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Digital Imaging-Assisted Characterization of Plants' Morphological Features for the Identification of Robusta Coffee Clones

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Abstract

Digital imaging can be a helpful tool to support the identification of Robusta coffee for breeding programs. This study aimed to identify Robusta coffee clones through morphological characterization assisted by digital image analysis. Five Robusta clones namely BP 308, BP 409, BP 534, BP 936, and BP 939, were evaluated. The trial was done using a completely randomized design with a single factor, namely a clone, with three replications. The results showed that the factor (Robusta coffee clones) had a significant effect on all quantitative characters except for the number of clusters per branch, leaf length, and fruit width. Significant differences were also shown in digital image characters, namely red and green values in the young and adult leaf phases. There was a distinct separation based on the clustering analysis results. The first group consisted of BP 939, and the second group consisted of BP 308, BP 409, BP 936, and BP 534. The second group had two subgroups with a dissimilarity distance of 30%. Subgroup 1 consisted of BP 308, while Sub-group 2 consisted of BP 409, BP 936, and BP 534

Keyword: Cluster analysis, multivariate analysis, Robusta breeding, PCoA

INTRODUCTION

Indonesia is the world's fourth largest coffee producer, with an annual production of 774,960 tons from a cultivated area of 1.26 million ha (BPS, 2023). Indonesia exported 437,560 tons of coffee, generating US$1,148.38 million in export value (BPS, 2023). On the other hand, domestic coffee consumption increased by 18.2% in 2023 compared to the previous year, making Indonesia the second largest coffee consumer in the Asia Pacific region, after Japan (ICO, 2023). Robusta coffee accounts for 73% of Indonesia's total coffee production (Ministry of Agriculture, 2022)

Coffea canephora is classified as a diploid (2n = 2x = 22) plant. It is self-incompatible and mostly pollinated by the help of insects and other animals (Charrier *et al*., 1985; Klein *et al*., 2003). Early isozyme analysis divided *C. canephora* into two distinct genetic groups: (i) the Guinean Group, comprising wild populations from the Ivory Coast, and (ii) the Congolese Group, consisting of accessions from the Central African Republic and Cameroon (Berthaud, 1986). On the other hand, Gomez *et al*. (2009) categorized the genetic diversity of *C. canephora* into five genetic groups (A, B, C, D, and E) through RFLP and SSR marker approaches. Geographically, genetic group A consists of wild populations from Congo and Cameroon, group B from east-central Africa, group C from west-central Africa, Cameroon, and northeastern Congo, group E from southern Congo and Cameroon, while Group D comprises wild populations from Ivory Coast and Guinea.

The development of Robusta coffee in Indonesia began in 1900s with BP 42, BP 234, BP 288, BP 358, BP 409, and SA 237 (Ministry of Agriculture, 2021). In the 2003-2004 period, BP 436, BP 920, BP 534, BP 936, BP 939, SA 203, and BP 308 were released (Ministry of Agriculture, 2021). Between 2014 and 2019, Sehasence, Sintaro 1, Sintaro 2, Sintaro 3, Hibiro 1, Hibiro 2, Hibiro 3, Hibiro 4, and Hibiro 5 were released (Ministry of Agriculture, 2021). These recommended clones have shown improvements in terms of yield, with a 50-70% increase compared to previously recommended clones (Hulupi, 2016). This is in line with global Robusta coffee breeding programs, which are aimed at achieving high productivity (AbacusBio, 2023). However, In Indonesia, Robusta coffee breeding programs are also focused on other important characteristics such as large bean size, good flavor, and resistance to biotic stress (Sumirat *et al.*, 2007; AbacusBio, 2023). Improving the superior traits of released Robusta coffee clones can be used as a direction to obtain new superior clones (Akbar *et al.*, 2022; Wibowo *et al.*, 2022). Therefore, information on the agronomic performance of existing clones is crucial for the selection of parental crosses. The crosses can serve as an initial step in obtaining broader genetic diversity.

Cluster analysis can be used to observe the genetic distance between parental Robusta coffee clones (Sumirat *et al.*, 2007; Rubiyo *et al.*, 2022). The morphological characteristics of plants are the parameters that can be used for cluster analysis. Digital imaging can assist in identifying morphological differences between Robusta coffee clones. This technology facilitates the accurate selection of large numbers of samples in a short time (Das Choudhury *et al.*, 2018). Several studies have reported the effective and efficient use of phenotype-based image selection methods in rice (Laraswati *et al.*, 2021; Guimaraes *et al.*, 2020) and maize. Li *et al.* (2021) reported the use of digital images for maize phenotyping at the seedling stage using end-to-end maize segmentation networks (Li *et al.*, 2021).

Studies on the use of digital imaging on coffee plants have been conducted to differentiate nitr-ogen concentrations in coffee leaves (Godoy *et al.*, 2022). This study employed regression and correlation analysis to evaluate the relationship between digital image variables and nitrogen content in coffee leaves. The results obtained showed that the hue approach and dark green color index (DGCI) could differentiate the nitrogen content in leaves (Godoy *et al.*, 2022). Digital images have also been used to identify coffee leaf rust (Jepkoech *et al.*, 2021; Soares *et al.*, 2022). This study used multispectral images obtained using a Mapir Survey3W camera, RGB, and unmanned aerial vehicle (UAV) to detect early leaf rust disease in coffee seedlings (Soares *et al.*, 2022). This study reported that multispectral images were able to accurately (>80%) distinguish *H. broadatrix* infection in coffee seedlings at the asymptomatic stage (15 days after inoculation) using the support vector machine (SVM) algorithm. This research aimed to identify Robusta coffee clones through digital imaging-assisted methods to provide better and faster characterization.

MATERIALS AND METHODS

Materials

Five Robusta coffee clones were evaluated in this study, consisting of BP 308, BP 409, BP 534, BP 936, and BP 939 (Table 1). The plants evaluated were 20 years old with BP 308 rootstock. The plant was planted at a spacing of 2.5 m × 2.5 m using *Leucaena leucocephala* as a shade tree. Plant maintenance was carried

Table 1. Description of origin of the Robusta clone samples

Clone	Origin
BP 308	Results of individual selection in the Robusta coffee population at the Kaliwining Experimental Garden in the 1930s.
BP 409	Primary clone of BP 42, selected at Kebun Dampar with selection number 01, re-selected at Dampar with number 10.
BP 534	The result of individual selection on the Robusta coffee population in Tugusari Plantation, with the parent tree number 6, was subsequently given the selection number BP 534.
BP 936	The result of individual selection on the offspring of a cross between SA 164-11 (seed origin) x BP 42 with the mother tree number SA mb 54.
BP 939	The result of individual selection on the offspring of a cross between BP 42 (from seed) x SA 1366, with the mother tree number SA mb 38.

out according to the Robusta coffee cultivation standards (Permentan, 2014). The plantation area was located at Sumber Asin Plantation (589 m asl.) Malang, East Java (8°16'40.4"S 112°42'37.8"E). Digital image analysis was carried out from May to November 2023 at the Plant Breeding Laboratory, Indonesian Coffee and Cocoa Research Institute, Jember, Indonesia (8°15'26.5"S 113°36'47.0"E).

Experimental Design

A completely randomized design with a single factor, namely clone, with three replications, was used in this study, resulting in 15 experimental units. Each experimental unit consisted of 20 plants. All morphological characters were observed according to the coffee description standards (IPGRI, 1996). A total of 10 quantitative characters were observed, including internode length, number of bunches per branch, number of fruits per bunch, number of fruits per branch, leaf length, leaf width, fruit length, fruit width, fruit thickness, and weight of 100 dry beans with a moisture content of 12.5%. A total of 18 qualitative characteristics were observed: overall appearance, plant habit, vegetative development, branch angle, leaf shape, leaf apex shape, leaf petiole color, leaf flush color, mature leaf color, stipule shape, firmness of leaf surface waves, firmness of leaf edge waves, inflorescence position, inflorescence on old wood, anther insertion, fruit shape, fruit-disc shape, and ripe fruit color.

Image Analysis

Leaf images were captured using a Nikon D90 RGB camera in a portable photo studio (40 cm × 40 cm × 40 cm). A white background with a single 14-watt white LED light in the studio and camera settings (8 F-stop, shutter speed 1/8, ISO 400, and no flash) was used. The observed characteristics were red (R), green (G), and blue (B) values on flush leaves, young leaves, and old leaves. RGB color was measured by averaging the RGB values of all pixels in the image. RGB values ranged from 0 (no color) to 255 (maximum color).

Data Analysis

The observed data were analyzed using analysis of variance. If there was a significant difference in treatments, a Duncan's multiple range test at the 5% level was conducted. Both analyses were performed using the STAR 2.0.1 IRRI software. Quantitative and qualitative traits were combined for cluster analysis using Gower's method and principal coordinate analysis (PCoA). Digital image traits were analyzed using Euclidean distance for cluster analysis. Cluster analysis and PCoA were performed using RStudio R 4.3.2. The cluster analysis used the 'cluster' package and the PCoA analysis used the 'ape' and 'vegan' packages. Subsequently, the results of the PCoA were plotted using orthogonal mapping according to the 'which-won-where' concept to determine the specific traits of each clone.

RESULTS AND DISCUSSION

Quantitative, Qualitative, and Digital Image Characteristics

Clone factor had a significant effect (p<0.05) on all parameters except for the number of clusters per branch, leaf length, and fruit width (Table 2). The coefficient of variation (CV) ranged from 0.65 to 12.70%. CV indicates heterogeneity in the population (Mattjik & Sumertajaya, 2013). The average values of the parameters are presented in Table 2. The internode length of the Robusta coffee clones varied from 6.4 to 8.3 cm. Clone BP 308 had the shortest internode length (6.5 cm), significantly different from those of other clones. Coffee plants with a wide internode length tend to have larger canopies. This affects the planting distance on the plantation. This parameter is important because determining the appropriate planting distance can optimize coffee plant growth. The number of fruits per bunch ranged from 15.4 to 24.9 fruits. BP 534 and BP 939 had significantly more fruits per bunch and fruits per branch compared to BP 308 and BP 936. This indicated that BP 534 and BP 939 were more productive than BP 308 and BP 936.

The character of the leaf and fruit of coffee clones were varied. Leaf length ranged from 22.3 to 24.4 cm, while leaf width ranged from 9.3 to 11.0 cm (Table 2). Robusta coffee had wider leaves compared to Arabica coffee. This was in agreement with a previous study (Hulupi, 2016). On the other hand, the fruit's size was characterized by a length of 1.5-1.7 cm, width of 1.3-1.4 cm and thickness of 1.1-1.2 cm. The weight of 100 beans ranged from 102.0 to 169.0 g. Fruit size affects the weight and volume of Robusta coffee fruit. Clones BP 534 and BP 936 had significantly higher 100-beans weights compared to BP 308, BP 409, and BP 939. On the other hand, the BP 308 clone had smaller fruit compared to other clones. This showed that BP 308 had the lowest potential productivity among the clones evaluated. However, BP 308 has another advantage to be used as a rootstock. This clone is resistant to nematodes and suitable to be used in areas endemic to nematode attacks.

Evaluation of qualitative characteristics of the clones showed that the five clones tested had similar performance. These qualitative characteristics include overall appearance, plant habit, vegetative development, branch angle, leaf shape, leaf apex shape, petiole color, mature leaf color, stipule shape, firmness of leaf surface waves, firmness of leaf edge waves, inflorescence position, inflorescence on old wood, anther insertion, fruit shape,

Table 2. Quantitative characters of Robusta coffee clones

Nr	Characters	MS Clone	CV (%)	BP 308	BP 409	BP 534	BP 936	BP 939
1	Internode length (cm)	1.8 **	6.50	6.4 b	8.3 a	7.7 a	7.5 a	8.2 a
2	Number of clusters per branch	0.9 ns	11.23	5.0	4.4	5.2	5.5	5.8
3	Number of fruits per cluster	49.3 **	11.51	15.4 c	19.0 bc	23.3 ab	17.1 c	24.9 a
4	Number of fruits per branch	2461.7 **	12.70	76.4 b	83.5 b	121.9 a	94.1 b	145.0 a
5	Leaf length (cm)	2.1 ns	5.01	23.2	23.8	22.3	24.2	24.4
6	Leaf width (cm)	1.9 **	3.81	9.3 b	10. a	9.6 b	11.0 a	10.8 a
7	Fruit length (cm)	0.1 *	3.68	1.5 b	1.7 a	1.6 ab	1.5 b	1.7 a
8	Fruit width (cm)	0.1 ns	7.90	1.3	1.4	1.4	1.4	1.3
9	Fruit thickness (cm)	0.1 **	3.10	1.1 c	1.1 c	1.2 ab	1.2 a	1.1 bc
10	100-bean weight (g)	2192.1 **	0.65	102.0 e	157.0 c	164.0 b	169.0 a	154.0 d

Note: MS = mean of square, CV = coefficient of variation, ** = significant at P <0.01, significant at P <0.05, ns = not significant at P < 0.05. Values followed by different letters in the same column were significantly different based on Duncan's multiple range test at α = 5%.

fruit-disc shape, and fruit color (Table 3). In general, the flush leaf color of the clones tested was green, except for clone BP 308 which had a brownish flush leaf color. The leaf shape of the clones was mostly elliptical except for BP 939 (oval leaf shape) and had an obovate fruit shape except for BP 939 (oblong).

The results of digital imaging analysis of Robusta coffee leaves are presented in Table 4. There was a significant difference in Red and Green values of young leaf and adult leaf between clones. BP 939 exhibited the highest and significantly different values of Red (141, $p < 0.05$) and Green (151, $p < 0.05$) in both the young and adult leaves compared to that of other clones. These indicated that clone BP 939 was distantly related to the other clones. Different levels of color gradation in young and mature leaves were also evaluated. This gradation might be influenced by environmental factors. Godoy *et al*. (2022), reported that fertilization affected the leaf color brightness. Visual differences in the flush appearance, although present, were not enough to provide significant differences in the RGB values (Figure 1). These results showed that the application of RGB observation for the characterization of leaf morphology will be effective if there are significant color differences. For example, the utilization of digital imaging-assisted analysis has been reported on the identification of coffee leaf rust (Jepkoech *et al*., 2021; Soares *et al*., 2022).In this case, coffee leaf rust caused significant alteration to the leaf color compared to the healthy ones for RGB analysis can be successfully conducted.

Tabel 3. Qualitative characters of recommended Robusta clones

Nr	Characters	BP 308	BP 409	BP 534	BP 936	BP 939
1	Overall appearance	Pyramidal	Pyramidal	Pyramidal	Pyramidal	Pyramidal
2	Plant habit	Shrub	Shrub	Shrub	Shrub	Shrub
3	Vegetative development	Sympodial	Sympodial	Sympodial	Sympodial	Sympodial
4	Branch angle	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect
5	Leaf shape	Elliptic	Elliptic	Elliptic	Elliptic	Ovate
6	Leaf apex shape	Acuminate	Acuminate	Acuminate	Acuminate	Acuminate
7	Leaf petiole color	Green	Green	Green	Green	Green
8	Leaf flush	Brownish	Green	Green	Green	Green
9	Mature leaf color	Green	Green	Green	Green	Green
10	Stipule shape	Triangular	Triangular	Triangular	Triangular	Triangular
11	Firmness of leaf surface waves	Present	Present	Absent	Present	Present
12	Firmness of leaf edge waves	Present	Present	Absent	Present	Present
13	Inflorescence position	Axillary	Axillary	Axillary	Axillary	Axillary
14	Inflorescence on old wood	Present	Present	Present	Present	Present
15	Anther insertion	Excluded	Excluded	Excluded	Excluded	Excluded
16	Fruit shape	Obovate	Obovate	Obovate	Obovate	Oblong
17	Fruit-disc shape	Not marked	Not marked	Not marked	Not marked	Not marked
18	Fruit color	Red	Red	Red	Red	Red

Table 4. RGB character at leaf flush, young leaf, and mature leaf

Nr	Characters	MS clone	CV (%)	BP 308	BP 409	BP 534	BP 936	BP 939
1	Red (leaf flush)	218.6 ns	7.43	120	130	131	125	143
2	Green (leaf flush)	262.3 ns	6.70	124	135	134	129	149
3	Blue (leaf flush)	146.9 ns	8.01	135	135	141	133	150
4	Red (young leaf)	750.3 *	10.53	108 b	118 b	110 b	104 b	144 a
5	Green (young leaf)	941.7 *	10.56	116 b	126 b	117 b	111 b	155 a
6	Blue (young leaf)	653.4 ns	11.33	122	119	117	111	149
7	Red (mature leaf)	879.9 *	13.60	109 b	108 b	102 b	97 b	141 a
8	Green (mature leaf)	1078.7 *	14.13	113 b	112 b	107 b	104 b	151 a
9	Blue (mature leaf)	957.7 ns	15.76	117	112	110	102	148

Notes: MS = mean of square, CV = coefficient of variation, *significant at $P < 0.05$, ns = not significant at $P < 0.05$. Values followed by different letters in the same column were significantly different based on Duncan's multiple range test at α = 5%

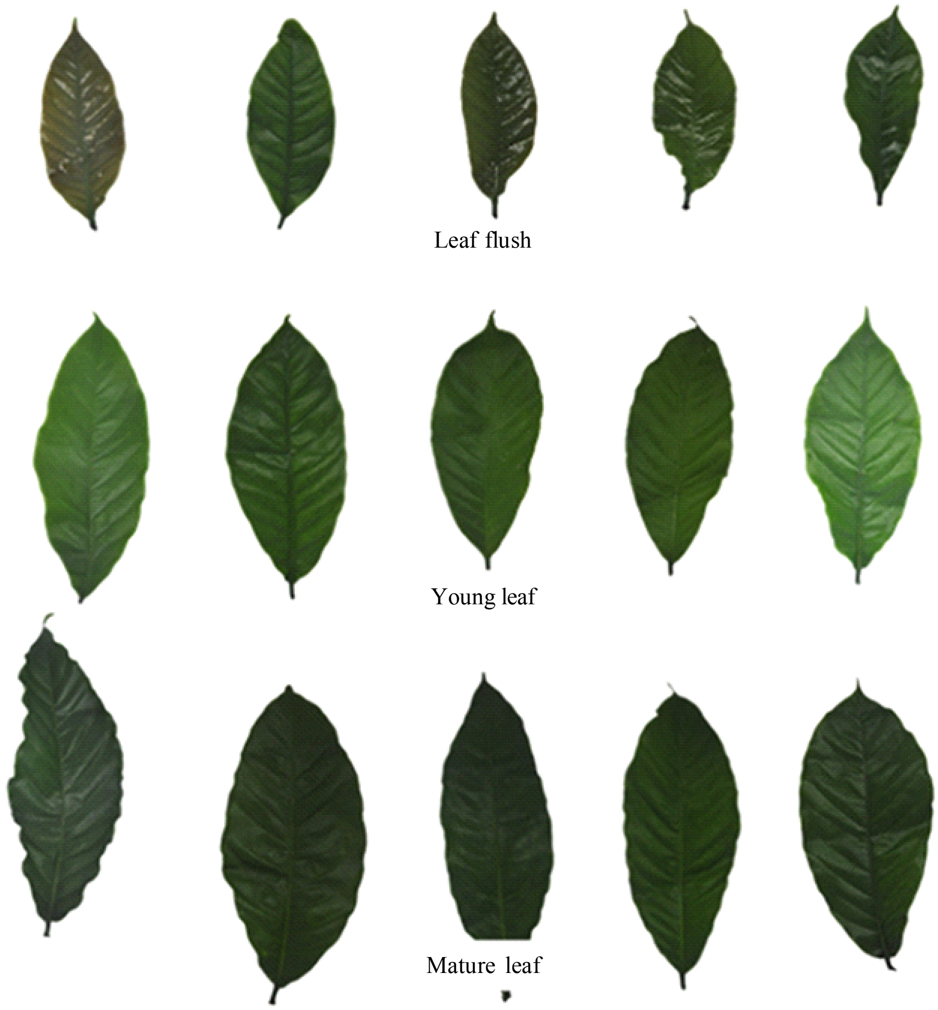

Figure 1. Visualization of leaf flush, young leaf, and mature leaf of five Robusta clones

Kinship Analysis Based on Cluster Analysis

Cluster analysis on both quantitative and qualitative parameters revealed two major groups with a dissimilarity coefficient of 0.5 (50%) (Figure 2). The first group consisted of BP 939, while the second group were BP 308, BP 409, BP 936, and BP 534. The second group was further divided into two subgroups with a dissimilarity coefficient of 0.3 (30%). BP 308 belonged to the first subgroup, while subgroup 2 included BP 409, BP 936, and BP 534. On the other hand, cluster analysis based on RGB data also resulted in two primary groups (Figure 3). The first group included BP 939, and the second group consisted of BP 308, BP 936, BP 409, and BP 534. The second group was further divided into subgroup 1 (BP 308 and BP 936) and subgroup 2 (BP 409 and BP 534). Cluster analysis results from both quantitative and qualitative characteristics (Figure 2) aligned with those from digital image characteristics (Figure 3). From these results, it was evident that clone BP 939 had a distant relationship with the other clones. Historically, BP 939 and BP 936 showed some similarity in genetic proximity due to their shared genetics from BP 42 (Table 1). BP 939 had a lineage tracing back to individual selection in the progeny of a cross between BP 42 (seed origin) and SA 1366. However, this high diversity might be caused by inherent factors in cross-pollinated Robusta coffee.

Generally, cluster analysis is used to visualize the relationship between objects based on their similarity or differences in various characteristics (Mattjik & Sumertajaya, 2013). In this context, this analysis was used to determine the genetic distance between each genotype of a population using available quantitative, and qualitative characters and their combination (Sumirat *et al*., 2007; Tounekti *et al*., 2017; Malau & Pandiangan, 2018; Akbar *et al*., 2022; Henry *et al*., 2015; Tessema *et al*., 2011; Rubiyo *et al*., 2022). Based on the results of the cluster analysis, BP 409 and BP 936 had a close relationship. Crossing between these coffees is not recommended because they share similar characteristics.

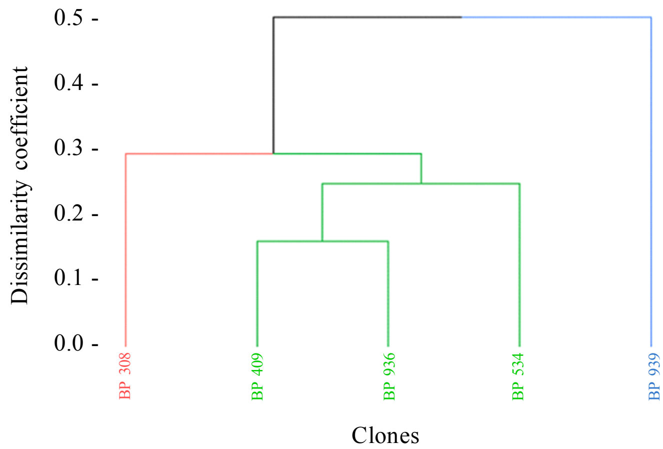

Figure 2. Cluster analysis of five Robusta clones based on quantitative and qualitative traits

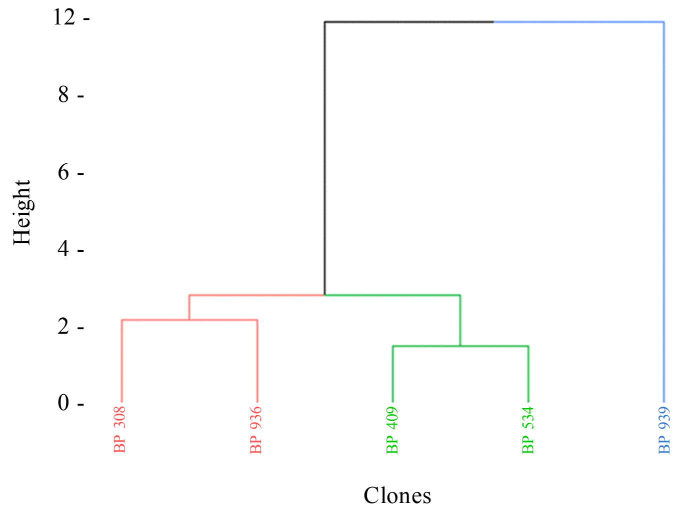

Figure 3. Cluster analysis of five Robusta clones based on digital image traits

Kinship Analysis Based on PCoA

PCoA was conducted to complement the results of cluster analysis. Cluster analysis can only identify the degree of relatedness, but it cannot explain how this relatedness is formed from many variables (Evgenidis *et al.*, 2011; Rubiyo *et al.*, 2022). PCoA fills this gap to comprehensively understand the clustering. Several studies have also reported this concept in complementing the information contained in cluster analysis (Evgenidis *et al.*, 2011; Tounekti *et al.*, 2017). PCoA allows various categorical data, especially those with many zeros, or a mixture of categorical and parametric data to be distributed linearly (Liu *et al.*, 2019). Based on cluster analysis and PCoA, there was diversity among the tested Robusta clones. This data has the potential to be used for crossing in breeding programs.

The PCoA biplot analysis results show that all clones are mapped in agreement with the result of cluster analysis (Figure 4). Specific differences between Robusta coffee clones were evaluated based on chosen character vectors. BP 939 (Group 1, Figure 2) was in quadrant IV, clearly separated from the other clones (Group 2, Figure 2). BP 939 clearly showed significant differences in the characteristics

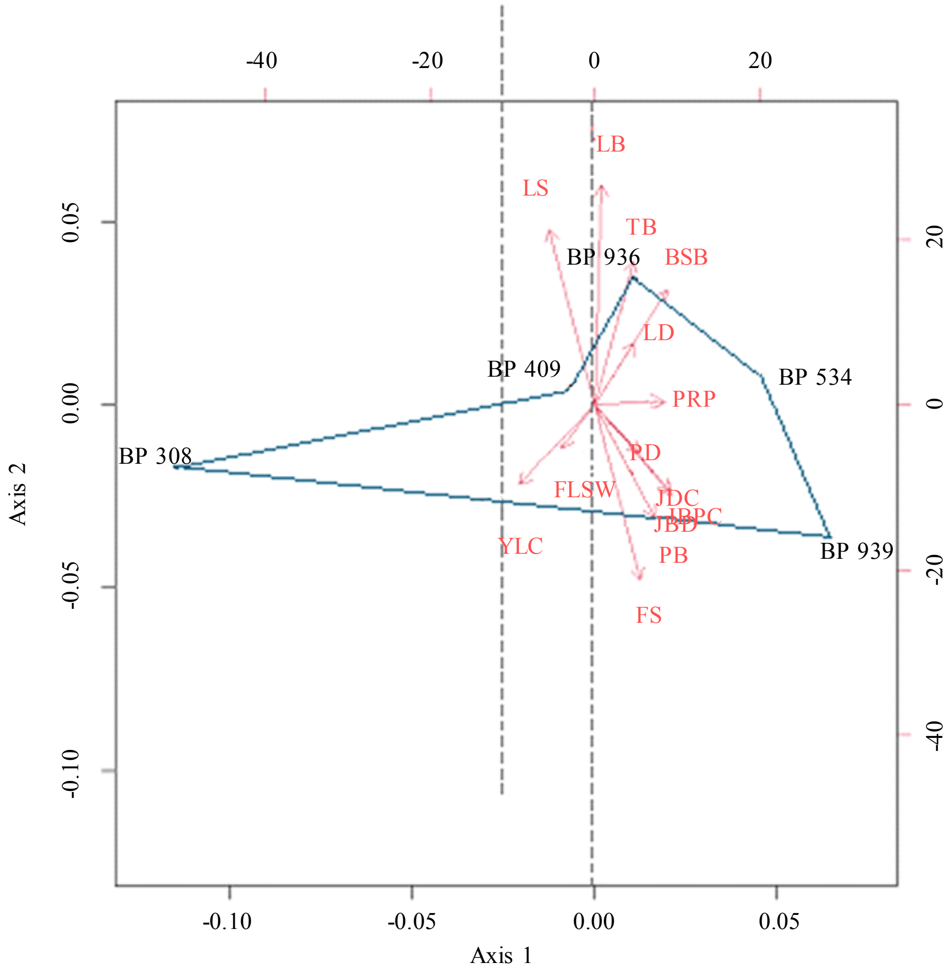

Figure 4. Principal coordinate analysis (PCoA) biplot analysis of five Robusta clones

Note: PRP = internode length, JDC = Number of cluster per branch, JBD = number of fruit per cluster, JBC = number of fruit per branch, PD:leaf length, LD:leaf width, PB:fruit length, LB:fruit width, TB:fruit thickness, BSB: 100-bean weight, LS = leaf shape, YLC = leaf flush, FLEW = firmness of leaf edge waves, FS = fruit shape of leaf length (PD), number of clusters per branch (JDC), number of fruits per bunch (JBD), number of fruits per branch (JBC), fruit shape (FS), and fruit length (FL) than other clones. BP 409, BP 534, and BP 936 were characterized by their fruit width (LB) and fruit thickness (TB). On the other hand, BP 308 was differentiated by its leaf flush color (YLC) and firmness of leaf edge waves (FLEW). The 'which-won-where' mapping which was based on connecting lines between the farthest clones showed that BP 409 and BP 936 were quite similar. BP 409 was obtained through single seed selection from BP 42, while BP 936 is a result of a cross between SA 164-11 (seed origin) as the female parent and BP 42 as the male parent. These two clones have a close genetic relationship due to the genetic influence of BP 42. On the other hand, BP 308 and BP 939 clones had a distant relationship based on PCoA analysis. BP 308 was obtained from individual selection in a garden endemic to parasitic nematodes, while BP 939 was produced from individual selection in the progeny of a cross between BP 42 (seed origin) as the female parent and SA 1366. Thus, it was reasonable for BP 308 and BP 939 to show a distant relationship due to no connection in the breeding origin among those clones.

CONCLUSIONS

The use of digital imaging could complement the characterization of the clones based on quantitative and qualitative characteristics. Five Robusta clones were successfully grouped based on their distinct characteristics. The first group consists of clone BP 939, which is specifically grouped based on characteristics such as leaf length, number of bunches per branch, number of fruits per bunch, number of fruits per branch, fruit shape, fruit length, green value in leaf flush, and blue value in leaf flush. The second group comprises clones BP 409, BP 534, BP 936, and BP 308, which are specifically grouped based on characteristics such as fruit width, fruit thickness, leaf flush color, and the sharpness of leaf margin waves.

REFERENCES

AbacusBio (2023). *Opportunities for Robusta variety innovation*. World Coffee Research, Portland, United States of America.

Akbar, M.R.; A. Wibowo; U. Sumirat; D. Nugroho & M.F. Anshori (2022) Yield performance evaluation of Arabica coffee progenies resulted from three way cross method. *Pelita Perkebunan*, 38(1), 10–19.

Berthaud, J. (1986). *Les ressources génétiques pour l'amélioration des caféiers africains diploïdes*. Collection Travaux et documents edn, Montpellier, France.

BPS (2023). *Statistik Kopi Indonesia 2022*. Badan Pusat Statistik, Jakarta, Indonesia.

Charrier, A.; J. Berthaud. (1985) Botanical Classification of Coffee. ***In***: *Coffee* (Clifford, M.N. & K.C. Willson, editors). Springer, Boston, US. pp. 13–47. https://doi.org/10.1007/978-1-4615-6657-1_2.

Das Choudhury, S.; S. Bashyam; Y. Qiu; A. Samal & T. Awada (2018). Holistic and component plant phenotyping using temporal image sequence. *Plant Methods,* 14, 1–21.

Ditjenbun (2019). *Statistik Perkebunan Indonesia komoditas kopi 2015-2020*. Sekretariat Direktorat Jenderal Perkebunan, Jakarta, Indonesia.

Evgenidis, G.; E. Traka-Mavrona & M. Koutsika-Sotiriou (2011). Principal component and cluster analysis as a tool in the assessment of tomato hybrids and cultivars. *International Journal of Agronomy*, 2011, 697879, 1–7.

Godoy, L.J.G.D.; A.M.D. Carvalho; A.F.D. Freitas; C.E. Botelho; J.L.T. Garibaldi & S. Ferrari (2022). Digital imaging of coffee leaves under different nitrogen concentrations applied to soil. *Revista Ceres*, 69(6), 648–658.

Gomez, C.; S. Dussert; P. Hamon; S. Hamon; A. de Kochko & V. Poncet (2009) Current genetic differentiation of *Coffea canephora* Pierre ex A. Froehn in the Guineo-Congolian African zone: cumulative impact of ancient climatic changes and recent human activities. *BMC Evolutionary Biology*, 9(167), 1–19.

Guimaraes, P.H.R.; I.P. Lima; A.P. Castro; A.C. Lanna; P.G.S. Melo & M. Raissac (2020). Phenotyping root systems in a set of japonica rice accessions: Can structural traits predict the response to drought. *Rice*, 13, 1–19.

Henry, D.; A.B. Dymnicki; N. Mohatt; J. Allen & J.G. Kelly (2015) Clustering methods with qualitative data: A mixed-methods approach for prevention research with small samples. *Prevention Science,* 16(7), 1007–1016.

Hulupi, R. (2016). Bahan Tanam Kopi. ***In:*** *Sejarah, Botani, Proses Produksi, Pengolahan, Produk Hilir, dan Sistem Kemitraan* (T. Wahyudi, Pujiyanto & Misnawi, eds). Gadjah Mada University Press, Yogyakarta, Indonesia.

ICO (2023). *Coffee Report and Outlook*. International Coffee Organization, London, England.

Jepkoech, J.; D.M. Mugo; B.K. Kenduiywo & E.C. Too (2021). Arabica coffee leaf images dataset for coffee leaf disease detection and classification. *Data in Brief*, 36, 107142.

Laraswati, A.A.; R. Padjung; M. Farid; N. Nasaruddin; M.F. Anshori; A. Nur & A.I. Sakinah (2021). Image based-phenotyping and selection index based on multivariate analysis for rice hydroponic screening under drought stress.

Li, Y.; W. Wen; X. Guo; Z. Yu; S. Gu; H. Yan & C. Zhao (2021). High-throughput phenotyping analysis of maize at the seedling stage using end-to-end segmentation network. PLoS One, 16(1), p. e0241528.

Liu, H.; Z. Hu; M. Zhou; J. Hu; X. Yao & H. Zhang; H. Zhang; Z. Li; L. Lou; C. Xi; H. Qian; C. Li; X. Xu; P. Zheng & B. Hu (2019). The distribution variance of airborne microorganisms in urban and rural environments. *Environmental Pollution,* 247, 898–906.

Malau, S. & S. Pandiangan (2018). Morphological variation in arabica coffee (Coffea *arabica* L.) growing in North Sumatra Indonesia. *Indonesian Journal of Agronomy*, 46(3), 314–321.

Mattjik, A.A. & I.M. Sumertajaya (2013). Design Experiment with SAS and Minitab Software. IPB Press, Bogor, Indonesia.

Kementerian Pertanian (2022). *Outlook Komoditas Perkebunan Kopi*. Pusat Data dan Sistem Informasi Pertanian, Sekretariat Jenderal, Kementerian Pertanian, Jakarta, Indonesia.

Klein, A.M.; I. Steffan-Dewenter & T. Tscharntke (2003). Bee Pollination and Fruit Set of *Coffea arabica* and *C. canephora* (Rubiaceae). *American Journal of Botany*. 90, 153–157.

Kepmentan (2021). *Keputusan Menteri Pertanian No.27/Kpts/KB.020/05/2021: Pedoman Produksi, Sertifikasi, Peredaran dan Pengawasan Benih Tanaman Kopi* (*Coffea* spp.). Kementerian Pertanian Republik Indonesia. Jakarta, Indonesia.

Permentan (2014). *Peraturan Menteri Pertanian No.49/Permentan/OT.140/4/2014: Pedoman Teknis Budidaya Kopi yang Baik* (*Good Agriculture Practices/ GAP on Coffee*). Kementerian Pertanian Republik Indonesia. Jakarta, Indonesia.

Rubiyo, R.; S. Sudarsono; M.F. Anshori; N. Nurdebyandaru; Y.A. Dewi & M.R. Akbar (2022). Determining kinship pattern of Robusta and arabica coffee clones using multivariate analysis. *Chilean Journal of Agricultural Research*, 82(2), 276–284.

Soares, A.D.S.; B.S. Vieira; T.A. Bezerra; G.D. Martins & A.C.S Siquieroli (2022). Early detection of coffee leaf rust caused by *Hemileia vastatrix* using multispectral images. *Agronomy*, 12(12), 2911.

Sumirat, U.; Priyono & S. Mawardi (2007). Selection of Superior Genotypes of Coffea Canephora Pierre on Controlled Hybrid Population Using Cluster Analysis Method. *Pelita Perkebunan*, 23(2).

Tessema, A.; S. Alamerew; T. Kufa & W. Garedew (2011). Genetic diversity analysis for quality attributes of some promising *Coffea arabica* germplasm collections in Southwestern Ethiopia. *Journal of Biological Sciences,* 11, 36–244.

Tounekti, T.; M. Mahdhi; T.A. Al-Turki & H. Khemira (2017). Genetic diversity analysis of coffee (*Coffea arabica* L.) germplasm accessions growing in the Southwestern Saudi Arabia using quantitative traits. *Natural Resources,* 8, 321–336.

Wibowo, A.; M.R. Akbar & U. Sumirat (2022). Heritability and Combining Ability of Some Vegetative and Yield Characteristics of Promising Arabica Coffee Varieties in Indonesia. *Pelita Perkebunan*. 38(1), 1–9.

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