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MOLECULAR CHARACTERIZATION OF THE CACAO F1 POPULATIONS WITH MORPHOLOGICAL VARIATIONS IN POD TRAITS

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SUMMARY

Cacao (*Theobroma cacao* L.) is an important export crop, requiring high-quality beans with sustainable production to meet market demands. For genetic enhancement, controlled hybridization provides a pathway to improving bean quality and increasing genetic variation in cacao. This study evaluated phenotypic variation in 16 quantitative traits of F1 progeny derived from four cross combinations and performed molecular characterization using 11 polymorphic markers. Data collection progressed in 2019–2021. Notably, the F1 hybrid TSH858 x DR1 emerged as promising by showing higher average pod weight and favorable bean counts per 100 g, aligning with AA/A quality grades. The F1 hybrid 5-1 (2) showcased the highest single dried bean weight and lowest pod index, indicating large bean sizes. Molecular characterization revealed the highest observed (0.64) and expected (0.56) heterozygosity, confirming the origins of hybrids. The average polymorphism information content (PIC) was 0.50, suitable for genetic studies. Distinct genetic relationships among the F1 progenies suggested that bean yield and quality variations stem from genetic variation. The results highlighted that strategic hybridization played a vital role in boosting genetic diversity and bean quality as key goals in cacao breeding programs.

Keywords: Cacao (*T. cacao* L.) breeding, bean quality, controlled hybridization, genetic variation, molecular characterization, heterozygosity, genetic relationship

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Key findings: This study integrates morphological, agronomic, and molecular data to comprehensively evaluate the cacao (*T. cacao* L.) F1 hybrids. The combined use of phenotypic traits and molecular characterization strengthens the selection strategies for breeding. Emphasis on pod index, yield traits, and quality standards aligns the findings with practical breeding goals and industry needs.

INTRODUCTION

Cacao (*Theobroma cacao* L.) beans have many uses as the raw material in global industries, such as food, confectionery, beverages, cosmetics, and pharmaceuticals. Cocoa beans are also the primary ingredient in chocolate. These beans, after harvest from cocoa pods, undergo fermenting, drying, and then roasting to develop their characteristic flavor and aroma. The processed beans can further continue being separated into cocoa butter and cocoa powder, used in various chocolate products and other foods. Bean quality is crucial in meeting industry demands that also directly affect the market value of the products. However, ensuring consistent quality has become challenging due to a limited supply of beans that meet required standards (Rodríguez-Carrasco *et al.,* 2018). The cacao genotypes, environmental conditions, maturity stage, and post-harvest procedures are the key factors influencing bean quality traits (Calvo *et al.,* 2021).

Selective crosses between superior and genetically distant cacao genotypes represent a vital strategy to improve bean yield, quality, and genetic diversity through heterotic effects (Izzah *et al.,* 2022). Genetic variation is fundamental in breeding programs, enabling the development of high-yielding cultivars (Shilpashree *et al.,* 2021). The ensuing F1 progenies gained morphological characterization to identify the traits linking to bean yield and quality traits. Therefore, morphological assessment remains crucial in conventional and modern breeding, supporting the selection of promising and well-adapted genotypes (Bekele and Phillips-Mora, 2019).

Although crossing two identical parental cultivars of cacao was viable, in fact, morphological variations in their F1 progenies were commonly visible, underscoring the need to assess their genetic relationship. Genetic variability assessment through morphological and molecular characterization has been a prevalent approach (Bekele and Phillips-Mora, 2019). Such evaluations are also essential for maintaining genetic diversity and guidance in effective breeding decisions (Karakaya *et al.,* 2023). However, morphological traits alone may not accurately reflect the genetic variations due to environmental influences (Deepashree *et al.,* 2023). Integrating molecular characterization helps overcome these limitations, offering obvious insights into genetic diversity (Bustamante *et al.,* 2022). Thus, the integration of morphological and molecular approaches provides a comprehensive evaluation of F1 cacao progenies.

Molecular DNA markers serve as reliable tools for discerning genetic relatedness within the plant species. Among them, SSRs are the best options due to their high polymorphism, codominance, multi-allelic nature, and broad genome coverage (Mekonen *et al.,* 2022). In cacao, the SSR markers have been extensively employed to evaluate the genetic diversity, support parental selection, and identify true male parents and key components of breeding programs (Everaert *et al.,* 2020; Silva *et al.,* 2023). Recognizing the pivotal role of both morphological and molecular characterization in elucidating genetic traits, the following study aimed to describe the phenotypic variations in cacao pod traits within F1 progenies. It also sought to assess their genetic relationships using SSR markers, with the goal of developing new cacao cultivars possessing improved bean quality and higher yield. The expected findings will yield promising cultivars with desirable traits that can positively influence cacao production in Indonesia.

MATERIALS AND METHODS

Plant material and procedure

This study evaluated 20 F1 cacao progenies derived from four distinct cross combinations, using DR 1, ICCRI 03, TSH 858, and SCA 6 as parental genotypes (Table 1). Parental genotype SCA 6 was a widely recognized genotype for its resistance to black pod disease, commonly used as a donor parent (Baruah *et al.,* 2024). In Indonesia, cacao genotypes SCA 6 and SCA 12 are often part of breeding programs due to their resistance to the vascular streak dieback (VSD) and black pod disease. In contrast, parental genotypes DR 1, ICCRI 03, and TSH 858 are the high-yielding and aromatic cultivars, recognized as promising candidate genotypes for developing fine-flavored cocoa (FFC) products (Sari *et al.,* 2022).

The presented experimental layout was a randomized complete block design with three replications, and each cross combination comprised five F1 progenies. The research proceeded at the Pakuwon Experimental Station, Sukabumi, West Java, Indonesia, with an altitude of 450 masl, climate type B based on Schmidt-Ferguson, and an inceptisol soil type, all of which align with the optimal conditions for cacao cultivation.

Cacao pod traits

The data on cacao pod traits attained recording based on the descriptors established by the International Plant Genetic Resources—IPGRI (Bekele and Butler, 2000). The study encompassed assessment of 16 quantitative traits, including pod length (cm), pod girth (cm), pod weight (g), and pod husk thickness by measuring the thickness of the V- and U-shaped exocarp (cm). Additionally, the research measured the fresh and dried weight of beans/pod (g), dried weight of a single bean (g), bean count, and pod index. The number of dark purple and purple beans/pod, light and pale purple beans/pod, and normal and defective cocoa beans/pod also entailed estimates. Cocoa beans sustained oven-drying with temperatures at 55 °C–60 °C for 40–50 hours to achieve a maximum moisture content of 7.5%.

Statistical analysis used the SAS version 9.1. Analysis of variance (ANOVA) succeeded in evaluating the performance of F1 genotypes for measured traits. By getting significant differences among means, the study applied the least significant difference ($LSD_{0.05}$) test for means comparison and separation.

Molecular characterization

Molecular characterization comprised examining the 20 F1 cacao progenies and their four parental genotypes (ICCRI 03, TSH 858, DR 1, and SCA 6). Genomic DNA extraction from leaf samples used a modified CTAB method (Izzah *et al.*, 2022). The DNA amplification performed employed 38 SSR markers, comprising 25 primers from the Indonesian Agricultural Genome Center (PGPI; http://genom.litbang.pertanian.go.id) and 13 markers previously employed by Izzah *et al.* (2022). The PCR amplification protocols followed the procedures as described by Izzah *et al.* (2022). Amplified products underwent separation on 6% non-denaturing PAGE gels. The polymorphic SSR markers' scoring relied on their allele types, with the resulting dataset analyzed using PowerMarker v3.25 to construct phylogenetic trees. Additionally, PowerMarker v3.25 program operations also helped calculate the allele number, major allele frequency, gene diversity, heterozygosity, and the polymorphic information content (PIC).

Table 1. Cacao F1 hybrids used in the study.

Cross combinations	F1 progenies
ICCRI 03 x TSH 858	1-1 (3), 1-3 (1), 1-7 (1), 1-10 (1), 1-11 (1)
ICCRI 03 x DR 1	2-1 (1), 2-7 (3), 2-8 (1), 2-8 (3), 2-11 (2)
TSH 858 x DR 1	5-1 (1), 5-1 (2), 5-3 (2), 5-4 (1), 5-6 (2)
DR 1 x SCA 6	9-1 (1), 9-2 (3), 9-5 (2), 9-6 (3), 9-1 (1)

RESULTS AND DISCUSSION

Pod trait variations

Analysis of variance revealed significant differences among the F1 progenies for five cacao pod traits, particularly pod length, pod girth, and pod weight (Table 2). The F1 progenies obtained from the cross TSH858 x DR1 showed the highest average pod girth and weight, ranging from 26.53 to 29.43 cm and 557.79 to 662.22 g, respectively. Several F1 progenies, 1-7 (1), 2-7 (3), 2-8 (3), 9-1 (1), and 9-6 (3) of the crosses, viz., ICCRI 03 x TSH858, ICCRI 03 x DR1, and DR 1 x SCA6, respectively, also exhibited at-par pod size and weight (Table 2). The mean pod girth and weight for each cross combination were 24.45 cm and 388.55 g (ICCRI03 x TSH858), 24.29 cm and 403.65 g (ICCRI03 x DR1), 27.87 cm and 606.74 g (TSH858 x DR1), and 24.36 cm and 463.8 g (DR1 x SCA6). In the presented study, the average pod weight from the TSH858 x DR1 cross combination exceeded values reported in previous studies on cacao trees in Côte d'Ivoire during the major harvest season. The mean weights were 582 g for stem pods and 406 g for canopy pods (Goudsmit *et al.,* 2023), underscoring the effectiveness of targeted parental genotypes in improving pod size and weight.

Table 2. Performance of 20 F1 progenies (obtained from four crosses) for cacao pod traits.

F1 hybrids	Progenies	Pod length (cm)	Pod girth (cm)	Pod weight (g)	V-shaped exocarp thickness (cm)	U-shaped exocarp thickness (cm)
ICCRI03 x TSH858	1-1 (3)	17.64	24.30	316.06	1.05	1.44
	1-3 (1)	20.21	24.15	388.01	1.15	1.58
	1-7 (1)	19.13	27.58	545.43	1.45	1.87
	1-10 (1)	17.63	22.03	305.44	0.67	1.33
	1-11 (1)	17.13	24.21	387.82	1.15	1.63
Population means		18.35	24.45	388.55	1.09	1.57
ICCRI03 x DR1	2-1 (1)	19.39	23.46	343.26	0.99	1.35
	2-7 (3)	25.53	26.93	596.13	1.40	1.80
	2-8 (1)	20.21	22.49	392.64	1.23	1.71
	2-8 (3)	21.67	27.33	439.63	1.11	1.72
	2-11 (2)	15.75	21.27	246.59	0.79	1.27
Population means		20.51	24.29	403.65	1.10	1.57
TSH858 x DR1	5-1 (1)	22.73	29.38	662.22	1.31	2.07
	5-1 (2)	20.92	29.43	627.95	1.41	1.73
	5-3 (2)	21.67	27.00	600.75	1.17	1.74
	5-4 (1)	21.08	26.53	585.00	1.40	1.80
	5-6 (2)	22.00	27.00	557.79	1.17	1.83
Population means		21.68	27.87	606.74	1.29	1.83
DR1 x SCA6	9-1 (1)	21.50	27.00	630.18	1.53	2.10
	9-2 (3)	18.27	21.86	315.52	1.01	1.48
	9-5 (2)	20.36	23.76	431.94	1.09	1.63
	9-6 (3)	22.25	25.95	575.33	1.52	2.13
	9-7 (1)	19.41	23.22	366.03	1.05	1.49
Population means		20.36	24.36	463.8	1.24	1.77
CV (%)		6.17	4.99	15.15	14.59	13.42
Means		20.22	25.24	465.69	1.182	1.69
$LSD_{0.05}$		2.06	2.08	116.65	0.28	0.37

The F1 progenies of the crosses ICCRI03 x DR1, TSH858 x DR1, and DR1 x SCA6 displayed the longest pods, with average lengths of 20.51, 21.68, and 20.36 cm, respectively. The results highlight the potential of specific F1 progenies to produce larger pods, which is a desirable trait in cacao breeding. Previous studies have shown a considerable correlation between pod size, pod weight, and bean content (Goudsmit *et al.,* 2023), emphasizing the pivotal role of larger pods in meeting breeding objectives. Modern cacao breeding strategies aim to improve both the pod number and size, as enhanced pod dimensions contribute to increased yield and better bean quality (Doaré *et al.,* 2020).

Assessment of the pod husk thickness in 20 F1 progenies revealed notable variations (Table 2). The V-shaped (furrow) exocarp thickness ranged from 0.67 to 1.45 cm, while the U-shaped (ridge) exocarp ranged from 1.27 to 2.13 cm. The six progenies, 9-1 (1), 9-6 (3), 1-7 (1), 2-7 (3), 5-1 (2), and 5-4 (1), of the F1 hybrids DR1 x SCA6, ICCRI 03 x TSH858, ICCRI 03 x DR1, and TSH858 x DR1, respectively, showed significantly thicker exocarp at both points. Additionally, three progenies, 5-1 (1), 5-6 (2), and 5-3 (2), of the F1 hybrid TSH858 x DR1, showed increased thickness specifically at the ridge. The mean exocarp thickness for the V-shaped and U-shaped measurements in each cross combination was 1.09 and 1.57 cm (ICCRI03 x TSH858), 1.10 and 1.57 cm (ICCRI03 x DR1), 1.29 and 1.83 cm (TSH858 x DR1), and 1.24 and 1.77 cm (DR1 x SCA6). In cacao breeding, exocarp thickness is a key trait due to its link with pests and disease resistance. Nyadanu *et al.* (2011) reported a significant negative correlation between exocarp thickness and lesion size, suggesting thicker husks confer greater resistance to *P. palmivora*. Similarly, Ando *et al.* (2015) demonstrated that thicker cuticles in cucumbers hinder pathogen growth, highlighting the importance of physical defense in plant resistance.

Cacao traits associated with bean yield and quality

Fresh and dried bean weights per pod are pivotal contributors to overall cacao production. In this study, the F1 progeny 5-1 (2) exhibited the highest fresh and dried bean weights at 78.06 and 44.43 g, respectively. Following this were three other F1 progenies: 5-6 (2) with 57.20 and 36.81 g; 2-7 (3) with 54.75 and 31.29 g; and 1-1 (3) with 57.56 and 25.23 g (Table 3). These results indicate effective transmission of favorable traits from selected parental genotypes. As detected, the yield potentials of the parental clones used in this study are as follows: DR 1=1,500 kg/ha/year; ICCRI 03=2,376 kg/ha/year; and TSH 858=1,760 kg/ha/year (Wahyudi *et al.*, 2015). Hence, the study expects the progenies derived from these crosses to possess higher yield potentials than their parents. Furthermore, the bean weight per pod emerges as a valuable selection criterion for high-yielding genotypes. Doare *et al.* (2020) emphasized the importance of the bean number and weight per pod in improving productivity in cacao. Identifying such superior progenies offers promising avenues for enhancing cacao yield performance.

Cocoa bean size and pod index are critical indicators of cacao productivity. The pod index reflects the cost efficiency and aids in selecting F1 hybrids with larger beans (Goenaga *et al.,* 2015). Previous studies have reported pod index values for several cacao varieties in different countries ranging from 10 to 30. For instance, the CCN-51 exhibits a pod index between 15 and 22, while Amelonado varieties showed a wider range of 10 to 30 (Jaimez *et al.*, 2022). Remarkably, the F1 progenies obtained in this study, 5-1 (2) displayed the largest bean size (69.41) and a low pod index (23.93), followed by 5-3 (2) with values of 77.60 and 27.17, respectively (Table 3). This observation aligns with the findings of Dinarti *et al.* (2015), who reported an

Table 3. Performance of 20 cacao F1 progenies (obtained from four crosses) for bean yield and quality traits.

F1 hybrids	Progenies	Fresh bean weight/pod (g)	Dried bean weight/pod (g)	Dried one bean weight (g)	Bean count	Pod index
ICCRI03 x TSH858	1-1 (3)	57.56	25.23	0.89	112.18	39.63
	1-3 (1)	37.24	22.74	0.94	106.95	44.90
	1-7 (1)	49.67	27.36	0.93	110.64	36.69
	1-10 (1)	38.04	22.14	0.69	145.57	45.16
	1-11 (1)	38.19	22.17	0.79	126.57	46.52
Population means		44.14	23.93	0.85	120.38	42.58
ICCRI03 x DR1	2-1 (1)	39.82	20.31	0.78	129.29	50.06
	2-7 (3)	54.75	31.29	0.99	100.99	32.48
	2-8 (1)	40.99	21.77	0.96	104.42	48.50
	2-8 (3)	38.06	24.66	1.19	83.91	43.98
	2-11 (2)	29.79	14.59	0.59	173.33	70.35
Population means		40.68	22.52	0.90	118.39	49.07
TSH858 x DR1	5-1 (1)	36.76	19.52	0.90	84.77	51.22
	5-1 (2)	78.06	44.43	1.47	69.41	23.93
	5-3 (2)	43.45	20.69	0.98	77.60	27.17
	5-4 (1)	37.54	20.80	0.89	87.76	48.07
	5-6 (2)	57.20	36.81	1.14	87.46	48.34
Population means		50.60	28.45	1.08	81.4	39.75
DR1 x SCA6	9-1 (1)	49.64	26.50	1.00	100.00	37.74
	9-2 (3)	34.20	19.56	0.76	130.39	67.79
	9-5 (2)	33.01	14.75	1.00	100.00	51.82
	9-6 (3)	50.72	23.54	1.11	89.73	42.49
	9-7 (1)	44.29	22.62	0.96	106.56	44.60
Population means		42.37	21.39	0.97	105.34	48.89
CV (%)		20.98	19.65	11.35	11.17	15.70
Means		44.45	24.07	0.95	106.38	45.07
$LSD_{0.05}$		15.41	7.82	0.19	19.64	11.70

association of a low pod index with a bigger bean size and reduced harvest costs in cacao. It emphasizes the value of selecting progenies with low pod index to improve yield efficiency.

In parallel, cocoa bean quality significantly affects its market value and global acceptance. Compliance with the Indonesian National Standards (SNI 01-2323-2008) ensures quality criteria related to flavor and safety (Ariyanti, 2017; Botutihe *et al.,* 2020). By studying factors influencing quality variation in cocoa (*T. cacao*) bean flavor profile, the key quality indicators were bean size, count, color, and acidity, which proved essential for cacao evaluation (Kongor *et al.,* 2016). Accordingly, this related study also examined these parameters to support selection for high-quality cacao beans.

Bean count per 100 g is an important quality indicator in cocoa classification, influencing grading into categories such as AA, A, and B (Ariyanti, 2017). In this study, all the F1 progenies of the cross TSH858 x DR1 showed bean counts below 100 per 100 g, aligning with AA/A grades (Table 3), and appeared consistent with their larger pod size. Likewise, other F1 progenies, such as 2-8 (3), 9-6 (3), and 9-1 (1), met the AA and A grade standards. These results emphasized the role of genetic factors and parental selection in determining bean count, though other variables (pod size, location, pollen quality, and environmental conditions) also contribute (Goudsmit *et al.,* 2023).

The cocoa bean quality further underwent assessment through color variations (Table 4). F1 progenies 1-1 (3) and 1-10 (1), obtained from the cross ICCRI03 x TSH858, had the highest counts of dark purple beans, while F1 progenies from the crosses DR1 x SCA

Table 4. Performance of 20 cacao F1 progenies (obtained from four crosses) for variations in bean color and the number of beans.

F1 hybrids	Progenies	Dark purple beans/pod	Purple beans/pods	Light purple beans/pod	Pale purple beans/pod	Normal beans/pod	Defect beans/pod
ICCRI03 x TSH858	1-1 (3)	21.38	12.56	6.29	2.00	33.14	3.36
	1-3 (1)	18.27	7.88	4.52	3.88	26.36	2.97
	1-7 (1)	13.12	12.99	9.78	3.08	28.63	2.82
	1-10 (1)	20.33	15.78	9.00	6.60	32.23	6.62
	1-11 (1)	14.39	10.47	7.64	5.34	30.31	3.75
Population means		17.49	11.94	7.45	4.18	30.13	3.90
ICCRI03 x DR1	2-1 (1)	13.44	8.19	4.95	6.50	29.13	1.84
	2-7 (3)	10.33	17.33	6.11	15.67	35.67	2.17
	2-8 (1)	5.52	6.88	12.25	6.32	25.44	2.78
	2-8 (3)	3.00	4.33	5.00	12.33	23.67	2.33
	2-11 (2)	5.75	10.50	4.83	11.33	27.83	4.50
Population means		7.61	9.45	6.63	10.43	28.35	2.72
TSH858 x DR1	5-1 (1)	15.33	18.50	3.00	4.00	40.83	2.00
	5-1 (2)	9.00	11.33	7.67	6.67	31.67	2.33
	5-3 (2)	14.13	11.25	11.13	7.71	44.21	4.22
	5-4 (1)	7.02	14.98	12.94	7.45	42.39	12.25
	5-6 (2)	16.00	nd	31.00	nd	47.00	2.00
Population means		12.29	14.01	13.15	6.46	41.22	4.56
DR1 x SCA6	9-1 (1)	12.00	12.00	Nd	6.00	30.00	1.00
	9-2 (3)	12.63	12.46	8.54	7.12	30.62	3.16
	9-5 (2)	12.67	20.40	5.00	nd	24.43	6.00
	9-6 (3)	2.00	16.50	14.00	1.00	28.00	10.00
	9-7 (1)	14.28	15.52	8.76	8.43	34.39	3.17
Population means		10.72	15.38	9.08	5.64	29.49	4.67
CV (%)		30.29	28.28	27.77	57.12	11.57	33.02
Means		12.29	12.62	9.07	6.71	32.29	3.96
$LSD_{0.05}$		1.19	0.89	1.17	1.34	6.17	2.16

6 and TSH858 x DR 1 displayed the higher purple coloration. Lighter shades were prevalent in the F1 progenies obtained from the crosses TSH858 x DR 1 and ICCRI03 x DR 1. Bean color reflected inheritance from parental types—Forastero (SCA 6) and Trinitario (TSH 858)—and favored the darker seeds, while parental cultivar DR1 contributed lighter hues. Bean color, size, and count were evidently the key FFC (fine-flavored cocoa) quality indicators (Kongor *et al.,* 2016; Sari *et al.,* 2022).

Normal and defective bean counts per pod also reflected the bean quality. The F1 progenies 5-6 (2), 5-3 (2), 5-4 (1), and 5-1 (1) from the cross TSH858 x DR1 displayed the most normal bean count (Table 4), supporting the association observed between pod size and bean number in cacao genotypes (Goudsmit *et al.,* 2023). In the presented study, the defective bean incidence was low across the F1 progenies, suggesting potential pest and disease resistance.

Molecular profiling of F1 populations

Twenty-four cacao genotypes (20 F1 progenies and four parental cultivars) succeeded in analysis using 11 polymorphic SSR markers (Table 5). These markers generated 35 alleles, averaging 3.18 alleles per locus. The major allele frequency ranged from 0.42 (mTcCIR33, mTcCIR1, and SSRCc_4-8) to 0.71 (mTcCIR24), with an average of 0.55. The mean gene diversity (He) was 0.56 (ranging from 0.44 to 0.69), occurring slightly lower than the observed heterozygosity (Ho) at 0.64 (ranging from 0.25 to 0.88). The polymorphic information content (PIC) spanned from 0.35 (mTcCIR184) to 0.63 (mTcCIR33), with an

Table 5. Summary statistics of 11 polymorphic SSR markers used to assess the allelic diversity in 20 F1 cacao hybrids and their parental genotypes.

Markers	Allele Number	Major Allele Frequency	Gene Diversity	Observed Heterozygosity	PIC
mTcCIR33	4	0.42	0.69	0.88	0.63
mTcCIR69	3	0.56	0.54	0.63	0.47
mTcCIR15	3	0.56	0.55	0.58	0.46
mTcCIR24	4	0.71	0.46	0.54	0.47
mTcCIR184	2	0.67	0.44	0.25	0.35
mTcCIR1	3	0.42	0.63	0.83	0.57
mTcCIR109	3	0.58	0.53	0.75	0.47
mTcCIR167	3	0.64	0.52	0.58	0.53
SSRCc_5-48	3	0.46	0.60	0.71	0.52
SSRCc_4-8	3	0.42	0.66	0.75	0.58
SSRCc_2-5	4	0.60	0.56	0.54	0.51
Means	3.18	0.55	0.56	0.64	0.50

average of 0.50. The Ho and He values align with previous reports based on Sulawesi cacao populations (Dinarti *et al.,* 2015) and emerged slightly higher than the values obtained at Jamaica (Lindo *et al.,* 2018). In addition, six markers had PIC values > 0.5, while five stretched from 0.35 to 0.47. Since markers with PIC > 0.5 were options, the markers with < 0.25 were undesirable (Serrote *et al.,* 2020). However, the 11 markers used in the timely study were notably appropriate for future genetic studies.

Molecular markers are essential tools in plant breeding, providing insights into genetic diversity, relatedness, and the genetic potential of the individual plant genotypes. This information accelerates the identification and selection of the promising cacao genotypes (Dinarti *et al.,* 2015). In this study, genetic relationships among the cacao F1 hybrids and their parental genotypes entailed visualization using 11 polymorphic SSR markers (Figure 1).

The phylogenetic tree divided all F1 progenies and their four parental genotypes into three main clusters. Group I comprised two parental genotypes and six F1 progenies. Progeny 9-7 (1) clustered with SCA 6, the male parent, while two progenies—2-1 (1) and 2-8 (1)—clustered with ICCRI 03, which served as the female parent in the crosses ICCRI 03 x DR 1.

Group II comprised the parental genotype DR 1 along with five F1 hybrids: 2-11 (2), 2-8 (3), 2-7 (3), 5-1 (2), and 9-1 (1). In this cluster, DR 1 functioned as both the female and male parent in the crosses ICCRI 03 x DR 1, TSH 858 x DR 1, and DR 1 x SCA 6. Group III included the parental genotype TSH 858 and nine F1 progenies. Among them, six hybrids—1-10 (1), 5-1 (1), 5-3 (2), 1-11 (1), 5-4 (1), and 5-6 (2)—closely clustered with TSH 858, which also served as both the female and male parent in the crosses TSH 858 x DR 1 and ICCRI 03 x TSH 858. The results highlighted the effectiveness of strategic parental genotypes in enhancing genetic diversity, which is an important aspect in cacao breeding. These outcomes support the previous findings emphasizing the role of genetic variation in the conservation of cacao (Lindo *et al.,* 2018).

Molecular characterization complements morphological data, offering better information on genetic relationships among the cacao genotypes. Morphological traits alone often fail to distinguish closely related genotypes, limiting the accuracy of genetic assessment. Previous studies have stated the values of molecular markers in clarifying genetic identity. For example, in Yunnan, China, tracing 59% of the parentage of 88 cacao accessions resulted in Amelonado and 17% to two Upper Amazon Forastero populations (Wang *et al.,* 2020). Molecular tools have emerged as effective in identifying duplicate germplasm accessions, highlighting the limitations of morphological descriptors (Lindo *et al.,* 2018). The genetic diversity

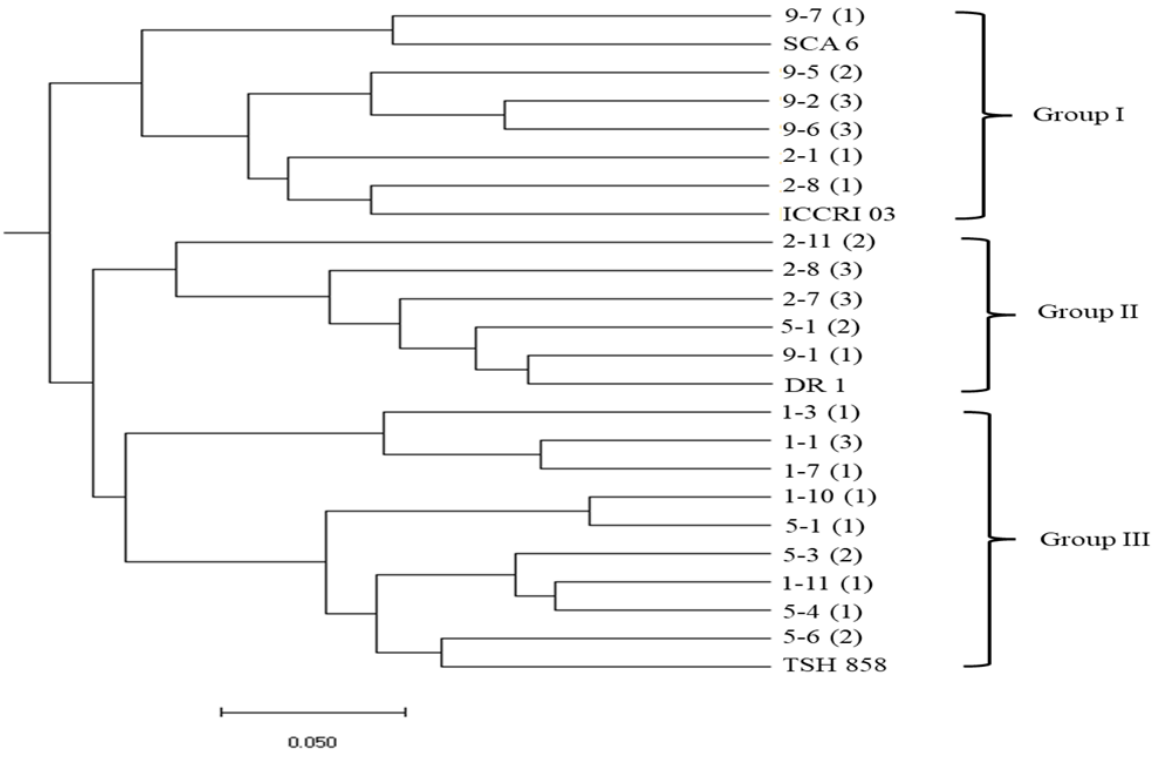

Figure 1. Genetic relationship among the four parental genotypes and 20 F1 cacao hybrids based on SSR markers.

observed in the F1 hybrids, characterized both morphologically and molecularly, can serve as a valuable resource for future cacao breeding efforts.

Genetic diversity data enables the assessment of genetic distance between the F1 progenies and their parental cultivars, aiding in the selection of optimal cross combinations. Greater genetic distance typically indicates more promising pairings. Understanding genetic distance is essential for selecting appropriate parental genotypes in cacao breeding. Bekele *et al.* (2022) emphasized choosing parental cultivars with favorable alleles and high predictive values can accelerate genetic gains. These findings highlighted the importance of integrating morphological and molecular data to update the breeding strategies aimed at enhancing bean yield with desirable quality in Indonesian cacao.

CONCLUSIONS

The latest study revealed substantial variations in pod traits, bean yield, and quality among the 20 F1 hybrids obtained from four cacao parental genotypes. Several hybrids exhibited superior pod size, husk thickness, and bean traits, reflecting effective inheritance of desirable traits. The SSR marker analysis expressed a high level of genetic diversity and heterozygosity in the genotypes studied. Phylogenetic clustering, genetic distance analyses, and morphological characterization collectively demonstrated the effectiveness of parental selection and identified promising cross combinations for future breeding. Integrating morphological and molecular data provides a strong basis for selecting elite genotypes to improve the cacao productivity and quality. These potential F1 hybrids could play a valuable role in cacao breeding

programs in Indonesia, particularly in producing high-quality cocoa beans and enhancing overall cacao production.

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REFERENCES

Ando K, Carr KM, Colle M, Mansfeld BN, Grumet R (2015). Exocarp properties and transcriptomic analysis of cucumber (*Cucumis sativus*) fruit expressing age-related resistance to *Phytophthora capsici*. *Plos One* 10(11): 1–20. https://doi.org/10.1371/journal.pone.0142133.

Ariyanti M (2017). Quality characteristics of cocoa beans (*Theobroma cacao* L) with time fermentation treatment based on ISO 2323-2008. *J. Ind. Has. Perk*. 12(1): 34–42.

Baruah IK, Shao J, Ali SS, Schmidt ME, Meinhardt LW, Bailey BA, Cohen SP (2024). Cacao pod transcriptome profiling of seven genotypes identifies features associated with post-penetration resistance to *Phytophthora palmivora.Sci. Rep.* 14(4175): 1–17. https://doi.org/10.1038/s41598-024-54355-8.

Bekele F, Butler DR (2000). Proposed list of cocoa descriptors for characterization. Working procedures for cocoa germplasm evaluation and selection. Proc. of the CFC/ICCO/IPGRI Project Workshop. 1998: 41–48.

Bekele F, Phillips-Mora W (2019). Cacao (*Theobroma cacao* L.) breeding. Springer International Publishing. In Advances in Plant Breeding Strategies: Industrial and Food Crops. Switzerland. https://doi.org/10.1007/978-3-030-23265-8_12.

Bekele FL, Bidaisee GG, Allegre M, Argout X, Fouet O, Boccara M, Saravanakumar D, Bekele I, Lanaud C (2022). Genome-wide association studies and genomic selection assays made in a large sample of cacao (*Theobroma cacao* L.) germplasm reveal significant marker-trait associations and good predictive value for improving yield potential. *Plos One* 17(10): 1–35. https://doi.org/10.1371/journal.pone.0260907.

Botutihe F, Yulita Kusumaningrum M, Jambang N (2020). Strategy to fulfill quality requirements of Indonesian National Standard (SNI) of fermented cocoa beans. *J. Tek. Per*. 21(3): 191–202.

Bustamante DE, Motilal LA, Calderon MS, Mahabir A, Oliva M (2022). Genetic diversity and population structure of fine aroma cacao (*Theobroma cacao* L.) from North Peru revealed by single nucleotide polymorphism (SNP) markers. *Front. Ecol. Evol.* 10(895056): 1–15. https://doi.org/10.3389/fevo.2022.895056.

Calvo AM, Botina BL, García MC, Cardona WA, Montenegro AC, Criollo J (2021). Dynamics of cocoa fermentation and its effect on quality. *Sci. Rep*. 11(16746): 1–16. https://doi.org/10.1038/s41598-021-95703-2.

Deepashree G, Raut N, Gasti VD, Manjunathagowda DC, Chittapur R, Gunnaiah R, Reddy SG, Kustagi G, Devan SR, Chinthan KN (2023). Molecular and morphological diversity among the cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] genotypes. *Genet. Resour. Crop. Evol*. 70(1): 159–168. https://doi.org/10.1007/s10722-022-01419-1.

Dinarti D, Susilo AW, Meinhardt LW, Ji K, Motilal LA, Mischke S, Zhang D (2015). Genetic diversity and parentage in farmer selections of cacao from southern Sulawesi, Indonesia revealed by microsatellite markers. *Breed. Sci*. 65(5): 438–446. https://doi.org/10.1270/jsbbs.65.438.

Doare F, Ribeyre F, Cilas C (2020). Genetic and environmental links between traits of cocoa beans and pods clarify the phenotyping processes to be implemented. *Sci. Rep*. 10(9888): 1–6. https://doi.org/10.1038/s41598-020-66969-9.

Everaert H, De Wever J, Tang TKH, Vu TLA, Maebe K, Rottiers H, Lefever S, Smagghe G, Dewettinck K, Messens K (2020). Genetic classification of Vietnamese cacao cultivars assessed by SNP and SSR markers. *Tree Genet. Genomes* 16(43): 2–11. https://doi.org/10.1007/s11295-020-01439-x.

Goenaga R, Guiltinan M, Maximova S, Seguine E, Irizarry H (2015). Yield performance and bean quality traits of cacao propagated by

grafting and somatic embryo-derived cuttings. *Hortscience* 50(3): 358–362.

Goudsmit E, Rozendaal DMA, Tosto A, Slingerland M (2023). Effects of fertilizer application on cacao pod development, pod nutrient content and yield. *Sci. Hort.* 313(2023): 1–15. https://doi.org/10.1016/j.scienta.2023.111869.

Izzah NK, Sulistiyorini I, Wicaksono INA (2022). Identification of true male parents in F1 populations of cacao using SSR markers. *IOP Conf. Ser: Earth Environ. Sci.* https://doi.org/10.1088/1755-1315/974/1/012051.

Jaimez RE, Barragan L, Fernández-Niño M, Wessjohann LA, Cedeño-Garcia G, Sotomayor Cantos I, Arteaga F (2022). *Theobroma cacao* L. cultivar CCN 51: A comprehensive review on origin, genetics, sensory properties, production dynamics, and physiological aspects. *Peer J.* 10: e12676. http://doi.org/10.7717/peerj.12676.

Karakaya O, Yaman M, Balta F, Yilmaz M, Balta MF (2023). Assessment of genetic diversity revealed by morphological traits and ISSR markers in hazelnut germplasm (*Corylus avellana* L.) from Eastern Black Sea Region, Turkey. *Genet. Resour. Crop. Evol.* 70(2):525–537. https://doi.org/10.1007/s10722-022-01444-0.

Kongor JE, Hinneh M, de Walle D Van, Afoakwa EO, Boeckx P, Dewettinck K (2016). Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavour profile - A review. *Food Res. Int*. 82(2016): 44–52. https://doi.org/10.1016/j.foodres.2016.01.012.

Lindo AA, Robinson DE, Tennant PF, Meinhardt LW, Zhang D (2018). Molecular characterization of cacao (*Theobroma cacao*) germplasm from Jamaica using single nucleotide polymorphism (SNP) markers. *Trop. Plant. Biol.* 11(2018): 93–106. https://doi.org/10.1007/s12042-018-9203-5.

Mekonen DA, Abraham A, Oselebe H, Afiukwa C, Ilesanmi O, Abebe TD (2022). Genetic diversity and population structure analysis of grass pea (*Lathyrus sativus* L.) accessions collected from North-Western Ethiopia using SSR markers. *Genet. Resour. Crop. Evol.* 69(3): 1247–1260. https://doi.org/10/1007/s10722-021-01302-5.

Nyadanu D, Assuah MK, Adomako B, Asiama YO, Adu-Ampomah Y (2011). Thickness of the cocoa pod husk and its moisture content as resistance factors to *Phytophthora* pod rot. *Int. J. Agric. Res*. 6(4): 310–322. https://doi.org/10.3923/ijar.2011.310.322.

Rodríguez-Carrasco Y, Gaspari A, Graziani G, Santini A, Ritieni A (2018). Fast analysis of polyphenols and alkaloids in cocoa-based products by ultra-high performance liquid chromatography and Orbitrap high resolution mass spectrometry (UHPLC-Q-Orbitrap-MS/MS). *Food. Res. Int.* 111(2018): 229–236. https://doi.org/10.1016/j.foodres.2018.05.032.

Sari IA, Murti RH, Misnawi, Putra ETS, Susilo AW (2022). Sensory profiles of cocoa genotypes in Indonesia. *Biodiversitas* 23(2): 648–654. https://doi.org/10.13057/biodiv/d230205

Serrote CML, Reiniger LRS, Silva KB, Rabaiolli SMS, Stefanel CM (2020). Determining the polymorphism information content of a molecular marker. *Gene* 726(2020): 1–4. https://doi.org/10.1016/j.gene.2019.144175.

Shilpashree N, Devi SN, Manjunathagowda DC, Muddappa A, Abdelmohsen SAM, Tamam N, Elansary HO, El-Abedin TKZ, Abdelbacki AMM, Janhavi V (2021). Morphological characterization, variability and diversity among vegetable soybean (*Glycine max* L.) genotypes. *Plants* 10(4): 671. https://doi.org/10.3390/.

Silva GS, de Santana Souza J, de Souza Junior JO, Villela-Dias C, Corrêa RX, Calle-Bellido J, Jia H, Ahnert D (2023). Mass selection of drought tolerant cacao in Bahia, Brazil: Morphological, genetic structure, and diversity analysis. *Trop. Plant Biol*. 16(2023): 53–66. https://doi.org/10.1007/s12042-023-09330-4.

Wahyudi T, Pujiyanto, Misnawi (2015). Cacao (*Theobroma cacao* L.): History, Botany, Production, Processing, and Trade. Yogyakarta, Gadjah Mada University Press, Indonesia.

Wang B, Motilal LA, Meinhardt LW, Yin J, Zhang D (2020). Molecular characterization of a cacao germplasm collection maintained in Yunnan, China using single nucleotide polymorphism (SNP) markers. *Trop. Plant Biol*. 13(4): 359–370. https://doi.org/10.1007/s12042-020-09267-y.