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Indonesia

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Contents

THE EFFECTIVENESS OF COLCHICINE AND ORYZALIN ON POLYPLOIDY INDUCTION IN TEAK (*Tectona grandis* Linn. f.) IN VITRO

Yusuf Sigit A. Fauzan[1*], Supriyanto[2], Joko Mulyono[3] and Teuku Tajuddin[1]

[1]Research Center for Applied Botany, BRIN, Jl. Raya Jakarta - Bogor Km. 46, Cibinong, Bogor, 16911, West Java, Indonesia,
[2]SEAMEO BIOTROP, Jl. Raya Tajur No.Km 6, Pakuan Bogor, 16134, West Java, Indonesia
[3]Micro Technics Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University Jl. Lingkar Kampus IPB, Darmaga Bogor 16680, West Java, Indonesia

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THE EFFECTIVENESS OF COLCHICINE AND ORYZALIN ON POLYPLOIDY INDUCTION IN TEAK (*Tectona grandis* Linn. f.) IN VITRO. The Indonesian government has launched the rehabilitation of a community plantation forest program in the entire country that would be beneficial for remedying the shortage in domestic demand for teak wood every year. This program needs to be supported by the availability of quality seed resources and quality seedlings utilizing polyploid teak (*Tectona grandis*). Our study aimed to examine the effectiveness of colchicine and oryzalin to modify diploid into polyploid *T. grandis* based on growth response, morphological, anatomical, and cytological alteration, as well as the acclimatization ability of polyploid plantlets. The materials used were aseptic lateral shoots consisting of nodal segments immersed in antimitotic agents at the concentrations of 0, 15, and 30 μM for 5 days, then cultured on regeneration medium until the 8th week and followed by acclimatization. The results showed that colchicine at a concentration of 30 μM was the most effective to induce polyploidy of plantlets in the parameter of high growth rate, length of internodes, number of leaf plantlets, leaf surface area, and significant chlorophyll index content compared to the control. Anatomical analysis of polyploidy was characterized by increasing leaf thickness, stomata size, decreased stomatal density, and increased chloroplast content in guard cells. Based on the cytological examination of polyploidy plantlets, there was an increase in the number of chromosomes in the cell nucleus. The acclimatization of polyploid plantlets successfully induced rooting and 100% survival rate of grown plantlets. Polyploid seedlings were able to grow and well adapted to the environment condition of acclimatization.

Keywords: Acclimatization, chromosome number, polyploidy, Tectona grandis, tetraploidy

EFEKTIVITAS KOLKISIN DAN ORYZALIN UNTUK INDUKSI POLIPLOIDI TANAMAN JATI (Tectona grandis Linn. f.) PADA KULTUR IN VITRO. Pemerintah Indonesia telah mencanangkan program rehabilitasi hutan tanaman rakyat di seluruh pelosok negeri yang dapat dimanfaatkan untuk pembangunan hutan berbasiskan kayu pertukangan sehingga dapat menambah suplai kayu jati atas permintaan tinggi industri setiap tahun. Program ini harus didukung tersedianya sumberdaya benih yang bermutu dan bibit yang berkualitas seperti jati (Tectona grandis) poliploid. Penelitian ini bertujuan untuk menguji efektivitas kolkisin dan oryzalin dalam mengubah jati diploid menjadi poliploid berdasarkan respon pertumbuhan, perubahan morfologi, anatomi dan sitologi serta kemampuannya dalam aklimatisasi. Bahan yang digunakan adalah pucuk lateral aseptik yang terdiri dari ruas nodul yang direndam dalam agen antimitotik pada konsentrasi 0, 15 dan 30 μM selama 5 hari, kemudian dikultur pada media regenerasi sampai berumur 8 minggu dan dilanjutkan untuk aklimatisasi. Hasil penelitian menunjukkan bahwa konsentrasi kolkisin 30 μM merupakan konsentrasi yang efektif untuk mengubah tingkat ploidi planlet jati berdasarkan parameter laju pertumbuhan tinggi, panjang ruas, jumlah planlet daun dan peningkatan luas permukaan daun serta jumlah klorofil yang signifikan dibandingkan dengan kontrol. Analisis anatomi poliploid ditandai dengan meningkatnya ketebalan daun, ukuran stomata, penurunan kerapatan stomata, dan peningkatan kandungan kloroplas pada sel penjaga. Berdasasarkan hasil uji sitologi planlet poliploid diperoleh

[*] Corresponding author: yusuf.sigit@bppt.go.id

peningkatan jumlah kromosom pada sel inti. Aklimatisasi planlet jati poliploid berhasil menginduksi akar dan persentase hidup planlet yang tumbuh mencapai 100%. Bibit poliploid mampu tumbuh dan beradaptasi dengan baik pada kondisi lingkungan aklimatisasi.

Kata kunci: Aklimatisasi, jumlah kromosom, poliploidi, Tectona grandis, tetraploidi

I. INTRODUCTION

Teak (*Tectona grandis* Linn. f.) is one of the important traded woody plant commodities in Indonesia and the world. The teak wood industry has a wide market share, both in the home country and abroad. Yet, the world teak wood production is much lower compared to its demand. In 2019, teak wood still dominated the raw material for the national domestic industry, amounting to 500,000 m^3 (Kominfo Jatim, 2019). During the same period, the teak wood production of the state-owned forestry company, *Perum Perhutani*, only reached to 396,500 m^3 (BPS Statistics Indonesia, 2019).

In accordance with the above issues, the need for seeds to rehabilitate community forests, especially wood for woodworking, is also huge. According to the Directorate General of Watershed Management and Forest Reserve of the Ministry of Environment and Forestry of the Republic of Indonesia, the total area of community forests that have not been rehabilitated is 3.96 million hectares (Kementerian Lingkungan Hidup dan Kehutanan, 2020). One of the programs was on community plantation forests, which allow the community to have an access to forest resources, particularly for wood-working-based forest development. As one of the teak wood producing countries, this opportunity is a challenge for Indonesia to increase the total production of teak wood, bearing in mind that Indonesia has huge potential, both inland and human resources, which can be used as assets for forestry development. Forestry development, especially in the timber sector, must be supported by the availability of quality seeds and seedlings in adequate amounts and continuously.

According to Monteuuis (2000), cconventional teak breeding using seed is still hindered by a number of difficulties, including: very limited seed production capability, asynchronous flowering time, low germination rate, high individual seed variability, not being economically feasible since it requires a large area for development, and a long period of time for preparation. In addition, seed qualification is difficult to be standardized due to compatibility issues during breeding. Moreover, the problem for teak plantation development with low productivity because the seeds and seedlings used by the farmer are generally not qualified.

New approach on teak quality improvement was initiated using mutation breeding strategy. Gamma-ray irradiation was used on Muna accession, which resulted in 10 putative mutants with superior characters (Zanzibar, Bramasto, Sianturi, Yuniarti, & Desmiati, 2015), and induction of 84% genetic diversity in in vitro plantlets (Parloangan, 2017). Another approach for stimulating mutation was polyploidy induction via in vitro culture. Polyploidy is generally the direction of the effect mutation, which increased in size (Rezende, Suzigan, Amorim, & Moraes, 2020), so it has the advantage of being more controlled than irradiation. Polyploidy can be induced by antimitotic agents on the organ, tissue or cells to obtain polyploid teak. So that it is expected to increase the character of teak to become superior, which can help improve the productivity and quality of teak wood as an industry related to bioresource material.

Various studies on the use of antimitotic agents for polyploidy induction have been done on many forestry plants. The purpose of those studies was to improve the quality and quantity of plants and plant products, such as *Eucalyptus grandis* (Silva, Carvalho, & Clarindo, 2019), *E. dunnii* (Castillo, Lopez, Tavares, Santinaque, & Dalla Rizza, 2020), *Aquilaria malaccensis* (Siti-Suhaila et al., 2020), *Acacia mangium* (Griffin, 2014; Viet et al., 2020; Le et al., 2021), and *T. grandis* (Nugraha, 2012; Ridwan, Handayani, Riastiwi, & Witjaksono, 2018).

Polyploidy research conducted on the *A. mangium* tree showed positive responses. The tetraploid *A. mangium* tree has a gigantism effect and showed 21% coarser and thicker bark, 20% thicker polyads, 28% longer wood fiber, 17% thicker leaves, 12% wider leaves, and longer stomata (24.3 µm) compared to the diploid tree (Griffin, 2014). Likewise, the tetraploid tree produced a larger seed and a higher flowering capacity than the diploid tree (Le et al., 2021). Additionally, da Silva Souza et al. (2021) reported that polyploid Eucalyptus clones derived from the crosses of *E. grandis* × *E. urophylla* produced a longer and thicker cell wall of wood fibers than the diploids, thus increasing the fiber strength of the paper products.

In our study, antimitotic agents, i.e. colchicine and oryzalin, were induced into the in vitro culture of *T. grandis* using sterile apical meristems. Colchicine is a toxic chemical extracted from the seeds, tubers, and flowers of *Colchicum autumnale* L., which grows in Europe and the United Kingdom. The seeds and tubers contain 0.2–0.8% and 0.1–0.5% colchicine, respectively, and a small amount is found in the flowers (Lin et al., 2020). Oryzalin is a dinitroaniline herbicide that has the same performance as colchicine (Chen, Yu, Patterson, Sayer, & Powles, 2021) and is often used as an alternative to colchicine. Both antimitotic agents act as metaphase inhibitors. During metaphase, the microtubule spindle emerges from the microtubule organizing center (spindle). This spindle, composed of a- and b-tubulin dimers, is important for the migration of chromosome poles during anaphase. Therefore, inhibition of chromosome segregation results in a cell with a complete double chromosome, which is called polyploidy. Polyploidy has many consequences for plant growth and development and is applied in plant breeding (Iannicelli & Escandon, 2022).

The benefits of this research were to produce new mutants with high quality of performance, high yields, and adaptation to the environment to help increase the productivity of *T. grandis*. The purpose of our study was to examine the effectiveness level of antimitotic agent concentrations to achieve the precise dose for increasing the ploidy of *T. grandis* plantlets in in vitro culture and obtaining polyploid *T. grandis* seedlings after acclimatization.

II. MATERIAL AND METHOD

A. Place and Time of Experiment

The study was carried out at the Plant Micropropagation Laboratory, Laboratory for Biotechnology, BRIN, the Science and Technology Area of BJ Habibie, South Tangerang City, Banten, and the Micro Technical Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Darmaga Campus, West Java. The study was conducted from January 2018 to June 2019.

B. Experimental Procedure

Source of explants and plant preparation

The source of the explants was obtained from the 2-year-old T. grandis mother plant of Muna accession planted in the mother plant collection park. The in vitro plants were initiated by culturing the axillary buds in the regeneration medium and multiplied until 8 weeks old with a height of 8 to 10 cm (Fauzan, Supriyanto, & Tajuddin, 2017a). Then, the shoots were cut to a size of 1 cm length for mutation induction. The shoots were planted on MS0 media and then immersed in antimitotic agents according to the treatment doses for 5 days. Subsequently, the shoot meristems were transferred into Murashige & Skoog regeneration media with the addition of BA (6-benzyladenin) and kinetin 0.5 mg/L each. The regeneration media used refers to Aguilar, Garita, Kim, Kim, and Moon (2019.

Antimitotic preparations and polyploidy induction

The preparation of sterile antimitotic solutions, colchicine and oryzalin, was done by making a 100 ppm stock solution. Antimitotic agent treatment was accomplished with concentrations of 0 (as a control), 15, and 30 µM. Induction of polyploidy was performed on the axillary buds consisting of 1 cm nodes and planted on the regeneration medium, followed by soaking in the antimitotic agents for 5 days (Fauzan, Supriyanto, & Tajuddin, 2017b). The experimental design in completely randomized design with 20 replicates for each treatment was used (Table 1).

Table 1. The experimental design for colchicine and oryzalin treatments

No	Treatments	
	Antimitotic Agent	**Concentration (µM)**
1.	Control	0 (AM0)
2.	Oryzalin	15 (AM1)
3.	Oryzalin	30 (AM2)
4.	Colchicine	15 (AM3)
5.	Colchicine	30 (AM4)

Acclimatization of Polyploid Teak

Acclimatization was carried out in a greenhouse with an average daily temperature of 24.9 – 32.1°C, a humidity of 71.5 – 77.6% and a light intensity of 533.16 – 2664.58 lux. The acclimatization phase was initiated with the process of hardening off the *T. grandis* plantlets in the greenhouse condition for a week. The plantlets were washed in running tap water and soaked in a 2 g/L bactericide and fungicide mixture solution for 10 minutes, then dried at room temperature. Root induction was done by the ex vitro technique, referring to Badilla, Xavier, Murillo, and de Paiva (2017). Plantlets were then planted on fine husk charcoal media and incubated in a plastic hoop house under 75% screen net shade until the plantlets induced roots and grew well. Subsequently, the plants were transferred into a plastic hoop house under the shade of a 55% double-screen net for development and adaptation. For maintenance, watering was carried out using clean water every two days so that the soil did not dry out.

C. Observation and Data Analysis

Polyploidy Induction

Observations were performed once a week for six weeks. The parameters observed consisted of 3 categories, those were growth, anatomical, and cytological analysis. The growth parameters were measured on height, number of leaves, length of internodes, leaf area, and chlorophyll content index. The plant height, number of leaves, and length of internodes were measured directly using a ruler. The leaf area was measured using the Image-J program. The chlorophyll content index was measured using the chlorophyll meter type CCM-200 plus (Apogee).

The anatomical analysis included stomata density and the number of chloroplasts in the stomata cell. Stomata density and chloroplast anatomical tests were referred to the method of Baker, Yarkhunova, Vidal, Ewers, and Weinig (2017). Observation of stomata density was performed three times in randomly selected fields of view, each covering an area of 0.19625 mm2. The stomata were then selected randomly to measure their length and width using a microscope type BX 51 (Olympus) and a DP 25 type camera with the DP2-BSW software (Olympus). Observation and calculation of chloroplasts were done directly on the stomata by selecting three stomata samples randomly.

Data analysis was accomplished using the following formulas:

1. Growth

$$Height\ (cm) = \frac{\sum observed\ plant\ height\ accumulation}{\sum total\ plant\ observed} \quad (1)$$

$$Length\ of\ internode\ (cm) = \frac{\sum the\ observed\ internode\ length\ accumulation}{\sum total\ plant\ observed} \quad (2)$$

$$Number\ of\ leaves = \frac{\sum the\ observed\ accumulation\ of\ number\ of\ leaves}{\sum total\ plant\ observed} \quad (3)$$

$$The\ wide\ of\ leaf\ area\ (cm2) = \frac{\sum the\ observed\ leaf\ area\ accumulation}{\sum total\ plant\ observed} \quad (4)$$

2. Anatomy

$$\text{The thick of leaf } (\mu m) = \frac{\sum \text{the observed leaf thickness accumulation}}{\sum \text{total plant observed}} \quad (5)$$

$$\text{The length of stomata } (\mu m) = \frac{\sum \text{observed plant stomata length accumulation}}{\sum \text{total plant observed}} \quad (6)$$

$$\text{The width of stomata } (\mu m) = \frac{\sum \text{observed plant stomata width accumulation}}{\sum \text{total plant observed}} \quad (7)$$

$$\text{Stomatal density } (n/mm^2) = \frac{\sum \text{stomata}}{\text{Wide field of view}} \quad (8)$$

$$\text{Number of chloroplasts per stomata} = \frac{\sum \text{chloroplasts in stomata}}{\text{Number of stomata observed}} \quad (9)$$

3. The Effectiveness of Ploidy and Cytology

The ploidy levels were analysed based on indirect estimation by comparing the amount of diploid control chloroplast plants with the putative polyploid plants (Robinson et al., 2018). Further analysis to confirm the occurrence of polyploidy was implemented by cytological analysis. The method of cytological analysis refers to the modified method of Zeng, Liu, Du, and Kang (2019).

$$\text{The percentage of diploid } (\%) = \frac{\sum \text{diploid plant observed}}{\sum \text{total plant observed}} \times 100\% \quad (10)$$

$$\text{The percentage of polyploid } (\%) = \frac{\sum \text{polyploid plant observed}}{\sum \text{total plant observed}} \times 100\% \quad (11)$$

4. Acclimatization

$$\text{The percentage of rooting } (\%) = \frac{\sum \text{rooting plant observed}}{\sum \text{total plant observed}} \times 100\% \quad (12)$$

$$\text{Percentage of survival } (\%) = \frac{\sum \text{life plant observed}}{\sum \text{total plant observed}} x\ 100\% \quad (13)$$

$$\text{Height } (cm) = \frac{\sum \text{the observed plant height accumulation}}{\sum \text{total plant observed}} \quad (14)$$

$$\text{Diameter } (cm) = \frac{\sum \text{the observed diameter accumulation}}{\sum \text{total plant observed}} \quad (15)$$

$$\text{Number of leaves} = \frac{\sum \text{the observed plant leaf accumulation}}{\sum \text{total plant observed}} \quad (16)$$

The experiment was designed as a single factor, completely randomized experimental design, which was the concentration of antimitotic agents.

D. Plant Growth Acclimatization Phase

Observation of growth parameters comprised of the number of life cultures, rooting, height of plant, number of leaves, and diameter of seedlings. The growth data were then statistically tested using the analysis of variance (ANOVA). Differences between treatments were determined by a Duncan multiple range test at the test level of $\alpha = 0.05$. Statistical analysis for observational data was carried out using the SAS program version 9.3.

III. RESULT AND DISCUSSION

A. Growth Response

The results showed that all explants survived and were regenerated. Figure 1 shows the process of mutation induction and plantlet regeneration *in vitro*.

Adding the antimitotic agents decreased the height and length of the internode of *T. grandis* plantlets and the number of leaves. On the contrary, these treatments increased the leaf surface area and chlorophyll content index of plantlets compared to control, as shown in Table 2. When the concentration of oryzalin increased from 15 to 30 µM, the height, internode length, and number of plantlets leaves decreased, from 3.37 to 2.92 cm, from 0.92 to 0.89 cm, and from 9.51 to 8.50 cm, respectively. However, these decreases were not significantly different. The treatment of oryzalin at 30 µM had the largest effect on the reduction of those parameters compared to the treatment of oryzalin at 15 µM and the control. The same phenomenon was seen in the effect of colchicine treatments on the plantlet height and the plantlet length of the internode. The higher the concentrations of colchicine, the shorter the height and internode length of plantlets (Table 2). The reduction in height and length might be associated with the polyploidy t stimulated by the antimitotic agents. According to Was et al. (2022), cell division was inhibited due to the doubled number of chromosomes. In some conditions, the chromosomes were multiplied or developed into polyploidy, increasing the level of complications in the chromosome pairing process (Syukur et al., 2019).

Comparing two treatments of colchicine and oryzalin at the same concentration of 15 and 30 µM, it showed that oryzalin has constantly lowered the average height and length of the

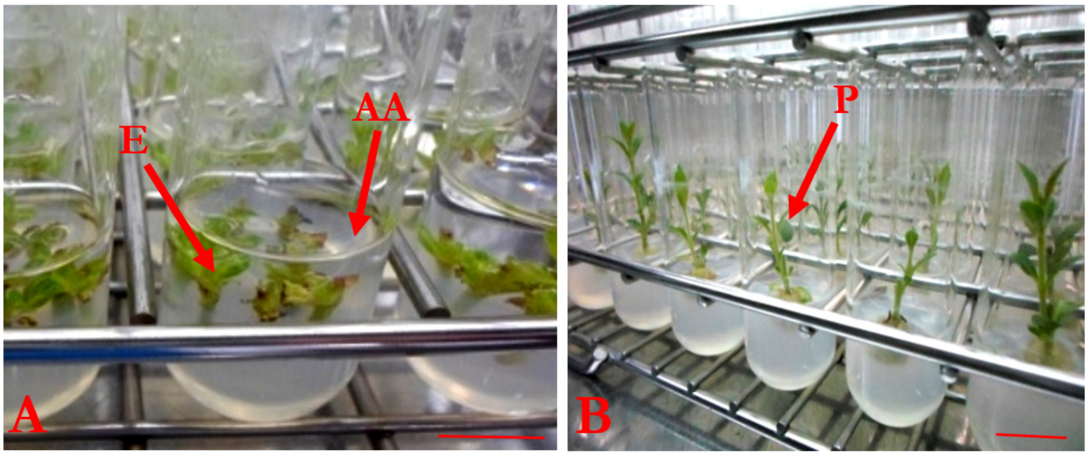

Figure 1. Explants on the in vitro media and immersing them in the antimitotic agents (A), regeneration of putative mutant shoots (B). AA: Antimitotic agent, E: Explants, P: Plantlets. Line Scale: 1 cm

Table 2. The growth response of plantlets *T. grandis* to various concentrations of antimitotic solution at 6 weeks after treatments

Treatment (μM)	Average Height (cm)	Average Length of Internode (cm)	Average Number of Leaves	Chlorophyll Content Index	Leaf Area (cm^2)
Control	5.06 a	1.17 a	10.58 a	7.37 c	1.03 b
Oryzalin 15	3.37 bc	0.92 b	9.51 ab	10.32 b	1.67 a
Oryzalin 30	2.92 c	0.89 b	8.50 b	10.74 ab	1.60 a
Colchicine 15	3.82 b	1.23 a	7.50 b	13.04 a	1.49 ab
Colchicine 30	3.13 bc	0.94 b	8.40 b	11.22 ab	1.79 a

Note: Numbers in the same column followed by the same letters are not significantly different based on the F-Test at a confidence level of 95%

internode. Nevertheless, both treatments were not significantly different on these parameters.

When the plantlets were subjected to 15 and 30 μM concentrations of oryzalin and colchicine, the results improved the chlorophyll content index and leaf area. The addition of oryzalin from the concentration of 15 to 30 μM showed an increase in chlorophyll content index; however, this did not occur in colchicine. Doubling the colchicine concentration from 15 to 30 μM increased the leaf surface area, yet decreased the chlorophyll content index, although not significantly different. It seems that an increase in the concentration of colchicine up to 30 μM damaged the chloroplast organelles, causing them to reduce the chlorophyll content (Cunha Neto et al., 2020; Kim et al., 2021). Adding colchicine at the concentrations of 15 and 30 μM, resulted a higher average of chlorophyll content index compared to oryzalin, though not widely different. Based on the parameters of growth responses in our study, it is assumed that oryzalin has a greater impact on the reduction of plantlet height, length of internode, and number of leaves than colchicine.

Table 2 also displays that increasing the antimitotic concentration was the cause of increasing the leaf surface area and the chlorophyll content of *T. grandis*. The use of colchicine and oryzalin at concentrations of 15 and 30 μM, resulted in a wider leaf surface (Figure 2) and higher chlorophyll content compared to the control. It is presumed that an increase in leaf surface area would increase the number of chloroplast organelles in the leaf cells. The more chloroplast organelles, the ability of the chloroplasts to produce chlorophyll pigments

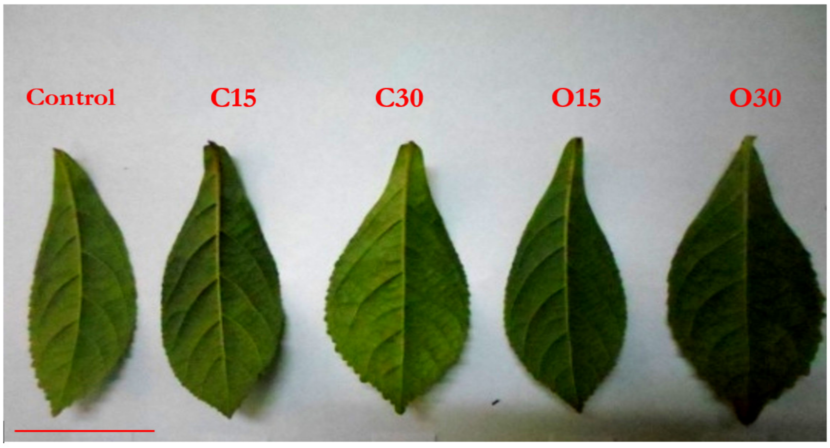

Figure 2. The leaf surface area at various treatments of control, colchicine (C15 and C30), oryzalin (O15 and O30). The leaves were obtained from the 7 days-old in vitro plantlets. Line Scale: 0.5 cm

Table 3. The anatomical response of *T. grandis* leaves at various concentrations of colchicine and oryzalin

Treatment (µM)	Leaf Thickness (µm)	Length of Stomata (µm)	Width of Stomata (µm)	Index (L/W Stomata)	Stomata density (per mm^2)	Number of Chloroplasts (per stomata)
Control	87.83 a	21.59 c	16.44 c	1.31 a	318.62 a	9.77 c
Oryzalin 15	93.43 a	23.97 bc	18.16 b	1.32 a	262.05 ab	11.37 bc
Oryzalin 30	102.81 a	27.82 b	19.62 b	1.42 a	202.03 cd	12.67 ab
Colchicine 15	99.77 a	24.85 bc	19.29 b	1.28 a	250.51 bc	10.88 bc
Colchicine 30	119.45 a	32.63 a	21.15 a	1.55 a	188.16 d	14.07 a

Note: Numbers in the same column followed by the same letters are not significantly different based on the F-Test at a confidence level of 95%

increased during the photosynthetic process, indirectly resulting in changes in leaf color that look darker green than the control. The result of our study coincided with that of Denaeghel et al. (2018). In their study on Escallonia illinita and *E. rubra*, they observed that one of the indicators of polyploidy was the increase in leaf area. Yan, Zhang and Zhang (2021) claimed that polyploid poplar (Populus euroamericana) had characteristics of increasing in leaf, thicker and rounder leaves, with a larger size of stomata and more of chloroplasts, but a lower stomatal index on abaxial leaf surfaces.

B. Anatomical and Cytological Responses

Anatomical and cytological analysis performed additional indirect and direct analysis of polyploid. The assessment was executed through observation of chloroplasts, stomata, and chromosome numbers. According to Manzoor, Ahmad, Bashir, Hafiz, and Silvestri, (2019), calculating the chloroplast content in stomata guard cells is an indirect method that has the advantage of being simple and fast for identifying ploidy levels. Furthermore, Tammu, Nuringtyas, and Daryono (2021) stated that the analysis has an accuracy rate of 90%. The results of Duncan's test on the anatomical response of leaves at various antimitotic concentrations are shown in Table 3.

It reveals that oryzalin and colchicine at concentrations of 15 and 30 µM increased the leaf thickness (Figure 3), length, and width of the stomata (Figure 4), as well as the number of chloroplasts in stomata guard cells (Table 3). Increasing the concentration of oryzalin and

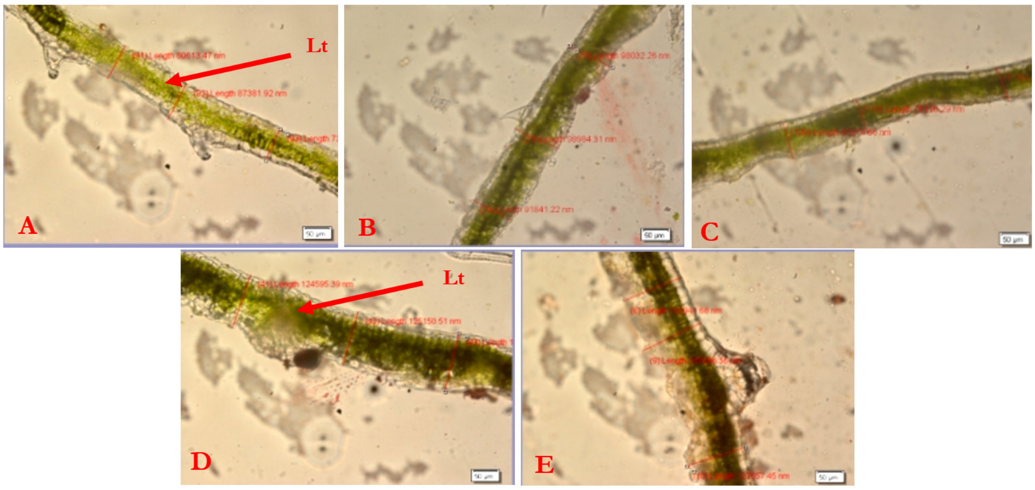

Figure 3. Anatomical leaf thickness of diploid and polyploid *T. grandis*. (A) Control, (B) Colchicine 15 μM, (C) Oryzalin 15 μM, (D) Colchicine 30 μM, (E) Oryzalin 30 μM.

Note: Lt=Leaf thickness. Line Scale: 50 μm

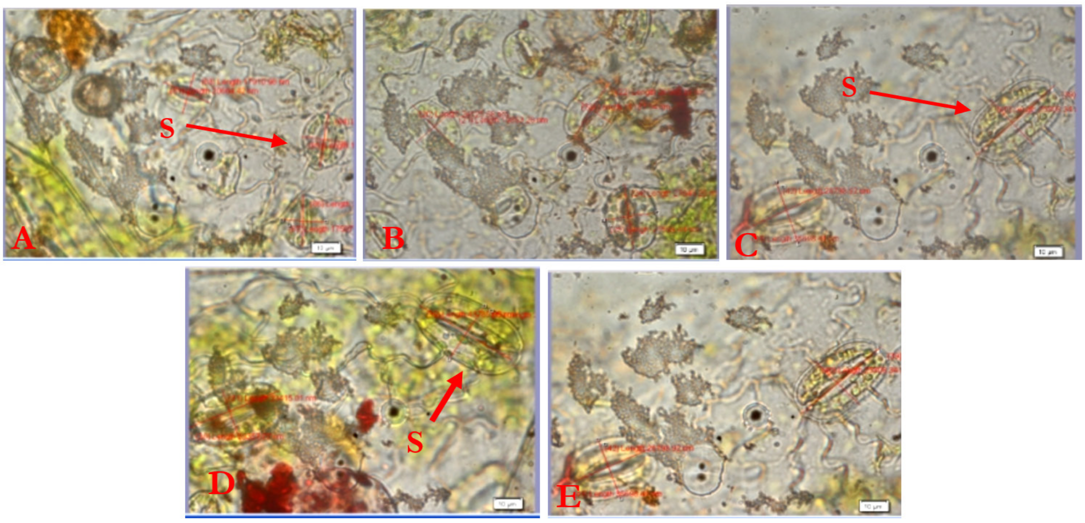

Figure 4. Leaf anatomical size of stomata of diploid and polyploid *T. grandis*. (A) Control, (B) Colchicine 15 μM, (C) Oryzalin 15 μM, (D) Colchicine 30 μM, (E) Oryzalin 30 μM.

Note: S=Stomata. Line Scale: 10 μm

colchicine from 15 to 30 μM tends to increase length and width of stomata considerably. Moreover, increasing the concentration from 15 to 30 μM also multiplied the significant number of chloroplasts, from 9.77 (control) up to 12.67 and 14.07, respectively. The concentration of oryzalin and colchicine at 30 μM influenced the alteration of the ploidy level from diploid to polyploid. These findings correspond to Oberprieler, Talianova, and Griesenbeck (2019) results on the Leucanthemum. In their experiment, the result showed that polyploid plants had more chloroplasts than diploids. The size of stomata is associated with the number of chloroplasts in stomatal guard cells. Increasing the number of chloroplasts per guard cell resulted in changes in the length and width of the stomata (Manzoor et al., 2019). Despite that, oryzalin and colchicine significantly reduced the stomata density per millimeter square (mm2) cell (Table 3). The stomatal L/W index shows the shape of the stomata, so the higher

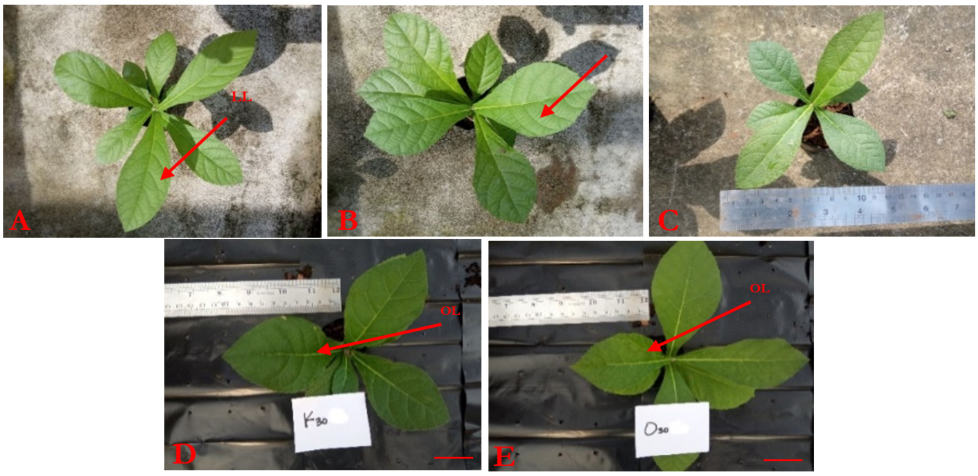

Figure 5. Leaf morphological performance of diploid and polyploid *T. grandis*.
The growth of 3 months-old plants after acclimatization. (A) Control, (B) Colchicine 15 µM, (C) Oryzalin 15 µM, (D) Colchicine 30 µM, (E) Oryzalin 30 µM.

Note: LL=Lancet Leaf, OL=Obovate Leaf.

Table 4. The percentages of polyploidy rate in *T. grandis* culture *in vitro*

Treatment		Life Culture (%)	(%) Ploidy	
Antimitotic agents	Concentration		Diploid	Polyploid
Control	0 µM	100	100 (9/9)	0 (0/9)
Oryzalin 15	15 µM	100	66.67 (6/9)	33.33 (3/9)
Oryzalin 30	30 µM	100	22.22 (2/9)	77.78 (7/9)
Colchicine 15	15 µM	100	55.56 (5/9)	44.44 (4/9)
Colchicine 30	30 µM	100	11.11 (1/9)	88.89 (8/9)

the stomata index, the more oval and larger the stomata shape. It is proven that increasing the concentration of oryzalin and colchicine can change the shape of the stomata from round to oval (Figure 4). Correspondingly, the shape of the leaves was also affected by the antimitotic treatments. The 30 µM concentration oryzalin and colchicine-treated *T. grandis* plants produced oval to obovate leaves, while the control plant had lancet-shaped leaves (Figure 5). The same phenomena were noticed on induced polyploidy plants, in which leaves were changed by the ploidy level (Manzoor et al., 2019; Wilson, Fradera-Soler, Summers, Sturrock, & Fleming, 2021).

The survival percentage and ploidy of T. grandis plantlets after antimitotic induction is presented in Table 4. The results showed that the immersion method at various concentrations of antimitotic agents combined with culturing on regeneration medium gave rise to a plant survival rate of 100% and a polyploidy rate ranging from 33.33 to 88.89%. It reveals that the higher the concentration of antimitotic added, the higher the percentage of polyploidy obtained. The largest polyploidy percentage was obtained at a colchicine concentration of 30 µM. At the same concentration, colchicine always produced a higher polyploidy rate compared to oryzalin. The results of our study showed that the polyploid *T. grandis* obtained was higher than that of Nugraha (2012), who combined the method of immersion and planting of *T. grandis* shoots in the antimitotic agent oryzalin at 5 µM. He generated an explant survival rate of 50-70% and a polyploidy success rate of 61.9%.

Comparing our results with Nugraha (2012), it was assumed that the highest percentage of polyploidy plantlets was obtained at a colchicine concentration of 30 μM. It showed that colchicine was more effective compared to oryzalin in generating polyploid *T. grandis*. It is believed that the method of culturing on the regeneration medium combined with immersion in the antimitotic solution directly impacts the antimitotic agents entering plant cells through the mechanism of absorption in the mitotic phase of the cell cycle. Syukur et al. (2019) verified that the application of antimitotic agents is more effective at the metaphase stage of mitotic cells, which affects the polyploidy in plants.

Further analysis to ensure alteration in the ploidy level was accomplished directly by looking at differences in the number of chromosomes in the cell nucleus. This direct observation and calculation are the most precise and accurate method. The number of chromosomes is easily calculated when the cell is in a late prophase before metaphase (Doyle & Coate, 2019). In this phase, the chromosomes are perfectly condensed, so it is easy to observe their morphology and quantity (Syukur et al., 2019).

The results demonstrated that the number of polyploid chromosomes was higher than the control diploid. Based on cytological examination, there was an increase in the ploidy level of *T. grandis*, from diploid to polyploid, resulting from the treatment of colchicine and oryzalin. The alteration in the number of *T. grandis* chromosomes from diploid (2n = 2x = 36) into polyploid was identified as follows: 1) tetraploid [2n = 4x = 72] after treatment of colchicine 30 μM; 2) polyploidy-1 [2n = 5x = 90] resulted from treatment of Oryzalin 15 μM; and 3) polyploidy-2 [2n = 9x = 162] resulted from treatment of Oryzalin 30 μM (Figure 3). Unfortunately, the chromosome numbers from colchicine 15 μM treatment specimens could not be identified, even after several attempts (Figure 6B). The reason is because the metaphase phase of mitosis in T. grandis cells was too short a time, so it was difficult to observe.

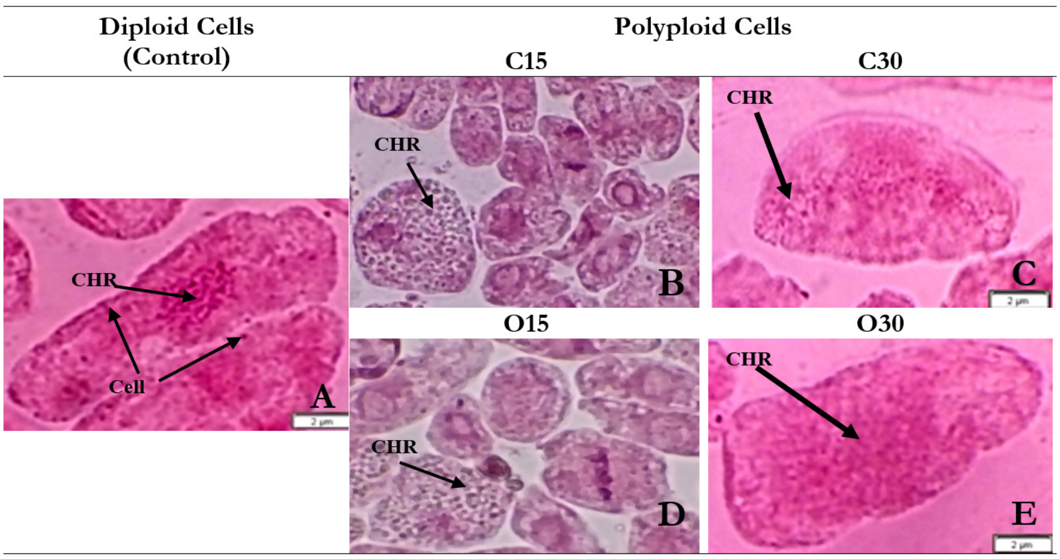

Figure 6. Image of polyploid cell of *T. grandis* under microscope resulted from various treatments of control, colchicine (C15 and C30), oryzalin (O15 and O30). A) Diploid cells [2n = 2x = 36]; B) Unidentified; C) Tetraploid cells [2n = 4x = 72]; D) Polyploid-1 cells [2n = 5x = 90]; E) Polyploid-2 cells [2n = 9x = 162]. Note: CHR=chromosomes. Line Scale: 2 μm.

Table 5. The growth of plantlets on germination medium at 6 weeks after acclimatization

Antimitotic Treatment (μM)	Rooting (%)	Survival (%)	Height (cm)	Stem Diameter (cm)	Number of leaves
Control	100 a	100 a	12.04 ab	0.151 a	11.11 ab
Oryzalin 15	100 a	100 a	9.45 c	0.141 a	9.33 b
Oryzalin 30	100 a	100 a	12.68 a	0.149 a	11.22 ab
Colchicine 15	100 a	100 a	10.65 bc	0.147 a	12.17 a
Colchicine 30	100 a	100 a	12.83 a	0.169 a	10.61 b

Note: Numbers in the same column followed by the same letters are not significantly different based on the F-Test at a confidence level of 95%

C. Acclimatization of Polyploid *T. grandis*

The results of the acclimatization and growth of polyploid *T. grandis* plantlets are revealed in Table 5.

Six weeks after acclimatization, we evaluated all plantlets resulting from antimitotic agent treatments. All the plantlets were developed successfully and were well-rooted. The average number of primary roots produced ranged from 3-5 roots per plantlet. Table 5 shows that the colchicine 30 (M) treatment had the best average plantlets height and stem diameter compared to other treatments. Nevertheless, it was not considerably different from the treatment with oryzalin (30 μM) and the control. Likewise, the number of leaves demonstrated the same phenomenon. Based on the evaluation of these parameters, it has been proven that polyploidy occurs in *T. grandis* seedlings, which is indicated by growing faster and bigger (Figure 7). Kardiman and Raebild (2018) argued that increasing the size of stomata leads to high rates of biomass accumulation, and consequently increasing plant growth.

Based on leaf morphology, particularly the edge of the *T. grandis* leaf was changed due to the ploidy level (shown in the Figure 7 by arrows). The usual diploid *T. grandis* leaf has entire to denticulate types of leaf margins, which are smooth to small-toothed edges. Increasing the ploidy level of *T. grandis* from diploid to polyploid resulted in change at the edge of the leaf. Polyploid *T. grandis* leaf has serrate to dentate types of leaf margin, which have a notched like a saw with teeth margin. The same occurrences were reported by other researchers on their polyploid plants (Baker et al., 2017; Corneillie et al., 2019; Mo et al., 2020).

Based on the data analysis in our study, it's believed that colchicine and oryzalin change the morphology and anatomy of teak seedlings from diploid to polyploid. The growth characteristics and wood performance could be recognized after stability tests and clonal tests at multiple locations in order to obtain more comprehensive data and information regarding changes in the genetic and physiological performance of teak seedlings from diploid to polyploid.

IV. CONCLUSION

Antimitotic agents affected the reduction of height, internode length and the number of leaves in *T. grandis* plantlets. They also increased the leaf surface area and chlorophyll content index. Additionally, oryzalin and colchicine increased the leaf thickness, length and width, and the number of chloroplasts in stomata, however, reduced the stomata density significantly. Among four treatments of antimitotic agents (oryzalin 15 μM, oryzalin 30 μM, colchicine 15 μM, and colchicine 30 μM), on the plantlets of *T. grandi*s the treatment of colchicine 30 μM, was the most effective to generate polyploidy plantlets, with a rate of effectiveness of 88.89%. The acclimatization of the *T. grandis* plantlet resulted in a high percentage of survival and rooting, as well as bigger and faster-growing plantlets. The polyploid *T. grandis* plantlets showed a fast

Figure 7. The growth of polyploid *T. grandis* plantlet during acclimatization after 15, 35, and 75 days at various treatments of control (A), colchicine 15 µM (B), colchicine 30 µM (C), oryzalin 15 µM (D) and oryzalin 30 µM (E). Line Scale: 1 cm.

growth and had more leaves compared to control. From this study we obtained the polyploid *T. grandis* as tetraploid [2n = 4x = 72], polyploid-1 [2n = 5x = 90], and polyploid-2 [2n = 9x = 162].

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THE POTENTIAL OF CARDAMOM LEAF IN THE AGROFORESTRY SYSTEM: ESSENTIAL OIL YIELD AND 1.8-CINEOL CONTENT

Dona Octavia[1,2], Nurheni Wijayanto[3*], Sri Wilarso Budi[3], Irmanida Batubara[4], and Sri Suharti[2]

[1]Post Graduate Study Program of Tropical Silviculture, Faculty of Forestry and Environment, IPB University, Bogor, Jawa Barat, 16680, Indonesia
[2]Research Center for Ecology and Ethnobiology, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta - Bogor KM. 46, Cibinong, Bogor, Jawa Barat, 16911, Indonesia
[3]Department of Silviculture, Faculty of Forestry and Environment, IPB University, Bogor, Jawa Barat, 16680, Indonesia
[4]Department of Chemistry, Faculty of Mathematics and Natural Sciences; Tropical Biopharmaca Research Center, IPB University, Bogor, 16880, Indonesia.

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THE POTENTIAL OF CARDAMOM LEAF IN THE AGROFORESTRY SYSTEM: ESSENTIAL OIL YIELD AND 1.8-CINEOL CONTENT. Cardamom (*Amomum cardamomum*), the 'Queen of Spices', is a native Indonesian spice and a type of potential biopharmaceutical currently prospective because of its high selling value, especially for its fruit, with various benefits and wide use. So far, cardamom essential oil comes from the use of its fruit, but production is limited. Therefore, its leaves have the potential to be developed as a source of essential oil since they are more abundant and available all year. In cardamom farming, low light intensity due to shading effects in agroforestry and low nutrients could stimulate the production of specific secondary metabolites. This study aimed to analyze the cardamom leaf essential oil (CLEO) yield and 1.8-cineol content of CLEO grown in agroforestry systems. The CLEO was obtained by steam-water distillation, while the 1.8-cineol content was analyzed by gas chromatography–mass spectrometry (GC-MS). The experimental design employed was a randomized block design with three cropping patterns, namely *Falcataria moluccana* + cardamom (FC), *F. moluccana* + cardamom + arrowroot (FCA), and Monoculture cardamom (MC) as treatment levels and three doses of bokashi manure as blocks. The results showed that the highest CLEO yield was generated in the FC agroforestry pattern of 3.16%, and the highest 1.8-cineol content in CLEO was generated in the FCA pattern of 47.23%. The lowest CLEO yield and 1.8-cineol content were found in the monoculture pattern of 2.02% and 43.16%, respectively. Compared to monoculture practices, agroforestry practices have the potential to increase the CLEO yield and 1.8-cineol content, which will be prospective in forest management to support forestry multi-business and social forestry programs.

Keywords: *Falcataria moluccana*, *Amomum cardamomum*, eucalyptol; medicinal plant; secondary metabolites

*POTENSI DAUN KAPULAGA DALAM SISTEM AGROFORESTRI: RENDEMEN MINYAK ATSIRI DAN KANDUNGAN 1,8-SINEOL. Kapulaga (*Amomum cardamomum*), sang 'Ratu rempah', merupakan rempah asli Indonesia, salah satu jenis biofarmasi potensial yang prospektif saat ini karena nilai jualnya yang tinggi, terutama buahnya, dengan beragam manfaat dan kegunaannya yang luas. Selama ini minyak atsiri kapulaga berasal dari pemanfaatan buahnya, namun produksinya terbatas. Oleh sebab itu, daun kapulaga berpotensi dikembangkan sebagai sumber minyak atsiri karena produksinya lebih banyak dari pada buah dan tersedia sepanjang tahun. Pada praktik budidaya kapulaga, rendahnya intensitas cahaya akibat efek naungan pada agroforestri dan rendahnya unsur hara dapat memacu produksi metabolit sekunder spesifik. Penelitian ini bertujuan untuk menganalisis rendemen minyak atsiri daun kapulaga (MADK) dan kandungan 1,8-sineol pada MADK yang ditanam pada sistem agroforestri. MADK diperoleh dengan distilasi uap-air, sedangkan kandungan 1,8-sineol dianalisis dengan kromatografi gas-spektrometri massa (GC-MS). Rancangan percobaan yang digunakan adalah Rancangan Acak Kelompok dengan tiga pola tanam sebagai perlakuan, yaitu *Falcataria moluccana *+ kapulaga (FK), *F. moluccana *+ kapulaga + garut (FKG), dan Monokultur kapulaga*

* Corresponding author: nurheniw@apps.ipb.ac.id

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(MK) serta tiga dosis pupuk bokashi sebagai blok. Hasil penelitian menunjukkan bahwa rendemen MADK tertinggi dihasilkan pada pola agroforestri FK sebesar 3,16%, dan kandungan 1,8-sineol tertinggi pada MADK dihasilkan pada pola agroforestri FKG sebesar 47,23%. Rendemen MADK dan kandungan 1,8-sineol terendah ditemukan pada pola monokultur masing-masing sebesar 2,02% dan 43,16%. Dibandingkan dengan praktik monokultur, praktik agroforestri berpotensi meningkatkan rendemen MADK dan kandungan 1,8-sineolnya yang prospektif dalam pengelolaan hutan lestari untuk mendukung program perhutanan sosial dan multi usaha kehutanan.

Kata kunci: Falcataria moluccana, Amomum cardamomum*, ekaliptol, tumbuhan obat, metabolit sekunder*

I. INTRODUCTION

Indonesia, 'a mega-biodiversity country', possesses the second-largest biodiversity, the second-highest number of indigenous medicinal plants, and the third-largest tropical forest area in the world (Yoganingrum, 2020; MOEFRI, 2022). Indonesia's tropical forests have great potential as a home for medicinal plants and food providers. However, Indonesia's tropical forest management encounters several challenges due to the high community need for forest resources, including their basic needs for food. More than 50% of villages are located on the edge and within forest areas (BPS, 2020). Currently, there are 19,410 villages located around forests. A total of 3.02% of villages are categorized as highly vulnerable to climate change, with a population of about 48.8 million people depending on the forest's resources (MOEFRI, 2022). Climate change also significantly influences crop production, leading to exacerbating the food crisis challenge.

The conversion of forests to agricultural land is the main cause of deforestation (FAO, 2021; FAO, 2022). Forest damage and land degradation directly impact the people living in these villages. Agroforestry becomes a land management system that can potentially reduce deforestation and soil degradation while ensuring food security and overcoming food crisis problems (Tomar et al., 2021). Furthermore, it provides environmental services and has good prospects for supporting sustainable food production (Rosso, Cantamessa, Chiarabaglio & Coaloa, 2021; Octavia et al., 2022; Bishaw, Soolanayakanahally, Karki & Hagan, 2022). Smart agroforestry (SAF) is a set of silviculture and agriculture knowledge and practices to improve environmental parameters, including soil and water conservation, biodiversity enhancement, climate change mitigation, and adaptation, as well as increase profits and resilience for farmers (Octavia et al., 2022). Climate SAF systems and practices provide a range of vital goods and services for human welfare, particularly for people living below the poverty line (Ntawuruhunga et al., 2023). Currently, agroforestry is encouraged to be developed in the Social Forestry (SF) program, particularly in tropical countries, as a solution to address several problems that cannot be solved just by forestry science (Gunawan et al., 2022). It can also significantly improve farmers' livelihoods due to better access to food, fodder, and fuelwood for livelihood capital (Jewel et al., 2022). SF schemes have been set as one of the priority activities that support the Poverty Alleviation Priority Program in Indonesia (Suharjito, Rahayu, Kartika, Arsyad & Meilantina, 2023). Agroforestry for rehabilitation activities is also encouraged by Indonesia's Forestry and Other Land Use (FOLU) Net Sink 2030 for mitigating and controlling climate change (MOEFRI, 2022).

Agroforestry systems have the potential to stimulate the production of secondary metabolites or bioactive compounds when the stress of water or soil nutrients occurs, as well as the stress of light intensity due to shading effects and a lack of soil fertility or other important resources needed by plants. Secondary metabolites are beneficial to assess the quality of therapeutic ingredients, which are used nowadays as important natural-derived drugs such as immune suppressants, antioxidants, antibiotics, anticancer drugs, and anti-diabetics. In addition, plants can produce a variety of secondary metabolites to manage the negative effects of stress (Jan, Asaf, Numan, Lubna & Kim, 2021; Pant, Pandey & Dall'Acqua, 2021). This potential serves the short-term benefit of compensating for the long-term benefit of planting trees in agroforestry sites. Leaves, branches, and twigs can be a potential source of secondary metabolites (Verma, Kumar & Saresh, 2021).

Regarding the high dependence of farmers on tropical forest services (Njurumana et al., 2020), optimizing forest land use and increasing its productivity are urgently needed to gain ecological and economic benefits for the community and to avoid pressure on the forest by diversifying species, including multipurpose tree species of legume groups, medicinal plants, and food crops. One of the fast-grown legume tree species with the multipurpose potential is 'sengon' (*Falcataria moluccana*), while the group of spice/medicinal plants and food crops that have the potential to be developed include cardamom (*Amomum cardamomum*) and arrowroot (*Maranta arundinacea*). *F. moluccana* is one of plantation forest commodities in Indonesia and is one of the most popular tree species as shade plants used by farmers. As a legume group, sengon provides nitrogen (N) that maintains soil fertility, can grow on marginal land and is easily cultivated and marketed (Hani & Octavia, 2020).

Cardamom fruit has a high economic value, while the production of the leaf is higher than that of the fruit in a cardamom clump. Therefore, the potential of other parts of the cardamom species, including the leaf, is an important one to study in its cultivation through agroforestry systems. Pruning leaves, an integral aspect of tending and leaf litterfall, yields enhanced value when processed into essential oil within agroforestry practices. Thus, this potential will be able to enhance farmers' livelihoods and augment the community's income, as well as expand the benefits to those residing in the SF area.

The content of 1.8-cineol in cardamom leaf has an interesting potential to be developed as a component of medicinal raw materials in the current COVID-19 pandemic era. The previous study revealed that among the seven *Zingiberaceae* species, *Elettaria cardamomum* leaf generated the highest yield of essential oil, which was 2.43%. This species also generated the best antibacterial activity, which found 1.8-cineol as the main compound responsible for antibacterial activity (Batubara, Wahyuni & Susanta, 2016). The antimicrobial (antifungal, antibacterial, anti-pathogenic) and immune-modulatory effects of cardamom essential oil have also been reported in several studies (Heimesaat et al., 2021).

The 1.8-cineol is classified as a monoterpenoid compound a secondary metabolite, which is extensively used in healthcare products and cuisine (Jan, Asaf, Numan, Lubna & Kim, 2021). The 1.8-cineol compound has fresh characteristics with a pungent aroma and sharp taste and has bioactivity with various benefits. Some studies found that the 1.8-cineol content with antiviral, anti-inflammatory, antioxidant, and antimicrobial activity can increase protection against influenza virus attacks (Bahrami, Yaghmaei & Yousofvand, 2023) as well as have the potential to be an alternative treatment to relieve symptoms of COVID-19 (Tshibangu et al., 2020).

In recent decades, demand for herbal medicines, including essential oil from the medicinal plant, has increased due to their lower side effects compared to conventional medicine. Cardamom is currently prospective because of its high selling value, with a variety of benefits and wide use, as well as being high needs, specifically in the pharmaceutical field (Nair, 2020). The long rotation period of trees becomes the main constraint on the spread of agroforestry. These benefits of secondary metabolites from leaves can be harvested many times during their growing period. Therefore, finding and analyzing the important secondary metabolites in leaves produced by plants in agroforestry systems is important in this research. This study aims to analyze the potential yield and 1.8-cineol content of cardamom leaf essential oil (CLEO) in agroforestry systems.

II. MATERIALS AND METHODS

A. Study Site

The research was conducted in October 2021 - September 2022 at the Cikabayan Forest, Bogor Agricultural University (IPB University), West Java, Indonesia. The study site was at an altitude of 150-200 meters above sea level with coordinate of latitude 6°32'48" S and longitude 106°42'58" E.

B. Tools and Materials

Planting materials used were cardamom seedlings (having at least 4-5 leaves and a height of 70-90 cm), arrowroot tubers (cultivar Creol), three-year-old sengon stands (which previously existing with a planting space of 1.5 m × 1.5 m), and manure (bokashi) fertilizer. Bokashi, 'fermented organic matter' containing microbial groups having the ability to create beneficial bioactive substances and enzymes as well as to execute many kinds of useful functions, including the breakdown of hazardous chemicals and waste. It also stands for Organic Material Rich Biological Sources and can be produced by the community themselves by utilizing the forest resources from the surrounding area (Kote, Lailogo, Purmanto & Hewe, 2021). The mixed planting of cardamom and arrowroot under the sengon stands (in an agroforestry pattern) was carried out on a 3 m × 3 m plot. Likewise, the monoculture plot in the opened area also uses the same size of plot, with a planting space of 1 m × 1 m and consisting of 16 seedlings or tubers in each plot (Figure 1). Cardamom seedlings used were local cardamom of red cultivars from Pamijahan sub-district, Bogor Regency, while the arrowroot tubers used were selected genotype and phenotype of West Java accessions from the germplasm collection in Dawuan experimental garden of National Research and Innovation Agency (BRIN) at Subang, West Java. The tools used were a GPS, lux meter, thermohygrometer, distillation unit, and gas chromatography-mass spectrometry (GC-MS).

C. Methods

1. Leaf sample preparation

Cardamom leaves were harvested from a ten-month-old cardamom plant and formed as a composite from cardamom plant in each treatment for the tested samples. Furthermore, the leaves were distilled by steam-water distillation method to generate essential oil.

2. Yield of cardamom leaf essential oil measurement

Steam-water distillation was applied to the samples of cardamom leaves, and the test method was based on SNI 01-3180-1992. Cardamom leaves were weighed before being put into the distillation kettle and measured for the water content. A total of 3 kg of leaves were distilled for each treatment. Heating was carried out at a temperature of 100 °C. The distillation time was calculated from the first drop for

FC FCA MC

Note: FC = *Falcataria moluccana* +cardamom, FCA=*F.moluccana* + cardamom + arrowroot, MC=monoculture cardamom

Figure 1. Planting patterns (cardamom and arrowroot) in agroforestry and monoculture plots

4-5 hours until no more oil drops were added. Subsequently, oil was collected in a measuring cup, and 2% of the oil volume was added with Na_2SO_4, then filtered using filter paper until pure CLEO was obtained. The essential oil yield of cardamom leaves was determined based on their dry weight. Oil yield was measured using

$$\text{Oil yield (\%)} = \frac{\text{Volume of oil produced (ml)}}{\text{Dry weight of processed material (g)}} \times 100\% \quad ..(1)$$

the following formula:

3. Analysis of 1.8-cineol content

For further analysis, the 1.8-cineol content of the CLEO were determined using the steam-water distillation method. Each treatment consisted of three replications. The 1.8-cineol content was determined by the GC-MS method concerning the method performed by Batubara, Wahyuni and Susanta (2016) with some adjustments. The CLEO was further analyzed by GC-MS (Shimadzu-QP-2010 Ultra, column: Rtx-5MS, 30m x 250 µm ID x 0.25 µm film thickness) and the temperature was programmed from 60 °C to 270 °C (for 28 minutes) at a rate of 10 °C per minute. The injection port temperature was 270 °C, meanwhile, the detector temperature was 250 °C. The inlet pressure was 8.23 psi, and the injection mode was split (200:1). Helium was employed as the carrier gas, with a flow rate of 0.83 mL per minute. The mass spectrometer conditions were set as follows: MS source temperature at 25 °C, MS quadrupole temperature at 150 °C, interface temperature at 270 °C, and ionization voltage at 70 eV. Cineol and other compounds were identified by comparing the mass spectra of cineol and other compounds with spectra from NIST library data in the literature.

4. Experimental design

The experimental design employed was the randomized block design, with three planting patterns by species combination as the treatment in agroforestry models as follows: A1: *F. moluccana* + cardamom (FC), A2: *F. moluccana* + cardamom + arrowroot (FCA), A3: Monoculture cardamom (MC), and doses of manure fertilizer as replication (block), which consisted of 3 levels (B1 = 0, B2 = 250, and B3 = 500 g for each plant), on a plot area of 1500 m^2.

D. Data analysis

The CLEO yield and 1.8-cineol content data were analyzed with analysis of variance (ANOVA) at a confidence level of 95% using SAS 9.4 software. Duncan's Multiple Range Test (DMRT) was used for further analysis when the variance (F-test) showed a significant effect. Origin 85 software was employed..(2) for analyzing and graphing chromatograph data of 1.8-cineol content. The 1.8-cineol content in leaves per plant was obtained as follows: ..(3)

1.8-cineol content in leaves per plant = 1.8-cineol in each g leaves × the leaf dried weight per plant

1.8-cineol in per g leaves = CLEO yield × 1.8-cineol content in the essential oil

III. RESULTS AND DISCUSSION

A. Results

1. Environmental condition of the planting site

In this research, cardamom grew up well at an altitude of 150-200 meters above sea level, where the average air temperature was 28.7 °C and air humidity was 79.5%. The shade intensity of 3-4- year- old *F. moluccana* amounts to an average of 65%, ranging from 45 – 73%, as shown in Figure 2. The soil texture was clay, with a clay content range of 54-60%, a cation exchange capacity (CEC) value in medium category of 16.1– 18.4 cmol(+)/kg, a N total before planting of 0.27 (medium category), and a N total after 10 months of planting of 0.34. The C-organic content was high (3.1%), equivalent to soil organic matter (SOM) of 5.2%.

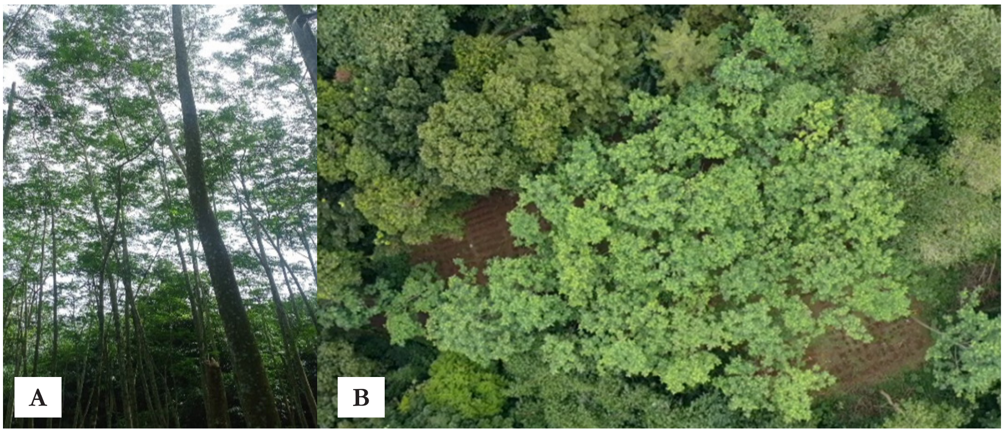


Figure 2. Canopy shade of sengon, viewed from under (A) and above (B) canopy

Table 1. The CLEO yield, 1.8-cineol content, and cineol quantity of CLEO in different planting patterns and doses of manure fertilizer

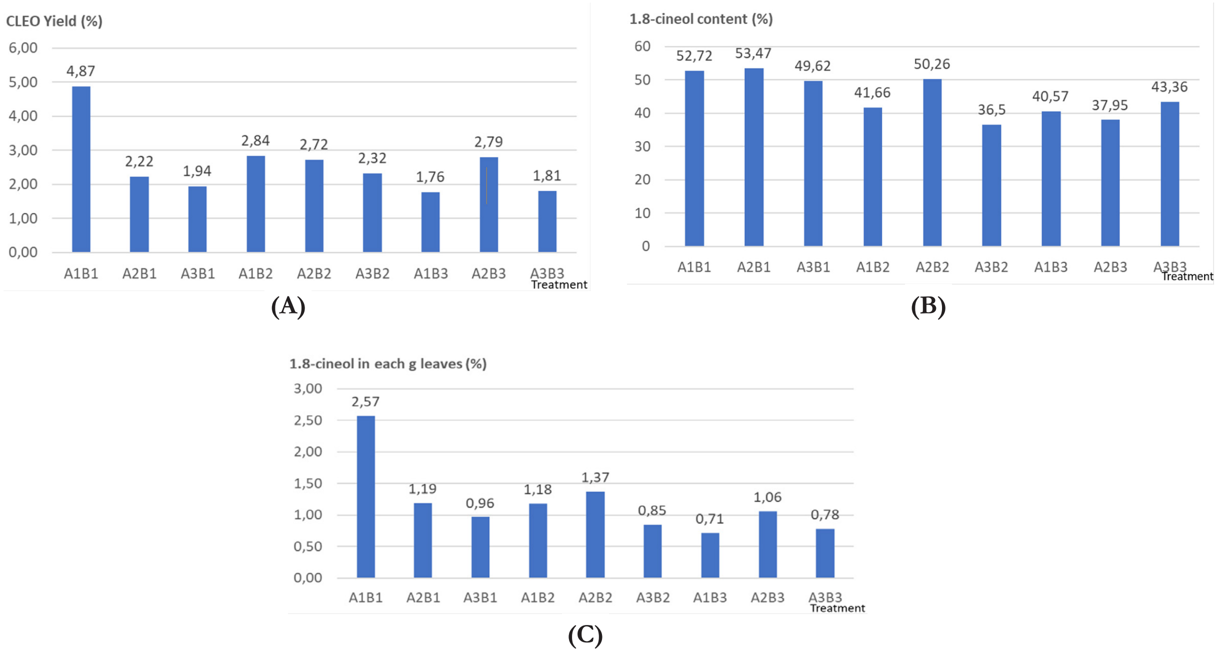
Treatments	Yield of CLEO (%, g essential oil in g dried leaves)	1.8-Cineol content (%, cineol in CLEO)	1.8-Cineol in each g leaves (%)	Fresh leaves weight per plant (g)	1.8-Cineol in leaves per plant (g)
Planting pattern					
A1 (FC)	**3.16**	44.98	**1.49**	**1249.60**	**17.78**
A2 (FCA)	2.58	**47.23**	1.21	1135.69	13.64
A3 (MC)	2.02	43.16	0.86	1211.87	10.60
Dose of manure					
B1 (0 g/plant)	**3.01**	**51.94a**	**1.57**	1212.34	**18.41**
B2 (250 g/plant)	2.63	42.81ab	1.13	1146.16	13.08
B3 (500 g/plant)	2.12	40.63b	0.85	**1238.66**	10.52

Note: Mean values followed by different letters within a column indicate that the treatment has a significantly different effect ($P < 0.05$). The mean value in bold is the highest value among the treatments. FC = *F. moluccana* + cardamom; FCA = *F. moluccana* + cardamom + arrowroot; MC = monoculture cardamom; CLEO = cardamom leaf essential oil.

2. Yield and 1.8 cineol content of cardamom leaf essential oil in an agroforestry system

Table 1 shows how the type of agroforestry planting and the amount of manure fertilizer changed the yield of CLEO, the amount of 1.8-cineol present, and the content of 1.8-cineol. Table 1 shows that agroforestry planting models generated a higher yield, 1.8-cineol content, and cineol quantity of CLEO (FC and FCA). Meanwhile, the lowest value of the three variables was gained from the monoculture planting pattern (MC). The highest yield, cineol content, and cineol quantity of CLEO were generated by the block of 0 g of manure fertilizer application (without applied manure fertilizer). The lowest value of the three variables was obtained in the block of 500 g of manure fertilizer application.

The fresh weight of leaves per plant shown in Table 2 is the real plant production, not the sample weight for the analysis of 1.8-cineol content. Likewise, the 1.8-cineol content did not have a positive correlation with leaf fresh weight. The 1.8-cineol content is correlated to the abundance of cineol compounds among the other bioactive compounds in CLEO. In Table 2, it can be seen that the highest fresh weight of leaves is found in the dose of 500 g of treatment (1238.66), but in contrast, they have the lowest 1.8-cineol content in each g leaf weight of 0.85%and also the lowest 1.8-cineol



Note: A1 = *F. moluccana*+cardamom; A2 = *F. moluccana*+cardamom+arrowroot; A3 = monoculture cardamom;
B1 = 0 g manure; B2= 250 g ; B3= 500 g

Figure 3. Yield of CLEO (A), 1.8-cineol content in CLEO (B), and 1.8-cineol in each g leaf (C) for each treatment

Table 2. Production of essential oil and 1.8-cineol from the pruned leaves in each treatment

Treatments	Yield (%, g essential oil in g dried leaves)	1.8-cineol in each g leaves (%)	Amounts of 1.8-cineol in leaves per ha (kg)	Amounts of CLEO per clump (ml)	Amounts of CLEO per ha (liter)	Income estimation per ha (IDR)
FC	3.16	1.49	37.25	7.89	78.9	316.000.000
FCA	2.58	1.21	30.25	6.45	64.5	258.000.000
MC	2.02	0.86	21.50	5.05	50.5	202.000.000

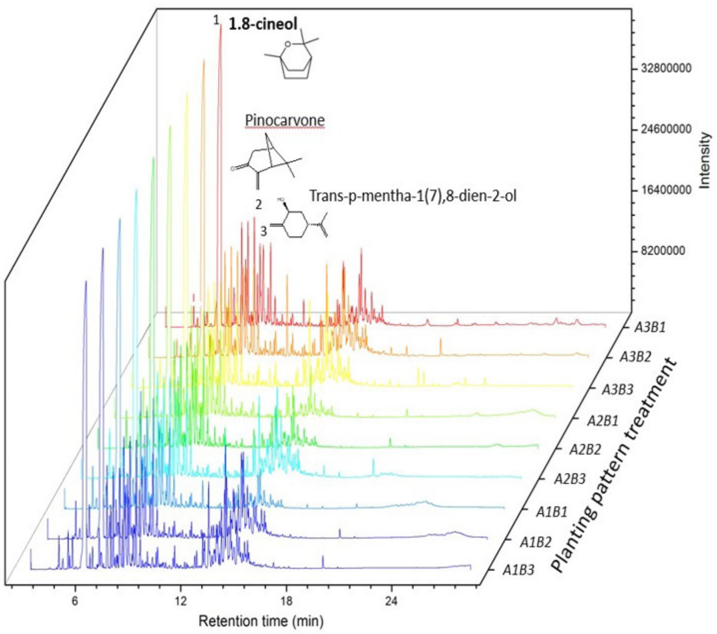
Note: 1 USD equal to IDR 14,500

content in CLEO of 40.63%. The effect of planting patterns at various doses of manure fertilizer on the CLEO yield and 1.8-cineol content is shown in Figure 3.

Figure 3 demonstrated that the FC agroforestry planting model with no manure fertilizer (A1B1) and FCA with no manure fertilizer (A2B1) produced the highest CLEO yield and 1.8-cineol content, respectively. Whereas, the lowest CLEO yield was attained in the FC planting pattern with 500 g of manure fertilizer (A1B3), which was 1.76%, even though it was not significantly different from that in monoculture (A3B3), which was 1.81%. Likewise, the lowest 1.8-cineol content in CLEO was obtained in the monoculture plot with 250 g of manure fertilizer (A3B2), which was 36.5%. The 1.8-cineol quantity referred to the 1.8-cineol content in each g of cardamom leaf weight in each treatment. The highest 1.8-cineol in each g of leaves was generated by the A1B1 treatment, amounting to 2.57%; meanwhile, the lowest value was generated in A1B3, amounting to 0.71 %; even though it was insignificantly different from that in monoculture (A3B3) amounting to 0.78%. Table 2 presents the production of essential oil and 1.8-cineol quantity from the pruned leaves of cardamom per hectare in planting spacing of 1 m × 1 m, with a number of 10,000 cardamom clumps per hectare when 1000 g of leaves were pruned per clump (the average leaf moisture content was 75%), resulting in dry leaf weight amounting to 250 g as distilled material.

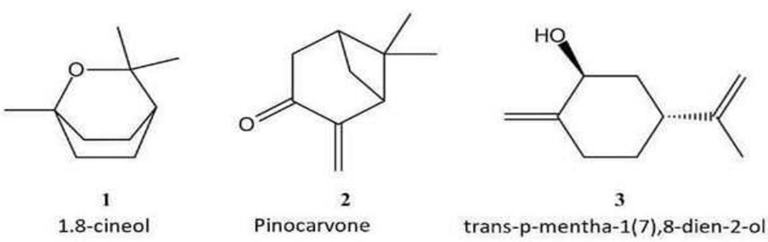
The chromatogram of CLEO in each treatment showed that 1.8-cineol was the one major compound found in CLEO at a retention time range of 6.133-6.147 minutes (Figure. 4). The other compounds in the second and third ranks of the area percentage and their chemical structure are shown in Figure 5.

Based on the GC-MS results, two other main bioactive compounds were found in addition to 1.8-cineol. Origin 85 software was used to draw their structures. In addition to the three compounds above, several other compounds were also found, among others lemnalol, santalol, alpha-pinene, linalool, carvone, gamma-terpinene, cyclohexen, trans-pinocarveol, (1R)-(-)-myrtenal, beta-santalol, (-)-trans-isopiperitenol, cis-p-mentha-1(7),8-diene-2-ol and (1R,2R,4S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylene for various benefits.



Note: A1 = *F. moluccana*+cardamom; A2 = *F. moluccana*+cardamom+arrowroot; A3 = monoculture cardamom; B1 = 0 g manure; B2= 250 g ; B3= 500 g

Figure 4. Chromatogram of *Amomum cardamomum* leaves essential oil in each treatment



Source: Primary data

Figure 5. Structure of three major compounds found in cardamom leaf essential oils

B. Discussion

1. Environmental condition of the planting site

The environmental conditions in cardamom planting plots are under the plant growth requirements. Cardamom is a plant species that requires shade throughout its life in fertile and loose soilat an altitude of 0-500 m above sea level with rainfall of 2500-4500 mm/year (Hani & Octavia, 2020). The total N content of the soil increased by 29% at 10 months after planting (MAP) in agroforestry plots (FC and FCA models). This is assumed because the litter of fallen leaves rapidly decomposes to become a nutrient source for plants.

Interplanting annual and perennial legumes can increase soil fertility by enhancing plant-available N and potassium (K) and plant yield, as well as farming profitability, so that the planting pattern can sustain the above-ground biomass cover (Tibasiima et al., 2023). The phosphorus (P) released from leaf litter was affected by the vegetation type, which was related to the favored soil microbial populations at tree roots during decomposition (Correa, Carvalhais, Utida, Oliveira & Scotti, 2016). Gunawan, Wijayanto and Budi (2019) reported that eucalyptus-based vegetable agroforestry planting patterns in some age classes improved the soil chemical fertility status for CEC, available P, and available K into high categories. Another study showed that complex agroforestry and natural forest land had similar physical soil quality (Briliawan, Wijayanto & Wasis, 2022; Purnama, Wijayanto & Wasis, 2022). Furthermore, multi-strata agroforestry can be a solution for improving soil quality on degraded lands. Multistrata agroforestry is an agroforestry planting system with a combination of plant species with a variety of canopy heights (high, medium, and low canopy), such as F. moluccana (high canopy) with cardamom (medium canopy) and grass (low canopy). Iskandar et al. (2022) also stated that revegetation served as a driver of physical and chemical soil property changes.

The SOM in the demonstration plot area was still high, reaching 5% under ten MAP. Despite there was a small decrease, the occurrence was lower in agroforestry plots compared to monoculture. This was caused by the positive influence of leaf litter, twigs, and root activity of *F. moluccana* trees which provided soil nutrients. The plants' combination found in agroforestry practices increases the chances of maintaining soil fertility resulting from litter decomposition to maintain soil organic C content, enhancing food security, land productivity, and biodiversity (Mulia & Phuong, 2021).

Another study revealed that soil organic C in *Acacia* stand plantations was subjected to changes that tended to decrease by increasing the age of the stand due to an increase in the ability of trees to absorb carbon. Furthermore, the youngest stand shows the highest soil organic C (Lee, Ong, King, Chubo & Su, 2015). The SOM is a crucial soil component that can accumulate as a component of a closed nutrient cycle with minimal nutrient losses, particularly in natural forests (Widyati et al., 2022). The agroforestry model produces more litter layers due to the presence of litterfall (twigs and leaves) on the soil surface, which can increase soil infiltration, minimize runoff, and maintain the availability of organic matter needed by plants. Besides that, this condition reduces the need for fertilizer and pesticide use in agroforestry systems, amounting to half of the fertilizer and pesticide requirements for monoculture farming (Nuddin, Arsyad, Putera, Nuringsi & Teshome, 2019). Reducing the use of fertilizers and pesticides is profitable for farmers, specifically those with limited start-up capital.

In agroforestry plots, earthworms are more commonly found, and this is an indicator of soil fertility. Hani and Suhaendah (2019) revealed that the number of soil macrofauna that become organic decomposers in agroforestry is higher than that in non-productive land. Therefore, land management with an agroforestry pattern has a positive impact on community livelihood and forest sustainability. According to Kainama, Matinahoru and Latumahina (2021), agroforestry provides opportunities for communities to increase their participation in forest management and obtain benefits from forests to increase social, economic, and ecological sustainability through social forestry programs. These above-mentioned conditions may provide promising benefits and are prospective for being applied in the social forestry area.

2. The potential yield and 1.8 cineol content of cardamom leaf essential oil (CLEO) in an agroforestry system

In this research, 1.8-cineol (in the commercial trade called 'eucalyptol') is the one major compound found in CLEO at a retention time range of 6.133-6.147 minutes, which was about 37-53% of the abundance of 1.8-cineol content. In line with another study on another species of cardamom, 1.8-cineol is also the major compound found in *Elettaria cardamomum* essential oil at a retention time of 8.781 minutes, about 83% in abundance. This compound serves as an antibacterial against *Streptococcus mutans* and for biofilm degradation (Batubara, Wahyuni & Susanta, 2016).

Another interesting finding from this study is that CLEO yield, 1.8-cineol content in CLEO and 1.8-cineol in each g of leaves, were higher in cardamom leaves at agroforestry plots (FC and FCA) than in monoculture planting patterns (MC). The highest value is also found in plant treatments without bokashi manure biofertilizer. This shows that the lack of light intensity under the sengon stands and fewer nutrients stimulate the production of CLEO and 1.8-cineol content. Light quality can impact the synthesis of secondary metabolites and bioactive compounds in plants (Jan, Asaf, Numan, Lubna & Kim, 2021; Pant, Pandey & Dall'Acqua, 2021). This was also confirmed in another study performed by Juliarti, Wijayanto, Mansur & Trikoesoemaningtyas (2022) on the other species that yield (2.84%) and 1.8-cineol content (50.70%) in cajuput leaf essential oil produced under agroforestry model is higher than that under the monoculture plot. The dosage of fertilizer had no significant effect on cajuput leaf essential oil yield and 1.8-cineol content. However, in this research, the dosage of fertilizer significantly affected the 1.8-cineol content of CLEO. Ramezani, Rezaei and Sotoudehnia (2019) also stated that nutrient effect on the chemical composition of EO varied among plant species, environmental conditions, and EO components.

The availability of nutrients had a significant effect on the 1.8 cineol content but not on the weight of fresh cardamom leaves. The highest weight of fresh leaves was found at agroforestry FC and application of bokashi 500 g. It was similarly discovered in another study that revealed the interaction of cropping pattern treatment and biofertilizer application did not significantly affect malapari growth, but the combination of the application of mycorrhiza biofertilizer and organic manure produced the highest growth after planting (Hani, Dendang & Pieter, 2021).

Another study on *Mahonia* ('well-known traditional Chinese' medicine) used for the treatment of several diseases also showed a higher yield of alkaloids under 50% of sunlight followed by 30% of sunlight rather than under 10% of sunlight and 100% of sunlight (Li, Kong, Liang & Wu, 2018). The opposite situation was also reported in traditional medicine *Flourensia cernua*, that used to treat various diseases, and indicated a higher content of total phenolic compounds under partial shade rather than under fully irradiated situations (Pant, Pandey & Dall'Acqua, 2021).

Subsequently, another study revealed that DNA methylation is probably responsible for changes in the content of the major secondary metabolites in a novel kind of tea cultivar 'Yujinxiang' in China recently, which is assumed to be related to the increased leaf chlorophyll level under the shading effects (Xu et al., 2020). The N, P, and K fertilizer applications affected essential oil biosynthesis in medicinal plants. They influence the levels of enzymes, which are very important for the biosynthesis of terpenoids (Verma & Shukla, 2015). Their effect on the chemical composition of EO varies among plant species, environmental conditions, and EO components (Ramezani, Rezaei & Sotoudehnia, 2019). For example, in *Ocimum basilicum* foliar, spraying of N increased the concentration of epicadinol and linalool but decreased 1.8-cineol, eugenol, and geraniol (Nurzyn´ska-Wierdak, Bogucka-Kocka, Sowa & Szymczak, 2012). Furthermore, eugenol, linalool, 1.8-cineol, and germacrene-D concentrations were not influenced by phosphorus application in sweet basil, while in *Chamomilla recutita*, α-bisabolol content increased with elevated phosphorus application (Rioba, Itulya, Saidi, Dudai & Bernstein, 2015). The other study showed that fertilization of 200 kg P ha^{-1} on *Vitex negundo* generated the highest essential oil yield and biomass, the highest number of volatile components, and the highest content of bioactive ingredients. Therefore, NPK fertilization treatment resulted in positive effects on the essential oil yield, biomass, and bioactive compounds of cultivated *V. negundo* (Peng & Ng, 2022). The primary and secondary metabolite content in plants is the main factor that determines their nutrition and health as well as promotes the value of the plant (Saleh, Selim, Jaouni & Abdelgawad, 2018).

Several factors can affect the composition of essential oil (EO) content, among them growth conditions, soil type, altitude, climate, cultivation and agricultural methods, including fertilization, plant part extraction, developmental stage, and harvesting time. Several factors determine the yield and chemical variability of EO for each plant species, including physiological and geographic variations, environmental conditions, genetic factors, and the amount of plant material (Figueiredo, Barroso, Pedro & Scheffer, 2008). The synthesis of secondary metabolites can be affected by fertilization activity (Ahmed, Shalaby & Shanan, 2011) and environmental changes, including several changes in daily temperatures, rainfall, drought, and the length and intensity of the sunlight (Marsic et al., 2011). The other study showed that effective environmental factors such as altitude and temperature were related to 1.8-cineol, menthone, and limonene content (Mollaei et al., 2020).

The light intensity and photoperiod also significantly affect the accumulation of plant secondary metabolites (Moghaddam & Mehdizadeh, 2017). Nitrogen and phosphorus are the main nutrients in fertilizers, serving as main factors in controlling the production of primary and secondary metabolites (Omer et al. 2014). Stress under certain environmental conditions, including drought, heat, and high or low light intensity in vegetation, encourages the production of high amounts of reactive oxygen species (ROS) through certain mechanisms, thus generate higher specific secondary compounds. ROS plays a key role in the plants' acclimation process to abiotic stress, which enables them to regulate and adjust their metabolism and fits a proper acclimation response in specific conditions (Verma, Kumar & Saresh, 2021; Pant, Pandey & Dall'Acqua, 2021).

Light-intensity stress under the shade effect can stimulate the production of secondary metabolites, which positively impacts the content of secondary metabolites in plants under the stands. Plants can adapt to changes in light radiation by accumulating and releasing different secondary metabolites, such as terpenoids and phenolic compounds, which have high economic value and use related to their antioxidant properties (Jan, Asaf, Numan, Lubna & Kim, 2021). Environmental conditions with limited light intensity under the shade of *F. moluccana* stands, and limited nutrients in the cultivation process without manure fertilizer application can stimulate and increase the secondary metabolite content and 1.8-cineol content of CLEO in this research.

Table 2 showed that the FC treatment in agroforestry systems generated the highest yield of the two other types of planting patterns, amounting to 78.9 liters of CLEO per hectare. Based on the selling price of around IDR 400 thousand or $27.6 per 100 ml of CLEO (price range according to the marketplace or online platform), the potential income earned from CLEO yield reaches IDR 316 million per hectare. This amount will significantly increase the farmers' livelihood. Meanwhile, the monoculture yielded amounting to 50.6 liters per ha, generating the lowest income of IDR 202 million per ha. This reveals that agroforestry practices can increase the yield and 1.8-cineol content of CLEO, providing a higher income. The production cost of CLEO in this study is low enough, around $0.88 per 10 mL, hence, the profits to the community are higher based on the selling price. It is lower than the production cost of *Cratoxylum formosum* leaf essential oil which reaches $ 4.84 in other studies (Hidayat, Fauzi, Saragih & Harianja, 2023). The use of pruned leaves from cardamom tending activities in agroforestry systems provides added value to increase farmers' income in addition to cardamom fruit with high selling value.

The existence of crop diversification in agroforestry systems, especially those developed in SF areas, increases the community's income from monthly, quarterly, and semi-annual yields. The higher the yield and 1.8-cineol content in CLEO under the agroforestry system, the greater the benefits will be obtained from the utilization of cardamom leaves, both from pruning leaves in tending activities and the litter fallen. This condition provides added value for its development in supporting SF implementation and forestry multi-business.

IV. CONCLUSION

The main results of this research could be summarized as follows: the highest yield of CLEO produced from the *F. moluccana* + cardamon (FC) agroforestry planting pattern was 3.16%, which was 27-56% higher than that from the monoculture cardamom (MC) planting pattern (2.02%). Likewise, the highest 1.8-cineol content was produced from the *F. moluccana* + cardamon + arrowroot (FCA) agroforestry pattern amounting to 47.2%, which was 4-9% higher than that in the MC planting pattern (43.2%). Interaction between FC agroforestry planting patterns (A1) without manure applications (B1) provided the highest yield of CLEO amounting to 4.82%. Likewise, interaction between FCA agroforestry planting patterns (A2) without manure applications (B1) provided the highest 1.8-cineol content of CLEO amounting to 53.47%. Agroforestry practices had the potential to increase the yield and 1.8-cineol content of CLEO which provided added value in its development with the potency to increase farmer's income. Furthermore, this agroforestry model also contributed to the success of social forestry program, by increasing the potential of community multi-business development. Based on these findings, it is important to perform further research to analyze the influence of agroforestry planting pattern on the content of bioactive compounds, including the oil yield and 1.8-cineol, in other parts of cardamom, such as the stem or fruit bunch. They were usually wasted in pruning and harvesting activities and not counted as crop yield, but had the opportunity to be further used.

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CULTURABLE ENDOSYMBIOTIC BACTERIA FROM THE INDIAN LAC INSECT, *KERRIA LACCA* (KERR)

Sweta Verma[1*], Hemant Kumar[2], Ranganathan Ramani[3], and Ramesh Chadra[1*]

[1]Department of Bioengineering and Biotechnology, Birla Institute of Technology, Mesra, Ranchi – 835215, India
[2]ICAR – National Institute of Secondary Agriculture, Namkum, Ranchi – 834010, India
[3]Department of Life Sciences, Central University of Jharkland, Manatu Ranchi – 835205, India

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CULTURABLE ENDOSYMBIOTIC BACTERIA FROM THE INDIAN LAC INSECT, *KERRIA LACCA* (KERR). The Indian lac insect, *Kerria lacca* (Kerr) (Coccoidea: Tachardiidae) is a commercially important phytosuccivorous and sessile scale insect. Lac insects are cultured on suitable host plants in India and some Southeast Asian countries to produce lac. The lac insect harbours a number of endosymbionts. Isolation of culturable microbial endosymbionts and their identification through 16S rRNA has revealed sex and host-related differences of microbial species. *Bacillus boroniphilus*, *Enterobacter cloacae* and *Staphylococcus* sp. were found only in the lac insects reared on the plant host *Cajanus cajan*, whereas *Bacillus firmus*, *Lysinibacillus xylanilyticus*, *Bacillus horneckiae* and *Bacillus velezensis* were recorded only from *Flemingia macrophylla*. *B. firmus* and *L. xylanilyticus* were female-specific and *B. horneckiae* and *B. velezensis* were male-specific with *Flemingia macrophylla* as host; *E. cloacae* was female-specific and *Bacillus boroniphilus* and *Staphylococcus* sp. were male specific with *C. cajan*. Biochemical characteristics of the isolates, their genetic relationship with their taxonomic kin and their probable role, based on the information available about these endosymbionts in other hosts, have been studied.

Keywords: *Kerria lacca*, endosymbiont, insect–host relationship, 16S rRNA

BAKTERI ENDOSIMBIOTIK YANG DAPAT DIBUDIDAYAKAN DARI SERANGGA LAC INDIAN, KERRIA LACCA *(KERR). Serangga lac Indian,* Kerria lacca *(Kerr) (Coccoidea: Tachardiidae) merupakan serangga skala yang penting secara komersial yang bersifat fitosukkivora dan bersifat sessil. Serangga lak dibudidayakan pada tanaman inang yang sesuai di India dan beberapa negara di Asia Tenggara untuk menghasilkan lak. Serangga lak menyimpan sejumlah endosimbiotik. Isolasi mikroorganisme endosimbiotik yang dapat dibudidayakan dan identifikasi mereka melalui 16S rRNA telah mengungkapkan perbedaan jenis kelamin dan terkait dengan inang dari spesies mikroba.* Bacillus boroniphilus, Enterobacter cloacae, *dan* Staphylococcus *sp. hanya ditemukan pada serangga lac yang dibesarkan pada tanaman inang* Cajanus cajan, *sedangkan* Bacillus firmus, Lysinibacillus xylanilyticus, Bacillus horneckiae, *dan* Bacillus velezensis *tercatat hanya dari* Flemingia macrophylla. B. firmus *dan* L. xylanilyticus *spesifik untuk betina dan* B. horneckiae *dan* B. velezensis *spesifik untuk jantan dengan tanaman inang* Flemingia macrophylla*;* E. cloacae *spesifik untuk betina dan* Bacillus boroniphilus *dan* Staphylococcus *sp. spesifik untuk jantan dengan* C. cajan. *Karakteristik biokimia dari isolat, hubungan genetik dengan kerabat taksonominya, dan peran yang mungkin, berdasarkan informasi yang tersedia tentang endosimbiotik ini pada inang lainnya, telah dipelajari.*

Kata kunci: Kerria lacca*, endosimbiotik, hubungan serangga–inang, 16S rRNA*

*Corresponding author: swetaverma86@gmail.com

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I. INTRODUCTION

The Indian lac insect, *Kerria lacca* (Kerr) (Coccoidea: Tachardiidae) is a beneficial insect, that yields commercially important resin, dye, and wax. It is phytosuccivorous, gregarious, and sessile in habit, thriving on specific plant hosts (Kapur, 1962) and deriving its nutrition from the phloem sap (Ahmad et al., 2012). *K. lacca* is represented by two infrasubspecific forms, kusmi and rangeeni, distinctly differing in their host preference (Kapur, 1962). India is the largest producer of lac and both *kusmi* and *rangeeni* forms of *K. lacca* are cultured on suitable host plants for lac production. A few other species of Kerria are used for producing lac in other countries, such as China, Thailand, and Indonesia (Yogi et al., 2022).

Phytosuccivorous hemipteran insects usually harbour obligate intracellular symbionts, especially to fulfill their essential amino acid requirements (Moran et al., 2008). Jing et al. (2020) demonstrated, in the weevil *Cryptorhynchus lapathi*, that the dominant role of gut bacteria is nutrient provisioning, followed by digestion and detoxification. The presence of the endosymbionts *Micrococcus varians*, *M. conglomeratus*, *Clostridium* sp., and *Bacillus subtilis* was reported from the Indian lac insect, based on microscopical evidence (Sharma & Jaiswal, 2011). Kandasamy et al. (2018) reported the endosymbionts in the *kusmi* form of *K. lacca*. *Wolbachia*, well known as a sex distorter found in a number of insect species was recorded in *K. lacca* also (Vashishtha et al., 2011) and its role in sex determination was revealed (Verma et al., 2023).

Recent studies on insect microbiomes have shown that they play an important role in the development, physiology, and evolution of their hosts. The ability to utilize a specific host plant by a phytosuccivorous insect could be endosymbiont-dependent, as exhibited by two species of the pentatomid bug, *Megacopta* (Hosokawa et al., 2007).

Thus, host-related variation in the endosymbiotic microbiota composition forms an interesting research domain. Frago et al. (2012) in their review, point out the lack of clarity on the role of endosymbionts on the host range of phytophagous insects and the need for more work on endosymbiont-insect-host associations. McLean et al. (2019) showed that the similarity of endosymbiont composition in aphid species is determined by the relatedness of their hosts, demonstrating the influence of host taxonomy on the endosymbiont composition.

The study of culturable endosymbionts is rewarding, as culturability facilitates the investigation of their role in the host. In view of the above, the present study was taken up to investigate the variations in the culturable endosymbionts of *Kerria lacca*, infrasubspecific form *rangeeni*, from two host species, *Flemingia macrophylla* and *Cajanus cajan*, which distinctly differ for their suitability as host. Sex-related variation was also included, as the sexes follow different developmental pathways; the lac insect males are neometabolous whereas the females are neotenic (Belles, 2011).

II. MATERIAL AND METHODS

A. Study Site/Location and/or materials

The lac insects used in this study were derived from the LIK004 collection of *Kerria lacca*, infrasubspecific form *rangeeni*, maintained at the germplasm facility of ICAR-National Institute of Secondary Agriculture, located 23°23' N latitude and 85°23'E longitude, at an altitude 650 m above the mean sea level. LIK004 was originally collected from Palamau, Jharkhand, India, in the prime lac-producing region of the country; the study was made in 2016–17 using this insect, cultured on the two host plant species as described below.

B. Methods

Isolation of endosymbionts

Male pupae and unfertilized females of K. lacca, infrasubspecific form *rangeeni*, were collected from the established lac insect cultures on potted hosts, *F. macrophylla* and *C. cajan*. Each male pupa was carefully taken out of the resinous shell, after removing the operculum, under aseptic conditions. Ten samples each of male pupae and females were collected and surface sterilized with 100% ethanol for 1 and 3 min and were then air dried in a sterile hood for 1 min. The samples were crushed in sterile peptone water and insect saline taken in 500 µl microcentrifuge tubes and suspended. Samples from this suspension were streaked on the prepared NA (nutrient agar) culture plates and incubated at 37°C for 24 – 48 hrs. Each bacterial colony was identified on the basis of colony morphology (Shamim et al., 2017). All the bacterial isolates that were obtained from sterilized and crushed lac insect samples were considered to be endosymbionts.

Molecular characterization

Genomic DNA isolation: The bacterial culture was inoculated in 5 ml liquid culture broth, which was grown overnight. A 1.5 ml aliquot of the culture was centrifuged at 10,000 rpm and pelletized. Tris-EDTA buffer (400 µl) was then added and mixed properly. Subsequently, 40 µl of 10% sodium dodecyl sulphate (SDS) and 5 µl of proteinase K (20 mg/ml) were added and incubated at 56 °C for 45 min. Tris-saturated phenol, pH-8.0 was then added and the mixture was centrifuged at 10,000 rpm for 10 min. The aqueous phase was then taken into a fresh tube and phenol:chloroform (24:1) was added to it and mixed properly. The aqueous phase was again taken out and chloroform: isoamyl alcohol (24:1) was added and mixed. It was then centrifuged at 10,000 rpm for 10 min. Aqueous phase was again taken out to which 0.1 volume of 3 M sodium acetate and 2 volumes of chilled absolute ethanol were added, then incubated at –20°C for 2 hrs. Centrifugation at 15,000 rpm was done for 30 min at 10-15°C resulting in pellet formation. The pellet was then washed with 70% ethanol by centrifugation at 10,000 rpm for 15 min at 10 –15°C. The supernatant was then discarded and the pellet was allowed to dry at room temperature. The DNA thus isolated was then resuspended in Tris-EDTA buffer. To remove the RNA contamination, RNAse (10 mg/ml) was added and incubated at 37°C for 30 min. The extracted DNA was then stored at –20°C for the study.

PCR and Gel Electrophoresis: Universal 16S rRNA primers were used for the amplification of bacterial DNA which amplifies 1.3 –1.5 kb of 16S rRNA gene. Primers used were: 16SF-5'AGAGTTTGATCCTGGCTCAG3' and 16SR-5'ACGGCTACCTTGTTACGACTT3'. The reaction mixture for the PCR was 25 µl with the following constituents: 1X Taq buffer (Thermo Fisher Scientific, Waltham, MA, USA), 2.5 mM $MgCl_2$, 10 mM dNTP mix, 10 picomoles of both the primers, 3 units of Taq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 20 ng of DNA. The reaction was carried out in a thermal cycler (SensoQuest GmbH, Germany) with the following PCR conditions: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, primer annealing at 57.3°C for 30 s and the DNA extension at 72°C for 1 min. The final extension of PCR products after the last cycle was then carried out at 72°C for 10 min. Gel electrophoresis was performed to check the amplification.

Sequencing and analysis: Sequencing of the PCR products was carried out at SciGenom Labs Pvt. Ltd., Cochin, India. To check the sequence quality, sequence analysis was carried out by using ABI sequence scanner ver 1.0. The ends of the sequences were trimmed and alignment was carried out by using Geneious Prime 2019 software, version 2019.0.4. A homology search for the 16S ribosomal RNA sequence was then carried out using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S ribosomal sequences were deposited in the GenBank bearing accession no. MN726499–MN726516 and MN822895– MN822896 (Table 1). The phylogenetic tree for each of the endosymbiotic genera isolated from lac insects was constructed using MEGA11 software and visualized with Figtree. The Neighbor-Joining (NJ) algorithm was used (Saitou & Nei., 1987; Tamura et al., 2021).

Standard biochemical tests were also performed for the characterization of the isolates: gram stain, catalase test, oxidase test, lysine decarboxylase test, urease test, methyl red, Voges-Proskauer test and indole test.

III. RESULT AND DISCUSSION

The details of twenty identified endosymbiont isolates from the lac insect reared on two host species have been furnished in Table 1. The composition of the endosymbiont species differed distinctly for the two hosts. *B. boroniphilus*, *E. cloacae* and *Staphylococcus* sp. were found only from the lac insects reared on *C. cajan*, whereas *B. firmus*, *L. xylanilyticus*, *B. horneckiae* and *B. velezensis* were recorded only from *F. macrophylla*. The *rangeeni* form of *K. lacca* is better adapted for *F. macrophylla* compared to *C. cajan*. Kandasamy et al. (2018) studied bacterial endosymbionts of the *kusmi* form of *K. lacca* from three lac hosts, kusum (*Schleichera oleosa*), ber (*Ziziphus mauritiana*) and *Flemingia semialata* and reported a host-associated variation of the species.

Sex-related variation is also seen for the endosymbionts recorded from both host plants. *B. circulans* was the only common endosymbiont that was present in both male and female samples collected from both the hosts taken for the study (Figure 1). Shamim et al. (2017) also showed that some of the endosymbionts were sex-specific and others were common for both sexes; they found that *Panibacillus barengoltzii*, *Pseudomonas fulva* and

Table 1. GenBank accession number, species name, and other details of the bacterial isolates from males and females of *Kerria lacca* (*rangeeni*) on *Flemingia macrophylla* and *Cajanus cajan*

Isolate No.	Accession No.	Identification (BLASTn)	Percent Identity	Amplicon length (bp)	Isolated from	Medium
Host: Flemingia macrophylla						
KL_1	MN726499	*Bacillus circulans*	100%	1344	Female	Peptone
KL_2	MN726500	*Bacillus circulans*	100%	1366	Female	Peptone
KL_4	MN726501	*Bacillus circulans*	98%	1344	Female	Peptone
KL_a	MN726502	*Bacillus firmus*	100%	1444	Female	Insect saline
KL_h	MN726503	*Lysinibacillus xylanilyticus*	100%	1425	Female	Insect saline
KL_I	MN726504	*Bacillus circulans*	100%	1434	Male	Insect saline
KL_II	MN726505	*Bacillus circulans*	100%	1387	Male	Peptone
Kl_IV	MN726506	*Bacillus circulans*	100%	1404	Male	Peptone
KL_VI	MN726507	*Bacillus horneckiae*	100%	1421	Male	Insect saline
KL_VII	MN726508	*Bacillus velezensis*	100%	1312	Male	Insect saline
Host: Cajanus cajan						
KL_R4	MN726509	*Bacillus boroniphilus*	100%	223	Male	Insect saline
KL_R11	MN726510	*Bacillus circulans*	100%	1339	Female	Insect saline
KL_R12	MN726511	*Bacillus circulans*	100%	1400	Male	Insect saline
Kl_R13	MN726512	*Bacillus circulans*	100%	1315	Male	Insect saline
KL_R15	MN726513	*Bacillus circulans*	100%	659	Female	Peptone
KL_R16	MN726514	*Bacillus circulans*	100%	1285	Female	Peptone
KL_R21	MN726515	*Bacillus circulans*	100%	611	Male	Peptone
KL_R22	MN726516	*Bacillus circulans*	100%	560	Male	Peptone
KL_R8	MN822895	*Enterobacter cloacae*	100%	740	Female	Insect saline
KL_R20	MN822896	*Staphylococcus* sp.	100%	687	Male	Insect saline

Pantoea amanatis were male-specific and *B. cereus*, *Solibacillus silvestris*, *Curtobacterium citreum*, *B. megaterium* and *Anthrobacter subterraneus*, female-specific. Developmental stage-linked variation has also been reported in the microbial diversity of *K. lacca*; the crawler (juvenile) stage was found to be distinct from the adult female stage (Kandasamy et al., 2022).

The endosymbionts identified in the present study were found to be at variance with the eight endosymbionts reported by Shamim et al. (2017) from *K. lacca*. Such variations in the results were attributed to qualitative and quantitative variations of endosymbionts among lac insect populations on different lac host plant species and/or sampling limitations. A very large-scale screening of different species of *Kerria* from a wide range of host plants is likely to provide good clarity of the associated endosymbionts in relation to the lac insect as well as host plant taxa.

It can be seen from Table 2 that all the endosymbionts isolated from *K. lacca*, with the exception of *B. boroniphilus* have been reported in other insect species. The role of some of them in the insects harbouring them has also been

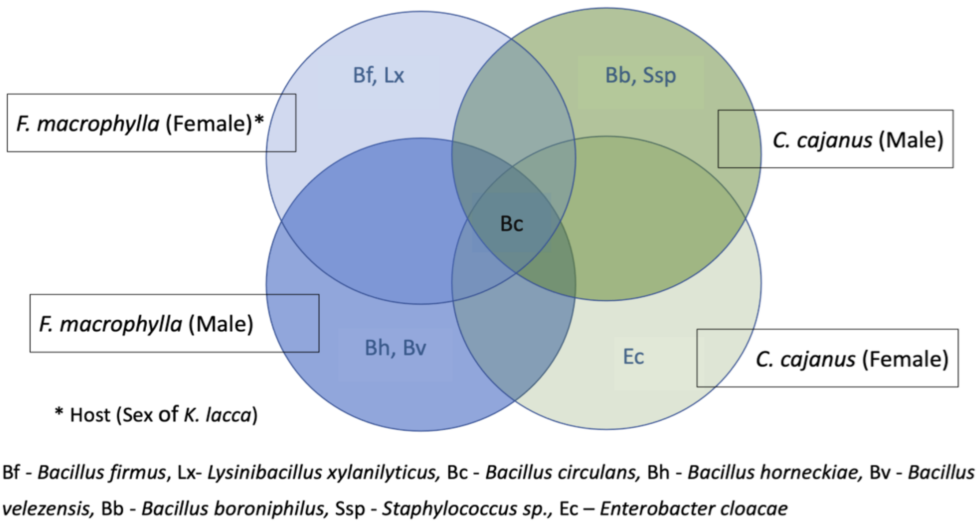

Figure 1. Endosymbiotic bacteria from Kerria lacca, in relation to the sex and the host species

Table 2. Occurrence and reported role of the endosymbionts recorded in K. lacca (rangeeni) from two lac hosts, in other insects/places

Endosymbiont	Occurrence	Description
Bacillus circulans	Gut of silkworm (Anand et al., 2010)	Has a remarkable impact on digestion. Helps in degrading starch, cellulose, xylan, and pectin.
	Gut of termite, Zootermopsis angusticollis (Wenzel et al., 2002)	It is a cellulolytic bacterium.
Bacillus firmus	An endophyte associated with the larvae of the beetle *Oryctes monocerus* (Banjo et al., 2006)	Non-pathogenic microbe isolated from the samples/organisms.
	Associated with cicadellids, *Acrogonia citrina* and *Dilobopterus costalimai* (Gai et al., 2011)	Isolated from two of the three species of the cicadellids studied.

Endosymbiont	Occurrence	Description
Lysinibacillus xylanilyticus	Midgut of Drosophila melanogaster larvae (Maji et al., 2012)	Plays an important role in larval development, under controlled environmental conditions.
	Midgut of the lepidopteran, Sesamia inferens (Reetha & Mohan, 2018)	Beneficial to the host.
	Forest humus (Lee et al., 2010)	Xylan degrading bacterium.
Bacillus horneckiae	Gut of *Apis mellifera* (Gasper et al., 2017)	Confers resistance to toxic metals and enables survival of chironomids in polluted environments.
	Isolated from the surface of PHSF located at the Kennedy Space Centre (Vaishampayan et al., 2010)	
	Chironomid egg masses and larvae (Senderovich & Halpern, 2013)	
Bacillus velezensis	Crop of the antlion *Myrmeleon bore* (Nishiwaki et al., 2007)	Shows slight insecticidal activity against cutworms.
	Isolated from rice root (Harun-Or-Rashid et al., 2018)	*Bacillus velezensis* and *B. oryzicola* have shown plant growth-promoting and antimicrobial activities (Hossain et al., 2016)
		Root drenching of *Arabisopsis* with *B. velezensis* resulted in a systemic response against green peach aphid (Rashid et al., 2017)
Bacillus boroniphilus	Isolated from the soil (Ahmed et al., 2007)	Highly boron tolerant and requires boron for its growth.
	Isolated from the marine ecosystem (Galaviz-Silva et al., 2018)	Inhibitory effects against *Staphylococcus aureus* and *Vibrio parahaemolyticus.*
Enterobacter cloacae	Isolated from the gut of Indian male cricket (Govindarajan et al., 2017)	Exhibits tannase activity, shielding the host insect from the antinutritional action of the plant tannins.
	One of the three highly abundant bacteria in the gut of diamondback moth, Plutella xylotella (Xia et al., 2017)	Metagenomic analysis indicates that they are involved in the production of enzymes involved in amino acid synthesis, digestion of plant products, and detoxification.
	E. cloacae, isolated from the whitefly, *Bemisia argentifolii* was reported to be mildly pathogenic to the insect (Davidson et al., 2000)	The odour emitted by the bacterium was found to attract fruit fly species *B. cucurbitae* and *B. papaya* (Narit & Anuchit, 2011). Phenolics released by *E. cloacae* metabolism help in defence and aggregation of the desert locust Schistocerca gregaria (Dillon & Charnley, 2002)
	Artificial rearing of transgenic Diamondback moth, *Plutella xylostella*	The fitness of the moth improved with inoculation of *E. cloacae* (Somerville et al., 2019)
Staphylococcus sp.	*B. subtilis*, *S. gallinarum*, and *S. saprophyticus* were isolated from the mealy bug *R. amorphophalli.* (Sreerag et al., 2014)	*S. saprophyticus* and *S. succinus* were among the dominant gut bacteria of the silkworm, *Bombyx mori* (Feng et al., 2011)
	Staphylococcus was found along with other gut flora in the honey bee, *Apis mellifera* (Anjum et al., 2018)	A number of *Staphylococcus* spp. have been reported from B and Q biotypes of the whitefly Bemisia tabaci and were dominant bacteria along with *Micrococcus*. (Indiragandhi et al., 2010)
	Potential as a biocontrol agent for the whitefly *Bemisia tabaci*	*Staphylococcus gallinarum* was found to be mildly toxic to the second nymphal instar of the fly (Ateyyat et al., 2009)

explored in a number of cases, which includes aiding digestion of specific substrates, growth, and development, enhanced resistance to biotic and abiotic factors, providing essential amino acids and detoxification of plant metabolites.

The biochemical characteristics of different isolates from *K. lacca* have been furnished in Supplementary Material, Table S1. Interestingly all the endosymbiotic bacteria, except *E. cloacea*, isolated from the Indian lac insect were gram-positive.

Separate phylogenetic trees were constructed for each of the four endosymbiotic genera isolated from the Indian lac insect and along with those available in GenBank. For each of the samples, GenBank accession number and its identification (isolation source) are indicated (Figures 2.1, 2.2, 2.3, and 2.4).

The study of insect endosymbionts has shown tremendous growth in recent years with the identification of a number of associated microorganisms and their role in the fitness of the host. When antibiotic was administered to lac insects cultured on pumpkin, the sex ratio and time taken for sexual maturity were affected, indicative of the influence of endosymbionts on lac insect development (Verma et al., 2023). Janson et al. (2008) pointed out the need for exploring the mutualistic organisms of herbivorous insects, which show an amazing diversity across the food plant range. Fukatsu & Hosokawa (2002) elegantly demonstrated how deprivation of the orally transmitted symbiotic bacteria led to retarded growth and development and other aberrations in the stinkbug *Megacopta punctatissima*. Obligate endosymbiont *Buchnera*

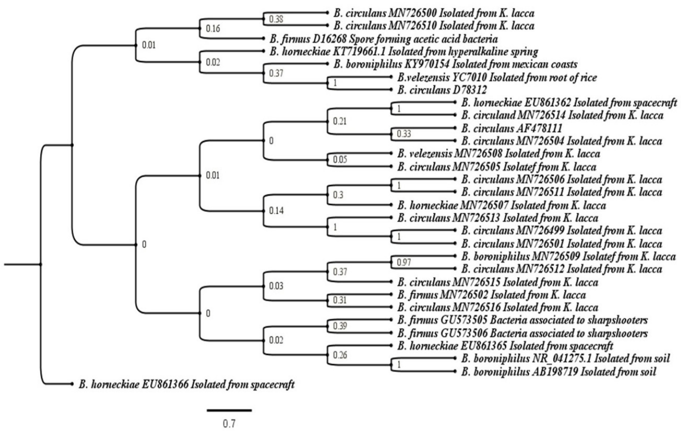

Figure 2.1. Phylogenetic tree of the culturable *Bacillus* species isolated from K. lacca with those isolated from other organisms and sources (data from GenBank) employing Neighbor-Joining (NJ); branches showing bootstrap values

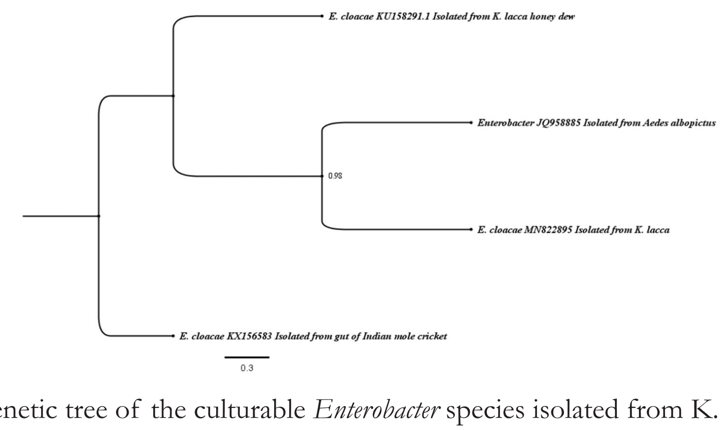

Figure 2.2. Phylogenetic tree of the culturable *Enterobacter* species isolated from K. lacca with those isolated from other organisms and sources (data from GenBank) employing Neighbor-Joining (NJ); branches showing bootstrap values

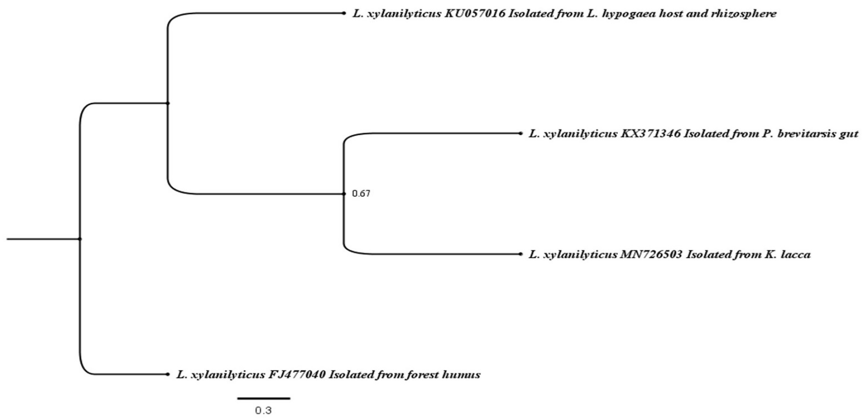

Figure 2.3. Phylogenetic tree of the culturable *Lysinibacillus* species isolated from K. lacca with those isolated from other organisms and sources (data from GenBank) employing Neighbor-Joining (NJ); branches showing bootstrap values

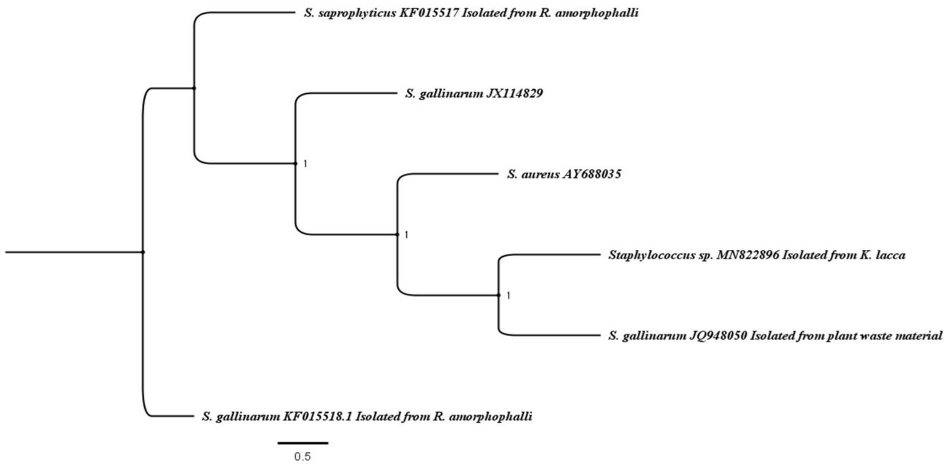

Figure 2.4. Phylogenetic tree of the culturable Staphylococcus species isolated from K. lacca with those isolated from other organisms and sources (data from GenBank) employing Neighbor-Joining (NJ); branches showing bootstrap values

confers thermal resistance to its aphid host (Dunbar et al., 2007).

Several studies suggest that facultative endosymbionts directly augment host plant usage of insects. When Tsuchida et al. (2011) injected *Regiella insecticola* (a facultative symbiont) from a clover-adapted pea aphid *Acyrthosiphon pisum* to *Megoura crassicauda*, a sympatric aphid species which does not normally feed on clover, the latter acquired the ability to utilize clover. This study further supports the role of endosymbionts on the ability of phloem-feeding insects to utilize a specific host plant in aphids (Frantz et al., 2009). Host-related endosymbiont variation in lac insects can be viewed from this perspective.

Bacillus and *Staphylococcus* sp. have the potential for producing sugars of medium length from sucrose thus leading to stickiness of the honeydew, secreted by many insects as reported in whiteflies (Indiragandhi et al., 2010). The *Bacillus* strains produce an amylase enzyme which helps in the conversion of cassava starch into simple sugars (Amund & Ogunsina, 1987; Oyewole & Odunfa, 1992). *B. circulans* has been isolated from both sexes of *K. lacca* on both the hosts; it probably assists the host in digestion. *B. firmus* produces serine protease (Sep 1) as a bioactive compound, which causes damage to the external barriers and degrades the gut epithelium of the insects. This enzymatic action is effective against insects whose body is covered by a cuticle (Geng et al., 2016). *B. velezensis*, an endophytic strain produces secondary metabolites which help in the suppression of pathogens and enhances plant growth simultaneously. *Enterobacter* present in the gut of the insects also plays a vital role. The dinitrogen reductase production in insects may be attributed to *Enterobacter* populations (Behar et al., 2005). *Enterobacter* presence in the gut microbiota increases the production of pheromone, thus enhancing sexual signaling and mating (Dillon et al., 2002). *E. cloacae* probably complements general functions such as amino acid synthesis, digestion, and detoxification as shown by Xia et al. (2017) in *P. xylotella*.

IV. CONCLUSION

The rangeeni form of K. lacca is widely distributed in India including areas where summer temperatures are high. The associated secondary endosymbionts may have a role in conferring resistance to high temperatures and increasing the insect's fitness. Based on the reported roles for the species isolated from *K. lacca* in other organisms, we suggest that these endosymbionts are likely to help the lac insect in host plant adaptation, nutrition, digestion, resistance, and other metabolic functions. Lac insect also produces sticky honeydew profusely, during feeding, with some sugars intermediated probably by *Staphylococcus* and *Bacillus*. *L. xylanilyticus* probably helps in normal development. *E. cloacae* is probably involved in digestion and detoxification mechanisms.

This study has shed some light on the culturable endosymbionts associated with the Indian lac insect in relation to two host plants, distinct for their suitability to the lac insect studied. Sex-related variation has also been observed in the endosymbiont species, which can be attributed to the difference in the mode of metamorphosis. Natural populations of lac insects have been reported on a wide range of host plant species over varied ecological regions. Further investigation on their diversity in relation to host plant and environmental conditions as well as experimentation to narrow down their role in the biology of lac insect would be rewarding.

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Table 1. Results of the biochemical tests done with the bacterial isolates from *Kerria lacca*, *rangeeni* on the host plants, *Flemingia macrophylla* and *Cajanus cajan*.

Name of isolate	Species	Gram stain	Catalase test	Oxidase test	Lysine decarboxylase test	Urease test	MR[1]	VP[2]	Indole test
KL_1	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_2	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_4	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_a	*Bacillus firmus*	+	+	+	-	-	+	-	-
KL_h	*Lysinibacillus xylanilyticus*	+	+	+	-	-	-	-	+
KL_I	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_II	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_IV	*Bacillus circulans*	+	+	+	-	-	-	+	-
KL_VI	*Bacillus horneckiae*	+	+	-	-	-	-	+	-
KL_VII	*Bacillus velezensis*	+	+	+	-	-	-	-	-
KL_R4	*Bacillus boroniphilus*	+	+	+	-	-	-	-	-
KL_R11	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R12	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R13	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R15	*Bacillus circulans*	+	+	+	-	-	--	-	-
KL_R16	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R21	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R22	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R8	*Enterobacter cloacae*	-	+	-	-	-	-	+	-
KL_R20	*Staphylococcus sp.*	+	+	-	+	+	+	+	-

[1] Methyl Red test
[2]Voges-Proskauer test

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THE SPECIFIC ORDINATION AND CLUSTERING OF MANGROVE ECOSYSTEM IN SEGARA ANAKAN

Endang Hilmi[1*], Teuku Junaidi[1], Arif Mahdiana[1], Norman Arie Prayogo[1], Rose Dewi[2], and Sri Rahayu[3]

[1]Program of Aquatic Resources Management and Magister SDA program, Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University.
Jl. Dr Soeparno, Purwokerto Utara, Banyumas 53122, Central Java, Indonesia
[2]Marine Science Program, Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University.
Jl. Dr Soeparno, Purwokerto Utara, Banyumas 53122, Central Java, Indonesia
[3]Freshgraduade student Aquatic Resources Management and Magister SDA Program, Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University,
Jl. Dr Soeparno, Purwokerto Utara, Banyumas 53122, Central Java, Indonesia

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THE SPECIFIC ORDINATION AND CLUSTERING OF MANGROVE ECOSYSTEM IN SEGARA ANAKAN. Mangrove ecosystem has specific ordination and clustering following the adaptation toward the environment properties and species competition. This research had purpose to analysis a specific ordination using the relation between mangrove density and environmental properties. The research was carried out with a multidimensional system using density and environmental properties with similarity and Euclidian distance indexes. The results showed that West Segara Anakan (WSAL) had 6 ordination areas, and East Segara Anakan (ESAL) had 5 ordinations with a range density of 68-3373 trees/ha and 550-2975 trees/ha. Based on environmental properties, WSAL had nitrate, phosphate, pyrite, water and soil pH, and water salinity levels of 10.57-31.44 mg/lt, 8.44-22.89 mg/lt, 1.03-1.57 %, 5.60-7.78, 6.58-7.03, and 24.15-33.85 ppt, respectively. In ESAL, nitrate, phosphate, pyrite, water and soil pH, and water salinity were within the range of 19.72-28.98 mg/lt, 10.83-19.72 mg/lt, 1.28-2.91%, 6.35-7.05, 5.91-6.23, and 18.00-32.33 ppt. Furthermore, specific ordination showed that *Rhizophora stylosa*, *Rhizophora apiculata*, *Avicennia marina*, and *Nypa frutican* had the highest level of adaptation to grow and life in Segara Anakan Lagoon (both of WSAL and ESAL.

Keywords: Environment properties, mangrove clustering, mangrove density, mangrove ordination, Segara Anakan Lagoon

ORDINASI DAN PENGELOMPOKAN YANG KHAS DARI EKOSISTEM MANGROVE DI SEGARA ANAKAN. Ekosistem mangrove memiliki ordinasi dan pengelompokan yang khas sebagai bentuk adaptasi dari karakteristik lingkungan dan kompetsisi antar jenis. Penelitian ini bertujuan untuk mengembangkan ordinasi spesifik menggunakan hubungan antara kerapatan mangrove dengan kondisi lingkungan. Penelitian dilakukan dengan sistem multidimensi menggunakan kerapatan dan karakteristik lingkungan dengan menggunakan indeks similaritas dan euclidian distance. Hasil penelitian menunjukkan bahwa Segara Anakan Barat (SAB) memiliki 6 wilayah ordonasi, dan Segara Anakan Timur (SAT) memiliki 5 wilayah ordonasi dengan kisaran kerapatan 68-3373 pohon/ha dan 550-2975 pohon/ha. Berdasarkan sifat lingkungan, SAB memiliki kadar nitrat, fosfat, pirit, pH air dan tanah, serta salinitas air masing-masing sebesar 10,57-31,44 mg/lt, 8,44-22,89 mg/lt, 1,03-1,57%, 5,60-7,78, 6,58-7,03, dan 24,15-33,85 ppt. Pada SAT, nitrat, fosfat, pirit, pH air dan tanah, serta salinitas air berada dalam kisaran 19,72-28,98 mg/lt, 10,83-19,72 mg/lt, 1,28-2,91%, 6,35-7,05, 5,91-6,23, dan 18,00-32,33 ppt. Selain itu, penahbisan spesifik menunjukkan bahwa Avicennia marina, Rhizophora stylosa, Rhizophora apiculata, *dan* Nypa frutican *memiliki tingkat adaptasi tertinggi untuk tumbuh dan hidup di Laguna Segara Anakan (SAB dan SAT).*

Kata kunci: Karakteristik lingkungan, pengelompokan mangrove, kerapatan mangrove, ordinasi mangrove, Laguna Segara Anakan

*Corresponding author: dr.endanghilmi@gmail.com

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I. INTRODUCTION

Segara Anakan Lagoon (SAL) is a semiclosed estuarine ecosystem that requires specific adaptation from mangrove vegetation as a dominant organism (Hilmi et al., 2021; 2022). The mangrove in Segara Anakan (SAL) must have ability to reduce the impact of salinity (water salinity between 0-40 ppt and soil salinity between 0-7,05 ppt), limited oxygen, muddy soil, and high organic matter (Cavalcante et al. 2021; Hilmi et al. 2021c). Specific adaptations of this vegetation to reduce impact of the environment is the ordination system, which represents as zonation and clustering pattern of the mangrove ecosystem. Furthermore, clustering and ordination pattern describe the adaptation and relationship between mangrove species with environment variables using the indicators of similarity, disimilarity, euclidian distance, and specific correlation among species (Haq et al. 2017; Hilmi et al. 2021c, 2022b). These concepts were developed using a dissimilarity index, which was analyzed with the Euclidian distance index (Haq et al. 2017; Hilmi et al. 2021c, 2022b). The cluster and ordination analyses also explain the multivariate analysis and hierarchical and non-hierarchical methods from the relation model of organisms with environmental properties.(Haq et al. 2017; Hilmi et al. 2021c, 2022b).

Based on previous reports, the dominant species in Segara Anakan Lagoon are Aegiceras *spp*. (*Aegiceras corniculatum*, and *A. floridum*) *Avicennia spp*. (*Avicennia alba*, *A. marina*, *A. officinalis*), *Bruguiera spp* (*Bruguiera gymnorrhiza*, *B. parviflora*, *B. sexangula*), *Callophylum inophylum*, and *Carbera manghas*. Furthermore, others include *Ceriops app* (*Ceriops decandra*, *C. Tagal*), *Rhizophora spp* (*Rhizophora apiculata*, *R. mucronata*, *R. stylosa*,) *Sonneratia spp* (*Sonneratia alba*, *S. caseolaris*), *Tespia pulpunea*, *Terminalia cattapa*, *Xylocarpus spp*. (*Xylocarpus granatum*, *X. moluccensis*) (Koswara et al. 2017; Hilmi et al. 2021c, b). The distribution of mangrove species in SAL follows the environmental conditions of water and soil salinity, soil texture, water inundation, microorganism diversity, and disturbance (Halwany and Andriani 2015; Hilmi et al. 2017). The distribution of the mangrove species in SAL explains the irregular cluster and zonation. The mangrove zonation refresent the cluster of mangrove to illustrate the adaptation of species to reduce impact of disturbance (Toosi et al. 2022), and climate change, such as storms (Cameron et al. 2019; Branoff 2020; Wang et al. 2020), sea level rise (Latiefa et al. 2018; Cherry and Cherry 2020; Cahoon et al. 2021), seawater inundation (Bullock et al. 2017; Hilmi et al. 2021a, 2022a), sea tide, freshwater and seawater supply (Sufyan et al. 2017; Hilmi et al. 2022c), industrial activity, water pollution, and anthropogenic activity(de Almeida Duarte et al. 2017; Nour et al. 2019). Based on previous studies, clustering, and specific ordination can describe the ecological connectivity of the mangrove ecosystem (d'Acampora et al. 2018), which plays a role in coastal protection, abrasion prevention, sedimentation, nutrient cycling, and mangrove conservation (Doughty et al. 2016).

The mangrove ordination in SAL both of W-SAL and E-SAL also explains the valuable adaptation from the health status, density, diversity, and protecting endangered species (d'Acampora et al. 2018), vertical and horizontal stand structures (Sreelekshmi et al. 2018), as well as species ordination and zonation (Amjad et al. 2014). Ordination shows that vegetation structure is a combination of habitat, environmental conditions, and species distribution (Amjad et al. 2014). It also explains the ability of mangrove vegetation to efficiently use and manage natural resources, as well as the effective approach of utilizing environmental properties to support life and growth (Chen et al. 2015). A previous study stated that ordination and clustering are useful in identifying the relationship between environment properties with organism in an ecosystem, as well as the the index of species diversity, heterogenity. species richness and evenness as adaptation species to reduce impact of environmental gradients (Haq et al. 2017). Furthermore, mangrove ordination can be used to analysis and record species composition, species richness and species adaptation across edaphic, climatic, aquatic ecosystem and topographic variables, to life in the mangrove ecosystem (Haq et al. 2017).

Mangrove ordination in SAL also explains the species distribution, cluster, landscape, and zonation following the adaptation pattern to reduce the unstable environment, and potential disaster to support their continued growth and survival. The mangrove ordination in SAL is the concept of the stability and adaptation of species, which allows them to reduce the effects of unstable environmental properties (Yanuartanti et al. 2015; Hilmi et al. 2019d), conserve carbon (Hilmi et al. 2019b; Azman et al. 2021), lower heavy metal pollution (MacFarlane et al. 2003; Kibria et al. 2016; Zhang et al. 2019; Shi et al. 2020), mitigation of biodiversity loss (Khan et al. 2021) and prevent coastal disasters (Hilmi 2018; Pham et al. 2019; Nur and Hilmi 2021). Therefore, this research had propuse to analysis and develop a specific ordination using the relation between mangrove density and environmental properties in Segara Anakan Lagoon.

II. MATERIALS AND METHODS

A. Study Site

This study was conducted in Segara Anakan Lagoon (as a specific ecosystem at the southern coast of Central Java (S 7°39 – S 7°43/E 108°50 – E 109°00)) which is divided into Western and Eastern regions (Holtermann et al. 2009; Nordhaus et al. 2019). Furthermore, the lagoon is home to a mangrove ecosystem that is distributed along several rivers, including Kali Panas (water outlet of Pertamina), Donan (main rivers as outlet system of Holcim and Pertamina), Sapuregel (estuary of Pelawangan Timur), Kembang Kuning (estuary of Pelawangan Timur) (East Segara Anakan Lagoon/ESAL) as well as Citanduy (main river in W-SAL_, Cikonde, and Cibeureum (West Segara Anakan Lagoon/WSAL) (Hilmi et al. 2021b, c). According (Hilmi et al. 2021c) writes that Segara Anakan has soil texture had clay, loam, loamy clay, mud, mud clay, soil nitrate between 0.010-0.22%, soil pyrite between 1.03-3.10%, soil phosphate between 6.85-17.65%,, soil salinity between 0-7.05, and water salinity between 0-40 ppt, soil pH between 5.7-6.92, water pH between 5.6-7.07,. The study site can be shown in Figure 1 and Table 1.

Table 1 shows that West and Segara Anakan have 22 and 20 stations, respectively, with each station containing 5-10 sampling plots. The plots were developed based on the abundance or density of mangrove species, which were dominated by *Rhizophora apiculata*, *R. mucronata*, *R. stylosa*, *Nypa frutican* and *Avicennia marina* (Hilmi, et al., 2021; 2022).

Table 1. The distribution of sample stations in Segara Anakan Lagoon

West Segara Anakan				East Segara Anakan			
Stations	number station	Coordinate		Stations	number station	Coordinate	
		Latitude (S)	Longitude (E)			Latitude (S)	Longitude (E)
River of Ujung Gagak	1 station	07◦40'13"	108◦48'43"	Donan	2 stations	070 40’ 33,98”	108 59’ 56,90”
River of Lorogan	1 station	07◦40'44"	108◦48'30"	Donan Kalipanas	1 station	070 42’ 10,17”	108 59’ 23,75”
River of Majingklak	1 station	07◦40'32"	108◦48'1"	Donan Pertamina	1 station	070 41’ 15,49”	108 59’ 43,22”
River of Mauara Cawitali	1 station	07◦41'46"	108◦47'41"	Kembang Kuning 1	3 stations	070 43’ 12,88”	108 55’ 42,21”
River of Kebuyutan	1 station	07◦41'13"	108◦47'45"	Kembang Kuning 2	4 stations	070 42’ 25,31”	108 54’ 53,56”
River of Batu Macan	1 station	07◦41'38"	108◦47'46"	Muara pelawangan Timur 1	1 station	070 43’ 48,07”	108 59’ 10,78”
River of Jongor	1 station	07◦40'23"	108◦48'20"	Muara pelawangan timur 2	1 station	070 43’ 20,95”	108 58’ 07,45”
River of Muara Legok	1 station	07◦39'48"	108◦48'13"	Sapuregel 1	1 station	070 42’ 54,20”	108 57’ 42,07”
River of Kayu Mati	1 station	07◦39'5"	108◦48'27"	Sapuregel 2	2 stations	070 41’ 53,33”	108 57’ 37,81”

West Segara Anakan				East Segara Anakan			
Stations	number station	Coordinate		Stations	number station	Coordinate	
		Latitude (S)	Longitude (E)			Latitude (S)	Longitude (E)
River of Langkap	1 station	07o38'48"	108o48'44"	Sleko	1 station	070 42' 46,06"	108 59' 29,10"
River of Karang Braja	1 station	07o40'59"	108o48'47"	Tritih	6 stations	070 40' 22,17"	109 0' 33,98"
River of Klaces	1 station	07o41'5"	108o49'47"				
River of Inti Ujung Gagak	1 station	07o40'34"	108o49'47"				
River of Muara Bagian	1 station	07o40'58"	108o51'42"				
River of Muara Masigitsela	1 station	07o41'24"	108o50'46"				
River of Pertigaan Ujung Alang	1 station	07o41'44	108o51'39"				
River of Ujung Alang	1 station	07o42'0"	108o51'42"				
River of Dermaga Ujung Alang	1 station	07o42'6"	108o51'53"				

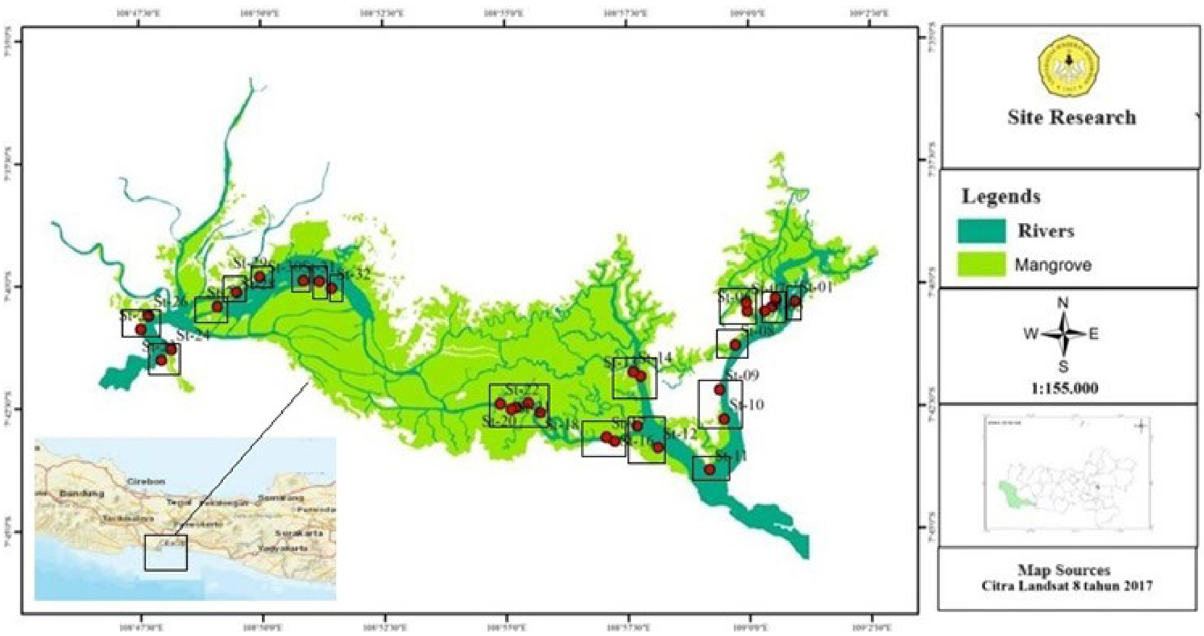

Figure 1. The research site

B. Sampling Techniques

This study used a two-stage cluster sampling method (Hilmi et al. 2017, 2021c), where the lagoon was divided into two clusters in the first stage, namely West and East Segara Anakan. In the second stage, West and East Segara Anakan (consisted 42 stations) (Hilmi et al. 2017, 2021d; Ismail et al. 2018) were further divided into 22 and 20 stations (following the characteristics of the mangrove ecosystem), respectively. Each station contains 5-10 sampling plots (size plot : 10 m x 10 m) following the species density soil and water salinity.

C. Study Procedures

Mangrove density. was measured using a quadrant and line transect with a sampling plot size of 10mx10m. The measurement was carried out on mangrove trees with a diameter of >4 cm (Hilmi et al. 2019c). Furthermore, the data obtained were analyzed using the vegetation analysis equation (Hilmi 2018; Xiong et al. 2018; Cooray et al. 2021), namely density $= \frac{Trees\ number\ of\ mangrove\ speies}{area}$. Mangrove density was then categorized with system analysis(Hilmi et al. 2019a).

Environment Properties. The analysis of environmental properties includes (a) the analysis of water and soil salinity (ppt) using the conductive-photometric method/Hand Refractometer (APHA, 2005; 2012), (b) the analysis of soil and water pH using the Potentiometric method/pH meter (APHA, 2005; 2012); (c) the analysis of Nitrate (NO) (mg 100 g) using the Brucine method (APHA, 2005; 2012), (d) the analysis of Phosphate (PO) (mg 100 g) using the ascorbic acid method (APHA, 2005; 2012); and (e) the analysis of pyrite (%) using the Calorymeter method.

D. Data Analysis

Mangrove density

The mangrove density was analyzed by equation: mangrove density = $\frac{\text{total number of mangrove trees from each species}}{\text{total area } (m^2 \text{ or } ha)}$ (Umroh et al. 2016; Hilmi et al. 2021c). The mangrove density was used to determine the degradation and stability of mangrove (Hilmi et al. 2020).

Mangrove Ordination

Mangrove ordination was analyzed using the dissimilarity index, euclidian distance index, and vegetation analysis (Haq et al. 2017). Furthermore, the stages involved the calculation of (1) IS (Similarity Index) using the equation, IS = $\frac{2W}{A+B}$ x 100 (w = the smallest individual value from the two stations; A = the sum of total individuals from station A; and B = the sum of total individuals from station B), (2) ID (index dissimilarity) with the formula, ID = 100 – IS, (3) ordination X using the equation X = $\frac{L^2+(dA)^2-(dB)^2}{2L}$, with (L = ID between AX and BX, dA = ID value from station A, dB = ID value from station B, (4) e2 value using the formula e2 = dA2 – X2 (5) ordination Y with the equation Y = $\frac{L^2+(dA)^2-(dB)^2}{2L}$.

The Specific Clustering of Mangrove Area and Density of Mangrove Species

The Specific Clustering of the mangrove area and mangrove species was determined using the mangrove species density (Hilmi et al. 2021c, b). The results obtained were visualized using scatter analysis. The clustering analysis had the option to analyze clusters using mangrove area or mangrove species following the grade of tree density in this ecosystem.

III. RESULTS AND DISCUSSION

1. Mangrove Clustering and Ordination in Segara Anakan

Ordination and clustering of mangrove areas in Segara Anakan Lagoon are presented in Figures 2 and 3. The data showed that the ecosystem in the area can be divided into two big clusters, namely West and East Segara Anakan, with 6 and 5 ordination classes, respectively. Ordination class in the WSAL cluster were estuary of Bagan and Klaces (ordo 1), Pertigaan Sudiro, Ujung Alang Port (ordo 2), Kayu Mati, Langkap (ordo 3), Legok, Pertigaan Ujung Alang, Ujung Alang River, Kali Semak, Estuary of Cawitali, Estuary of Masikitsela, Batu Macan (ordo 4), Majingklak, Karang Braja, Ujung Gagak, Lorongan, (ordo 5), Core of Ujung Gagak, Jongor, Kebuyutan (ordo 6). The characteristics of mangrove ordination explain the similarity of mangrove species and mangrove density. The data in Figure 2 showed that the Estuary of Bagan and Kalces have a high similarity of species distribution and species density. The Estuary of Bagan and Klaces have a high distance with the core of Ujung Gagak and Kebuyutan. Meanwhile, Estuary of Pelawangan Timur 2, Tritih (ordo 1), Kembang Kuning 1, Kembang Kuning 2 (ordo 2), Sapuregerl 1, Sapuregel 2, Pertamina Donan (ordo 3), Kalipanans Donan, Sleko (ordo 4) and Donan dan Estuary Pelawangan Timur 1 (ordo 5) were found in the ESAL cluster.

The clustering showed the similarity of mangrove species dominant and similarity of environmental properties. For example, the estuary of Bagan and Klaces have similarities of species dominant, that are *Sonneratia caseolaris*, *Rhizophora mucronata*, *Aegiceras corniculatum*, *Ceriops tagal*, and similarity of soil nitrate, soil phosphate , soil pyrite, pH (water and soil) and

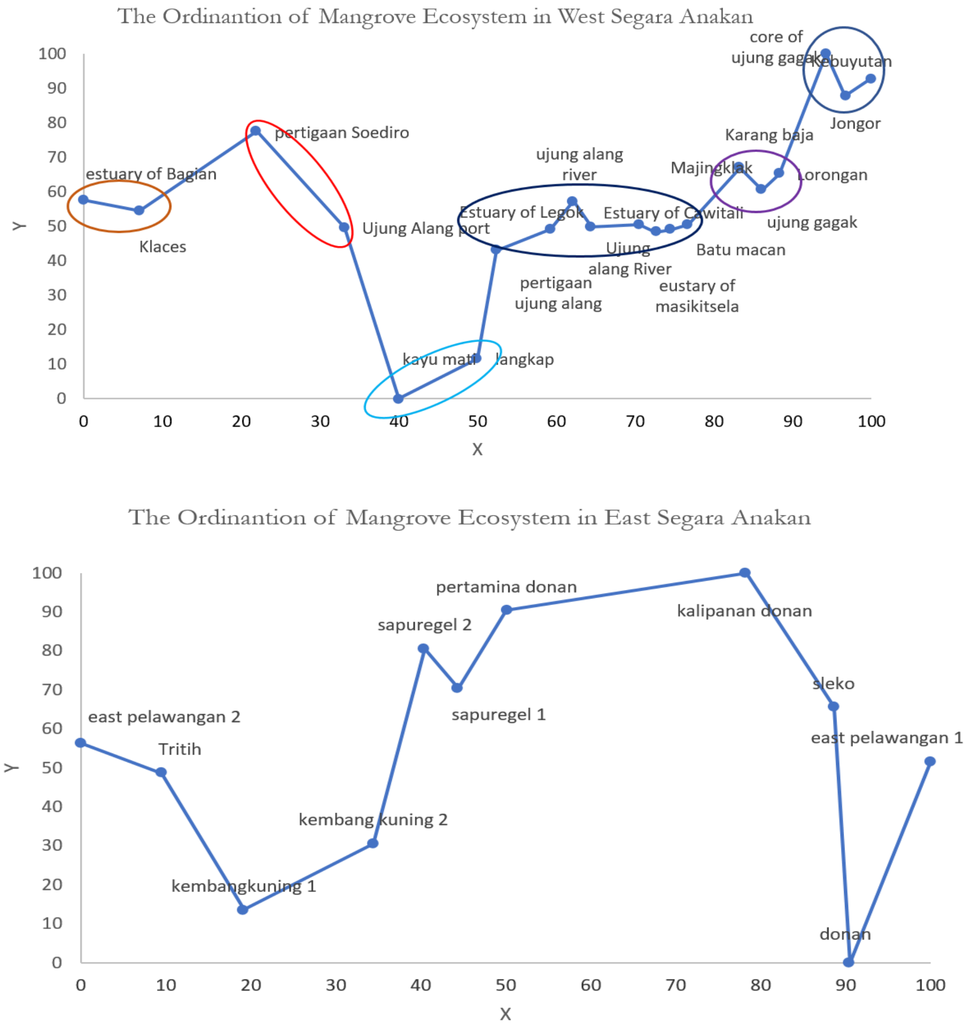

Figure 2. The ordination of the mangrove area in Segara Anakan Lagoon

salinity (water and soil). The other condition, in East Segara Anakan also gave data that Estuary of Pelawangan Timur 2, Tritih has similarity of mangrove species that are *Avicennia marina*, *A. alba*, *Rhizophora apiculata*, *R. mucronata*, *R. Stylosa*, *Sonneratia caseolaris*, *S. alba*, *Nypa frutican*, *Bruguiera sexangula*, *Aegiceras corniculatum* and similarity of environmental properties.

Ordination of mangrove areas in SAL were influenced by several factors, including species distribution and dominance, environmental properties, mangrove association (Barreto et al. 2016; Datta and Deb 2017), species competition (Masuda et al. 2017; Bathmann et al. 2021), solar energy, nutrient, mineral, and water (Amjad et al. 2014; Sreelekshmi et al. 2018; Xiong et al. 2018). Furthermore, the existence of *Rhizophora mucronata*, *R. apiculata*, *R. stylosa*, *Sonneratia caseolaris*, *Avicennia marina*, and *Nypa frutican* supported the development of mangrove ordination, clustering and zonation.

2. Ordination and Mangrove Clustering Based on Environmental Properties

Table 2 provides an overview of mangrove clustering in Segara Anakan. Clustering in this region was developed by several factors, including environmental properties, such as phosphate, pyrite, pH, and salinity. The data showed that West Segara Anakan had nitrate, phosphate, pyrite, water pH, soil pH, and salinity range of 10.57-31.44 mg/l, 8.44-22.89

Note : A. the lagoon and mangrove ecosystems in WSAL, B = The lagoon and mangrove ecosystems in ESAL, C= The beach ecosystem in WSAL, D = the sedimentation factor in the mangrove ecosystem,25

Figure 3. The viewing ordination of the research location in Segara Anakan Lagoon

mg/l, 1.03-1.57 %, 5.60-7.78, 6.58-7.03, and 24.15-33.85 ppt, respectively. Meanwhile, East Segara Anakan had nitrate, phosphate, pyrite, water pH, soil pH, and water salinity range of 19.72-28,98 mg/l, 10.83-19.72 mg/l, 1.28-2,91 %, 6.35-7.07, 5.91 – 6.23, and 18.00-32.33 ppt, respectively.

The environmental properties in the study location showed that soil pH, soil salinity, and phosphate have a significant correlation (but only have a score between 0.15-0.22/ weak correlation) with vegetation life and growth. These properties were also crucial factors that supported the development of mangrove species association and clustering (Datta and Deb 2017). But in Segara Anakan Cilacap voth of WSAL and ESAL are not clear clustering and zonation pattern, because the distribution of environment properties haven't specific clustering. Basically, the ordination, clustering, and zonation also are influenced by the distance from the ocean and wind buffers area, mangrove canopy cover, the height of vegetation above the soil surface, water inundation, tidal inundation, pollution, and the mangrove degradation (Osland et al. 2019; Hilmi et al. 2022c). Similar to this condition, Radabaugh et al. (2020) also highlighted the critical role of soil properties in the strengthening of species in fringe mangrove and basin forests.

Similar to Segara Anakan, Sullivan et al. (2021) also reported that the salinity and NO_2 levels in mangrove swamps were 25.24–28.33 ppt and 0.006-0.011 mg/L. In Myanmar, The water salinity ranged from 14.4±3.0-28.8±0.5 PSU (Win et al. 2019). The water salinity and NO_2 give a high impact on the mangrove ecosystem because of the reduction of N assimilation rate for NO_3^- and NH_4^+ as well as inhibit the association enzyme (Shiau et al.

Table 2. The ordination of environment properties in Segara Anakan Lagoon

Ordi-nation	mangrove area	mangrove density		Environment properties					
				Soil Nitrate	Soil Phosphate	Soil Pyrite	pH		Water salinity
		trees/ha		mg/l	mg/l	(%)	Water	Soil	(ppt)
		min	max						
West Segar Anakan									
1	estuaty of bagian and klaces	145-176	633-1300	10,57-20,86	10,67-18,22	1,12-1,41	7,40-7,65	6,67-6,99	28,85-31,15
2	pertigaan sudiro, ujung alang port,	68-363	599-1899	13,43-22,86	11,38-21,78	1,03-1,39	7,50-7.78	6,58-6,93	30,15-31,85
3	kayu mati, langkap	83-279	800-1567	14,06-26,86	16,22-22,89	1,20-1,34	7,40-7,60	6,60-6,72	26,85-30,85
4	estuary of legok, pertigaan ujung alang, Ujung Alang River, Kali Semak, Estuary of Cawitali, Estuary of Masikitsela, Batu macan	47-600	600-3373	12,00-28,57	12,00-21,33	1,04-1,38	5,90-6,12	6,58-6,83	25,65-33,15
5	Majingklak, Karang Braja, Ujung Gagak, Lorongan,	10-422	433-2167	16,86-31,44	12,44-21,56	1,26-1,57	5,60-5,72	6,67-7,03	24,15-29,85
6	Core of Ujung Gagak, Jongor, Kebuyutan	79-732	832-3367	17,47-24,57	8,44-18,04	1,24-1,49	5,60-5,72	6,56-6,68	27,65-33,85
East Segara Anakan									
1	Estuary of Pelawangan Timur 2, Tritih	631-919	2150-2553	19,77-28,91	13,24-17,33	1,38-2,89	6,67-7,07	6,01-6,20	25,33-31,00
2	Kembang Ku ning 1, Kembang Kuning 2	550-810	1425-1833	21,08-28,63	10,83-19,72	1,63-2,88	6,70-6,90	6,00-6,09	28,00-30,00
3	Sapuregerl 1, Sapuregel 2, Pertamina Donan	630-880	1933-2800	23,61-28,73	16,45-19,22	1,83-2,82	6,35-6,97	5,94-6,23	28,33-31,00
4	Kalipanans donan, Sleko	702-850	2763-2975	20,82-27,12	16,55-18,91	1,28-2,91	6,76-6,83	5,91-6,03	18,00-28,67
5	Donan dan Estuary Pelawangan Timur 1	680-792	2208-2756	19,72-28,98	13,14-17,33	1,40-2,89	6,54-7,05	6,03-6,22	18,00-32,33

2017b, a; Wang et al. 2018). Mangrove species must have the adaptation patterns to eliminate the ieffect of environmental conditions, including water salinity that can affect fine root function (Ahmed et al. 2021), as well as develop a relationship with microbial respiration activity (Davies et al. 2017).

Other properties, the distributions of pyrite percent of soil in Segara Anakan between 1.28-2,91 % more than the potential of pyrit from (Ding et al. 2014) between 1.0770.41 wt% and 0.6270.32 wt% also give impact to support clustering pattern of mangrove species. Basically, pyrite existed in three forms, including framboidal aggregates, minute crystals, and large solitary crystals. The observation showed that the dominant forms were minute crystals (<2 pm) and framboidal aggregates (Ding et al. 2014). Ding et al. (2014) writes that this condition give influence for the distribution of mangrove species, such as *Bruguiera cylinderica*, *Kendelia candel*, *Sonneratia apetala*, *Nypa fruitican*, *Xylocarpus granatum*, *Avicennia alba*, *Cerbera odollam*, *Erythrina indica*, *Heritiera littoralis*, *Kendelia candel*, *Rhizophora apiculata*, *Sonneratia caseolaris*, *Sapium indicum*, *Brownlowia tersa*.

3. Mangrove Ordination and Clustering Using the Indicator of Mangrove Density

Mangrove clustering and ordination based on the indicator of species density are presented in Table 3. Species density data showed that *Rhizophora stylosa*, *R. apiculata*, *Avicennia marina* and *Nypa frutican* were the dominant species. The second dominant were *Avicennia alba*, *Rhizophora mucronata*, *Sonneartia alba*, *S. caseolaris*, and *Bruguiera gymnorrhiza*, and co-dominant

Table 3. The mangrove ordination based on species density in Segara Anakan Lagoon

Ordination	Stations	Mangrove species	Mangrove density	
			Density	Class density (Hilmi et al. 2020)
		West Segara Anakan		
1	estuaty of bagian and Klaces	*Sonneratia caseolaris, Rhizophora mucronata, Aegiceras corniculatum, Ceriops tagal*	145-1300	very rare- rare
2	pertigaan sudiro, ujung alang port,	*Avicennia marina, Avicennia officinalis, Ceriops tagal, Bruguiera gymnorrhiza, Rhizophora apiculata, Rhizophora mucronata, Sonneratia caseolaris,.*	68-1899	very rare-moderate
3	kayu mati, langkap	*Avicennia alba, Avicennia marina, Sonneratia caseolaris*	83-1567	very rare- rare
4	estuary of legok, pertigaan ujung alang, Ujung Alang River, Kali Semak, Estuary of Cawitali, Estuary of Masikitsela, Batu macan	*Aegiceras corniculatum, Aegiceras florisum, Avicennia officinalis, Avicennia alba , Avicennia marina, Ceriops tagal, Sonneratia caseolaris, Sonneratia alba,, Xylocarpus granatum*	47-3373	very rare-very dense
5	Majingklak, Karang Braja, Ujung Gagak, Lorongan,	*Avicennia alba, Avicennia marina, Rhizophora stylosa,, Sonneratia caseolaris, Sonneratia alba, Xylocarpus granatum*	10-2167	very rare-moderate
6	Core of Ujung Gagak, Jongor, Kebuyutan	*Avicennia marina, Aegiceras corniculatum, Bruguiera gymnorrhiza, Ceriops tagal, Rhizophora mucronata, Sonneratia alba*	79-3367	very rare-very dense
		East Segara Anakan		
1	Estuary of Pelawangan Timur 2, Tritih	*Avicennia marina, Avicennia alba, Aegiceras corniculatum, Bruguiera sexangula, Nypa frutican,, Rhizophora apiculata, Rhizophora mucronata, Rhizophora Stylosa, Sonneratia caseolaris, Sonneratia alba,*	631-2553	rare-dense
2	Kembang Kuning 1, Kembang Kuning 2	*Avicennia marina, , Bruguiera gymnorrhiza, Aegiceras corniculatum, Ceriops tagal, Ceriops tagal, Excoecaria agallocha, Hibiscus tiliaceus, Nypa frutican,, Rhizophora apiculata, Rhizophora mucronata, Rhizophora Stylosa, Xylocarpus granatum,*	550-1833	rare-moderate
3	Sapuregerl 1, Sapuregel 2, Pertamina Donan	*Aegiceras corniculatum, Bruguiera gymnorrhiza, Ceriops tagal, Ceriops tagal, Heritiera litoralis, Nypa frutican , Rhizophora apiculata,Rhizophora mucronata, Rhizophora Stylosa,*	630-2800	rare-dense
4	Kalipanans donan, Sleko	*Avicennia marina, Avicennia alba, Aegiceras corniculatum,, Bruguiera gymnorrhiza, Bruguiera sexangula, Ceriops tagal, Rhizophora apiculata, Rhizophora mucronata, Rhizophora Stylosa, Sonneratia alba, Nypa frutican,*	702-2975	rare-dense
5	Donan dan Estuary Pelawangan Timur 1	*Avicennia marina, Avicennia alba, Aegiceras corniculatum,, Bruguiera gymnorrhiza, Bruguiera sexangula, Ceriops tagal, Nypa frutican,, Rhizophora apiculata, Rhizophora mucronata, Sonneratia caseolaris, Sonneratia alba,*	680-2756	rare-dense

were followed by *Bruguiera sexangula*, *Ceriops tagal*, *Ceriops decandra*, *Aegiceras corniculatum* and *Xylocarpus granatum*. Furthermore, the recessive species included *Heritiera littoralis*, *Hibiscus tiliaceus*, *Excoecaria agallocha*, *Avicennia officinalis*, and *Xylocarpus mollucensis*.

The mangrove distribution using the ordination system has a different pattern from Sreelekshmi et al. (2018) who noted that the Southwest Coast of India has been dominated by *Avicennia marina*, *A. alba*, *Avicennia officinalis*, *Acrostichum aureum*. *Aegiceras corniculatum*, *Bruguiera sexangula*, *B. cylindrica*, *Ceriops tagal*, *Kandelia candel*, *Lumnitzera racemosa*, *Excoecaria agallocha*, *E. indica*, *Bruguiera gymnorrhiza*, *Rhizophora mucronata*, *R. Apiculata*, *Sonneratia alba* and *S. caseolaris*, Cooray et al. (2021a) reported that Rekawa Lagoon had been dominated by *Lumnitzera racemosa* (32.6%), *Avicennia marina* (21.0%), *Ceriops tagal* (15.4%) and *Excoecaria agallocha* (11.1%), while Excoecaria agallocha (34.7%), *Rhizophora apiculata* (23.3%), and *Rhizophora mucronata*

(15.2%) dominated the other area in Rekwa Lagoon. Furthermore, Puttalam-Kalpitiya was dominated by *Avicennia marina* (83.8%), and the Batticaloa Lagoon was dominated by *Excoecaria agallocha* (71.7%). Different conditions were also shown by the mangrove dominant in Negombo Lagoon which was dominated by *Avicennia marina* (42.1%), *Rhizophora mucronata* (21.1%), and *Lumnitzera racemosa* (19.2%)

The ordination analysis using the Principal Component Analysis (PCA) aims to determine specific locations dominated by certain and dominant species (Chen et al. 2015). Datta and Deb (2017) reported that mangrove in Indian Sunderbans has extensive mixed plantations, such as *Avicennia marina*, *Ceriops tagal*, and *Excoecaria agallocha* (dominant species). The co-dominant group comprised *Xylocarpus varieties*, *Nypa fruticans*, *Phoenix paludosa*, and *Aegiceras corniculatum*. Meanwhile, the recessive group consisted of *Avicennia alba*, *Ceriops tagal*, *Excoecaria agallocha*, and *Bruguiera gymnorhiza* (Datta and Deb 2017). The other area, Myanmar has different clusters and ordination which was dominated by *Bruguiera cylinderica*, *Kendelia candel*, *Sonneratia apetala*, *Nypa fruitican*, *Xylocarpus granatum*, *Avicennia alba*, *Cerbera odollam*, *Erythrina indica*, *Heritiera littoralis*, *Rhizophora apiculata*, *Sonneratia caseolaris*, *Sapium indicum*, and *Brownlowia tersa*.

4. The Spesific Clustering of Mangrove Species

The Specific Clustering of Mangrove Area

The specific clustering of mangroves in SAL is presented in Figure 4. The data showed that the ESAL had nine clusters of mangrove species density, but, the WSAL had six clusters. The different clustering of mangrove areas in East and West Segara Anakan described the mangrove ability to grow and live in specific environments because these areas have different oceanography (Karl and Church 2017), salinity (Junaidi et al. 2022), soil texture, sea tide, sea current, sedimentation (Sari et al. 2016; Hilmi et al. 2021d), and water pollution (Costa-Böddeker et al. 2020; Chai et al. 2020). West Segara Anakan had higher sediment potency, water salinity, sea tide, and sea current, but its water pollutant was lower compared to East Segara Anakan. Various environmental conditions served as trigger factors for the survival and growth of different species. the different conditions also affected the activity of mangrove species to develop zonation, cluster, association, and ordination.

The mangrove area in ESAL had a specific cluster of mangrove areas that was lower than WSAL. The specific cluster of mangrove area in ESAL had 6 clusters, while in WSAL had 9 clusters. The different numbers of clusters were influenced by the different dominant species, the characteristics of water and soil salinity, water inundation, and potential sedimentation (Sari 2016; Hilmi et al. 2021a). The specific ordination of mangrove areas also explains the adaptation pattern of mangrove species to reduce the effects of environmental conditions in Segara Anakan.

The clustering of mangrove area can be divided into (1) East Segara Anakan is developed by *Aegiceras corniculatum* (species cluster 1), *Avicennia alba* and *Avicennia marina* (species cluster 2), *Bruguiera sexangular*, *B. parviflora* and *B. gymnorrhiza* (species cluster 3), *Ceriops tagal* and *Ceriops decandra* (species cluster 4), *Excoecaria agallocha-Heritiera litoralis* (species cluster 5), *Nypa frutican-Rhizophora apiculata* (species cluster 6), *Rhizophora mucronata-Rhizophora stylosa* (species cluster 7), *Sonneratia caseolaris-Sonneratia alba* (species cluster 8) and *Xylocarpus granatum-Xylocarpus molucensis* (species cluster 9). (2) West Segara Anakan is developed by *Aegiceras corniculatum* (species cluster 1), *Avicennia alba-Avicennia marina* (species cluster 2), *Bruguiera gymnorrhiza* and *Ceriops tagal* (species cluster 3), *Rhizophora apiculata*, *R. mucronate* and *R. stylosa* (species cluster 4), *Sonneratia caseolaris-Sonneratia alba* (species cluster 5) and *Xylocarpus granatum-Xylocarpus molucensis* (species cluster 6).

Species clusters show similar adaptations, requirements for environmental factors such

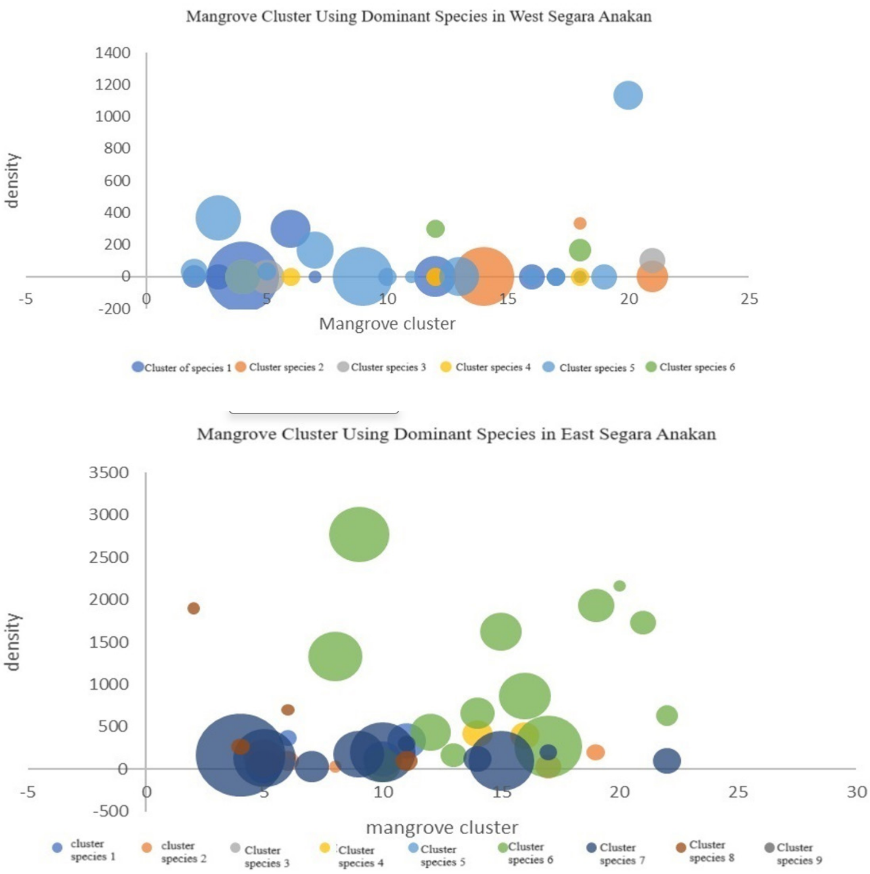

Figure 4. The specific clustering of mangrove areas in Segara Anakan

as salinity, pH, soil fertility, pyrite (Kayalvizhi and Kathiresan 2019; Bomer et al. 2020; Khan et al. 2021), growth ability, and mangrove affinity (Hilmi et al. 2021c; Marlianingrum et al. 2021). Species clusters also explain mangrove succession and mangrove zonation, indicating mangrove regeneration in Segara Anakan Lagoon.

The specific clustering of mangrove species

Specific clustering of mangrove species using species density in Segara Anakan Lagoon is shown in Figure 5. The data in Figure 5 showed that the mangrove species density ranged from <100–400 trees/ha (cluster 1/very rare), 600–800 trees/ha (cluster 2/rare), 800–1000 trees/ha (cluster 3/rare) and >1000 trees/ha (cluster 4/moderate). The Segara Anakan was dominated by the rare category, namely 100-400 trees/ha. The dominance and density of mangrove species were influenced by mangrove damage, delayed mortality, and early recovery (Radabaugh et al. 2020). Species density and species distribution in Segara Anakan were different from those in the Mekong Area of Vietnam, which was dominated by *Avicennia*, *Aegiceras corniculatum*, and *Nypa fruticans*, followed by *Sonneratia* and *Nypa frutican* as second and third dominants, respectively (Bullock et al. 2017). East Segara Anakan had dominant species *Nypa frutican* between 433-2775 trees/ha, *Rhizophora apiculata* with a density between 275 – 1.067 trees/ha, and Rhizophora mucronata with 233 – 1633 trees/ha. But West Segara Anakan was dominated by Sonneratia caseolaris with 133 – 700 trees/ha), Sonneratia alba with 100 – 1133 trees/ha, and Avicennia marina with 100 – 1000 trees/ha.

The number and species density of mangrove ecosystems in ESAL was larger

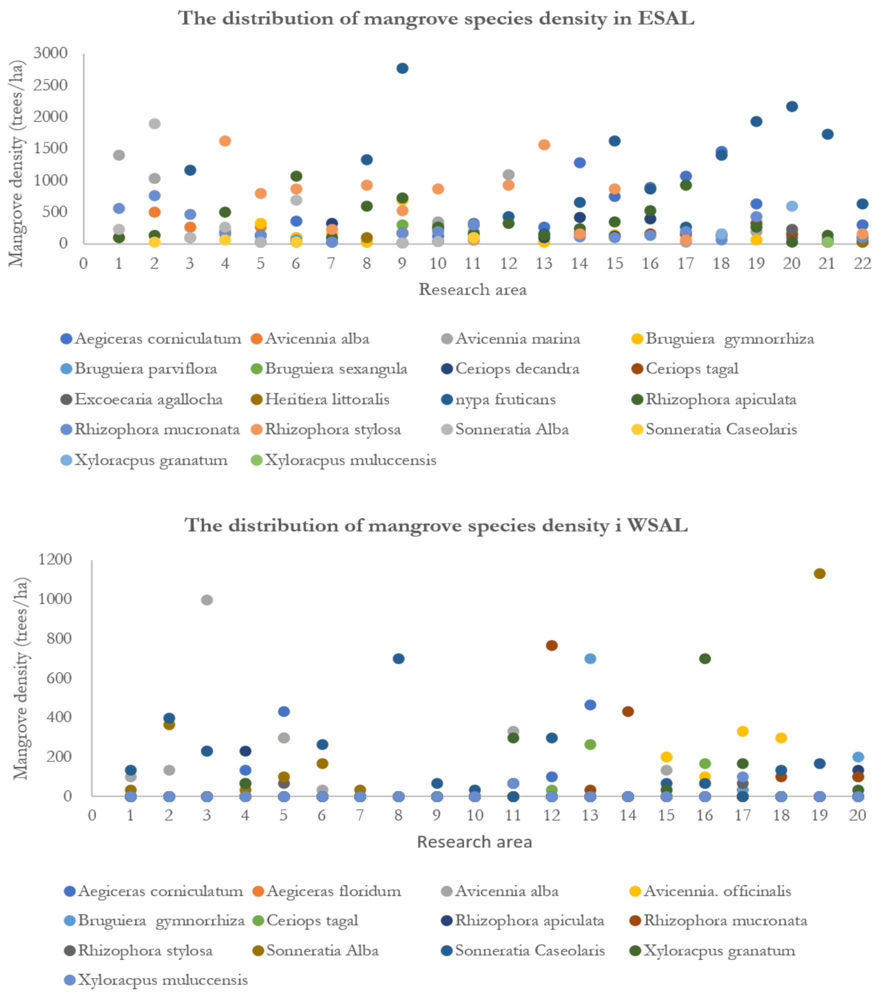

Figure 5. The clustering distribution of species density in Segara Anakan Lagoon

than that in WSAL. The data also explained that *Avicennia marina*, *Nypa frutican*, *Rhizophora styllosa* and *Rhizophora apiculate* were dominant species, in both WSAL or ESAL The clustering and ordination of mangrove species reflected the ability of mangrove species to live and growth in the specific environment properties in WSAL and ESAL.

IV. CONCLUSION

Mangrove ordination and clustering in Segara Anakan explain the relationship between species density, water quality, and soil quality, which represented a specific adaptation pattern to life and growth in the lagoon ecosystem. *Avicennia marina*, *Nypa frutican*, *Rhizophora mucronata*, *Rhizophora apiculata*, *Rhizophora stylosa* and *Sonneratia caseolaris* as dominant species which supported the development of mangrove ordination, clustering, and zonation. The ordination of the mangrove ecosystem in WSAL has 6 ordos, but the mangrove ecosystem in ESAL has only 5 ordos. The specific ordination of mangrove species is dominated by trees density ranging from <100–400 trees/ha (very rare).

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STRATEGIC MANAGEMENT OF TALAS BENENG (*Xanthosoma undipes*) AGROFORESTRY IN CIAMIS REGENCY, INDONESIA

Suhartono[1], Dwiko Budi Permadi[2*], and Sanudin[3]

[1]Research Center for Ecology and Ethnobiology, Badan Riset dan Inovasi Nasional, Jl. Raya Jakarta-Bogor Km. 46, Cibinong, Bogor, Indonesia 16911

[2]Faculty of Forestry, Universitas Gadjah Mada, Jl. Agro No. 1 Bulaksumur Yogyakarta, Indonesia 55281*

[3]Research Center for Population, Badan Riset dan Inovasi Nasional, Jl. Jenderal Gatot Subroto No 10, Jakarta, Indonesia 12710

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STRATEGIC MANAGEMENT OF *TALAS BENENG* (*Xanthosoma undipes*) AGROFORESTRY IN CIAMIS REGENCY, INDONESIA. Recently, the Indonesian government has sought to secure food production from forestlands by implementing agroforestry in rural areas. *Talas beneng* or tall elephant ear plant (*Xanthosoma undipes*) is a potential species for agroforestry that produces medicinal tubers and leaves. It has high productivity and export opportunities, but its management strategy under community forestland needs to be more widely studied. In Ciamis Regency, *talas beneng* has been cultivated for the last two years, but the progress has not been significant. This study aimed to formulate strategic management for improving its business model by identifying internal and external factors. Two groups of farmers in Ciamis Regency were chosen as the case study. Observation and in-depth interviews with farming group leaders as key informants were employed to gather the existing model business. Internal and external factors were analyzed using a business model canvas (BMC) framework, SWOT analysis and quantitative strategic planning matrix (QSPM). Results show that the market channels between the two groups of *talas beneng* producers are slightly different. SWOT analysis shows that both groups have positions in which product development and market penetration are required as strategies to improve future management. Specific strategies that need to be prioritized include 1) improving farming intensification, 2) improving *talas beneng* product competitiveness, and 3) expanding the business partnership to access market information better.

Keywords: Business Model Canvas, product development, market penetration, SWOT, QSPM

*MANAJEMEN STRATEGIS AGROFORESTRI TALAS BENENG DI KABUPATEN CIAMIS, INDONESIA. Saat ini, pemerintah Indonesia berusaha manambahkan produksi pangan dari kawasan hutan dengan menerapkan agroforestri di pedesaan. Talas beneng (*Xanthosoma undipes*) merupakan jenis potensial untuk agroforestri yang menghasilkan umbi dan daun. Jenis ini memiliki produktivitas dan peluang ekspor yang tinggi, tetapi strategi pengelolaannya secara agroforestri belum dipelajari secara luas. Di Kabupaten Ciamis, talas beneng telah dibudidayakan dalam dua tahun terakhir, namun perkembangannya belum signifikan. Studi ini bertujuan merumuskan manajemen strategis untuk meningkatkan model bisnisnya dengan mengidentifikasi faktor internal dan eksternal. Dua kelompok petani di Kabupaten Ciamis dipilih sebagai studi kasus. Observasi dan wawancara mendalam dengan ketua kelompok tani sebagai informan kunci digunakan untuk mengumpulkan model bisnis yang ada. Faktor internal dan eksternal dianalisis menggunakan kerangka kerja business model canvas (BMC), analisis SWOT dan analisis Quantitative Strategic Planning Matrix (QSPM). Hasil menunjukkan bahwa alur pemasaran antara kedua kelompok produsen talas beneng sedikit berbeda. Analisis SWOT menunjukkan bahwa kedua kelompok tersebut memiliki posisi bisnis di mana pengembangan produk dan penetrasi pasar diperlukan sebagai strategi utama untuk meningkatkan pengelolaannya jangka panjang. Strategi khusus yang layak mendapat prioritas antara lain 1) meningkatkan intensifikasi usahatani; 2) meningkatkan daya saing produk talas beneng; dan 3) memperluas kemitraan bisnis untuk akses informasi pasar yang lebih baik.*

Kata kunci: Model Bisnis Canvas, pengembangan produk, penetrasi pasar, SWOT, QSPM

*Corresponding author: dbpermadi@ugm.ac.id

I. INTRODUCTION

The three main challenges in realizing food availability in many countries include limited agricultural land, declining land carrying capacity, and increasing population (García-Oliveira et al., 2022; Winara et al., 2022). In Indonesia, the government has sought several solutions to address this problem when dealing with limited agricultural land, one of them being the distribution of 4.1 million ha of forest area land for agrarian reform and 12.7 ha of forest area land for social forestry programs (Ministry of Environment and Forestry, 2020). This policy will have an effect in the longer term. While in short and medium terms, the government is also encouraging the intensive use of private forestland through agroforestry cropping patterns to increase food production and generate rural income. Agroforestry is an effective, efficient, and sustainable land-use pattern to help increase food production and reduce the negative impacts of climate change on agricultural productivity (Papa et al., 2020; van Noordwijk et al., 2021; Winara et al., 2022).

Currently, agroforestry not only produces food for subsistence purposes (Achmad et al., 2022) but has also shifted into agricultural business activities (Parthiban et al., 2022; Syaifudin, 2020). *Talas beneng* (*Xanthosoma undipes*) is one of the superior national varieties of tubers being developed to strengthen food security and commercial purposes (Budiarto & Rahayuningsih, 2017). It also has a common name, called the "tall elephant's ear" plant as its leaves are quite big enough like an elephant's ear. Along with being adaptive to shaded environments (Rusbana et al., 2016), *talas beneng* also has high tuber productivity (Susilawati et al., 2021), sufficient nutritional content, economic value (Hermita et al., 2017; Rostianti et al., 2018), and export opportunity (Maulana, 2020; Wiyanto, 2021). Its demand is increasing along with the development of its use for food (Fatmawaty et al., 2019).

In Indonesia, *talas beneng* tubers are widely cultivated in Pandeglang Regency, Banten Province and have penetrated various West Java Province regions, including the Ciamis Regency. Ciamis Regency represents regions in which the size of small-scale community forests has been significantly developed in West Java Province (Siarudin et al., 2022). The private land for agroforestry in Ciamis covers an area of 20,866.47 ha (Statistics Ciamis, 2022), potentially supporting the development of *talas beneng* agroforestry cultivation. In this regency, it has been cultivated just recently, for the last two years, but the progress has yet to be widely studied.

Some research has been conducted on *talas beneng*, especially concerning tuber postharvest products, such as modified *talas* starch (Yuliani & Herawati, 2022), talas starch nanoparticle (Agustina et al., 2022), talas flour quality (Putri et al., 2021), talas amylographies (Pamela et al., 2019), *nata de talas beneng* (Maulani & Hakiki, 2018), and talas brownie cake (Haliza et al., 2017). Moreover, a few studies also have been performed on cultivation and nurseries (Maulina et al., 2022; Sari et al., 2019), phytochemical analysis of talas leaves (Fatmawaty et al., 2019), and the economic value of using talas leaves (Astuti, 2022). Information on the results of *talas beneng* research as a business is still limited. For example, research on market chains, business strategies, internal and external factors influencing *talas beneng* farming using agroforestry patterns.

The strategic management theory approach can be applied to bridge this research gap on the business model of Agroforestry *talas beneng*. It is also needed to formulate, implement, and re-evaluate farming to make it more profitable and sustainable. Information on internal and external farming environments is needed (David, 2011). Strengths, Weaknesses, Opportunities, and Threats (SWOT) is an analysis commonly used to formulate strategies that maximize strengths and opportunities but simultaneously minimize weaknesses and threats (Setyorini & Santoso, 2017). A business unit can use the business model canvas (BMC) framework to identify internal and external factors. BMC discusses business from nine aspects: customer segments, customer relationships, channels, value propositions, revenue streams, key activities, key partners, key resources, and cost structures (Osterwalder & Pigneur, 2015; Herawati et al., 2019; Rastryana, 2021).

Several research cases have used the BMC approach to studying small-scale farming. Rahayu (2019) studied the dragon fruit business strategy with the BMC approach, yielding several alternative strategies, including developing markets, increasing product competitiveness, increasing human resource capacity, increasing partnerships, and increasing business capital. Maftahah et al. (2022) explored mushroom cultivation, resulting in recommendations for an aggressive strategy with a new design for its canvas business model. Other studies have concluded that the digital marketing strategy for cut roses using the BMC approach increases business profits during pandemics (Manalu & Utami, 2021). Our study aimed to formulate strategic management for developing *talas beneng* farming based on the canvas business model framework, internal and external environmental conditions, and the current farm position. The results were expected to become references for farmers and related stakeholders, especially local government and market players for developing more profitable and sustainable *talas beneng* farming.

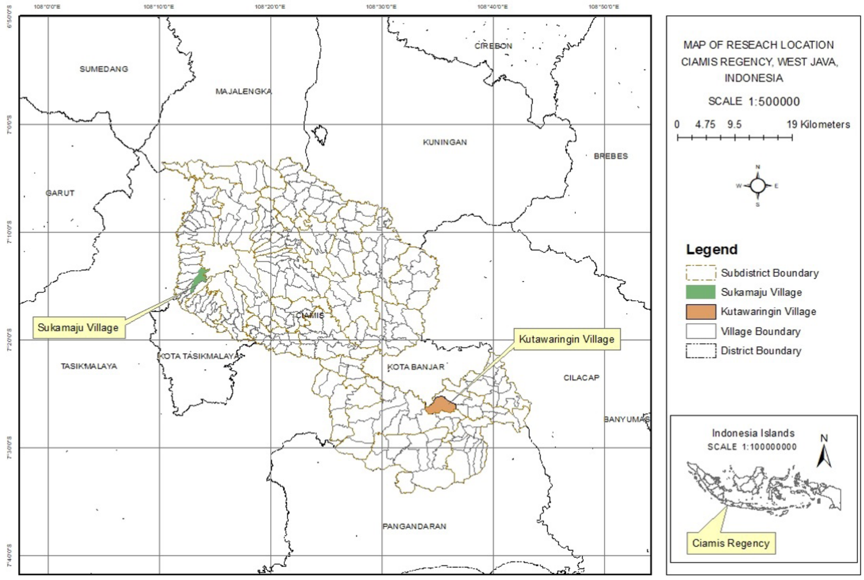
II. MATERIAL AND METHOD

A. Study Site

This research was conducted from May to August 2022 using a case study approach to *talas beneng* farming in the community forest in Sukamaju Village and Kutawaringin Village, Ciamis Regency. These two locations were chosen because *talas beneng* is available in this area. The development of taro farming in both locations began when several farmers collectively ordered taro seeds from 2019-2020. Farmers utilize community forestland by planting *talas beneng* between trees, fruit trees, and plantation crops. Currently, there are 51 *talas* farmers in both villages.

B. Data Collection Methods

The first stage of research data collection involved direct observation of the phenomenon of *talas beneng* farming and preliminary interviews with collectors, members of the Indonesian *talas beneng* Farmers Association (Pertabenindo), taro leaf choppers, and farming



Source: RBI Map 2019

Figure 1. Map of the study site

group chairs to identify the main issues and gain an understanding of the business. The main data collection comprised nine BMC aspects: customer segments, value propositions, channels, customer relationships, revenue streams, key activities, key resources, key partners, and cost structures. The nine aspects were classified into internal and external factors. Each factor was weighted and rated, including the relative attractiveness of the strategy to internal and external factors. The stages of data collection in this study were as follows.

1. BMC data collection was conducted through open-ended in-depth interviews with two key informants (farming group chairs). Furthermore, the classification is carried out into internal and external factors as well as the formulation of a strategy based on the SWOT analysis,
2. Assessment of the weight and rating of internal and external factors was carried out by filling out a questionnaire by two chairs and five administrators of *talas beneng* farming group, and
3. The attractiveness score of each strategy for internal and external factors was assessed by filling out a questionnaire with two farming group chairs, one manager of a village-owned enterprise that cultivates *talas beneng*, three researchers who investigated *talas beneng*, two academics, and three forestry officers.

The operational definitions and measurement methods for BMC variables are presented in Table 1.

C. Data Analysis

Farming business model data were analyzed descriptively, starting by reducing the data, presenting and finally concluding it so that a list of SWOT could be compiled. Furthermore, formulating the strategic management was arranged through three data processing stages, namely, the input stage (data with the internal factor evaluation [IFE] matrix and external factor evaluation [EFE] put into the matrix), the matching stage (building the internal–external [IE] matrix and SWOT matrix), and decision stage (applying the Quantitative Strategic Planning Matrix [QSPM]) (David, 2011). Each stage is explained in the following paragraphs.

Input stage

Evaluating internal and external factors begins with compiling a list of internal and external factors of taro farming, followed by weighting and rating. The weight indicates how important a factor influences taro farming

Table 1. Operational definitions and measuring methods of BMC variables

BMC variable	Definition	Measuring method/question
Customer segments	Talas beneng consumers who want to reach	Who and where are the current consumers of *talas beneng* and what are their characteristics?
Value propositions	*Talas beneng* products with economic values	What value products are offered to *talas beneng* consumers?
Channels	Media to inform *talas beneng* products to consumers	What channels to reach consumers, how, and which channels are most efficient?
Customer relationships	Types of relationships with consumers	What types of relationships do consumers expect and what types have been built?
Revenue streams	Income sources from t*alas beneng* farming	How much cost (in general) are the *talas beneng* products and how much does it contribute to farm income?
Key resources	Main resources needed for *talas beneng* farming	What are the main resources needed to run *talas beneng* farming?
Key activities	Main activities of *talas beneng* farming	What are the key activities to run *talas beneng* farming?
Key partnerships	Main partners who support *talas beneng* farming	Who is the *talas beneng* business partners?
Cost structures	All costs to run *talas beneng* farming	What are the most essential costs in the *talas beneng* farming business?

success. Meanwhile, the rating shows how well an organization responds to internal and external factors. The weight value starts from a scale of 5 (very important) to a scale of 1 (very unimportant), whereas the rating value starts from a scale of 4 (very good) to a scale of 1 (not good) (Rangkuti, 2016). This study's weight and rating values were obtained from the interview of two heads and five administrators/movers of taro farming groups. The next stage was to multiply the relative weight by rating to obtain the total score of IFE and EFE matrices.

Matching stage

Two data processing steps are taken in the matching stage: preparing the IE matrix and the SWOT matrix. The IE matrix aims to see the strategic position of *Talas beneng* farming in the matrix, which is arranged based on two key dimensions, namely, the total IFE score on the X axis, the total EFE score on the Y axis. It comprises nine cells that are grouped into three major sections with different strategy implications (Table 2).

a) Cells I, II, and IV are the grow and build categories. The main strategies supporting this position are backward integration, forward integration, horizontal integration, market penetration, market development, and product development.
b) Cells III, V, and VII are the hold and maintain categories. The main strategies supporting this position are market penetration and product development.
c) Cells VI, VIII, and IX are the harvest or divest categories. The main strategies supporting this position are retrenchment and divestment.

The SWOT matrix aims to develop alternative strategies based on a logic that maximizes strengths and opportunities while minimizing existing weaknesses and threats to support the main strategies for developing taro farming. The stages of compiling the SWOT matrix are as follows: 1) compiling a list of taro farming SWOT, 2) compiling a strength–opportunity (S–O) strategy based on matching strength and opportunity factors, 3) compiling a weakness–opportunity (W–O) strategy based on matching weakness and opportunity factors, 4) compiling a strength–threat (S–T) strategy based on matching strength and threat factors, and 5) compiling a weakness–threat (W–T) strategy based on matching weakness and threat factors.

Decision stage

After compiling several alternative functional strategies based on the SWOT matrix, priority strategy selection was carried out through the QSPM approach with the following steps: 1) listing internal and external factors in the left column of the QSPM; 2) assigning a weight value to each factor as in the IFE and EFE matrices; 3) compiling a list of strategic alternatives on the top row; 4) giving attractiveness score (AS), which indicates the relative attractiveness of each factor to alternative strategies with a value of 1 (not attractive), 2 (rather interesting), 3 (quite interesting), 4 (very interesting); and 5) calculating the total attractiveness score by multiplying the weight against the AS value. In

Table 2. Internal–external matrix for SWOT analysis

	I / E	Internal factor		
		Strong 3–4	Medium 2–2.9	Weak 1–1.9
External factor	High 3–4	I	II	III
	Medium 2–2.9	IV	V	VI
	Low 1–1.9	VII	VIII	IX

Source: (David, 2011)

this study, an assessment of the attractiveness of alternative strategies for internal and external factors was carried out by informants comprising two *talas beneng* farming group heads, a *talas beneng* business manager in a Badan Usaha Milik Desa enterprise, three researchers from the National Research and Innovation Agency, two academics from the Galuh University, two forestry practitioners from the West Java Forestry Department, and a forestry practitioner from the Center of Standard and Instrument Implementation of Environmental and Forestry Ciamis.

III. RESULTS AND DISCUSSION

A. Business Model Canvas of Talas beneng

Talas beneng is cultivated to produce leaves and tubers. However, only leaves have been produced and marketed in the research locations. Although the target consumers of both groups refer to wholesalers/exporters, a slight difference exists between the two *talas beneng* business groups in implementing their business models. Farming group 1 (Sukamaju Village) processes talas leaves into dried chopped leaves and sells them to wholesalers/exporters. Farming group 2 (Kutawaringin Village) sells wet talas leaves to collectors who produce dried chopped leaves and sell them to wholesalers/exporters. However, this difference has affected the other aspects of BMC between the two groups. An overview of the complete business model implementation by the two farming groups is presented in Table 3.

Information about *talas beneng* is disseminated to consumers and the public through social media and agricultural fairs. Proceeds from the sales of dry chopped and wet taro leaves are the main income sources for *talas beneng* farming. The main general activities of *talas beneng* farming are plantation, maintenance, leaf harvesting, leaf processing, and marketing. These activities differ slightly from those of farming groups in Kutawaringin Village who do not carry out postharvest activities because they sell wet taro leaves directly to collectors. The relationships between groups and consumers are carried out personally through direct and contract sales. To develop their business, farming groups in Sukamaju Village have Received Support from the village government and private companies from Semarang, Central Java. However, farming groups in Kutawaringin Village have yet to formally cooperate in developing their business. Some main farming activities that require direct

Table 3. BMC implementation of Talas beneng farming with agroforestry patterns by farmers

No.	BMC aspect	Farming group Sukamaju Village	Farming group Kutawaringin Village
1	*Customer segments*	Wholesalers/exporters	Collectors/choppers of taro leaves
2	*Value propositions*	Dried chopped taro leaves, taro tubers	Wet taro leaves, taro tubers
3	*Channels*	Private/direct, social media	Private/direct, agriculture fairs
4	*Customer relationships*	Direct sales, sales by contract	Direct sales
5	*Revenue streams*	Sales of dried chopped taro leaves, taro tubers	Sales of wet taro leaves, taro tubers
6	*Key resources*	Land, labor, farming tools, postharvest technology, farming capital, farming group institutions	Land, labor, farming tools, farming capital
7	*Key activities*	Plantation, maintenance, harvesting, processing, marketing	Plantation, maintenance, harvesting, marketing
8	*Key partners*	Village governments, private companies, production input providers	Production input providers
9	*Cost structures*	Direct costs (land tax, seed, chopper machine, postharvest infrastructure, postharvest labor) and indirect costs (family labor for planting and harvesting)	Direct costs (land tax, seed) and indirect costs (family labor for planting and harvesting)

costs include seed procurement, postharvest facility, postharvest labor, and land tax.

B. Internal and External Factors of *Talas beneng* Farming

The results of the SWOT analysis assessment of the nine BMC aspects of *talas beneng* farming reveal internal and external factors that affect the current *talas beneng* farming, as presented in the SWOT matrix in Table 4.

Farmers cultivate *talas beneng* as an intercrop among annual crops on community forestland. The potential for community forests in Ciamis Regency is extensive, as 20,866.47 ha spread over 27 subdistricts (Statistics Ciamis, 2022), making it a production factor potential for *talas beneng* development. It is also supported by the labor production factors available and simple cultivation methods. The number of farmers in Ciamis has reached 133,109 people (Ciamis Local Government, 2019). Farmers can cultivate *talas beneng* only with simple farming tools such as hoes, scrapers, and sickles. The production potential of up to 40 kg/stem at >2 years allows farmers to earn additional income. Moreover, the availability of chopper machines owned by farming groups and partners (collectors) is one of the supports for increasing the added value of *talas beneng* cultivation.

The low awareness among farmers regarding the maintenance of plants and limited product diversification are two causes of farming productivity at the research location needing to be more optimal. The limited farmer capital to buy seeds, which are expensive, and limited cooperation also significantly affect taro development. It is exacerbated by the farming management implementation that can be more optimal. The high demand for *talas beneng* leaves supported by the available marketing channels is still the main attraction for *talas beneng* farming. The revenue potential of *talas beneng* farming is relatively high because leaves have economic value in addition to producing large tubers. *Talas beneng* farming productivity increases with the opportunity to increase the added value from tuber processing. The weights of *talas beneng* tubers reach 2.4–15 kg in 8–12 months with potential revenue of IDR 20 million/ha per year (Susilawati et al., 2021). Government and private support and facilitation are needed to optimize these potentials and opportunities.

The impacts of similar businesses, such as Nampu taro (Halmonela javanica) and Kajar taro (Colocasia gigantea), are felt by *talas beneng* farming groups because both types can be used as substitutes for *Talas beneng* leaves. Access to market information is also an obstacle experienced by *talas beneng* farming groups. These groups have no strong bargaining position in determining selling prices. Plant-disturbing organisms and rainy season factors are also obstacles to *talas beneng* farming. Wild boar is a potential threat to *talas beneng* plants far from settlements. By contrast, rainy weather factors can reduce the quality of dry chopped taro leaves because the drying of leaves can be improved.

Table 4. Internal and external factors of talas beneng farming with agroforestry patterns

Strengths	Weaknesses
1. Sufficient land available	1. Limited product diversification
2. Sufficient labor available	2. Low intensification
3. Farmer equipment available	3. Limited capital
4. High production potential	4. Less partnership
5. Postharvest technology available	5. Poor farming management
Opportunities	**Threats**
1. High market demand	1. Beneng taro leaf substitution product
2. Marketing channel available	2. Limited access and market information
3. High-income potential	3. Monopoly selling price by trader
4. Non-leaf utilization potential	4. Plant-disturbing organism
5. Government/Private support	5. Rainy weather

C. *Talas Beneng* Farming Position

The responses of farming groups in utilizing strengths and minimizing existing weaknesses are in the average category, as shown by the total IFE matrix score of 2.76 in Figure 2.

Farmer optimism about the high production potential, leaf processing technology availability, and community forestland availability are the main strengths of *talas beneng* farming. Meanwhile, low farming intensification, limited product diversification, and limited farmer capital are the main areas for improvement in *talas beneng* farming development. Farmers can obtain additional income because of the high potential for *talas beneng* production, supported by adequate postharvest technology and sufficient land availability. The yield of taro tubers reaches 17.500 kg per ha with an average tuber weight of 40 kg/stem at >2 years (Agricultural Research Agency, 2016). Leaf processing technology availability and land adequacy are capital to increase the added value of *talas beneng* farming. Postharvest technology is quite helpful in adopting agricultural businesses (Mariyono, 2019). Meanwhile, land area determines income level (Nopitasari et al., 2019) and positively affects production, profit, efficiency, and farming sustainability (Arimbawa & Widanta, 2017; Benedetti et al., 2019; Ren et al., 2019).

Less intensive cultivation, low product diversification, and limited farmer capital cause *talas beneng* farming production and development to be less than optimal. Intensification is crucial to increase farming productivity (Mariyono, 2019) and labor absorption (Achmad et al., 2015). Opportunities to increase business productivity are still possible by increasing the variety of products harvested. Besides leaf products, the main tubers and mini tubers of *talas beneng* also have economic value (Susilawati et al., 2021). Thus, additional production input capital is needed, which is sufficient to increase farming income (Hermawan, 2019).

Apart from strengths and weaknesses, *talas beneng* farming has main opportunities and threats. The responses of farming groups in managing their opportunities and potential threats are in the moderate category, as shown by the total EFE matrix score of 2.93 in Figure 3.

Talas leaf demand, marketing channel availability, and the potential to increase added value from other leaf products are the main opportunities in *talas beneng* farming. Meanwhile, farmer limitations in accessing market information, the monopoly in selling prices by traders, and rainy weather factors are the main obstacles to its development. Market demand can influence farmers' selection of commodities (Setyaningrum & Banowati, 2020) and technology (Wardana & Alzarliani, 2019). The *talas beneng* business is increasingly attractive with the availability of institutions and marketing channels and opportunities to increase added value from tuber processing. However, the limitations of farming groups in accessing market information, the low bargaining positions of farmers in determining selling prices, and the weather factors during the rainy season are also obstacles in the current *talas beneng* farming development. In the digital era,

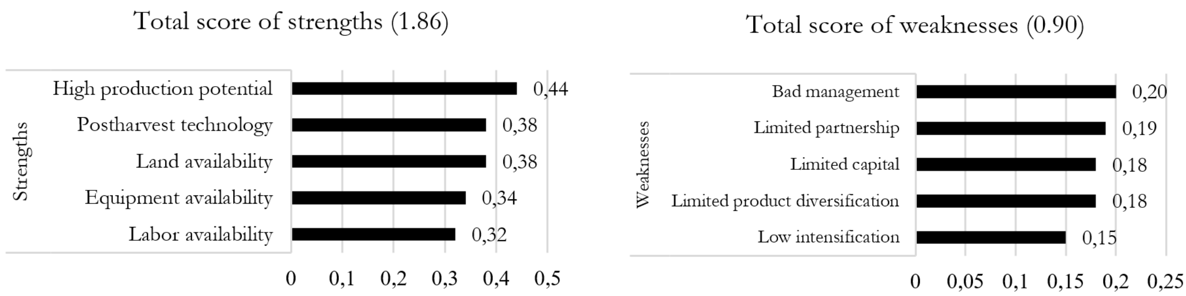


Figure 2. IFE matrix assessment of *Talas beneng* farming with agroforestry patterns

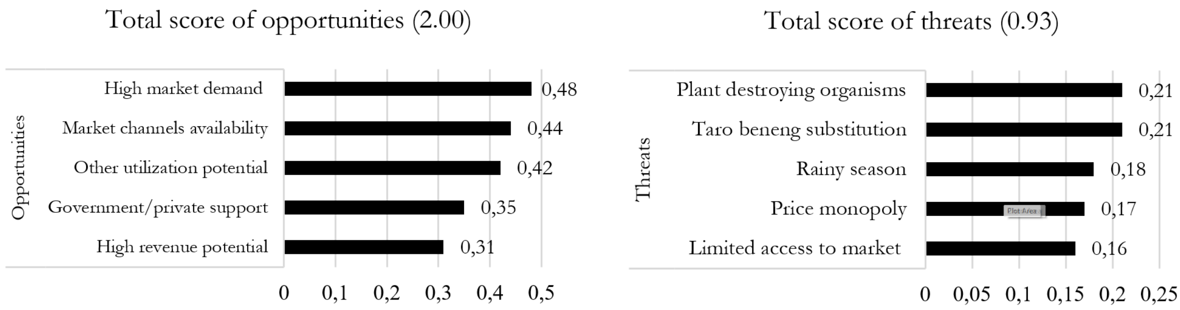


Figure 3. EFE matrix assessment of *Talas beneng* farming with agroforestry patterns

easy access to market information significantly affects agricultural business performance (Andriani et al., 2019; Isman, 2022) and farmer income (Syairozi, 2020). Price monopoly practices are also detrimental because farmers tend to obtain unfair prices (Sukri et al., 2018). Additionally, the rain factor is often the cause of decreased product quality due to drying problems (Apriliawan et al., 2018).

The evaluation results of internal and external factors show that *talas beneng* farming have more strengths than weaknesses and more significant opportunities than threats. Based on the IE position matrix, *talas beneng* farming in Ciamis Regency is in Cell V with a total score of IFE 2.75 and EFE 2.94. It is in the hold and maintained category. The main strategies recommended in this condition are market penetration and product development (David, 2011).

D. Formulating Strategic Management

It is suggested the main strategic management of both farmer groups should increase market penetration and product development in the near future. To support the main strategy in the current farming position, several alternative functional strategies were arranged by matching the strengths, weaknesses, opportunities, and threats through the SWOT matrix approach, as presented in Table 5.

The S–O strategy uses land, labor, agricultural equipment, processing technology, available market channels, and government/private support to increase taro farming productivity. Agricultural extensification and government/private facilitation can be alternative strategies to increase farming productivity. Meanwhile, the W–O strategy is formulated to reduce product diversification, farming intensification, working capital, partnership, and farming management weaknesses to gain better market opportunities. Increasing product diversity, farming intensification, increasing working capital, and expanding partnerships can be considered as alternative strategies to gain better market opportunities.

The S–T strategy is formulated to reduce the negative impacts of similar business development, price monopoly, pest disturbance, and weather factors that potentially threaten *talas beneng* farming sustainability. Optimizing available land, production, labor, equipment, and processing technology is an alternative strategy to reduce threat impact. Meanwhile, the W–T strategy is a defensive way to reduce internal weaknesses and external threats. Increasing product competitiveness, expanding partnerships, and improving farm management can be alternative strategies to minimize plant-destroying organisms and weather factors.

Three priority strategies are worth considering to support the main strategy for developing *talas beneng* farming in the Ciamis Regency based on the QSPM assessment. These strategies are: 1) improving farming intensification, increasing farming capital, and cooperating with the government/private sector; 2) improving *Talas beneng* product competitiveness; and 3) improving access to

Table 5. SWOT matrix of *Talas beneng* farming with agroforestry patterns

IFE / EFE	**Strengths (S)** Sufficient land available, adequate labor available, farming equipment available, high production potential, and processing technology available	**Weaknesses (W)** Limited product diversification, low intensification, limited capital, partnership, and poor management
Opportunities (O) High market demand, marketing channel available, high potential revenue, utilization potential other than leaves, and government/ private support	S–O Strategy (1) Farming extensification to increase production and sales; (2) Seeking government/private support for business development to increase production and sales;	W–O Strategy (3) Increasing product diversification to obtain better market opportunity; (4) Improving farming intensification, capital and government/private cooperation; (5) Government/Private facilitation to improve farming management;
Threats (T) Talas beneng substitution, limited access, market information, selling price monopoly, plant-destroying organism, and rainy season	S–T Strategy (6) Optimizing land and production potential to obtain better market opportunity, (7) Optimizing resources and technology to overcome plant-destroying organisms (OPT) and the rainy season	W–T Strategy (8) Improving farming competitiveness to obtain better market opportunities; (9) Expanding partnerships to better access market information; and (10) Improving farm management to overcome OPT and rainy season

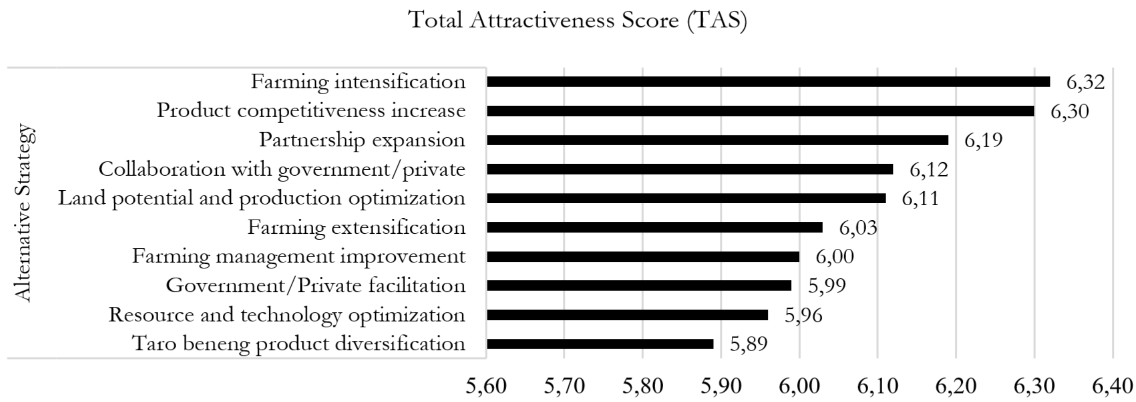


Figure 4. QSPM assessment of the *Talas beneng* farming strategy

market information through government/ private facilitation, as illustrated in Figure 4.

First, one of the causes of low productivity is that farmers need to take care of plants more intensively. Thus, farming intensification supported by capital and government/private facilitation is needed to increase business productivity. This strategy supports the main strategy (product development). Farming intensification affects increased production, income, and labor productivity (Emran et al., 2021; Gathala et al., 2021; Xie et al., 2019). Capital facilitation and cooperation by the government/private sector are expected to improve *talas beneng* farming intensification. It is in line with several research results that place intensification strategy, easy access to capital, and increased cooperation as priority strategies for overcoming farming problems (Fikri et al., 2019; Halimah et al., 2020; Rahayu et al., 2020).

Second, improving product competitiveness is necessary for developing *talas beneng* farming in the Ciamis Regency. Competitiveness shows how superior *talas beneng* products are so that consumers like them. Strategies to increase *talas beneng* product quality and quantity are needed to support the main strategy (market penetration). Such a strategy has been widely used and made a priority in various studies on farming strategy development, as described in previous research results (Ariyanti & Suryantini, 2019; Malik et al., 2018).

Third, improving cooperation with the government/private sector and expanding access to market information are needed to support the main product development strategies and market penetration. The government has become a potential institution as a facilitator of counseling and capacity building for farming groups (Atuahene-Gima & Amuzu, 2019). Meanwhile, the private sector can support production supply facilities and crop sales. The more comprehensive the business partnership, the more it minimizes business risk and increases the profit potential (Osterwalder & Pigneur, 2015).

Furthermore, the selected priority strategy is expected to support the main strategy and strengthen the current farming position to be further profitable and sustainable. *Talas beneng* farming intensification supported by capital, networking, product competitiveness, and adequate access to market information is expected to increase *talas beneng* value proposition, providing solutions for consumers and contributing significantly to farming income.

IV. CONCLUSION AND RECOMMENDATION

The BMC analysis shows the *talas beneng* farmers in Ciamis regency can be distinguished into two groups based on product and market channel types. The first group (Sukamaju Village) sells dry chopped taro leaves directly to wholesalers/exporters. In contrast, the second group (Kutawaringin Village) sells wet taro leaves directly to collectors who produce dried chopped taro leaves for sale to wholesalers/exporters. IFE and EFE matrices show that the *talas beneng* farming position in both groups is in Cell V (medium), indicating the hold and maintained categories with the two main strategies: product development and market penetration.

The SWOT analysis results in 10 alternative strategies based on matching internal and external factors that make it possible to support the main strategies in the current position of *talas beneng* farming. Based on the QSPM, three of 10 strategies should be prioritized, namely, 1) strategies to improve farming intensification; 2) strategies to increase product competitiveness; and 3) strategies to expand the business partnership.

This research suggests improving *talas beneng* technology intensification with agroforestry patterns and forming cooperation that benefits the farmers. The government of Ciamis Regency and related parties can help strengthen farming group institutions, facilitate capital, and improve farming management skills to ensure farming sustainability. Farmer groups are advised to increase production to supply *talas beneng* demand by optimizing resources, improving intensification, implementing better farming management, and expanding cooperation with the government and or market players. In the near future, local government and market players should have a road map to enhance the adoption of *talas beneng* as an understorey plant grown in the community forest areas outside the research areas. It has potentially improved the farmers' income and rural economic development while keeping the community forests more sustainable.

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AN ATTEMPT TO CONSERVE A VULNERABLE TREE SPECIES OF *Santalum album* L. THROUGH MICROPROPAGATION

Manikandan Gurusamy[1*], Karolinsobina Charlie[2], Kalaimathi Palanisamy[2], Divya Chelladurai[3], and David R Katerere[1]

[1]Department of Pharmaceutical Sciences, Tshwane University of Technology, Arcadia Campus, Pretoria, South Africa
[2]Department of Botany, Sri Kaliswari College (Autonomous), Sivakasi, TN, India
[3]PG and Research Department of Botany, Vivekanandha College of Arts and Sciences for Women, Tiruchengode, TN, India

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AN ATTEMPT TO CONSERVE A VULNERABLE TREE SPECIES OF *Santalum album* L. THROUGH MICROPROPAGATION. A rare kind of tropical plant in the Santalaceae family is Santalum album. The active ingredient in *S. album*, santalol, is also referred to as sandalwood oil and is highly prized in the fragrance business for its fixative qualities and pleasant, enduring scent. Out of all the species in the genus Santalum, *S. album* has the greatest oil concentration (about 6%). The wild plants are overharvested for their wood, which is used to make santalol, as well as for other uses including woodcarving and traditional medicine. *S. album* is an easily hurt plant. Thus, the creation of an in vitro mass propagation protocol for this valuable species is necessary in order to generate homozygous clones with large yields for the establishment of sandalwood plantations. In this study, a full-strength MS medium supplemented with varying concentrations of BAP and Kn (0.5-2.5 mg/l) was used to cultivate the shoot tip and intermodal portions of *S. album* that were collected from the wild. The maximum shoot development (4.50±0.50) occurred at a BAP concentration of 1.5 mg/l. IBA and IAA were added to the rooting medium along with the developing shoots. IBA (2.0 mg/l) had the highest mean number of roots (4.90±0.25) and root length (5.75±0.47 cm). Shoots that had been successfully rooted were moved to the field to harden. According to the current study, MS medium with 1.5 mg/l of BAP and 2.0 mg/l of IBA is an appropriate technique for micropropagating and conserving *S. album* is fragile tree species.

Keywords: Vulnerable, shoot tip, internode, BAP, IBA, conservation

UPAYA KONSERVASI JENIS POHON RENTAN Santalum Album L. *MELALUI MIKROPROPAGASI. Jenis tumbuhan tropis yang langka dalam famili Santalaceae adalah Santalum album. Bahan aktif dalam* S. album *juga disebut sebagai minyak cendana dan sangat dihargai dalam bisnis wewangian karena kualitas fiksatifnya serta aromanya yang menyenangkan dan tahan lama. Dari semua spesies dalam genus* Santalum, S. album *memiliki konsentrasi minyak terbesar (sekitar 6%). Tanaman liar dipanen secara berlebihan untuk diambil kayunya, yang digunakan untuk membuat santalol, serta untuk keperluan lain termasuk ukiran kayu dan obat tradisional.* S. album *merupakan tanaman yang mudah terluka. Oleh karena itu, pembuatan protokol perbanyakan massal in vitro untuk spesies berharga ini diperlukan untuk menghasilkan klon homozigot dengan hasil yang besar untuk pendirian perkebunan cendana. Dalam penelitian ini, media MS berkekuatan penuh yang dilengkapi dengan berbagai konsentrasi BAP dan Kn (0.5-2.5 mg/l) digunakan untuk mengolah pucuk pucuk dan bagian antarmodal* S. album *yang dikumpulkan dari alam. Perkembangan tunas maksimum (4.50±0.50) terjadi pada konsentrasi BAP 1.5 mg/l. IBA dan IAA ditambahkan ke media perakaran bersamaan dengan tunas yang sedang berkembang. IBA (2.0 mg/l) memiliki rata-rata jumlah akar tertinggi (4.90±0.25) dan panjang akar (5.75±0.47 cm). Tunas yang telah berhasil berakar dipindahkan ke lapangan untuk dilakukan pengerasan. Menurut penelitian saat ini, media MS dengan 1.5 mg/l BAP dan 2.0 mg/l IBA merupakan teknik yang tepat untuk perbanyakan mikro dan konservasi* S. album *adalah spesies pohon yang rapuh.*

Kata kunci: *Rentan, pucuk pucuk, ruas, BAP, IBA, konservasi*

[*] Corresponding author: rgmani.19@gmail.com

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I. INTRODUCTION

The eastern Himalayas and the Western Ghats, two of the 38 global biodiversity hotspots, are located in India, one of the most populous countries in the world. Along with countless endemic and Rare Endangered Threatened plant species, these places are home to some of the most important gene pools of medicinal plants, wild cultivable crop variants, and other species of commercial relevance (Roy et al.,2012). A number of endemic plant species, including 5,725 Angiosperm species, 10 Gymnosperm species, 193 Pteridophytes, 678 Bryophytes, 466 Lichens, 3,500 Fungi, and 1,924 Algae, are found in India's diverse flora (Sanjappa, 2005). One of the only remaining areas of great plant biodiversity are tropical forests, which are home to valuable, uncommon, and endangered commercial and medicinal tree species that must be immediately conserved for future generations and sustainable use.

The foundation of traditional medicine, medicinal plants, has been the focus of extensive pharmacological research in recent years. The recognition of the importance of medicinal plants as possible sources of novel compounds with therapeutic potential and as sources of novel compounds for drug development has led to this. Medicinal plants are employed for their antiviral, antibacterial, and antifungal properties in various parts of the world. Drugs generated from plants are used as a prototype for safer and more effective pharmaceutical products (Manikandan et al., 2017, 2019, & 2021). For millennia, plants have been a vital source of medicinal materials. The usage of plants to treat of numerous diseases datesback to prehistory and people of all continents has this historical tradition (Manikandan et al., 2020). Since plant-derived medications have greatly improved human health and well-being, plants have served as an inspiration for new medicinal molecules. They serve as a phytomedicine that may be utilised to treat illnesses as well as a source of essential chemical structure for the creation of novel antibacterial medications (Manikandan & Ramasubbu, 2020; Manikandan et al., 2022). They promote excessive collection of therapeutic plants, which results in extractive exploitation and puts the species in danger of going extinct. Because medicinal plants can be propagated in vitro to replace the supply chain for medications and other pharmaceutical items, there is evidently growing interest in this process. In vitro culture techniques provide extremely useful resources for conserving germplasm and mass-producing several endangered plant species (Pan et al., 2003).

One valuable tropical plant species that is a member of the Santalaceae family is *Santalum album* L., also known as Indian sandalwood (Rai, 1990). There are 29 genus in this family and nineteen of the nearly 400 species in this family are unique to the *Santalum* genus (Fox, 2000; Harbaugh, 2007; Harbaugh & Baldwin, 2007; Harbaugh et al., 2010; Butaud, 2015). The sandal oil found in the aromatic heartwood of *S. album*, an evergreen, hemi root parasitic tree of medium size, is highly prized in the fragrance, cosmetics, medical, and agarbatti (incense sticks) sectors (Srinivasan et al., 1992). It is indigenous to the subcontinent of India. The active ingredient in *S. album*, santalol, is also referred to as sandalwood oil and is highly prized in the fragrance business for its fixative qualities and pleasant, enduring scent (Jain et al., 2003). Of the species in the genus *Santalum*, *Santalum album* has the greatest oil content (6%) (Srinivasan et al., 1992).

Due to centuries of overexploitation, several species in the genus Santalum have been extinct, and three more are considered threatened. Due to the industrial exploitation of sandalwood's aromatic, oil-rich heartwood, the species' biodiversity has decreased. The IUCN designated *S. album* as vulnerable in 1998 due to its rapidly diminishing status (Awasthi, 2007). Biotechnology offers a viable and efficient technique of propagating members of this species in order to preserve the existing germplasm. Using clonal material with a comparable genetic background throughout cultivation is crucial for improving the homogeneity of essential oils and for producing economically significant metabolites in large quantities (Teixeira da Silva et al., 2016).

According to Sanjaya et al. (2003), *Santalum album* is resistant to both in vivo and in vitro multiplication, with only patchy results to yet. The primary means of artificial and natural multiplication are seeds. Conversely, grafting, air layering, and root suckers are methods used in vegetative multiplication; however, clone creation is a laborious and insufficient process (Srimati et al., 1995). Sandalwood is spread via seeds in the wild. But the germination success rate of seeds is quite low (Viswanath et al., 2009). After storage, seeds lose their viability in six to nine months. As per the IUCN status (2021.1), *S. album* is considered to be a vulnerable tree species. Thus, it's critical to create an in vitro strategy for the mass multiplication of this valuable species in order to generate homozygous clones with large yields for the establishment of sandalwood plantations. This work has been designed to support the conservation efforts on the fragile *S. album* L. (Indian Sandalwood) tree by micropropagation investigations, based on the foregoes.

II. MATERIAL AND METHOD

S. album, a 20-year-old tree, was harvested for its explants (shoot tips and nodal portions) from the Sirumalai hills in Dindigul, Tamil Nadu, India (1200 m asl; 10°11'05.8"N 78°01'04.7"E). After being submerged in running water for half an hour, the explants were treated with a commercial liquid detergent called Tween 80 (1%) and surface sterilised for five minutes using 0.1% Mercuric chloride. Afterwards, rinse with distilled sterile water. The explants were once again sterilised for three minutes using 4% sodium hypochlorite, and then they were washed with sterile distilled water. After a final three minutes of surface sterilisation with 70% ethanol, the material was cleaned with sterile distilled water, and the explants used a sterile blade to cut the tip end (Ramasubbu et al., 2015&2016; Manikandan et al., 2017; Thiri Bhuvaneswari et al., 2020).

The explants were infused with varying concentrations (0.5 - 2.5 mg/l) of BAP and kinetin (0.5 - 2.5 mg/l) in the MS medium (Murashige & Skoog, 1962). The culture rack in the room held the inoculated glass tubes at a suitable temperature of 25 ± 2ºC. The light source was a cool white fluorescent lamp with an average brightness of 2000 lux, and the photoperiod was 16 hours of day and 8 hours of night. Following 30 to 45 days, the number and length of shoots that emerged from the explants were also carefully monitored, along with the development of the shoots from the culture. Then, auxillary shoots that were grown in culture with cytokinin were typically rootless. To get whole plant, grown shoots were transferred to rooting medium supplemented with varying concentrations (0.5 - 2.5 mg/ml) of IAA and IBA. Half strength of MS medium is best for rooting shoots in vast number of plants

To prevent excessive water loss, rooted shoots with four to five completely developed leaves were planted in plastic pots filled with a 3:1:1 v/v mixture of sand, soil, and vermin compost. The pots were covered with polyethylene bags and watered twice a week during the first fifteen to twenty days of growth. For three to four weeks, plantlets were housed at 25°C (±2°C) in artificial light (16 hours photoperiod) supplied by white fluorescent light. After that, the pots were moved outside into the sun. Plantlets that had successfully established themselves were then moved to field conditions after three to four months.

III. RESULT AND DISCUSSION

A. Induction and Multiplication

Explants with a predetermined meristem, like nodes or shoot tips, are typically used in the in vitro propagation of plant species. These explants can come from mature trees or from in vitro or ex vitro germinated seedlings; other explants, like internodes, stem segments, or leaves of leaf discs, are typically used to induce adventitious shoots or somatic embryos. The explants employed for sandalwood included nodes and shoot tips from seedlings or old trees (Pearis & Senarath, 2015; Radhakrishnan et al., 2001; Singh et al., 2015; Ilah et al., 2002; Primawati, 2006; Revathy & Arumugam, 2011), The author of the current work employed the shoot tip and the internodal portion to induce the shoot and root of *S. album*.

Hartini and Endang (2016) state that the Woody Plant Medium is a type of medium that is commonly used for the in vitro cultivation of hardwood plant species, whereas MS media is most commonly utilised for the *in vitro* culture of callus and shoots induction for all plants. Since MS medium was higher in mineral salts and N compounds like ammonium and nitrate than Woody Plant Medium medium, it was more effective at inducing shoots in the propagation of sandalwood (Indrianti, 2003). These elements stimulated the growth of sandalwood. According to Bhargava et al. (2018), Murashige and Skoog's (MS) medium was previously used to propagate plants using *S. album* nodal explants.

In the current study, explants of shoot tip developed the highest number of shoots (4.50±0.50) and shoot length (4.12±0.50 cm), while explants of internodal at 1.5 mg/l concentration of BAP and 2.0 mg/l concentration of Kn formed 5.00±0.20 shoots and 4.70±0.35 cm length (Fig. 1 & 2; Plate 1 & 2). When compared to the Kn, the 1.5 mg/l concentration of BAP had noteworthy

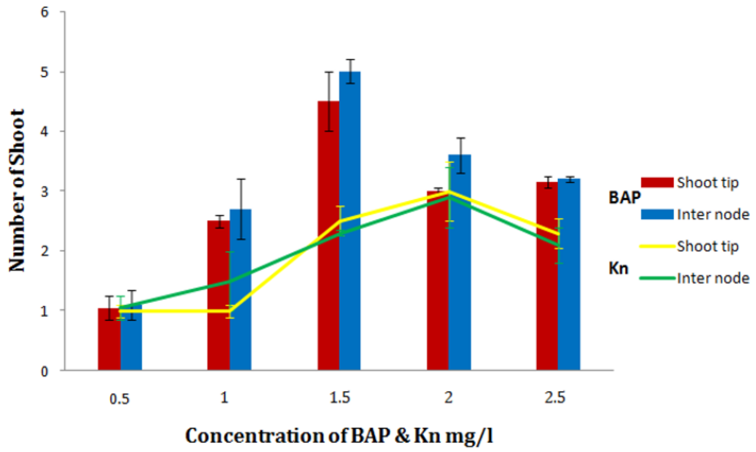

Figure 1. Number of shoot induction in various concentrations of BAP & Kn

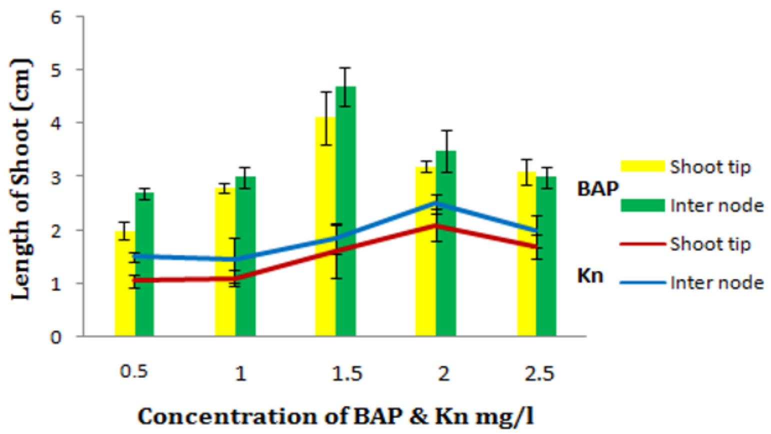

Figure 2. Length of shoot induction in various concentrations of BAP & Kn

outcomes. According to earlier research by Hartini and Endang (2016), MS medium enhanced with 2 mg/l BAP was the most effective medium for inducing shoots from Sandalwood. The 1.5 cm shoot with four leaves formed in MS0 medium. One hypocotyl explant produced twelve shoots after two milligram's per litre of BAP was added to the MS medium. The *S. album* explants that were examined for multiple shoot induction and intermodal segments yielded positive findings. The species reacted favourably to MS Medium out of the two media—MS and White media. The optimal combination of MS with 5.0 mg/l of Kn and 2.0 mg/l of BAP was found to be effective in inducing numerous shoots in shoot tip explants (Krishnakumar & Parthiban, 2018).

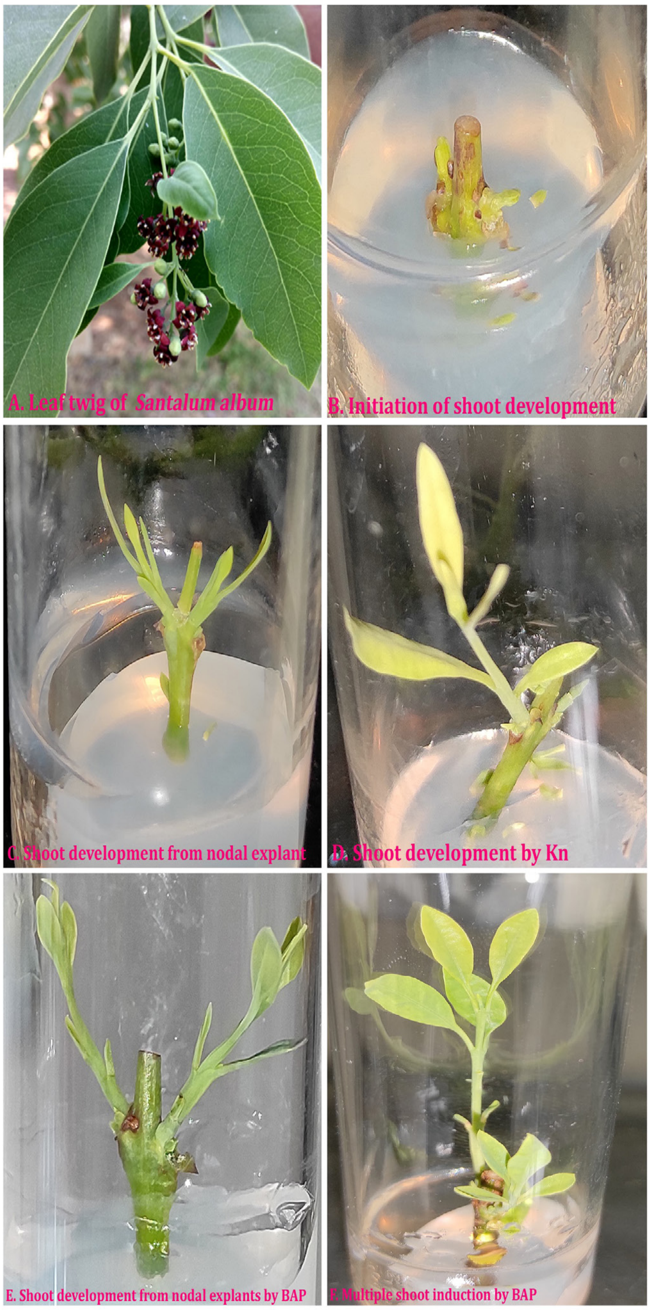

Figure 3. Shoot induction in various concentrations of BAP & Kn

B. Rooting

IBA and IAA were added to the rooting medium along with the developing shoots. IBA (2.0 mg/l) had the effective mean number of roots (4.90±0.25) and length of root (5.75±0.47 cm). In IAA (2.0 mg/l), the greatest mean number of roots (3.10±0.19) and root length (2.80±0.25) were found (Fig. 4; Fig. 5). When compared to IAA, the 2.0 mg/l concentration of IBA produced noteworthy outcomes. The current study was corroborated by a prior study, which found that there was a strong rooting response on MS medium with IBA (Janarthanam & Sumathi, 2011).

The most effective treatment for optimising sprouting, cutting rooting, shoot length, and root length was to root shoot tip cuttings of *S. album* 3 mg/L IBA (Krishnakumar & Parthiban, 2018). On woody plant media with 1.5 mg/l BAP and 1.5 mg/l IBA, nodal explants of *Syzygium densiflorum* produced the greatest number of shoots (7.7 ± 0.08). In half strength woody plant media supplemented with 0.5 mg/1 IBA, the maximum number of roots (3.83±0.53) and root length (3.4 ± 0.05 cm) were obtained (Ramasubbu & Divya, 2000). The 0.5 strength MS medium supplemented with 1.48 gM IBA produced the highest root induction (75%) and survival (80%) in *Holostemma annulare* (Sudha et al., 1998).

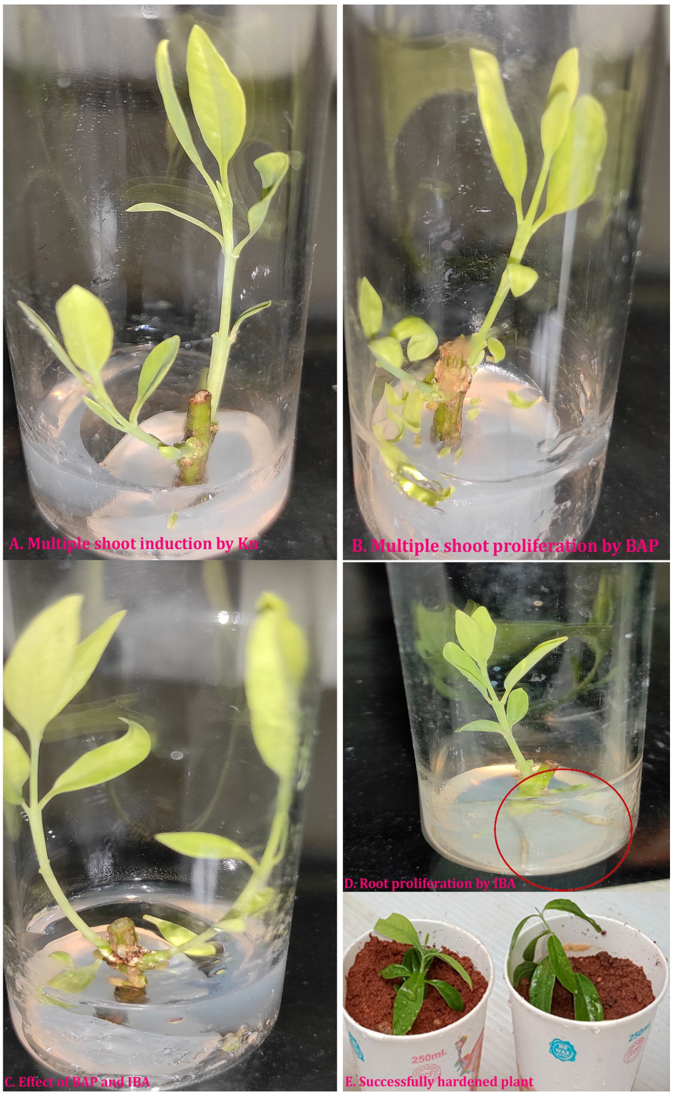

Figure 4. Shoot induction and root formation of *S. album*

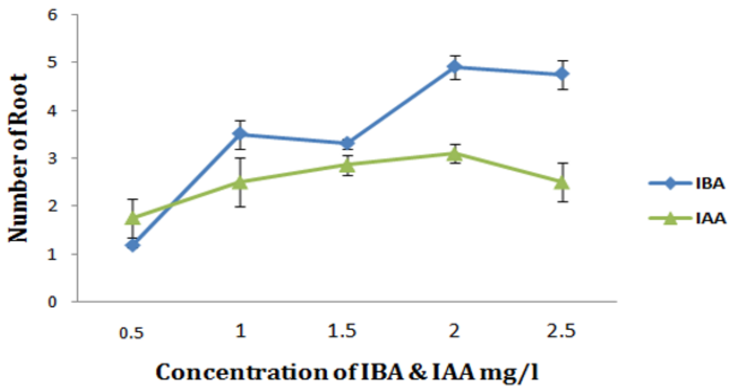

Figure 5. Shoot induction and root formation of *S. album*

C. Acclimatization

Shoots that had been successfully rooted were moved to the field to harden. Rooted shoots with four to five completely developed leaves were placed in plastic pots filled with a 3:1:1 sand, soil, and vermicompost. The pots were covered with polyethylene bags and watered twice a week for fifteen to twenty days in order to minimise excessive water loss (Fig. 4). For three to four weeks, plantlets were housed at 25°C (±2°C) in artificial light (16 hours photoperiod) supplied by white fluorescent tubes. After that, the pots were moved outside into the sun. Plantlets that had been successfully developed after three to four months were then moved to field conditions, where they survived 70% of the time. According to Soumen et al. (2010), shoots were able to root on half-strength MS medium that was enhanced with 2% sucrose and 1.5 mg/1 IBA. When well-developed plantlets were placed in plastic containers with soil and vermiculite (1:1), 81.1% of them survived. From shoot tip and nodal explants of *Morus nigra* L. several shoots multiplied when cultivated on Murashige and Skoog's media supplemented with BAP or K (0.5–5.0 mg l−1 each). After being moved to rooting media, shoots were given 0.25–1.0 mg l−1 IBA. Plantlets took four weeks to root. Plantlets that had been regenerated and rooted had been successfully inserted into the ground (Yadav et al., 1990).

IV. CONCLUSION

The current investigation established that the best technique for *S. album* micropropagation was MS medium containing 1.5 mg/L of BAP and 2.0 mg/L of IBA. As a result, this procedure can be utilised to conserve and propagate *S. album* tree species that are at risk.

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IMPACT OF FOREST FIRES ON PORTUGUESE FOREST ECOSYSTEM AND ITS NATIONAL EMISSIONS BUDGET

Muha A. Al Pavel[1*], Mário Marques[2] and K. N. Mukta[3]

[1]Forest Research Centre (CEF), School of Agriculture (ISA), University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal
[2]Institute of Social Science, University of Lisbon, Av. Professor Aníbal de Bettencourt, 9 1600-189 Lisbon, Portugal
[3]Department of Physics, Jahangirnagar University Bangladesh, Savar, Dhaka-1342, Bangladesh

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IMPACT OF FOREST FIRES ON PORTUGUESE FOREST ECOSYSTEM AND ITS NATIONAL EMISSIONS BUDGET. The study highlights the significance of the forestry industry in Portugal, and delves into the economic, social, and environmental ramifications of forest fires on this vital sector. With a specialization in forest services, the country's healthy and sustained growth is heavily reliant on this sector. The study is a literature review analysis of carbon sink-source relationships and related uncertainty in the Portuguese forest ecosystem. We found wide literature research that Portugal lost 4% of its total tree cover due to forest fires in 2016-2017. The study concludes that the drought and forest fires suffered by the country throughout the years have had a considerable impact on the national emissions budget. Forest fires have wielded a significant impact on the nation's overall carbon emissions, representing 10% during the years with the highest incidence and averaging 2.7% over 25 years. Prior to 2014, the country was making significant progress towards achieving its National Plan on Climate Change (PNAC) 2020 reduction target, with emissions projected to fall between 18-23% below 2005 levels. These factors are putting country in a non-compliance position with the PNAC 2020 commitment target (which is the same target as for the Kyoto Protocol 2nd Commitment Period).

Keywords: GHG emission, carbon flux, forest fires, climate mitigation and adaptation, and Mediterranean forest

DAMPAK KEBAKARAN HUTAN TERHADAP EKOSISTEM HUTAN PORTUGIS DAN ANGGARAN EMISI NASIONAL. Studi ini menekankan pentingnya industri kehutanan di Portugal, dan membahas dampak ekonomi, sosial, dan lingkungan dari kebakaran hutan terhadap sektor vital ini. Dengan spesialisasi dalam layanan kehutanan, pertumbuhan sehat dan berkelanjutan negara sangat bergantung pada sektor ini. Studi ini adalah analisis tinjauan literatur tentang hubungan penyerapan-karbon dan sumber-karbon serta ketidakpastian terkait dalam ekosistem hutan Portugal. Kami menemukan penelitian literatur yang luas bahwa Portugal kehilangan 4% dari total tutupan pohonnya akibat kebakaran hutan pada tahun 2016-2017. Studi ini menyimpulkan bahwa kekeringan dan kebakaran hutan yang dialami oleh negara selama bertahun-tahun telah memberikan dampak yang signifikan terhadap anggaran emisi nasional. Emisi dari kebakaran hutan telah memberikan dampak yang signifikan terhadap total emisi negara, dengan kontribusi sebesar 10% selama tahun-tahun dengan kejadian terbanyak dan rata-rata 2,7% selama periode 25 tahun. Sebelum tahun 2014, negara tersebut telah membuat kemajuan signifikan dalam mencapai target pengurangan Rencana Nasional untuk Perubahan Iklim (PNAC) 2020, dengan perkiraan emisi turun antara 18-23% di bawah tingkat tahun 2005. Faktor-faktor ini menempatkan negara dalam posisi yang tidak sesuai dengan target komitmen PNAC 2020 (yang merupakan target yang sama dengan Periode Komitmen Kedua Protokol Kyoto).

Kata kunci: Emisi GHG, aliran karbon, kebakaran hutan, mitigasi dan adaptasi iklim, dan hutan Mediterania

*Corresponding author: pavel.sust@gmail.com /maapavel@isa.ulisboa.pt

I. INTRODUCTION

The concentration of carbon dioxide (CO2) in the atmosphere has steadily increased since the early stages of industrialization (Dlugokencky & Tans, 2019; Quéré et al., 2019). This is primarily due to the emissions from fossil fuels and changes in land use, which have caused an increase in atmospheric carbon concentrations (Ciais et al., 2013; Quéré et al., 2017). The definition of land use and land cover modifications should be broad and inclusive, taking into account all types of land management activities, including those caused by humans, such as shifting cultivation, fertilizer use, logging, and fire presence, among others (Houghton & Nassikas, 2012).

The decline in land coverage seems to be caused primarily by forest fire (Alencar et al., 2011). In tropical rain forests, fire scarcely occurs naturally; due to human activity, fire deals with extreme temperatures and drought (Goldammer, 2016). In 2017, the fire trend increased since El Niño's international impact would last for two years and triggered drought throughout the tropics belt (Silva et al., 2018). Human influences also make forest more vulnerable to burning by the local climate (WMO, 2018). El Niño is also active in boreal and humid forests, where fires are more common, but global climate change raises fire intensity and costs (Weisse & Goldman, 2017; Ribeiro-Kumara et al., 2020). Forest combustion will emit huge quantities of carbon into the atmosphere (Weisse and Goldman, 2017). Blazes in Brazil, California, Portugal and elsewhere have recently been shown that forest fires do not go away–they just intensify because the global warming (BBC, 2019). Over the last two years (2016-2017), Portugal has had half of all forest areas (approximate 165.8 thousand ha) burnt in the EU because of incorrect policies of forest management, poor land use management and fire prevention activities (Weisse & Goldman, 2017; BBC, 2019; GFW, 2023). During this context, our study is, therefore, to review the carbon sink-source relationship and related uncertainty within the Portuguese forest ecosystem; discuss what the economic, social, and environmental importance of forests for Portugal is; and consider the implications that forest fires are having in Portugal's CO2 reduction targets. The objective of this study is to know carbon sink-source relationships and related uncertainties within the local forest ecosystem in Portugal through the review of obtainable literature. Intending to guide us throughout the development of this study and fulfil the proposed objectives, we set the subsequent research questions: (a) What has been the carbon sink and source evolution in recent years within the Portuguese forest ecosystems by taking into consideration the relevant uncertainties (e.g., forest fires, drought, etc.)? (b) What are the CO2 emissions related to forest fires and consequent impacts on GHG reduction targets for Portugal?.

II. MATERIAL AND METHODS

The research has been explored supported literature search by combination of the specific keyword using "logical" AND "operator" with period between 2005-2020 on search engine by Google, Google Scholar, Scopus, and Web of Science for the peer-reviewed article, news, case studies, report, on the issues of carbon sinks and sources within the forest ecosystem of Portugal. For searching, we used keywords like "objective" AND "problem" AND "scale". We also administered a literature-screening focus in the target objectives. We have been found total 92 literature to be focused of our objectives, and we have been short listed 25 articles. We categorized literature supported the combinations of the used word. We have read the abstract and content of selected articles to critically identify the various approaches to treat carbon sinks and sources within the Portuguese forest ecosystem (Figure 1.). Our literature review was examined consistent with specific predefined objectives (Vieites-Blanco & González-Prieto, 2020).

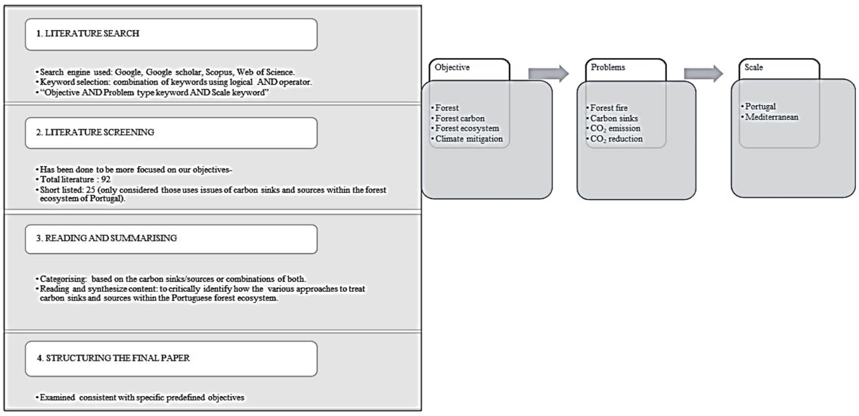


Figure 1. Sketched mapped for the literature review process.

III. RESULT AND DISCUSSION

1. Present Scenario of Portuguese Forests and their climate condition

Due to a group of important forest protection measures and impactful reforestation actions that were implemented during the past four centuries, Portugal has currently one among the most important forest areas in Europe – 35.4% or 3.2 million ha (Figure 2, Figure 3.) (ICNF, 2013a). The eucalyptus tree is the predominant specie in Portugal with a 26% occupation or 812 thousand hectares. The cork oak tree follows up with a 23% territorial presence, equivalent to 737 thousand hectares. In third place comes the pine tree with 22% or 714 thousand ha of forest areas (Figure 4.) (Nunes et al., 2019).

Geographically speaking, the eucalyptus species is primarily concentrated in the Beirã region (specifically, Baixo Vouga and Beira Interior Sul), as well as in Tâmega, Central Tagus, the West, and Alto Alentejo. The eucalyptus is comparatively recent in Portugal and coincides with the establishment and emergence of the paper industry (Figure 5.). The oak trees are abundant across Portugal, with various species found throughout the country. The cork oak tree dominates the south coast, while the holm

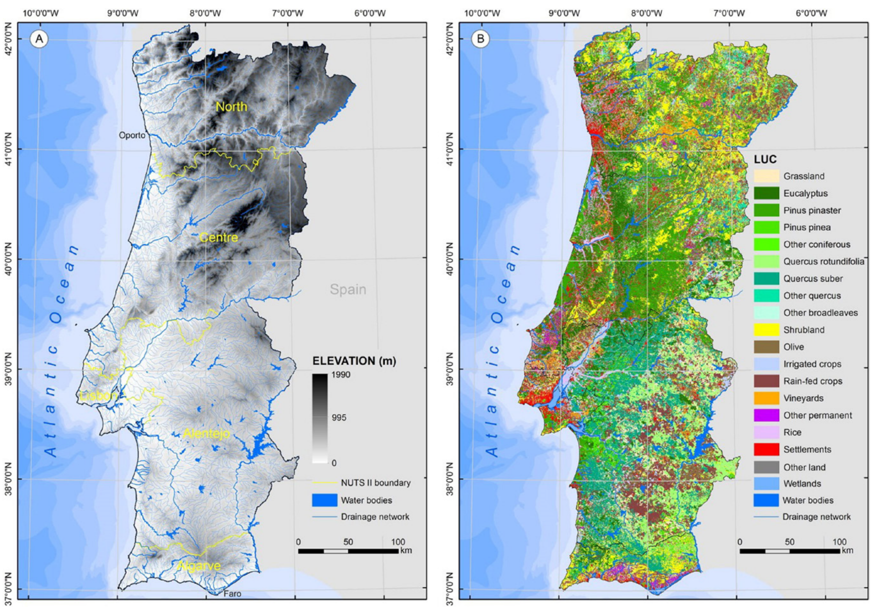


Figure 2. Elevation and land cover map of Portugal (Meneses et al., 2018).

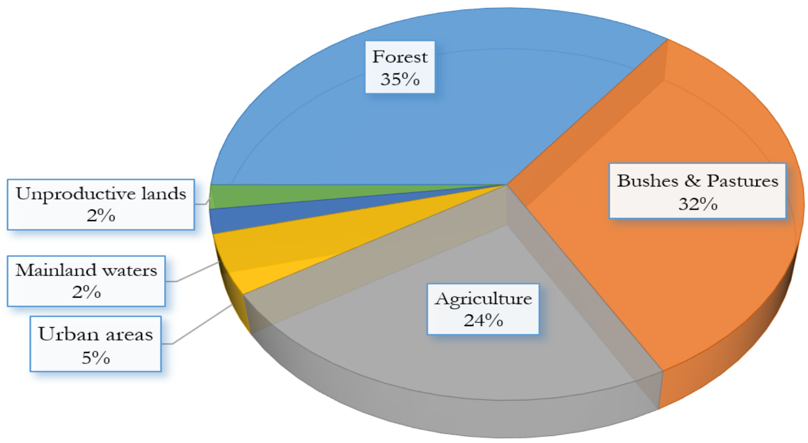


Figure 3. Landscape distribution in Portugal (Público, 2017).

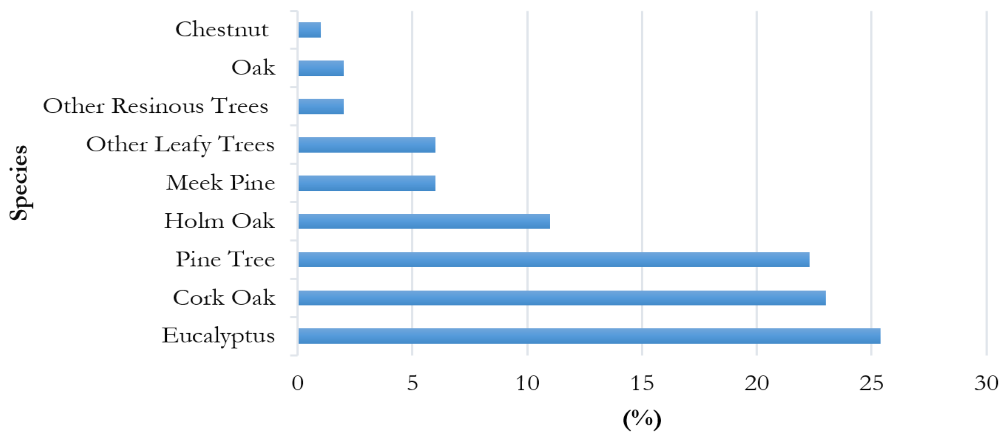
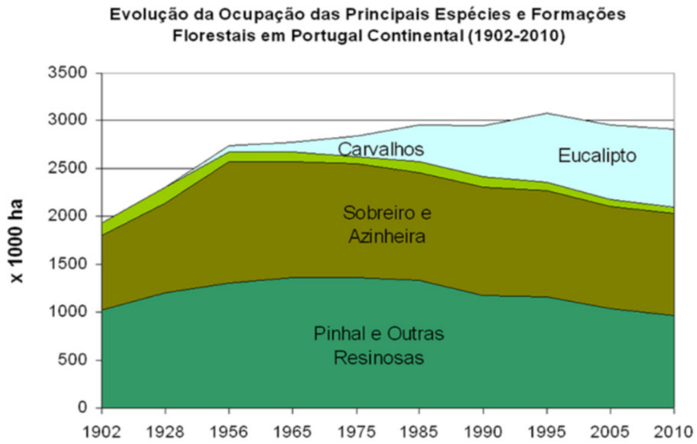


Figure 4. Forest areas distribution in Portugal (PEFC, 2017).

oak is frequently seen in the country's interior. The pendunculate oak is primarily concentrated in the north-east region, spanning throughout the Minho-Leiria coast band, which is known for its mild temperatures and high humidity. The black oak is located in the Beira Interior and Trás-os-Montes regions, and the meek oak is particularly present in the Setubal region, which is situated in the centre of the country.

Riparian species such as the pine-oak, willow, poplars, alders, elm, and ash trees are found within the northern part of the Tagus River and more rarely within the South in smaller agglomerates or sub-regions of the inner land (ICNF, 2013b). Most of the Portuguese forest property is owned by privately or entities. About 84.2% of the total forest area, equivalent to 2.8 million hectares, is owned by industrial businesses, while small household-level owners own the rest. The public ownership corresponds to 15.8% of the total forest of which 2% is owned by the state (lowest figure in the EU). It is estimated that in total there is the maximum amount as half a million forest owners (ICNF, 2013a). Despite the high number of forest owners and therefore the small dimension of forest properties, the resources that forests provide sustain an important and integrated industrial chain that is exports-based. Forestry is a significant contributor to Portugal's



In Sky Blue: Eucalyptus

In Green: Oaks

In Olive green: Cork oak and Holm oak

In Aqua: Pine Forest and Other Resinous

Figure 5. The main forest species occupation and forest formation between 1902-2010 in Portugal (Anonymous, 2014).

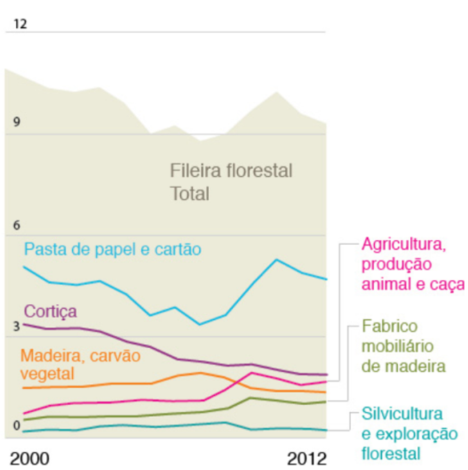
economy, and the country is well-known for its expertise in the industry. In comparison to the EU average, the forestry sector significantly contributes to Portugal's national GDP (PEFC, 2017). Despite its significant economic importance, the national forest in Portugal plays an equally crucial role in the environment and society more broadly.

The impact of climate change is significant in the region of the Atlantic Ocean and the Mediterranean Sea. This is due to the transition between the climatic conditions of the Mediterranean and the Atlantic. The amount of rainfall that falls in the region varies across the area. In the southern and north-eastern areas, the mean annual precipitation is less than 500 mm. In the northwest, however, it exceeds 2000 mm. The mean annual rainfall typically increases with higher latitudes, elevations, and proximity to the Atlantic Ocean. In the summer, the region experiences dry conditions, particularly in June, July, and August. Most of the rainfall is concentrated between October and March (Meneses et al., 2018).

2. Importance of Portuguese Forests

Forests play a crucial role in the Portuguese economy and society, as they contribute 10% of the country's total national exports and 2% of the Gross Domestic Product (GDP) (ICNF, 2017). The Portuguese forestry sector's most important products include paper and cardboard, paper folders, cork, wood, resin, and furniture products, which not only contribute significantly to the economy but also have environmental benefits, highlighting the multi-dimensional importance of Portuguese forests (Figure 6.). In addition to the significant economic benefits, Portuguese forests also provide valuable non-timber forest products such as dry fruits like chestnuts and pine nuts, support sport hunting, and facilitate outdoor activities such as tourism and recreation, further underlining the significance of forests in Portuguese society.

Portuguese forests also contribute significantly to the country's external trade, with a net value of the balance of payments representing a total of €2,267 million, which further emphasizes the economic importance of the forestry sector (ICNF, 2017). Portuguese forests play a critical role in the job market, providing employment opportunities to 101,700 direct workers, which corresponds to 1.8% of the active population, demonstrating the significance of the forestry sector for the country's workforce (Público, 2017). All these figures indicate that the sector is of greater importance in comparison with the EU (Table 1.). The strength of the Portuguese forestry



In Blue: Paper and cardboard pulp.
In Purple: Cork.
In Pink: Agriculture, livestock production and hunting.
In Yellow: Wood, coal, vegetal.
In Green: Wood furniture
In Turquoise: Forestry and forest exploration

Figure 6. The forest subsectors as a share of the total forest exports (Público. 2017)

Table 1. Contribution of employment, GDP, forest cover, and ownership in the forest sector of Portugal (Lopes and Cunha-e-Sá, 2014)

Forestry sector	Portugal (%)	Europe (%)
Contribution of Employment (2006)	1.6	1.1
Contribution of GDP (2006)	1.7	1.0
Forest Cover (2010)	38	45
Private Ownership	86	60

Carbon Sequestration	**Regulating services**	Climate regulation	Climate regulation	Mediation by biota, sequestration
Watershed Protection		Water regulation	Water purification	Mediation by ecosystems
Protection from Soil Erosion		Erosion regulation	Erosion prevention	Mediation of mass flows
Recreation	**Cultural Services**	Recreation	Recreation	Physical and experiential interactions
Landscape		Aesthetic values	Aesthetic information	Intellectual and representational interactions
Biodiversity		Supporting services	Habitat services (Gene pool protection)	Maintenance services (Gene pool protection)

Figure 6. Environmental importance of forests (Lopes and Cunha-e-Sá, 2014

sector is not only limited to the national level but also extends to the EU-wide level, as evidenced by its significant contribution to the economy and society, further cementing its position as a vital sector in the country.

Despite efforts to monetize forest services, the true value of Portuguese forests remains invaluable due to their unique, complex, and vulnerable nature, emphasizing the need to prioritize their preservation and protection for the benefit of both present and future generations. Portuguese forests offer numerous environmental benefits as listed (Figure 7.). Watershed protection is crucial for ensuring the quality of drinking water, balanced water and nutrient flow, and soil conservation (Postel &

Thompson, 2005). It involves keeping forests intact and investing in green infrastructures instead of building water treatment plants. This approach reduces property damage and future costs of providing clean water and treating wastewater, and it has become more cost-effective over time (EPA, 2012). The devastating fires of 2017 in Portugal have led to significant problems with water quality and contamination in certain regions, highlighting the urgent need for effective measures to prevent and mitigate the impact of future forest fires on the environment and communities. (Anonymous, 2017).

In recent years, the gradual forest cover decline in Portugal has also reduced biodiversity (Figure 8.). Some species are in danger and others have already disappeared from the country (Tomas, 2017).

The problem is that without an accurate human intervention in burnt forest areas, native species will not be able to reinstall themselves within the region because invasive tree species with rapid growth will tend to occupy these lands (i.e., eucalyptus). These same forest fires also created open-air and unprotect land areas. Raindrop splash makes soil particles detach in nutrient-rich lands. As a result of such fires, soils degrade and are unable to retain and purify water. This might considerably increase the risk of floods in surrounding communities (depending on location) and further reduce water quality. For instance, the dramatic forest fires during the summer of 2016 within the highlands of Funchal have significantly increased the risk of floods within the city. Funchal is surrounded by mountains that used to have forests widespread. Its tropical climate means that precipitation could also be intense sometimes. These surrounding forests served as water and land retainer-like thus prevented big floods and landslides. Since big parts of those forests were burnt in 2016, the city is today highly susceptible to inundation and rock collapse (Anonymous, 2016b). Finally, CO_2 emissions increase as a result of forest devastation. Because it is understood, forests are an important carbon sink and without them, the atmosphere would present much higher levels of greenhouse emission concentrations. The annual occurrence of fires has an impression of an impact on Portugal's national emissions target because it is discussed within the following section.

3. Impact of Fires in Portuguese Forests

Since the beginning of the new millennia, Portugal has suffered from a severe forest loss (Figure 9.). This is often mainly due to an increasing number of forest fires. The numbers are striking within the last 17 years, the country lists a total burnt forest area of 1.023 million ha (including the dramatic fires of 2017) (Assembleia da Républica, 2017). The numbers are not the same in every source. As an example, Global Forest Watch (GFW) states that for the

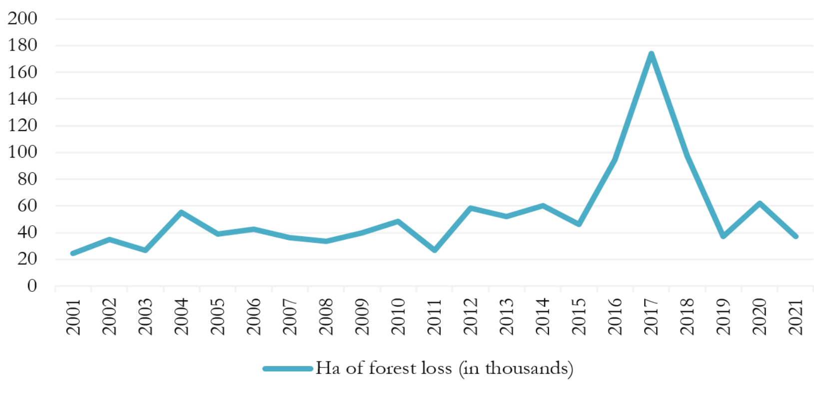


Figure 8. The gradually forest cover decline in last ten years (GFW, 2023).

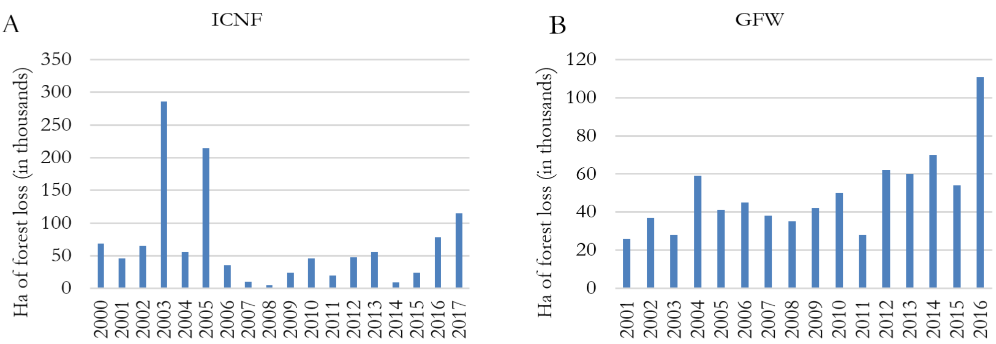


Figure 9. Burnt forest area in Portugal by ICNF (Institute for Nature Conservation and Forests) (A) and GFW (B).

same period a total of 0.79 million ha of forest were lost (excluding 2017 fires estimated at 0.12 million ha and including other sources of forest loss – logging and deforestation) (GFW, 2017); the national government stands for 0.15 million ha (excluding 2017 fire events) (Anonymous, 2016a). Altogether these three assertions, only forest loss is being considered as bush loss is outside the scope of this analysis. The previous two being the foremost independent sources.

Portugal has also made significant progress in increasing its tree cover, with a gain of 0.29 million hectares between 2001 and 2012, according to data from Global Forest Watch (GFW, 2017). Another report indicates that this same gain was about 0.2 million ha from 1995 to 2010 (Caetano et al., 2017). While it's important to note that the figures may not be entirely comparable due to variations in research methodology and content date, the overall trend suggests that losses in forest cover over the past two decades have outpaced gains. Forest fires are unequivocally the basis explanation for this outcome.

4. Impact of Forest Fires in Meeting National GHG Emission Targets

Over time, the frequency of forest fires has been on the rise. As previously discussed, these forest fires have pre-occupying effects on the economy, society, and environment. These effects are expected to aggravate over time due to global climate change. In Portugal, burnt forest area has increased in recent decades due to increasing dimension of forest fires (Figure 10.) (Assembleia da Républica, 2017).

When exclusively considering the impact of forest fires on the country's annual emissions, we can observe a noteworthy impact. Capable of significantly deviating Portugal's trajectory from achieving the ultimate GHG targets set under the National Plan on Climate Change (PNAC) 2020/2030 on an isolated year. The impact is lower when forest fire emissions are considered on average terms. As we can observe, emissions from forest fires can count at most with approximately 10% of the national total as it happened after the catastrophic years of 2003 and 2005 (expected to reach similar levels in 2017); and a minimum of with 0.2% like in 2008 and 2014 (Figure 11.). On average, from 1990 to 2015, forest fire emissions have contributed to 2.7% of Portugal's total emissions budget. This average figure is expected to increase after the 2017 events.

PNAC 2020/2030 has set a twin goal of reducing emissions by 18% to 23% by 2020, and 30% to 40% by 2030, having the 2005 emission levels as a baseline. When referring to the 2020 target (in line with the national target under the Kyoto Protocol 2nd Commitment Period), emissions from forest fires were not so important until 2014 when Portugal's emissions were at its lowest. Today, forest emissions (both on average and yearly) have the potential to vociferously undermine Portugal's commitments. Together may observe, in 2017 forest fires have abruptly

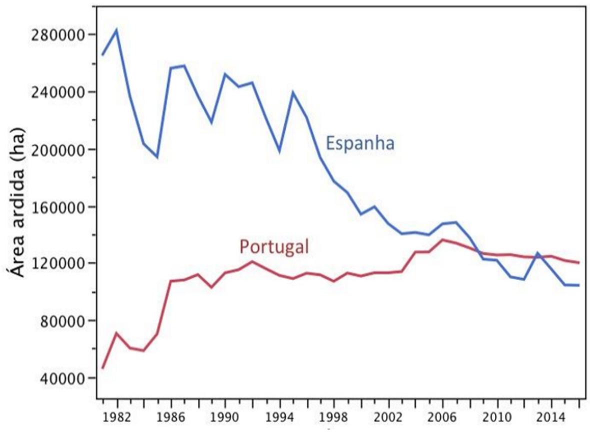
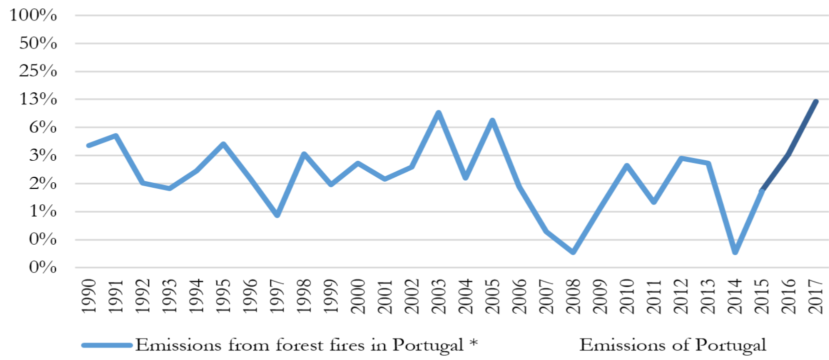


Figure 10. Evolution of forest fires over time in Portugal and Spain (Assembleia da Républica, 2017).



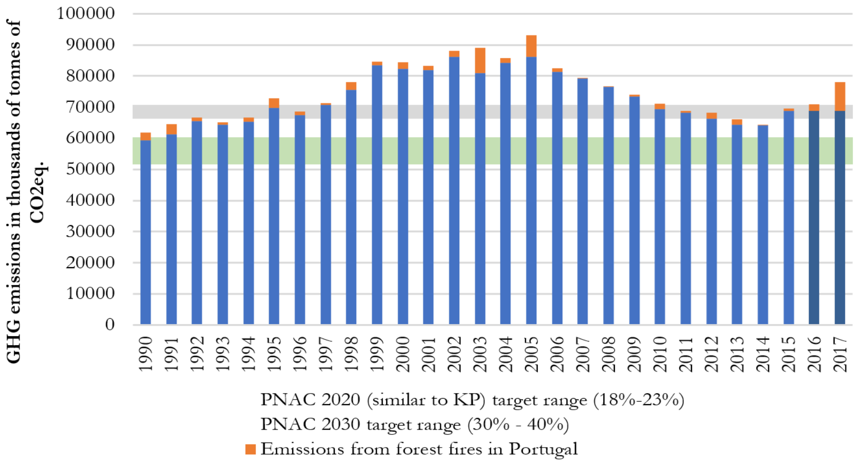
Note: *The ***dark blue*** line covering the 2016 and 2017 years is the forest fire emissions - published by the Portuguese Environment Agency (APA, 2017a, b) - as a percentage of the 2015 emissions since the figures for those years are not yet available. National emissions for 2016 and 2017 (excluding CO2 from forest fires) are expected to be higher than the 2015 reference due to the unprecedented droughts. This is an exercise intended to merely help the reader understand and have a clear view of the impact of 2017 forest fires.

Figure 11. Forest fire emissions as a (%) of total national emissions (APA, 2017a, b.

increased national emissions to levels that put Portugal in nonconformity regarding its reduction commitments (Figure 12.).

On the other hand, forest fires are not so relevant when considering that Parties under the Kyoto Protocol are allowed to leave 'abnormally high' emissions from natural phenomena unaccounted in their National Inventory Report (NIR) documents. This option can be pursued as long as regeneration and reforestation of the burnt areas are not used as negative emissions (sink) (APA, 2017a, b). For the time being, this seems to be a plausible possibility, and a decisive one is as long as the national emissions are increasing steadily since 2014. As an example, in 2017 the inclusion of forest fire emissions within the national total puts Portugal during a non-compliant position, but not any longer if equivalent emissions are excluded.

As to the PNAC 2030 goal, Portugal has committed to reducing its emissions by 30% to 40%. The foremost recent NIR report submitted by Portugal to the UNFCCC shows that Portugal has significantly increased its emissions in 2015 due to the severe droughts that the country has faced (APA, 2017a, b). These emissions are expected to further increase in 2016 and 2017 given the intensified droughts (Público, 2017). Due to these extreme events, Portugal is distancing itself from the



Note: *The two ***dark blue*** columns for 2016 and 2017 are the national emissions of the 2015 emissions given that the figures for the two most recent years are not yet available. National emissions for 2016 and 2017 (excluding CO2 from forest fires) are expected to be higher than the 2015 reference due to the unprecedented droughts. This is an exercise intended to merely help the reader understand and have a clear view of the impact of 2017 forest fires.

Figure 12. CO2 emissions and the impact of forest fires in Portugal (APA, 2017a, b).

2030 target (Figure 9.). Nevertheless, for this specific commitment, the state has enough time (13 years) to curb its emissions right down to promised levels with adequate investments in renewable energy, efficient technology, forest fire-prevention measures, and reforestation. Also, improve forest fire prevention measures and combat interventions (Weisse & Goldman, 2017; APA, 2022).

IV. CONCLUSION

Half all-forest areas (approximate 165.8 thousand ha) burned within the European Union occurred within the last two years (2016-2017) of Portugal which one of the most important forest areas in Europe (GFW, 2017; 2023). This is truly worrying given the economic, social, and environmental importance this sector has for the country. It concluded that these same forest fires have a considerable impact on the country's annual emissions. The impact of large-scale forest fires is significant, as they have the potential to significantly deviate Portugal from achieving its greenhouse gas (GHG) targets set under the National Plan on Climate Change (PNAC) 2020/2030 in a single year. Prior to 2014, Portugal was making good progress towards achieving its PNAC 2020 reduction target of reducing emissions by 18-23% below 2005 levels. In contrast, since that time, there has been an alarming increase in national emissions. The emissions' trajectory in the years of 2016 and 2017 is expected to be one of continued ascendance, up to levels that put Portugal in a non-compliance position with the PNAC 2020 commitment target (which is the same as the national target under the Kyoto Protocol 2nd Commitment Period). For the future, it is important to keep tracking the emissions growth trajectory and estimate what impact the increasing occurrence and intensity of forest fires and droughts may have in the years preceding the 2020 national CO2 reduction target. It is equally important to insist on a wide and broad discussion because of improving and upgrading adaptation plans and forest fire prevention and combat policies. To address the situation in Portugal effectively, it is crucial to focus on two key strategies (Weisse & Goldman, 2017; APA, 2022; GFW, 2023): controlling forest fire occurrences and reducing GHG emissions.

1. Controlling forest fire occurrences:
   - Enhance prevention efforts: Implement rigorous forest management practices, including regular monitoring, early detection systems, and firebreaks.
   - Improve firefighting capabilities: Invest in advanced firefighting equipment, enhance training programs for firefighters, and establish efficient coordination mechanisms among different agencies.
   - Increase public awareness: Educate communities about fire safety measures, promote responsible behavior in forested areas, and encourage the reporting of potential fire hazards.
2. Reducing GHG emissions:
   - Transition to renewable energy sources: Transitioning to renewable energy is vital not only in tackling the challenges of our dependency on fossil fuels but also in mitigating greenhouse gas emissions. Investing in renewable infrastructure, such as solar and wind power, will help achieve this goal.
   - Improve energy efficiency: Implementing energy-saving measures in buildings, industries, and transportation sectors can decrease energy consumption and associated emissions. Improving energy efficiency is a necessary step towards a sustainable future.
   - Promote sustainable practices: Encourage sustainable agriculture techniques, afforestation projects, and the use of cleaner technologies to reduce emissions from agricultural activities.
   - Support public transportation and electric vehicles: Improve public transportation systems, incentivize the use of electric vehicles, and develop charging infrastructure to reduce emissions from the transportation sector.

By prioritizing these strategies, Portugal can make significant progress in controlling forest fires and reducing GHG emissions, contributing to a more sustainable and resilient future. Additionally, international collaborations and partnerships can further support these efforts through knowledge sharing and resource mobilization.

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PHYSIOLOGICAL ACTIVITY ANALYSIS AND GROWTH OF EBONY SEEDLINGS (*Diospyros celebica* Bakh) TREATED BY VARIOUS BIOFERTILIZER COMBINATIONS

Siti H. Larekeng[1,2*], Sukriati A. Lamanda[3], and Muh. Restu[1]

[1]Biotechnology and Tree Breeding Laboratory, Forestry Faculty, Hasanuddin University. Makassar, South Sulawesi, Indonesia

[2]Natural Heritage and Biodiversity Research Center, Hasanuddin University. Makassar, South Sulawesi, Indonesia

[3]Postgraduate Faculty of Forestry, Hasanuddin University, Makassar, South Sulawesi, Indonesia

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PHYSIOLOGICAL ACTIVITY ANALYSIS AND GROWTH OF EBONY SEEDLINGS (*Diospyros celebica* Bakh) ON VARIOUS BIOFERTILIZER COMBINATIONS. Ebony is one of slow-growing species), that of suspected to be the triggering factors to prone to extinction. In addition, the nature of ebony seeds is recalcitrant, so they cannot be stored for a long time. Other factors affecting seed germination are the degree of maturity, size, and weight of the seeds. Seeds harvested before the physiological maturity level would not have high viability and even could not germinate well due to the in-sufficient of food reserves, and un-perfect embryo formatio. Bio-fertilizer is used to help accelerate the growth of ebony. This study aims to analyze the combination of bio fertilizers which would be giving a better effect on the physiological activity and growth of ebony seedlings. Morphological and physiological variables measurement were used in this study. The research design used *a Completely Randomized Design* at a real level of 5%. If the fingerprint results obtained have a real effect, then a further test of the *Duncan Multiple Range Test* is carried out. Research results showed that seed coating treatment of 45% *Penicillium* microbial solution + seed coating) gave the best results in each variable of both growth and physiology of ebony seedlings, while in the treatment without *seed coating*, *Sargassum* microbial solution 30% indicates good results in each variable of growth and physiology of ebony seedlings. Treatment with the immersion of seeds in microbes can produce a high percentage in each variable.

Keywords: Viability, germinate, morphological, seed coating

ANALISIS AKTIVITAS FISIOLOGIS DAN PERTUMBUHAN BIBIT EBONI (Diospyros celebica BAKH) PADA BERBAGAI KOMBINASI BIOFERTILIZER. Eboni merupakan salah satu jenis yang mempunyai pertumbuhan lambat(, yang menjadi salah satu faktor pemicu jenis tersebut rawan kepunahan. Selain itu sifat biji eboni rekalsitran, sehingga tidak dapat disimpan dalam waktu yang lama. Faktor lain yang mempengaruhi perkecambahan biji adalah tingkat kemasakan, ukuran, dan bobot biji. Biji yang dipanen sebelum tingkat kemasakan fisiologis tercapai tidak mempunyai viabilitas tinggi, bahkan tidak dapat berkecambah, dikarenakan belum mempunyai cadangan makanan yang cukup dan pembentukan embrio belum sempurna. Untuk mempercepat pertumbuhannya maka digunakan Biofertilizer untuk membantu mempercepat pertumbuhan eboni. Penelitian ini bertujuan untuk menganalisis pengaruh kombinasi bioferlilizer terhadap aktivitas fisiologis dan pertumbuhan bibit eboni. Variabel yang digunakan dibagi menjadi 2 karakter, yaitu morfologi dan fisiologi. Rancangan penelitian menggunakan rangcangan acak lengkap pada taraf nyata 5%. Apabila hasil sidik ragam yang diperoleh berpengaruh nyata, maka dilakukan uji lanjut Uji Wilayah Berganda Duncan . Hasil penelitian didapatkan pada perlakuan seed coating hasil yang terbaik di setiap variabel pertumbuhan dan fisiologi bibit eboni yaitu pada larutan mikroba Penicillium 45% + seed coating, sedangkan pada perlakuan tanpa seed coating hasil yang terbaik di setiap variabel pertumbuhan dan fisiologi bibit eboni yaitu pada larutan mikroba Sargassum 30%, perlakuan dengan perendaman benih dalam mikroba mampu menghasilkan persentasi tertinggi pada setiap variabelnya.

Kata kunci: Viabilitas, perkecambahan, morfologi, lapisan benih

*Corresponding author: sittihalimah@unhas.ac.id

I. INTRODUCTION

The development of the field of biotechnology has supported the level of public awareness of the negative impacts of the use of chemicals, which encourages the development of alternative products that are more environmentally friendly such as biofertilizers. Bio-fertilizer can be interpreted as an inoculum made from active living organisms that serve to add certain nutrients or facilitate the availability of soil nutrients for plants (Saraswati, 2012). The use of bio-fertilizers plays a role in influencing the availability of macro and micro-nutrients, the performance of enzyme systems, and increasing metabolism, plant growth, and yield. Bio-fertilizers are able to increase nutrient absorption efficiency, improve growth and production yields, and increase resistance to pest and disease attacks. The practice of using chemical fertilizers is harmful in the long run, so the alternative of using biofertilizers in agriculture and forestry can be used sustainably (Haggag et al., 2014).

Biofertilizers are usually applied by sprinkling on the soil at the stem and spraying on plants (Kalay et al., 2020), mixing with organic materials such as compost or manure (Shokibatun, 2019), and soaking seeds (Julfajri, 2019). Seed soaking causes microbes to colonize the seeds so that it can cause rapid plant growth or protect plants from pathogen attacks, so that plant growth becomes maximum. The results of the study of Agustina and Syamsiah (2018) showed that the length of seed soaking with a solution of local microorganisms for 16 hours gave the best effect in each observation parameter, namely on the percentage of seed germination, percentage of normal seedlings, average seedling height, average number of leaves, average root length, average fresh weight of seedlings.

The big role of bio-fertilizers is to help plant growth and development, with the help of living elements being one of the factors planned for bio-fertilizers derived from superior isolates produced from previous studies. Based on previous research (Restu et al., 2019), it shows that the superior isolate that has the potential to degrade litter is the *Penicillium ochrocloron* isolates. So, for the next stage, organic fertilizer based on *Penicillium ochrochloron* isolates will be made, and one isolate from the research results of (Masniawaty et al., 2019), namely *Bacillus*, as well as humic acid fertilizers and *Sargassum*. This study was conducted to compare physiological and growth activities of ebony seedlings treated by various biofertilizers.

Based on research conducted previously (Rohmanah, 2016), biological fertilizers have a very noticeable effect on the growth and productivity of green bean plants (*Vigna radiate* L.). In addition, there is an influence of brown algae liquid fertilizer (*Sargassum polycystum* L.) on the availability of N, P, and K soils in *Eleutherine americana* (Meliala, 2018). There is a noticeable effect of humic acids on the growth of cocoa seedlings (Santi, 2016). Application of Bacillus sp. strain consortium biofertilizer to the growth of nutmeg seedlings (*Myristica fragrans* Houtt) (Kalay et al., 2020a). Application of *Diospyros kaki* chitinase exhibited antifungal activity towards the pathogenic fungus *Trichoderma viride*. Chitinases with antifungal properties can be used as biocontrol agents replacing chemical fungicides (Zhang et al., 2013).

Ebony is (slow-growing species), that of suspected as triggering factors for this type to be prone to extinction (Mayasari et al., 2012). In addition, ebony seeds are recalcitrant seeds, so they cannot be stored for a long time (Sumiasri & Setyowati, 2006a). The seeds are rapidly germinated, that can reach germination percentage of 90%, while for those that have been stored in the refrigerator for 2 weeks, the percentage of germination drops to 20%. Ebony seeds stored in the room with temperate 25°C can no longer germinate (Suhartati & Alfaizin, 2020). To maintain their germination, they should be stored in wet charcoal powder (stored for 12 days, the percentage of germination can still reach 70%), Intact fruit of ebony is also good to keep the seeds viable (Santoso & Chairil, 2002). Other factors affecting seed germination are the degree of maturity, size, and weight of the seeds. Seeds harvested before the physiological maturity level do not have high viability and cannot even germinate due to in-sufficient food reserves, and in-perfectly embryo formation. In addition, the size of the seeds affects their ability to germinate, as this indicates the content of carbohydrates, proteins, fats, and minerals. Such materials are necessary as energy raw materials for embryos at the time of germination. So, it can be said that the size of the seeds shows a positive correlation with the protein content in the seeds. Seed weight also affects the speed of growth and production (Sumiasri & Setyowati, 2006; Sutopo, 1988).

The technology of extending the life of ebony seeds has not been mastered until now, so it has become an obstacle to their cultivation. This problem should be immediately studied to obtain a simple technology that can be applied so that the life of ebony seeds can be extended. This assessment also needs to be carried out so that the ebony stands grown are of high quality. The availability of a quality seed source of ebony is urgently needed. Seed sources can be chosen from well-looking natural stands or have grown well in their development areas. The current source of seeds, both in area, quality, and location of management, does not support large-scale ebony cultivation (Santoso & Chairil, 2002). There seems to be still unresolved related to the problem of ebony germination and there has been no research related to the use of microbes that can accelerate the process of ebony seed germination. This study aims to analyze the combination of bio fertilizers which would be giving a better effect on the seed physiological activity and growth of ebony (*Diospyros celebica*) seedlings.

II. MATERIAL AND METHOD

A. Materials

The materials used in this study were ebony seeds, microbial solution, CMC (Carboxymethyl cellulose) flour, water, kaolin, benzoate, hand spoon latex, sterile planting media, polybags, writing utensils, and measuring paper. The equipment used in this study were autoclave, analytical balance, oven, plastic tray, 70% shading net, camera, chlorophyll meter (SPAD-502), calliper, ruler, and soil meter).

B. Methods

Preparation of Microbial Solutions and Seed Coatings

The ebony seeds used are seeds derived from ripe fruits, then wash the seeds thoroughly under running water until the surface of the seeds is slippery and clean from the remnants of the fruit, then drain. Prepares microbial solutions of *Penicillium ochrochloron*, *Bacillus*, *Humic Acids*, and *Sargassum*. Soak the seeds with microbial solutions for 1 hour. The coating material is dissolved according to the concentration in the ratio of 1 gr (CMC flour) and 0.1 liters of water. Furthermore, additives, namely kaolin dan benzoates, are mixed in a ratio of 1: 1. Carrier materials (kaolin and benzoates) are used as a source of nutrients, and can maintain moisture so that microbes can grow. Each microbial solution is made, soaking the ebony seeds for 1 hour, then half of the seeds thet have been soaked in the *seed coating* manually. The *seed coating* is done as a carrier for additives, such as antioxidants, antimicrobials, antagonistic microbes, growth regulators, and substances with osmotic potential. Seeds without microbial solution soaking treatment and seed coating as a control. The total treatments in this study were P0 = (Control), PP1 = (*Penicillium* 30 ml / 1000 ml), PP2 = (*Penicillium* 45 ml / 1000 ml), PP3 = (*Bacillus* 30 ml / 1000 ml), PP4 = (*Humic Acid* 30 ml / 1000 ml), PP5 = (*Sargassum* 30 ml / 1000 ml), SP1 = (*Penicillium* 30 ml / 1000 ml + *Seed Coating*), SP2 = (*Penicillium* 45 ml/1000 ml + *Seed Coating*), SP3 = (*Bacillus* 30 ml/1000 ml + *Seed Coating*), SP4 = (*Humic Acid* 30 ml/1000 ml + *Seed Coating*), and SP5 = (*Sargassum* 30 ml/1000 ml + *Seed Coating*). The total experimental units were 33 units derived from 11 treatments x 3 replications.

Preparation of planting media

The planting media used are soil, compost, and rice husks that have passed the sterilization process with an autoclave. The sterilization process was carried out with an autoclave so that microorganisms or unwanted pathogens in the planting media died and the planting media was completely sterile. The heat generated from the evaporation process can naturalize proteins in microorganisms so that the microorganisms are depressed and then die (Sembiring, 2020). The sterilized planting media was then put into polybags size of 15 cm × 20 cm. Sterilized soil, compost, and rice husks (1:1:1, by volume) were then put in as much as 2/3 of the volume of polybags (900 g).

Seedling care

Care is carried out, including watering and weeding weeds. Watering was carried out at intervals of 2 days, once every morning and evening. Weeding was carried out once a week or when weeds appear. Weed control is simple, namely by pulling out the grass growing in polybags so as do not disturb the ebony seedlings.

Observation variables

The observation variables are divided into 2, namely the morphological character of the seedling and the physiological of the seedling. Morphological observations of ebony seedlings were carried out after planting seedlings in polybags for 12 months by observing several variables, consisting of:

a. The height of the seedlings is measured using a rule. Measured from the soil surface to the shoots.
b. The diameter of the seedlings is measured using a caliper that should be perpendicular to the stem and slightly pressed with constant pressure but not cause damage to the seedling. The diameter of the stem is measured at the bottom (± 2 cm above ground level).
c. The number of leaves in each plant is carried out by counting the number of leaves that grow until the end of the observation.
d. Root shoot ratio (RSR) is the ratio of dry weight of shoots (stems and leaves) to dry weight of the roots measured at the end of the observation with the formula: RSR = Dry weight of shoots (g)/ Dry weight of roots (g) (Darwo & Sugiarti, 2008).
e. The sturdiness quotient of seedlings (SS) is calculated using the formula: SS = Seedling height (cm)/ Seedling diameter (cm) (Sudrajat et al., 2019).
f. The seed quality index (SQI) is calculated using the formula SQI = (A+B)/ ((C/D)+(A/B)), where A = dry weight of stems + leaves (grams), B = dry weight of roots (grams), C = height (cm) and D = diameter (cm) (Kurniaty & Budiman, 2010).
g. The volume of the roots is measured at the end of the observation. The mensuration of roots volume started by washing the roots thoroughly, then the roots are cut and put in a measuring cup and observing the difference in the volume of water when inserted the roots with the initial volume of water.
h. The length of the roots is carried out using a crossbar. Measured from the beginning of the appearance of the roots to the tip of the roots.

The physiological observation variables of seedlings consist of the following:

1. The moisture content is tested using the oven method. Ebony seedlings are weighed for the initial weight (wet weight), then inventory the sample at a temperature of 105°C for 30 minutes and dried at a temperature of 80°C for 40 hours until constant weight, then re-weighed using analytical scales to determine the dry weight (Zhao et al., 2020). The formula moisture content (%) = ((initial weight – dry weight)/ initial weight) x 100%.
2. Biomass mensuration are carried out by harvesting the seedling and measured for

fresh weight, then placed the seedling into envelope and put it in an oven at 105°C for 30 minutes and dried at 80° C for 40 hours until they reach a constant weight during dry weighs (Zhao et al., 2020). Samples were weighed to determine the dry weight of the leaves, stems, and roots (Herpinawati et al., 2010).

3. Carbon can be measured based on the results of biomass calculations. Biomass data is entered in the formula per count of the number of carbon reserves. The amount of stored carbon comes from 50% biomass, so the calculation of carbon can be used in the equation of C = B x 0.5, where C= Carbon, and B= Biomass (Herpinawati et al., 2010).
4. Specific leaf area (SLA) is the ratio between the surface area of the leaf and the dry weight of the leaf. Leaf measurement is carried out by measuring the total area of the leaves with millimeter block paper. Next, the leaves are dried in the oven so that they get their dry weight. SLA values are expressed in cm2/g (Prihastanti, 2011).
5. Leaf area index (LAI), calculated according to the formula (Gardner et al., 1992):

$$LAI = \frac{L_{A2} + L_{A1}}{2} x \left(\frac{1}{G_A}\right) \quad \text{.........................(1)}$$

Where:
LAI = Leaf Area Index
LA = Leaf Area
GA = Surface Area

6. Leaf chlorophyll can be measured using a chlorophyll meter tool (SPAD-502). The observed leaf samples were whole leaves on ebony seedlings. Measurements are carried out at three points on the leaf, namely the base, middle, and tip, and do not hit the leaf bone, which is then averaged. The value of the number of leaf chlorophyll is calculated using the formula Y = 0.0007x – 0.0059, where Y = chlorophyll content and x = the value of the chlorophyll meter measurement result (SPAD-502) (Sudrajat & Siagian, 2014).

C. Data Analysis

Data analysis using the application programs STAR and Microsoft Excel. The observational data were analyzed using a Completely Randomized Design (CRD) at a real level of 5%. If the fingerprint results obtained have a real effect, then a further test of the Duncan Multiple Range Test (DMRT) is carried out. The statistical test model in this study is as follows (Malau, 2005):

$$Yij = \mu + \tau i + \varepsilon ij \text{ or } Yij = \mu i + \varepsilon ij \quad \text{...........(2)}$$

Where:
Yij = Observations on the i-th treatment and j-th observation
μ = General average
τi = Effect of i-th treatment = μ i-μ
εij = Random effect on the i-th treatment of the j-th observation

III. RESULT AND DISCUSSION

A. Morphological Character of Seedlings

Morphology is the form or structure of an organism or some of its parts (Haase, 2008a). Morphological characters are parameters that can describe an ideal seedling that always starts with parameters such as height, diameter, number of leaves, seedling weight, root shoot ratio (RPA), index the sturdiness of the seedling, the quality index of the seedling, the volume of the roots, and the length of the roots. The results of the analysis that have been carried out show that immersion with microbial solutions and seed coatings has a significant influence on the morphological character of ebony seedlings.

Seedling Height

The height of the seedling is measured by the crossbar from the base of the stem to the terminal end of the shoot. The results of the analysis showed that soaking treatment with microbial solutions and seed coatings had a very noticeable effect on the variable height of seedlings. Duncan's further test results (Table 1.) showed that the highest average seedling height (39.97cm) was found in SP2 (45% *Penicillium*

Table 1. Duncan's test of average seedling height of ebony treated by microbial solution treatment with and without seed coating

Treatment	Height (cm)
P0 = Control	16.53^{d}
PP1 = 30% *Penicillium* Microbial Solution	22.37^{cd}
PP2 = 45% *Penicillium* Microbial Solution	22.63^{cd}
PP3 = 30% *Bacillus* Microbial Solution	26.23^{bc}
PP4 = 30% Humic Acid Microbial Solution	27.37^{bc}
PP5 = 30% *Sargassum* Microbial Solution	37.00^{a}
SP1 = 30% *Penicillium* Microbial Solution + Seed Coating	22.90^{cd}
SP2 = 45% *Penicillium* Microbial Solution + Seed Coating	39.97^{a}
SP3 = 30% *Bacillus* Microbial Solution + Seed Coating	27.30^{bc}
SP4 = 30% Humic Acid Microbial Solution + Seed Coating	32.67^{ab}
SP5 = 30% *Sargassum* Microbial Solution + Seed Coating	27.13^{bc}

Description: Numbers followed by the same letters are not significantly different, and different letters are significantly different based on Duncan's test at the 0.05 significance level

microbial solution +Seed coating) treatment and PP5 (30% Sargassum microbial solution) without seed coating (37.00 cm) compared to control. The lowest seedling height (16.53 cm) was found in P0 (Control).

The SP2 and PP5 treatments did not differ markedly and gave higher average height of the seedlings compared to other treatments. This shows that soaking with 45% *Penicillium* microbial solution + seed coating and Sargassum microbial solution tends to give a good seedling height.

Ebony seedlings are more responsive to SP2 (45% *Penicillium* microbial solution + seed coating) and PP5 (30% Sargassum microbial solution) treatment than other treatments. It is concluded that microbial solution soaking treatment works optimally to stimulate the height growth of the seedling. The absorption of nutrients will greatly affect the speed of plant growth both vegetatively and generatively(Siregar, 2002)

Seedling Diameter

The diameter of the seedling is a morphological measure commonly used in the selection of seedlings in the seedbed. The results of the analysis showed that soaking treatment with microbial solutions and seed coatings had a significant effect on seedlings diameter. Duncan's further test results (Table 2.) show that the highest diameter of ebony seedlings was found after treating with SP2 (45% *Penicillium* microbial solution + seed coating) that is 0.57 cm compared to other treatments, The lowest seedling diameter is found in P0 (Control) with an average value of 0.29 cm.

The SP2 treatment gives the highest (0.57 cm) average diameter of the seedlings when compared to other treatments. This shows that soaking of 45% *Penicillium* microbial solution + seed coating tends to provide the best seedling diameter.

SP2 treatment (30% sargassum microbial solution) gave a good result to the diameter of the seedling, thus it works optimal. Accelerating the growth of the diameter of ebony seedlings requires sufficient nutrients that can be absorbed by seedlings. The microbes present at the roots of seedlings help the roots in the absorption of nutrients needed for the growth of the diameter of the seedling. Nutrient absorption will greatly affect the speed of plant growth both vegetatively and generatively (Siregar, 2002).

In general, larger diameter indicates better seedlings (Haase, 2008). The diameter of the stem is considered as a good estimator for

Table 2. Duncan's test average seedling diameterof ebony treated by microbial solution treatment with and without seed coating

Treatment	Diameter (cm)
P0 = Control	0.29e
PP1 = 30% *Penicillium* Microbial Solution	0.39d
PP2 = 45% *Penicillium* Microbial Solution	0.45cd
PP3 = 30% *Bacillus* Microbial Solution	0.43d
PP4 = 30% Humic Acid Microbial Solution	0.46cd
PP5 = 30% *Sargassum* Microbial Solution	0.56ab
SP1 = 30% *Penicillium* Microbial Solution + Seed Coating	0.48bcd
SP2 = 45% *Penicillium* Microbial Solution + Seed Coating	0.57a
SP3 = 30% *Bacillus* Microbial Solution + Seed Coating	0.45cd
SP4 = 30% Humic Acid Microbial Solution + Seed Coating	0.54abc
SP5 = 30% *Sargassum* Microbial Solution + Seed Coating	0.48bcd

Description: Numbers followed by the same letters are not significantly different, and different letters are significantly different based on Duncan's test at the 0.05 significance level

Table 3. Duncan's test of the average number of leaves of ebony seedlings treated by microbial solution treatment with and without seed coating

Treatment	Number of leaves (strands)
P0 = Control	13.67d
PP1 = 30% *Penicillium* Microbial Solution	17.33cd
PP2 = 45% *Penicillium* Microbial Solution	17.33cd
PP3 = 30% *Bacillus Microbial* Solution	19.33bcd
PP4 = 30% Humic Acid Microbial Solution	21.00bc
PP5 = 30% *Sargassum Microbial* Solution	24.00b
SP1 = 30% *Penicillium* Microbial Solution + Seed Coating	14.67d
SP2 = 45% *Penicillium* Microbial Solution + Seed Coating	30.33a
SP3 = 30% *Bacillus* Microbial Solution + Seed Coating	15.67cd
SP4 = 30% Humic Acid Microbial Solution + Seed Coating	19.00bcd
SP5 = 30% *Sargassum* Microbial Solution + Seed Coating	24.00b

Description: Numbers followed by the same letters are not significantly different, and different letters are significantly different based on Duncan's test at the 0.05 significance level

seedling survival in the field. A large diameter also indicates a good root system and a large volume of rod.

Number of Leaves

The results of the analysis showed that the soaking treatment of microbial solutions with seed coating had a noticeable effect on the number of leaves. Duncan's test results (Table 3.) showed that the highest average number (30.33) of seedling leaves was found in the SP2 (*Penicillium* microbial solution 45%+Seed Coating) treatment. The number of leaves is found in P0 control) was 13.67.

The result of Duncan test is in Table 3. The SP2 treatment provides an average of more leaves when compared to other treatments. This shows that soaking a of 45% *Penicillium* microbial solution + seed coating tends to provide the greatest number of leaves.

SP2 treatment (45% *Penicillium* microbial solution + seed coating) gave the microbial solution soaking treatment work optimally to increase the number of leaves. The growth process of the number of leaves requires nutrients that can be absorbed by the seedlings so that the microbes contained in the roots of ebony seedlings help the roots in the absorption of nutrients needed for vegetative and generative plant growth (Siregar, 2002).

The large number of leaves is directly proportional to the height and diameter of the seedling. A large number of leaves will result in more shading. Shading also tends to increase auxin content which can affect the length of the internodes so as to increase the height of the seedlings (Siregar, 2002). Leaves are one of the plant organs where the photosynthesis process takes place that produces food for plant and as a food reserve, leaves are also very related to photosynthesis activity because they contain chlorophyll needed by plants in the process of photosensitive and the number of leaves, the results of photosynthesis are higher so that plants grow well (Ekawati, 2007).

Root Shoot Ratio (RSR)

The results of the various analysis showed that the treatment of soaking microbial solutions with seed coating had no noticeable effect on the ratio of root shoots. The results of Duncan's test (Figure 1.) showed that the ratio of the highest ebony seedling root shoots was found in the PP1 treatment (30% *Penicillium* microbial solution), i.e 5.15 when compared to other treatments. Meanwhile, the lowest root shoot ratio was found in P0 (Control) treatment with an average root shoot ratio value of 2.37.

Calculation of the ratio of root shoots is one way to find out the quality of seedlings. Eligible seedlings height are 10-40 cm, RSR = 1:1 or 2:1, wood around the hard root neck, symmetrical header, and solid root system (Torey et al., 2014). The seedlings assessed are ready for planting if the range of RSR is 2-5 (Jumadi & Hartono, 2015). Ebony seedling at the age of 12 months has already qualified to be ready for planting, which can be seen in the graphic (Figure 1.) with a range of RSR values of ebony seedlings between 2-5.

The RSR indicates the physiological condition of the plant because RSR reflects the total value of growth production, namely the dry weight of the shoots and root. The large dry weight of the shoots will limit the RSR value. More leaf growth will increase the value of RSR (Prananda & Riniarti, 2014). In addition, the size of the RSR indicates that seedlings can be moved to the field (Danu & Kurniaty, 2013).

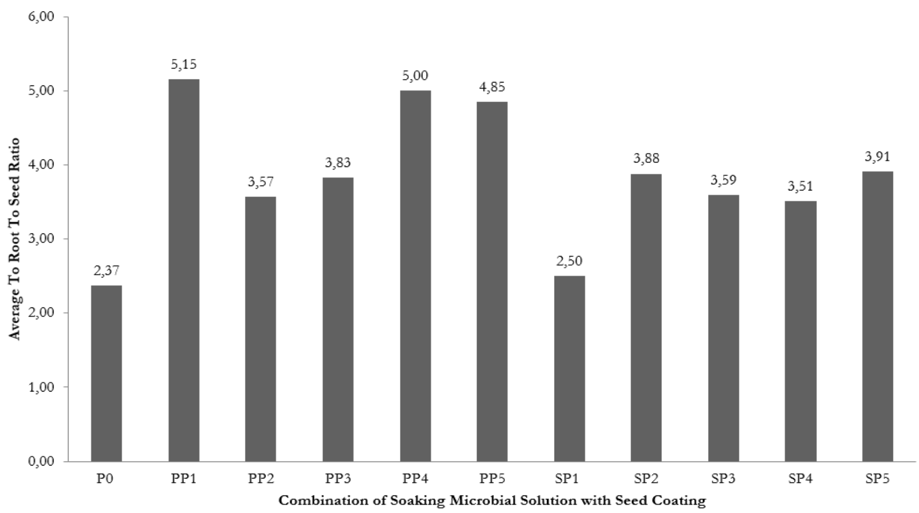

Figure 1. Root shoot ratio diagram of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

Seedling sturdiness quotient (SS)

The sturdiness quotient parameter of seedlings (SS) is a comparison between height (cm) and diameter (cm). A high ratio indicates relatively tall seedlings are thin, while a low ratio indicates sturdy seedlings. The results of the analysis showed that the treatment of soaking microbial solutions with seed coating had a noticeable effect on the SS of seedlings. The result of Duncan test (Table 4.) showed the highest value of sturdiness of ebony seedlings found in the SP2 treatment (45% *Penicillium* microbial solution + seed coating) i.e 70.04. Meanwhile, the lowest value of seedling sturdiness is found in the SP1 treatment (30% *Penicillium* microbial solution + seed coating), with an average SS value of 47.73.

The result of Duncan test is in Table 4. The result shows that soaking a 45% *Penicillium* microbial solution + seed coating tends to provide a high SS value of the seedlings.

The SS indicators gave significantly different results in the SP2 treatment (*Sargassum* microbial solution 30%) compared to SP1 (30% *Penicillium* microbial solution + seed coating). It can be expected that the microbial solution soaking treatment worked optimally to the SS of the seedlings. The sturdiness of seedlings is determined by the magnitude and variety of height and diameter of seedlings. The sturdiness value of seedlings at all treatments ranges from 47.73-70.04. Seedlings with a SS value of more than 60 is not expected to be planted. The smaller the value of SS, the stronger the seedlings(Pamungkas & Rini, 2013). Optimum SS is close to the value of 40-50 (Adinugraha, 2012). It can be said that seedlings with PP3, PP4, PP5, SP2, SP3, and SP4 treatments do not meet the optimum SS value of seedlings because they have a SS value of more than 60. The treatment of P0, PP1, PP2, SP1, and SP5 were resulting the value ranges from 40 to 50 that indicates optimum SS of ebony seedling to be planted.

Seedling quality index (SQI)

The quality index is designed to evaluate a number of combinations of morphological parameters to estimate the performance of seedlings after planting in the field. The results of the analysis showed that the treatment of soaking microbial solutions with seed coating had no noticeable effect on the SQI. Duncan's test results (Figure 2.) showed the highest ebony SQI found in the SP2 treatment (45% *Penicillium* microbial solution + seed coating) i.e 0.20. Even though, this value of SQI is not different with those untreated (control) seedlings (0.08).

Table 4. Duncan's further test of the average sturdiness quotient of ebony seedlings treated by microbial solution treatment with and without seed coating

Treatment	The Sturdiness of Seedlings
P0 = Control	56.43[bc]
PP1 = 30% *Penicillium* Microbial Solution	56.81[abc]
PP2 = 45% *Penicillium* Microbial Solution	49.35[c]
PP3 = 30% Bacillus Microbial Solution	60.53[abc]
PP4 = 30% *Humic* Acid Microbial Solution	60.10[abc]
PP5 = 30% *Sargassum* Microbial Solution	66.08[ab]
SP1 = 30% *Penicillium* Microbial Solution + Seed Coating	47.73[c]
SP2 = 45% *Penicillium* Microbial Solution + Seed Coating	70.04[a]
SP3 = 30% *Bacillus* Microbial Solution + Seed Coating	60.28[abc]
SP4 = 30% Humic Acid Microbial Solution + Seed Coating	60.31[abc]
SP5 = 30% 30% *Sargassum* Microbial Solution + Seed Coating	55.66[bc]

Description: Numbers followed by the same letters are not significantly different, and different letters are significantly different based on Duncan's test at the 0.05 significance level.

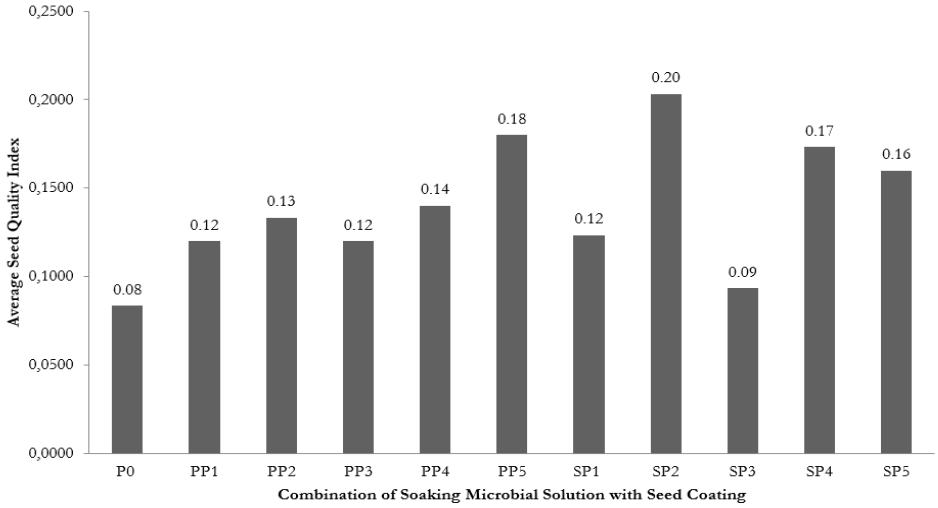

Figure 2. Seed quality index diagram of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

Ebony seedlings in each combined treatment of microbial solution soaking with seed coating have good seedling quality except for the control. A good quality seedling has an average value of seedling quality index greater than 0.08 (Djamhuri, 2012). Thus, refer to the result of SQI of ebony (Figure 2.), there needs some treatments to get good quality seedlings.

The SQI is a comparison between the total dry weight and the sturdiness quotient of the seedling, and the ratio of the root canopy. The quality index of seedlings can be used as a parameter because it can describe the morphological and physiological properties (Adinugraha, 2012). The availability of nutrients in the soil, soil structure, and good soil air system greatly affects the growth and development of roots, as well as the ability of roots to absorb nutrients. In addition, the availability of sufficient sources of carbon and energy in the soil seems to make microbes grow and develop well in the soil. Therefore, bacteria can associate with plant roots in tethering and dissolving nutrients for plants (Altaf, 2021).

Root volume

The results of the analysis showed that the treatment of soaking microbial solutions with *seed coating* had a noticeable effect on the volume of roots. Duncan's test results (Table 5.) showed that the highest average volume of ebony seedlings was found in the SP2 (45%*Penicillium* microbial solution + seed coating) treatment of 11.83 cm3 The lowest root volume is found in the PP1 treatment (30% *Penicillium* microbial solution), with an average root volume value of 6.80 cm^3. This value is significantly different with those seedlings treated by SP2 (45% *Penicillium* microbial solution + seed coating).

Plants faced with dry conditions, there are two kinds of responses that can improve water status. Including plants changing the distribution of new assimilates to support root growth at the expense of the canopy so as to increase the capacity of the roots to absorb water and inhibit the expansion of leaves to reduce transpiration. Plants will regulate the degree of opening of the stomata to inhibit water loss through transpiration (Setiawati & Syamsi, 2019). The absorption of water and nutrients is absorbed by the tip of the roots. Large absorption of water and nutrients causes root development so that there is a balance of root volume with plant growth (Kalay et al., 2020). The low amount of water will lead to limited root development, so the absorption of nutrients by the roots of the plant will be disturbed (Scharwies & Dinneny, 2019).

Table 5. Duncan advanced test average root volume of ebony seedlings treated by microbial solution treatment with and without seed coating

Treatment	Root Volume (cm^3)
P0 = Control	7.23[c]
PP1 = 30% *Penicillium* Microbial Solution	6.80[c]
PP2 = 45% *Penicillium* Microbial Solution	7.30[c]
PP3 = 30% *Bacillus* Microbial Solution	8.27[bc]
PP4 = 30% Humic Acid Microbial Solution	8.20[bc]
PP5 = 30% *Sargassum* Microbial Solution	9.17[abc]
SP1 = 30% *Penicillium* Microbial Solution + Seed Coating	8.37[bc]
SP2 = 45% *Penicillium* Microbial Solution + Seed Coating	11.83[a]
SP3 = 30% *Bacillus* Microbial Solution + Seed Coating	6.87[c]
SP4 = 30% *Humic Acid Microbial* Solution + Seed Coating	10.97[ab]
SP5 = 30% *Sargassum Microbial* Solution + Seed Coating	8.60[abc]

Description: Numbers followed by the same letters are not significantly different, and different letters are significantly different based on Duncan's test at the 0.05 significance level

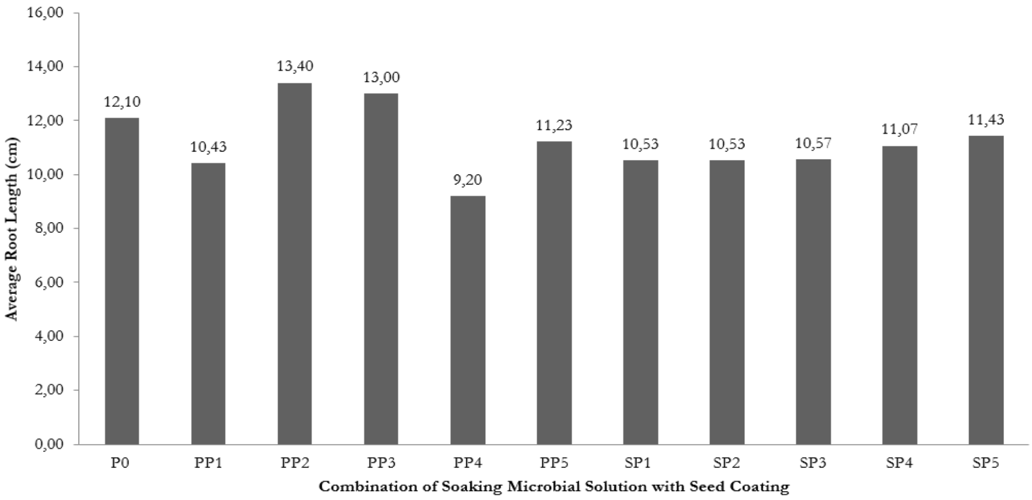

Figure 3. Diagram Root length of ebony seelings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

Root length

The results of the analysis showed that the treatment of soaking microbial solutions with seed coating had no noticeable effect on the length of the roots. Figure 3. shows that the highest average root length of ebony seedlings was found in the PP2 treatment (45% *Penicillium* microbial solution) of 13.40 cm, and the lowest root length is found in the PP4 treatment (30% *Humic Acid Microbial* Solution), with an average root length value of 9.20 cm.

Based on the combined results of the study on the variables of height, diameter, number of leaves, and root volume, it has a noticeable effect of the used treatments on the measured variables. The best results produced in each variable are found in the treatment of SP2 (45% *Penicillium* microbial solution + seed coating) and PP5 (30% *Sargassum* microbial solution). Treatment by soaking seeds in the suspension of biological agents (microbes) is able to produce the highest percentage in each variable. Based on previous research, SP2 is produced from the best microbes that produce the growth hormone indole acetate acid (IAA), which can spur growth. Microbes used as boosters in seedling growth can be through mechanisms such as fixing nitrogen, dissolving phosphates, and producing IAA, gibberellin, and cytokinin.

Seed coating is one of the preventive control techniques for pathogenic attacks consisting of carrier materials, adhesives, and biological agents(Wiyono, 2012). The carrier material used in this study is kaolin. Kaolin content is in the form of micro and macronutrients such as Mg, Na, Fe, and Cu, which can be a source of nutrients (Trivana & Pradhana, 2017). Besides, it can maintain moisture so that microbes can still grow (Garinas, 2012).

The use of carrier materials and other materials as seed coatings not only affects microbial growth but also affects seed viability. The selection of carrier materials, nutrient providers, and adhesives needs to be considered because it can affect the imbibition process so that germination would be disturbed. The use of CMC adhesive resulted a lower germination percentage compared to *gum Arabic* adhesive in cucumber seeds (Ikrarwati et al., 2015). The best results produced in each variable are found in the existing treatment due to the role of coating materials and microbes that are able to produce growth hormones.

B. Physiological Character of Seedlings

Measurement of the physiological activity of seedlings can provide more accurate results in estimating the quality of seedlings (Nurhasybi et al., 2020). Physiological tests have been practiced on coniferous species in several nursery locations operationally by the USDA forest service and have produced more tangible results. Although the physiological test is more reflective of the ability of seedlings to grow after planting, the test relatively takes time to employ (Koryati et al., 2021).

Moisture content

Moisture content of leaves

The average value of moisture content of the leaves is in Figure 4. It shows that SP5 has the highest moisture content of 55.77%, while the lowest is found in the SP3 treatment at 49.14%. The results of a complete randomized design analysis shows that moisture content of ebony leaves having an unreal effect among treatments. SP5 treatment, indicates a high average leaf moisture content value compared to other treatments, so it is assumed that SP5 treatment has a good character of leaf moisture content.

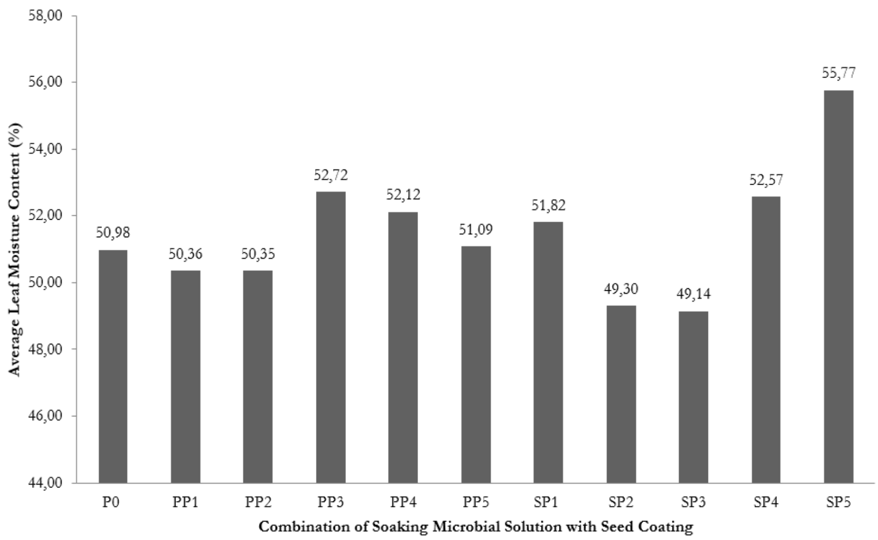

Figure 4. Diagram of leaf moisture content of ebony seedling treated by microbial solution treatment with seed coating. See Table 1 for treatment information

Moisture content of the stem

Based on Figure 5, it can be seen that the highest stem moisture content is found in the PP3 treatment at 50.58%, while the lowest is found in the PP1 treatment at 41.00%. The results of the analysis showed that the moisture content of the ebony stem having an unreal effect among treatments.

Based on Figure 6, it can be seen that the highest root moisture content is found in the PP3 treatment at 39.08%, while the lowest is found in PP2 treatment at 31.21%. The results of the analysis show that the moisture content of ebony roots having an unreal effect among treatments.

Based on the combined graph of moisture content on the leaves, stems, and roots of various treatments, it can be seen that the moisture content of the leaves is high compared to the moisture content of the stems and roots. This happens because leaf production is more abundant compared to stems and roots, which identify a greater moisture content of the leaves. In addition, it can show better forage quality. This statement can be supported by (Syafrida et al., 2018), which state that the higher the leaf portion of a plant and the smaller the portion of the stem, the ratio of the dry weight of the leaf to the dry weight of the stem will be higher.

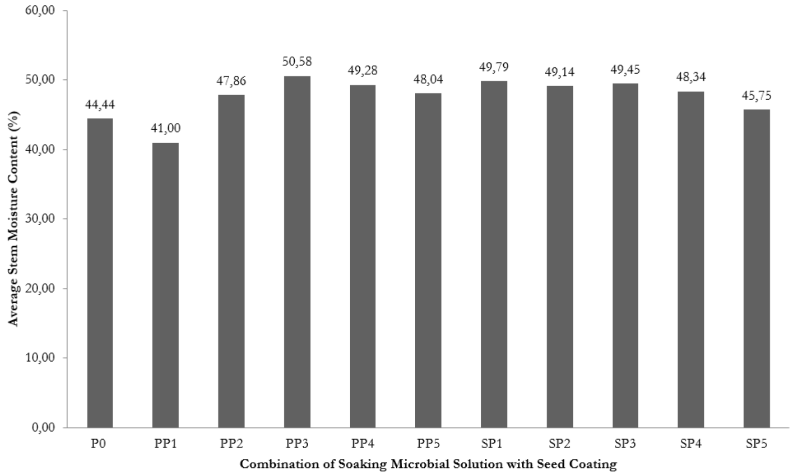

Figure 5. Diagram of the stem moisture content of ebony seedlings treated by the treatment of microbial solutions with and without seed coating. See Table 1 for treatment information

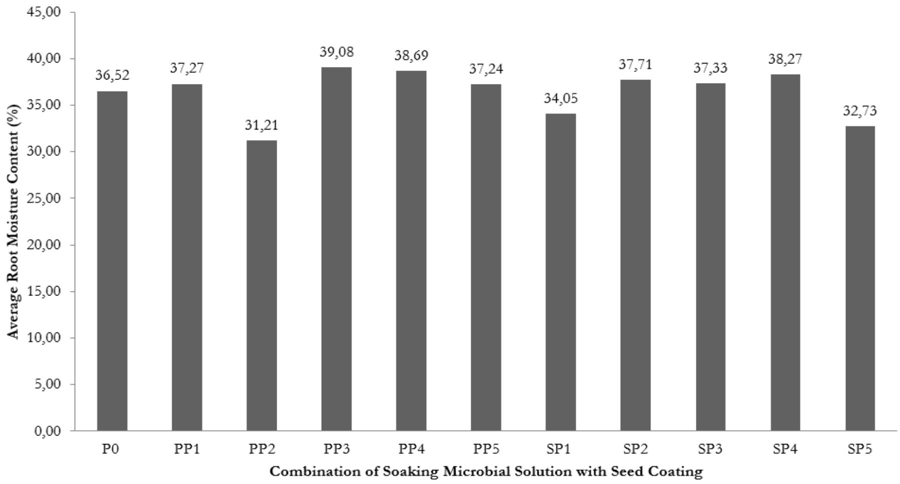

Figure 6. Diagram of root moisture content of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

Leaves have a high moisture content compared to stems and roots. Where the leaf part is the easiest part to dry compared to other parts, this is due to the tighter thickness of the stems and roots compared to the leaves. In addition, it is the dimensional difference between the leaves, stems, and roots that accelerate the moisture content that the material can achieve.

The water status of the leaves is usually an interaction between the potential of leaf water and the conductance of the stomata, where drought will induce a root signal to the canopy to reduce the rate of transpiration so that the stomata close when the water supply decreases. High relative water content (leaves) is a mechanism of plant resistance to drought, and this high relative moisture content is the result of excessive osmotic regulation or reduction of elasticity of cell walls (Nurhasybi et al., 2020).

Biomass

Biomass (dry weight), as a result of the representation of the wet weight of the plant, is a plant condition that expresses the amount of organic matter contained in the plant without moisture content. Based on Table 6. it was seen that the highest biomass (14.90 g), found in SP2 treatment, while the lowest (4.72 g) was found in P0 (control). The analysis showed a real effect between those treatments. The SP2 treatment has a high average biomass value compared to other treatments. This might be the relatively fast growing of the seedling makes, the photosynthesis process works better, which ultimately affects the increase in plant biomass. Photosynthesis is a very important metabolic process in plants, where the things needed for photosynthesis process such as light, CO_2, O_2, chlorophyll, and water are available abundantly after being treated with SP2.

Plant growth and development are influenced by the availability of water. The growth of a plant can be measured through its dry weight and relative growth rate. The dry weight of plants, commonly called total biomass, is a manifestation of metabolic processes that occur in plants. Plant biomass consists of photosynthesis metabolite (glucose and oxygen, nutrient uptake, and water. Dry weight can indicate plant productivity because 90% of photosynthesis results are found in the form of dry weight (Fabre et al., 2020).

Carbon

Carbon dioxide is an early product of the process of photosynthesis. The mass value of carbon dioxide produced during

Table 6. Duncan advanced test of Average Biomass of ebony seedlings treated by microbial solution treatment with and without seed coating

Treatment	Biomass (g)
P0 = Control	4.72[d]
PP1 = 30 % *Penicillium* Microbial Solution	7.33[bcd]
PP2 = 45% *Penicillium* Microbial Solution	7.08[bcd]
PP3 = 30% *Bacillus* Microbial Solution	7.74[bcd]
PP4 = 30% Humic Acid Microbial Solution	8.95[bcd]
PP5 = 30% *Sargassum* Microbial Solution	12.70[ab]
SP1 = 30% *Penicillium* Microbial Solution + Seed Coating	6.29[cd]
SP2 = 45% *Penicillium* Microbial Solution + Seed Coating	14.90[a]
SP3 = 30% *Bacillus* Microbial Solution + Seed Coating	5.69[cd]
SP4 = 30% Humic Acid Microbial Solution + Seed Coating	11.06[abc]
SP5 = 30% *Sargassum* Microbial Solution + Seed Coating	8.56[bcd]

Description: Numbers followed by the same letters are not significantly different, and different letters are significantly different based on Duncan's test at the 0.05 significance level photosynthesis lasts in proportion to the mass of carbohydrates. The mass of carbohydrates is high, the mass of carbon dioxide in plants will be high, while if the carbohydrate mass is low, the absorption is low so that it can be said to be directly proportional (Purwaningsih, 2007).

The results of the analysis showed that soaking treatment of microbial solutions with *seed coating* had a very noticeable effect between the highest and the lower values of carbon mass. Duncan's test results (Table 7.) showed that the highest average carbon (7.45) of ebony seedlings was found in the SP2 (45% *Penicillium* microbial solution + seed coating) treatment, and the lowest carbon mass was found in P0 (control), with an average value of 2.36.

Carbon dioxide absorption in plants is the ability of a plant to absorb carbon dioxide through the stomata pores that are widely found on the surface of the leaves. Carbon dioxide is one of the materials used in the process of photosynthesis to obtain energy and convert it into the form of sugar and oxygen groups with the help of sunlight. Determination of the mass of carbohydrates produced during photosynthesis can determine the mass of carbon dioxide absorbed by plants. Each type of plant has a different absorption capacity, and this is influenced by many factors, including leaf area, the thickness of the relative thickness of the leaves, the number of stomata, the age of the plant, and the environmental factors (Chen et al., 2021).

Specific leaf area

Specific leaf area (SLA) is the ratio between leaf area and dry weight (Gusmayanti, 2015). The average SLA value of leaves can be seen in Figure 7. which shows that SP2 has the highest SLA value of 153.22 cm^2 / g, while the lowest is found in the SP4 treatment i.e 123.97 cm^2 / gr. The results of the analysis showed that the specific leaf area of ebony did not influence by the treatments

The specific leaf area values of ebony seedlings show values ranging from 123.97 cm^2/gr to 153.22 cm^2/g (figure 7). As long as the treatments have no real effect on the value of SLA there might be due to the availability of soil nutrients and biased to be absorbed by the roots so that they can still support their growth. Generally, in drier soil conditions, ion loss will occur quickly, and ion diffusion to the roots is more inhibited. Drought conditions with lower cell water potential can limit cell enlargement, causing growth to decrease. Decreased soil moisture decreases dry weight production and also leaf size (Tavakol & Pakniyat, 2007; Zheng et al., 2008).

Table 7. Duncan's Advanced Carbon Mean Test

Treatment	Carbon (C)
P0 = Control	2.36^{d}
PP1 = 30 % *Penicillium* Microbial Solution	3.67^{bcd}
PP2 = 45% *Penicillium* Microbial Solution	3.54^{bcd}
PP3 = 30% *Bacillus* Microbial Solution	3.87^{bcd}
PP4 = 30% Humic Acid Microbial Solution	4.48^{bcd}
PP5 = 30% *Sargassum* Microbial Solution	6.35^{ab}
SP1 = 30% *Penicillium* Microbial Solution + Seed Coating	3.15^{cd}
SP2 = 45% *Penicillium* Microbial Solution + Seed Coating	7.45^{a}
SP3 = 30% *Bacillus* Microbial Solution + Seed Coating	2.85^{cd}
SP4 = 30% Humic Acid Microbial Solution + Seed Coating	5.54^{abc}
SP5 = 30% *Sargassum* Microbial Solution + Seed Coating	4.28^{bcd}

Description: Numbers followed by the same letters are not significantly different, and different letters are significantly different based on Duncan's test at the 0.05 significance level

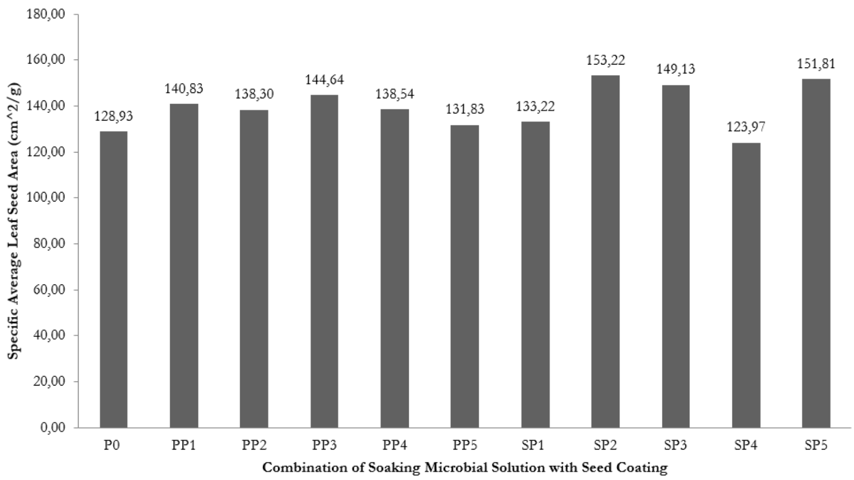

Figure 7. Specific leaf area of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

Leaf area index (LAI)

The leaf area index (LAI) shows the ratio of the leaf surface to the area of the land occupied by the plant. The results of the analysis showed that the soaking treatment with microbial solutions and seed coatings had a very noticeable effect on the variable leaf area index. Duncan's test results (Table 8.) showed that the highest average LAI was found in the SP2 (45% *Penicillium* microbial solution + seed coating) treatment of 29.42 when compared to other treatments, including control. Meanwhile, the lowest leaf area index was found in the P0 (Control) treatment, with an average value of the number of leaves 7.38.

The result of Duncan test (Table 8.) showed that SP2 treatment (soaking a 45% *Penicillium* microbial solution + seed coating) tended to provide the LAI of the seedlings. Ebony seedlings are more responsive to SP2 treatment (45% sargassum microbial solution) than other treatment. It can be suspected that the seeds treated by 45% microbial solution work optimally by assisting the roots in the absorption of nutrients needed for the high growth of seedlings.

Table 8. Duncan's follow-up test average Leaf Area Index (LAI)

Treatment	Leaf Area Index (LAI)
P0 = Control	7.38^{e}
PP1 = 30% *Penicillium* Microbial Solution	15.35^{bcde}
PP2 = 45% *Penicillium* Microbial Solution	11.97^{cde}
PP3 = 30% *Bacillus* Microbial Solution	15.60^{bcde}
PP4 = 30% Humic Acid Microbial Solution	17.71^{bcd}
PP5 = 30% *Sargassum* Microbial Solution	22.43^{ab}
SP1 = 30% *Penicillium* Microbial Solution + Seed Coating	9.46^{of}
SP2 = 45% *Penicillium* Microbial Solution + Seed Coating	29.42^{a}
SP3 = 30% *Bacillus Microbial* Solution + Seed Coating	10.81^{cde}
SP4 = 30% Humic Acid Microbial Solution + Seed Coating	16.95^{bcd}
SP5 = 30% *Sargassum* Microbial Solution + Seed Coating	18.94^{bc}

Description: Numbers followed by the same letters are not significantly different, and different letters are significantly different based on Duncan's test at the 0.05 significance level

Chlorophyll leaf content

Chlorophyll is the main component of chloroplasts for photosynthesis. The average value of leaf chlorophyll content can be seen in Figure 8. SP2 has the highest leaf chlorophyll content value of 0.04, while the lower important is found in the P0 (control) treatment of 0.03. The analysis resulted that the given treatments had not noticeable effect towards chlorophyll content of ebony leaves.

The chlorophyll content value of ebony seed leaves ranges from 0.03 – 0.04 (Figure 8). Good vegetative growth of plants can result in better metabolic processes, especially in the process of photosynthesis. Better metabolic processes in the vegetative period will greatly affect the subsequent processes, namely the process by which plants enter the generative period (Tariq et al., 2022). In general, the age of the leaves is fraught with the chlorophyll content. The greener the color of the leaves, the higher the chlorophyll content, so the green color of the leaves is closely related to the chlorophyll content. Differences in leaf color also indicate differences in the types of pigments contained in the leaves. Chlorophyll in young leaves is still a proto-chlorophyll, and the leaves turn green after the transformation of pro-chlorophiles (Paembonan et al., 2021). The amount of chlorophyll content is influenced by the amount of pigment and the surface area of the leaves. The size of the leaf area also plays a role in the photosynthesis that occurs in the leaves. The result of photosynthesis per plant unit is determined by the area of the leaves (Sumenda, 2011a).

The larger surface area of the leaves makes it possible to capture better light so that it has a higher photosynthetic value. As the leaves age, the chlorophyll content and leaf area also increase. This is in accordance with the statement of Musyarofah et al. (2007) that the chlorophyll content is also influenced by the morphological and anatomical structure of a plant. The larger the size of the leaf, the more chlorophyll it contains, and vice versa. However, the older the leaf, the less its ability to photosynthesize due to the reduced function of chlorophyll. Chlorophyll will increase in line with the development of the leaf area, where the amount of chlorophyll per unit of leaf area will reach the maximum level before the leaf finally stop growing (Sumenda, 2011b). Photosynthesis that runs optimally will affect the accumulation of assimilates of reproductive and vegetative organs (Suryatmana & Sobardini, 2016).

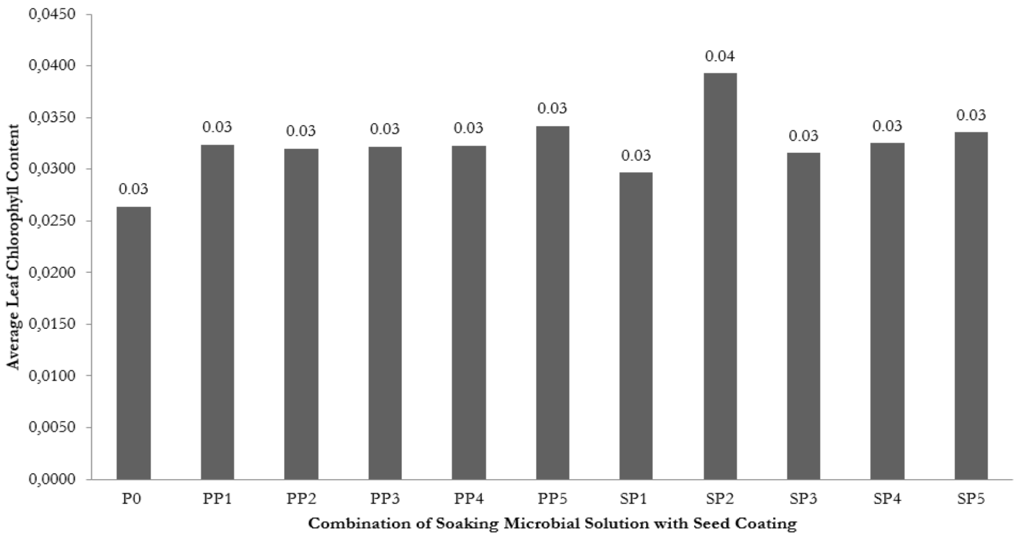

Figure 8. Diagram of leaf chlorophyll content of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

The chlorophyll content in the vegetative phase is still low, and it will increase, but as the leaves age will decrease. This is in accordance with the pattern of chlorophyll synthesis and chlorophyll overhaul due to the synthesis of cytokinin hormones when young, followed by an increase in ethylene which spurs chlorophyll enzyme activity. The application of soaking microbial solutions and seed coatings does not significantly affect the chlorophyll content of the leaves but this in line with the area of the leaves (Suryatmana & Sobardini, 2016;Jarecki, 2022).

Microbes used as boosters in seedling growth can be through mechanisms such as fixing nitrogen, dissolving phosphates, and producing IAA, gibberellin, and cytokinin (Gusmiaty et al., 2021). Seed coating consists of carrier materials, adhesives, and biological agents. The carrier material used in this study is kaolin. The content of kaolin in the form of micro and macro-nutrients such as Mg, Na, Fe, and Cu can be a source of nutrients. Besides that, it can maintain moisture so that microbes can still grow. The use of carrier materials and other materials as seed coatings not only affects microbial growth but also affects seed viability. The selection of carrier materials, nutrient providers, and adhesives needs to be considered because it can affect the imbibition process so that germination can be disturbed. Therefore, treatment with seed coating mostly has a noticeable influence because of the role of coating materials and microbes that affect each other. This is consistent with research on seed coating technology that combines nutrients and bio-stimulants to increase seedling growth and has the potential to facilitate the formation of cover crops in agriculture and land reclamation(Qiu et al., 2020)

IV. CONCLUSION

Based on the results of the study, it can be concluded that seed coating treatment of SP2 (45% *Penicillium* microbial solution + seed coating), gave the best results in each variable of growth and physiology of ebony seedlings. The treatment without seed coating of PP5 (30% Sargassum microbial solution) also resulted good performance in every variable of growth and physiology of ebony seedlings. Treatment with seed immersion in microbes would be able to produce higher percentage of seedling in each variable.

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ASSESSING THE WILLINGNESS TO PAY FOR CONSERVATION AND MANAGEMENT OF WETLANDS AT JAGDISPUR RESERVOIR IN KAPILVASTU DISTRICT OF NEPAL

Rama Gyawali[1*], Gyan Bandhu Sharma[1], and Jiban Shrestha[2]

[1]Faculty of Forestry, Agriculture and Forestry University, Hetauda, Nepal
[2]Nepal Agricultural Research Council, National Plant Breeding and Genetics Research Centre, Khumaltar, Lalitpur, Nepal

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ASSESSING THE WILLINGNESS TO PAY FOR CONSERVATION AND MANAGEMENT OF WETLANDS AT JAGDISPUR RESERVOIR IN KAPILVASTU DISTRICT OF NEPAL. Wetlands are one of the world's most productive ecosystems. They have significant ecological, economic, cultural, and recreational significance. However, wetlands are being misused and degraded; therefore, wise and sustainable use of wetlands, conservation and management of wetlands resources are critical at the local level. Community-based conservation is a method of managing wetlands near settlements sustainably. The objective of this study was to determine local people's willingness to pay (WTP) for community-based conservation and management activities, as well as the factors which influence it, at the Jagdispur Reservoir in Nepal's Kapilvastu district. A total of 62 households were surveyed. Household surveys and key informant interviews were conducted. Contingent valuation was used to elicit WTP, while regression analysis identified significant determinants. Thirteen provisioning, regulating, cultural and supporting services were identified, including irrigation, fisheries, tourism and biodiversity. Of 62 households, 67% expressed WTP averaging NRs 3,351 (US $ 25.38) annually. WTP was higher for proximal households, reflecting greater dependence. Cash payments dominated, but labor contributions were also substantial. Age, income, participation in conservation, and distance from wetlands positively and significantly influenced WTP. Whereas education, gender, ethnicity and number of family members did not. The findings indicate a high value placed on sustaining wetland services, justifying investment in conservation and wise use. Follow-up research on governance, benefit sharing and sustainable financing is recommended to translate WTP into effective action. Overall, the results provide important baseline data to guide policies and collective action for wetland stewardship.

Keywords: Conservation and management, wetland, wetland goods and services, willingness to pay, contingent valuation method

PENILAIAN KESEDIAAN PEMBAYARAN KONSERVASI DAN PENGELOLAAN LAHAN BASAH DI WADUK JAGDISPUR, KAPILVASTU NEPAL. Lahan basah adalah salah satu ekosistem dunia yang paling produktif karena memiliki peran signifikan terhadap ekologi, ekonomi, budaya, dan rekreasi. Lahan basah sering disalahgunakan dan mengalami degradasi, sehingga studi terkait pemanfaatan lahan basah secara bijaksana dan berkelanjutan sangat perlu dilakukan mulai dari tingkat lokal. Konservasi berbasis masyarakat merupakan salah satu strategi pengelolaan lahan basah di dekat pemukiman secara berkelanjutan. Tujuan dari penelitian ini adalah untuk menentukan kesediaan masyarakat untuk membayar (Willingness to Pay/WTP) kegiatan konservasi dan pengelolaan berbasis masyarakat, serta faktor-faktor yang mempengaruhinya. Penelitian dilakukan di Waduk Jagdispur, Distrik Kapilvastu, Nepal melalui survei. Survei dilakukan terhadap 62 rumah tangga. Pengumpulan informasi dilakukan melalui pemberian kuesioner dan wawancara dengan responden dan informan kunci. Penentuan WTP dilakukan dengan menggunakan Contingent Valuation Method (CVM), dan faktor-faktor yang mempengaruhi WTP diidentifikasi menggunakan analisis regresi. Dalam ukuran nilai rata-rata kesediaan rumah tangga untuk membayar tunai dan barang untuk konservasi dan pengelolaan Waduk Jagdispur adalah sebesar NRs 3351 (US $ 25.38). Umur, pendapatan bulanan, jarak dari lahan basah, dan partisipasi

*Corresponding author: gyawalirama43@gmail.com

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dalam kegiatan konservasi semuanya memiliki pengaruh yang nyata (p<0,05) terhadap kesediaan rumah tangga untuk membayar perlindungan dan pengelolaan waduk. Berdasarkan data yang diperoleh dalam survey, perkiraan kesediaan untuk membayar jasa konservasi waduk dan kegiatan pengelolaan dapat dibenarkan secara ekonomi dan lingkungan. Penelitian lanjutan mengenai tata kelola, pembagian manfaat dan pembiayaan berkelanjutan direkomendasikan untuk menerjemahkan WTP menjadi tindakan yang efektif. Secara keseluruhan, hasil penelitian ini memberikan data dasar yang penting untuk memandu kebijakan dan tindakan kolektif dalam pengelolaan lahan basah.

Kata kunci: Konservasi dan manajemen, lahan basah, barang dan jasa lahan basah, kemauan membayar, willingness to pay, contingent valuation method

I. INTRODUCTION

Wetlands provide critical ecosystem services such as water filtration, flood control, biodiversity habitat, and opportunities for recreation that benefit local communities (Mitsch et al., 2015). However, wetlands around the world are being degraded and lost at an alarming rate due to land conversion for agriculture, pollution, and development pressures (Davidson, 2014).

Nepal's wetland has a total area of 743,563 ha, accounting for 5% of the country's total land area MFSC (2014). Wetlands provide local people with a variety of ecological goods and services, and habitat for riparian and aquatic animals (Lamsal et al., 2015b). In Nepal specifically, rapid population growth and land use changes have put pressure on critical wetland areas like the Jagdispur Reservoir in the Kapilvastu district (Thapa et al., 2016). This reservoir is the largest in the country and was recognized as a Wetland of International Importance under the Ramsar Convention in 2003, mainly for its rich biodiversity, including threatened mammals and migratory waterbirds (IUCN, 2004). Jagdispur Reservoir lies well north of tropical latitudes and lacks a coastal location or cold boreal climate with waterlogged soils; based on the cited definitions (Mitsch et al., 2015) it does not contain the habitat criteria needed to develop mangrove swamps or peatlands. It likely qualifies as an inland freshwater lake wetland instead.

Many wetlands have been classified as wastelands in the past, and have been drained or otherwise converted to agricultural, industrial, and private land uses (Barbier, Acreman & Knowler, 1997). This implies that humans are unable to take advantage of the diverse benefits provided by wetlands (Roberts & Leitch, 1997). Consequently, by underestimating its real values, an opportunity to achieve benefits is put at risk. Sustainable wetlands management is critical for reducing the rate of wetlands loss and degradation worldwide. Nearby communities are becoming more aware of the importance of wetlands. If they benefit from wetlands preservation, conservation, and management, they will change their behaviour to support conservation and management efforts (Sibanda & Omwega, 1996). The research in developing nations has used contingent valuation methodologies to indicate a willingness to pay for wetland biodiversity conservation and other natural attractions (Pearce, 2001).

Assessing willingness to pay (WTP) provides quantitative information on how much the surrounding community values the wetland's services (Carson & Mitchell, 1993), which can inform policy decisions on whether investments in wetland conservation and restoration make economic sense and if community members would support such efforts (Hanley & Barbier, 2009). The concept of payment for ecosystem services (PES) schemes builds on this WTP to conserve natural areas like wetlands by having beneficiaries compensate landowners or managers for providing those services (Engel et al., 2008). As governments and NGOs in Nepal look to expand PES programs to fund protected area conservation (ICIMOD, 2016), understanding local WTP is an important first step.

While some research has valued ecosystem services of wetlands in Nepal (Baral et al., 2016; Lamsal et al. 2015; Asadi et al. 2014), factors affecting willingness to pay have not been previously assessed for the significant Jagdispur Reservoir. Most of Jagdispur Reservoir's study is on water birds and biodiversity conservation. Factors like income, age, education level, distance from the wetland, and use of wetland resources have been found to influence WTP in previous CVM studies on wetlands (Lamsal et al. 2015; Asadi et al. 2014). This suggests WTP for wetland conservation may vary between communities depending on their characteristics. Therefore, this study aims to estimate WTP to conserve and sustainably manage wetlands for communities around Jagdispur Reservoir using CVM. It will also analyze factors affecting WTP, such as demographics, wetland use, and participation in conservation activities. Conducting this assessment empowers local voices in determining the future of this important site and provides an economic model that could guide decision-making at other threatened wetlands in Nepal. The results can inform future payment for ecosystem service programs to fund wetland conservation in the area.

II. MATERIAL AND METHOD

A. Study area

The Jagdispur Reservoir is located in Nepal's western region. It is located between 27°37' N and 83°06' in the Kapilvastu Municipality ward number 10 of Kapilvastu District with an elevation of 197 meters above mean sea level. Because of its international importance for threatened species and habitat conservation, the Jagdispur Reservoir is recognized as an Important Bird Area (IBA). In 2003, the wetland area was designated as a Ramsar Site, mostly due to migrating waterbirds and threatened animals. With a core size of 157 hectares and

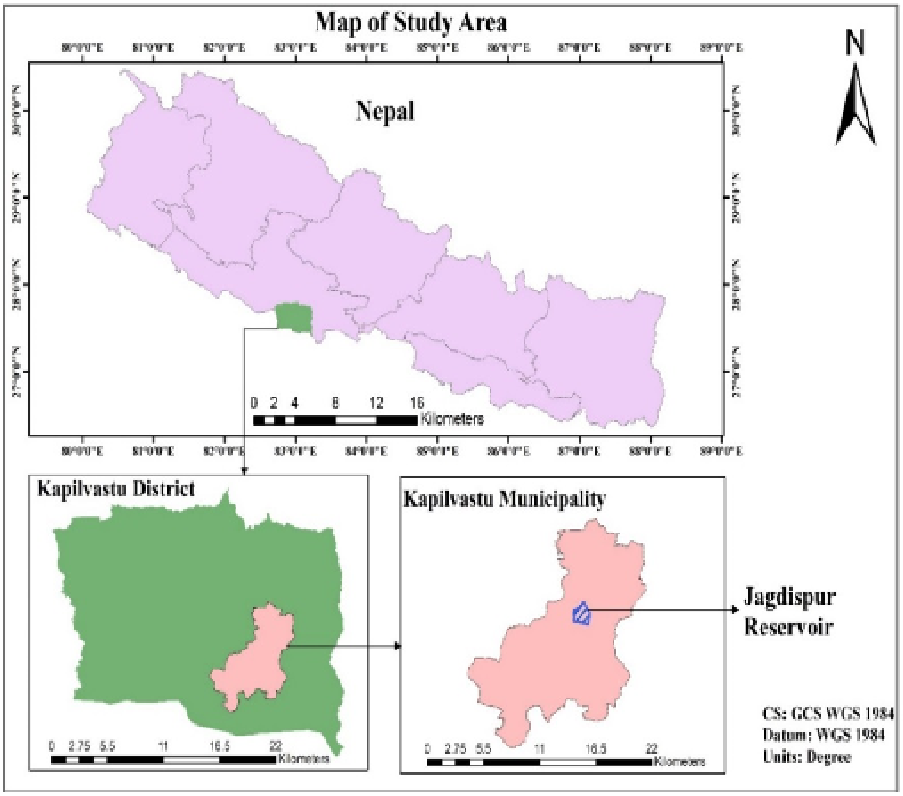

Figure 1. Map of the study area

surrounding wetlands totalling around 225 ha, this reservoir is currently the largest reservoir in Nepal and an important wetland site. It was built in early 1970 to provide irrigation for Jakhira Lake and the adjacent agricultural land. The neighbouring Banganga River feeds the Reservoir, a catchment in the Churia Hills. This study was conducted in 2020 (July to November).

B. Data collection

The purposive sampling method was carried out to select sample households for primary data collection. This sampling method was adopted because the researcher has field-level experience, and the studies are confined to a small number of household inquiries (Kothari, 2004). Different three villages, i.e. Jagdispur (located within 1 km of the wetlands), Birata (located within 2 km of the wetlands), and Jahadi (located within the vicinity of 3 km of wetlands), were selected because of the communities' dependency and direct access with the Jagdispur reservoir. The villages were sparsely populated. Therefore, a sampling intensity of 30% was utilized for each village. A survey was conducted on 20, 15, and 27 households of respective villages out of 66 houses from Jagdispur village, 50 households from Birata village, and 90 households from Jahadi village. The purpose of the household survey was to gather detailed information about wetlands and their users. Typically, the household head (eldest family member) was interviewed. The main questionnaires in the household survey included five-point Likert scale response options for collecting responses from respondents on reservoir conservation and management. Apart from the respondents interviewed from each village, in-depth interviews were also conducted with 6 key informants who represented the local communities regarding social status, ethnicity, economic well-being, knowledge, and ecological regions.

The study's data and information were gathered from secondary sources. To obtain supporting data related with existing knowledge, technical aspects and policy developed in the research area, secondary data was gathered from research articles, case studies, papers, journals, and records from the District Forest Office in Kapilvastu and wetland operational plans.

C. Contingent Valuation Method (CVM)

The Contingent Valuation Method calculated Willingness to Pay for reservoir conservation and management. In Nepal and elsewhere, WTP is often used to estimate option and existence value. CVM is a survey method in which respondents are asked how much they would be ready to pay or donate in cash and labor to conserve natural goods, with their preferences presumed to be dependent on alternative goods available in a hypothetical market (Hoevenagel, 1994). Using a bidding game, the overviewed families were asked about the amount they were willing to contribute in cash and deliberate labor for the reservoir's conservation. The labor contribution was then converted to a monetary value using the study site's average wage rate (NPR. 500/day). The average monetary value was then calculated.

D. Regression analysis

Regression analysis was carried out to determine the factors affecting the household's WTP for the conservation and management of the reservoir. The multiple linear regression model used was in Eq. 1:

$$y = \beta0 + \beta1X1 + \beta2X2 + \beta3X3 + \beta4X4 + \beta5X5 + \beta6X6 + \beta7X7 + ẹ \quad \text{..........................(1)}$$

Where:

y = Willingness to Pay for the community-based conservation activities

β0 = Constant Term & β1, β2, β3, β4, β5, β6, β7, β8, β9, β10 = Beta coefficients,

X1 = Age

X2 = Level of education

X3 = Gender

X4 = Participation in conservation

X5 = Monthly income

X6 = Distance from wetland

X7 = Number of household members

X8 = Ethnicity
e = Error Term

Here, y is a dependent variable that depends upon the independent variables (X1, X2, X3, X4, X5, X6, X7, X8, X9, and X10). As there are more than two independent comparison groups to compare the means of a continuous variable, hence we use analysis of variance (ANOVA) as an appropriate test of hypothesis (Eneji et al., 2009).

E. Data analysis

Data were collected in both qualitative and quantitative forms, which were evaluated using descriptive statistics including percentage, mean and frequency. Simple tables, charts, and graphs were used to convey certain data. Data were imported into Microsoft Excel 2016 and analyzed using Statistical Package for Social Sciences (SPSS ver.19). The study employed both descriptive and inferential statistics.

III. RESULT AND DISCUSSION

Results from direct observation, key informant interviews, and household surveys reveal that wetland provides both consumptive and non-consumptive goods and services. Irrigation, fish, driftwood, medicinal plants, firewood, livestock bathing, water, tourism and recreation, biodiversity conservation, attractive terrain, fresh air, water regulation, and a clean environment are some of the major goods and services provided by Jagdispur Reservoir.

Socioeconomic status of the respondents

The total number of responses was 62 (N=62). The Socio-Demographic Profile of Sampled Households is shown in Table 1. Gender, ethnicity, educational status, conservation awareness, primary source of income, and age group are all socioeconomic factors that directly and indirectly affect community-based conservation efforts to conserve and manage the reservoir. These variables affect the willingness to pay (WTP) for the conservation of wetland biodiversity. The findings of this study were similar to those of Bhatt, Shah and Abdullah (2014), who found that analyzing respondents' socioeconomic status is important in assessing WTP for conserving wetland biodiversity. Income and education are two variables that directly affect the respondents' WTP in Shadegan International Wetland (Kaffashi et al., 2015).

Table 1: Socio-demographic status of respondents (N=62)

Variable	Percentage (%)	Frequency(n)
Gender		
Male	55	34
Female	45	28
Ethnicity		
Brahmin/Chhetri	18	11
Tharu	44	27
Made	27	17
Others	11	7
Age group		
Below 20	8	5
20-30	13	8
40-59	45	28
Above 60	34	21
Educational Status		
No formal education	38	24
Below Secondary Education Examination (SEE)	26	16
SEE	13	8

Table 1. Continued

Variable	Percentage (%)	Frequency(n)
Above SEE	23	14
Primary income sources		
Agriculture	56	35
Business	10	6
Foreign remit	16	10
Services	10	6
Others	8	5
Awareness of conservation		
Yes	55	34
No	45	28
Family size(number)		
Below 5	24	15
5	13	8
Above 5	63	39
Participation in conservation activities		
Yes	56	35
No	44	30

The reaction of the respondents to willingness to pay

The reaction of respondents regarding willingness to pay is given in Figure 2. Out of the 62 respondents, 69% respondents were ready to pay for the conservation and management of wetlands at 81%, 66% and 59% respectively for Jagdispur Village, Birata Village, Jahadi Village. Meanwhile, 31% of respondents stated that they were not ready to pay, respectively 19%, 34% and 41% from Jagdispur Village, Birata Village and Jahadi Village.

A 2010 study by Chand on the Ghodaghodi wetland in Nepal found a higher rate of willingness to pay, with 79% of respondents willing to pay. In contrast, a study by Lamsal et al. (2015a) on the Ghodaghodi Lake complex found only 48% were willing to pay cash for conservation activities. A 2014 study by Asadi et al. on an Iranian wetland had a comparable 67% willingness to pay rate. The key reasons for unwillingness to pay in this Nepal study were lack of conservation policies and awareness. This matches the findings of Lamsal et al. (2015a) who found many people may not fully understand the importance of wetlands and their role in ecosystem health. In summary, the share of households willing to pay varies considerably across studies from 48% to 79%, likely reflecting differences in site-specific factors.

Reaction of respondents regarding the reason for willingness and unwillingness to pay

The goods and services provided by the Jagdispur Reservoir benefited all responders. Figure 2 shows that 69 % of respondents were ready to pay in cash or labour for reservoir conservation and management. The willingness to pay was mostly motivated by the goods and services obtained from Jagdispur Reservoir and for the good and benefit of the future generation. Local people have placed a high value on wetlands conservation and management, primarily for the ecosystem services and goods provided by the reservoir and to maintain the reservoir for the next generations. 31% of respondents claimed they couldn't afford to pay for reservoir conservation and management. Figure 3 shows the main reason for unwillingness to pay in three different villages due to policy

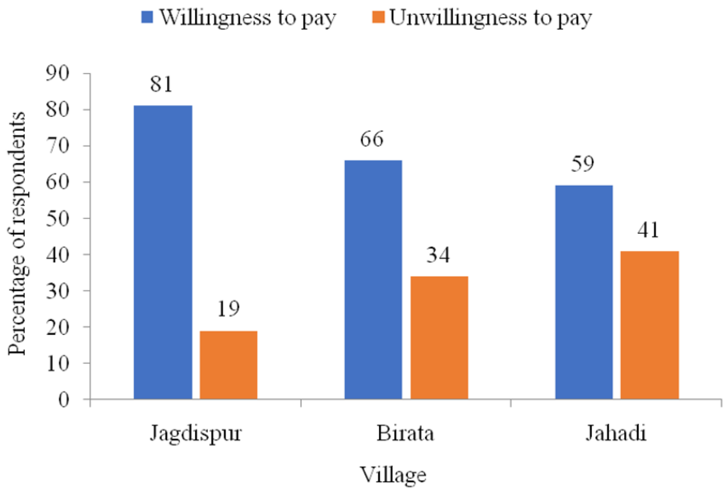

Figure 2. Reaction of the respondents for willingness to pay

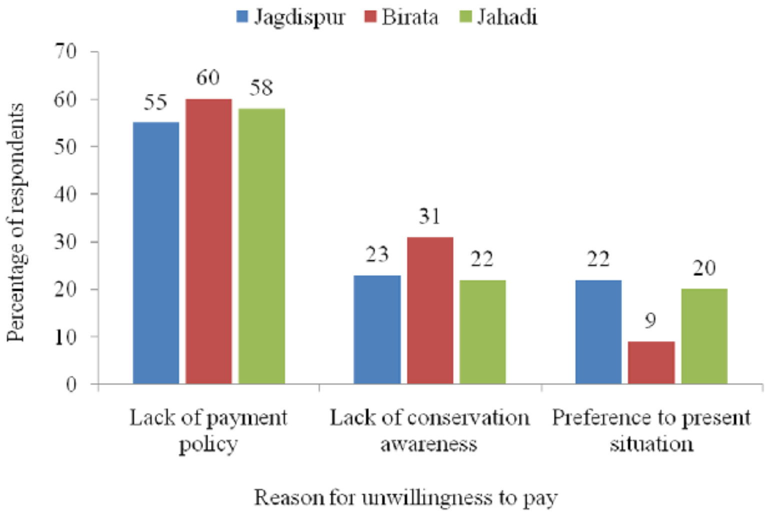

Figure 3. Reaction regarding the reason for unwillingness to pay

gaps and lack of awareness. Similarly, Lamsal et al. (2015a) identified a lack of policy and knowledge and a lack of awareness as the main reason for unwillingness to pay Asadi et al. (2014) found distrust in authorities managing payments also reduced willingness to pay in their Iran wetland study. In summary, the reasons given for unwillingness to pay and preferred payment forms can vary considerably across sites based on policies, awareness, trust, and economics.

Reaction of respondents regarding the form of payment

Figure 4 shows the form of payment in three different villages. Out of 69% of respondents who were ready to pay, 44% of respondents were ready to pay by cash, 21% of respondents were ready to contribute as voluntary labor, and 35% of respondents were ready to pay in both forms on average of three villages. Here, most respondents preferred payment in cash. This aligns with Lamsal et al.'s (2015) Nepal study, where the most preferred cash payment.

However, Asadi et al. (2014) found higher willingness for labor than cash payment in Iran, perhaps reflecting affordability. Do and Bennett (2007) in Vietnam actually found a preference for payment in kind rather than cash.

Value of willingness to pay

Our results found that the community in the research sites are willing to pay in cash and labor for the conservation and management of the reservoir. On average, each household is willing to pay NRs 518 per year in cash and provide voluntary labor of 5.7 days per year, which is equivalent to NRs 2833 per year, calculated by local wage rates. Willingness to pay by cash or voluntary labor was higher in Jagdispur village than in Birata and Jhahadi villages. This reveals that people around the Jagadishpur Reservoir give high importance to the conservation and management of the reservoir for future use and wetland goods and services.

This study found the average total willingness to pay (WTP) for conservation and management of the Jagdispur Reservoir wetland to be NRs 3,351(US $ 29) per household annually. This WTP was comprised of cash payments (average of NRs 518 (US $4.48) per household) as well voluntary labor contributions valued at NRs 2,833(US $ 24.52) per household.

Compared to findings from other wetland valuation studies in Nepal, WTP by cash in the research area is higher than the NRs 378 (US$5.4) found for the community-based conservation of Ghodaghodi Lake (Lamsal et al., 2015a). Total WTP is slightly lower than the NRs 3,135 value local communities placed on conservation of the same Jagdishpur Reservoir in previous research (Baral et al., 2016). The reasons for the high WTP found in this study and other Jagdishpur studies are likely linked to the high dependence and utilization of

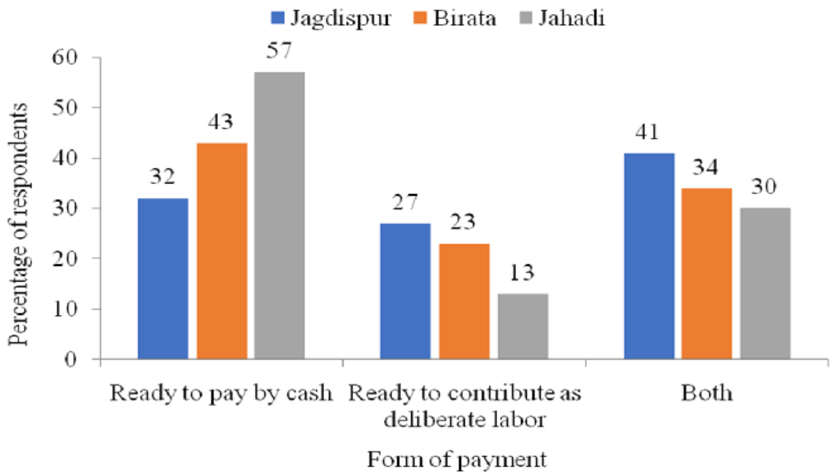

Figure 4. Reaction of respondents regarding the form of payment

Table 2: Total willingness to pay for conservation and management

Village	Willingness to pay by cash (NRs/HH)	Contribute as labor		Total
		Day per year	Monetary form (NRs/HH)	
Jagdispur	737	7	3500	4237
Birata	512	6	3000	3512
Jhahadi	306	4	2000	2306
Total	518	5.7	2833	3351

Note: US$ 1 ≈ 115.52 NRs

this wetland by local communities for fishing, driftwood collection, livestock grazing and other activities (Chaudhary, 2021).

Factors affecting a household's willingness to pay

A regression analysis was carried out to establish whether these factors influence households' willingness to pay for wetlands conservation. Several factors might influence are age, gender, participation in conservation, source of income, number of household members, monthly income, ethnicity, and the result is presented in Table 3. To determine the factors affecting households' WTP for the conservation and management of Jagdispur Reservoir, a null hypothesis was formulated thus: there are no factors affecting households' WTP for the conservation and management of Jagdispur Reservoir (H0).

Analysis of variance (ANOVA) of the multiple regressions in Table 4 shows that the P-value is 0.000, less than the 0.005 significance level, meaning that we can reject the null hypothesis, and the F calculated value is 4.48, while the critical F value is 2.29. The calculated F value of 4.48 is greater than the critical F value of 2.29 at a 5% significant level; hence, the result is significant at a 5% significant level. A significant F test indicated that we would reject the null hypothesis, which states that there are no factors affecting households' WTP for the conservation and management of Jagdispur Reservoir.

Subsequently, Table 5 shows that age (P<0.00), participation in conservation activities (P<0.00), monthly income (P<0.00), and distance from wetland (P<0.00) factors have p-values less than 0.05. These factors were significant, which means that these factors contribute to the variability of the dependent variable i.e., the household's willingness to pay for conservation and management of the reservoir. Similarly, the level of education, gender, household size, and ethnicity factors were insignificant (p >0.05), which means these factors do not contribute to the variability of the dependent variable, i.e., the household's willingness to pay for conservation and management of the reservoir. The WTP was positively and significantly associated with age, conservation participation, and monthly income parameters. It shows that older people with more money are more eager to contribute to the conservation and management of the environment than younger people with less money. People who participate in more activities are more likely to contribute to the reservoir's conservation and management. WTP is inversely proportional to the distance from a wetland. People who live close to the wetland are ready to pay more for the reservoir's conservation and management than people who live further away.

Table 3: Strength of model

Model	R	R Square	Adjusted RSquare	Std. The error ofthe Estimate
1	0.606[a]	0.367	0.285	0.6232

[a] = Willingness to pay for conservation and management of Jagdispur Reservoir

Table 4: Analysis of variance of the multiple regressions

Model	Sum of squares	df	Mean Square	F	Significance
Regression	2354643	7	336377	4.48	0.000
Residual	4052653	54	75049		
Total	6407297	61			

Table 5: Multiple linear models of the total amount of WTP for conservation and management of the reservoir

Socioeconomic variable	Coefficient estimate	Standard error	t-value	p-value
(Constant)	147.44	51.3	2.87	0.02
Age (years)	6.32	14.7	0.42	0.00*
Level of education	-5.8	8.1	-0.71	0.46
Gender	-16.63	47.13	-0.35	0.66
Participation in conservation Activities	9.73	22.1	0.44	0.02*
Monthly income (NRs)	105.6	12.9	8.18	0.00*
Distance from wetland (in km)	-12.57	19.41	0.64	0.00*
Number of household members	2.52	5.21	0.48	0.35
Ethnicity	14.59	47.13	0.30	0.43

*Significant at 5% level of significance.
The established multiple linear regression equation becomes:
y = 147.44 + 6.32X1 +9.73X4 + 106.6X5 - 12.57X6

There is a significant correlation between the amount of WTP for ecosystem services and monthly income, but no correlation between the amount of WTP for ecosystem services and education level (Bhandari et al., 2016). The positive influence of age on WTP aligns with a

study by Asadi et al. (2014) on an Iranian wetland, which also found that higher age led to greater WTP. As the authors suggested, older people likely have more experience benefiting from wetlands; therefore, they are more willing to pay. Many studies have found income is positively associated with WTP for wetlands, like Bhandari et al. (2009) in Nepal, Lankia et al. (2014) in Finland, and Bennett and Thang (2005) in Vietnam. Higher-income logically provides a greater ability to pay. The negative effect of distance on WTP makes sense since proximity increases wetland use and awareness. This finding is in accordance with the study from

Lankia et al. (2014) who found that distance reduced WTP for managing recreational forests in Finland. Past participation in conservation increased WTP is consistent with findings of Do and Bennett (2007) in Vietnam and Blomquist and Whitehead (1998) in the USA.

IV. CONCLUSION

The study found that the Jagdispur Reservoir provides several important ecosystem goods and services to local communities, including irrigation, fish, driftwood, medicinal plants, livestock bathing, tourism, recreation, biodiversity conservation, flood control, and aesthetic beauty. A total of 13 key goods and services were identified through household surveys and key informant interviews. Out of 62 respondents surveyed, 67% were willing to pay for wetland conservation and management activities. The average willingness to pay was NRs 3,351(US $ 29) per household annually, comprising an average of NRs 518 (US $4.48) in cash and NRs 2,833 (US $ 24.52) worth of labor. Willingness to pay was higher among those who live closer to the wetland, reflecting greater dependence and benefits obtained from the wetland. Multiple regression analysis found that age, monthly income, distance from the wetland, and participation in conservation activities significantly influenced willingness to pay positively. In contrast, education level, gender, number of household members and ethnicity did not show a significant effect. This indicates that those who are older, wealthier, actively participate in conservation and live close to the wetland are willing to pay more. This is not only driven by greater awareness of the importance of Jagdispur Reservoir but also an understanding of the greater benefits that can be obtained from the ecosystem services.

Overall, the high willingness to pay values reveals significant local dependence on wetland resources and appreciation of the ecosystem services provided. The findings provide evidence that investment in conserving and managing the Jagdispur Reservoir would be economically justified, given people's reliance on wetland resources. This information can help justify and direct government policies and programs aimed at protecting the Jagdispur Reservoir and similar wetlands. More research on governance, benefit sharing, and sustainable finance is needed to put WTP into action. Overall, the findings of this study provide critical baseline data for guiding policy and community action in wetland management.

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V. CONCLUSION

A brief summary of the possible clinical implications of your work is required in the conclusion section. Conclusion contains the main points of the article. It should not replicate the abstract, but might elaborate the significant results, possible applications and extensions of the work.

ACKNOWLEDGEMENT

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REFERENCES

At least 10 references are listed according to American Psycological Association (APA) referencing style, 6th edition. References must be listed in alphabetical order by another name. Eighty percent of references should be cited from primary sources and published in the last five years. To properly credit the information sources, please use citation tools such as Mendeley or EndNote to create a bibliography, references and in-text citations. Mendeley is a free reference manager that can be downloaded at https://www.mendeley.com/download-mendeley-desktop/.

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