. K13-K21. K13-K22. K14-K15. K14-K16. K14-K19. K15-K16. K15-K17. K15-K18. K15-K20. K15-K21. K15-K22. K16-K17. K16-K18. K16-K19. K16-K20. K16-K21. K16-K22. K17-K19. K18-K19. K19-K20. K19-K21. K19-K22

DISCUSSION

The correlation between RAPD and phenotype was carried out by correlation analysis between genetic similarity matrices. This test was performed using Z Mantel statistics with the MXCOM program on NTSYSpc version 2.02. The results obtained show the correlation value r = -0.41865. The value of this correlation is based on the criteria of goodness of fit, namely the level of harmony of matrix values in the RAPD data and the phenotype data interpreted very weakly (very poor fit; r <0.7). Based on the statistical test of coat α 0.05, it was found that the correlation was not real, because at the level of correlation it was obtained p = 0.0001, where the correlation value obtained is not real. Regression analysis of phenotype data between taxonomic distance as estimating value (X) and genetic similarity of RAPD data as an independent variable (Y) obtained linear regression equation Y = 15.67 - 0.05 X and determination coefficient R2 = 0.254.

The low value of the determination coefficient (R2) shows that the linear regression equation is not good, which means that the average taxonomic distance from the phenotype data cannot be used to predict the genetic similarity derived from the RAPD data. Only about 25.4% of the data on phenotype similarity can predict genetic similarity. Some factors that cause the small correlation or inconsistency between these two markers include; 1) the character of the observed phenotype and the amplified profile of the RAPD tape are not one part that is interconnected or only partially related; 2) the large percentage of the genome area that has not been detected by the primary used; 3) inaccurate interpretation of the reading of DNA band sequences. Many studies have been conducted in testing the alignment of grouping between morphological and molecular markers, but most of them failed to obtain harmonious groupings. For example, grouping based on morphological markers with molecular on wheat with a correlation r = 0.47. p <0.01 [8], in Populus nigra with a correlation r = 0.27 p <0.001, and on Orchid with a correlation r = -0.381. p <0.0008 [16].

Relationship analysis on 22 cassava accessions based on phenotypic characters produced similarity coefficients ranging from 0.39-0.83. As many as 94% of accession pairs have a low similarity coefficient and 6% has a fairly high similarity coefficient. Analysis based on RAPD markers produces a similarity coefficient of 0.5-1.0 or 55-100%. The majority of accession pairs of 74% has a low similarity coefficient. The correlation between genetic similarity matrix and phenotype similarity matrix shows a very weak correlation. Testing the coefficient of determination shows the results (R2) = 0.254, which means that only 25.4% of the genetic similarity value is determined by the similarity of the phenotype.

CONCLUSION

The genetic relationships were analyzed using similarity coefficients and phenotypic genetic distances. Relationship analysis of 22 cassava accessions based on phenotypic characters yielded similarity coefficients ranging from 0.39 to 0.83. Relationship analysis of 22 cassava accessions based on RAPD markers, resulted in a similarity coefficient of 0.5-1.0, or 55 to 100%.

ACKNOWLEDGMENTS

This work was supported by the Post Graduated Project, Ministry of Research and Technology, No. 1839//UN25.3.1/LT/2017 on 15 December 2017, Cq. Dr. Ir. Sholeh Avivi, M.Si.

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