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IDENTIFICATION OF SUPERIOR DWARF COCONUT (*COCOS NUCIFERA* L.) PARENTAL CULTIVARS FOR HYBRID BREEDING

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SUMMARY

Indonesia has a high level of coconut (*Cocos nucifera* L.) germplasm diversity. However, information about the superiority and genetics of each dwarf coconut accession remains limited. This research aimed to obtain information that is important for choosing the appropriate coconut material for breeding programs and industrial use. Eight coconut cultivars were evaluated at the morphological level on the basis of 12 traits and at the molecular level by using 10 SSR primers were carried out during 2017 and 2018. Morphological observations performed at the Mapanget Experimental Garden, IPCRI, Manado, North Sulawesi, Indonesia. Molecular characteristics were studied at the Department of Genetics and Plant Breeding, Universitas Gadjah Mada, Yogyakarta, Indonesia. The cultivars 'Sagerat Orange Dwarf' (SOD) and 'Waingapu Red Dwarf' (WRD) showed desirable fruit and yield characters. Genetic structure analysis showed that dwarf coconut cultivars could be divided into two groups, and multivariate analysis revealed the relationship of the individual palms with one another, thus enabling the selection of palms with genetic integrity. Coconut cultivars with large genetic distances and superior characters have a good chance of producing hybrids with heterosis for fruit and yield characters. The cultivars SOD and WRD were identified as the most superior parents for the production of dwarf × dwarf coconut hybrids.

Keywords: Diversity, morphology, genetics, genetic structure, dwarf coconut cultivars, *Cocos nucifera* L.

Key findings: This study describes the application of genetic and morphological information in coconut breeding programs, especially in the selection of parental candidates that can be applied effectively. Results are expected to be a material consideration in the utilization of Indonesian coconut germplasm in breeding programs.

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INTRODUCTION

Indonesia has a high level of coconut (*Cocos nucifera* L.) diversity because it is one of the centers of coconut genetic diversity in the world (Vavilov, 1951). Coconut varieties are divided into two large groups on the basis of the speed of their first flowering: tall and dwarf coconut varieties. Dwarf coconut cultivars account for approximately 5% of the coconut population worldwide (Perera *et al*., 2016). The area of origin and location of the spread of coconuts remain debatable (Clement *et al*., 2013; Baudouin *et al*., 2014; Neto *et al*., 2016; Perera *et al*., 2016). In the 2012 coconut collection database, which involves 39 COGENT member countries, the number of Indonesian dwarf coconut cultivars is the highest, followed by that of Philippine and Indian dwarf coconut cultivars.

Coconut palms are widely developed in Indonesia. Although Indonesian farmers mainly cultivate tall coconut cultivars, some also cultivate dwarf coconut varieties. Generally, coconut hybrid cultivars are cultivated by private companies. In Indonesia, most hybrid coconuts that were developed are mostly dwarf × tall or tall × tall hybrids, whereas intervariety dwarf × dwarf (D × D) hybrid coconuts are poorly developed because they are thought to have low production potential (Tenda, 2004; Novarianto, 2008). This situation is not entirely true. The right parental selection method accompanied by good cultivation technology will provide optimal benefits. Dwarf coconut cultivars have several advantages over tall coconuts. Tight pacing and early bearing are the advantages that can be obtained from the cultivation of dwarf coconuts and D × D hybrids. Dwarf coconut varieties have short trunks that facilitate harvesting and fruits with sizes that are suitable for individual consumption and delicious flavors. They can be a promising commodity for supplying raw material for coconut-based industries.

This study provides an overview of the phenotypic and genetic diversity of dwarf coconut germplasm in Indonesia, one of the centers of dwarf coconut diversity in the world. However, the technology for the utilization of plant genetic information based on molecular markers and the diversity of morphological characters in the assembly of hybrid coconuts is very limited. Molecular markers have been extensively used in population studies, genetic diversity analysis, associations, and hybrid testing (Rajesh *et al*., 2014; Perera *et al*., 2016; Geethanjali *et al*., 2017; Mahayu and Taryono, 2019). This study describes a holistic method of utilizing genetic and morphological information, which has rarely been discussed, in supporting coconut breeding programs.

MATERIALS AND METHODS

The coconut research material was provided by the Mapanget Experimental Garden, Indonesian Palms Crop Research Institute (IPCRI), Manado, North Sulawesi, Indonesia, from an *ex-situ* garden (Table 1). Eighty-four palms were randomly selected to represent eight dwarf coconut cultivars, 10 'Sweet Green' dwarf (SGD) palms, 10 'Sagerat Orange' dwarf (SOD) palms, 15 'Tebing Tinggi' dwarf (TTD) palms, 15 'Jombang Green' dwarf (JGD) palms, nine 'Bali Yellow' dwarf (BYD) palms, 10 'Nias Green' dwarf (NGD) palms, 10 'Nias Yellow' dwarf (NYD) palms, and nine 'Waingapu Red' dwarf (WRD) palms. The first cultivar (SGD) originated from Thailand, whereas the rest are local Indonesian cultivars. Two Indonesian dwarf coconut cultivars (SOD and WRD) originated from the central region of Indonesia, whereas five cultivars (NYD, NGD, TTD, JGD, and BYD) were from western Indonesia (Figure 1). Two of the seven dwarf coconut cultivars from Indonesia, namely, NYD and BYD, are local cultivars that have been officially released by the Indonesian Ministry of Agriculture, Indonesia.

Table 1. Dwarf coconut (*Cocos nucifera* L.) cultivar names, collection year, total number of palms, number of palms used in the study, and origin.

No.	Cultivars	Collection year	Total number of palms	Number of palms used in the study	Origin of the cultivars
1	Sweet Green Dwarf	2008	32	10	Thailand
2	Sagerat Orange Dwarf	1985	20	10	Sagerat, North Sulawesi/ Indonesia
3	Tebing Tinggi Dwarf	1978	51	15	Tebing Tinggi, North Sumatera/ Indonesia
4	Jombang Green Dwarf	1977	57	15	Jombang, East Java/Indonesia
5	Bali Yellow Dwarf	1976	54	9	Bali Island/ Indonesia
6	Nias Green Dwarf	1977	64	10	Nias Island, North Sumatera/ Indonesia
7	Nias Yellow Dwarf	1976	79	10	Nias Island, North Sumatera/ Indonesia
8	Waingapu Red Dwarf	1997	16	5	Waingapu, East Nusa Tenggara/ Indonesia

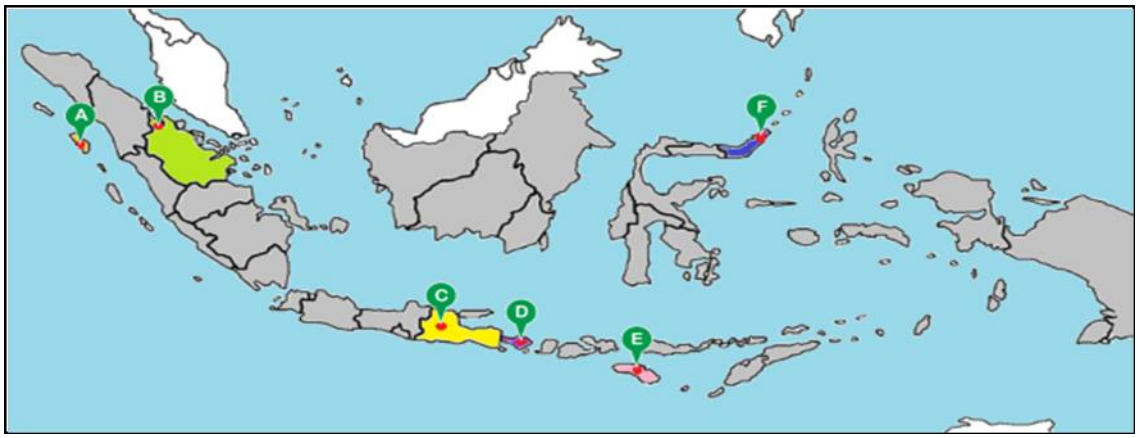

Figure. 1. Collection sites of the dwarf coconut accessions in Indonesia. A: Nias Island, North Sumatra (NYD and NGD); B: Tebing Tinggi, North Sumatra (TTD); C: Jombang, East Java (JGD); D: Bali Island (BYD); E: Waingapu, East Nusa Tenggara (WRD); and F: Sagerat, North Sulawesi (SOD).

The study consisted of two aspects: morphological and molecular observations were carried out during 2017 and 2018. The morphological observations performed at the Mapanget Experimental Garden, IPCRI, Manado, North Sulawesi, Indonesia. Observations on fruit and yield characters were recorded on the basis of the Manual on Standardized Research Technique in Coconut Breeding COGENT (Santos *et al*., 1996). Molecular characteristics were studied at the Department of Genetics and Plant Breeding, Universitas Gadjah Mada, Yogyakarta, Indonesia.

DNA isolation and amplification via polymerase chain reaction

Young coconut leaves collected as samples from the Mapanget Experimental Garden, Manado, North Sulawesi, were cleaned with 70% alcohol and prepared for DNA extraction based on the CTAB method (Doyle and Doyle, 1990) with modified PVP, CTAB, and mercaptoethanol

Table 2. Characteristics of the 10 SSR primers used in PCR.

No.	Markers	Oligonucleotide sequences	Size (base)	TA (°C)
1	CnCir 2	F: AGAACCCTTGCTCCAC	16	55
		R: TCCAGCCATTCCATC	15	
2	CnCir 87	F: AACCAGAACTTAAATGTCG	19	55
		R: TTTGAACTCTTCTATTGGG	19	
3	CnCir 121	F: TTGGTCTATTGCATGTTC	18	55
		R: TGGCATTGAGAGGGT	15	
4	CnCir 123	F: TCATTCAGAGGACAAAAGTT	20	46
		R:TAAAAATTCATAAAGGTAAAA	21	
5	CnCir 167	F: GGTGGGTAAGTGAACATC	18	57
		R: GTGATACAACGAACCCTC	18	
6	CnCir A9	F: GGACACTGGGTTCTGTT	17	55
		R: CTCTGTAATCTGCGGG	16	
7	CnCir C5	F: CTGAAGATATGTGTTTATGC	20	52
		R: TGTTCCAGATTGAGGTT	17	
8	CnCir C9	F: ATGTTTTAGCTTCACCATGAA	21	55
		R: TCAAGTTCAAGAAGACCTTTG	21	
9	CnCir J2	F: CCATTGTCATTGTTATTTTG	20	52
		R: GTCACCATCTTCTCAGTTTC	20	
10	CNZ 51	F: AAAGTGAAGTGGATAATGTG	20	55
		R: AGAGAGGATCTAGGGTTGT	19	

TA: annealing temperature

concentrations. Genomic DNA was quantified by using 1.5% agarose in 1× TBE buffer added with florosafe as the DNA dye and then electrophoresed for 60 min at a voltage of 100 V, 400 mA with PowerPac Basic TM Bio-Rad. The bands of the palm genome were observed under UV light and documented with a digital camera. The purity of the DNA solution was measured by using a UV light spectrophotometer. The DNA purity value is determined by using the ratio λ 260/280. Pure DNA has a ratio of approximately 1.8.

The selected primers were those with PIC value > 0.4; those with PIC <0.4 are less informative markers (Okoye *et al*., 2016). In this research, 10 SSR primers were used, i.e., CnCir 2, CnCir 121, CnCir 123, CnCir 167, CnCir 87, CnCir A9, CnCir C5, CnCir C9, CnCir J2, and CNZ 51, and are presented along with their nucleotide sequences and annealing temperatures in Table 2 (Rivera, 1999; CIRAD, 2002). The polymerase chain reaction (PCR) for microsatellite analysis was performed with a total reaction volume of 11.5 µl that consisted of 6.25 µl of KAPA2G Fast Ready Mix, 0.25 µl of 0.1 mM MgCl20, 2.35 µl of nuclease-free water, 0.65 µl of 0.8 mM forward and reverse primers, and 2 µl of (±2 ng/µl) DNA working solution. Amplification was done by using a PCR machine Bio-Rad T 100TM Thermo Cycler. The PCR method applied in this research referred to that used by Pesik *et al*. (2015).

PCR products were run in 1.5% MetaPhor™ electrophoresis agarose gel (3:1) in 1× TBE buffer at a constant power of 100 V, 400 mA for 1 h. DNA bands were visualized under UV light and documented with a digital camera. *GelRed* DNA staining and DNA markers were used for the confirmation of the position of PCR products to determine the allele size of the DNA bands that had been amplified by SSR markers. The DNA band that appeared in the electrophoresis agarose gel was assessed manually as a codominant marker.

The descriptive statistical data of fruit and yield characters were analyzed by using MS Office Excel. Principal component analysis (PCA) based on production and fruit component characters was carried out with Statistical Tool for Agricultural Research software. On the

basis of genetic distance, principal coordinate analysis was used to map the distribution of the dwarf coconut palms representing the eight dwarf coconut cultivars studied in a dimension that was calculated with GenAlEx 6.5 software (Peakall and Smouse, 2012). STRUCTURE V2.3.4 software (Pritchard *et al*., 2000) was used to estimate the population structure of dwarf coconut cultivars with a burning period of 5000 and 50 000 (three times iteration) MCMC repetitions after burning. Furthermore, the average value and variety plotted in "likelihood per K" were calculated via the Evanno method (Evanno *et al*., 2005).

RESULTS

Fruit and yield characters of dwarf coconut cultivars

PCA revealed that the eight coconut cultivars were scattered in four quadrants, indicating different fruit and yield characters (Figure 2). The PCA biplot captured 82.92% of the total variation among cultivars. The PCA biplot identified the primary fruit and yield characters that contributed to the total variation. Fruit equatorial circumference, fruit polar circumference, fruit weight, dehusked fruit weight, and dehusked fruit equatorial circumference showed the longest vectors in PC1, whereas the weight of coconut meat per palm per year showed the longest vectors in PC2. These results indicated that these traits contributed to most of the variation.

The PCA biplot showed that the SOD cultivar had the highest number of bunches and number of fruits per bunch and, therefore, had the highest productivity. Among all cultivars, WRD had the largest fruit polar circumference; heaviest husk weight; and the highest coconut meat weight, coconut water volume, and coconut husk value. Among all cultivars, SGD had the lowest productivity. However, SGD was assumed to not have yet reached production stability when this research was conducted.

Cultivars SOD and WRD were, therefore, candidate parents for the development of hybrids with high production potential. Moreover, TTD, BYD, and NGD were close to the center of the ordinate. This position indicated that these values of these cultivars were comparable with the average value of the characters observed in the dwarf coconut cultivars.

Morphological markers for the assessment of legitimate hybrids

The eight dwarf coconut cultivars had round canopies with green leaves. Petiole color showed differences among cultivars (Figure 3). NYD and BYD showed greenish-yellow petiole colors; JGD, TTD, SGD, and NGD had green petioles colors; and SOD and WRD have greenish orange and orange petiole colors, respectively.

Genetic characteristics of dwarf coconut cultivars

The grouping of palms representing the eight cultivars was inferred on the basis of SSR data (Figure 4) with STRUCTURE software by using an ad hoc quantity (ΔK) program (Evanno *et al*., 2005). A clear peak was shown at the true value of K (K = 2) (Figure 5).

On the basis of their genetic structure, the dwarf coconut palms were divided into two groups (Figure 6A). The first group consisted of cultivars SGD, SOD, and NGD with dominant genetic proportions that were represented in red. The second group consisted of cultivars TTD, JGD, BYD, NYD, and WRD cultivars with genetic proportions that were distinguished in green. However, within cultivars, a few palms, such as the cultivars SOD, TTD, BYD, NGD, NYD, and WRD (Figure 6B), showed doubtful genetic composition.

The pair-wise population matrix (Table 3) also illustrates the relationship of the cultivars with one another and supports the findings on the population

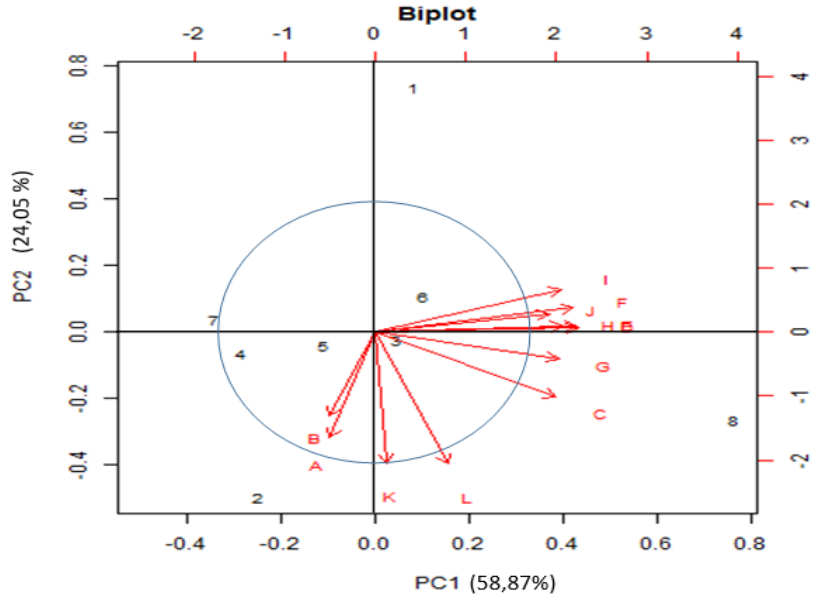

Figure 2. Main characteristics of the dwarf coconuts in Indonesia based on fruit components and production. Accessions: 1: SGD, 2: SOD, 3: TTD, 4: JGD, 5: BYD, 6: NGD, 7: NYD, and 8: WRD; Characters: A: number of bunches, B: number of fruits per bunch, C: fruit polar circumference, D: fruit equatorial circumference, E: fruit weight, F: dehusked fruit weight, G: husk weight; H: dehusked fruit polar circumference; I: dehusked fruit equatorial circumference; J: weight of coconut shell, K: volume of coconut water, and L: weight of coconut meat per palm per year.

Figure 3. Diversity of petiole colors in the eight dwarf coconut accessions in Mapanget Experimental Garden, IPCRI, North Sulawesi, Indonesia. 1: SGD, 2: SOD, 3: TTD, 4: JGD, 5: BYD, 6: NGD, 7: NYD, and 8: WRD.

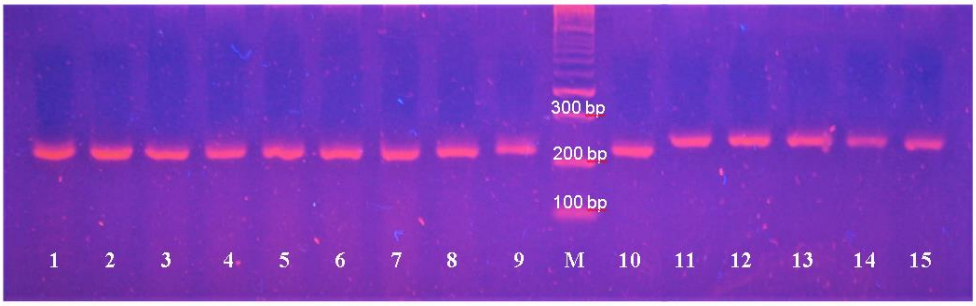

Figure 4. JGD (1–10) and BYD (11–15) coconut DNA amplified based on CnCir C5 SSR primer in MetaPhor™ agarose gel electrophoresis. M = Marker

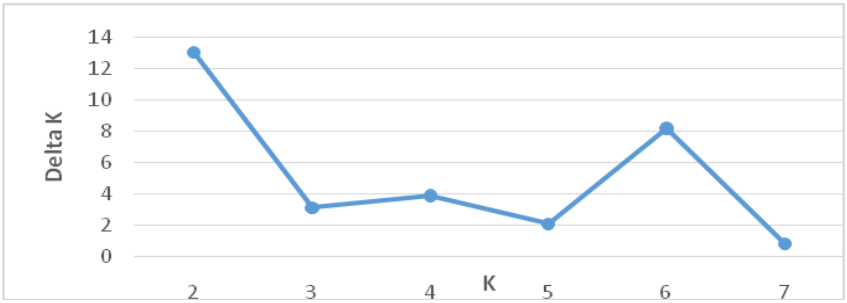

Figure 5. Delta K of eight dwarf coconut accessions.

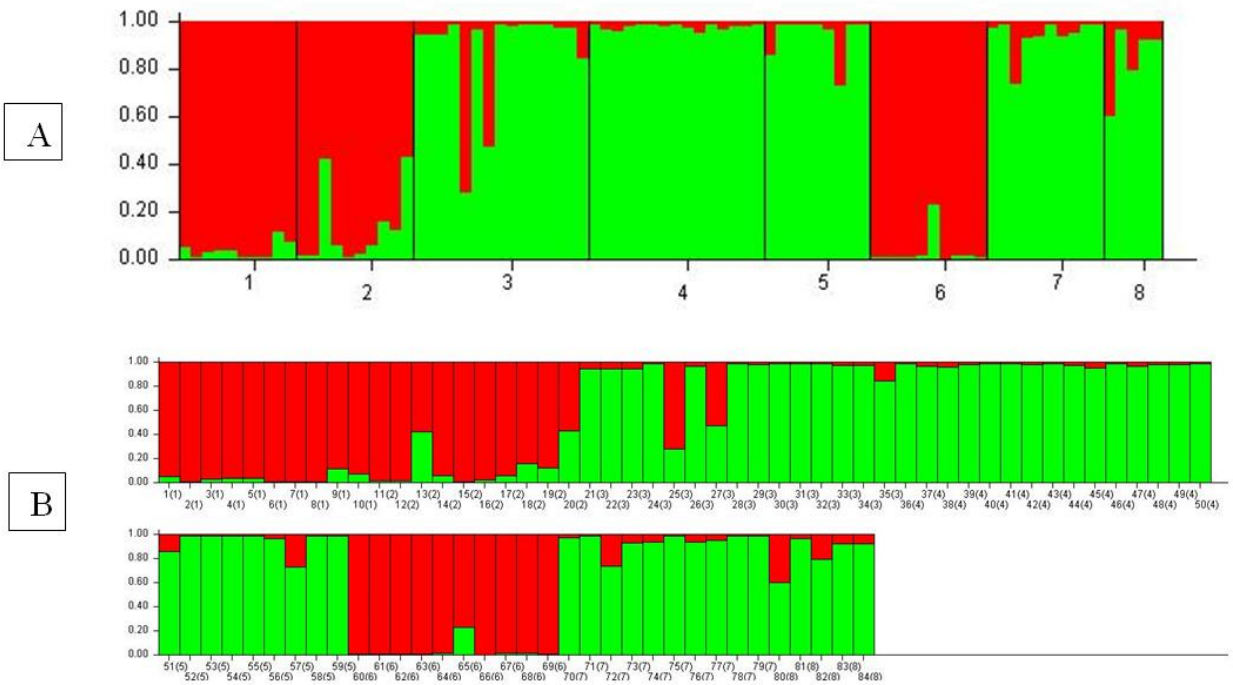

Figure 6. Genetic structure of the dwarf coconut cultivars determined by using STRUCTURE V.2.3.4 software. The red and green colors represent genetic structure groupings. (A) Membership of the cultivars in genetic structure groups: 1: SGD, 2: SOD, 3: TTD, 4: JGD, 5: BYD, 6: NGD, 7: NYD, and 8: WRD. (B) Some cultivars have palms that showed doubtful genetic compositions: SOD (13, 20), TTD (25, 27), BYD (57), NGD (65), NYD (72), and WRD (80).

Table 3. Pairwise population matrix of Nei's genetic identity among the dwarf coconut cultivars.

Accessions	SGD	SOD	TTD	JGD	BYD	NGD	NYD	WRD
SGD	1.000							
SOD	0.642	1.000						
TTD	0.667	0.610	1.000					
JGD	0.416	0.542	0.699	1.000				
BYD	0.486	0.546	0.703	0.547	1.000			
NGD	0.533	0.499	0.318	0.406	0.277	1.000		
NYD	0.315	0.526	0.524	0.625	0.499	0.409	1.000	
WRD	0.328	0.369	0.551	0.428	0.549	0.457	0.529	1.000

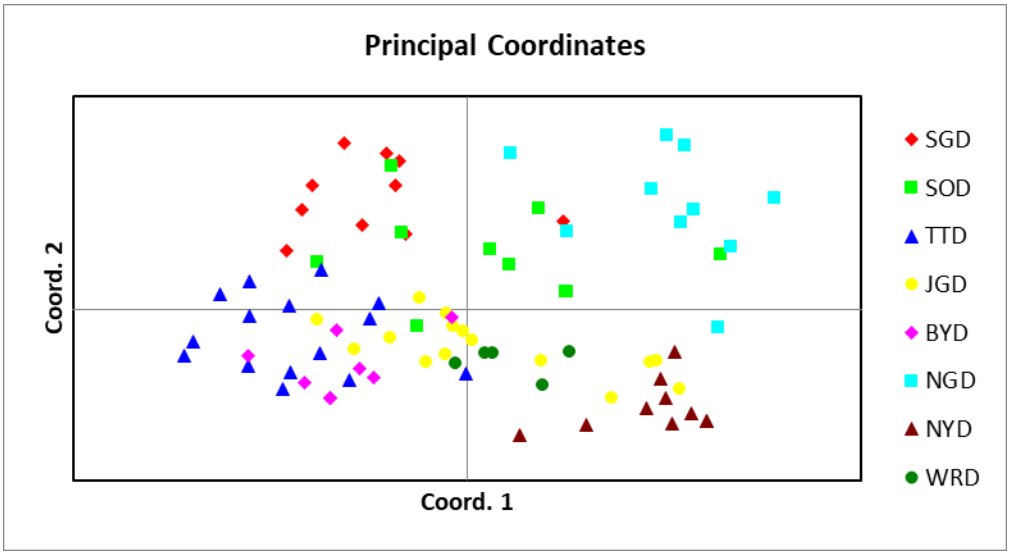

Figure 7. Multivariate analysis of the dwarf coconut palms based on SSR markers.

Table 4. Selected palms within cultivars with superior fruit and yield characters and genetic integrity based on genetic structure.

No.	Accessions	Superior palms with genetic integrity	Superior palms with doubtful genetic composition	Superior fruit and yield characters
1	SGD	1, 5, 6, 7, 8	-	fruit equatorial circumference, fruit weight, dehusked fruit weight
2	SOD	11, 14, 15, 24	13, 20	number of bunch
3	TTD	21, 29, 30, 31, 33	25	husk weight, fruit polar circumference
4	JGD	36, 37, 38, 42	-	number of fruits/bunches
5	BYD	53, 54, 59	-	number of bunches
6	NGD	60, 61, 66, 67, 69	65	fruit polar circumference, dehusked fruit polar circumference, fruit equatorial circumference
7	NYD	70, 74, 75, 77, 78	72	fruit polar circumference, fruit weight, dehusked fruit weight
8	WRD	81, 84	-	weight of coconut meat/palm/year, weight of coconut water, fruit weight, dehusked fruit weight, husk weight

structure. Cultivars TTD and BYD were the most genetically similar cultivars (0.703). Large genetic distances were observed between the cultivars NGD and BYD (0.277), SGD and NYD (0.315), NGD and TTD (0.318), SGD and WRD (0.328), and SOD and WRD (0.369).

SSR marker data were used to further infer the relationship of each dwarf coconut palm with one another via multivariate analysis (Figure 7). SOD palms appeared to be dispersed among the clusters of SGD and NGD palms, which collectively represented the first group on the basis of population structure. JGD palms appeared to be dispersed among the clusters of the NYD, WRD, BYD, and TTD cultivars, which collectively represented the second group on the basis of population structure. Given that some JGD and SOD palms had alleles that were common with the alleles of some palms of the other dwarf coconut cultivars, they appeared to be dispersed among other cultivars. The high genetic diversity within the cultivars was exhibited by the extensive dispersion of the palms in the graph.

The cultivars with large genetic distances between them have a good chance of producing hybrid progenies that exhibit heterosis for fruit and yield characters. Selection within cultivars identified superior palms with genetic integrity. Superior palms with doubtful genetic composition based on the genetic structure analysis may be discarded as candidate parents (Table 4).

DISCUSSION

This study combined conventional breeding and molecular tools to identify dwarf coconut cultivars with a good chance of producing hybrid progenies that exhibit heterosis for fruit and yield characters given that minimal breeding work has been conducted on these materials. Heterosis is the phenomenon wherein the progeny of diverse varieties of a species or between species exhibit increased biomass, speed of development, and fertility than both parents (Birchler *et al*., 2010).

Dwarf coconut cultivars show divergent characters. The result of the PCA illustrated that the SOD and WRD cultivars have the highest production potential. SOD was characterized by the highest number of bunches and number of fruits per bunch. WRD was characterized by the heaviest husk weight and weight of coconut meat per palm per year, largest fruit polar circumference, and highest coconut water production. Therefore, these two cultivars have the best potential when used as parents for hybridization. TTD, BYD, and NGD cultivars showed average characters that can be improved by individual palm selection when used in breeding programs. Selection can be carried out on characters that have moderate to high variance. In this study, the characters with a high coefficient of variance were characterized by long vectors.

Petiole color is a morphological marker that is generally used in assessing the identity of coconut hybrids (Rajesh *et al*., 2014). Petiole color is equal to stem color in the coconut palm seedling phase and can thus be used in the evaluation of hybrid identity in the initial growth phase. This morphological marker can be used if the parents have different petiole colors (Batugal and Bordeix, 2005).

The grouping of cultivars based on molecular marker data is not influenced by collection sites and not always related to phenotypic similarities. This study showed that a large genetic distance existed between NGD and BYD, SGD and NYD, NGD and TTD, SGD and WRD, and SOD and WRD cultivars. The combination of these dwarf coconut parents can potentially produce hybrids that exhibit heterosis in terms of fruit and yield characters. Moreover, considering that most ecotypes or varieties that differ from each other exhibit some level of heterosis, interspecific crosses show greater heterosis than intraspecific crosses.

This study showed that polymorphism existed among palms within a cultivar and that a few palms of the

cultivars had doubtful genetic composition. On the basis of genetic structure analysis, some palms may be discarded as parents in the development of hybrids. The doubtful genetic composition of a palm can be due to genetic contamination, which occurred because dwarf coconut palms in Indonesia are generally cultivated along with other dwarf and tall coconut palms. Therefore, out-crossing may have caused genetic contamination.

Dwarf coconuts are predominantly pure lines because they are naturally self-pollinating palms (Kamaral *et al*., 2014). Outcrossing between dwarf coconut cultivars should be relatively low. The genetic uniformity of the parental palm is necessary to obtain the uniform performance of the hybrid. The same alleles shared by palms of different cultivars caused the clustering of palms among different cultivars. The present results were in line with the findings of past analyses on coconut germplasm with isozymes (Parthasarathy *et al*., 2004) and RAPD (Upadhyay *et al*., 2004) and SSR (Pesik, 2016; Mahayu and Taryono, 2019) markers.

Dwarf coconut has an important role as a source of industrial raw materials or as breeding material. Thus, evaluating dwarf coconut germplasm is important. Dwarf coconut cultivars with huge genetic distance or narrow genetic identity can be used as candidates for hybrid parents. Parents with good genetic combining ability produce superior offspring. Cluster analysis helps obtain genetically divergent parents, while multivariate analysis helps identify superior palms in each cultivar. Genetic structure helps select legitimate palms of the cultivars. Furthermore, a holistic application method with accuracy in cultivar selection based on the evaluation of genetic characters and superiority followed by individual selection will result in the optimal benefit of hybrid coconuts for cultivation and industry. Utilizing morphological and genetic markers simultaneously will increase the chances of success and effectiveness in conducting crop evaluations (Perdani *et al*., 2018) for the development of hybrid coconut (D × D) with high potential production in Indonesia.

CONCLUSIONS

As concluded from the above findings, conventional breeding must be accompanied by molecular analysis to strengthen evaluation. The said evaluation provided information regarding the identification of superior coconut genotypes with genetic integrity and desirable traits. The coconut cultivars SOD and WRD were identified as the potential parental genotypes to produce promising coconut hybrids (D × D) with high yielding ability.

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