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Morphological Variation in The Leaf Shape of Banana Kepok Tanjung (*Musa paradisiaca* L.) Derived From Tissue Culture

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Abstract

Banana seedlings produced through tissue culture exhibit both narrow and broad leaves shape. Assessing these leaf shapes in field conditions is limited regarding their morphological changes, growth, and yield. This research undertook a comprehensive morphological analysis to investigate the development of narrow and broad leaves in banana Kepok Tanjung, from the seedling phase to field conditions. The study was conducted in Solok, West Sumatra, from January 2024 to July 2025. The study involved transplanting tissue-cultured narrow and broad leaves banana Kepok Tanjung seedlings into the field, totaling 70 plants (35 with narrow leaves and 35 with broad leaves) after the emergence of 5-8 leaves. Sixty morphological traits, including the pseudostem, leaves, inflorescence, and fruit, were observed following guidelines established by the International Plant Genetic Resources Institute (IPGRI). The findings revealed that variations in narrow and broad leaves morphology were evident in five vegetative growth parameters: plant height, stem diameter, leaf length, leaf width, and leaf number. In contrast, 55 qualitative morphological traits and reproductive features remained consistent between the two phenotypes. The genetic similarity based on leaf shape morphology of banana Kepok Tanjung was 0.957. The UPGMA cluster dendrogram formed a single cluster with a genetic distance of 0.96, indicating close genetic relationships. This research helps in identifying key leaf traits that improve the classification and selection of banana Kepok Tanjung seedlings derived from tissue culture for better growth. It is recommended that users study these traits further to enhance breeding programs and improve management practices for banana cultivation.

Keywords: Characteristics selection, Field-grown, In vitro, Leaf characteristic, Seedlings

1. Introduction

Bananas Kepok Tanjung are valued for their consistent resilience to environmental stresses and their economic potential, making them a popular choice among consumers (Ray et al., 2021; Adhikary et al., 2024; Tripathi et al., 2024). This banana variety serves as a nutritious food source and is widely utilized both fresh and as a primary ingredient in various processed food products (Ribeiro-Duthie et al., 2020; Luo et al., 2024; Saensouk et al., 2025). Furthermore, bananas Kepok Tanjung hold cultural and social significance, contributing to biodiversity conservation and enhancing food security (Aryal et al., 2018; Azizi et al., 2021; Pillai et al., 2024).

Morphological characterization is a method used to identify plant development stages and differentiate taxonomic groups within the Musa genus (Tripathi et al., 2024). Vegetative morphological traits, particularly those related to leaves, are closely linked to seedling quality in nurseries and can serve as indicators of plant adaptability and yield potential under varying environmental conditions (Saravanapandeeswaran & Vanitha, 2018). Variations in leaf shape, such as narrow versus broad leaves, directly affect photosynthetic efficiency, metabolic processes, light absorption, and tolerance to abiotic stress (Hazarika et al., 2020). These morphological differences significantly impact banana growth, productivity, and photosynthetic capacity (Meya et al., 2020).

Morphological studies are crucial for identifying and

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classifying banana varieties and understanding genetic diversity within banana plants (Inta et al., 2023). Leaf morphological characteristics are influenced by the interplay of genetic factors, hormonal regulation, environmental conditions, and serve as important indicators of seedling quality. Assessing these traits demonstrates seedling adaptability to various soil and environmental conditions, thereby supporting management strategies aimed at enhancing productivity and plant resilience to stress (Pillay, 2024).

Traditional banana propagation using suckers frequently yields low genetic diversity and increased disease susceptibility (Tumuhimbise & Talengera, 2018). Conversely, tissue culture is recognized as an effective method for producing uniform, vigorous plants with reduced pathogen presence (Omari et al., 2024). The success of this technique relies on the culture medium, controlled environmental conditions, and the expertise of laboratory personnel (Abdalla et al., 2022). In addition to enhancing shoot multiplication rates, tissue culture also promotes improved root system development, particularly through micropropagation systems, which enhance the overall quality of plantlets (Prabhuling & Sathyanarayanak, 2017; Omari et al., 2024). Banana seedlings propagated via tissue culture begin to exhibit morphological traits, growth patterns, and developmental characteristics during the early stages of the acclimatization phase (Lin et al., 2022).

Recognizing the effects of environmental factors and morphological changes following acclimatization and field planting is vital for evaluating plant adaptation (Costa et al., 2020). This stage is crucial for establishing seedling vigor and quality, factors that subsequently influence growth success (Thanonkeo et al., 2024). Although tissue culture supports large-scale production with reduced variation, challenges related to maintaining genetic uniformity and successful acclimatization remain (Carrera et al., 2021; Dong et al., 2020).

Leaf shape variation is a significant trait in banana Kepok Tanjung plants propagated through tissue culture. Broad leaves generally exhibit greater flexibility than narrow leaves, which are typically thicker, stiffer, and possess slightly pointed tips (Fritz et al., 2018; Ding et al., 2020; Ouyang et al., 2022). Morphological changes during the seedling phase can present challenges for large-scale production aimed at yield enhancement (Yuan et al., 2017; Masmoudi et al., 2023). Furthermore, field conditions influence vegetative traits, including leaf size, suckers, and fruit structure, thereby directly affecting plant growth and productivity (Al-Pavel et al., 2023). Analyzing morphological characteristics facilitates the evaluation of a variety's competitiveness through comparison of its traits and agronomic potential. However, research on the dynamics of morphological changes and their implications for banana production remains limited (Herriman et al., 2016; Stuepp et al., 2020).

Banana Kepok Tanjung plants grown through tissue culture have better genetic uniformity and disease resistance than those from traditional methods (Tumuhimbise & Talengera, 2018). Most research has focused on yield and disease control, with less focuse to how the plants change from seedlings to mature field plants (Xiang et al., 2023). More studies are needed to improve farming methods, increase productivity, and understand growth stages better to support these valuable local bananas (Wahome et al., 2021; Feyso & Menses, 2021; Aeberli et al., 2023).

One challenge in setting selection criteria is the limited leaf traits that reliably distinguish narrow from broad leaves during growth, making breeding and cultivation decisions difficult (Batte et al., 2017). Therefore, data on leaf traits are crucial to improve seedling growth by leaf type and to identify the best leaf characteristics for environmental adaptation and yield improvement (Donato et al., 2018; Malikongwa et al., 2022).

This study examines the morphological traits and growth stages of tissue-cultured banana Kepok Tanjung seedlings with narrow and broad leaves during their field growth. By studying the morphology of these tissue-cultured seedlings once they are established in the field, the study is expected to enhance our understanding of their characteristics and growth patterns, support breeding initiatives, and facilitate the selection of superior seedlings to boost the productivity and resilience of banana Kepok Tanjung cultivation.

2. Material and Methods

The research was conducted in Solok, West Sumatra (0.7431511° S100.6208869°E and 455 m above sea levels), from January 2024 to July 2025. The materials required in this research were banana Kepok Tanjung seedlings, polybags, soil, husk, manure, urea, SP36, and KCL. The tools required for this study included a camera, rope, a hoe, meters, analytical scales, and other equipment.

This study used a descriptive method, focusing on the seedling phase and field-grown plants (Figure 1). Tissue-cultured seedlings of banana Kepok Tanjung variety were planted in polybags measuring 10 × 15 cm, utilizing a planting medium composed of soil, manure, and husks in a 3:1:1 ratio (Figure 2). Seedlings were selected when they were 1 to 1.5 months old following transfer to the polybags. Once the plants had developed 5-8 leaves, they were transplanted to the field and planted in holes measuring 50 × 50 × 50 cm, spaced 3 meters apart.

Fertilization was conducted four times annually, with each plant receiving 350 g of urea, 400 g of SP36, and 450 g of KCl. Observations focused on morphological traits, including pseudostems, leaves, flowers, and fruits, following guidelines from the International Plant Genetic Resources Institute (IPGRI, 1996). A total of 70 plants were observed in the field, comprising 35 narrow-leaved and 35 broad-leaved specimens. The collected data were analyzed for these traits, and genetic similarity matrics and cluster analysis were performed using NTSYSpc version 2.11a to generate dendrograms.

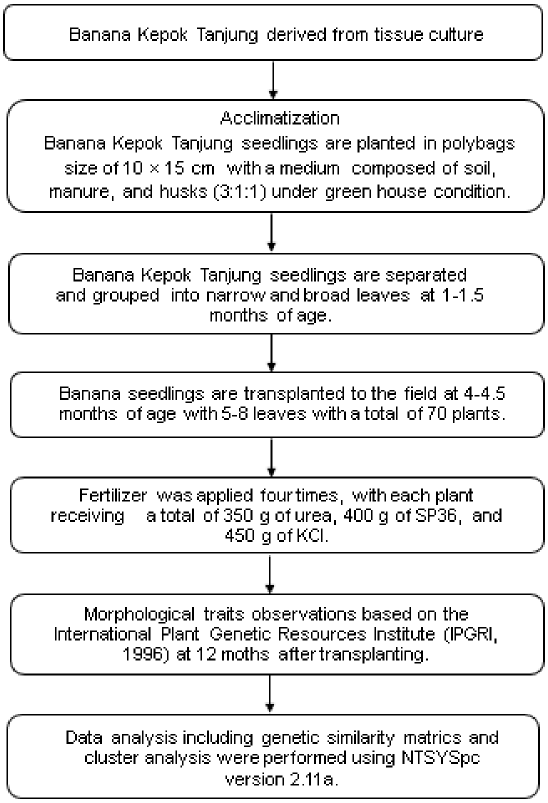

Figure 1. Flow diagram the study of morphological variation in the leaf shape of banana Kepok Tanjung derived from tissue culture.

3. Results and Discussion

3.1. Morphological Characteristics of Banana Kepok Tanjung Leaf Shape Under Field Conditions

Morphological traits are essential for the classification of fruit plants, including bananas (Probojati et al., 2021; Osorio-Guarin et al., 2024). These traits are crucial for distinguishing species in other fruit plants, such as seed shape, fruit size, and leaf anatomy (Christelová et al., 2016; Dhivya et al., 2020; Šimoníková et al., 2020; Rijzaani et al., 2021). In plant taxonomy, features such as flower structure, color, fruit shape, leaf morphology, and seed morphology aid in differentiating between genera, species, and varieties. For example, a study by Inta et al. (2023) highlighted that male flower morphological traits are distinguishing features in the genus Musa, alongside phylogenetic and molecular data in the *Musaceae* family.

At 12 months post-planting in the field, leaf morphological traits exhibited uniformity in both qualitative and reproductive characteristics (55 traits) (Table 1 and Figure 3). However, significant differences were observed in five vegetative growth traits: plant height, pseudostem diameter, leaf length, leaf width, and the number of leaves. This consistency in qualitative and reproductive traits likely reflects genetic factors, given that the same banana variety, Kepok Tanjung, was utilized in this study (Boonsrangsom et al., 2023; Inta et al., 2023). These findings align with research by Premjet et al. (2022), which demonstrated morphological uniformity and reproductive traits in the Musa (ABB) 'Mali-Ong' cultivar, alongside low genetic variation. Another study of several banana cultivars found uniformity among six closely related cultivars (Malikongwa et al., 2022). In addition, the research conducted by Martanti et al. (2022) showed uniformity in the qualitative morphological and reproductive traits observed in *Musa acuminata* (AA) cv. Pisang Rejang.

Variations in vegetative growth traits highlight the banana plant's capacity to adapt to environmental conditions and stressors in the field (Chen et al., 2023; Sun et al., 2024). Indicators such as plant height, stem diameter, and leaf size reveal phenotypic plasticity, influenced by interactions among genetic and environmental factors, including soil fertility, water availability, and light intensity (Hofhansl et al., 2021; Zhang et al., 2022; Hočevar et al., 2025). Previous research investigating phenotypic plasticity, driven by genetic and environmental interactions, has demonstrated its effects on plant height (Mu et al., 2021), height and flowering time (Chen et al., 2025), and modifications in leaf morphology, resulting in smaller and thicker leaves (Wang et al., 2021; Lafont Rapnouil et al., 2023; Aneja et al., 2024), as well as stem diameter (Rosique-Esplugas et al., 2021). Furthermore, studies employing genetic mapping and genome analysis in bananas have identified several candidate genes governing morphological variation, growth, and fruit quality, thereby contributing to the phenotypic diversity observed in the field (Huang et al., 2023; Osorio-Guarin et al., 2024; Miao et al., 2025).

Figure 2. Morphological features of narrow (left) and broad leaves (right) banana Kepok Tanjung plants grown from tissue at seedlings phase.

Table 1. Morphological characteristics of narrow and broad leaves banana Kepok Tanjung seedlings in the field at 12 months of age.

Morphological characteristics	Narrow leaf	Broad leaf
1. Plant height of pseudostem (cm)	212.17±8.26	268.89±6.61
2. The colour of pseudostem	Green-red	Green-red
3. The appearance of pseudostem	Shiny (not waxy)	Shiny (not waxy)
4. Pseudostem dominant colour	Light green	Light green
5. Pseudostem pigmentation	Pink	Pink
6. Pseudostem sap colour	Watery	Watery
7. Stem diameter of psuedostem (cm)	68.37±1.60	79.77±1.36
8. Leaf habit	Intermediate	Intermediate
9. Petiole canal leaf	Straight with erect margins	Straight with erect margins
10. Margins of petiole	Winged,not clasping the pseudostem Not dry	Winged, not clasping the pseudostem
11. Leaf wing type	≤1 cm	Not dry
12. Margin width of petiole (cm)	104.31±3.26	≤1 cm
13. Length of leaf blade (cm)	74.57±6.91	123.49±5.87
14. Width of leaf blade (cm)	Dark green	96.31±8.36
15. Leaf colour upper surface	Shiny	Dark green
16. Leaf upper surface appearance		Shiny
17. Leaf colour lower surface	Green	Green
18. Leaf lower surface appearance	Dull	Dull
19. Leaf blade insertion point on petiole	Asymmetric	Asymmetric
20. Leaf blade shape at base	One side rounded, one pointed	One side rounded, one pointed
21. Leaf number	41.37±0.53	40.48±0.37
22. Leaf corrugation	Few stripes	Few stripes
23. Midrib colour of dorsal surface	Light green	Light green
24. Midrib ventral surface colour	Green	Green
25. Cigar leaf colour of dorsal surface	Green	Green
26. Leaves blotches of water suckers	Without blotches	Without blotches
27. Peduncle colour	Green	Green
28. Position of bunch	Hanging at angle 45°	Hanging at angle 45°
29. Shape of buch	Spiral	Spiral
30. Fruits position on the crown	Biseriate	Biseriate
31. Bract apex shape	Slightly pointed	Slightly pointed
32. Bract colour at external face	Brown-red	Brown-red
33. Bract colour at internal face	Red	Red
34. Bract apex colour	Not tinted with yellow (colour is uniform until apex)	Not tinted with yellow (colour is uniform until apex)
35. Bract stripes colour	With discoloured lines or stripes on the external face	With discoloured lines or stripes on the external face Lifting one at a time
36. Male bract lifting	Lifting one at a time	Revolute (rolling)
37. Behaviour bract before falling	Revolute (rolling)	Falling with the bract
38. Behaviour of male flower	Falling with the bract	Yellow
39. Colour of compound tepal base	Yellow	Cream
40. Colour of lobe compound tepal	Cream	Translucent white
41. Colour of free tepal	Translucent white	Oval
42. Shape of free tepal	Oval	White
43. Colour of filamen	White	Yellow
44. Colour of anther	Yellow	Cream
45. Colour of pollen sac	Cream	Arched
46. Shape of ovary	Arched	Cream
47. Colour of ovary base	Cream	Cream

Morphological characteristics	Narrow leaf	Broad leaf
48. Dominant colour of male flower	Cream	Curved upward (obliquely, at a 45°angle upward)
49. Position of fruit	Curved upward (obliquely, at a 45°angle upward)	Straight (or slightly curved)
50. Fruit shape	Straight (or slightly curved)	Slightly ridged
51. Fruit transverse section	Slightly ridged	Pointed
52. Fruit apex	Pointed	Hairless
53. Surface of pedicle	Hairless	Dark green
54. Peel colour of immature fruit	Dark green	Bright yellow
55. Peel colour of mature fruit	Bright yellow	Fruit peels easily
56. Fruit peel adherence	Fruit peels easily	Without cracks
57. Cracks in fruit peel	Without cracks	Cream
58. Pulp colour before maturity	Cream	Cream
59. Pulp colour at maturity	Cream	Soft
60. Flesh texture	Soft	

Note: data are presented as mean ± standard error.

a b c d e f g h

Figure 3. The morphological traits of bananas kepok tanjung observed in the field: pseudostem (a), leaves and leaf sheaths (c), bract (e), flowers (g) with narrow leaves derived from tissue culture, pseudostem (b), leaves and leaf sheaths (d), bract (f), flowers (h) with broad leaves from tissue culture.

3.2. Genetic Similarity Matrix and Dendrogram Based on The Morphological Characteristics of Banana Kepok Tanjung Leaves

Environmental factors significantly influence leaf morphology (Wen et al., 2024). Genetic diversity, encompassing epigenetic variation, somatic mutations, and polyploidy, contributes to the phenotypic diversity observed in plants and affects leaf morphology (Pillay, 2024). Previous research has demonstrated that leaf size and shape vary in response to environmental conditions, including nutrient levels, water supply, and light intensity. For instance, phenotypic plasticity in leaf characteristics allows plants to adapt to environmental variability, thereby influencing their growth and survival (Zhang et al., 2022).

Figure 4 presents a dendrogram illustrating that bananas Kepok Tanjung are categorized into a single cluster based on leaf morphological characteristics. The high genetic similarity value of 0.957 (Table 2) indicates a strong relationship between the two leaf morphological types. This close relationship suggests that variation between narrow and broad leaves is attributable to limited phenotypic variation, influenced by the interaction of genetic and environmental factors in the expression of leaf morphology.

This finding is consistent with some studies on bananas that reveal a constrained genetic diversity within certain cultivars, despite evident morphological differences. For instance, some banana cultivars demonstrate genetic similarities that enable their classification into two primary clusters, highlighting both diversity and close genetic connections within these clusters (Slameto, 2023). In a similar study of Boonsrangsom et al., (2023) showed that variations in genetic similarity among banana cultivars indicating that morphological differences can arise even among genetically similar groups. Although banana varieties generally possess a limited genetic background, they were categorized into distinct groups, reinforcing the concept that morphological differences often originate from restricted genetic variation, which is further influenced by environmental factors (Noor et al., 2022). In addition, phenotypic plasticity was observed to have low genetic diversity due to factors such as somatic mutations, epigenetics, and genome plasticity (Pillay, 2024). Furthermore, the diversity in Musa leaves results from minor genetic variations interacting with environmental influences, aligning with the close genetic ties and phenotypic adaptability highlighted in the study (Miao et al., 2025).

Table 2. Genetic similarity matrix based on the morphological characteristics of banana Kepok Tanjung leaves.

Leaf morphological	Narrow leaf	Broad leaf
Narrow leaf	1.00	
Broad leaf	0.957	1.00

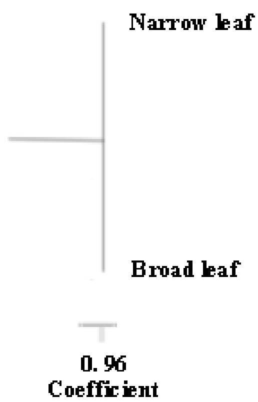

Figure 4. A dendrogram according to the morphological traits of the leaf shape of the banana Kepok Tanjung.

The minimal phenotypic differences observed between the two leaf types in banana Kepok Tanjung are likely attributable to genetic variation and phenotypic plasticity in response to environmental factors, including light intensity, water availability, and nutrient availability (Hofhansl et al., 2021; Hočevar et al., 2025). Phenotypic plasticity in leaf morphology involves alterations in parameters such as specific leaf area, leaf thickness, and stomatal density, which are crucial for regulating photosynthesis and water use in response to environmental conditions (Hočevar et al., 2025). Furthermore, some studies suggest that the interaction between phenotypic plasticity and genetic variation results in relatively minor variations in leaf shape, reflecting the plants' adaptive response to their environment (Solé-Medina et al., 2022; Saito et al., 2024).

4. Conclusion

The study's findings indicate that banana Kepok Tanjung possesses consistent qualitative and reproductive traits, confirming that the studied plants belong to the same variety. Morphological and cluster analyses revealed high genetic similarity (0.957) among leaf characteristics, while variations in vegetative traits indicate phenotypic plasticity. These findings strengthen the use of morphological traits for banana Kepok Tanjung identification. Future research should expand the sample size, include different banana varieties, and consider diverse environmental conditions to better understand morphological variation and plant adaptation.

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