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Evaluation of Grafting Success and Morphological Traits of Liberica Coffee Clones MKL 1, MKL 5, MKL 6, and MKL 7 for Optimized Rootstock Selection

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Abstract

This study evaluated four Liberica coffee clones MKL 1, MKL 5, MKL 6, and MKL 7 for their morphological traits, grafting compatibility, and genetic characteristics to identify optimal rootstocks for coffee cultivation. Morphological analysis identified MKL 6 as the tallest clone, significantly surpassing MKL 7 in plant height, while showing comparable stem diameter and root length to MKL 1 and MKL 5. Grafting trials using MKL 8 scions demonstrated that MKL 6 achieved the highest grafting success, with a 93% success rate and an 88% survival rate 90 days post-grafting, outperforming the other clones. Molecular analysis using SSR markers revealed notable genetic variations among the open-pollinated MKL 5, MKL 6, and MKL 7 rootstocks compared to their parent plants. Despite these genetic differences, seedling performance and graft compatibility remained largely unaffected, highlighting the robustness of these clones for coffee production. The findings emphasize the importance of selecting rootstocks based on both morphological traits and grafting performance to optimize coffee cultivation practices. Future research should investigate broader genetic studies to elucidate the mechanisms underlying graft compatibility and further refine rootstock selection strategies. Additionally, exploring environmental impacts on morphological traits and genetic expression will enhance understanding of the adaptability of Liberica coffee clones across diverse agroecological conditions. This comprehensive evaluation offers valuable insights for improving coffee crop management through informed rootstock selection, contributing to sustainable and efficient coffee production systems.

Keywords: Liberica Coffee; Rootstock Selection; Grafting Compatibility; Morphological Traits; Genetic Variation; Coffee Cultivation Optimization

1 Introduction

The world of coffee extends beyond the familiar Arabica and Robusta varieties, encompassing a diverse array of over 100 documented species [1,2]. Despite this rich diversity, only three species Arabica, Robusta, and Liberica have achieved significant recognition and commercial use. Among these, Liberica, which currently accounts for just 1% of global coffee production, is experiencing a resurgence in popularity [2]. Between 1992 and 2023, the Malaysian Agricultural Research and Development Institute (MARDI) has introduced 10 distinct coffee clones. The most recent releases, MKL 8, MKL 9, and MKL 10, in 2023, represent a significant step forward in diversifying coffee cultivation, with the potential to transform Malaysia's coffee industry. This growing interest in and experimentation with alternative coffee varieties has heightened the need to improve seedling production. Government-funded initiatives aimed at

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expanding coffee cultivation areas have increased the demand for planting materials. However, producing these materials, particularly for Liberica coffee, poses several challenges. For instance, establishing a single hectare of coffee plantation requires approximately 1,280 planting materials, and producing grafted clonal planting materials for Liberica takes between 8 to 12 months [3]. To address the demand for Liberica clonal planting materials, MARDI employs MKL 1, a polyhybrid variety, as rootstock. The MKL 1 plot at MARDI Kluang features a mix of other high-performing Liberica plants, whose seeds are used to produce polyhybrid planting materials coffee plants grown exclusively from seed without grafting. However, the performance of plants derived from polyhybrid seeds can be variable and lacks uniformity. The compatibility between coffee scion and rootstock varieties is critical for enhancing the productivity and quality of Liberica coffee. Optimizing this relationship is essential for the overall health and yield of coffee plants. Therefore, this study aims to evaluate Liberica coffee seedlings produced from MARDI's clonal Liberica coffee seeds (MKL 1, MKL 5, MKL 6, and MKL 7) to determine their suitability as rootstock for Liberica seedling production.

2 Material and methods

2.1 Assessment of MKL 1, MKL 5, MKL 6, and MKL 7 Liberica Coffee Seedlings

In this study, four Liberica coffee clones MKL 1, MKL 5, MKL 6, and MKL 7 were evaluated. Fresh fruits were harvested from the coffee plot at MARDI Kluang and transported to MARDI Headquarters in Serdang, Selangor. The fruits, which ranged in color from orange-red to full-red, had their exocarps manually removed. The seeds, with the mesocarp and endocarp still intact, were soaked in water at 30°C for 24 hours, allowing the mucilaginous mesocarp to be easily washed away. Once dried, the seed coats were manually removed, and the seeds were sown in germination boxes filled with sand as the growth medium. Seedlings were subsequently transferred to 8-inch x 10-inch polybags and cultivated for six months. During this period, growth parameters such as plant height, stem diameter, and root length were recorded every two months. The experiment was designed as a completely randomized design (CRD) with four replications per clone, and the collected data were used to assess the performance of each clone.

2.2 Determination of Grafting Performance of MKL 8 Scion on MKL 1, MKL 5, MKL 6, and MKL 7 Coffee Seedlings

Scions of the MKL 8 clone were sourced from MARDI Kluang and grafted onto six-month-old seedlings of MKL 1, MKL 5, MKL 6, and MKL 7 rootstocks using the top wedge grafting technique. This method involved creating a V-shaped incision at the apex of the rootstock and a corresponding cut at the base of the scion. The graft was then secured with tape, and the seedlings were maintained in a controlled environment to facilitate healing and growth. Grafting success was evaluated 30 days post-grafting by monitoring new shoot production on the scion. The survival rate of the grafted seedlings was recorded 90 days post-grafting.

2.3 Statistical Analysis

Data collected were analysed using ANOVA in the SAS software (Version 9, SAS Institute Inc., Cary, North Carolina, USA). Differences between treatment means were compared using Tukey's Honest Significant Difference (HSD) test at $P \leq 0.05$.

2.4 Molecular Assessment of MKL 5, MKL 6, and MKL 7 Coffee Seedlings

Leaf samples from MKL 5, MKL 6, and MKL 7 seedlings were collected for DNA extraction following the protocol described by [4]. The extraction process was performed in two replicates, and the resulting DNA from each replicate was analyzed to determine the highest quality samples. Prior to extraction, samples were stored at -80ºC. The leaves were lysed using a TissueLyserII (Qiagen, Germany), and the supernatant was separated using a Beckman Coulter liquid handler (Beckman Coulter, USA). DNA quantification was carried out with an Epoch BioTek microplate spectrophotometer (Agilent Technologies, USA), and DNA quality was assessed using 0.8% agarose gel electrophoresis. The extracted DNA was subsequently utilized for PCR amplification. Sixteen SSR primers [5] were employed for PCR amplification in a final reaction volume of 10 µl, containing 10X PCR buffer, 50 mM MgCl2, 10 mM dNTP, 10 µM forward and reverse primers, 5 µM M13 primer, 20 mg/ml BSA, and 5 U/µl Taq. Amplification was performed using a GeneAmp® PCR System 9700 (Applied Biosystems, USA), with an initial denaturation at 94°C for 2.5 minutes, followed by 34 cycles of 94°C for 45 seconds, 58.3°C for 45 seconds, and 72°C for 45 seconds, with a final extension at 72°C for 5 minutes. The resulting amplicons were analyzed for SSR genotyping using the ABI 3730XL DNA Analyzer. Genotyping data were analyzed using GeneMapper Software 5 (Thermo Fisher Scientific, USA). The data were scored and tabulated in Microsoft Excel, and allelic frequency analysis was performed using PowerMarker V3.25 [8]. Genetic distance was

calculated based on the method described by [6], and a dendrogram was constructed using the UPGMA method in PowerMarker software [7].

3 Results and discussion

3.1 Assessment of MKL 1, MKL 5, MKL 6, and MKL 7 Liberica Coffee Seedlings

Among the evaluated clones, MKL 6 seedlings exhibited the greatest height, reaching 70.67 cm, which was significantly taller than MKL 7 seedlings, measuring 49.87 cm. However, the height of MKL 6 did not differ significantly from that of MKL 1 (64.87 cm) and MKL 5 (61.87 cm) (Figure 1A). Stem diameter measurements revealed no significant differences between MKL 1 (7.2 mm), MKL 5 (6.99 mm), and MKL 6 (6.97 mm). In contrast, MKL 7 showed a significantly smaller stem diameter of 5.47 mm (Figure 1B). Root length was consistent across all clones, with MKL 1 measuring 29.5 cm, MKL 5 at 31.78 cm, MKL 6 at 30.56 cm, and MKL 7 at 31.33 cm (Figure 1C). These observed differences in plant height and stem diameter among the clones suggest that these morphological traits may be influenced by both genetic and physiological factors [8]. The significantly greater height of MKL 6 compared to MKL 7 may indicate that MKL 6 has a potential advantage as a rootstock in promoting vertical growth. The absence of significant differences in stem diameter among MKL 1, MKL 5, and MKL 6 suggests a similarity in stem development. On the other hand, the reduced stem diameter observed in MKL 7 may indicate distinct physiological characteristics that could affect its overall plant architecture. The uniformity in root length across all clones suggests a consistent pattern in root development, indicating stability in this aspect of growth among the clones studied.

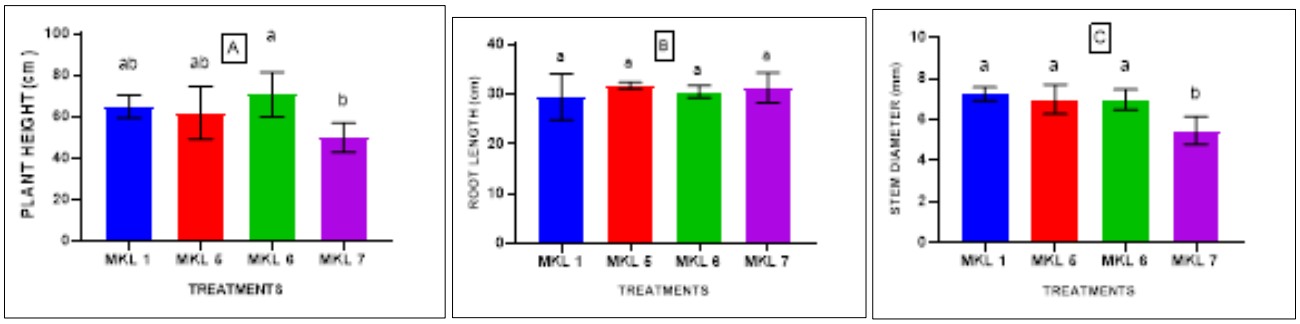

Figure 1 Plant Height (A), Stem Diameter (B), and Root Length (C) at 6 months. Means with different letters in each graph indicate significant differences at P≤0.05 according to HSD

3.2 Grafting Performance of MKL 8 Scion on MKL 1, MKL 5, MKL 6, and MKL 7 Coffee Seedlings

Thirty days post-grafting, the success rates of the rootstocks varied significantly. MKL 6 achieved the highest success rate at 93%, surpassing MKL 1 (78.25%), MKL 5 (73%), and MKL 7 (81.5%) (Figure 2A). Ninety days after grafting, MKL 6 maintained the highest graft independence rate at 88%, which was significantly greater than MKL 1 (64.5%), MKL 5 (54.5%), and MKL 7 (71.25%) (Figure 2B).

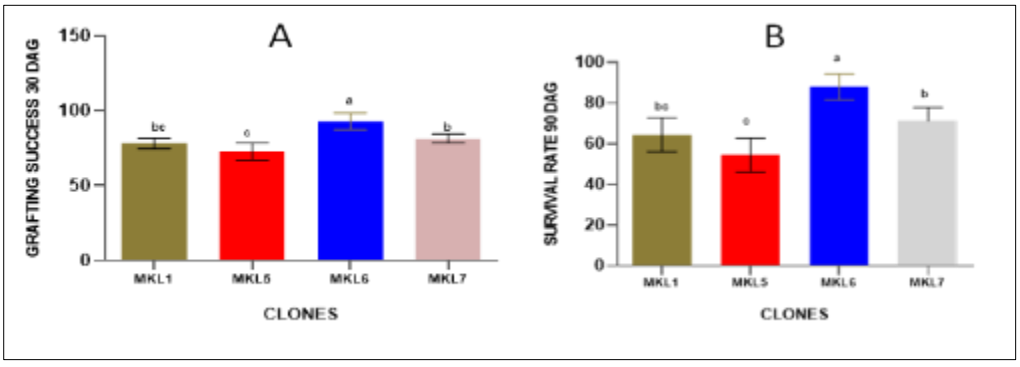

Figure 2 Grafting success at (A) 30 days and (B) 90 days post-grafting of MKL 8 scion on MKL 1, MKL 5, MKL 6, and MKL 7 rootstocks. Means with different letters in each graph indicate significant differences at P≤0.05 according to HSD.

According to [9], grafting compatibility is assessed based on the successful establishment of the graft union and the long-term survival and functionality of the grafted plant. Intraspecific grafts, where rootstock and scion belong to the same species, generally demonstrate high compatibility [10]. The superior performance of MKL 6 in this study suggests a strong correlation between its genetic and physiological characteristics, highlighting the significance of careful rootstock selection for successful grafting in coffee cultivation. These results offer valuable insights into grafting compatibility and underscore the practical implications of optimizing rootstock choices to improve coffee crop management.

3.3 Molecular Assessment of MKL 5, MKL 6, and MKL 7 Coffee Seedlings Compared to Parent DNA

The molecular analysis revealed significant genetic variations between the open-pollinated rootstocks (MKL 5, MKL 6, and MKL 7) and their respective parents (Figure 3). However, these genetic differences did not significantly impact the seedling performance of the rootstocks. The seedlings showed consistent growth and development regardless of the genetic variations observed. The genetic variation between the open-pollinated rootstocks and their parents suggests the potential for unique genetic combinations. However, the limited impact on seedling performance and graft compatibility aligns with [11], who reported that the genotype of Arabica coffee seedlings did not significantly affect their performance.

One possible explanation for the limited influence of genetic variation on seedling performance could be the adaptability and resilience of the studied rootstocks. These rootstocks may possess inherent mechanisms that buffer against potential negative effects of genetic differences, ensuring consistent seedling growth. Rootstock species are often closely related to but genetically distinct from the scion species they support [12]. Furthermore, the grafting compatibility observed in this study supports the practical application of these rootstocks in Liberica coffee seedling production. Despite genetic variations, the rootstocks were compatible with MKL 8 scion, making them a viable option for grafting.

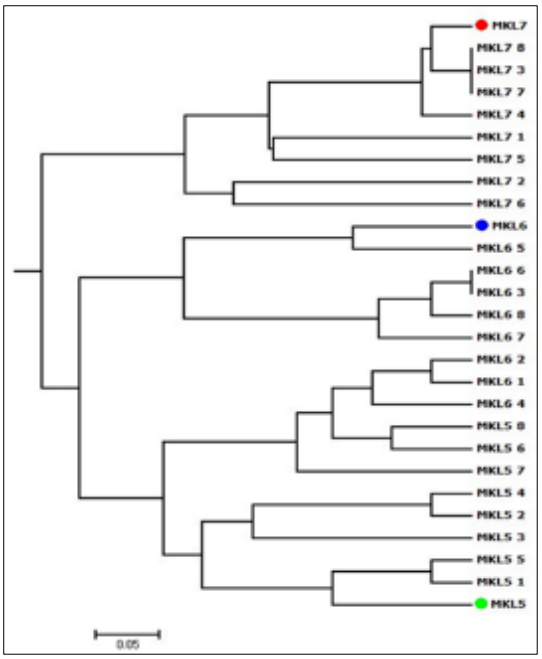

Figure 3 Dendrogram of MKL 5, MKL 6, and MKL 7 genetic distances of rootstocks compared to SSR markers of the clones.

4. Conclusion

In conclusion, the evaluation of morphological and grafting parameters among the examined clones (MKL 1, MKL 5, MKL 6, and MKL 7) has provided valuable insights into their growth characteristics and compatibility as rootstocks. While there were minor differences in growth parameters, MKL 6 stood out with a remarkable grafting success rate of 93%, surpassing the other clones. MKL 6 also achieved the highest graft survival rate at 88%, highlighting its potential as a superior rootstock. Moreover, the molecular analysis revealed significant genetic variation between the open-pollinated rootstocks (MKL 5, MKL 6, and MKL 7) and their respective parents. Despite this genetic diversity, the variations did not significantly affect the seedling performance or graft compatibility of the rootstocks. These findings underscore the

practical implications of selecting MKL 6 as a preferred rootstock for enhancing Liberica coffee cultivation. Future research could focus on long-term field trials to assess the performance and yield of coffee plants grafted onto MKL 6 under various environmental conditions. Additionally, further molecular studies could explore the specific genetic traits that contribute to the superior performance of MKL 6, potentially guiding the development of even more robust rootstock varieties. Investigating the interaction between different scion and rootstock combinations could also provide deeper insights into optimizing grafting techniques and improving overall coffee plant health and productivity.

Compliance with ethical standards

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Disclosure of conflict of interest.

No conflict of interest to be disclosed.

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