Pelita Perkebunan 39(2) 2023, 104–118
DOI: 10.22302/iccri.jur.pelitaperkebunan.v39i2.576
ISSN: 0215-0212 / e-ISSN: 2406-9574

Stability Analysis of Fat and Polyphenol Content of Five Cocoa Clones Grown in Different Environment in Indonesia

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Received: 24 January 2023 / Accepted: 12 March 2023

Abstract

Fat and polyphenols are functional compounds in cocoa beans that determine product quality and are highly influenced by environmental factors. Information regarding the stability of the character of the polyphenol and fat content of the cocoa plant is still limited, therefore it needs further study. This study aimed to determine the stability of fat and polyphenol content in several cocoa clones at three different growing locations. The study used a nested design with a randomized block design with field design consisting of five cocoa clones, three different growing locations and each combination treatment was repeated three times. The cocoa clones used were ICCRI 09, MCC 02, Sulawesi 1, KW 516, and KW 562, planted in three different growing locations namely, Kaliwining Experimental Station, Jember, East Java; Sekampung Udik, East Lampung, Lampung; and Harapan Jaya, Pesawaran, Lampung. The combined analysis of variance indicated that there was a genetic interaction with the environment for the character of fat and polyphenol content. Based on the stability analysis, it is known that all cocoa clones fall into the stable category according to the concept of static stability. Furthermore, based on addtive main effect and multiplicative interaction (AMMI) analysis, the clones that can be recommended for Jember, East Lampung and Pesawaran locations for the character of fat content are clone KW 516, while for the characters of high and stable polyphenol levels at the three locations are clones MCC 02, KW 516, and KW 562. In addition, the most recommended clone based on fat content at Jember was the MCC 02 clone, KW 562 at East Lampung, and KW 516 clone at Pesawaran. The existence of the phenomenon of genetic interaction and the growing environment for the characters of fat and polyphenols in cocoa plants provides important information, especially in considering the development of cocoa for specific purposes, namely parameters of fat and polyphenols.

Keywords: *Theobroma cacao*, clones, fat, polyphenol, environment

INTRODUCTION

Fat and polyphenols are the two functional compounds of cocoa beans widely used in health, beauty, and medicine (Mustiga *et al.,* 2019; Pinilla, 2015). Cocoa butter is the primary vegetable fat as a dispersion matrix of sugar and cocoa particles; it also determines the final product of cocoa beans. Cocoa fat content directly affects the quality of commercial beans, representing increased costs during the milling process for cocoa beans with a low-fat content (Wood & Lass, 1985). Many polyphenolic compounds are

vital in the mechanism of cocoa resistance to disease stress; they are also known to contribute to the taste and aroma of the final product of cocoa beans (Cerri *et al*., 2019). The currently known fat content of cocoa beans from the 10 clones grown in the Kaliwining Experimental Station of the Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, ranges of 47-58%, with polyphenol levels ranging of 4-7% (Sari *et al*., 2022). Fat and polyphenol levels were affected by bean maturity level, genotype, growing environment, and harvest time (Saldana *et al*., 2002; Aidoo *et al*., 2014).

Cocoa plant breeding has focused on increasing yields and disease resistance (Lockwood & Yin, 1993). However, creating clones that focus on increasing the functional levels of cocoa beans is an opportunity for cocoa plant breeders. Identification of genetic diversity is the first thing to do to find out the characteristics of each clone. Adaptability and the effect of genetic and environmental interactions are crucial for cocoa breeding activities. ICCRI has tested superior and promising clones of cocoa grown in areas with different agroclimatic types, including Sulawesi 1, MCC 02, ICCRI 09, KW 562, and KW 516 clones. However, information on plant adaptability, especially the fat and polyphenol content of cocoa beans from these clones, is still needed to support the cocoa breeding program at ICCRI. Analysis of plant suitability and adaptability to various environmental conditions is crucial to explore before being cultivated commercially (Susilo *et al.,* 2011).

Multilocations experiments can provide plant stability and adaptability information in different environmental conditions. Using only one stability analysis method is not enough to determine the actual performance of a genotype because it will only provide the right decision on the one hand and not be confident on the other analysis method. Plant breeders typically use several suitability and adaptability methods to analyze and interpret genotype recommendations. Therefore, using several methods can help breeders make the right decisions about genotypic stability by comparing existing statistical relationships (Goksoy *et al.,* 2019; Herawati *et al.,*2021). The primary factors affecting plant breeding programs are genetics (G), environment (E), and the interaction between the two (G × E). By minimizing the interaction effect of genetics with the environment, accurate information on plant stability and adaptability can be generated. Thus, to reduce the effect of the G × E interaction, it is necessary to evaluate the adaptability and stability of each genotype to predict its behavior and response to environmental variations under specific and general conditions (Neto *et al*., 2022). Stability analysis of fat and polyphenols quality can be used as a basis for releasing clones; it will also add to the body of know-ledge on the quality profile of superior cocoa clones.

MATERIALS AND METHODS

The study took place from August 2022 to December 2022 with a randomized block design with clone as single factor and three replications in each environment. The clone factor had five levels, consisting of five ICCRI cocoa clones, thus in total 18 experimental units in each location. The size of each experimental unit was 3 m x 3 m. All plants cultivated follow the cocoa cultivation standards of ICCRI. The five cocoa clones used in this study are listed in Table 1.

Data on the average temperature, humidity, and rainfall of the study locations confirm that the locations have a suitable climate for cocoa plants which grow optimally at a temperature of 26-28 ^{0}C, relative humidity of 78.8%, and rainfall at 1250-3000 mm per year (Basri *et al.,* 2021).

Table 1. List of names, origins, character traits, fat content, and polyphenol content of cocoa clones

Names of clones	Origin	Character traits*	Fat content**	Polyphenol content**
ICCRI 09	TSH 858 x Sulawesi 01 cross	Pod is elliptical, and the young one is dark red.	50.23%	4.80%
Sulawesi 1	East Kalimantan exploration	Pod is oblong, and the young one is dark red.	51.73%	4.33%
MCC 02	South Sulawesi exploration	Pod is rounded and elliptical, and the young one is dark red.	55.50%	6.87%
KW 516	Pabatu exploration, North Sumatera	Pod is rounded and elliptical, and the young one is light green.	53.73%	6.63%
KW 562	Pabatu exploration, North Sumatera	Pod is elongated and elliptical, and the young one is dark red.	-	-

Notes: * = Clone description refers to the germplasm clones of ICCRI , ** = Sari *et al.* (2022).

Table 2. Data on the average temperature, relativev humidity, rainfall, sunshine duration and altitude of the study locations (during 1 July 2021 – 1 July 2022)

Locations	Climate				
	Temperature (^{0}C)	Relative humidity (%)	Rainfall (mm month^{-1})	Day length (hours day^{-1})	Altitude (m asl.)
Jember (Kaliwining Experimental Station)	27	82	204	6.72	45
Lampung Timur (Sekampung Udik)	27	84	181	6.59	29
Pesawaran (Padang Cermin)	26	85	194	6.59	148

Analysis of Fat and Polyphenol Content

Fat content analysis was done following the standard procedures used by ICCRI (SNI, 2009). The fat content analysis began with grinding the cotyledon (without shell) and drying the beans to reach ±7.5% moisture content. Grinding was done using a blender machine to crush the beans into ±150 µm powder. A total of 5 g per sample was taken for hydrolysis using 25% (w w^{-1}) HCl. Then, the hydrolyzed sample was extracted for its fat content using a non-polar organic solvent (petroleum benzene; boiling point 40–60 0C). Fat content is calculated as follows:

$$\frac{100\ (M2-M1)}{M0} \times \frac{100}{(100-KA)}$$

In which:
M_0 = Weight of the tested sample (g)
M_1 = Weight of soxhlet flask and boiling stone (g)
M_2 = Weight of soxhlet flask, boiling stone, and fat (g)
K_A = Water content of the tested sample (%)

Total polyphenol was analyzed based on standardized laboratory procedures used by ICCRI (SNI, 2009). We took ±5 g of cocoa powder of each experimental unit to reduce the fat content to 10-12% in a soxhlet machine using petroleum benzene. The process was done ±6 hours until no more fat dripped into the soxhlet machine from the flask. The sample was dried until it was free of petroleum benzene compounds. A total of 250 mg of cocoa powder mixed with 40 mL of 80% acetone was then sonified for 30 minutes in cold conditions. After sonification, the sample was transferred to a 50 mL volumetric flask in filtered liquid and vacuumed using a vacuum machine up to 50 mL with the addition of 80% acetone. The sample was then calibrated up to 50 mL. The sample was taken as much as 1 mL to be dissolved in 70 mL of distilled water, added with 5 mL of Folin-Ciocalteu reagent, and 15 mL of 20% saturated Na_2CO_3 mixture. The mixed sample was then allowed to stand for two hours at room temperature and measured using spectrophotometer at a wavelength of 765 nm. Estimating the absorbance value of the sample was done using the standard percentage (%).

Data Analysis

Data analysis was done on the five clones grown in three different environments. The analysis was done using combined ANOVA. The GE interaction was examined using the stability analysis, including the one by Francis & Kannenberg (1978), referring to the conventional variance (CV) and Shukla (1972). We also referred to Finlay-Wilkinson (1963) on the regression coefficient value (b_i), in which b_i >1 refers to below-average stability, b_i = 1 refers to average stability, and b_i<1 refers to above-average stability, while the average clone value on all environments (Y_i) was considered as a component of the desired outcome. Wricke (1962) mentions that this method uses equivalence (W_i^2) as a stability parameter. Ecovalence measures the contribution of each clone to the square of total interaction between the clone and the environment. The formula is:

$$W_i^2 = \sum_{j=1}^{q} (X_{ij..} - \bar{X}_{j..} + \ldots)^2$$

The concept of Eberhart-Russell (1966) refers to the value of b_i and the regression deviation (s^2_{di}) (if s^2_{di} = 0 and b_i = 1, then the clone is stable, b_i>1 refers to adaptability in a productive environment, and b_i<1 refers to adaptability in a marginal environment). We also did non-parametric stability analysis, including Nassar-Huehn's method (1987), which refers to the value of $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, and $S_i^{(6)}$ as the parameter of non-parametric stability analysis. $S_i^{(1)}$ is the mean value of the difference in the absolute positions of the clones at several locations. $S_i^{(2)}$ is the variance between ranks in the location. $S_i^{(3)}$ is the sum of the absolute deviations, and $S_i^{(6)}$ is the sum of the squares of the ranks for each clone relative to the mean of the rank. The formula for each of those values is:

$$S_i^{(1)} = 2 \sum_{j}^{n-1} \sum_{(j'=j+1)}^{n} |rij - rij'|/[n(n-1)]$$

$$S_i^{(2)} = \sum_{j}^{n-1} (rij - \bar{r}i.)^2/(n-1)$$

$$S_i^{(3)} = \frac{\sum_{j=1}^{n} (rij - \bar{r}i.)2}{\bar{r}i.}$$

$$S_i^{(6)} = \frac{\sum_{j=1}^{n} |rij - \bar{r}i.|2}{\bar{r}i.}$$

Fox (1990) mentions that this method divides the genotyping results into three layers: top, mid, and low at each location. The frequency of a clone being in the Top layer is used as a stability index value for ranking each clone.

Thenarassu (1995) mentions that this method employs non-parametric stability indices ($NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$) as stability parameters. The value of the nonparametric stability index is obtained from the corrected average ranks of the genotypes or, in this case, the clones at each location, with the following calculation:

$$NP_i^{(1)} = \frac{\sum_{j=1}^{n} |r * ij - M * di|}{n.}$$

$$NP_i^{(2)} = \frac{1}{n}\left(\sum_{j}^{n-1} |r * ij - M * di|/Mdi\right)$$

$$NP_i^{(3)} = \frac{\sqrt{\sum r * ij} - \bar{r} * i/n}{\bar{r}i}$$

$$NP_i^{(4)} = \frac{2}{n(n-1)}\left(\sum_{j=1}^{n-1} \sum_{(j'=j+1)}^{n} |r * ij - r * j'/\bar{r}i|\right)$$

Addtive main effect and multiplicative interaction (AMMI) is a method often used for multilocation tests under the assumption that the errors are normally distributed. AMMI stages are (1) compiling a matrix of the interaction effect of strains and locations, (2) performing a bilinear breakdown of the matrix through SVD (singular value decomposition), (3) determining the number of principal fundamental components I (*Komponen Utama I* – KUI) through postdictive success, and (4) making AMMI biplots (Sujiprihati *et al.*, 2006).

Genotype main effect and genotype by environment interactions (GGE) biplot analysis is a method that partitions the genotype main effect (G) and genotype by experiment interactions (GE) components as the main components evaluated together; they have meaning for cultivars and reduce the environment (E) component to obtain expected clones with number ranks (Ysi). Component E is considered to lack explanation because it has extensive data but is not relevant to the cultivar being evaluated.

RESULTS AND DISCUSSION

Fat and Polyphenol Content

The results showed a significant interaction between genetics and environment on the fat and polyphenol characteristics in the five cocoa clones at three different study locations (Table 3). Environmental factors did not influence the fat characteristic of cocoa beans. Meanwhile, apart from being influenced by the interaction of clones and the environment (GE), polyphenol characteristic was also individually influenced by genetic and environmental factors. Stability tests were only carried out for characteristics influenced by genetic and environmental interactions (G × E) because the test was carried out to interpret G × E interactions in all environments (Parimala *et al*., 2019; Aboughadare *et al.,* 2022).

The analysis showed that Sulawesi 1 planted at Jember and Pesawaran, ICCRI 09 at East Lampung, and MCC 02 at Pesawaran had the lowest fat content, while MCC 02 at Jember and Pesawaran, and KW 516 at East Lampung had the highest polyphenol content. In general, the highest fat and polyphenol content was produced by the clones planted in Kaliwining Jember. Table 4 shows that polyphenol content has significant correlation with rainfall. The analysis showed that Sulawesi 1 planted at Jember and Pesawaran, ICCRI 09 at East Lampung, and MCC 02 at Pesawaran had the lowest fat content, while MCC 02 at Jember and Pesawaran, and KW 516 at East Lampung had the highest polyphenol content. In general, the highest fat and polyphenol content was produced by the clones planted in Kaliwining Jember. Table 4 shows that polyphenol content has significant negative correlation with relative humidity and significant positive correlation with sunshine duration. This is presumably due to the different humidity and sunshine duration in the Jember location, where the relative humidity is lower than the other two locations, while the sunshine duration is longer than the two locations. Therefore, relatively dry environmental conditions tend to produce plants with high levels of polyphenols due to the response of plants to drought. The same thing was found by Kabtni *et al.* (2020) where plants that are in a semi-arid environment (dry environment) tend to produce high total polyphenols. The types of clones also affect the fat and polyphenol of cocoa.

Table 3. Combined ANOVA results on fat characteristics and polyphenol characteristics of the five clones at three different study locations (environments)

Source of diversity	df	Fat			Polyphenol		
		SS	MS	F count	SS	MS	F count
Environment	2	40.15	20.07	2.52 ^{ns}	91.32	45.66	122.02 **
Replication	6	47.74	7.95	1.62 ^{ns}	2.24	0.37	0.88 ^{ns}
Genotype	4	125.14	31.28	6.39 *	30.48	7.62	4.07 *
G x E	8	129.88	16.23	3.31 *	14.96	1.87	4.40 **
Error	24	117.50	4.89		10.18	0.42	
Total	44	460.43					

Notes: df = degree of freedom; SS = sum of squares; MS = mean square; *,** = significantly different at 5% and 1%; ns = not significantly different.

Table 4. Correlation among fat content, polyphenol content, rainfall, air temperature, relative humidity and sunshine during study period

Variable	Correlation	P-value	Note
Air temperature vs rainfall	-0.343	0.777	ns
Relative humidity vs rainfall	-0.744	0.466	ns
Sunshine duration vs rainfall	0.822	0.386	ns
Fat content vs rainfall	-0.129	0.918	ns
Polyphenol content vs rainfall	0.791	0.419	ns
Relative humidity vs air temperature	-0.372	0.757	ns
Sunshine duration vs air temperature	0.254	0.837	ns
Fat content vs air temperature	0.976	0.140	ns
Polyphenol content vs air temperature	0.303	0.804	ns
Sunshine duration vs relative humidity	-0.992	0.079	ns
Fat content vs relative humidity	-0.566	0.617	ns
Polyphenol content vs relative humidity	-0.997	0.047	*
Fat content vs sunshine duration	0.459	0.696	ns
Polyphenol content vs sunshine duration	0.999	0.033	*
Polyphenol content vs Fat content	0.505	0.663	ns

Notes: ns = not significant; * = significant at 0.05 probability level.

Protein, carbohy drates, polyphenols, fat content, and enzymatic activity of cocoa beans are also affected by genotype (Sari *et al.,* 2022). In addition, dif ferences in fat and polyphenols for each clone are influenced by genetics and envi ronment. The fat characteristic of cocoa is strongly influenced by genetics and the environment (Mustiga *et al.,* 2019).

The types of clones also affect the fat and polyphenol of cocoa. Protein, carbohydrates, polyphenols, fat content, and enzymatic activity of cocoa beans are also affected by genotype (Sari *et al.,* 2022). In addition, differences in fat and polyphenols for each clone are influenced by genetics and environment. The fat characteristic of cocoa is strongly influenced by genetics and the environment (Mustiga *et al.,* 2019).

Fat and Polyphenol Content Stability

Singh & Chaudray (1979) group the coefficients of variance into four: (1) low = 0-25%, (2) moderate = 25-50%, (3) quite high = 50-75 %, and (4) high = 75-100%. Sulawesi 1, KW 516, and KW 562 clones had a quite stable polyphenol content, with coefficients of variance of 43.8%, 41.68%, and 38.41%, respectively (Table 7), the values are included in the moderate variance (25-50%). These clones can be assumed to have relatively stable polyphenol content with a static stability group—this kind of stability is important in suboptimal lands or lands with minimal input (Syukur *et al.,* 2018). Francis & Kannenberg (1978) mentioned that genotypes with a low coefficient of variation (*CV*) and high yields are considered stable. Genotypes with high *CV* but below-average results are undesirable which according to Francis-Kannenberg's stability is categorized into static stability (Lin *et al*., 1986). In this method, all clones tested were stable for fat content (%) because the coefficient of variation was very low, ranging from 1.09 to 6.68 (Table 6).

Finlay & Wilkinson (1963) assess stability based on the regression coefficient value (b_i) and the general average of genotypes in all environments; they state that an increase in the regression coefficient value ($b_i = 1$) is the limit of the average stability, an increase in the regression coefficient value ($b_i > 1$) means increased adaptation sensitivity to environmental changes or responses to environments with high yields, and a decrease in the regression coefficient value ($b_i < 1$) means increased adaptation ability to environmental changes. Based on this stability

Table 5. The average percentage of fat and polyphenol characteristics per study location

Clone	Kaliwining Experimental Station Jember		Sekampung Udik East Lampung		Padang Cermin Pesawaran		Average	
	F	PF	F	PF	F	PF	F	PF
ICCRI 09	55.68 [a]	4.95 [c]	50.56 [b]	1.70 [c]	53.06 [a]	2.06 [b]	52.93 [b]	2.90 [c]
MCC 02	57.30 [a]	7.93 [a]	54.63 [ab]	3.06 [abc]	50.15 [b]	4.10 [a]	54.03 [ab]	5.03 [a]
Sulawesi 1	51.10 [b]	4.92 [c]	55.09 [ab]	2.15 [bc]	50.82 [b]	2.83 [ab]	52.34 [b]	3.30 [bc]
KW 516	56.68 [a]	6.71 [b]	56.73 [a]	4.71 [a]	55.64 [a]	2.77 [ab]	56.35 [a]	4.73 [ab]
KW 562	54.75 [a]	5.16 [c]	58.51 [a]	3.41 [ab]	55.58 [a]	2.38 [ab]	56.28 [a]	3.65 [abc]

Notes: F = Fat (%), PF = polyphenol (%), the numbers in each variable column followed by the same letter are not significantly different in the LSD test at the 5% level.

model, clones with above-average stability in terms of fat content are ICCRI 09, KW 516, and KW 562, while MCC 02 and Sulawesi 1 belong to the below-average stability (Table 6). For polyphenol content, the clones with (b_i <1) regression coefficients are KW 562 and Sulawesi 1, and the two clones have above-average stability. ICCRI 09 and KW 516 have average stability, while MCC 02 has below-average stability (Table 7).

Meanwhile, according to Eberhart & Russell (1966), stability analysis combines the value of the regression coefficient (b_i = 1) and the square of the regression deviation value (s^2_{di} = 0) as a parameter of the average stability of a genotype. When this parameter is associated with a high average yield value, the genotype is categorized as having general adaptability; conversely, when this parameter is associated with a low average yield value, the genotype is categorized as having low adaptation to all environments. A regression coefficient above 1 means that genotypes have a quite high sensitivity to environmental changes and are suitable for optimum growing environments. Conversely, if genotypes have a coefficient regression below 1, they are adaptive to environmental changes and suitable for suboptimum growing environments. Using this method, the clones with high adaptability and stability for fat content are ICCRI 09, KW 516, and KW 562 (Table 6). ICCRI 09 are categorized as having average stability with a regression coefficient of 1.01 and a squared deviation value of 0.00 for its polyphenolic content (Table 7). KW 562 and Sulawesi 1 are categorized as having high adaptability and stability in a suboptimum environtment, while MCC 02 has a high sensitivity to environmental changes in terms of poliphenol content (Table 7).

Wricke's (1962) concept of stability uses the smallest ecovalence value. Using this method, the small ecovalence values for fat content are MCC 02, Sulawesi 1, and KW 562 (Table 6). From polyphenol content, ICCRI 09, Sulawesi 1, and KW 562 are the stable clones with small ecovalence values (Table 7); thus, these three clones are classified as having dynamic stability or being able to respond positively to any environmental changes. This stability is included in the concept of dynamic or agronomic stability; dynamic stability means a positive response to the environment and can appear above or below average in different environments (Sabaghnia *et al*., 2014). Dynamic stability is the state of a genotype that is able to adapt to environmental conditions (Lin *et al*., 1986; Becker & Leon, 1988).

Based on Spearman's correlation analysis on fat content, the parameter D_i significantly correlates with s^2d_i, while Wricke's stability parameter (W^2_i) significantly correlates with Shukla's stability variance ($\acute{o}^2_i$) and Kang's stability parameter (YS_i), while the two parameters are also correlated (Table 8). For polyphenol content stability based on Shukla's

variance ($ó^2_i$) positively correlates with Wricke's stability parameter (W^2_i) and Hanson's stability parameter (D_i) (Table 9). Significant correlations between stability parameters confirm that these parameters measure the same stability aspects, and using those stability parameters is possible (Kusumah, 2010).

Nonparametric stability analysis measures the stability of genotypes based on rankings of genotypes in each environment. Genotypes with the same ranking in each environment are not required to be categorized as stable genotypes. Nassar & Huehn (1987) assess stability based on a correlated ranking where the

Table 6. Parametric stability analysis of fat of the five cocoa clones in three different environments

Clones	Y_i	CV_i	b_i	P_b_i	S^2_{di}	$P\_s^2_{di}$	$ó^2_i$	W^2_i
ICCRI 09	52.93	4.35	-0.19	0.18	8.90	0.01	30.41	14.33
MCC 02	54.03	6.68	2.85	0.05	2.78	0.11	28.4	0.59
Sulawesi 1	52.34	4.56	1.22	0.80	5.82	0.04	13.53	7.28
KW 516	56.35	1.09	0.53	0.58	-1.63	0.99	-3.95	13.53
KW 562	56.29	3.51	0.60	0.63	5.21	0.05	12.78	7.58

Notes: Y_i = average fat content of each clones in three different environments (%); CV_i = coefficient of variation; W_i = wricke ecovalence; b_i = genotype regression coefficient; P_b_i = the value of P for b_i; $ó^2_i$ = Shukla's stability variance.

Table 7. Parametric stability analysis of polyphenol of the five cocoa clones in three different environments

Clon	Y_i	CV_i	b_i	P_b_i	S^2_{di}	$P\_s^2_{di}$	$ó^2_i$	W^2_i
ICCRI 09	2.91	61.24	1.01	0.96	0.00	0.32	-0.26	0.14
MCC 02	5.03	50.99	1.42	0.05	0.71	0.02	4.23	1.56
Sulawesi 1	3.3	43.83	0.80	0.30	0.20	0.13	0.86	0.75
KW 516	4.73	41.68	1.01	0.95	1.42	0.00	3.28	1.94
KW 562	3.65	38.41	0.76	0.23	0.26	0.10	1.24	0.6

Notes: Y_i = Average fat content of the five cocoa clones in three different environments (%); CV_i Coefficient ofi variation; W_i = Wricke ecovalence; b_i = genotype regression coefficient; P_b_i = the value of P for b_i; $ó^2$ = Shukla's stability variance.

Table 8. Spearman correlation between parametric stability parameters of fat characteristics of the five cocoa clones in three different environments

	Y_i	CV_i	b_i	s^2d_i	W^2_i	D_i	$ó^2_i$
CV_i	0.70						
b_i	-0.20	0.30					
s^2d_i	0.80	0.30	-0.20				
W^2_i	0.70	0.70	0.50	0.70			
$D\text{-}_i$	0.80	0.30	-0.20	1.00**	0.70		
$ó^2_i$	0.70	0.70	0.50	0.70	1.00**	0.70	
YSi	0.70	0.70	0.50	0.70	1.00**	0.70	1.00**

Notes: Y_i = Average fat content of each clone of the five cocoa clones in three different environments; CV_i = Coefficient of variation; W_i = Wricke ecovalence; S^2b_i = Genotype regression coefficient; P_b_i = the value of P for b_i; D_i = Hanson's stability parameter; YS_i = Kang's stability parameter; $ó^2_i$ = Shukla's stability variance; *, ** = Each is significantly different with 1 at 0.05 and 0.01.

Table 9. Spearman's correlation between parametric stability parameters of fat content of the five cocoa clones in three different environments

	Y_i	CV_i	b_i	s^2d_i	W^2_i	D_i	$ó^2_i$
Y_i							
CV_i	0.30						
b_i	-0.70	-0.30					
s^2d_i	-0.90*	-0.50	0.40				
W^2_i	-1.00**	-0.30	0.70	0.90*			
$D\text{-}_i$	-0.90*	-0.50	0.40	1.00**	0.90*		
$ó^2_i$	-1.00**	-0.30	0.70	0.90*	1.00**	0.90*	
YSi	0.11	0.11	-0.78	0.22	-0.11	0.22	-0.11

Notes: Y_i = Average fat content of each clone of the five cocoa clones in three different environments; CV_i = Coefficient of variance; W_i = Wricke ecovalence; b_i = Genotype regression coefficient; P_b_i = the value of P for b_i; D_i = Hanson's stability parameter; YS_i = Kang's stability parameter; $ó^2_i$ = Shukla's stability variance; *, ** = Each is significantly different with 1 at 0.05 and 0.01.

smallest nonparametric stability index (NSI) value indicates a stable genotype than genotypes with a higher NSI value (Rahadi *et al.,* 2013). The clone with stable fat content in this study is KW 516 because it has thesmallest values for $S_i^{(1)}$ (0.66) $S_i^{(2)}$ (0.33) $S_i^{(3)}$, and (0.4). $S_i^{(6)}$ (0.8) (Table 10). ICCRI 09 is the most stable clone seen from its polyphenol content with $S_i^{(1)}$ (1.33) $S_i^{(2)}$ (1.33) $S_i^{(3)}$, and (0.14) $S_i^{(6)}$ (0.28) (Table 11). Estimation of $S_i^{(1)}$ comes from all possible differences in pairings between all environments for each genotype, where $S_i^{(1)}$ is based on the variance in rankings of environments for each genotype in each environment. The next stable clone is Sulawesi 1.

Thennarasu's method (1995) proposes the nonparametric stability based on the indices of $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$, and $NP_i^{(4)}$, in which a sable genotype is the one that tends to stay at the same ranking in each environment. The stability index based on Thennarasu in this study is KW 516 because $NP_i^{(1)}$ (0.33), $NP_i^{(2)}$ (0.16), $NP_i^{(3)}$ (0.28), and $NP_i^{(4)}$ (0.4) have the smallest index values (Table 10). The unstable clone is KW 562 since it has the highest stability indices in almost all parameters. For polyphenol content, the clone with the smallest nonparametric stability index and stays at the highest ranking is ICCRI 09 with $NP_i^{(1)}$ (0.66), $NP_i^{(2)}$ (0.13), $NP_i^{(3)}$ (0.20), dan $NP_i^{(4)}$ (0.28) presented in Table 11. The clone with the lowest ranking for polyphenol content is MCC 02.

Table 10. Non-parametric stability analysis of fat content of the five cocoa clones in three different environments

Clones	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	TOP	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$
ICCRI 09	2.66	4.33	0.72	0.72	2	1.33	0.44	0.46	0.72
MCC 02	2.66	4	2.6	1.4	1	1.33	0.33	0.48	0.8
Sulawesi 1	2	3	0.5	0.5	1	1	0.25	0.35	0.5
KW 516	0.66	0.33	0.4	0.8	3	0.33	0.16	0.28	0.4
KW 562	2	0.27	2	1.42	2	1	0.5	0.60	0.85

Notes: TOP (Fox *et al.,* 1990); $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $S_i^{(6)}$ (Nassar & Huehn, 1987); $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{()}$, $NP_i^{(4)}$ (Thennarasu, 1995).

Table 11. Nonparametric analysis of polyphenol of the five cocoa clones in three different environments

Clone	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	TOP	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$
ICCRI 09	1.33	1.33	0.14	0.28	0	0.66	0.13	0.20	0.28
MCC 02	2.66	4	1.6	1.6	3	1.33	1.33	0.97	1.6
Sulawesi 1	2	2.33	1.27	0.90	1	1	0.25	0.34	0.54
KW 516	2.66	4	1	1	3	1.33	0.66	0.81	1.33
KW 562	2	2.33	0.66	0.66	2	1	0.33	0.41	0.66

Notes: TOP (Fox *et al.,* 1990); Si(1), Si(2), Si(3), Si(6) (Nassar and Huehn, 1987); NPi(1), NPi(2), NPi(), NPi(4) (Thennarasu, 1995). Nassar & Huehn (1987) propose that Si(1) and Si(2) parameters correlate with static stability.

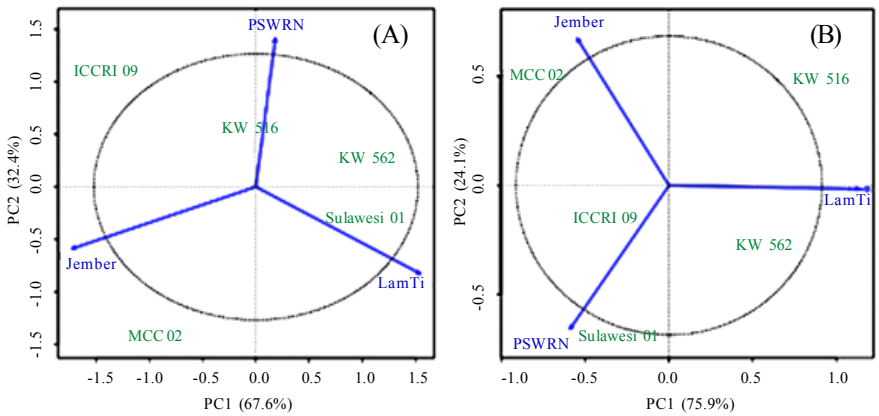

Figure 1. AMMI biplot PC1 vs. PC2 for (A) fat content (B) polyphenol content of the five cocoa clones in three different environments

Rahadi *et al.* (2013) and Sabaghnia *et al.* (2014) report the same things by grouping the Nassar & Heuhn's method and Thennarsu's method as static stability concepts. This method can be used in selecting the best genotype, but it is also necessary to consider the targeted components. Results in this concept can also change at any time, along with the high diversity of environmental conditions or wider testing environments. The concepts of static and dynamic stability rely on the genotype comparison method—the static concept compares the genotype performance in each environment, while the dynamic concept compares the performance of genotypes tested.

Fox *et al.* (1990) measure stability parameters based on the top three of the TOP rankings in each environment. In this study, all clones are included in the stable category for their fat content because they have a non-parametric stability index value which is in the highest TOP category with the following order of KW 516 (3), KW 562 (2), ICCRI 09 (2), Sulawesi 1 (1), and MCC 02 (1). As for the polyphenolic content, MCC 02 (3), KW 516 (3), KW 562 (2), and Sulawesi 1 (1) are clones that fall into the top 3 of the TOP category, which is classified by Fox *et al*. (1990) as clones that are able to adapt to their environments (Table 10). Meanwhile, ICCRI 09 is ranked as an unwanted clone, as described by Mut *et al*. (2009). A high TOP value (the highest genotype in the top three) represents a genotype that can be widely adopted (Aboughadareh *et al*., 2019).

AMMI analysis can be performed if the interaction between genotype and environment is significant. AMMI uses the biplot as a visualization tool to see stable genotypes in all test environments or, specifically, in specific environments. Environment-specific genotypes are far from the main axis but are close to the environmental line, while stable genotypes are close to the main axis (0.0) (Mattjik & Sumertajaya, 2006; Rashidi *et al.,* 2013). Thus, the stable genotype in three environments for fat content is KW 516, while the most suitable for the East Lampung region are KW 562 and Sulawesi 1 (Figure 1). As for polyphenolic content, ICCRI 09 is the closest to the main axis (0.0). It can be categorized as a stable genotype but is classified as the lowest compared to the average of the other clones (2.90%).

The contribution of interaction diversity that can be explained by each component of AMMI 1 and AMMI 2 on fat content is 67.6% and 32.4%. These values dominate in explaining interaction diversity effects by 100%. For polyphenol content, AMMI 1 contributes 75.9%, while AMMI 2 contributes 24.1%, so the dominant diversity has been able to explain 100% of the interaction. AMMI uses a principal component effect to visualize GE interactions in a biplot diagram (Erdemci, 2018). The greatest proportion of interaction diversity of genetic x environment is found in the main component (PC1) (Mattos *et al*., 2013; Regis *et al*., 2018). AMMI is used to identify genotypes with high averages and adaptation values at the desired location through ANOVA analysis and describes the largescale environment (Gauch, 2013; Hongyu *et al*., 2014). Becker & Leon (1988) and Jambormias & Riry (2008) suggest that the stability analysis of Finlay-Wilkins on, Eberhart-Russel, and AMMI is expressed as stability analysis results classified as dynamic stability.

GGE biplot analysis classifies genotypes as stable based on statistical models of main genetic influence or genotype and genetic × environment interaction (Yan, 2001). The GGE biplot produces a graphical visualization that describes genotype performance in specific environments and genotype adaptability in several different environments, identifies the best genotypes in each environment, visualizes environments on a small scale and large scale, and average genotype performance and stability. GGE biplots are also able to display

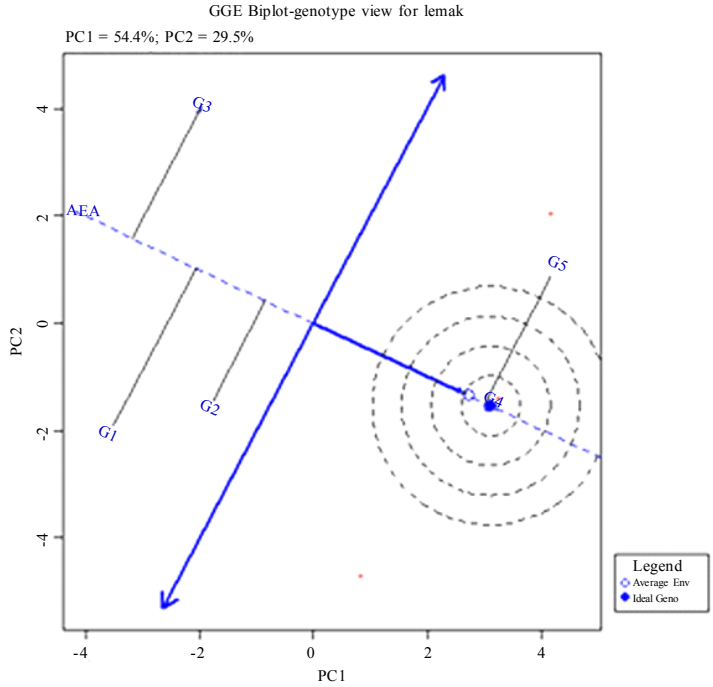

Figure 2. GGE biplot presenting fat stability of five cocoa clones in three different environments. E1 = Kaliwining ES, Jember; E2 = Sekampung Udik, East Lampung; E3 = Padang Cermin, Pesawaran; G1 = ICCRI 09; G2 = MCC 02; G3 = Sulawesi 1; G4 = KW 516; G5 = KW 562.

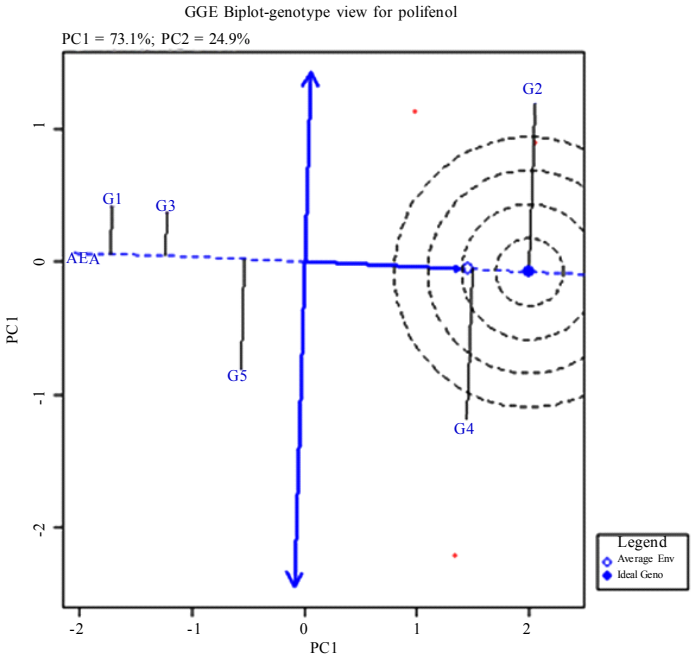

Figure 3. GGE biplot presenting polyphenol stability of five cocoa clones in three different environments. E1 = Kaliwining ES, Jember; E2 = Sekampung Udik, Lampung East; E3 = Padang Cermin, Pesawaran; G1 = ICCRI 09; G2 = MCC 02; G3 = Sulawesi 1; G4 = KW 516; G5 = KW 562.

the best genotype with the highest yield in each test environment and are able to show the ideal genotype and environment (Farshadfar *et al*., 2013; Farshadfar & Sadegi, 2014; Susanto *et al*., 2015).

The AEA axis (X axis) provides an overview of average yields, while the Y axis is a line perpendicular to the AEA axis and through the point of origin of the biplot, which provides an overview of agronomic stability (Yan & Tinker, 2005). Clones to the right of the Y axis have higher yields than the average of all genotypes, while those to the left of the Y axis have yields below the average of all genotypes, and the farther the genotype is from the X axis, the more unstable the genotype will be (Priyanto *et al*., 2017). This concept shows that KW 516 and KW 562 give the highest average results for fat content. In addition, KW 516 is the most stable clone in all test environments because it is closest to the X axis (Figure 3). As for polyphenol content, KW 516 and MCC 02 have the highest average for all genotypes; conversely, ICCRI 09 yields below average (Figure 4).

Our data confirm that all five cocoa clones fall into the stable category according to the concept of static stability. However, there is a clone that can be recommended for cultivation in Jember, East Lampung, and Pesawaran based on fat content, namely KW 516. If we are looking for stable and high polyphenol content, MCC 02, KW 516, and KW 562 can be recommended. In addition, the most recommended clone based on fat content is MCC 02 in Jember, KW 562 in East Lampung, and KW 516 in Pesawaran. These clones have a high-fat content and a relatively low amount of polyphenols at their respective cultivation locations. High-fat content and relatively low polyphenol content is an excellent combination to produce cocoa beans that taste good and are beneficial for health. The high levels of polyphenols in cocoa beans can spoil the taste, such as a high astringent taste for a long time (Sari *et al*., 2022).

CONCLUSIONS

The five cocoa clones are able to produce quality cocoa with high-fat content and relatively moderate polyphenol content. Based on parametric and nonparametric stability tests, ICCRI 09, KW 516, and KW 562 have the most stable fat content, while MCC 02 and Sulawesi 1 are categorized as having specific stability to the environment (MCC 02 in Jember and Sulawesi 1 in Pesawaran) as revealed by AMMI. Sulawesi 1, KW 516, and KW 562 have stable polyphenol content according to the concept of static stability; the two clones are also categorized as clones with above-average stability based on dynamic stability.

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