Acclimatization of The Rare Orchid *Dendrobium sutiknoi*: An Ex-Situ Conservation Effort for Rare Papuan Orchids

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ABSTRACT

Indonesia possesses one of the world's richest orchid diversities, including numerous endemic species that are increasingly threatened by habitat degradation, illegal collection, and environmental disturbances. *Dendrobium sutiknoi*, a rare orchid species native to Papua, requires effective conservation strategies to prevent further population decline. In vitro propagation through tissue culture offers an efficient method for producing large numbers of plantlets; however, the acclimatization stage remains a critical factor determining the successful transition of plantlets from in vitro to ex vitro conditions. This study aimed to evaluate the effects of environmentally friendly organic acclimatization media on the growth and survival of *Dendrobium sutiknoi* plantlets as part of an ex situ conservation effort. A single-factor Randomized Block Design was employed using seven acclimatization media treatments consisting of black moss, tree fern fiber, charcoal, coconut coir, black moss + tree fern fiber, black moss + charcoal, and black moss + coconut coir, with five replications. Parameters observed included acclimatization success percentage, number of leaves, and plant height during 35 days after transplanting. The results demonstrated that acclimatization media significantly affected plantlet growth and survival. Coconut coir produced the highest acclimatization success rate (55%), while the combination of black moss and tree fern fiber resulted in a 40% survival rate. Black moss generated the highest average number of leaves (5.47 ± 0.49 leaves), whereas coconut coir produced the greatest plant height among single media treatments (5.99 ± 0.53 cm). Among combined media, black moss + charcoal yielded the highest plant height (5.92 ± 0.84 cm). The findings indicate that organic waste-based substrates can serve as effective and sustainable acclimatization media for *D. sutiknoi*, thereby supporting ex-situ conservation programs and contributing to biodiversity preservation in accordance with Sustainable Development Goal 15.

Keywords: *Dendrobium sutiknoi*, acclimatization, ex-situ conservation, organic media, Papua, tissue culture

INTRODUCTION

Indonesia has one of the richest orchid germplasm resources in the world after Brazil, with 6,347 records of orchid species in 200 genera based on the POWO and GBIF databases spread across the country (Wati, et al., 2023). Many of these species are endemic and naturally inhabit Indonesian forests. However, the diversity of native orchids is increasingly threatened by forest fires, illegal logging, and excessive collection by orchid enthusiasts without considering conservation aspects in their natural habitats (Mullin, et al., 2022). The conservation status of orchids is highly dependent on habitat quality; therefore, preserving their natural ecosystems is essential for maintaining orchid diversity.

Orchid propagation through seeds is generally difficult because orchid seeds lack functional endosperm, resulting in slow germination. Germination begins with the formation of protocorms, spherical structures that have not yet developed leaves, stems, or roots (Handini & Puspitaningtyas, 2020). Consequently, in vitro propagation through plant tissue culture has become an effective approach for orchid multiplication. Successful in vitro propagation depends on environmental factors and culture media composition. After several subcultures, seedlings develop into complete plantlets that are ready for acclimatization under external conditions (Maulia, et al., 2025). Acclimatization is a critical stage in orchid cultivation, but its success is often species-specific and many plantlets fail to survive during this transition. Therefore, appropriate acclimatization techniques are needed for rare orchid species such as *Dendrobium sutiknoi*.

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The genus Dendrobium is one of the largest and most economically important genera within Orchidaceae. Its species are highly valued because of their diverse flower colors, shapes, patterns, and fragrances. Dendrobium species are widely distributed from South Asia to Southeast Asia and are commonly cultivated in countries such as Thailand, Indonesia, and the Philippines. As epiphytic orchids, they possess fibrous roots covered by velamen, which facilitates water absorption, and exhibit sympodial growth with pseudobulbs (Kurniawan & Mustika, 2021). The species used in this study, *Dendrobium sutiknoi*, naturally occurs in Papua, Papua New Guinea, and the Solomon Islands but has become increasingly rare due to unfavorable environmental conditions. According to Dressler & Dodson (2000), *D. sutiknoi* belongs to the family Orchidaceae and the genus Dendrobium. This species is a member of the Spatulata section and is characterized by golden yellow and brown flowers with elongated, slightly twisted petals. It was first described by P. O'Byrne in 2005 (Orchidroots, 2022).

This study aimed to acclimatize *Dendrobium sutiknoi* plantlets produced from previous seed isolation and inoculation through tissue culture by using environmentally friendly media derived from organic waste materials. Specifically, the study sought to evaluate the effects of different acclimatization media on the growth of D. sutiknoi and to identify the most effective medium for supporting plantlet development. The findings are expected to contribute to ex situ conservation efforts for this rare Papuan orchid species and support the achievement of Sustainable Development Goal 15 concerning terrestrial biodiversity conservation.

Plant tissue culture has become an important ex situ conservation approach for rare and endangered orchids because it enables the rapid production of large numbers of genetically uniform plantlets under controlled environmental conditions. Previous studies have demonstrated that tissue culture techniques are effective in propagating various orchid species and reducing pressure on natural populations. Nevertheless, despite the successful production of plantlets under in vitro conditions, the transition from laboratory conditions to natural environments remains one of the most critical stages in orchid propagation.

The acclimatization process represents a major challenge because plantlets produced in vitro possess anatomical and physiological characteristics that differ from those of naturally grown plants. In vitro plantlets generally have poorly developed cuticles, non-functional stomata, and weak root systems, making them highly susceptible to water loss, pathogen infection, and environmental stress after transfer to ex vitro conditions (Utami et al., 2022). Therefore, the selection of suitable acclimatization media is essential to improve plant survival and promote healthy growth.

Traditionally, orchid acclimatization media include materials such as sphagnum moss, tree fern fiber, charcoal, and coconut coir. These materials differ in their physical and chemical properties, including water-holding capacity, aeration, drainage, and nutrient availability, all of which influence the growth performance of orchid seedlings. Furthermore, the utilization of renewable and organic waste-based substrates offers an environmentally friendly alternative that supports sustainable agricultural practices while reducing dependence on non-renewable resources.

Among organic materials available in abundance in Indonesia, coconut coir and other agricultural residues possess considerable potential as sustainable growing media. These materials not only improve moisture retention and root aeration but also contribute to waste recycling and environmental management. Their utilization is consistent with the principles of circular economy and sustainable resource use.

The conservation of rare orchid species is closely aligned with the United Nations Sustainable Development Goals (SDGs), particularly Goal 15 (Life on Land), which emphasizes the protection, restoration, and sustainable use of terrestrial ecosystems and biodiversity. Ex situ propagation and acclimatization of endangered orchids contribute directly to biodiversity conservation by safeguarding valuable genetic resources and supporting future restoration efforts.

Therefore, this study aimed to evaluate the effectiveness of several environmentally friendly organic acclimatization media on the survival and growth of *Dendrobium sutiknoi* plantlets derived from tissue culture. The findings are expected to provide scientific information regarding appropriate acclimatization techniques for rare orchids and contribute to sustainable ex situ conservation programs for Indonesia's endemic plant biodiversity.

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MATERIALS AND METHODS

This study was conducted over one year and consisted of two sequential stages. The first stage involved exploratory and experimental activities to propagate the rare Papuan orchid *Dendrobium sutiknoi* through plant tissue culture. Seed explants were isolated and inoculated to produce in vitro plantlets. Plantlets selected for acclimatization possessed complete organs, healthy green shoots, balanced root and leaf development, and uniform plant height ranging from 3 to 4 cm.

The second stage focused on the acclimatization of in vitro-derived plantlets using environmentally friendly substrates. The experiment employed a Randomized Block Design with one factor, namely acclimatization medium, using five replications. Seven media treatments were evaluated: black moss, tree fern fiber, coconut coir, charcoal, black moss + tree fern fiber, black moss + coconut coir, and black moss + charcoal. The parameters measured consisted of survival which was calculated by the percentage of plantlet survival after acclimatization at 35 DAT, the number of leaves and height of the plantlets were calculated starting from 7 DAT, 14 DAT, 21 DAT, 28 DAT, and 35 DAT.

Plantlets were removed from culture bottles, cleaned of agar residues, washed under running water with detergent, soaked in a fungicide solution for one hour, and transplanted into community pots containing the designated media. The pots were covered with transparent plastic to reduce transpiration and maintained under laboratory conditions with routine watering, fertilization, vitamin application, and regular observations.

The parameters observed during acclimatization included the number of leaves, plant height, and the number of surviving plantlets in each pot, which were used to determine the percentage of acclimatization success. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test. Prior to ANOVA, normality and homogeneity assumptions were tested using the One-Sample Kolmogorov-Smirnov test and the Test of Homogeneity of Variances, respectively. Descriptive quantitative and qualitative analyses were also performed.

RESULTS

This study was conducted over one year and began with an exploratory stage involving descriptive and experimental approaches to prepare the rare orchid *Dendrobium sutiknoi* for propagation through tissue culture using seed explants. Successful in vitro plantlets obtained from the first stage were subsequently subjected to acclimatization under ex-vitro conditions using environmentally friendly media based on abundant organic materials as part of an ex-situ conservation effort supporting SDG 15 (Life on Land).

Overall, the results showed that different acclimatization media significantly affected the growth performance of *Dendrobium sutiknoi* plantlets during the acclimatization period. Observations were conducted at 7, 14, 21, 28, and 35 days after transplanting (DAT). The parameters evaluated included acclimatization success percentage, number of leaves, and plant height. The results and statistical analyses for each growth parameter are presented in the following sections.

Acclimatization Success Percentage

Table 1 present the acclimatization success of *Dendrobium sutiknoi* plantlets, expressed as the percentage of plantlets that successfully adapted to ex vitro conditions and developed into viable orchid seedlings.

Table 1. Effect of acclimatization media on the survival percentage of *Dendrobium sutiknoi* plantlets.

Acclimatization Medium	Survival Percentage (%)
Black moss	35
Tree fern fiber	5

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Charcoal	15
Coconut coir	55
Black moss + tree fern fiber	40
Black moss + charcoal	15
Black moss + coconut coir	15

The type of acclimatization medium influenced the survival of *Dendrobium sutiknoi* plantlets during the transition from in vitro to ex vitro conditions. The highest acclimatization success was obtained using coconut coir medium, with a survival rate of 55%, followed by the combination of black moss and tree fern fiber (40%) and black moss alone (35%). The lowest survival rate was observed in tree fern fiber medium, which resulted in only 5% successful acclimatization. These results indicate that coconut coir provided the most favorable conditions for plantlet adaptation under ex vitro conditions.

Number of Leaves

The number of leaves increased progressively throughout the observation period in all treatments, indicating that all acclimatization media supported plant growth. However, differences among media were observed.

Table 2. Number of leaves of *Dendrobium sutiknoi* plantlets during acclimatization.

Medium	**7 DAT**	**14 DAT**	**21 DAT**	**28 DAT**	**35 DAT**
Black moss	4.99	5.13	5.42	5.58	6.25
Tree fern fiber	4.00	4.20	4.54	4.83	4.99
Charcoal	4.75	5.03	5.33	5.52	5.81
Coconut coir	4.37	4.79	4.93	5.09	5.34
Black moss + tree fern fiber	4.02	4.55	4.72	4.98	5.30
Black moss + charcoal	4.01	4.12	4.41	4.76	4.93
Black moss + coconut coir	4.25	4.55	4.94	5.13	5.32

According to the data presented in Table 2, the number of leaves increased continuously up to 35 days after transplanting (DAT) in all acclimatization media. Among the single media treatments, black moss produced the highest number of leaves (6.25 leaves), followed by charcoal (5.81 leaves), coconut coir (5.34 leaves), and tree fern fiber (4.99 leaves). For the combined media treatments, the highest leaf number was recorded in black moss + coconut coir (5.32 leaves), followed by black moss + tree fern fiber (5.30 leaves) and black moss + charcoal (4.93 leaves). The increase in leaf number from the beginning of acclimatization until 35 DAT indicates that all media treatments positively supported the growth of *Dendrobium sutiknoi* plantlets.

Normality and homogeneity tests indicated that the data were normally distributed and homogeneous. One-way ANOVA showed significant differences among treatments ($P < 0.05$), indicating that acclimatization media significantly affected leaf production. Duncan's Multiple Range Test revealed that black moss produced the highest average number of leaves.

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Table 3. Duncan's Multiple Range Test for average number of leaves of *Dendrobium sutiknoi*.

Medium	**Mean ± SD**
Black moss	5.47 ± 0.49^{c}
Tree fern fiber	4.51 ± 0.41^{a}
Charcoal	5.29 ± 0.41^{bc}
Coconut coir	4.90 ± 0.36^{abc}
Black moss + tree fern fiber	4.72 ± 0.49^{ab}
Black moss + charcoal	4.45 ± 0.40^{a}
Black moss + coconut coir	4.84 ± 0.43^{ab}

Note: Means followed by the same letter (a, b, or c) are not significantly different, whereas means followed by different letters indicate significant differences among treatments according to Duncan's Multiple Range Test

Based on Duncan's Multiple Range Test, black moss produced the highest average number of leaves (5.47 ± 0.49 leaves) and differed significantly from several other treatments. In contrast, tree fern fiber resulted in the lowest average leaf number (4.51 ± 0.41 leaves), which was not significantly different from the black moss + charcoal treatment (4.45 ± 0.40 leaves). Charcoal medium showed the second-highest average leaf number (5.29 ± 0.41 leaves), while coconut coir produced an intermediate response (4.90 ± 0.36 leaves).

Among the combined media treatments, black moss + coconut coir resulted in the highest average number of leaves (4.84 ± 0.43 leaves), followed by black moss + tree fern fiber (4.72 ± 0.49 leaves) and black moss + charcoal (4.45 ± 0.40 leaves). Overall, black moss was identified as the most effective single acclimatization medium for promoting leaf development, whereas the combination of black moss and coconut coir was the best-performing mixed medium for *Dendrobium sutiknoi* plantlets.

Plant Height

Plant height increased steadily throughout the acclimatization period in all treatments. Differences among media became more evident at later observation stages.

Table 4. Plant height (cm) of *Dendrobium sutiknoi* plantlets during acclimatization.

Medium	**7 DAT**	**14 DAT**	**21 DAT**	**28 DAT**	**35 DAT**
Black moss	4.56	4.62	4.75	5.05	5.58
Tree fern fiber	4.00	4.36	4.49	4.62	5.19
Charcoal	5.08	5.53	5.70	5.97	6.20
Coconut coir	5.23	5.84	5.92	6.31	6.64
Black moss + tree fern fiber	4.40	4.58	5.76	6.17	6.34
Black moss + charcoal	5.04	5.33	5.61	6.65	6.95
Black moss + coconut coir	5.18	5.25	5.50	5.94	6.29

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According to the plant height data presented in Table 4, all acclimatization media supported the growth of *Dendrobium sutiknoi* plantlets up to 35 days after transplanting (DAT). Among the single media treatments, coconut coir produced the tallest plantlets (6.64 cm), followed by charcoal (6.20 cm) and black moss (5.58 cm), whereas tree fern fiber resulted in the lowest plant height (5.19 cm).

For the combined media treatments, black moss + charcoal produced the greatest plant height (6.95 cm), followed by black moss + tree fern fiber (6.34 cm) and black moss + coconut coir (6.29 cm). These results indicate that all acclimatization media were capable of supporting the growth and development of *Dendrobium sutiknoi* plantlets during the acclimatization period.

ANOVA analysis demonstrated significant differences among treatments. Subsequent multiple comparison analysis indicated that both coconut coir and the combination of black moss and charcoal promoted superior plant height development.

Table 5. Duncan's Multiple Range Test for plant height of *Dendrobium sutiknoi*.

Medium	**Mean ± SD (cm)**
Black moss	4.91 ± 0.42 ^{Ab}
Tree fern fiber	4.53 ± 0.43 ^{A}
Charcoal	5.70 ± 0.43 ^{Bc}
Coconut coir	5.99 ± 0.53 ^{C}
Black moss + tree fern fiber	5.45 ± 0.90 ^{Bc}
Black moss + charcoal	5.92 ± 0.84 ^{C}
Black moss + coconut coir	5.63 ± 0.47 ^{Bc}

Note: Means followed by identical letters are not significantly different according to Duncan's Multiple Range Test.

Based on the average plant height data, the combination of black moss and charcoal produced the highest mean plant height (5.92 ± 0.84 cm) and differed significantly from tree fern fiber, which resulted in the lowest average height (4.53 ± 0.43 cm). Among the single media treatments, coconut coir produced the highest average plant height (5.99 ± 0.53 cm), followed by charcoal (5.70 ± 0.43 cm), while black moss showed a lower average height (4.91 ± 0.42 cm).

Among the combined media treatments, black moss + charcoal was identified as the most effective medium, followed by black moss + coconut coir (5.63 ± 0.47 cm) and black moss + tree fern fiber (5.45 ± 0.90 cm). Overall, coconut coir was the best single medium for promoting plant height, whereas the combination of black moss and charcoal provided the best growth performance among the mixed media treatments for *Dendrobium sutiknoi* plantlets.

DISCUSSION

The results demonstrated that acclimatization media significantly affected the acclimatization success, leaf production, and plant height of *Dendrobium sutiknoi* plantlets throughout the observation period. Among the single media treatments, coconut coir produced the highest acclimatization success (55%). This performance may be attributed to its lightweight structure, good aeration, moisture retention capacity, and natural nutrient content, particularly potassium, which support root development and plant adaptation.

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Although coconut fiber media proved superior in terms of aeration, moisture retention, and nutrient content, resulting in the highest plantlet survival rate (55%) compared to other treatments, the results also showed that nearly half of the plantlets failed to survive the acclimatization process. The superiority of the media alone appears insufficient to sustain plantlet survival during the acclimatization process. This high mortality rate indicates that the acclimatization phase remains the most critical stage in the success of orchid tissue culture. Acclimatization success is influenced not only by the type of growing medium used but also by the plantlets' physiological ability to adapt to environmental changes that differ significantly between in vitro and ex vitro conditions, such as differences in light intensity, humidity, and nutrient availability (Putri & Ermawati, 2023).

Tissue culture plantlets grow in an in vitro environment with a high relative humidity, low light intensity, sterile conditions, and receive a direct supply of sucrose from the culture medium. When transferred to an ex vitro environment, the plantlets must cope with reduced humidity, increased light intensity, and temperature fluctuations, as well as begin to independently absorb water and nutrients through their root systems. These sudden environmental changes often cause physiological stress, leading to high plantlet mortality during the acclimatization stage (Arianbach et al., 2023). Izuddin et al. (2018) found that the long-term success of epiphytic orchid reintroduction is largely determined by the suitability of microhabitats and the support of high relative humidity in tropical urban environments.

The incomplete development of plantlet anatomical and physiological structures is also a major cause of low plantlet survival rates, such as malfunctioning and suboptimal stomata, and weak root systems (Grzelak et al., 2024). In vitro plantlet leaves generally have stomata that are unable to open and close normally in response to changing environmental conditions (Saeedi et al., 2023). Consequently, after being transferred to an ex vitro environment, the transpiration rate increases drastically, resulting in water loss that outpaces the root's ability to absorb water. Furthermore, the cuticle layer on tissue culture plantlet leaves is generally thin due to the high humidity conditions during culture. An incompletely developed cuticle increases water evaporation through the leaf surface, making the plantlets susceptible to dehydration and ultimately death (Din et al., 2020).

In addition to leaf factors, root system development also determines the success of acclimatization. Plantlet roots formed during in vitro culture are often not optimally developed, both in terms of root hair number and water and nutrient absorption capacity (Targu, M., & Kumaria, S., 2025). In the early phase of acclimatization, this condition causes the plant's water needs to be unable to be optimally met, increasing the risk of wilting and death. At the same time, the plantlets must also undergo a physiological transition from a heterotrophic state, obtaining carbon sources from sucrose in the culture medium, to an autotrophic state that relies entirely on photosynthesis. This metabolic adaptation process takes time, making the early phase of acclimatization the period most vulnerable to environmental stress.

Despite this, coconut fiber still exhibits a higher survival rate than other media. The fine fiber structure of coconut fiber facilitates water and nutrient absorption, contributing to a healthier root system. The availability of adequate oxygen and the media's ability to retain moisture help reduce drought stress during initial acclimatization. The fine fibrous structure of coconut coir also facilitates water and nutrient uptake, contributing to healthier root systems and improved survival. In addition, the utilization of coconut coir as an agricultural by-product supports SDG 15 and promotes sustainable waste management. The combination of black moss and tree fern fiber also resulted in relatively high survival (40%), likely due to the complementary properties of both materials. Black moss possesses excellent water retention capacity, while tree fern fiber improves drainage and aeration, creating favorable conditions for orchid root development (Ambarwati, 2016; Tini et al., 2019; Susanto, 2018).

However, the superior physical properties of this medium have not fully overcome the physiological limitations of tissue cultured plantlets. Therefore, the survival rate of only 55% indicates that successful acclimatization is not solely determined by the growing medium but also by the physiological condition of the plantlets and the acclimatization method used.

Several studies on Dendrobium orchids report that the success rate of acclimatization can be increased by a strengthening process (hardening off) in vitro plantlet, namely by transferring bottled plantlets from the culture room to a room temperature environment with indirect sunlight exposure with higher light intensity for two weeks before acclimatization (Yasmin et al., 2018). This is done to assist the initial adaptation process to ex

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vitro conditions. The results of research by Sudartini & Diantini (2020) also found that the use of a transparent cover method (humidity dome) had a very significant positive impact on the success of orchid seedling acclimatization Dendrobium. This technique aims to reduce water loss through transpiration so that the stomata and cuticles develop more perfectly.

In addition to humidity regulation, light intensity and temperature are also important factors in successful acclimatization because they are closely related to the transpiration and photosynthesis processes of plantlets. da Silva et al. (2017) reported that the use of shading techniques to regulate light intensity and air humidity during the orchid acclimatization period can determine the success rate of orchid plantlets. Liu et al. (2021) also reported that a difference in day-night temperature (DIF) of ±10°C in *Dendrobium aphyllum* can significantly increase root length and chlorophyll content. Gradual increases in light intensity help plantlets increase their photosynthetic capacity without experiencing stress due to photoinhibition or excessive increases in leaf temperature.

For leaf development, black moss produced the highest number of leaves among single media treatments, whereas the combination of black moss and coconut coir gave the best results among mixed media. Black moss is known to retain moisture effectively and contains approximately 2–3% nitrogen with small amounts of phosphorus, nutrients that are important for vegetative growth and leaf formation (Ambarwati, 2016). The additional nutrients naturally present in coconut coir further enhanced nutrient uptake and root performance, resulting in improved leaf development. These findings indicate that the interaction between adequate water retention, aeration, and nutrient availability plays an important role in supporting vegetative growth during acclimatization.

Plant height was also significantly influenced by acclimatization media. Coconut coir produced the greatest plant height among single substrates, while the combination of black moss and charcoal resulted in the highest plant height among combined media. Plant height reflects irreversible growth resulting from primary meristematic cell division and elongation (Prabawardani et al., 2025). According to Kinasih et al. (2025), charcoal media are more effective in promoting plant height than tree fern fiber. Furthermore, organic components contained in moss and charcoal may function as supplementary nutrient sources that enrich the growing medium and enhance plant development (Erfa et al., 2019). These results indicate that environmentally friendly organic media can effectively support the ex situ conservation and sustainable propagation of the rare Papuan orchid *\bium sutiknoi.*

CONCLUSION

The environmentally friendly organic waste-based acclimatization media used in this study, consisting of seven treatments (four single media and three combined media), significantly affected the acclimatization parameters of the rare orchid *Dendrobium sutiknoi*, including acclimatization success percentage, number of leaves, and plant height.

Among the media tested, coconut coir was the most effective single medium for acclimatization success, resulting in a survival rate of 55%, while the combination of black moss and tree fern fiber produced the highest success rate among the combined media treatments (40%) at 35 days after transplanting (DAT).

For leaf production, black moss was identified as the best single acclimatization medium, producing 6.25 leaves per plantlet, whereas the combination of black moss and coconut coir yielded the highest number of leaves among the mixed media treatments (5.32 leaves) at 35 DAT.

Regarding plant height, coconut coir produced the greatest height among the single media treatments (6.64 cm), while the combination of black moss and charcoal resulted in the highest plant height among the combined media treatments (6.95 cm).

Further research is recommended to evaluate the development of the root system, such as the number of roots and root length, as well as optimizing acclimatization conditions, including humidity regulation, light intensity, and gradual adaptation techniques, so that the factors that influence the success of acclimatization can be identified.D. sutiknoi can be understood more comprehensively.

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