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COCOA (*THEOBROMA CACAO* L.) CHARACTERIZATION FOR MORPHOLOGICAL TRAITS AND DROUGHT RESISTANCE GENES IN BANGKA BELITUNG, INDONESIA

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SUMMARY

This research aimed to determine the morphological and genetic diversity in 12 local cocoa (*Theobroma cacao* L.) accessions obtained from Bangka Belitung, Indonesia. The results revealed that cocoa accessions observed with the highest variations for morphological and productivity traits, including fruit and seed weight and fruit skin thickness, are selection opportunities for yield improvement. All accessions exhibited favorable upright branching. Accessions of the Lampur and Pedindang series displayed superior profiles, combining good vigor, upright branching, and heavy fruiting. Cluster analysis yielded three groups, and Clusters 1 and 2 owned the accessions with the highest vigor and potential for low-risk crossbreeding, while Cluster 3 (Cupat and Teladan) offered unique genetic diversity. According to molecular analysis, 10 out of 12 accessions (except RB and PDH) were notable with the *DREB2* gene, indicating greater potential for drought resistance. The results confirmed that the Bangka Belitung cocoa germplasm has a promising genetic potential. This research aims to conduct selection that combines superior morphological characters and *DREB2* gene expression, as well as develop a crossing strategy based on genetic cluster proximity to produce adaptive, productive, and drought-resistant cocoa.

Keywords: Cocoa (*T. cacao* L.), genetic resources, morphological and genetic diversity, cluster analysis, coefficient of variance, *DREB2* gene, mahalanobis

Key findings: The Bangka Belitung cocoa (*T. cacao* L.) accessions emerged with the highest yield potential and the *DREB2* drought resistance gene, such as accessions of the Lampur, Pedindang, and Bembeng series.

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a bean-producing plant that provides the primary raw material for chocolate and its derivatives in various food, beverage, pharmaceutical, and cosmetic industries (Rojo-Poveda *et al.*, 2020; Dzandu *et al.*, 2021). The chocolate product consumption will continue to rise with population growth and rising living standards. Therefore, optimizing cocoa development through intensification and extensification programs is crucial to accelerate the cocoa production both in producing areas and other potential regions, including Bangka Belitung (Directorate of Food Crops, Horticulture, and Plantation Statistics, 2025).

Cocoa is a listed vital plantation commodity in the Bangka Belitung economy, mainly based on its production. Bangka Belitung's dried cocoa bean products owned a significant share in national cocoa production (Directorate of Food Crops, Horticulture, and Plantation Statistics 2025). Cocoa production is primarily prevalent at the smallholder plantations spread across several producing areas, including Bangka Regency, Central Bangka Regency, South Bangka Regency, and West Bangka Regency (Zasari and Sitorus, 2022; Central Statistics Agency of the Bangka Belitung Islands, 2025). Cocoa farming is a mainstay source of income for the farming community in Bangka Belitung, Indonesia. Its development is quite promising with the availability of local cocoa germplasm with the highest genetic potential in Bangka Belitung (Zasari and Sitorus, 2022).

Local Bangka Belitung cocoa accessions are generally 'lindak' cultivars, namely the Forastero group, which have a less distinctive flavor; however, they have shown to be highly productive and tolerant to diseases (Viet-Ha *et al.*, 2016). Therefore, the search for promising local cocoa genetic material can take place through phenotypic and genotypic characterization, thus enabling more efficient utilization of superior genetic material (Mariduena-Zavala *et al.*, 2016; Bekele *et al.*, 2019). Characterization of cocoa accessions, clones, and cultivars is mainly dependent on phenotypic and plant morphological traits.

Agronomic parameters derived from leaves, flowers, fruits, and seed morphology appeared to be crucial information used to study the genetic variability, identify the genotypes, and conserve the genetic resources (Olivia-Cruz *et al.*, 2021) and facilitate the breeders in selecting the high-yielding cocoa plant accessions (Viet-Ha *et al.*, 2016). Confirmation of plant trait variability through molecular analysis enables the collection of data inconsistent with phenotypic observations, as plant phenotypes mostly gain influences from diverse environmental factors (Thottathil *et al.*, 2016).

Molecular marker technology is gaining popularity for genetic identification in cocoa plants in the present era. Various molecular markers have succeeded in their development and implementation to characterize germplasm collections (Sulystiyorini *et al.*, 2018; Osorio-Guarin *et al.*, 2018). These include markers for genes controlling various traits in cocoa plants, which have reached wide detection using different molecular markers (Guiraud *et al.*, 2018; Dzandu *et al.*, 2021). The conduct of characterization holistically was successful by combining phenotype and genotype approaches. The phenotype approach examined morphological traits and visual responses to drought, while the genotype approach detected the *DREB2* gene. Both underwent integration to comprehensively select drought-tolerant cocoa varieties. Information on morphological diversity and drought resistance genes will be helpful to facilitate mapping of cocoa breeding programs in Bangka Belitung, Indonesia.

MATERIALS AND METHODS

Experimental site and genetic materials

The research began during 2025 at the Cimanggu Biomolecular Plantation and Laboratory, Bogor, West Java, Indonesia (106°43'0.01" E, -6°34'59.99" S). Observations of all morphological traits transpired directly on the field of smallholder cocoa plantations in Bangka Belitung, with coordinates of 107°58'00" E and 03°15'00" S.

Table 1. Cacao accessions and their regional origins.

No.	District	Accession	Code
1		Lampur	LMP
2		Bembeng	BMB
3		Pedindang Merah	PDM
4	Bangka Tengah	Pedindang Hijau	PDH
5		Pedindang Merah Hijau	PDMH
6		Kampung Jeruk A	KJA
7		Kampung Jeruk B	KJB
8		Jurung	JRG
9	Bangka	Rebo	RB
10		Deniang	DND
11	Bangka Barat	Cupat	CPT
12	Bangka Selatan	Teladan	TLD

The genetic material comprised 12 local cocoa (*T. cacao* L.) accessions obtained from Bangka Belitung Province, Indonesia (Table 1).

Traits measured

Quantitative traits observed in the fruit and seeds were as follows: fruit length (cm)—measured using a ruler by placing the fruit next to the ruler from the tip to the base of the fruit; fruit circumference (cm)—measured on the widest section of the fruit by wrapping a measuring tape around the fruit, cutting from the top to the bottom; fruit weight (g)—measured by weighing physiologically ripe cocoa fruit using a digital scale with a capacity of 0.5 kg; and fruit exocarp thickness (cm)—measured on the same section of the fruit cut as the fruit diameter, measuring exocarp thickness using a vernier caliper, placed from the outer to the inner side of the fruit skin. The number of seeds per fruit entailed counting. The wet and dry seed weight per fruit (g) underwent weighing and recording.

For traits where higher values are desirable (fruit length, circumference, weight, seeds per fruit, wet seed weight, and dry seed weight):

$$\text{Score} = ([X_i - X_{\min}] / [X_{\max} - X_{\min}]) \times 100$$

For fruit skin thickness, where lower values are desirable:

$$\text{Score} = ([X_{\max} - X_i] / [X_{\max} - X_{\min}]) \times 100$$

The computation of the overall selection index (I) for each accession then continued as the weighted sum of these standardized scores:

$$I = 0.10(\text{Length}) + 0.05(\text{Circ.}) + 0.25(\text{Fr. Wt.}) + 0.05(\text{Skin}) + 0.15(\text{Seeds}) + 0.15(\text{Wet Wt.}) + 0.25(\text{Dry Wt.})$$

Qualitative traits observed by Engels (1986) and Zasari and Sitorus (2022) included vigor, branching type, fruiting rate, color appearance, fruit size, ripe fruit color, unripe skin color, fruit shape, bottleneck, fruit tip, texture, furrow depth, and furrow anthocyanin. These also comprised skin thickness, flowering ability, flowering period, stalk color, staminode shape, staminode anthocyanin, sepal anthocyanin, petal anthocyanin, leaf shape, flush color, leaf base, leaf tip, leaf texture, petiole pulvini, seed shape, seed surface, and cotyledon color.

Molecular analysis

The study performed total DNA extraction using a modified cetyltrimethyl ammonium bromide (CTAB)-based method (Wicaksono *et al.*, 2017). The testing of DNA quantity and quality used a Nanodrop 2000 spectrophotometer. The PCR amplification, as performed, used primers for the *DREB2* gene. PCR template DNA optimization proceeded from each individual using a pair of primers based on the *DREB2* gene mRNA sequence.

Primer design using the Basic Local Alignment Search Tool (BLAST) program on the National Center for Biotechnology Information (NCBI) website yielded the following primers—Forward (F): ATGAGAGGGAAGGGAGGTCC and Reverse (R): CTGCCCAAATGGAGTCGTCT. The PCR took place with a total volume of 10 µl, consisting of 2 µl DNA template, 1 µl primer mix (10 pmol), 5 µl PCR Ready Mix (Biolen), and 2 µl ultrapure water (ddH₂O). PCR proceeded with 35 cycles: pre-denaturation at 95 °C for 3 min, denaturation at 95 °C for 15 s, annealing at 57.5 °C for 15 s, extension at 72 °C for 20 s, and a final extension at 72 °C for 5 min.

Statistical analysis

All the quantitative and qualitative traits data received analysis for genetic distance using the Tocher method in the R 4.3.0 software package biotools—dendrogram visualization using ITOL (Interactive Tree of Life, <https://itol.embl.de/itol.cgi>). The CV calculation used a Python software version with packages (scipy.stats). Genetic distances (Jaccard) based on combined data ranged from 0 to 1. Intra- and inter-cluster distances are available for parent selection guidance processed with R software 4.6.0 with the Micropan package.

RESULTS AND DISCUSSION

Quantitative characters

In cocoa (*T. cacao* L.) accessions, the most stable fruit character was fruit circumference (CV = 3.19%), followed by fruit length (CV = 11.62%), indicating a consistent fruit shape across the studied accessions (Sitorus *et al.*, 2020). Conversely, the most variable character was fruit skin thickness (CV = 149.84%), wherein the highest variation could provide an opportunity for selection to achieve ideal skin thickness (Susilo *et al.*, 2019). In cocoa, the yield-related traits, such as fruit weight (CV = 18.65%), fresh bean weight (CV = 19.70%), and dry bean weight (CV = 20.53%), showed considerable variations, owing to the potential for significant improvement in the fruit yield (Table 2). The bean number variation also suggested a possible trade-off between the bean size and number, a common finding in cocoa (Colonges *et al.*, 2021). However, selection for highly variable traits, such as skin thickness, should have support from multi-location evaluation with sufficient replications to minimize the influence of environmental factors (End *et al.*, 2021).

Table 2. Quantitative traits of fruits and seeds in cocoa accessions.

Accessions	Fruit length (cm)	Fruit circumference (cm)	Fruit weight (g)	Fruit skin thickness (cm)	Seeds per fruit	Wet seed weight per fruit (g)	Dry seed weight per fruit (g)
Lampur	15.00	26.80	450	1.40	37	80	45.50
Bembeng	17.00	26.30	500	1.40	37	90	37.60
Pedindang Merah	15.60	26.50	460	1.35	38	75	43.00
Pedindang Hijau	16.20	26.90	480	1.42	38	77	45.00
Pedindang Merah Hijau	15.00	25.40	450	1.40	37	70	42.00
Kampung Jeruk A	15.00	26.80	450	1.40	38	76	42.50
Kampung Jeruk B	15.80	25.60	480	1.42	36	73	42.00
Jurung	15.20	26.30	485	1.39	40	60	38.00
Rebo	12.40	27.30	350	1.40	47	50	32.00
Deniang	15.30	25.80	390	1.40	45	60	37.20
Cupat	12.00	25.30	300	1.40	30	50	22.00
Teladan	11.70	24.40	260	1.20	28	50	24.50
stdev	1.71	0.83	78.57	3.64	5.25	13.31	7.72
average	14.68	26.12	421.25	2.43	37.58	67.58	37.61
CV(%)	11.62	3.19	18.65	149.84	13.96	19.70	20.53

Qualitative characters

Qualitative traits data from 12 cocoa accessions revealed the highest uniformity in branching type, and all accessions exhibited upright branching, which is also beneficial for intensive cultivation (Lahive *et al.*, 2018). Vigor varied from moderate to good; however, it did not always correlate directly with fruiting rate, as observed in the Deniang accessions (Sitorus *et al.*, 2020). The phenotypic coefficient of variation was the topmost for pod weight, seed weight per pod, and dry seed weight, while the number of seeds per pod and pod length expressed moderate variations among the accessions (Olalekan *et al.*, 2025).

Fruiting rate revealed apparent variations into high, medium, and low categories. Accessions, such as Lampur and Cupat, observed with their high fruiting rates, owned the genetic potential for breeding programs. There were indications of a trade-off between fruit quantity and quality, as observed in the accession Cupat, which produced abundant but small fruits (Susilo *et al.*, 2019). The cocoa accessions with a combination of better vigor, upright branching, and the optimum fruiting (like in accessions Lampur and Pedindang) have an ideal profile for superior selection (Tables 1 to 5). Index-based selection that integrates qualitative and quantitative traits proved necessary to optimize genetic gain in the genotypes (End *et al.*, 2021). Selection index analysis (Table 6) showed a wide variation in index values among

accessions (2.6–88.2), indicating high genetic diversity for fruit and seed traits. Bembeng (I = 88.2) was the selected best accession due to its superior balance of traits (fruit weight, fresh seed weight, and thin skin), followed by Lampur (I = 82.2), which excelled in dry seed weight, and Pedindang Hijau (I = 81.9). Interestingly, Rebo with the highest number of seeds (47 seeds) gained the 9th rank (I = 46.5) due to its very low fresh and dry seed weights, confirming that selection based on seed number alone is misleading. In contrast, Teladan (I = 2.6) performed inferiorly in almost all traits. Thus, the multi-trait selection index proved effective in identifying quantitatively superior genotypes, and Bembeng, Lampur, and Pedindang Hijau emerged as recommended potential parents for cocoa breeding programs.

Observations on 12 durian accessions showed the flowering period was uniform (continuous), which was in accordance with the characteristics of tropical plants (Subhadrabandhu and Ketsa, 2001). However, the flowering capacity among these accessions varied, namely, heavy, medium, or low, and this variation appeared to be related to productivity potential (Brown, 1997). Anthocyanin pigment patterns were highly consistent across the plant organs, with staminodia being the most intensely colored, potentially serving as pollinator attractants and genus markers (Nanthachai, 1994). However, considerable variations were prominent in stalk color and staminodia shape. Accessions with

Table 3. Qualitative traits of flower in cocoa accessions.

Accessions	Flowering ability	Flowering period	Stalk color	Staminode shape	Staminode anthocyanin	Sepala anthocyanin	Petala anthocyanin
Lampur	Abundant	Continuous	Reddish green	Straight	Intensive	Less	Absence
Bembeng	Abundant	Continuous	Reddish	Open	Intensive	Less	Absence
Pedindang Merah	Abundant	Continuous	Red	Open	Intensive	Less	Absence
Pedindang Hijau	Abundant	Continuous	Red	Open	Intensive	Less	Absence
Pedindang Merah Hijau	Moderate	Continuous	Red	Open	Intensive	Less	Absence
Kampung Jeruk A	Moderate	Continuous	Reddish	Open	Intensive	Less	Absence
Kampung Jeruk B	Moderate	Continuous	Reddish green	Straight	Intensive	Less	Absence
Jurung	Moderate	Continuous	Green	Open	Intensive	Less	Absence
Rebo	Moderate	Continuous	Reddish green	Open	Intensive	Less	Absence
Deniang	Moderate	Continuous	Green	Straight	Intensive	Less	Absence
Cupat	Moderate	Continuous	Green	Open	Intensive	Less	Absence
Teladan	Less	Continuous	Green	Open	Intensive	Less	Absence

Table 4. Qualitative traits of vigor, branch types, and pollination rate in cocoa accessions.

Accessions	Color appearance	Fruit size	Ripe fruit color	Rawhide Color	Shape	Bottle neck	Fruit tip	Texture	Groove depth	Anthocyanin in streamline	Skin thickness
Lampur	Glossy	Medium	Orange	Green	Rounded Ellipse	Unclear	Nippled	Slightly smooth	Shallow	Absence	Thick
Bembeng	Glossy	Large	Yellow	Light Green	Rounded Ellipse	Absence	Nippled	Slightly smooth	Shallow	Absence	Thick
Pedindang Merah	Glossy	Large	Red	Red	Rounded Ellipse	Absence	Blunt	Slightly smooth	Shallow	Absence	Thick
Pedindang Hijau	Glossy	Large	Orange	Green	Rounded Ellipse	Absence	Blunt	Slightly smooth	Shallow	Absence	Thick
Pedindang Merah Hijau	Glossy	Medium	Reddish green	Green	Rounded Ellipse	Absence	Blunt	Slightly smooth	Shallow	Absence	Thick
Kampung Jeruk A	Glossy	Medium	Red	Red	Rounded Ellipse	Absence	Blunt	Smooth	Shallow	Absence	Medium
Kampung Jeruk B	Glossy	Medium	Yellow	Light Green	Rounded Ellipse	Absence	Blunt	Kasar	Shallow	Absence	Medium
Jurung	Glossy	Medium	Yellow	Green	Rounded Ellipse	Absence	Nippled	Smooth	Shallow	Absence	Medium
Rebo	Glossy	Medium	Orange	Green	Rounded Ellipse	Absence	Blunt	Smooth	Shallow	Absence	Medium
Deniang	Glossy	Large	Yellow	Light Green	Rounded Ellipse	Absence	Blunt	Smooth	Shallow	Absence	Medium
Cupat	Glossy	Small	Yellow	Green	Oblong	Absence	Blunt	Rough	Medium	Absence	Medium
Teladan	Glossy	Medium	Reddish Yellow	Red	Oblong	Absence	Blunt	Slightly rough	Deep	Intensive	Medium

Table 5. Qualitative traits of seed shape, seed surface, and cotyledon color in cocoa accessions.

Accessions	Seed shape	Seed surface	Cotyledon color
Lampur	Oblong	Flat	Dark Purple
Bembeng	Ovate	Flat	Dark Purple
Pedindang Merah	Oblong	Flat	Dark Purple
Pedindang Hijau	Oblong	Flat	Dark Purple
Pedindang Merah Hijau	Ovate	Flat	Dark Purple
Kampung Jeruk A	Ovate	Flat	Dark Purple
Kampung Jeruk B	Ovate	Flat	Dark Purple
Jurung	Ovate	Flat	Dark Purple
Rebo	Ovate	Flat	Dark Purple
Deniang	Ovate	Flat	Dark Purple
Cupat	Oblong	Flat	Dark Purple
Teladan	Ovate	Flat	Dark Purple

abundant flowering capacity and striking morphological characteristics, such as Lampur and Bembeng, were notable as prime candidates for further selection.

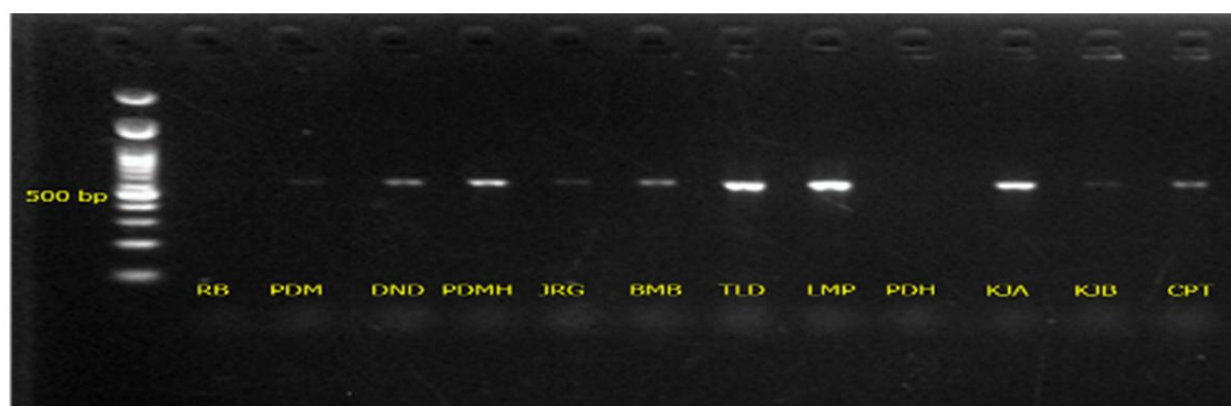
Presence of the *DREB2* gene

Molecular analysis of 12 cocoa accessions identified varying distribution patterns of the *DREB2* (dehydration-responsive element-

binding protein 2) gene. Two accessions (RB and PDH) did not contain the *DREB2* gene, while the other 10 accessions (PDM, DND, PDMH, JRG, BMB, TLD, LMP, KJA, KJB, and CPT) showed the presence of the gene (Figure 1). In general, the presence of the *DREB2* gene in these accessions revealed potential drought tolerance, consistent with its primary function as a transcription factor that manages the abiotic stress responses (Li *et al.*, 2020a,

Table 6. Standardized scores and selection index (I) for cocoa accessions based on fruit and seed traits.

Accession	Fruit length (cm)	Fruit circumference (cm)	Fruit weight (g)	Fruit skin thickness (cm)	Seeds per fruit	Wet seed weight per fruit (g)	Dry seed weight per fruit (g)	Index (I)	Rank
Bembeng	100	73	100	100	69	100	71	88.2	1
Lampur	62.3	91.5	79.2	100	69	75	100	82.2	2
Pedindang Hijau	85	95	91.7	0	76	67.5	97.9	81.9	3
Pedindang Merah	73.6	80.5	83.3	58	76	62.5	89.4	78.1	4
Kampung Jeruk A	62.3	91.5	79.2	100	76	65	87.2	77.8	5
Jurung	66	73	93.8	71	92	25	68.1	72	6
Kampung Jeruk B	77.4	45.5	91.7	0	61	57.5	85.1	71.7	7
Deniang	67.9	53	54.2	100	100	25	64.7	61.3	8
Rebo	13.2	100	37.5	100	100	0	21.3	46.5	9
Ped. Merah Hijau	62.3	37	79.2	100	69	50	85.1	71	10
Cupat	5.7	34	16.7	100	15	0	0	18.3	11
Teladan	0	0	0	0	0	0	10.6	2.6	12

**Figure 1.** Presence of the *DREB2* gene in cocoa accessions.

2020b). These results got support from the recent studies of Onele *et al.* (2023a, 2023b), who reported the significant induction of the *DREB2* gene expression by various abiotic stresses, including drought and extreme temperatures, thus elucidating the molecular mechanisms of plant adaptation.

However, interpretation of *DREB2* gene detection results requires caution. The presence of the gene does not always guarantee optimal functional expression and superior phenotypic resistance. Zhang *et al.*'s (2024) findings disclosed that the effectiveness of the *DREB2* gene in conferring tolerance was highly dependent on post-transcriptional

regulation and its interaction with specific cofactor proteins. Allelic variations and single-nucleotide polymorphisms (SNPs) in the coding regions of the *DREB2* gene can also affect protein stability and DNA-binding affinity, ultimately modulating the resistance levels (Kidokoro *et al.*, 2022). Therefore, the 10 *DREB2*-positive cocoa accessions in this study could enunciate the varying resistance spectra, depending on the type of *DREB2* allele they possess and their genetic background.

Furthermore, resistance responses were the result of complex gene networks. The *DREB2* gene is typical in interacting synergistically with other transcription factors

from the NAC, MYB, and WRKY families in a regulatory network to coordinate the overall stress response (Movahedi *et al.*, 2022). Therefore, the absence of the *DREB2* gene in accessions RB and PDH does not necessarily render them completely susceptible. These two accessions may have the potential to rely on alternative resistance pathways mediated by other genes, as also reported by Hussain *et al.* (2021) in several plants that exhibited drought tolerance through mechanisms independent of the *DREB2* gene.

Practically, the identification of these *DREB2*-carrying accessions provides a basis for marker-assisted selection (MAS) in drought tolerance breeding programs (Singh *et al.*, 2023). The cocoa accessions, such as TLD and CPT, in addition to *DREB2*, also exhibited the highest genetic diversity based on previous cluster analysis and could serve as potential and unique sources of *DREB2* alleles. Further validation through gene expression assays in stress conditions and direct phenotypic evaluation is essential to confirm the functional contribution of the detected *DREB2* gene alleles to the resistance of each accession.

Cluster analysis

Cluster analysis grouped the 12 cocoa accessions into three genetic clusters, each demonstrating distinct breeding strategies. Among the three clusters, Cluster 3 showed the highest morphological diversity for fruit peel thickness (CV = 10.87%), much higher than Cluster 1 (1.75%) and Cluster 2 (0.78%). For fruit weight, Cluster 2 gave the highest CV (12.86%), followed by Cluster 3 (10.10%) and Cluster 1 (4.43%). Thus, Cluster 3 is an important source of genetic diversity for the fruit peel thickness trait, while Cluster 2 is for fruit weight. Cluster 1 (intra-cluster distance = 43.41) was homogeneous, indicating uniform genetic traits in the accessions. The determination of Cluster 1 as a cluster with the potential for stable varieties was reliant on several parameters. First, the smallest intra-cluster distance (43.41) signifies genetic closeness among accessions within this cluster. Second, the low standard deviation values for key quantitative traits (fruit weight, dry seed

weight, and seed number) imply minimal phenotypic variation. Third, the uniformity of qualitative traits (fruit shape, color, and skin texture) reinforces the consistency of performance among accessions. The combination of these parameters stipulates that accessions in Cluster 1 have a relatively uniform and predictable performance, making them suitable for developing superior varieties. However, high homogeneity also has consequences. Low genetic diversity can reduce the population's adaptive capacity to environmental changes or new pests and diseases (Kumar *et al.*, 2021; Al-Turki and Basahi, 2022). Therefore, although Cluster 1 holds promise for the development of stable varieties, breeding programs still need to maintain genetic diversity by combining genetic material from other clusters to ensure long-term resilience.

Cluster 2 (intra-cluster distance = 49.48) was genetically close to Cluster 1 (inter-cluster distance = 107.41) (Figure 2). The identified closeness among the clusters further allows relatively easy crosses among accessions of both clusters to combine superior traits with minimal genetic barriers. Such a phenomenon can be favorable as an effective strategy for the development of superior cultivars (N'Danikou *et al.*, 2021; Amas *et al.*, 2023). The genetic distance between genotypes from Cluster 1 and Cluster 2 ranged from 0.58 to 0.62 (Table 7), which seemed moderate, still allowing for crosses with minimal genetic barriers. In contrast, the genetic distance between genotypes from Cluster 1 or Cluster 2 and Cluster 3 ranged from 0.72 to 0.78, which is considerably far. This large genetic distance has the potential to cause genetic incompatibilities, such as unstable segregation, low hybrid viability, and difficulty in obtaining desired genotypes in the next generation (Monden *et al.*, 2019; Okechukwu *et al.*, 2023). Therefore, crosses between genotypes from Cluster 1 and Cluster 2 are better options for conventional breeding programs, while the use of Cluster 3 requires biotechnological approaches, such as marker-based selection or bridge crosses (Devi *et al.*, 2022; Singh *et al.*, 2023).



Figure 2. Phylogenetic morphological traits and presence of the *DREB2* gene in cocoa accessions.

Table 7. Genetic distance (dissimilarity) between genotypes recommended as crossing parents.

Cross Combination	Cluster Origin	Genetic Distance	Category
LMP × BMB	Cluster 1 × Cluster 1	0.28	Close (intra-cluster)
LMP × PDM	Cluster 1 × Cluster 1	0.35	Close (intra-cluster)
LMP × KJA	Cluster 1 × Cluster 1	0.41	Moderate (intra-cluster)
BMB × PDH	Cluster 1 × Cluster 1	0.44	Moderate (intra-cluster)
DND × JRG	Cluster 2 × Cluster 2	0.42	Moderate (intra-cluster)
DND × PDMH	Cluster 2 × Cluster 2	0.48	Moderate (intra-cluster)
JRG × KJB	Cluster 2 × Cluster 2	0.52	Moderate (intra-cluster)
TLD × CPT	Cluster 3 × Cluster 3	0.55	Moderate (intra-cluster)
BMB × DND	Cluster 1 × Cluster 2	0.58	Moderate (inter-cluster)
LMP × JRG	Cluster 1 × Cluster 2	0.62	Moderate (inter-cluster)
PDM × DND	Cluster 1 × Cluster 2	0.6	Moderate (inter-cluster)
KJA × KJB	Cluster 1 × Cluster 2	0.58	Moderate (inter-cluster)
BMB × RB	Cluster 1 × Cluster 2	0.65	Distant (inter-cluster)
LMP × TLD	Cluster 1 × Cluster 3	0.72	Distant (inter-cluster)
BMB × CPT	Cluster 1 × Cluster 3	0.75	Distant (inter-cluster)
DND × TLD	Cluster 2 × Cluster 3	0.78	Distant (inter-cluster)
JRG × CPT	Cluster 2 × Cluster 3	0.76	Distant (inter-cluster)

Cluster 3 (comprising accessions CPT and TLD) exhibited a unique profile. This cluster revealed the highest internal diversity (intra-cluster distance of 67.81), indicating its potential as a source of diverse alleles for trait improvement. However, this cluster was genetically very distant from other clusters (inter-cluster distance > 105). The inter-cluster distance of 105 is notably wide because it is three times higher than the highest intra-

cluster distance (67.81). A large inter-cluster distance implies significant genetic differences between groups (Mohammadi and Prasanna, 2003). In a breeding context, excessively large genetic distances can lead to cross incompatibilities, unstable segregation, low hybrid viability, and difficulty in recovering desired genotypes in subsequent generations (Monden *et al.*, 2019; Okechukwu *et al.*, 2023). Therefore, although Cluster 3 has value

as a genetic resource, its utilization in direct crossing programs requires special strategies, such as genetic bridging and marker-based selection, to introgress desired chromosome segments while minimizing the influence of unwanted genes (Devi *et al.*, 2022; Singh *et al.*, 2023).

The results also revealed the complexity of the relationship between gene presence and phenotypic expression. Although detecting the *DREB2* gene, the actual level of drought tolerance can vary between accessions, depending on other factors, such as gene expression regulation, interactions with other stress signaling pathways, and the genotypes' genetic background (Kidokoro *et al.*, 2022; Zhang *et al.*, 2024). Therefore, the detection of the *DREB2* gene in accessions, such as TLD and CPT in the Cluster 3, and the accessions within Cluster 2, needs a follow up with phenotypic drought tolerance evaluation. Likewise, a confirmation in the field and greenhouse is vital to validate the gene's function and quantify its contribution to the overall resistance.

Identifying the specific characteristics of each cluster provides strategic guidance for cocoa breeding programs. Cluster 1, with its high dry bean weight and moderate homogeneity, is suitable for development as a superior variety with predictable performance. Superior accessions in this cluster are BMB (dry bean weight = 37.6 g) and LMP (dry bean weight = 45.5 g), which can be effective as parents for improving dry bean productivity.

Cluster 2 offers the advantage of high seed number per pod, particularly RB with 47 seeds per pod, but its dry bean weight is low (32.0 g). This suggests that selection based solely on seed number can be misleading, as was also seen with the Rebo accession in previous research. The DND and JRG accessions in this cluster have more balanced potential, with seed number 40–45 and dry bean weight 37.2–38.0 g. A multi-trait selection index approach is essential to balance seed number and dry bean weight in this cluster.

Cluster 3, with its TLD and CPT accessions, represents a unique source of genetic diversity, primarily due to its thin-

skinned fruit and *DREB2* positivity. However, its very low dry grain productivity (22.0–24.5 g) is a major constraint. Utilizing this cluster in breeding programs requires biotechnological approaches, such as marker-based selection or bridge crossing to introgress the *DREB2* allele and thin-skin trait without introducing the low-productivity trait (Devi *et al.*, 2022; Singh *et al.*, 2023).

Overall, BMB, LMP, DND, and JRG are the recommended accessions as potential parents for conventional breeding programs, while TLD and CPT can be options as unique gene sources for biotechnological approaches. RB and PDH, which are *DREB2*-negative, remain valuable as benchmarks or alternative gene sources for *DREB2*-independent drought tolerance pathways.

CONCLUSIONS

Twelve local Bangka Belitung cocoa (*T. cacao* L.) accessions exhibited the highest morphological diversity in productivity traits, such as fruit weight and skin thickness. Detecting 10 out of 12 accessions were distinct in possessing the *DREB2* gene, which has the potential to confer drought resistance. Cluster analysis grouped the accessions into three genetic groups, with the accessions Cupat and Teladan belonging to a group with unique diversity but observed with a large genetic distance from the other groups. Accessions Lampur, Pedindang Merah, and Bembeng emerged as recommendable superior candidates due to their better vigor, upright branching, highest productivity, and presence of the *DREB2* gene. The recommended breeding strategy was index-based selection that combines superior morphological traits with drought resistance genes and utilizes genetic distance between groups to develop adaptive and productive cocoa cultivars.

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