

J-SuNR

Vol. 5 No. 1 (2024) 20-34 https://publisher.uthm.edu.my/ojs/index.php/j-sunr

Rooted Cutting of *Gymnostoma Sumatranum* **for Land Reclamation in Degraded Ultramafic Forests in Sabah**

Richard J. Majapun1*, Dzulyana Idhamsah1, Alviana Damit1, Reuben Nilus1

¹ Forest Research Centre, Sabah Forestry Department, P.O. Box 1407, 90715 Sandakan, Sabah, MALAYSIA

*Corresponding Author: Richard.Majapun@sabah.gov.my DOI: https://doi.org/10.30880/jsunr.2024.05.01.003

Article Info Abstract

Received: 10 April 2024 Accepted: 26 June 2024 Available online: 30 June 2024

Keywords

G. sumatranum, rooted cutting, nodulation, potting media, ultramafic forests

Gymnostoma sumatranum, a member of the Casuarinaceae family, is a resilient tree species well-adapted to nutrient-deficient soils. In Sabah, it stands out among the three widely distributed Casuarinaceae species, holding promise for land reclamation initiatives in degraded ultramafic forests. This comprehensive study addresses three primary objectives for effective *G. sumatranum* propagation, 1) sprig selection for rooted cutting, 2) impact of commercial rooting hormones on growth and nodulation, and 3) identification of suitable potting media for seedling development. The results indicated that the middle-part sprig is preferable for rooted cutting, with a higher rooting percentage (75.9%) compared to the upper-part sprig (57.5%). Additionally, the use of Seradix-3 as a growth hormone treatment yields significant improvements in explant length and root production. However, variations in root nodulation, influenced by different treatments, suggested limitations in commercial rooting hormones, with only 31% of rooted explants exhibiting nodulation. This underscores the need for further research to comprehend factors influencing nodulation in this context. In terms of potting media, river sand (R-sand) emerges as the most effective for *G. sumatranum* seedling development in the nursery stage based on S-index (healthy seedlings = 31.5%) and growth index (Height = 51.14 ± 14.82 mm; N-Branch = 51.14 ± 14.82), emphasising the critical role of proper potting medium selection for optimal seedling growth and survival. The study also highlights previous restoration efforts by the Sabah Forestry Department, utilising *G. sumatranum* and other pioneer species to restore degraded ultramafic areas in Sabah. Overall, these results contribute to the long-term goal of restoring the functioning capacity of valuable ultramafic ecosystems and promoting sustainable reforestation practices.

1. Introduction

The genus *Gymnostoma* belongs to the family Casuarinaceae, along with the other genera namely *Allocasuarina, Casuarina,* and *Ceuthostoma* [1]. These genera are well-known as multipurpose trees and shrubs that are naturally distributed throughout the equatorial and sub-equatorial regions of the world, including South-East Asia, Malaysia, Australia, and the Melanesian and Polynesian regions of the Pacific [2]. These Casuarinaceae are easily recognisable due to their conifer-like appearance, characterised by distinctive foliage with tiny teeth on green, needle-like branchlets [3]. Casuarinaceae species are valued for their various uses and play important roles in agroforestry, providing timber and fuelwood; and contributing to landscape and dune stabilisation efforts [4]. They are particularly well-adapted to grow in nutrient-deficient soils, making them suitable for a wide range of environmental conditions in the regions where they naturally occur [5].

It is estimated about 100 species of Causarinaceae occurring in Southeast Asia to Australia and Polynesia [6]. However, in Sabah, there are only four species that have been recorded, namely *Casuarina equisetifolia, Ceuthostoma terminale, Gymnostoma sumatranum,* and *Gymnostoma nobile* [6, 7]. These species have been widely distributed and can grow in a wide range of different environments and elevations. Vernacularly known as *pokok aru* or rhu, these species are largely planted as ornamental trees for urban beautification in places like schools, parks, seashores, government and private company buildings, roadsides, and even housing areas [8]. Most of these Casuarinaceae species exhibit strong resilience to challenging edaphic and climatic conditions, including high temperatures and strong winds. They are adept at stabilising coastal and sand dunes, often serving as effective windbreaks and providing a consistent source of fuelwood. These trees continuously produce flowers and fruits, with wind facilitating their pollination. Importantly, these plants form a symbiotic relationship with the soil bacterium *Frankia* (Frankiaceae) within their root systems, allowing them to create nodules that fix atmospheric nitrogen [9]. Recent studies have explored the integration of these Casuarinaceae species into agroforestry systems as intercrops alongside tree plantation species to enhance growth yield and soil fertility, although many aspects of this application remain unexplored [10]. This ability to harness atmospheric nitrogen plays a pivotal role in the large-scale cultivation of Casuarinaceae for land reclamation in degraded areas.

In addressing this issue, the Sabah Forestry Department (SFD) has been actively involved in a comprehensive rehabilitation program aimed at restoring degraded forests resulting from a history of poor logging practices, recurrent forest fires, and continuous encroachments over the decades. The overarching objective of this endeavour is to reinstate the operational capacity of these forests in terms of water production, retention, and utilisation. The department has planted various tree species as part of these restoration initiatives, encompassing species such as Kapur Paji (*Dryobalanops lanceolata*), Kapur Gumpait (*Dryobalanops keithii*), Seraya Majau (*Shorea johorensis*), and fast-growing species like Sentang (*Azadirachta excelsa*), Angsana (*Pterocarpus indicus*), Batai (*Paraserianthes falcataria*), Eucalyptus as a trial, Laran (*Neolamarckia cadamba*), Binuang (*Octomeles sumatrana*), Ketapang (*Terminalia catappa*), Pulai (*Alstonia* spp*.*), and Bayur (*Pterospermum javanicum*) [11]. However, it is crucial to note that none of these species are well-suited for restoring the infertility of ultramafic forests. Ultramafic soils are notably distinguished by significantly high concentrations of magnesium and nickel, both of which unfortunately possess phytotoxic properties. Additionally, they exhibit low water retention capacity, limited phosphorus availability, partly due to phosphorus-immobilizing ferric sesquioxides, and reduced levels of essential plant nutrients such as nitrogen and potassium [12].

In Sabah, approximately 3,500 km² of land is covered by Malaysia's largest expanse of ultramafic forests [12], [13], [14]. These ultramafic sites stretch from the west coast to the east coast. Notable areas include Mt. Tambuyukon, Bukit Hampuan F.R., Morou-Porou, the Meliau Range, Bidu-Bidu F.R., Mt. Tawai F.R., Mt. Tingkar F.R., Mt. Silam F.R., and Marai Parai (Appendix A: Plate 1). A substantial portion of these areas, specifically 102,167.45 hectares, which accounts for 51.5% of the total ultramafic areas in Sabah, falls under the protection of the Sabah Forestry Department and Sabah Parks [15]. These protected areas are vital guardians of the distinctive ultramafic ecosystems and contribute to their conservation and restoration endeavours. The Sabah Forestry Department has undertaken significant restoration projects, including the utilisation of *G. sumatranum* and other pioneer species for land reclamation, thereby contributing to the reforestation and rehabilitation of ultramafic and degraded areas. In 2007, a research plot was established at Tawai Forest Reserve to initiate the restoration of the ultramafic forest, which had previously succumbed to fires. *G. sumatranum*, along with a total of 1260 individual seedlings, was selected with seven other native species for the project, alongside *Aquilaria beccariana, Palaquium rostratum, Toona sureni, Hopea pentanervia, Alstonia angustiloba*, and *Shorea multiflora* [16]. An assessment in 2010 revealed that *G. sumatranum* had recorded 83.3% of survival rate with an average height increment of 1.0 meter per annum (Appendix A: Plate 2), [17]. This demonstrates *G. sumatranum*'s resilience in nutrient-poor, highlighting its crucial role in restoring the ultramafic ecosystem. However, in 2011, a migratory pack of elephants predominantly destroyed this plot, presenting significant challenges to ongoing ecological restoration efforts.

Gymnostoma sumatranum, commonly known as Sempilau Bukit, makes it an ideal choice for reclamation efforts in degraded ultramafic forests. Given the imperative for mass propagation of *G. sumatranum* as a crucial tool for enhancing productivity to support ecological restoration initiatives, several challenges emerge. One significant challenge is the limited availability of seed resources, necessitating precise timing for seed collection. Additionally, concerns arise regarding seed viability and dormancy. Current practices within the department involve collecting wildings from mother trees, which are restricted to post-fruiting events. Moreover, there is a lack of silviculture information regarding the species' phenology, as indicated by SFD silviculturist, Alexander Hastie (personal communication, March 27, 2024). Most of the existing studies on *G. sumatranum* date back to the 1990s, while others primarily focus on the *Casuarina* genus, providing general information on propagation techniques such as seed cultivation, vegetative cuttings, and in-vitro multiplication. Although in-vitro propagation

for clonal planting stock may incur high development costs, conventional rooted cuttings are likely more costeffective for mass production.

Therefore, this study was conducted to enhance rooted cutting techniques at the nursery stage, aiming to establish *Gymnostoma sumatranum* as a potential restoration species in degraded areas, particularly in ultramafic forests. The paper comprises three distinct sub-studies, each targeting a specific objective of nursery management, namely:

- i)Selecting the best segment of sprigs for rooted cutting.
- ii)Investigating the influence of rooting hormones on growth and nodulation.
- iii)Selecting potting media for seedling development.

2. Methodology

2.1 Study Site

This study was conducted from January 2013 to August 2014 at the Forest Research Centre (FRC) nursery, Sepilok. The resources for the experiment were collected from nursery stock plants of *G. sumatranum* that were cultivated from the wildings within the forest reserves. Propagation was conducted inside a misting room within the elevated sowing bed and watered using automated misters, operating at 10-minute intervals every hour. This setup is based on a closed system to maintain optimal humidity levels and prevent moisture loss, which is crucial for the survival of the cutting explants.

2.2 Selecting The Best Segment of Sprigs for Rooted Cutting

A total of 109 cuttings were collected from 2-year-old stock plants of *G. sumatranum* at FRC Nursery (Appendix A: Plate 3). These cuttings were divided into two groups: a) the upper-part sprig (UPS), characterised by a cluster of needles from the top shoot, and b) the middle-part sprig (MPS), representing the woody tissue segment (Appendix A: Plate 3). Each cutting measured between 13 mm to 200 mm in length. To encourage rooting development, the basal end of each cutting was treated with rooting hormone before being placed in a sand bed for the germination stage (Appendix A: Plate 4). The sand medium was treated with sodium hypochlorite and subsequently rinsed using tap water to prevent the growth of harmful fungi and bacteria that could negatively affect the cuttings. After 12 weeks, the length of roots for each group was measured by carefully pulling out the cuttings from their media bed. The collected data was then subjected to analysis using descriptive statistics and Mann-Whitney U-Test to compare the rooting performance between the upper and middle-parts of the cutting sprigs.

2.3 Investigating The Influence of Rooting Hormones On Growth and Nodulation

A total of 400 middle-part sprigs (MPS) were selected. The cuttings, averaging 150 mm to 200 mm in length, were divided into four groups, each containing 100 cuttings. These groups received different treatments: three groups had the basal end of the cuttings dipped into different commercial rooting hormones, while the fourth group served as a control (Table 1). After the hormone treatment, the cuttings were placed in a sand bed using fine river sand (Appendix A: Plate 5). Data collected at 10 months included measurements of explant length (mm) and the number of roots (Appendix A: Plate 6). Additionally, nominal binary-dependent data such as survival, rooting, and nodulation were recorded. Non-parametric Kruskal-Wallis Test and post-hoc analysis using Dunn-Bonferroni were conducted to assess differences between groups. A Mann-Whitney U-Test was performed to determine differences in metric variables in response to nodulation. Furthermore, a Point-Biserial correlation was carried out to examine the influence of nodulation on the number of roots and explant length.

Table 1 *Commercial rooting hormones are used as root promoters*

2.4 Selecting Potting Media for Seedling Development

A total of 171 successful rooted cuttings from MPS that were achieved (subsection 2.3), were transplanted into perforated polythene bags measuring 20cm x 12cm, using four different types of potting media (Table 2). Media were selected based on *G. sumatranum's* adaptable habitat to mimic these natural conditions with all four of the selected media comprised low-nutrient soil with a mix of sandy and clay elements. The heath sand used in the experiment was obtained from the kerangas forest at Sepilok Kabili Forest Reserve. Other potting media such as nursery soil and river sand were purchased from a local supplier. The seedlings were placed under 70% shade at the main nursery and manually watered once a day. Variables on growth, including the seedling height and the number of branches, were recorded at the end of the 60-day period. Additionally, binary data (Table 3) on the condition of the cuttings were indexed into three categories: 1) healthy, 2) dying, and 3) dead.

	. . ີ		.	
Media	Ratio	Composition	No. of cuttings	
N-soil		Nursery soil	30	
K-sand	1	Heath sand	30	
M-soil	1:1	Nursery soil: heath sand	30	
R-sand		Fine river sand	81	

Table 2 *Comparison of potting media for G. sumatranum cutting propagation*

Note: N-soil = nursery soil, K-sand= kerangas soil, M-soil = mixed soil, R-sand = river sand.

Table 3 *S-INDEX for G. sumatranum cutting propagation*

<i>I</i> -value		Description			
Healthy		Leaves appear green, turgid and exhibit vigorous growth			
Dying		50% of the leaves discolored with stunted growth			
Dead		Cutting shows all leaves dried up and withered			

Note: S-INDEX = Survival index, *I* = Index, *I*-value = Index value.

2.5 Data Analysis

Basic data processing and descriptive statistics were performed using MS Excel (MS Office Professional Plus 2019). All parameters were assessed for normality with the Shapiro-Wilk test using PAST 3.22 program [18]. Further statistical tests were performed using DATAtab Statistics Calculator (DATAtab e.U., Graz, Austria). A twosided p-value < 0.05 was considered statistically significant. The experimental design of the study is briefly outlined in Fig. 1.

Fig. 1 *Experimental design*

3. Results and Discussion

3.1 Selecting The Best Segment of Sprigs for Rooted Cutting

The Upper-part sprig (UPS) recorded 46 cuttings (57.5%) exhibiting root growth, while 30 cuttings (37.5%) showed no growth after the 12-week period. Additionally, four cuttings (5%) in the UPS did not survive and were recorded as dead (Fig. 2). The Middle-part sprig (MPS) recorded 22 rooted cuttings (75.9%) and six non-rooted cuttings (20.7%), while one cutting (3.4%) was recorded as dead (Fig. 2). Overall, the MPS showed a higher percentage of rooted cuttings and a lower percentage of non-rooted cuttings compared to the UPS. Both segments had a relatively small proportion of cuttings that did not survive the 12-week period.

Table 4 *The number of rooting for Upper-and-middle-part sprigs of G. sumatranum in 12-week period*

Cuttings	No. of rooted \boldsymbol{N}		No. of non- rooted	Dead
UPS	80	46	30	4
MPS	29	22		

Note: *N* = number of cuttings, UPS = Upper-part sprig, MPS = Middle-part sprig

Fig. 2 *Percentage of growth between upper- and middle-part sprigs in 12-week period*

3.2 Variation in Rooting Behavior Between UPS and MPS of *G. Sumatranum*

UPS exhibited a wide range of root lengths from 1.74 mm to 91.12 mm, resulting in a mean root length for this group of approximately 30.46 ± 24.87 mm. While MPS, having fewer numbers of cuttings (29 cuttings), exhibited root length measurements spanning from 2.18 mm to 108.93 mm, with a mean root length of approximately 33.13 ± 29.2 mm. Both UPS and MPS showed a significant departure from normality, as indicated by the Shapiro-Wilk normality test ($p < .001$; Table 5). However, there was no strong evidence to suggest that the root lengths observed in the UPS and MPS groups were significantly different from each other based on the non-parametric Mann-Whitney U-Test, $U = 480$, $p = .739$, $r = 0.04$.

These results were important for comparing rooting characteristics of different segments of *G. sumatranum* cuttings for mass propagation. The results suggested that MPS may be more conducive to root growth, as evidenced by the higher mean root length observed in the MPS group. Furthermore, MPS showed a higher number of explants with consistent rooting performance and a lower number of explants producing dwarf roots compared to UPS (Fig. 3). The findings could serve as a baseline strategy in planning mass propagation for nursery practices of *G. sumatranum*. Additionally, future research could explore other factors that might influence rooting in different segments of this species to enhance our understanding of the significance of the observed root length variations and characteristics.

 $R -$

Fig. 3 *Rooting percentage on upper- and middle-part sprigs of G. sumatranum in 12-week period*

3.3 Effects of Rooting Hormones On the Growth and Nodulation

The results showed consistent mean values for the different commercial rooting hormones applied in terms of the number of roots produced and the length of explants (Fig. 4). Among the treatments, the use of Seradix-3 resulted in the highest mean values for both the length of explants (46.04±40.58 mm) and the number of roots (4.2±4.53), followed by Spectra (Length = 36.63 ± 40.81 mm; N_root = 2.91 ± 4.12), Seradix-2 (Length = 31.25 ± 38.38 mm; N_root = 1.93 ± 3.06), and the Control group (Length = 28.87 ± 35.12 mm; N_root = 1.81 ± 2.99). Statistical analysis using Kruskal-Wallis test showed that there was a significant difference between treatment groups concerning the dependent variables Length (mm) and N_root, respectively (H (3) = 11.14, $p = .011$; H (3) = 21.83, $p < .01$). Post-hoc using Dunn-Bonferroni test revealed that the pairwise group comparisons of Seradix-2 - Seradix-3 and Seradix-3 – Control, indicated that these groups were significantly different from each other in pairs (Table 6). The results showed that the use of Seradix-3 yielded promising outcomes in terms of both explant length and the number of roots produced. Statistical analyses confirmed significant differences between certain treatment groups, further supporting the selection of Seradix-3 as a suitable root promoter for further seedling development using the rooted cutting technique of *G. sumatranum.*

Fig. 4 *Bar chart of rooting hormone treatments applied to explant length and number of roots*

Note: Mean followed by the same letter within each growth parameter do not differ significantly using Dunn-Bonferroni test; *p* < 0.05.

Metric variable	Treatments	Freq.	Mean ± Std.	W
	Spectra	100	36.63 ± 40.81	$W=0.84*$
	Seradix-2	100	31.25±38.38 ^a	$W=0.79*$
Root Length (mm)	Seradix-3	100	46.04 ± 40.58 ^b	$W=0.88*$
	Control	100	28.87±35.12 ^a	$W=0.77*$
	Spectra	100	$2.91 + 4.12$	$W=0.74*$
	Seradix-2	100	1.93 ± 3.06^a	$W=0.69*$
N root	Seradix-3	100	4.2 ± 4.53 ^b	$W=0.85*$
	Control	100	1.81 ± 2.99 ^a	$W=0.67*$

Table 6 *Mean and normality test of metric variables of the number of roots produced and the length of explants with rooting hormone*

Note: means followed by the same letter within each growth parameter do not differ significantly following the Dunn-Bonferroni test; *p* < 0.05. Data Mean ± Standard deviation (Std.). N_root = Number of roots, *N* = Number of sample, W = Normality test using Shapiro-Wilk, * *p* <.001

3.4 Association Between Nodulation and Growth Variables

Nodulation plays a significant role in the growth and development of certain plants, through a symbiotic relationship with nitrogen-fixing bacteria. The nodules formed on the roots enable the plants to fix atmospheric nitrogen, which is essential for their growth and overall development [19]. Hence, in this experiment, data from explants with nodules were compared to data from explants without nodules to determine whether there is a significant impact of nodulation on the growth variables, specifically the explant's length and the number of roots.

The study showed that explants with root nodules demonstrated superior performance, exhibiting higher growth variables compared to explants without root nodulation (Table 7). Explants with nodules showed a maximum length of 174 mm and had 18 roots, whereas non-nodulated explants had a maximum length of 134 mm with 16 roots. The mean length of explants with nodules was substantially higher (81.76±27.58 mm) than that of explants without root nodulation (21.35±30.2 mm). Similarly, the mean number of roots in explants with nodules (6.81 ± 4.13) was significantly higher than in non-nodulated explants (1.44 ± 2.69) . Statistical analysis using Mann-Whitney U-Test showed that the difference between binary data on nodulation $(0 =$ without nodule and $1 =$ with nodule) and the dependent variables for root length (mm) and number of roots, were statistically significant (*U* = 2418, *p* < .001, *r* = 0.65; *U*=3301, *p* < .001, *r* = 0.61). To further support this evidence, a point-biserial correlation was run to determine the relationship between growth variables and the event of nodulation. There was a positive correlation between N_root and Nodulation, which was tested statistically significant ($r_{\rm pb}$ = 0.6, $p < .001$). Additionally, there was a positive correlation between Length (mm) and Nodulation, which was also statistically significant (r_{pb} = 0.66, $p < .001$). We could conclude that growth variables were positively correlated with nodulation, as the number of roots and the length (mm) increase with nodulation. These results indicated that explants with nodules contributed to better growth of explants development at nursery stage.

We also noticed variations in nodulation within the roots, which can be attributed to the different treatments used. The application of commercial rooting hormones may have played a role in promoting nodulation by nitrogen-fixing bacteria to some extent, as published by previous studies [20], [21]. The formation of nodules is crucial for legume plants to adapt to low-nutrient environments, as similar media condition using sterilized sand was used in this experiment. The nodulation results align with the previously described success ranking of growth hormones. However, it is essential to note that out of the 400 cuttings involved in the experiment, only 31% of the explants with roots showed nodulation. This indicated that not all rooted explants exhibited nodulation, highlighting a limitation in this study. Further research is necessary to gain a better understanding of the factors that influence nodulation in this specific context.

Variable	*Nod	N	Med	Max	Mean ± Std.	MW
Root Length		305		134	21.35±30.21	$U=2418, p=<.001, r=$
(mm)		95	79	174	81.76±27.58	0.65
N root	0	305	θ	16	$1.44 + 2.69$	$U=2418, p=<.001, r=$
		95		18	6.81 ± 4.13	0.65

Table 7 *Descriptive and Mann-Whitney U-Test on nodulation with growth variables*

Note: N_root = Number of roots, N = Number of samples, Med = median, Max = Maximum. *Nod = Nodulation (Binary data; $0 =$ without nodule and $1 =$ with nodule). Data Mean \pm Standard deviation (Std.). MW = Mann-Whitney U-Test

3.5 Selecting Potting Media for Seedling Development

The result based on Table 9 showed seedlings that were grown using K-sand exhibited the highest mean value corresponding to height (mm) with 54.69 ± 11.7, followed by R-sand (51.14±14.82), N-soil (48.94±13.95), and Msoil (46.97±16.01). The potting media with R-sand yielded the highest mean value in response to the number of branches produced, with 14.81±6.93. This was followed by K-sand (11.47±6.58), M-soil (10.4±6.45), and N-soil (6.37±4.52). Statistical analysis using Kruskal-Wallis test showed that there was a significant difference between the groups and growth variable for number of branches, $H(3) = 17.81$, $p < .001$, but no significant difference was recorded for variable height, $H(3) = 4.55$, $p = 0.208$. A post-hoc analysis using the Dunn-Bonferroni Test was conducted to further examine differences between groups and the growth variable for number of branches. The pairwise comparison between M-soil and R-sand revealed an adjusted p-value of less than 0.05, indicating that the two groups differ from each other (Table 9).

Table 8 of S_INDEX showed variable success using different groups of potting media. The result demonstrated that R-sand recorded the highest Healthy index by 54 seedlings, followed by N-soil (19 seedlings), K-sand (15 seedlings), and M-soil (10 seedlings). K-sand recorded the highest Dying index by 12 seedlings, while R-sand also recorded the highest Survival index for Dead by 17 seedlings. This suggests that R-sand achieved more than 50 percent of the ideal seedling condition, but it also recorded the highest dead index (10%) among the treatments. The study indicated that R-sand (river sand) provided the most satisfactory results as a potting medium for the development of *G. sumatranum* seedlings at the nursery stage. Despite the high mortality rate categorised by S-INDEX, it still showed the best option among the groups. While K-sand (kerangas soil) demonstrated the highest mean value for the growth variable of height, it did not show any significant advantage compared to the other treatment options. This suggests that river sand (R-sand) may be the most suitable potting medium for promoting the growth development of *G. sumatranum* seedlings in a nursery setting.

	Treatment	N-soil	K-sand	M-soil	R-sand	Total
S_INDEX	$1 =$ Healthy	19	15	10	$*54$	98
	$2 = Dying$		$*12$		10	40
	$3 = Dead$				$*17$	33

Table 8 *Survival index of seedlings for each treatment group*

Note: S_INDEX = Survival Index, N-soil = nursery soil, K-sand= kerangas soil (heath sand), M-soil = mixed soil (1:1 nursery soil, heath sand), R-sand = river sand. *Highest frequency value

Growth Variable	Treatment	\boldsymbol{N}	Min	Max	Mean ± Std.
HEIGHT, mm	R-sand	54	28	90	51.14 ± 14.82
	N-soil	19	29.4	75	48.94±13.95
	K-sand	15	40.5	77.7	$*54.69 \pm 11.7$
	M-soil	10	25.5	70	$*46.97 \pm 16.01$
N_BRANCH	R-sand	54	$\overline{4}$	35	$*14.81 \pm 6.93$ ^a
	N-soil	19	1	14	$*6.37 \pm 4.52$
	K-sand	15	2	27	$*11.47 \pm 6.58$
	M-soil	10	2	21	$*10.4\pm6.45^{\circ}$

Table 9 *Descriptive statistic on growth variables with treatment group*

Note: Means followed by the same letter within each growth parameter do not differ significantly following the Dunn-Bonferroni test; $p < 0.05$. Data mean \pm Standard deviation (Std.). HEIGHT = Seedlings height (mm), N_BRANCH = Number of branches, *N* = Number of samples, Min = minimum, Max = maximum. *Normality test using Shapiro-Wilk showed a significant departure from normality, *p* < 0.01.

4. Project Contribution

In 2014, approximately 300 wildlings of *G. sumatranum* were sourced from ultramafic forests (Mt. Tingkar and Bidu-Bidu Forest Reserves), and 50 rooted cuttings from this study were planted out at Bukit Hampuan Forest Reserve, alongside other selected species such as *Agathis borneensis, Shorea laxa, Calophyllum* sp., and Sabah's endemic *Dipterocarpus ochraceus* (P. Butin, personal communication, November 04, 2023). The planted sites consisted of 1.4 hectares of partially sloped and degraded area and underwent a restoration program, which involved the establishment of two plots measuring 140 meters by 50 meters. The restoration method employed a 1-meter triangle cluster planting approach, with three seedlings per cluster. A random assessment conducted in April 2015 recorded that *G. sumatranum* had a survival rate of 82.7% with an average height of 1.3 meters (Unpublished data by P. Butin, personal communication, November 04, 2023). However, in June 2015, these restoration plots suffered significant devastation caused by the catastrophic Mount Kinabalu earthquake. This event triggered a massive landslide and impacted a substantial portion of the restoration sites and other adjacent hills connected to the Crocker Range [22].

4.1 Challenges of Restoring Degraded Ultramafic Forests in Sabah

Restoring degraded ultramafic forests in Sabah presents numerous challenges, as evidenced by available references. These unique ecosystems are characterised by harsh edaphic conditions, with low nutrient content and high heavy metal concentrations in the soil. This poses a significant limitation in selecting suitable restoration species, as many tree species struggle to adapt and thrive in such conditions. The lack of native species adapted to ultramafic soils further complicates restoration efforts.

One of the major challenges faced in the restoration process is the risk of wildfires. Ultramafic forests are susceptible to fire outbreaks, particularly during dry periods, which can severely damage newly established seedlings and set back restoration progress. Besides, human activities, such as illegal settlements and agriculture, also threaten the degraded ultramafic forests, leading to encroachment and habitat destruction. This ultramafic vegetation, particularly outside the Kinabalu Park boundary, is threatened by massive land clearing, uncontrolled encroachment, and the potential climate change effect which induces droughts and fires [12]. Restoration projects require substantial resources, including funding, skilled labor, and equipment, which can be challenging to secure for large-scale initiatives. Moreover, the long-term success of restoration efforts relies on continuous monitoring and maintenance, particularly in the initial stages of establishment [23].

Another critical aspect is maintaining genetic diversity within restored populations to enhance ecosystem resilience and adaptability. However, limited genetic diversity in small, isolated populations can pose challenges to restoration success [24]. Lastly, engaging stakeholders, including local communities and policymakers, is crucial for successful restoration. Collaboration and effective communication strategies are vital to gain local support and ensure the long-term success of restoration efforts in degraded ultramafic forests in Sabah [25]. A holistic approach combining scientific research, community involvement, and adaptive management practices is necessary to address these challenges and conserve these unique and ecologically important ecosystems.

5. Conclusion

The study has contributed valuable knowledge on improving rooted cutting techniques for *G. sumatranum*, a potential species for restoring degraded ultramafic forests in Sabah. These findings can aid in successfully rehabilitating degraded areas by and contributing to the long-term goal of restoring the functioning capacity of the forest ecosystem in the region. The use of *G. sumatranum* as a pioneer tree species holds promise in improving soil constitution and addressing fire setbacks in degraded ultramafic areas. By employing the most effective propagation methods, understanding nodulation, and selecting suitable potting media, successful restoration efforts can be realised to the recovery of degraded ultramafic forests and promoting forest rehabilitation practices in Sabah.

Acknowledgements

The authors would like to thank Martin Tuyok and the UMS (Universiti Malaysia Sabah) trainees, Siti Azmah Jambo, Jocelyn Joachim, and Eryati Derman, for their assistance in the nursery. The authors also extend their gratitude to Alexander Hastie and Petrus Butin for sharing their knowledge. Special thanks are due to Eyen Khoo, Head of the Biotechnology Section, for her support, and we acknowledge the Chief Conservator of Forests and the

Deputy Chief Conservator of Forests (R&D), Datuk Frederick Kugan and Dr. Arthur Chung respectively, for approving the study.

Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Richard Majapun; data collection: Dzulyana Idhamsah; analysis and interpretation of results: Richard Majapun, Alviana Damit; draft manuscript preparation: Richard Majapun, Reuben Nilus. All authors reviewed the results and approved the final version of the manuscript.

Appendix A: Related Photos

Plate. 1 *Map of ultramafic forests in Sabah, Malaysia [12]*

Plate 2 *Growth of G. sumatranum at Tawai Forest Reserve after 3 years. Photo taken on March 20, 2011*

Plate 3 *Left - A healthy top sprig, 150mm in length, was selected from adult G. sumatranum seedlings using a steel cutter. Right - The explants were later excised into two sections: the upper- and middle-part sprigs for subsequent experiments*

Plate 4 *All explants were treated with rooting hormone before being placed in the sand bed for the germination stage*

Plate 5 *A: Planting cuttings from the middle-part sprigs in the sand germination bed following rooting hormone treatments. B-C: Extracting out cuttings for growth data assessment after 10 months. D-E: Examples of root and shoot development after 10 months. F: Transferring cuttings into polybags for seedling stocks at FRC nursery as part of the forest restoration program*

Plate 6 *G-H: Measuring rooted cuttings from both upper- and middle-part sprigs. I-J: Ranges of maximum, average, and minimum measurements of rooted cuttings from both parts of G. sumatranum sprigs, with nodulation observed for J. K-L: Transferring the rooted cutting into polybags for seedling stock at FRC nursery, to be later utilized in the forest management program*

References

- [1] Laplaze, L., Bon, M.C., Sy, M. O., Smouni, A., Allonneau, C., Auguy, F., Frutz, T., Rio, M., Guermache, F., Duhoux, E., & Franche, C. (2000). Molecular biology of tropical nitrogen-fixing trees in the Casuarinaceae family. In *Molecular Biology of Woody Plants: Volume 1* (pp. 269-285). Dordrecht: Springer Netherlands. https://doi.org /10.1007/978-94-017-2311-4_10.
- [2] Britannica (n.d). Casuarinaceae. In *Encyclopedia Britannica*. Retrieved May 27, 2016, from https://www.britannica.com/plant/Casuarinaceae

- [3] Johansen, K. I., Musil, K. J., & Forster, P. I. (2015). The generic delimitation of Allocasuarina L.A.S. Johnson (Casuarinaceae) based on morphological and molecular evidence, *Australian Systematic Botany*, *28(4-5),* 259-271.
- [4] Viglizzo, E. F., Pordomingo, A. J., Castro, M. G., Lértora, F. A., & Funes, F. (2003). The value of conserving biodiversity in the Argentine pampas region: a spatial econometric analysis of land use change, *Ecological Economics, 45(3),* 445-457.
- [5] Smith, T. E. (2008). *Taxonomy of New Guinean Casuarinaceae*. [Doctoral dissertation, University of Adelaide].
- [6] Pungga, R. S. (1996). Casuarinaceae. In: E. Soepadmo, K. M. Wong & L. G. Saw (eds), *Tree Flora Sabah & Sarawak* (Vol. 2, pp. 119–127). Forest Research Institute Malaysia, Kuala Lumpur.
- [7] Sugau, J. B. & Damit, A., comps. (2021). *A Checklist of Trees of Sabah*. Sandakan: Sabah Forestry Department.
- [8] Wong, T. M. (1982). *A Dictionary of Malaysian Timbers*. Revised by Lim, S. C. & Chung, R. C. K. Malayan Forest Records No. 30 (pp. 201). Forest Research Institute Malaysia, Kuala Lumpur.
- [9] Torrey John G. & Dale Callaham (1982). Structural features of the vesicle of Frankia sp. CpI1 in culture. *Canadian Journal of Microbiology, 28(7),* 749-757.
- [10] Quintela-Sabarís, C., Auber, E., Sumail, S., Masfaraud, J. F., Faucon, M. P., Watteau, F., Saad, R. F., Van der Ent, A., Repin, R., Sugau, J., & Nilus, R. (2019). Recovery of ultramafic soil functions and plant communities along an age-gradient of the actinorhizal tree *Ceuthostoma terminale* (Casuarinaceae) in Sabah (Malaysia), *Plant and Soil, 440,* 201-218, https://doi.org/10.1007/s11104-019-04085-y
- [11] SFD (2016). *Forest Restoration*. Sabah Forestry Department. https://forest.sabah.gov.my/ulukalumpang/Forest_Restoration.html
- [12] Van der Ent, A., Repin, R., Sugau, J., & Wong, K. M. (2015). Plant diversity and ecology of ultramafic outcrops in Sabah (Malaysia). *Australian Journal of Botany, 63(4)*, 204-215, https://doi.org/10.1071/BT14214
- [13] Proctor, J., Anderson, J. M., Chai, P., & Vallack, H. W. (1988). Patterns of plant species diversity in the rainforest of Ranomafana National Park, Madagascar. *Biological Journal of the Linnean Society, 31(4),* 327- 353.
- [14] Repin, R. (1998). Preliminary survey of serpentine vegetation areas in Sabah. *Sabah Parks Nature Journal, 1*, 19-28.
- [15] Sugau, J. B., Middleton, J. J., Koshy, M., & Corlett, R. T. (2012). Distribution and Conservation Status of the Ultramafic Flora of Sabah, Malaysia. *Gardens' Bulletin Singapore, 64*, 1-20.
- [16] Sabah Forestry Department (2007). *Annual report 2007*. Sabah Forestry Department, Sandakan. https://forest.sabah.gov.my/images/pdf/publication/annualreport/ar20.pdf
- [17] Sabah Forestry Department (2010). *Annual report 2010*. Sabah Forestry Department, Sandakan. https://forest.sabah.gov.my/images/pdf/publication/annualreport/ar2010.pdf
- [18] Oyvind, H., David, H., & Paul, R. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica, (4),* 1-9.
- [19] Ngom, M., Oshone, R., Diagne, N., Cissoko, M., Svistoonoff, S., Tisa, L. S., Laplaze, L., Sy, M. O., & Champion, A. (2016). Tolerance to environmental stress by the nitrogen-fixing actinobacterium Frankia and its role in actinorhizal plants adaptation. *Symbiosis, 70*, 17-29, https://doi.org/10.1007/s13199-016-0396-9
- [20] Marappa, N., Dhanasekaran, D., & Nooruddin, T. (2021). *Root Nodule Microbiome from Actinorhizal Casuarina Plant.* CRS Press. https://doi.org/10.1201/9781003037521-22.
- [21] Velandia, K., Reid, J. B., & Foo, E. (2022). Right time, right place: The dynamic role of hormones in rhizobial infection and nodulation of legumes. *Plant Communications, 3*, 1-14, https://doi.org/100327.10.1016/j.xplc.2022.100327.
- [22] Wang, Y., Wei, S., Wang, X., Lindsey, E. O., Tongkul, F., Tapponnier, P., Bradley, K., Chan, C. H., Hill, E. M., & Sieh, K. (2017). The 2015 M w 6.0 Mt. Kinabalu earthquake: an infrequent fault rupture within the Crocker fault system of East Malaysia. *Geoscience Letters*, *4(1),* 1-12, https://doi.org/10.1186/s40562-017-0072-9.
- [23] Gann, G. D., McDonald, T., Walder, B., Aronson, J., Nelson, C. R., Jonson, J., Hallett, J. G., Eisenberg, C., Guariguata, M. R., Liu, J., & Hua, F. (2019). International principles and standards for the practice of ecological restoration. Second edition. *Restoration Ecologym 27*(S1), S1-S46, https://doi.org/27. 10.1111/rec.13035.
- [24] Hvilsom, C., Ekblom, R., Fischer, M. C., & Segelbacher, G. (2022). Selecting species and populations for monitoring of genetic diversity. *International Union for Conservation of Nature*. https://doi.org/10.2305/IUCN.CH.2022.07.en
- [25] Fernandes, A. A., Adams, C., de Araujo, L. G., Romanelli, J. P., Santos, J. P. B., & Rodrigues, R. R. (2022). Forest Landscape Restoration and Local Stakeholders: A Global Bibliometric Mapping Analysis. *Sustainability, 14*, 16165, https://doi.org/10.3390/su142316165.

