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Morphological And Agronomic Characteristics And Genetic Stability Of MANGO (*Mangifera Indica* L.) Accession Of Bikul

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ABSTRACT
This research which aimed to acquire basic data of the morphological and agronomic characteristics and genetic stability of bikul mango plants produced by vegetative (shield budding) and generative (seed) propagation was conducted from October 2019 to July 2020. On-farm characterization was carried out in Menyali Village, Sawan District, Buleleng Regency. Other than in the field, agronomic observation was also performed at the Food Laboratory of the Faculty of Agricultural Technology of Universitas Udayana. DNA preparation, PCR, and microsatellite analysis were conducted at the Laboratory of Plant Breeding and Genetics of the Faculty of Agriculture of Universitas Gajah Mada. The research results showed that, morphologically, elliptical leaves 4.5–5.4 cm wide and 20.2–34.6 cm long, oblong, mouse-like fruit shape, yellowish green ripe fruit color, yellowish orange ripe fruit flesh color, and flat (ngumpen), tapered, oval-shaped seed are the chief characteristics of bikul mango. The most obvious agronomic characteristics include sweet fruit flesh flavor, fragrant aroma, high sugar level (64.94% on average), fairly high vitamin C level (up to 2.48%), edible fruit portion reaching 83.44%, and average weight per fruit of 89.2 g. The genetic stability of bikul mango is categorized as quite good with a potential for genetic trait change of 15–30% if generatively propagated.

KEYWORDS: *flat, genetic stability, cluster, bikul mango, microsatellite.*

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I. INTRODUCTION

Bali Island is a tropical island which over the time is recognized for its tourism. Tourists' interest in coming to this island originates not only in its unique culture and breathetaking natural panorama but also in the tropical plant specific diversity existing there. One of the plant species is mango (*Mangifera indica* L.). Bikul mango is a unique mango germplasm native to Bali Island, but it has yet to acquire wide recognition. On a limited scale, this mango has a fairly fanatic enthusiast segment for its tasty flavor and flat (*ngumpen*) seed which gives it a constantly high price reaching up to Rp30,000 kg^{-1}. In the light of this objective condition, bikul mango is tauted as an exotic mango in Bali Island. It is cultivated in a small area in Bali, that is, Menyali Village, Sawan District, Buleleng Regency.

Over the time, the uniqueness and superiority of bikul mango is restricted only to the enthusiasts' perceptions. Such perceptions open up an opportunity for bikul mango development as local superior mango. However, to further develop this mango, in-depth scientific studies are required. Some of the information pieces needed are related to the morphological and agronomic characteristics and genetic stability of the mango.

Many morphological and agronomic studies to find out about the unique characteristics and superiority of mango accessions have been conducted in various places, including the study by [1] on mangoes growing in Egypt, the study by [2] on mango cultivars around Shendi, the study by [3] on mango germplasms in Bhagalvar, India, the study by [6] on mangoes in Bengkulu Province, the study by [10] on mango plants in Mexico, the study by [11] on mango plants in Nigeria, the study by [12] on mango cultivars in West Nigeria, the study by [15] on mangoes in Rimba Jaya Village of Merauke Regency, the study by [18] on Cantek, Ireng, Empok, and Jempol mango plants in Tiron Village, Banyakan District, Kediri Regency, the study by [19] on mango accessions in Brazil, the study by [21] on Harumanis Mango in Malaysia, the study by [23] on mango varieties in Banyumas Regency, and the study by [26] on the fruits and leaves of the Imbu mango cultivar.

Many genetic stability tests have been carried out to figure out the consistency of plant progenies generated through generative or vegetative propagation, particularly on individuals propagated through tissue culture, cell culture, and protoplasm fusion, as those conducted in the study [4] on new Indonesian melon

cultivars, the study by [8] on synthetic wheat accessions, the study by [9] on some wheat genotypes, the study by [14] on banana plants regenerated from floral axis, and the study by [16] in oak plants produced from somatic embryogenic cultures.

The information on morphological and agronomic characteristics and genetic stability obtained will serve as a consideration for the development of strategies of the conservation, breeding, management, and utilization of bikul mango genetic resources as well as the propagation techniques.

This research aimed to collect basic data on the morphological and agronomic characteristics and genetic stability of bikul mango plants produced by vegetative (shield budding) and generative (seed) propagation.

II. MATERIALS AND METHODS

2.1 Research Location and Time

On-farm characterization was carried out in Menyali Village, Sawan District, Buleleng Regency. Other than in the field, agronomic observation was also performed at the Food Laboratory of the Faculty of Agricultural Technology of Universitas Udayana. DNA preparation, PCR, and microsatellite analysis were conducted at the Laboratory of Plant Breeding and Genetics of the Faculty of Agriculture of Universitas Gajah Mada. This research was conducted for 10 months from October 2019 to July 2020.

2.2 Research Materials and Tools

2.2.1 Morphological and Agronomic Characterization

The materials used included fresh mango leaves, flowers, fruits, and seeds. The on-farm characterization tools used included GPS, digital camera, mango descriptor book, scissors, calipers, measuring tape, scale, labeling paper, plastic rod, brown paper, sack, plastic bag, hook, cool box, and writing instruments. Tools used for morphological and agronomic characterization included Olympus microscope, digital scale, calipers, refractrometer, and mango descriptor.

2.2.2 Genetic Stability Analysis

The materials used included the following: young leaves from 88-year-old parent plant 1 (I1), 20-year-old parent plant 2 produced by shield budding using a bud from parent plant 1 (I2), 10-year-old plant seed-propagated from parent plant 1 (F1.1), 3-year-old plant seed-propagated from parent plant 1 (F1.2), and 3-month-old plant seed-propagated from parent plant 1 (F1.3). A diagram is provided in Figure 1. Other materials included extraction buffer, EDTA, ice, lysis buffer, dithiotreitol, chloroform isoamylalcohol, sodium acetate, isopropanol, 70% ethanol, PCR buffer, forward primer, reverse primer, and Taq DNA Polymerase. DNA analysis tools included mortar, centrifuge, micro-pipette, micro-tube, oven, MJ Research PCT-100 PCR device, gel electrophoresis, and genetic analyzer (Beckman Coulter® CEQ-8000).

2.2.3 Determination of Morphological and Agronomic Characteristics

The morphological characteristics observed following a mango descriptor guide included the crowns, stems, branching, leaves, flowers, fruits, and seeds (IPGRI, 2009). The sugar level was measured by the Anthrone method, total acidity by the alkalinity titration method, vitamin C content by the alkalinity titration method, and total antioxidant content by the 2,2-diphenyl-1 picryl hidrazil (DPPH) method.

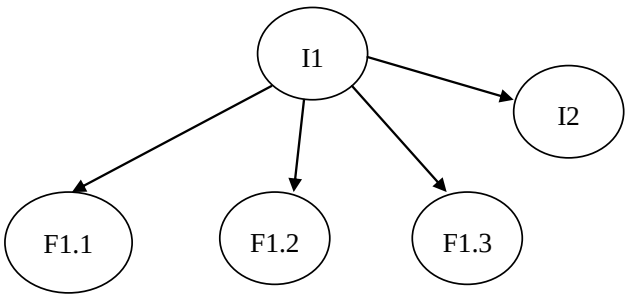

Figure 1: Sample Bikul Mango Plant for Genetic Stability Analysis

Description:
I1 = first parent tree, aged 88 years
I2 = second parent tree, propagated vegetatively (shield budding) using a bud from the first parent tree, aged 20 years since planting
F1.1 = plant seed-propagated from the primary parent, aged 10 years since planting
F1.2 = plant seed-propagated from the primary parent, aged 3 years since planting
F1.3 = plant seed-propagated from the primary parent, aged 3 months since seedling

2.3 Genetic Stability Analysis

2.3.1 Sample and Microsatellite Primer Preparation

A sample of three young leaves was extracted from each sample plant, wrapped in aluminum foil, placed in a cool box containing dry ice, and then carried to the laboratory. Molecular characterization used 10 pairs of microsatellite primers previously tried on a mango plant [22, 24]. A list of sequences and microsatellite primer repetition types is provided in Table 1.

Tabel 1: List of Sequences and Microsatellite Primer Repetition Types Used in the Research

Primer	Forward	Reverse	Repetition
AY942818	CCACGAATATCAACTGCTGCC	TCTGACACTGCTCTTCCACC	(CT/AG)11
AY942821	TGTAGTCTCTGTTTGCTTC	TTCTGTGTCGTCAAACTC	(GTT/AAC)6
AY942825	CGAGGAAGAGGAAGATTATGAC	CGAATACCATCCAGCAAAATAC	(CGG/CCT)7
AY942827	GTTTTCATTCTCAAAATGTGTG	CTTTCATGTTCATAGATGCAA	(CT/AG)15
AY942828	CTCGCATTTCTCGCAGTC	TCCCTCCATTTAACCCTCC	(AG/CT)9
AY942829	GAACGAGAAATCGGGAAC	GCAGCCATTGAATACAGAG	(GTT/AAC)8
AY942831	TTTACCAAGCTAGGGTCA	CACTCTTAAACTATTCAACCA	(GA/TC)15
AJ635165	GATGAAACCAAAGAAGTCA	CCAATAAGAACTCCAACC	(TG)10
AJ635168	TTCTAAGGAGTTCTAAAATGC	CTCAAGTCCAACATACAATAC	(GT)9
AJ635176	TGCGTAAAGCTGTTGACTA	GACAAGATAAACAACTGGAA	(TG)11

Source: Scanell *et al*. (2005); Tasliah *et al*. (2013)

2.3.2 DNA Isolation and PCR Analysis

As much as 1–2 g of young leaves from each accession was extracted until DNA samples were obtained. DNA isolation was carried out with the cetyl trimethyl ammonium bromide (CTAB) extraction buffer following [5]. Dithiotreitol was added to prevent phenolic compound oxidation during extraction. PCR was conducted in a total volume of 20 µl.

The DNA duplication steps were as follows: denaturation initiation at 95 °C for 3 minutes, followed by 34 cycles which contained denaturation at 94 °C for 1 minute, primer attachment at 50 °C for 1 minute, and base elongation at 72 °C for 2 minutes [22]. The final step in the PCR process was final elongation at 72 °C for 5 minutes and incubation at 4 °C. To see whether amplified DNA occurred, electrophoresis was performed with 2% agarosa gel. The primers which produced ribbons on the agarosa gel were used for DNA fragment analysis.

2.3.3 DNA Fragment Analysis

DNA fragments were detected on genetic analyzer (Beckman Coulter® CEQ™ 8000). The PCR product sample storage procedure for loading and the CEQ 8000 procedure followed the protocol standardized by [25]. The samples were analyzed with multiplexing by mixing the PCR amplification products with two types of primers, fluorescent green and blue in color, into one well. PCR product in a certain volume (according to the optimization results) from each primer was mixed in a well, then added with 0.5 µl of internal size standard CEQ (400 bp), labeled with red, and SLS, with a final volume of 40 µl.

The fragment sizes from the CEQ 8000 analysis were processed with CEQ Fragment Analysis Software. This was followed by binning, that is, grouping of DNA fragments (alleles) by number of certain DNA motif repetations (e.g., thread repetition from two, three, or four base pairs), flanked by a pair of microsatellite primers.

2.4 Data Analysis

2.4.1 Morphological and Agronomic Characteristics

Morphological and agronomic data are in the form of qualitative and quantitative data. Quantitative data included results of measurements and counting of stems, leaves, flowers, fruits, and seeds (on average). The agronomic characteristics observed are as follows: fruit shape, fruit weight, edible fruit proportion, sugar level, texture, vitamin C content, total acidity, and antioxidant content.

2.4.2 Genetic Stability

DNA concentration and purity was measured using GeneQuant 1300 spectrophotometer. The DNA concentration obtained was high (2,697 ng/µl). DNA purity in comparison to impurities classified as proteins was measured at a wavelength of λ260/280, while DNA purity in comparison to impurities in the form of RNA was measured at a wavelength of λ260/280. Good DNA purity ranges between 1.8 and 2.2.

Mango germplasm accessions cluster analysis was performed based on the between-accessions allele similarity using the genetic distance formula described by [17]. A dendogram is developed based on the binary data from the mango plant molecular scoring by the unweight pair-group method with arithmetic mean using NTSYS 2.02 software.

III. RESULTS AND DISCUSSION

3.1 Bikul Mango Morphological Characteristics

Based on the results of identification on 24 morphological characteristics, it was found that bikul mango belongs to the species *Mangifera indica* L. Despite already reaching its 88 years of life, the parent plant was observed as still healthy and productive. The cultivation technique applied by the owner was considered simple and environment-friendly as the only fertilizer used was manure and no pesticide was employed. Specific morphological characteristics can be seen in the leaf shape, fruit shape, fruit flesh color, and seed shape (see Table 2).

Table 2: Morphological Characteristics of Bikul mango

No.	Morphological Characteristics	Description	No.	Morphological Characteristics	Description
1.	Plant height	13 m	13.	Leaf edge	Non-wavy
2.	Stem cross-sectional shape	Cylinder	14.	Flower panicle shap	Widened, pyramidal
3.	Stem color	Grayish brown	15.	Flowering time	July–August
4.	Plant crown shape	Oblong	16.	Harvest time	October–December
5.	Growth behavior	Upright	17.	Fruit shape	Oblong
6.	Branching density	Medium	18.	Fruit apex shape	Unpointed
7.	Leaf shape	Oval	19.	Fruit base shape	Slightly rounded
8.	Leaf tip shape	Pointed	20.	Beak type	Slightly pointed
9.	Leaf base shape	Pointed	21.	Fruit peel color	Yellowish green
10.	Leaf size	20.2–34.6 cm long, 4.5–5.4 cm wide	22.	Fruit flesh color	Yellowish orange
11.	Upper-part leaf color	Dark green	23.	Seed shape	Dented and ellipsoid, flat (*ngumpen*)
12.	Lower-part leaf color	Green	24.	Seed color	White

According to Table 2, bikul mango leaves are oval and relatively narrow, sized 4.5–5.4 cm in width and 20.2–34.6 cm in length, and they are green both at the upper and lower parts. The fruit is oblong, with the fruit apex being unpointed and the base slightly rounded. On closer inspection, the fruit is mouse-like in shape. It is assumed that due to such a shape the mango earned its name *poh bikul* (mouse) mango from local community. The young fruit is green, and the ripe one is yellowish green. Meanwhile, the flesh is yellowish orange. The seed is ellipsoid and dented, and it is flat to the point that the fruit appears seedless. In Bali, such flatness is termed *ngumpen*.

The morphological characteristics of bikul mango are distinct from other mangoes planted around there, e.g., arumanis, golek, manalagi, lalijiwa, and Brazil mangoes, to name a few. Hence, the morphological characteristics of the leaves, fruits, and seeds can be used as markers in bikul mango development. The use of morphological characteristics as markers in the development of mango accessions was recommended by some mango researchers such as [11] as well as [19].

3.2 Agronomic Characteristics of Bikul Mango

From the observation of 15 agronomic characteristics, as presented in Table 3, some uniqueness of bikul mangoes was identified. Some clear agronomic characteristics include sweet flesh, fragrant aroma, high sugar level of 63.94% on average, rather high vitamin C content of 2.48%, and edible fruit portion reaching up to 83.44%. Despite its relatively small dimensions (5.5–8.1 cm long, 3.3–4.6 cm wide, and 89.2 g in weight per fruit), but due to its flat (*ngumpen*) seed and yield per tree of up to 192 kg every year, this mango possesses a high economic value.

The various agronomic characteristics described above constitute uniqueness to bikul mango in comparison to mangoes of other types. Hence, agronomic charactersitics of flesh flavor, sugar level, vitamin C content, edible fruit portion, and seed shape can be used as markers in bikul mango development. This bikul mango development is oriented toward consumption rather than rootstock resource.

Table 3: Agronomic Characteristics of Bikul mango

No.	Agronomic Characteristics	Description	No.	Agronomic Characteristics	Description
1.	Fruit length	5.5–8.1 cm	9.	Texture	32.90 N on average
2.	Fruit width	3.3–4.6 cm	10.	Total acidity	1.78% on average
3.	Fruit thickness	3.1–4.1 cm	11.	Vitamin C content	2.48% on average
4.	Fruit flesh thickness	0.82 cm on average	12.	Total antioxidant content	0.025% on average
5.	Quality of fiber in fruit flesh	Medium	13.	Weight per fruit	89.2 g on average
6.	Fruit flesh flavor	Sweet	14.	Edible fruit portion	83.44%
7.	Fruit aroma	Moderate (fragrant)	15.	Yield per tree per year	192 kg
8.	Sugar level	63.94%			

3.3 Genetic Stability

The dendogram analysis of five bikul mango samples—I1, I2, F1.1, F1.2, and F1.3—yielded a coefficient of similarity in the 0.7–0.95% range (Figure 2) from the 10 tested marker/primer pairs as presented in Table 4. The microsatellite profile in the five samples can be seen in Figure 3. It is shown that the individuals with the highest similarity are I1 and F1.1 with a coefficient of 0.95. F1.3 is farthest apart from the four other samples, and the farthest from two other F1 samples (F1.1 and F1.2). All the coefficents of genetic similarity of the five individuals are presented in Table 5.

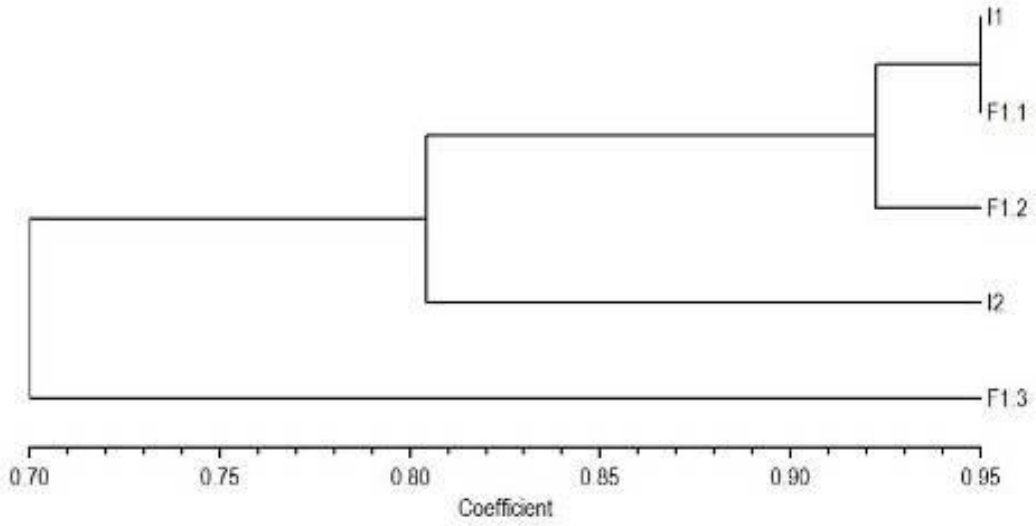

Figure 2: Dendogram of Five Bikul Mango Samples (I1, I2, F1.1, F1.2, and F1.3)

Table 4: Number of Ribbons Produced by the Five Mango Samples on 10 Primers

Sample	Primer AY18	AY21	AY25	AY27	AY28	AY29	AY31	AJ65	AJ68	AJ79	Number
I1	1	2	6	3	2	2	2	2	4	4	28
I2	1	1	7	3	1	2	2	3	3	5	28
F1.1	1	2	7	3	2	2	2	2	4	4	29
F1.2	1	2	6	3	2	2	2	2	4	4	28
F1.3	1	1	4	1	2	2	1	2	4	5	23
					Total						136

Table 5: Matrix of Coefficients of Genetic Similarity of the Bikul Mango Samples

Coefficient of Similarity	I1	I2	F1.1	F1.2	F1.3
I1	1.00				
I2	0.805	1.00			
F1.1	0.95	0.805	1.00		
F1.2	0.93	0.805	0.92	1.00	
F1.3	0.70	0.70	0.70	0.70	1.00

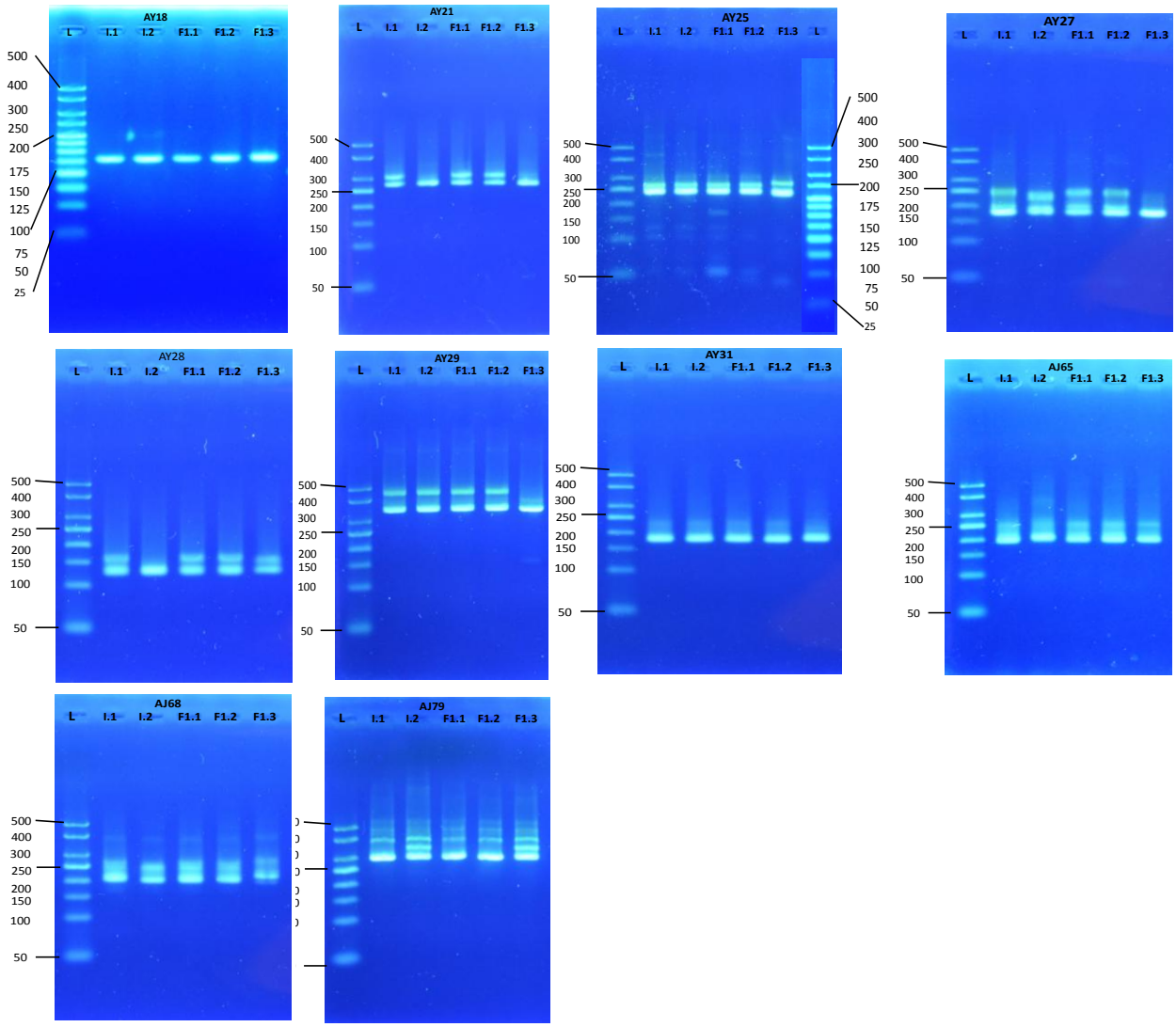

Figure 3: Microsatellite Profile of the Five Bikul Mango Samples on the 10 Primers Used

According to the data in Figure 2 and Table 5, based on the consistency of the coefficients of genetic similarity of the five bikul mango samples, the genetic stability of bikul mango can be categorized as fairly stable with a potential for genetic trait change of 15–30%. This has an implication for the plant propagation methods used. Plant propagation with seed has a potential of genetic trait change of about 30%, meaning that the potential of environmental factor influence on plant phenotypic appearance reaches 30%. Meanwhile, vegetative propagation (shield budding) has a greater stability level, with a potential for genetic trait change of about 19.05%. In relation to the foregoing, further investigation is needed to find out the factors causing genetic trait differences between I1 and the progenies resulted by vegetative propagation. It is strongly assumed that these differences are caused by rootstock influence. Therefore, a study on the influence of rootstock type on the genetic stability of bikul mango seedlings is required. The existence of variation in coefficients of similarity in bikul mango progenies propagated by seed is presumed because bikul mango has a potential for open pollination. As a matter of fact, around the bikul mango planting area there are other types of mangoes as well, such as, arumanis, sanih, lalijiwa, and golek. Such an objective condition supports the potential for pollen contamination in the fertilization of some bikul mango flowers.

Althouh I2 was propagated by shield budding from I1, there is a difference in DNA ribbon pattern after shield budding.This ribbon pattern difference generates genetic similarity of 0.805. From the main coordinate analysis presented in Figure 3, it can be seen that I1, F1.1, and F1.2 are positioned near to each other in a cluster, while I2 and F1.3 are farther separated (Figure 4).

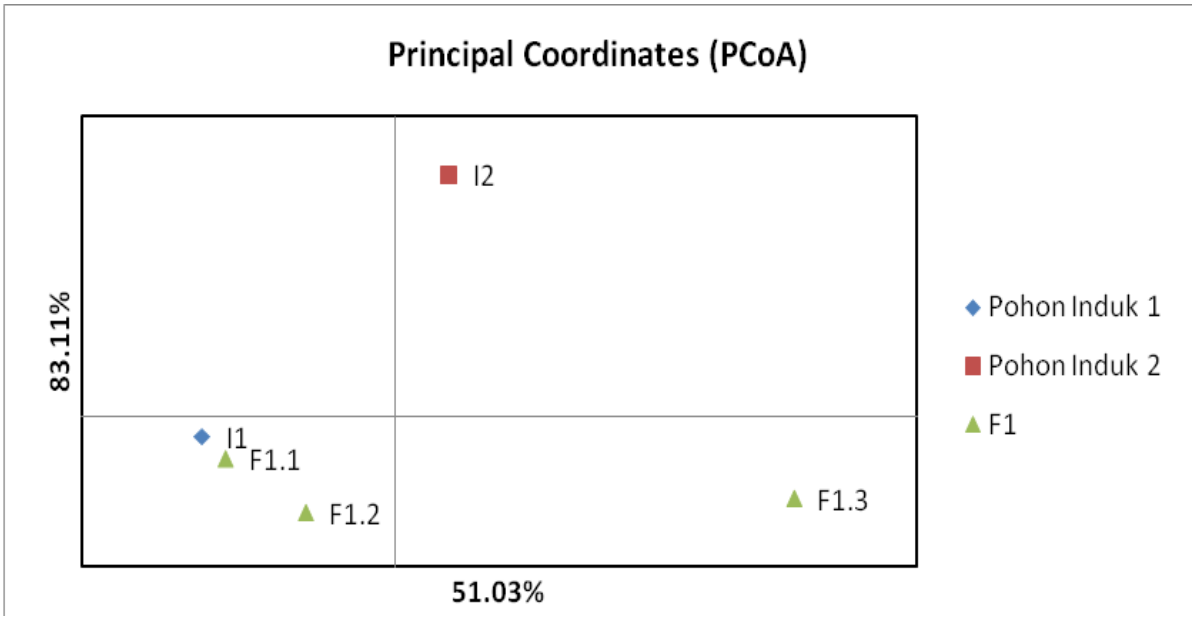

Figure 4: Main Coordinate Analysis of the Five Mango Samples

IV. CONCLUSIONS

From this research the following conclusions were drawn: (1) morphologically, bikul mango has main characteristics including oval, relatively narrow leaves 4.5–5.4 cm wide and 20.2–34.6 cm long, green in color both at the upper and lower part. The fruit is oblong in shape, with unpointed fruit apex and slightly rounded fruit base. The young fruit is green, and the ripe one is yellowish green. The ripe fruit flesh is yellowish orange. It has ellipsoid and dented seed, flat to the point that the fruit appears seedless (*ngumpen*), (2) the obvious agronomic markers include sweet fruit flesh flavor, fragrant aroma, high sugar level of 63.94% on average, fairly high vitamin C content of up to 2.48%, edible fruit portion of up to 83.44%, and weight per fruit of 89.2 g on average, and (3) the genetic stability of bikul is categorized as fairly good with a potential for genetic trait change of 15–30% if propagated generatively.

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