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Combination of Acclimatization Media for The Primary Hardening Phase of *Musa paradisiaca* L. var. Kepok Tanjung Plantlets

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ABSTRACT

Acclimatization is the last and critical stage in the in vitro culture of *Musa paradisiaca* L. var. kepok tanjung. The primary hardening phase of acclimatization is carried out so that plantlets can adapt from heterotrophic conditions to autotrophic conditions. This study aims to optimize the acclimatization media for *M. paradisiaca* L. var. kepok tanjung plantlets, an important factor in the acclimatization process. A completely randomized design was applied in this study using 13 different combinations of soil, cocopeat, and charcoal husk media. The observation parameters included morphological parameters (height, number of leaves, leaf area, pseudo-stem diameter, and survival rate) and physiological parameters (wet weight, dry weight, relative water content (RWC), and chlorophyll content index (CCI), which were analyzed using ANOVA or Kruskal-Wallis according to data assumptions. The results showed that all media combinations produced a 100% survival rate, with the best results shown by the cocopeat: charcoal husk (1:1) combination. This media combination provided the highest increase in height (1.05 ± 0.11 cm), pseudo-stem diameter (1.15 ± 0.12 mm), number of leaves (0.75 leaves), leaf area (121.06 ± 20 cm²), fresh weight (8.23 ± 2.99 g), dry weight (0.45 ± 0.16 g), CCI (23.2 ± 3.63), and the highest relative water content (98.47%). The combination of cocopeat and husk charcoal (1:1) was able to maintain moisture and provide good aeration for plantlet growth. Therefore, the combination of cocopeat and charcoal husk (1:1) is recommended as the most effective media for acclimatizing *M. paradisiaca* L. var. kepok tanjung plantlets in the primary hardening phase.

1. Introduction

Musa paradisiaca L. var. kepok tanjung is one of the banana varieties in Indonesia that has high economic value (Sparta & Emilda, 2020). This variety has several advantages, including resistance to bacterial wilt (bacterial blood disease) and shows potential as an alternative food resource with yields of up to 40 kg per plant. (Febmita & Putri, 2023). Demand for this banana continues to increase, especially in large cities, so farmers need to increase production (Latunra *et al*., 2017). Improving the seedling production system is a crucial first step in increasing the productivity of *M. paradisiaca* var. kepok tanjung, because high productivity is greatly influenced by the availability of high-quality seedlings (Febmita & Putri, 2023), which are produced in large quantities and of the same quality (Supristiwendi *et al*., 2024).

Micropropagation or in vitro culture is considered one of the most effective techniques for the rapid production of superior seedlings. (Gupta *et al*., 2020). In vitro cultivation is an aseptic plant propagation technique capable of producing a large quantity of uniform and high-quality seedlings within a short period of time, which then supports strong growth in the next stage of development (Nurzaman *et al*., 2022). According to Justine *et al*. (2022), this technique consists of four stages, with the acclimatization stage being the final and crucial stage because of the significant differences between the low-stress in vitro environment and the more complex and fluctuating field conditions (Setyowati *et al*., 2024). The success of acclimatization determines the overall success of tissue culture, as plant seedlings must adapt and grow under in vivo conditions (Agustin *et al*., 2022).

The acclimatization process for banana seedlings consists of two stages: the pre-nursery or primary hardening stage, and the polybag or secondary hardening stage (Wong *et al*., 2017). Primary hardening is the initial adaptation process in which seedlings removed from culture bottles are transferred to a highly porous and moist growth medium under in vivo conditions (Rahmi *et al*., 2021). This stage aims to transition seedlings from a heterotrophic to an autotrophic state. Secondary hardening is the next adaptation phase in which strengthened seedlings are transferred to soil-based media (Rahmi *et al*., 2021). One of the factors influencing the success of acclimatization is the selection of growth media (Rineksane *et al*., 2023). The physical, chemical, and biological characteristics of the media, as well as the addition of supplemental nutrients, can significantly affect the physiological response of plants (Couto *et al*., 2022), plant anatomy, especially root structure (Li *et al*., 2022), and the morphological development of roots and stems (Alessa & Earnhart, 2000).

Several studies have been conducted to find media for banana acclimatization during the primary hardening phase. Samanhudi *et al*. (2024), used 1 husk charcoal : 1 humus : 1 sterile sand during the primary hardening phase of banana plantlet var. kepok. Kepok bananas var. unti sayang (ABB) used a mixture of 1 charcoal husk : 1 compost media for primary hardening (Rachmi *et al*., 2020). In the acclimatization of banana cavendish by Augustien *et al*. (2020), they used 1 cocopeat : 1 charcoal husk : 2 sand as the media. Setyowati *et al*. (2024), used 100% husk charcoal in the primary hardening stage of barangan merah. Research by Dwiyani *et al*. (2024), stated that the acclimatization of banana var. raja used a combination of cocopeat and soil with a ratio of 1:1 in the primary hardening phase. The differences in the use of acclimatization media during the initial hardening phase for several banana varieties form the basis for finding the optimal media specific to the acclimatization of *M. paradisíaca* L. var. kepok tanjung during the initial hardening phase in order to increase success and produce high-quality seeds.

2. Methodology

2.1 Materials

Plantlet of *M. paradisiaca* var. Kepok Tanjung was taken from the Indonesian Tropical Fruits Research Institute (IP2TP), Ministry of Agriculture, Subang Regency, Indonesia, while the media was taken from PD. Nusa Indah, Bandung, Indonesia.

2.2 Experimental Design and Data Analysis

A completely randomized design (CRD) was applied in this study with soil, cocopeat, and rice husk charcoal as the media (P), resulting in 13 treatment combinations with three replication. The composition of soil, cocopeat, and husk charcoal used in this study is shown in Table 1.

Table 1. Media Combinations for Acclimatization Of *M. paradisiaca* L. Var. Kepok Tanjung Plantlet in Primary Hardening

Code	Media
P0	Soil
P1	Cocopeat
P2	Husk Charcoal
P3	1 Soil : 1 Cocopeat
P4	1 Soil : 1 Husk Charcoal
P5	1 Cocopeat : 1 Husk Charcoal
P6	1 Soil : 1 Cocopeat : 1 Husk Charcoal
P7	1 Soil : 2 Cocopeat
P8	1 Soil : 2 Husk Charcoal
P9	2 Cocopeat : 1 Husk Charcoal
P10	1 Cocopeat : 2 Husk Charcoal
P11	2 Soil : 1 Cocopeat
P12	2 Soil : 1 Husk Charcoal

Data were analyzed using IBM SPSS Statistics version 30.0. Variables meeting the assumptions of normality and homogeneity were examined using ANOVA, followed by Duncan’s test when significant differences were detected ($\alpha = 0.05$). Variables that did not meet these assumptions were analyzed using the Kruskal–Wallis test, followed by pairwise Mann–Whitney tests with Bonferroni correction.

2.3 Experimental Procedure

2.3.1 *Plantlet Transfer*

Seedlings that are ready for acclimatization are taken out from the culture flasks and rinsed under running water to remove any remaining agar, then soaked in a fungicide solution (0.01%) for 5 minutes (Sarkar *et al*., 2023). Plantlets that have been soaked in fungicide are planted in primary hardening media. Plantlets that have been transferred to the media are sprayed with water until moist but not flooded, then covered with transparent plastic for 14 days (Ahmed *et al*., 2014). After 14 days, the cover is removed, and maintenance and watering are carried out every morning (06 : 00 - 08 : 00 WIB) with a volume according to the media field capacity.

2.3.2 *Parameter Measurement*

Parameter measurement in the primary hardening phase is morphology and physiology, which begins when the cover is removed and continues for 4 weeks. Morphological parameters consist of survival rate (%) (Setyowati *et al*., 2024) , plantlet height, number of leaves, pseudo-stem diameter (Dwiyani *et al*., 2024), and leaf area (Kumar *et al*., 2002). Physiological parameters consist of wet weight, dry weight (Rodrigues *et al*., 2022), relative water content (RWC) (Efeoğlu *et al*., 2009), and chlorophyll content index.

3. Results and Discussion

3.1. Environmental Conditions

Environmental conditions at the research site were measured before and after the installation of the shade net. Environmental conditions at the research site are shown in Table 2.

Table 2. Environmental Conditions at The Research Site Before and After Shading

Enviromental Parameters	Before Shading	After Shading
Light Intensity	19.400 lux	13.589 lux
Temperature (^oC)	30.3 ^oC	28.1^oC
Air Humidity	53%	60%

The environmental conditions at the research site had a light intensity of 19,400 lux, with an average environmental temperature of 30.3°C and air humidity of 53%. After being shaded by paranet, the light intensity decreased to 13,589 lux, with an average environmental temperature of 28.1°C and air humidity of 60%. These environmental conditions are optimal according to Pinar *et al*. (2020), which acclimatization of banana planlet at the primary hardening stage requires environmental conditions with a temperature of 20°C-28°C, humidity of 60%-90%, and shade coverage of 70%.

This condition is adjusted to prevent plantlets from suffering stress, as one weakness of in vitro culture plantlets is their root structure and the vascular tissue from the roots to the shoots, which are not yet fully developed. This makes in vitro culture plantlets very sensitive to water loss due to transpiration if light intensity and temperature are too high and humidity is too low (Zulkarnain *et al*., 2023). In banana plantlets, water stress can cause an imbalance between light energy absorption and photosynthesis, leading to a decline in cellular metabolism, lipid oxidation, protein, and nucleic acid levels.

3.2 Morphological Parameters

The results showed that the percentage of live plantlets in the primary hardening phase of *M. paradisiaca* L. var. kepok tanjung acclimatization was 100% in all acclimatization media (Table.3). The use of different media in the primary hardening phase of *M. paradisiaca* L. var. kepok tanjung banana acclimatization showed significant results. According to the results, the combination of cocopeat : husk charcoal (1:1) in *M. paradisiaca* L. var. kepok Tanjung acclimatization during the primary hardening phase showed the optimal result in all parameters. This showed that the combination of cocopeat: husk charcoal (1:1) is appropriate for the *M. paradisiaca* L. var. kepok tanjung acclimatization during yhe primary hardening phase. The growth of *M. paradisiaca* L. var. kepok tanjung is shown in Figure 1.

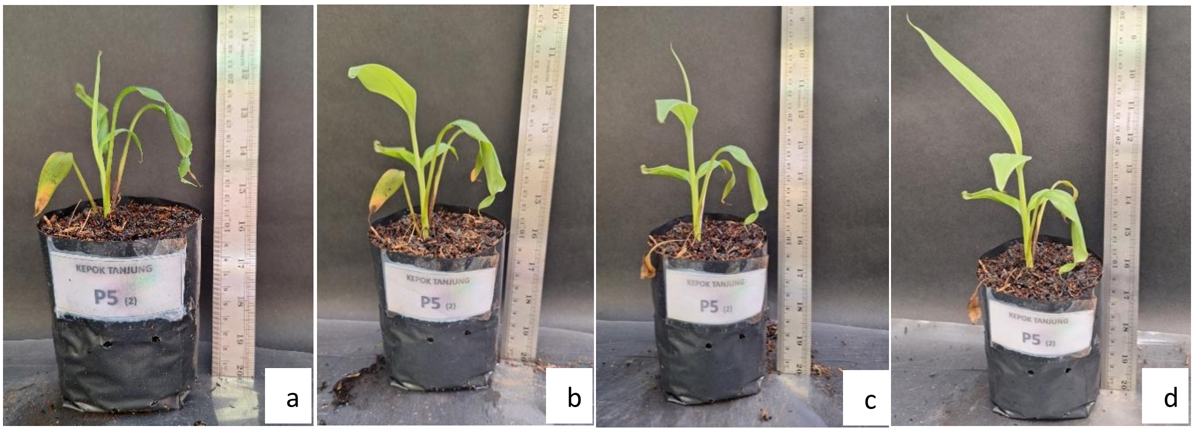

Figure 1. Growth of *M. paradisiaca* L. var. kepok tanjung plantlets during the primary hardening phase of acclimatization. (a) week 1, (b) week 2, (c) week 3, (d) week 4.

The combination of cocopeat and husk charcoal (1:1) is optimal for the acclimatization of *M. paradisiaca* L. var. kepok tanjung, as indicated by morphological parameters such as plant height increase, stem diameter increase, number of leaves increase, and leaf area, which have the highest values compared to other media combinations. The growth of plant height and pseudo-stem diameter of kepok tanjung banana plantlets is shown in Table 3.

Table 3. Survival rate, plant height increase, and pseudo-stem diameter increase *M. paradisiaca* var. kepok tanjung plantlet

Media	Survival Rate (%)	Plant Height Increase (cm)	Pseudo-stem Diameter Increase (mm)
Soil	100	0.13 ± 0.12^{g}	0.43 ± 0.05^{cde}
Cocopeat	100	0.47 ± 0.08^{ef}	0.51 ± 0.04^{cd}
Husk Charcoal	100	0.23 ± 0.02^{fg}	0.12 ± 0.04^{f}
1 Soil : 1 Cocopeat	100	0.42 ± 0.30^{fg}	0.52 ± 0.15^{cd}
1 Soil : 1 Husk Charcoal	100	0.56 ± 0.18^{de}	0.40 ± 0.05^{cde}
1 Cocopeat : 1 Husk Charcoal	100	1.05 ± 0.11^{a}	1.15 ± 0.12^{a}
1 Soil : 1 Cocopeat : 1 Husk Charcoal	100	0.66 ± 0.08^{cd}	0.32 ± 0.03^{e}
1 Soil : 2 Cocopeat	100	0.93 ± 0.17^{ab}	0.85 ± 0.16^{b}
1 Soil : 2 Husk Charcoal	100	0.86 ± 0.20^{ab}	0.47 ± 0.10^{cde}
2 Cocopeat : 1 Husk Charcoal	100	0.70 ± 0.12^{cd}	0.37 ± 0.03^{de}
1 Cocopeat ; 2 Husk Charcoal	100	0.76 ± 0.08^{bc}	0.74 ± 0.08^{b}
2 Soil ; 1 Cocopeat	100	0.32 ± 0.12^{fg}	0.56 ± 0.05^{c}
2 Soil : 1 Husk Charcoal	100	0.89 ± 0.24^{ab}	0.83 ± 0.15^{b}

*Duncan's post hoc test showed significant differences at the 95% confidence level. Mean values followed by the same letter indicate no significant differences between treatments ($P < 0.05$).

The growth of the number of leaves of *M. paradisiaca* L. var. kepok tanjung plantlets. base on Pairwise Mann–Whitney analysis with Bonferroni correction. indicated significant differences among treatments ($P < 0.05$). An increase in the number of leaves of *M. paradisiaca* L. var. kepok tanjung plantlets can be seen in Figure 2.

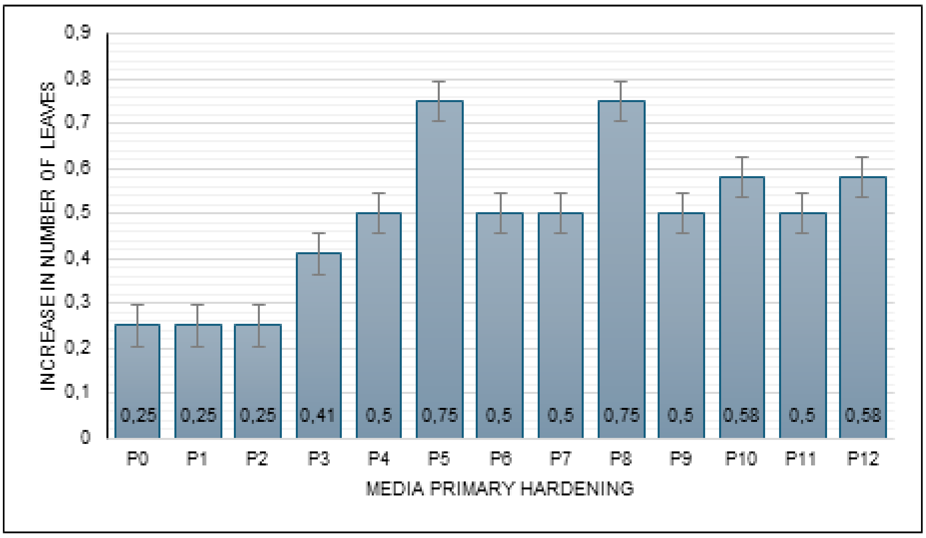

Figure 2. Increase In the Number of Leaves of *M. paradisiaca* L. Var. Kepok Tanjung Plantlets.

Leaf area of *M. paradisiaca* L. var. kepok tanjung plantlets. base on Pairwise Mann–Whitney analysis with Bonferroni correction. indicated significant differences among treatments ($P < 0.05$). Leaf area of *M. paradisiaca* L. var. kepok tanjung plantlets can be seen in Figure 3.

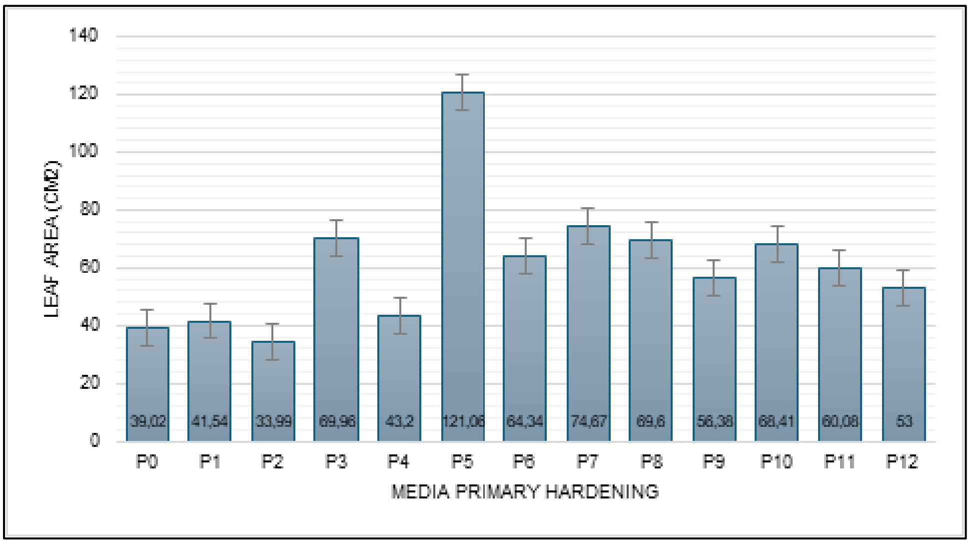

Figure 3. Leaf Area of *M. paradisiaca L.* var. Kepok Tanjung Plantlets

The combination of cocopeat and husk charcoal (1:1) provided the highest increase in height (1.05 ± 0.11 cm). pseudo-stem diameter (1.15 ± 0.12 mm). number of leaves (0.75 leaves). and leaf area (121.06 ± 20 cm^2) which is significantly different from media soil. cocopeat. and husk charcoal when used alone. The comparison of the growth *M. paradisiaca* L. var. kepok tanjung between soil. cocopeat. husk charcoal. and cocopeat: husk charcoal (1:1) is shown in Figure 4.

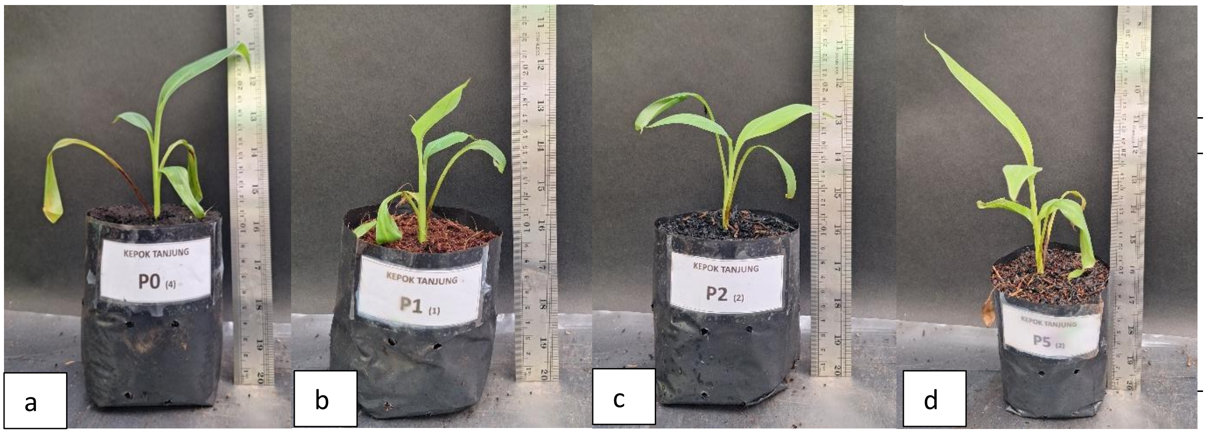

Figure 4. Comparison of the growth *M. paradisiaca* L. var. kepok tanjung plantlet. (a) soil, (b) cocopeat, (c) husk charcoal, (d) cocopeat: husk charcoal (1:1)

Several studies found that the utilizing cocopeat and husk charcoal as media acclimatization is effective for the acclimatization of several types of bananas. Research by Augustien *et al*. (2020). on Cavendish banana acclimatization stated that the combination of cocopeat : husk charcoal : sterile sand (1:1:2) had the best result for the acclimatization. A previous study was carried out by Aprianto *et al*. (2023). that utilizing cocopeat 25% and 75% top soil provide effective results for red baranang banana plantlets throughout the acclimatization period. This is different from the study conducted by Setyowati *et al*. (2024). which utilized 100% charcoal husk media during the primary hardening phase of red baranang banana plantlets.

Cocopeat is appropriate as media in acclimatization during primary hardening due to the characteristics that can bind and store water strongly (Aprianto *et al*.. 2023). Meanwhile. husk charcoal has a porous structure with good circulation and good aeration. enabling it to assist plants in absorbing nutrients (Sugianto & Jayanti. 2021). The characteristic of cocopeat and husk charcoal are suitbale for root growth especially in primary hardening phase. which emphasize root initiation and shoot development (Chamling & Bhowmick. 2021). because plantlets that have just been transferred from culture bottles have root structure and vascular tissue that are not fully developed. make plantlets very sensitive to water loss due to transpiration (Zulkarnain *et al*.. 2023).

Combination of cocopeat: husk charcoal (1:1) is appropriate for *M. paradisiaca* L. var kepok tanjung acclimatization during the primary hardening phase. because the combination has high water retention. enabling it to maintain more stable moisture for plantlet root development. Jeong *et al*. (2021). explain that media with good water retention can improve root development. while media with low field capacity will interfere with water absorption. causing stress on the roots and inhibiting root growth. The presence of a well-developed root system promotes healthy shoot growth and plant development (Jeong *et al*.. 2021). The good result of the combination cocopeat and husk charcoal (1:1) in the morphology parameter is also influenced by the physiological parameter. which has a good result in this combination.

3.3 Physiological Parameters

The differences among the combination of acclimatization media for the primary hardening phase of *M. paradisiaca* L. var. kepok tanjung in physiological parameters showed significant differences. Relative water content (RWC) of *M. paradisiaca* L. var. kepok tanjung plantlets based on Pairwise Mann–Whitney analysis with Bonferroni correction indicated significant differences among treatments (P < 0.05). Relative water content (RWC) of *M. paradisiaca* L. var. kepok tanjung plantlets can be seen in Figure 5.

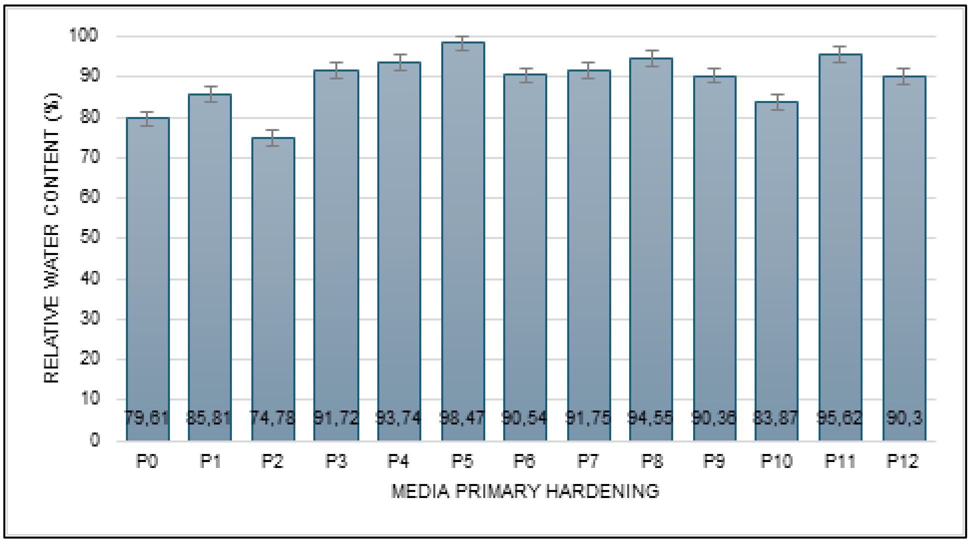

Figure 5. Relative Water content (RWC) of *M. paradisiaca* L. var. Kepok Tanjung Plantlets.

The characteristic of cocopeat and husk charcoal to retain water is evident in the relative water content parameter. In this study combination of cocopeat: husk charcoal (1:1) has the highest RWC value of 98.4% compared to other media. especially with soil and husk charcoal when used alone (Figure 5). This finding is in line with the explanation by Qayyum *et al*. (2021). that media capable of retaining water can maintain the relative water content in plant tissue. RWC is a measure of plant water status related to water absorption by roots and water loss through transpiration (Sharma *et al*.. 2021).

When the relative water content is high in plants. it can increase plant biomass (Chen & Sun. 2025). The fresh weight and dry weight of *M. paradisiaca* L. var. kepok tanjung plantlet that acclimatized with cocopeat : husk charcoal (1:1) during primary hardening have the heaviest weight. the fresh weight was 8.23 □ 0.16 g and the dry weight was 0.45 □ 0.16 g. which is significantly different from media soil. cocopeat. and husk charcoal when used alone. The fresh weight and dry weight of the plant can be seen in Table 4.

Table 4. Fresh Weight and Dry Weight of *M. paradisiaca* L. var. Kepok Tanjung Plantlet

Media	Fresh Weight (g)	Dry Weight (g)
Soil	2.90 ± 0.19^{e}	0.18 ± 0.02^{e}
Cocopeat	3.82 ± 0.78^{cde}	0.18 ± 0.02^{e}
Husk Charcoal	3.19 ± 0.41^{de}	0.17 ± 0.04^{e}
1 Soil : 1 Cocopeat	5.23 ± 1.24^{bc}	0.21 ± 0.03^{cde}
1 Soil : 1 Husk Charcoal	4.94 ± 1.69^{bcd}	0.27 ± 0.66^{bcde}
1 Cocopeat : 1 Husk Charcoal	8.23 ± 2.99^{a}	0.45 ± 0.16^{a}
1 Soil : 1 Cocopeat : 1 Husk Charcoal	5.06 ± 0.66^{bcd}	0.24 ± 0.02^{bcde}
1 Soil : 2 Cocopeat	5.87 ± 0.63^{bc}	0.33 ± 0.02^{bc}
1 Soil : 2 Husk Charcoal	4.61 ± 1.11^{cde}	0.20 ± 0.04^{de}
2 Cocopeat : 1 Husk Charcoal	3.80 ± 1.19^{cde}	0.20 ± 0.09^{de}
1 Cocopeat ; 2 Husk Charcoal	5.13 ± 1.6^{bcd}	0.32 ± 1.1^{bcd}
2 Soil ; 1 Cocopeat	3.77 ± 0.94^{cde}	0.22 ± 0.06^{cde}
2 Soil : 1 Husk Charcoal	6.83 ± 2^{ab}	0.36 ± 0.07^{ab}

*Duncan's post hoc test showed significant differences at the 95% confidence level. Mean values followed by the same letter indicate no significant differences between treatments ($P < 0.05$).

The high relative water content in combination media cocopeat : husk charcoal (1:1) resulted in a high chlorophyll content index in the plantlet (23.2 ± 3.63 CCI). which is significantly different from cocopeat and husk charcoal when used alone. Chlorophyll content index (CCI) of *M. paradisiaca* L. var. kepok tanjung plantlets based on Pairwise Mann–Whitney analysis with Bonferroni correction indicated that the treatments differed significantly ($P < 0.05$). Chlorophyll content index (CCI) of *M. paradisiaca* L. var. kepok tanjung plantlets can be seen in Figure 6.

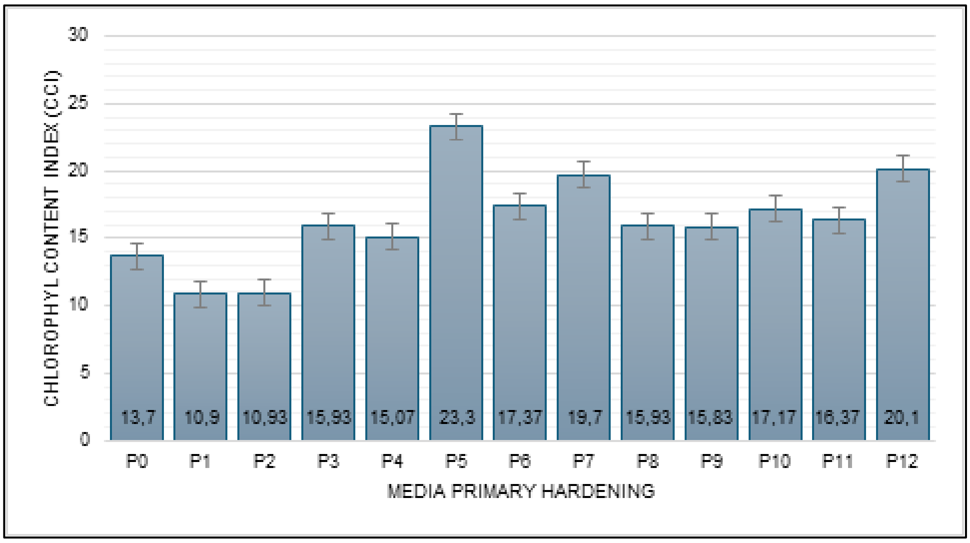

Figure 6. Chlorophyl Content Index (CCI) of *M. paradisiaca* L. var. Kepok Tanjung Plantlets.

This result is in line with the research by Aref *et al*. (2014). that a decrease in relative water content in Juniperus procera was followed by 37% decrease in chlorophyll content index. A decrease in relative water content in a plant will decrease the chlorophyll content in the plant as a protective mechanism against photo-oxidative damage that can inhibit the photosynthesis process (Khanna. 2022). The relatively high chlorophyll content index in the plantlets that acclimatized with cocopeat: husk charcoal (1:1) during primary hardening indicates that chlorophyll a and chlorophyll b content in these seedlings is high. This occurs because CCI is positively correlated (R^2 = 0.87) with chlorophyll a and chlorophyll b content in leaves (Padilla *et al*.. 2018).

The best result in growth occurred because plantlets that acclimatized in cocopeat: husk charcoal (1:1) had the highest chlorophyll content index (Figure 6). The high content of macronutrients potassium (K). nitrogen (N). and phosphorus (P). as well as micronutrients such as calcium (Ca). magnesium (Mg). and sodium (Na) present in cocopeat (Siregar *et al*.. 2020) and the high carbon content (31%) in husk charcoal according to Saputra & Sutriana (2022). also help increase chlorophyll formation in leaves. The chlorophyll content in plantlets acclimatized with this medium will optimize the use of light energy. allowing the plantlets to continue to adapt and promote growth (He *et al*.. 2022). The higher level of chlorophyll content index can increase the rate of photosynthesis. resulting in higher photosynthetic products that can lead to better plant growth (Tini *et al*.. 2019).

4. Conclusion

Based on the study. it can be concluded that the combination of cocopeat: husk charcoal (1:1) is the optimal media for the acclimatization of *M. paradisiaca* L. var. kepok tanjung plantlets during primary hardening. as seen in increasing plant height. increasing pseudo-stem diameter. increasing in the number of leave. the highest in leaf area and having the best result in physiological parameters such relative water content. fresh weight. dry weight. and cholorphyl content index. This combination can be used as a reference for *M. paradisiaca* L. var kepok tanjung acclimatization during primary hardening for the farmer in the future. Further research is needed on optimizing the acclimatization media for *M. paradisiaca* L. var. kepok tanjung in the secondary hardening.

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