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Evaluation of Phenotypic and Genetic Characteristics of *Dryobalanops aromatica* (Dipterocarpaceae) Seedlings Growing in Peat and Mineral Media

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ABSTRACT

The selection of tree species suitable for restoration in diverse site conditions is still a challenge. Therefore, this study aimed to analyze the phenotypic and genetic characteristics of *Dryobalanops aromatica* seedlings growing in mineral soil media and peat media. Phenotypic characteristics were evaluated by analyzing the growth performance, leaf morphological characteristics, leaf color, and chlorophyll content, while genetic characteristics were evaluated by using microsatellite markers. The study revealed that seedlings planted in peat media exhibited greater height and shoot length growth, although the difference was not statistically significant compared to those in mineral soil media. However, seedlings in mineral soil media displayed a significantly higher survival rate than those in peat media. Peat-grown seedlings had higher chlorophyll content in their leaves, while mineral soil-grown seedlings displayed a lighter green leaf color based on digital analysis. Genetic analysis indicated a high genetic diversity of overall *D. aromatica* seedlings (*He* = 0.635) and unclear genetic structure between the two media. Overall, these findings demonstrate the successful adaptation of *D. aromatica* seedlings to peat media, positioning them as a promising choice for peatland restoration.

1. Introduction

Tropical peat swamp ecos ystems are vital for water storage and critical habitats for diverse flora and fauna species (Djufri *et al.* 2016; Yuwita *et al.* 2021). However, extensive deforestation, repeated burning, illegal logging, and land use changes for agriculture have led to the degradation of peat swamps in Indonesia, resulting in improper water management and loss of forest areas (Munarti and Suharti 2018; Yuwita *et al.* 2021). To conserve and restore these degraded areas within the biosphere reserve, it is crucial to preserve the remaining forest remnants. Seedling transplantation is a valuable restoration technique, but limited information exists on which species or combinations are most suitable for restoring degraded land (Florentine and Westbrooke 2004). Therefore, studying promising species is essential to understanding their characteristics and regeneration processes in the remaining peat swamp forest. Presently, restoration efforts primarily focus on fast-growing non-dipterocarp plants like *Calophyllum* sp., *Palaquium* sp., *Combretocarpus* sp., *Dyera* sp., and *Alstonia* sp. (Banjarbaru Forestry Research Unit *et al.* 2014). However, improved management strategies should emphasize the selection of multi-purpose tree species that possess both economic and conservation

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values, such as camphor (*Dryobalanops aromatica* C.F.Gaertn.) or locally known as Kapur from the Dipterocarpaceae family.

Dryobalanops aromatica produces precious timber that can be used for construction, and its expensive crystal camphor is used for incense, perfume, and medicine (Ashton 2004; Wahyuni *et al.* 2021). However, the species faces increasing vulnerability in its natural habitat, leading to its classification as a "vulnerable" species on the IUCN (International Union for Conservation of Nature and Natural Resources) Red List since 2018 (Barstow and Randi 2018). It is predominantly found in the lowland mixed dipterocarp forests of Malaya, Sumatra, the Riau Archipelago, and Borneo, particularly on deep, humid, yellow sandy soils (Ashton 1964; Ashton 2004). Recently, its presence was discovered in shallow peat swamp forests in the Singkil Wildlife Reserve (Suaka Margasatwa Singkil), Aceh Province, Sumatera Islands, Indonesia (Siregar *et al.* 2020). This expansion into peatlands presents an opportunity for considering *D. aromatica* as a promising species for future large-scale restoration programs. However, questions remain regarding its long-term adaptation to this habitat, as well as its growth, phenotypic characteristics, and genetic characteristics.

Environmental factors affect the regulation of plant growth and development (Quero *et al.* 2006; Demmig-Adams *et al.* 2008), and higher plants typically cope with varying environmental conditions through changes in both the entire plant and the individual tissues and organs (Walter and Schurr 2005). The influence of environmental factors on plant growth can be either direct, via the impact of physical conditions on primary growth processes, or indirect due to developmental adaptation (Choat *et al.* 2007). Leaf characteristics can serve as indicators of plant responses to environmental changes and adaptations (He *et al.* 2008). Typically, individual plants exhibit consistent leaf shapes in their mature leaves. However, certain plants may display variations in mature leaf shapes within the same individual, resulting from different growth stages or shifts in environmental conditions (Zhai *et al.* 2020).

Genetic variation influences phenotypic traits that enable tree species to adapt and thrive in specific environments. Identifying adaptive genetic variation helps us understand a tree's potential for rapid evolutionary adaptation. Therefore, studying the phenotypic and genetic response of *D. aromatica* to its local environment and different habitats is crucial for its evolution. This study aimed to analyze the phenotypic (morphological leaf and growth performance) and genetic characteristics of *D. aromatica* seedlings in mineral soil and peat media. The findings will provide valuable information for selecting economically valuable and adaptive species like *D. aromatica* to restore degraded peat swamp forests in Indonesia.

2. Materials and Methods

2.1. Plant Material and Growing Media

A total of 59 seedlings of one-year-old *Dryobalanops aromatica* were collected from Lae Kombih Forest Park, Aceh, Indonesia, and transported to the greenhouse of the Silviculture Department, Faculty of Forestry and Environment, IPB University, Bogor, Indonesia, in August 2018. Those seedlings were then planted in polybags 20 cm × 30 cm, in which 34 seedlings were planted in mineral soil media, and another 25 seedlings were planted in air-dried peat media. Acclimatization was carried out for 1 month in a closed, dark, and humid location inside the greenhouse, then moved to a place that was more exposed to sunlight.

Mineral soil media were obtained from an area in the district of Dramaga, Bogor, West Java, Indonesia, while the peat media were collected from the peat swamp area in Peatland Hydrology Units of Sungai Mendahara-Sungai Batanghari (KHG Sungai Mendahara-Sungai Batanghari), East Tanjung Jabung, Jambi, Indonesia. Mineral soil and dry peat were sieved using a 1 × 1-m screen with a galvanized 8 × 8 square wire mesh and a 1.00-mm sieve opening to remove rocks and soil impurities, obtaining a fine planting medium (Rahmah *et al.* 2023). The fine peat media was moistened to restore its water content before being distributed into the polybags.

The peat and mineral soil media used in this study differed in pH, water content, and texture (Supplementary Table 1). Peat media had an acidic pH of 4.0, while mineral soil media had a pH of 5.0 (Supplementary Table 1). The water content was 135.32% for peat media and 32.09% for mineral soil media. Peat media was classified as fibric or least mature, while mineral soil media had a clay loam texture (Rahmah *et al.* 2023).

2.2. Seedling Growth Measurements

A completely randomized design (CRD) was used to study the growth of *D. aromatica* seedlings in peat and mineral soil media. Seedling growth was observed for eight months after planting, focusing on parameters of height, new shoot length, and survival rate. To determine significant differences in media treatment, the data on seedling height growth, new shoot length growth, and survival rate were analyzed using a Variance Test. The type of Variance Test was determined based on the normality of the data, which was assessed using the Shapiro-Wilk test (Hanusz *et al.* 2014). Normally distributed data were tested using the ANOVA test (Park *et al.* 2009; McFarquhar 2019). Non-normally distributed data were tested using the Friedman test as an alternative (St. Laurent and Turk 2013).

2.3. Leaf Morphological Characteristics Measurements

Leaf morphological characteristics of *D. aromatica* seedlings were measured eight months after planting (April 2019) following the method by Kremer *et al.* (2002) with modifications from Wu *et al.* (2007) and Ellis *et al.* (2009). Sinus width (SW) was not measured due to differences in leaf form. One branch with at least five leaves was measured for lamina length (LL), petiole length (PL), lobe width (LW), length of lamina at the widest part (WP), number of secondary veins (NSV), and angle between primary and secondary veins (AV) (Supplementary Figure 1A). Apex shape (AS) and base shape (BS) were observed and scored based on Ash *et al.* (1999) (Supplementary Figure 1b and Supplementary Figure 1C). The measured leaf variables were used to calculate leaf area (LA), the circumference of leaves (CL), aspect ratio (AR), form factor (FF), and perimeter ratio of diameter (PR) following the methods by Wu *et al.* (2007).

Principal Component Analysis (PCA) was conducted to simplify the leaf morphology data and present the results in a biplot. Comparative analysis was performed using an independent t-test to determine differences in leaf morphological variables. PCA and biplot analysis were carried out in MS Excel 2010 using XLSTAT 2014.5 software, while the t-test analysis was performed using SPSS Ver 25 (IBM Corp 2017).

2.4. Chlorophyll Content and Leaf Color Measurement

Leaf chlorophyll content of *D. aromatica* seedlings grown in peat and mineral soil media was measured using a Konica Minolta SPAD-502 Chlorophyll Meter by calculating the average chlorophyll of all seedling leaves in both media and comparing the means. Leaf color measurements were conducted following the quantifying plant colors method by Kendal *et al.* (2013).

For leaf color measurement, five leaves from every seven seedlings in each medium were photographed using a Canon EOS 1000D DSLR camera with a wide-angle lens (35-36 mm focal length) and a black cloth background. Scoring was performed during the day to avoid shadow bias and temperature fluctuations. The captured RAW (.CR2) images were processed using RawTherapee 5.5 software, adjusting temperature, tint, exposure, and black point standards with ColorChecker support. ImageJ 1.52 (Schneider *et al.* 2012) was used to isolate the leaf from the background. The leaf color was converted to the 1976 Commission Internationale de l'Eclairage (CIE) value (L * a * b *) (CIE 1986) using the convertColor function in R Statistics version 3.6.0 (R Core Team 2020) with the grDevice package's GUI. The L *, a *, and b * values in CIELAB were assumed to follow a normal distribution. The Kolmogorov-Smirnov test from the ks.test package in the stats function was utilized to determine the significance of the difference between the modeled color distribution and the Gaussian standard normal distribution.

Intra-specific color variation differences were mapped using non-metric multidimensional scaling analysis (nMDS) based on Euclidean distances in the isoMDS function of the MASS package. Earth Mover's Distance (EMD) was employed to measure color differences between distributions, calculated using the emd function in the emdist package of R Statistics version 3.6.0. The obtained CIELAB 1976 color data was visualized back into RGB (Red Green Blue) and Munsell Chart using the methods described by Ohta and Roberson (2006) and Mufarhatun *et al.* (2023) for graphical representation.

2.5. Microsatellite Genotyping

Four polymorphic nuclear microsatellite loci (Dra187, Dra428, Dra426, Dra519) developed for *D. aromatica* (Nanami *et al.* 2007) were used in this study. The forward primer was labeled with universal primer for PCR fragment fluorescent labeling, namely Tail A (6-FAM), Tail B (VIC), Tail C (NED), and Tail D (PET) from Applied Biosystems Japan, Tokyo (Roche Applied Science 2006; Blacket *et al.* 2012). A Type-it Microsatellite PCR kit (QIAGEN Japan, Tokyo, 2019) was used to amplify microsatellite loci. Multiplex PCR amplification was performed in a volume of 10 µl, containing 1 × Type-it Multiplex PCR Master Mix, 0.2 µM of forward and reverse primers, fluorescent-labeled universal primer, and about 40 ng of genomic DNA. An Applied Biosystems 2720 thermal cycler was used for the PCR amplification under the following conditions:

initial denaturation at 95°C for 5 min, then 35 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 1 min 30 s, and extension at 72°C for 30 s, followed by a final incubation at 60°C for 30 min. Fragment sizes were determined using an ABI PRISMTM 3500 Genetic Analyzer and visualized using Gene Mapper 3.0 software (Applied Biosystems Japan, Tokyo, 2002).

Basic statistics of genetic diversity, including the number of alleles per locus (*Na*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and fixation index (*F*), were calculated using GenAlEx software version 6.41 (Peakall and Smouse 2006). Deviations from Hardy-Weinberg (HW) equilibrium were analyzed for each locus in each population using GENEPOP 4.7.5 (Raymond and Rousset 1995; Rousset 2008). Population structure was analyzed using The Bayesian model-based clustering method implemented in STRUCTURE software version 2.3.4 (Pritchard *et al.* 2000) to estimate the number of genetically homogeneous populations (K) and determine the genetic structure of the sampled populations. A burn-in of 20,000 steps followed by 100,000 steps of MCMC (Markov Chain Monte Carlo) simulation with 10 replications each, for a range of K-values from 1 to 10, was performed on the entire data set. STRUCTURE HARVESTER Web v0.6.94 (Earl and vonHoldt 2012) was further used to determine the appropriate number of clusters (K), which the statistic ΔK (Evanno *et al.* 2005) was calculated based on the rate of change in the log probability of data between successive K-values.

3. Results

3.1. Seedling Growth Performance

In this study, *D. aromatica* seedling growth was demonstrated by height growth, new shoot length growth, and survival rate. The result of height growth analysis (Supplementary Figure 2) and new shoot length growth analysis (Supplementary Figure 3) showed that the seedlings planted on peat media have a higher height growth (average = of 3.63 cm /month) and new shoot length growth (average 5.74 cm/month) compared with the height growth (average = 2.26 cm/month) and new shoot length growth (average = 4.78 cm) of the seedlings planted in mineral soil media. However, variance test calculations using ANOVA for the height growth and new shoot length growth of seedlings planted on peat and mineral soil media did not show a significant difference at p-value < 0.05 (Supplementary Table 2).

In the final month of observation (Supplementary Figure 4), the survival analysis results indicated a slightly higher survival rate of *D. aromatica* seedlings in mineral soil media (61.03%) compared to those in peat media (60%). The Friedman test, which analyzed the variance, confirmed the significant difference in survival rates between seedlings planted in mineral soil and peat media, with a Chi-square value of 8.00 and a significance level of 0.005 (Supplementary Table 3).

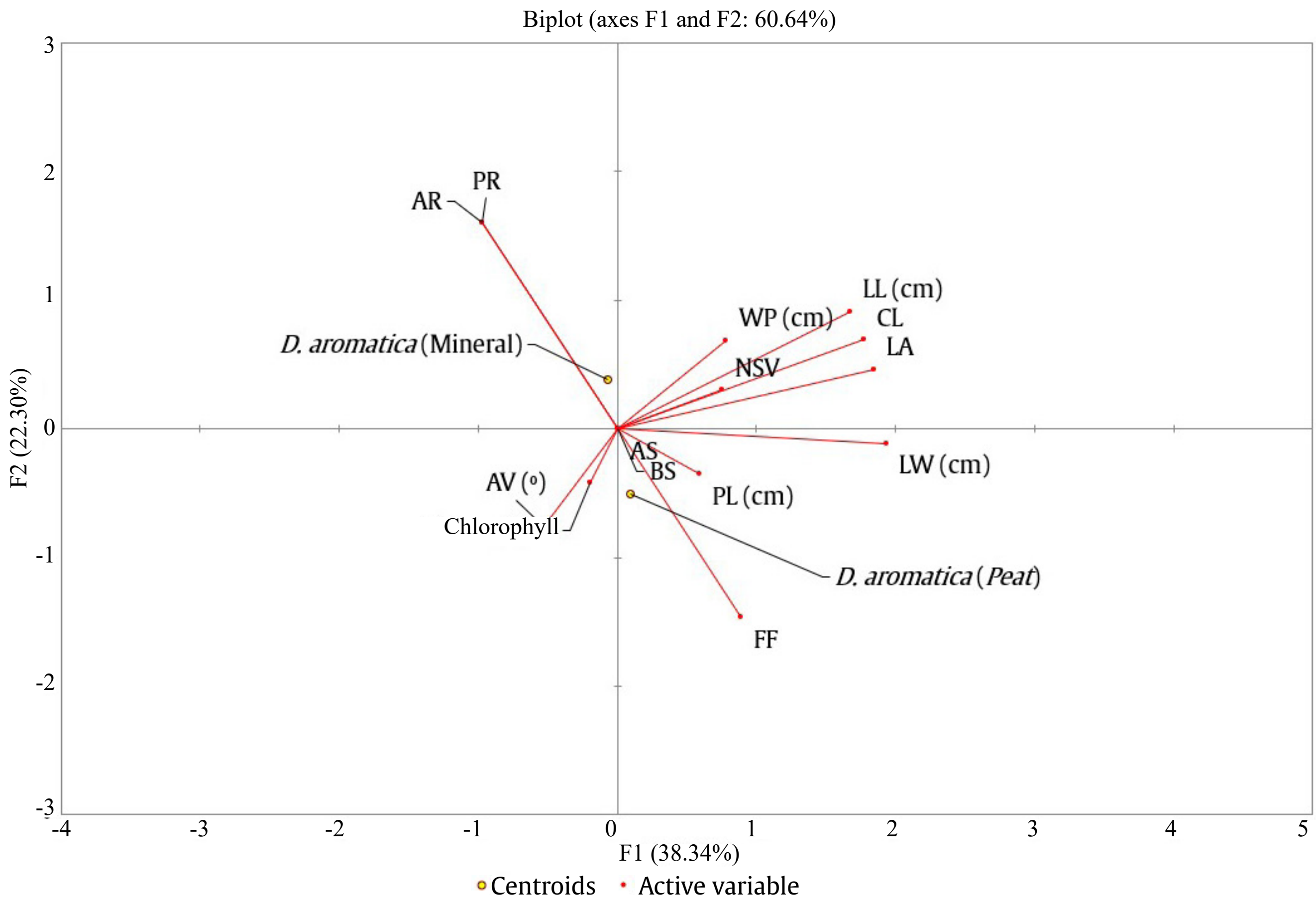
3.2. Morphological Variation of The Leaves

The biplot showed that *D. aromatica* on peat media was dominant to the FF, chlorophyll, SD, and PT variables (Figure 1). Still, FF has a strong negative correlation with AR and RR, which means that *D. aromatica* on peat media has a small value in AR and PR variables. Meanwhile, *D. aromatica* in mineral soil has a value that is inversely proportional to *D. aromatica* in peat soil, where minerals were dominant to AR and PR variables but had a small value for the FF variable (Figure 1).

Significant differences in seedling leaf characteristics between the two types of media were observed for petiole length (PL), an angle between the primary and secondary veins (AV), aspect ratio (AR), and perimeter ratio of diameter (PR) (Table 1). Seedlings growing on peat media exhibited greater petiole length (PL = 0.94 cm) and angle between primary and secondary veins (AV = 18.32°) compared to seedlings on mineral soil media (PL = 0.86 cm, AV = 16.78°). Leaves on mineral soil media were longer and oval-shaped, as indicated by higher average values of aspect ratio (AR = 2.49) and perimeter ratio of diameter (PR = 5.48) compared to peat media (AR = 2.37, PR = 5.28).

3.3. Leaf Color Characteristic

Leaf color on mineral soil media exhibits a lighter and more yellowish shade compared to leaves on peat media, as observed digitally (Figure 2). However, both media show negative a* values in the 1976 CIE color space, indicating a green color. The greenness of peat media (-16.57±3.13) is lower than that of mineral media (-20.68±3.48) (Table 2). The extracted Lab* color values are considered sufficient to represent the actual color (Table 2) and follow a normal distribution according to the Kolmogorov-Smirnov test for both peat and mineral soil leaf color. These average values can serve as standard models for leaf color in *D. aromatica* seedlings grown in both media.



Leaf morphological characteristics:

LL	:Lamina Length	AV	:The Angle Between the Primary and Secondary Veins	CL	:Circumference of leaves
PL	:Petiole Length	AS	:Apex Shape	AR	: Aspect Ratio
LW	:Lobe Width	BS	:Base Shape	FF	: Form Factor
WP	:Length of Lamina at The Widest Part	LA	:Leaf Area	PR	: Perimeter Ratio of Diameter
NSV	:Number of Secondary Veins				

Figure 1. Biplot of leaf morphological characteristic variables of *D. aromatica* seedlings based on PCA analysis

Table 1. Two-tailed test parameters for leaf morphological characteristics of *D. aromatica* seedlings

Variable	Peat	Mineral	T
LL	9.93	10.05	0.619
PL	0.94	0.86	0.000*
LW	4.20	4.08	0.275
WP	3.48	3.99	0.276
NSV	21.43	20.87	0.494
AV	18.32	16.78	0.001*
AS	6.00	6.00	-
BS	3.00	3.00	-
LA	65.98	65.36	0.843
CL	21.96	22.16	0.703
AR	2.37	2.49	0.012*
FF	1.66	1.62	0.149
PR	5.28	5.48	0.011*

Lamina length (LL), petiole length (PL), lobe width (LW), length of lamina at the widest part (WP), number of secondary veins (NSV), an angle between the primary and secondary veins (AV), apex shape (AS), base shape (BS), leaf area (LA), circumference of leaves (CL), aspect ratio (AR), form factor (FF), and perimeter ratio of diameter (PR). * Significantly different at p-value <0.05

The Earth Mover's Distance reveals a significant distance (8.133) between leaf color values on peat media and mineral soil media. To facilitate on-field display, leaf color was transformed from CIELAB values to RGB models and Munsell Charts (Table 3). This result aligns with the analysis of chlorophyll content, which indicates higher levels in the leaves of seedlings grown on peat media (37.50 nmol/cm) compared to those on mineral soil media (32.19 nmol/cm) (Supplementary Figure 5). Consequently, the seedling leaves on peat media appear darker in green color than those on mineral soil media.

3.4. Genetic Variation and Structure

Twenty-eight individuals from mineral media and 22 individuals from peat media were successfully

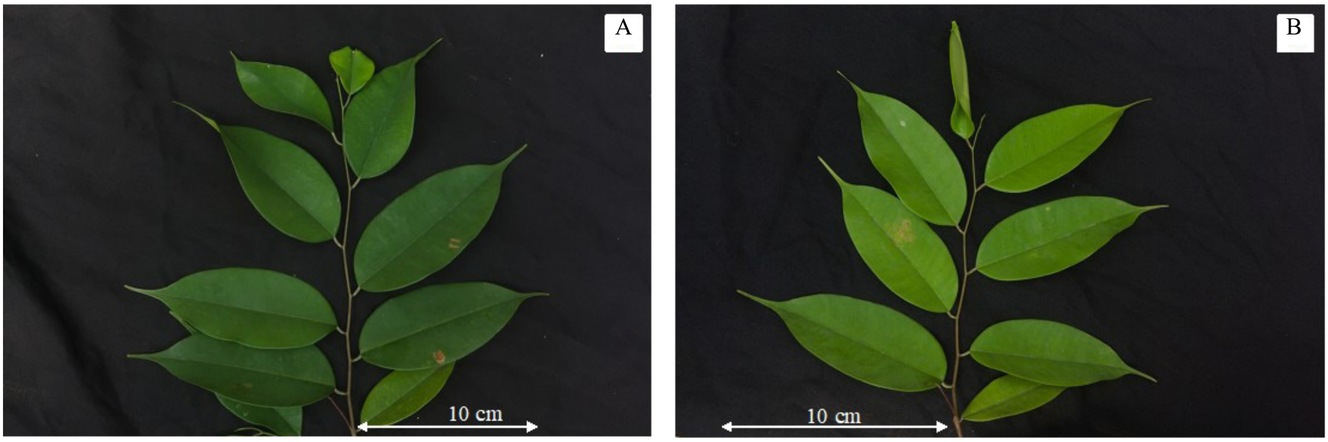


Figure 2. Comparison of actual leaf color of *D. aromatica* seedlings grown on (A) peat and (B) mineral media

Table 2. The average color and Z-value of the Kolmogorov-Smirnov test in 1976 CIE value (L * a * b *) for *D. aromatica* seedlings. The L* values can range from 0 (black color) to 100 (white color). Positive a* values correspond to red and negative a* values exhibit green. Positive b* values mean yellow and negative b* values mean blue (Rhee *et al.* 2008; Jitchoom *et al.* 2013)

Media	Average color			Kolmogorov-smirnov		
	L*	a*	b*	L*	a*	b*
Mineral	49.14±3.17	-20.68±3.48	38.19±3.08	0.18	0.23	0.65
Peat	42.27±5.73	16.57±3.13	30.26±5.17	0.17	0.18	0.17

**significantly different at Z-value <0.05

amplified using the four polymorphic microsatellite primers employed in this study. The mineral population exhibited a higher mean number of alleles per locus (Na = 8.250) than the peat population (Na = 5.750). Similarly, the expected heterozygosity was slightly higher in the mineral population (He = 0.660) compared to the peat population (He = 0.611) (Table 4), indicating greater genetic diversity among seedlings planted in mineral media. Nonetheless, the overall grand mean of expected heterozygosity (He) for *D. aromatica* seedlings was 0.635, signifying a high level of genetic diversity.

The fixation index (F = 0.307) of seedlings grown in mineral media was positive and significantly larger than zero, indicating an excess of homozygotes. Conversely, the fixation index (F = 0.138) of seedlings grown in peat media was positive but not significantly different from zero, suggesting no deviation from Hardy-Weinberg Equilibrium. However, the grand mean of the fixation index for *D. aromatica* seedlings in both media was positive and significantly larger than zero (F = 0.223), indicating an excess of homozygotes.

The STRUCTURE analysis showed that ΔK reached its maximum value (ΔK = 49.10) at K = 3 (Figure 3A), indicating that the 50 individual seedlings grown on two different media most likely belonged to the three main genetic clusters (Figure 3B). Cluster One mainly included individuals from the peat media population, Cluster Two mainly included individuals from the mineral media population, and Cluster Three was a mixture of individuals among the populations.

Table 3. RGB values and Munsell Chart modeled colors of *D. aromatica* seedlings grown on peat and mineral media

Media	RGB			HEX code	Munsell
	R	G	B		
Mineral	102	125	48	#667D30	5.2GY 4.8/6.3
Peat	89	106	48	#596A30	5GY 4.1/5

4. Discussion

4.1. Phenotypic Characteristics

Analysis of seedling growth performance showed that different growing media did not cause differences in height growth and new shoot length growth; however, they affected the survival rate. *D. aromatica* planted with mineral soil media was slightly higher than the survival rate of seedlings on peat media. During the observation period, there was a decrease in new shoot length growth, particularly in peat media, due to shoot death during the acclimatization process from September to October 2018. A similar trend was observed in seedling survival, with a higher number of

Table 4. Genetic diversity values in *Dryobalanops aromatica* seedling grown on peat and mineral media

Media	Locus	*N*	*Na*	*Ne*	*Ho*	*He*	*F*
Mineral	DRA 187	28	7.000	3.824	0.393	0.739	0.468***
	DRA 428	28	13.000	4.115	0.429	0.757	0.434***
	DRA 519	28	8.000	2.034	0.250	0.508	0.508***
	DRA 426	28	5.000	2.736	0.750	0.635	-0.182^{ns}
	Mean	**28**	**8.250**	**3.178**	**0.455**	**0.660**	**0.307*****
	SE	0	1.702	0.483	0.106	0.057	0.164
Peat	DRA 187	22	4.000	2.109	0.318	0.526	0.395^{ns}
	DRA 428	22	10.000	3.396	0.591	0.706	0.163^{ns}
	DRA 519	22	5.000	2.272	0.500	0.560	0.107^{ns}
	DRA 426	22	4.000	2.890	0.727	0.654	-0.112^{ns}
	Mean	**22**	**5.750**	**2.667**	**0.534**	**0.611**	**0.138**^{ns}
	SE	0	1.436	0.296	0.086	0.041	0.104
	Grand Mean	**25**	**7.000**	**2.922**	**0.495**	**0.635**	**0.223*****
	SE	1.134	1.134	0.279	0.065	0.034	0.095

N number of samples, *Na* mean number of alleles, *Ne* mean number of effective alleles, *Ho* mean observed heterozygosity, *He* Nei's mean expected heterozygosity, and *F* fixation index with the significance level for deviation from the Hardy-Weinberg Equilibrium (HWE): $p < 0.001$ (***), not significant (ns)

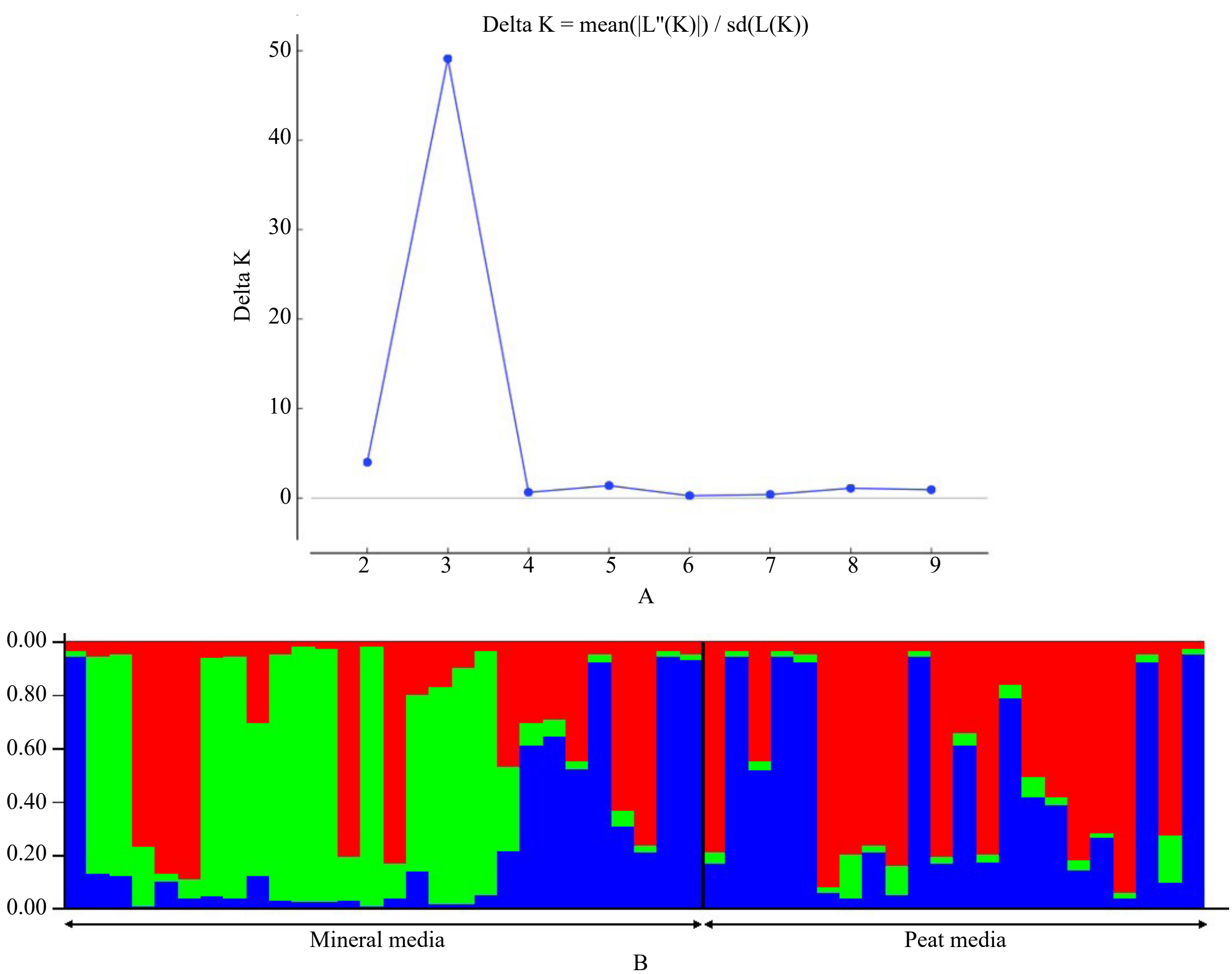


Figure 3. Clustering of *Dryobalanops aromatica's* seedling grown in mineral and peat media obtained from STRUCTURE analysis. (A) STRUCTURE estimation of population number from K values ranging from 1 to 10, by delta K (ΔK) values, and (B) population structure from K = 3

D. aromatica seedling deaths, especially in peat media, during the acclimatization period itself (September to October 2018) compared to the subsequent months. During this stage, the plants experienced stress as they adapted to the new media, which significantly differed from the original mineral soil media in their native habitat in Aceh province. Plant stress refers to deviations in plant physiology, development, and function, which can pose a threat to plant survival and cause severe damage (Sopandie 2013). Additionally, differences in survival rates between the two media after the acclimatization period could be attributed to variations in acidity, water content, and nutrient levels between peat soil and mineral soils (Dariah *et al.* 2014), leading to significant stress for *D. aromatica* seedlings.

Variations in leaf morphology in both media can also occur due to the adaptation of seedling leaves; for example, the photosynthetic rate and transpiration rate were reported to be higher in peat media than mineral soil media in *D. aromatica* seedlings (Rahmah *et al.* 2023), indicating that differences in media can affect the physiological processes of leaves (Kenzo *et al.* 2007), which may be due to changes in leaf characteristics. The water content of the media also gradually causes an adjustment in the characteristics of the leaves so that the seedlings can compensate for the excess water supply they receive through transpiration through the leaves (Pita and Pardos 2001).

D. aromatica seedlings planted on peat and mineral media also showed differences in leaf color, where seedlings on mineral soil media have a lighter green color than leaves on peat media based on digital appearance. This pattern was also supported by the results of chlorophyll content analysis, which showed a higher leaf chlorophyll content in peat media than in mineral soil media. This could be due to the high organic matter in peat soil, especially nitrogen, which can significantly increase chlorophyll levels (Moilanen *et al.* 2010; Zhang *et al.* 2013; Dariah *et al.* 2014). Kiang *et al.* (2007) also reported that plant chlorophyll absorbs light with wavelengths ranging from 680-700 nm or is classified as red-blue light waves and emits green light waves so that the leaves appear green in color.

4.2. Genetic Characteristics

In this study, the high genetic diversity of *D. aromatica* seedlings (*He* = 0.635) illustrated the original population of the seedlings, namely Lae Kombih Forest Park, Aceh province, Indonesia when compared to other natural populations of *D. aromatica* in Indonesia such as Singkohor, Aceh province (*He* = 0.1760), Danau Paris, Aceh province (*He* = 0.2066), and Barus, North Sumatera province (*He* = 0.2134) populations, as assessed by Ritonga *et al.* (2018) using the RAPD marker and compared to other Malaysian populations such as Kanching (*He* = 0.598), Similanjau (*He* = 0.571), Lambir (*He* = 0.623), and Limbang (*He* = 0.575), as assessed by Dwiyanti *et al.* (2015) using microsatellite marker. However, this value was lower than the grand mean of expected heterozygosity of *D. aromatica* from other Indonesian populations such as Mursala Island, North Sumatera province (*He* = 0.725) and Lingga Island, Riau Archipelago province (*He* = 0.689), as well as another Malaysian population i.e. Gunung Panti (*He* = 0.729), which were also analyzed by Dwiyanti *et al.* (2015) using microsatellite marker.

The grand mean fixation index of *D. aromatica* seedlings in both media (*F* = 0.223) was comparable to the Gunung Panti population, known for its high genetic diversity and fixation index (*He* = 0.729, *F* = 0.289) (Dwiyanti *et al.* 2014). The significantly positive inbreeding coefficients observed could be attributed to factors such as null alleles, biparental inbreeding or self-fertilization, moderate pollen dispersal, or the Wahlund effect (Papi *et al.* 2012). While null alleles and the Wahlund effect were not assessed in this study, biparental inbreeding or self-fertilization could explain the relatively high fixation index in *D. aromatica* seedlings from Lae Kombih Forest Park. Despite the high fixation index, the Lae Kombih population exhibited relatively high genetic diversity, indicating that before extensive inbreeding occurred, the forest had a rich source of genetic diversity. This can be attributed to the presence of numerous mother trees (123 individuals) in the forest (Muslih *et al.* 2022). Consequently, this population holds significant value as a genetic diversity source for future plant breeding and conservation efforts in Indonesia.

The analysis of genetic structure using microsatellite markers in this study did not provide clear discrimination of genetic characteristics between the two media. Similar results were obtained for *Shorea balangeran* growing in a peat swamp and heath forests with RAPD markers (Indriani *et al.* 2019). These findings indicate that both microsatellite and RAPD markers are not sufficient for analyzing genetic differences in tree species that naturally grow in different forest ecosystems within the same area or in seedlings treated with different media when the mother trees originate from the same population. RNA sequencing (RNA-Seq) transcriptome analysis in previous studies using the same species of *D. aromatica* (Siregar *et al.* 2020; Yosie *et al.* 2021) revealed that MYB26, NAC043, and NAC73 genes are potential transcription factors involved in responding to peat stress, while DREB2a and MYB16 are responding to mineral stress. However, these gene effects were not observed in the genetic structure analysis of seedlings between the two media in this study. Nonetheless, the overall good growth of the seedlings suggests their successful adaptation to both mineral and peat environments.

4.3. Implication for Conservation and Restoration Strategy

Efforts to restore the peatlands face huge challenges concerning socio-economic and ecological dynamics, and there is still a lack of knowledge on the adaptation of promising tree species. This study demonstrated that *D. aromatica* showed good seedling growth performance in peat media in terms of height growth, shoot length growth, and leaf chlorophyll content when compared to mineral media. Although this study could not show any genetic differences in the seedlings in the two media, it could be concluded that the seedlings have good adaptation to the peat media if observed from their growth performance (survival rate). Therefore, this species can be recommended as a potential species for mass planting on peatlands to support restoration efforts of degraded peatlands and also to conserve the species itself. In addition, the population of *D. aromatica* from Lae Kombih Forest Park, Aceh Province, used in this study is also recommended as a source of genetic diversity for this species for future plant breeding and other conservation activities in Indonesia.

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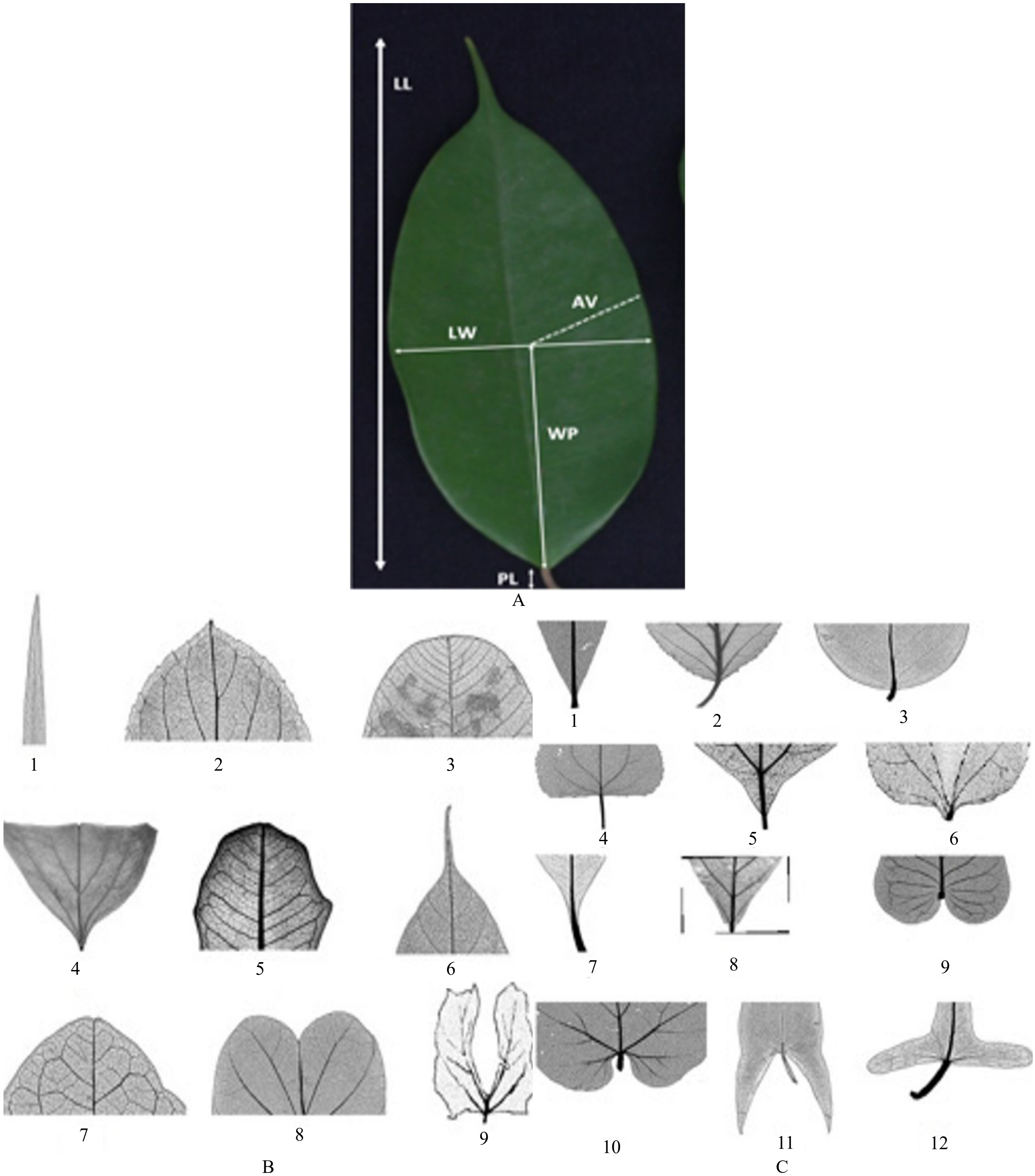
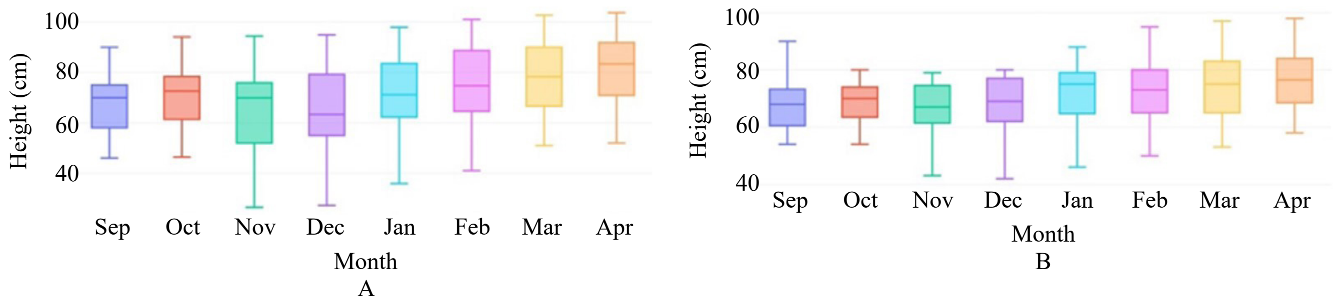
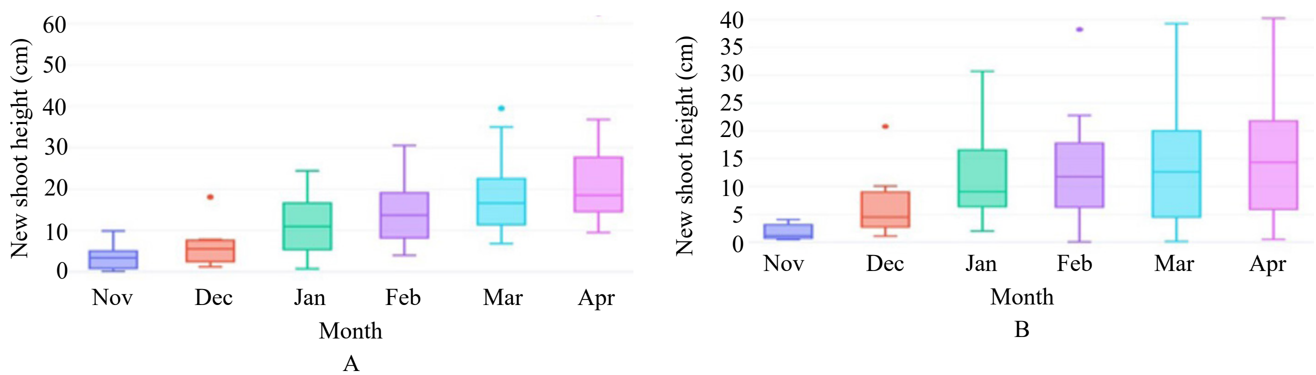
Supplementary Materials



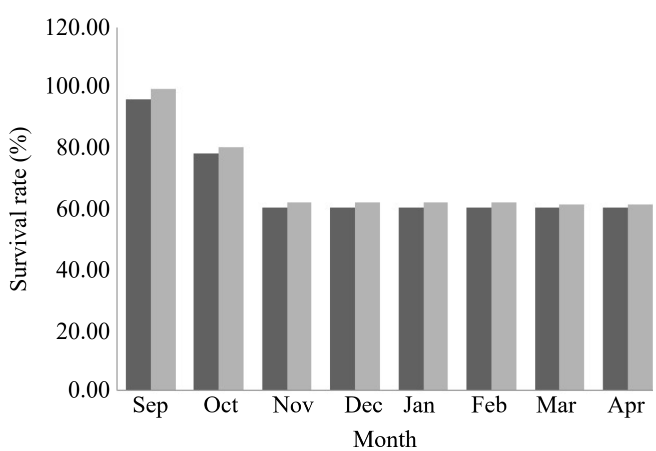
Supplementary Figure 1. Presentation of the assessed leaf morphological variables (A) lamina length (LL), lobe width (LW), an angle between the primary and secondary veins (AV), length of lamina at the widest part (WP), and petiole length (PL), and the observed leaf morphological variables, (B) the score of apex shape, and (C) the score of base shape (Ash *et al.* 1999)



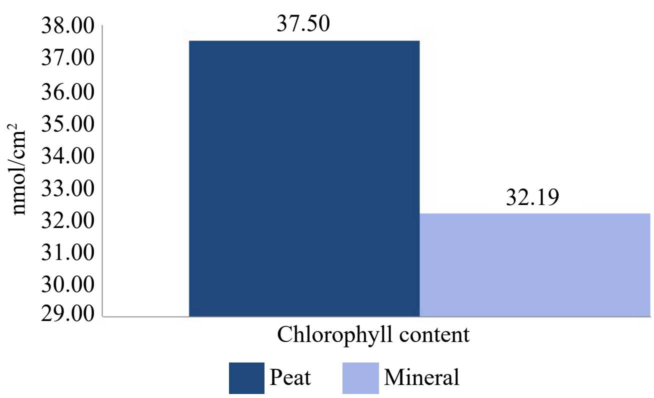
Supplementary Figure 2. Seedlings height growth of *D. aromatica* in peat media (A) and in mineral soil media (B) for 8 months



Supplementary Figure 3. New shoot length of *D. aromatica* seedlings in peat media (A) and in mineral soil media (B) for 6 months



Supplementary Figure 4. Survival rate of *D. aromatica* seedlings planted on peat media (dark grey) and mineral soil media (light grey) for 8 months



Supplementary Figure 5. Leaf chlorophyll content in *D. aromatica* seedling grown on peat and mineral media

Supplementary Table 1. Results of peat and mineral media analysis

Media	pH	Water content (%)	Texture/ maturity level
Peat	4.0	135.32	Fibric
Mineral soil	5.0	32.09	Clay loam

Supplementary Table 2. ANOVA results for the effect of media on the seedling height and new shoot length

Source	Type III sum of squares	df	Mean square	F	Sig.
Height	250.174	1	250.174	13.259	0.171[ns]
New shoot length	137.572	1	137.572	52.950	0.087[ns]

ns = not significantly different at p-value <0.05

Supplementary Table 1. Friedman test of survival rate of *D. aromatica* seedlings on peat and mineral soil media

	Score
N	8
Chi-square	8.000*
df	1
p-value	0.005**