Phenotypic Diversity in First Plagiotropic Branch of Caffeine-Producing Robusta Coffee Population in Gunung Betung, Bogorejo

Winarno[1], Priyambodo[1], Elly Lestari Rustiati[1], Septi Wahyu Lestari[1], Shifa Sandra[1], Natasya Thesalonika[1], Minanti Mayda Ashari[1], Yuliana Andriyani[1], Annisa Lidya Maharani[1], Aril Afandi[1], Muhammad Febriansyah[1], Dian Neli Pratiwi[2], Nindy Permatasari[3], Suhada[3]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia*
[2]*Yayasan Akar Lestari Indonesia, Bandar Lampung, Indonesia*
[3]*Department of Cultivation of Plantation Crops, Politeknik Negeri Lampung, Indonesia*

Corresponding author: *ely_jazdzyk@yahoo.com*

Keywords: Phenotype, Plagiotropic, Purposive Sampling, Robusta Coffee

Abstract: Coffee has caffeine content that may be beneficial for health in the appropriate dosage use. Robusta coffee has a higher caffeine content compared to other types of coffee. Robusta coffee is the most widely cultivated type of coffee in Indonesia as it is more tolerant of certain pests and is suitable for the climate in Indonesia. Supporting the germplasm diversity management, identification of robusta coffee diversity is essential, both in terms of phenotypic and genotypic characters. This research has been conducted as a comprehensive part of phenotyping identification of robusta coffee populations in Gunung Betung, Bogorejo, Pesawaran, Lampung. This investigation has aimed to determine the diversity of robusta coffee phenotypes according to leaf width on plagiotropic branches. Samples were taken using purposive sampling method on 30 individual robusta coffee plants. Identification has been done based on leaf morphology, width of the first leaf on the first plagiotropic branch. Leaf width can be utilised as an indicator to identify different robusta coffee varieties or genotypes. The phenotypic character of the width of the first leaf on the first plagiotrope branch is crucial because this branch is the first point where the crop shows an early response to its environment, which may include factors such as light, water, and nutrients. Leaf width has been measured directly in the field using a standardised measuring instrument on the widest part of the first leaf on the lowest plagiotrope branch. The results have demonstrated significant diversity, with a range of leaf widths varying from 6.0 cm to 15.0 cm with an average of 9.21 cm. This variation has indicated the existence of genetic diversity based on phenotypic characters among members of the robusta coffee population at Gunung Betung, Bogorejo, Pesawaran.

1. INTRODUCTION

Robusta coffee (*Coffea canephora*) is an economically significant coffee species worldwide, accounting for approximately 20% of the total coffee trade in the global market (Roonprapant *et al*., 2021). Indonesia is one of the largest coffee-producing countries in the world, with the majority of its coffee plantations dominated by robusta coffee (Rosiana *et al*., 2018). Lampung, Indonesia is known as one of the largest coffee-producing regions in the country, with plantation areas exceeding 155,017 hectares (BPS, 2022).

The robusta coffee plant exhibits stem dimorphism, characterised by branches that grow upwards (orthotropic) and branches that grow sideways (plagiotropic). On plagiotropic branches, the internode distance can reach 8–15 cm (van Steenis et al., 2008). These horizontally growing plagiotropic branches play a crucial role as sites for flower and fruit emergence (Pohlan and Janssens, 2010).

Robusta coffee leaves are broad and elongated, measuring between 20–30 cm in length and 10–16 cm in width. They are dark green with a glossy, thick surface and a stiff, rough texture, indicating adaptation to hot and humid environments. The robusta coffee leaves are also more resistant to diseases and insects due to their thick texture and higher caffeine content, unlike other coffee types such as arabica, which are more susceptible to diseases like leaf rust. This resistance is one reason why robusta coffee is more widely cultivated in Indonesia (Rahayu, 2016).

One of the most abundant compounds in coffee is caffeine, an alkaloid derivative of xanthine (purine base) naturally found in coffee (Fatoni, 2015). The caffeine content in coffee has health benefits such as stimulating the nervous system, relaxing smooth muscles, particularly bronchial smooth muscles, and stimulating cardiac muscles, thereby enhancing stamina. It can be used as a supplement and in medications. However, excessive caffeine consumption, overdose, can cause nervousness, restlessness, insomnia, nausea, and seizures. The maximum allowable caffeine intake in food and beverages is 150 mg/day and 50 mg/serving (Maramis et al., 2013). The caffeine content in raw robusta coffee beans is higher compared to raw arabica coffee beans and other coffee types.

Robusta coffee is the most widely cultivated coffee in Indonesia, including in Lampung, particularly in Bogorejo, Gunung Betung. Characterising local Lampung robusta coffee plants is crucial as it helps inventory and determine the genetic diversity of local robusta coffee. Morphological characterization allows for the identification of traits, variability, and relationships among coffee plants (Zasari et al., 2023). Information on the genetic diversity of local robusta coffee is useful for improving and increasing production, quality, and mapping coffee breeding programs, especially in Lampung province.

Previous research on robusta coffee was conducted in Brazil by Vitória et al (2024) to obtain leaf area data from robusta coffee plants which also recorded the width of robusta coffee leaves on plagiotropic branches, similar research has never been conducted in Bogorejo, so the potential genetic variation of robusta coffee in the area is still not widely known.

Leaves on plagiotropic branches often exhibit more distinct morphological variations compared to leaves on orthotropic (vertical) branches. These variations can include leaf shape, size, and colour, reflecting phenotypic differences. To predict genetic diversity, leaves are the plant tissue where nutrient concentration contributes most significantly to diversity among genotypes (Santos et al., 2021). Leaves on plagiotropic branches are more exposed to environmental conditions such as light and humidity, so adaptive changes in these leaves can reflect specific genetic adaptations to particular environments. According to research (Randriani et al., 2014), a small number of innovative farmers who have rehabilitated coffee plants using plagiotropic bud grafting techniques from selected genotypes will see faster flowering and fruiting.

The genetic diversity of robusta coffee is influenced by two factors: genotype and phenotype. Phenotype is the result of a complex interaction between genotype and environment, which can be observed physically. Two organisms with the same genotype can have different phenotypes if grown in different environmental conditions. The physical appearance of an organism includes body structure, size, shape, and colour. Utilising plant genetic resources requires knowledge of superior plant genetic material, with the presence of superior genotypes obtained through exploration and characterization of cultivated plants (Zasari et al., 2023). Characterization of coffee accessions, clones, and/or varieties is generally based on the morphological or phenotypic traits of the plants (Haniefan & Basunanda, 2022). Morphological differences are the most easily observed traits, especially in mature plants. Morphological characterization is useful for collecting or assembling coffee plant germplasm (Ramadiana et al., 2018).

Morphological descriptors serve as a reference for identifying plant morphological characteristics according to established criteria. These descriptors facilitate the identification of morphological traits in plants, such as coffee plants. Specific morphological descriptors for coffee plants have been published by *Internassional Plant Genetic Resources* (IPGRI, 1996).

2. METHODS

This research was conducted from May to June 2024. Data collection was carried out using purposive sampling with exploration and characterization methods on 30 coffee plants in the coffee plantation area of Bogorejo, Gedong Tataan, Pesawaran, Lampung (Figure 1). The location of the studied coffee plantation is at an altitude of 360 m asl.

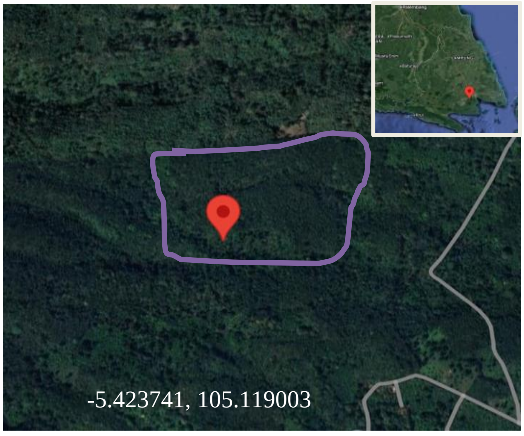
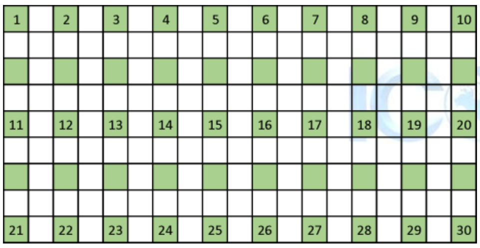


Figure 1. Research site, robusta coffee (*Coffea Canephora*) at Bogorejo, Gedung Tataan, Pesawaran Lampung.

Purposive sampling is a technique for deliberately selecting sample locations based on specific criteria or considerations. (Dananjaya, 2020). In this study, 10 plants each were taken on the right and left outer lanes, and 10 plants on the centre lane of the plantation (Figure 2). A total of 30 samples were then characterised based on IPGRI guidelines.

Figure 2. Sampling site for 30 individuals robusta coffee (*Coffea Canephora*). The green-coloured squares indicate the coffee plants in the studied plantations.



Samples were taken using purposive sampling from 30 individual robusta coffee plants out of the total population. Identification was based on leaf morphology, with a specific focus on the width of the first leaf on the first plagiotropic branch (IPGRI, 1996). Measurements were conducted by measuring the widest part of the first leaf on the first plagiotropic branch of the coffee tree using a ruler. (Akpertey *et al*., 2019).

Figure 3. Measurement of leaf width robusta coffee (*Coffea Canephora*)

3. RESULTS AND DISCUSSION

Randriani *et al*. (2014) examined leaf width in a plant positively correlated with fruit production/tree/year. A positive correlation between leaf width and fruit production has also been observed in several other plant species. The more extensive a leaf is, the greater the area of the leaf is likely to be. This causes the chloroplasts it contains to multiply. This condition has implications for the increase in photosynthesis that occurs, so that it can show a positive correlation for fruit production in these plants.

Based on the 30 individual robusta coffee plants whose leaf widths were measured (Table 1), they were grouped into three size ranges: 6 to 9 cm, 9.1 to 12 cm, and 12.1 to 15 cm, with 14, 13, and 3 plants in each range, respectively. The widest leaf measured 15 cm, the smallest 6 cm, with an average size of 9.21 cm. The size range of 12.1 to 15 cm had the fewest individuals, only 3, compared to the other two ranges, which had nearly the same number of individuals, 14 and 13, respectively.

Table 1 : Robusta leaves' width (N=30)

No	Tree Number	Width Leaf (cm)
1	Individu 1	12.5
2	Individu 2	7
3	Individu 3	9.5
4	Individu 4	6
5	Individu 5	9.5
6	Individu 6	11.5
7	Individu 7	7
8	Individu 8	6.5
9	Individu 9	12.5
10	Individu 10	11
11	Individu 11	10
12	Individu 12	10
13	Individu 13	9.8
14	Individu 14	7.5
15	Individu 15	7
16	Individu 16	15
17	Individu 17	6.5
18	Individu 18	9
19	Individu 19	9
20	Individu 20	8
21	Individu 21	9.6
22	Individu 22	6.2
23	Individu 23	10.9
24	Individu 24	6.5
25	Individu 25	10.7
26	Individu 26	11
27	Individu 27	7.9
28	Individu 28	10.7
29	Individu 29	7
30	Individu 30	11.1
	Rerata	9.21

According to van Steenis et al. (2008), the leaves of robusta coffee on plagiotropic branches in Indonesia generally have a width between 10 to 16 cm. In comparison, the plagiotropic leaves of robusta coffee in Bogorejo have a smaller size range, between 6 to 15 cm. External factors such as light, water, nutrients, and variety can cause differences in the leaf size range of robusta coffee in Indonesia.

When comparing the average width of plagiotropic robusta coffee leaves in Bogorejo with a study on the leaf width of robusta coffee in Brazil conducted by da Vitória et al. (2024), the average width in Bogorejo is significantly larger at 9.21 cm, whereas the average width in Brazil is only 5.79 cm. External factors including light, water, nutrients, and variety cause differences in leaf width.

External factors that affect plant growth, including the physical appearance of the plant, can be environmental stress. Environmental stress significantly influences a plant's physiology and its physical characteristics, highlighting the importance of regular assessments across pertinent categories (Van Leeuwen, 2009). The effect of light on the morphological growth of leaflets can be correlated with the amount of canopy cover in the plant. The investigation regarding the impact of canopy stratification was conducted by Castellarin et al. (2007), who documented a reduction in the leaf layer count attributable to hydric stress, albeit exclusively during the terminal phase of the vegetative season. The research underscored that while the quantity of leaves remained constant, their dimensions underwent alterations, potentially leading to discrepancies in size at the canopy tier.

It is also commonly recognized that plants display unique functional traits and utilise diverse strategies for growth and adaptation to their environments. Previous investigations conducted by Qin et al. (2024) suggested that variations in life history strategies among leaf habits may be elucidated through the interactions between traits and environmental factors. Moreover, the findings presented by Zheng et al. (2024) indicated that consistent trait-trait associations can be discerned in images of coarse resolution; however, trait-trait associations at local scales demonstrate insensitivity to wide-ranging abiotic and biotic influences.

Leaf width in plants certainly affects the rate of photosynthesis. According to Adams & Terashima (2018), leaves are organs optimised to capture sunlight and safely use that energy through photosynthesis to drive plant productivity. Leaves that do not grow optimally will hinder the plant's ability to produce secondary metabolites, such as caffeine (Putri, 2022).

The relationship between environmental factors has both physiological and morphological functions. Plants respond to specific needs during their life cycle if the growing environment is not supportive. This is evident from morphological and physiological changes. Even with the same genotype, plants will appear different in different environments. Genotypic variation occurs due to small, imperceptible shifts from one individual to another (Muhsanati, 2012). Sudden changes in environmental factors, such as temperature, relative humidity, solar radiation, and wind, will result in short-term responses. However, if these changes persist over one or more developmental periods, plants will gradually alter their physiological processes. Genetic diversity based on phenotypic characteristics is necessary for the identification of robusta coffee varieties or genotypes in the Bogorejo, Gunung Betung.

4. CONCLUSIONS

The plagiotropic leaves of robusta coffee in Bogorejo tend to be small compared to other locations in Indonesia. This variation indicates genetic diversity based on phenotypic characteristics among the robusta coffee population in Bogorejo, Pesawaran, Lampung.

5. ACKNOWLEDGEMENT

We extend our gratitude to BLU LPPM Universitas Lampung for research. The owner of the Bogorejo coffee plantation, Gunung Betung, for allowing us to access the coffee plantation.

6. REFERENCES

Adams III, W. W., & Terashima, I. (Eds.). (2018). *The leaf: a platform for performing photosynthesis* (Vol. 44). Cham: Springer International Publishing.

Akpertey, A., Anim-Kwapong, E., & Ofori, A. (2019). Assessment of genetic diversity in Robusta coffee using morphological characters. *International Journal of Fruit Science*, *19*(3), 276-299.

Castellarin, S. D., Pfeiffer, A., Sivilotti, P., Degan, M., Peterlunger, E., & Di Gaspero, G. (2007). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant, Cell & Environment*, *30*(11), 1381-1399.

Dananjaya, I. G. A. N. (2020). Pengaruh Integrasi Ternak Kambing Dan Tanaman Kopi Terhadap Pendapatan Kelompok Tani Ternak Satwa Amerta, Di Desa Mundeh, Kecamatan Selemadeg Barat, Kabupaten Tabanan. *dwijenAGRO*, *10*(1), 53-60.

da Vitória, E. L., Júnior, A. O. N., Ribeiro, L. F., Dubberstein, D., & Partelli, F. L. (2024). Leaf area estimation in Coffea canephora genotypes by neural networks and multiple regression1. *Brazilian Journal of Agricultural and Environmental Engineering*, *28*(9), e279246.

Fatoni, A., 2015. Analisa Secara Kualitatif dan Kuantitatif Kadar Kafein Dalam Kopi Bubuk Lokal Yang Beredar Di Kota Palembang Menggunakan Spektrofotometer UV-Vis, Laporan Penelitian Mandiri, Lembaga Penelitian dan Pengabdian Kepada Masyarakat Sekolah Tinggi Ilmu Farmasi Bhakti Pertiwi, Palembang.

Haniefan, N, and P Basunanda. 2022. Eksplorasi dan identifikasi tanaman kopi Liberika di Kecamatan Sukorejo, Kabupaten Kendal. *Vegetalika*. 11(1): 11–18.

[I-PGRI] International Plant Genetic Resources Institute. 1996. Descriptors for Coffee (*Coffea spp. and Psilanthus spp*.). Biodiversity International. Rome.

Maramis, R.K., Citraningtyas G., & Wehantouw F., (2013). Analisis Kafein Dalam Kopi Bubuk Di Kota Manado Menggunakan Spektrofotometri UV-Vis. *Jurnal Ilmiah Farmasi* –UNSRAT, Vol. 2, No. 04, 122 –128.

Pohlan, H.A.J. & Janssens, M.J.J. (2010). Growth and production of coffee.Dalam : Verheye, W.H (ed). Soils, Plant Growth and Crop Production – Volume III. Nottingham: EOLSS Publishers.

Prastowo, B. Karmawati, E. Rubijo. Siswanto. Indrawanto, C. Munarso, S.J. 2010. Budidaya dan Pasca Panen: Kopi. Bogor: Pusat Penelitian dan Pengembangan Perkebunan.

Putri, M. (2022). Pengaruh Daerah Tempat Tumbuh Terhadap Kadar Kafein Biji Kopi Robusta (Coffea canephora). *Jurnal Ilmu Kesehatan Bhakti Setya Medika*, *7*(1), 33-42.

Qin, Y., Wang, C., Zhou, T., Fei, Y., Xu, Y., Qiao, X., & Jiang, M. (2024). Interactions between leaf traits and environmental factors help explain the growth of evergreen and deciduous species in a subtropical forest. *Forest Ecology and Management*, *560*, 121854.

Rahayu, S. S. M. (2016). Pengaruh Fase Perkembangan Embrio Somatik Kopi Robusta *(Coffea canephora Pierre ex A. Froehner)* Terhadap Keberhasilan Perkecambahan Dan Aklimatisasi Secara Langsung (Doctoral dissertation, Universitas Muhammadiyah Purwokerto).

Ramadiana, S, D Hapsoro, and Y Yusnita. 2018. Morphological variation among fifteen superior Robusta coffee clones in Lampung Province, Indonesia. *Biodiversitas*. 19(4):1475–1481.

Randriani.E, Dani,Tresniawati.C, dan Syafaruddin. (2014). Hubungan Antar Karakter Vegetatif, Komponen Hasil, Dan Daya Hasil Kopi Robusta Hasil Sambung Tunas Plagiotrop. *J. TIDP* 1(2), 109-116.

Roonprapant, P., Arunyanark, A., & Chutteang, C. (2021). Morphological and physiological responses to water deficit stress conditions of robusta coffee (Coffea canephora) genotypes in Thailand. *Agriculture and Natural Resources*, *55*(3), 473-484.

Rosiana, N. R., Nurmalina, R Winandi, dan A Rifin. 2018. Dynamic Of Indonesian Robusta Coffee Competition Among Major Competitor. *Jurnal Tanaman Industri dan Penyegar*. 5(1): 1-10.

Santos, M. M. D., Silva, C. A. D., Oza, E. F., Gontijo, I., Amaral, J. F. T. D., & Partelli, F. L. (2021). Concentration of nutrients in leaves, flowers, and fruits of genotypes of Coffea canephora. *Plants*, *10*(12), 2661.

Van Leeuwen, C., Trégoat, O., Choné, X., Bois, B., Pernet, D., & Gaudillère, J. P. (2009). Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes?. *Oeno One*, *43*(3), 121-134.

van Steenis, C.G., Den Hoed, G.J.,&Eyma, P.J. (2008). Flora Untuk Sekolah di Indonesia. Jakarta: PT Pradnya Paramita.

Zasari, M., Kartika, K., & Altin, D. (2023). Eksplorasi-Karakterisasi Morfologi Kopi Robusta Lokal di Pulau Bangka. *J. Agrikultura*, *34*(2), 200-209.

Zheng, T., Ye, Z., Singh, A., Desai, A. R., Krishnayya, N. S. R., Dave, M. G., & Townsend, P. A. (2024). Variability in forest plant traits along the Western Ghats of India and their environmental drivers at different resolutions. *Journal of Geophysical Research: Biogeosciences*, *129*(3), e2023JG007753.