# 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

Received: 28 March 2023, Revised: 26 June 2023, Accepted: 20 March 2024

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

 doi:10.59465/ijfr.2024.11.1.105-125

## 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 25ºC 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 | 0.29$^{e}$ |
| PP1 = 30% *Penicillium* Microbial Solution | 0.39$^{d}$ |
| PP2 = 45% *Penicillium* Microbial Solution | 0.45$^{cd}$ |
| PP3 = 30% *Bacillus* Microbial Solution | 0.43$^{d}$ |
| PP4 = 30% Humic Acid Microbial Solution | 0.46$^{cd}$ |
| PP5 = 30% *Sargassum* Microbial Solution | 0.56$^{ab}$ |
| SP1 = 30% *Penicillium* Microbial Solution + Seed Coating | 0.48$^{bcd}$ |
| SP2 = 45% *Penicillium* Microbial Solution + Seed Coating | 0.57$^{a}$ |
| SP3 = 30% *Bacillus* Microbial Solution + Seed Coating | 0.45$^{cd}$ |
| SP4 = 30% Humic Acid Microbial Solution + Seed Coating | 0.54$^{abc}$ |
| SP5 = 30% *Sargassum* Microbial Solution + Seed Coating | 0.48$^{bcd}$ |

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

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 | 13.67$^{d}$ |
| PP1 = 30% *Penicillium* Microbial Solution | 17.33$^{cd}$ |
| PP2 = 45% *Penicillium* Microbial Solution | 17.33$^{cd}$ |
| PP3 = 30% *Bacillus Microbial* Solution | 19.33$^{bcd}$ |
| PP4 = 30% Humic Acid Microbial Solution | 21.00$^{bc}$ |
| PP5 = 30% *Sargassum Microbial* Solution | 24.00$^{b}$ |
| SP1 = 30% *Penicillium* Microbial Solution + Seed Coating | 14.67$^{d}$ |
| SP2 = 45% *Penicillium* Microbial Solution + Seed Coating | 30.33$^{a}$ |
| SP3 = 30% *Bacillus* Microbial Solution + Seed Coating | 15.67$^{cd}$ |
| SP4 = 30% Humic Acid Microbial Solution + Seed Coating | 19.00$^{bcd}$ |
| SP5 = 30% *Sargassum* Microbial Solution + Seed Coating | 24.00$^{b}$ |

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).

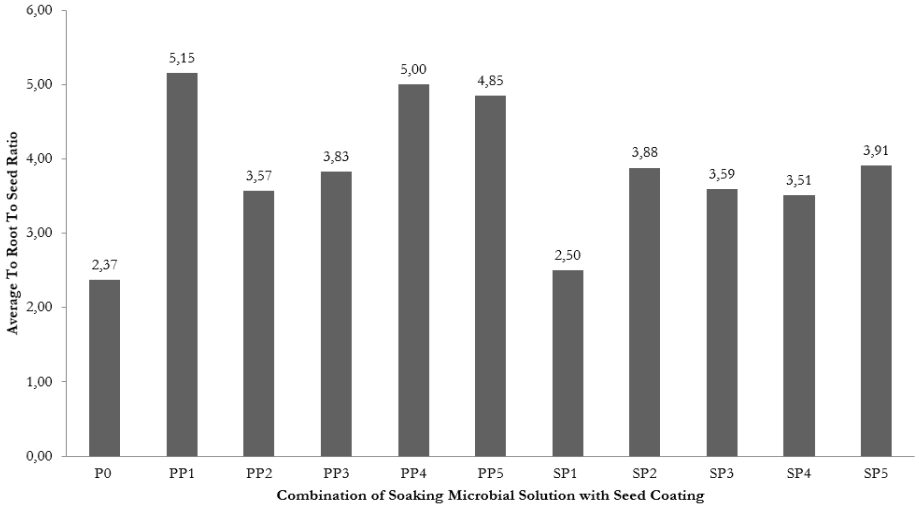


Figure 1. Root shoot ratio diagram of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

**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.

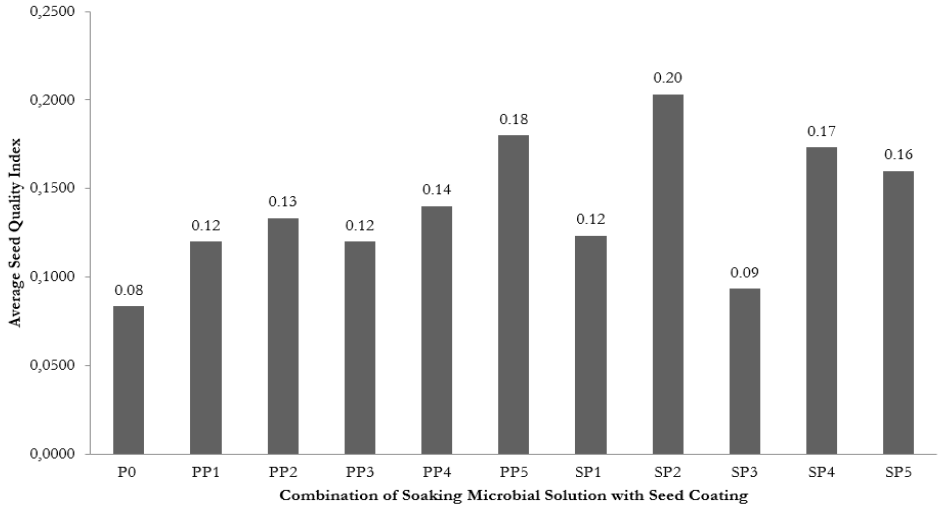


Figure 2. Seed quality index diagram of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

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

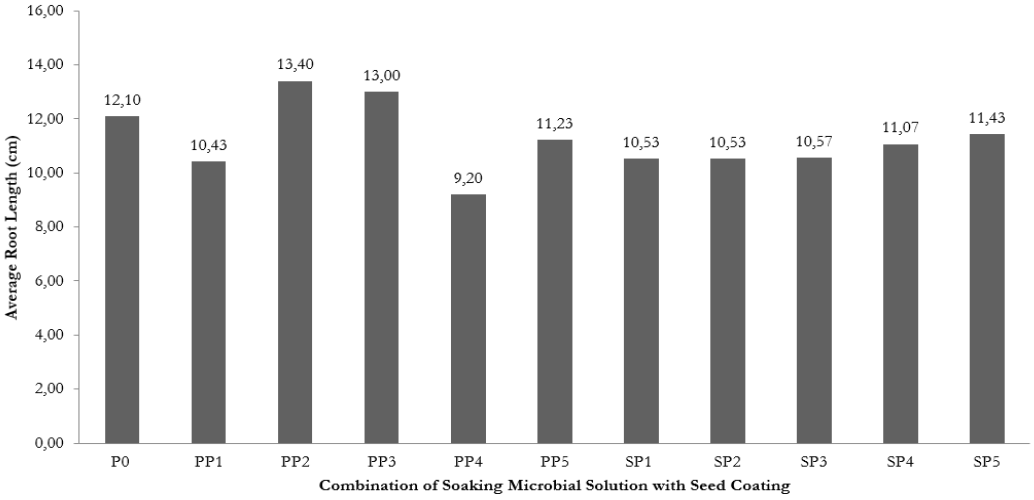


Figure 3. Diagram Root length of ebony seelings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

**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.

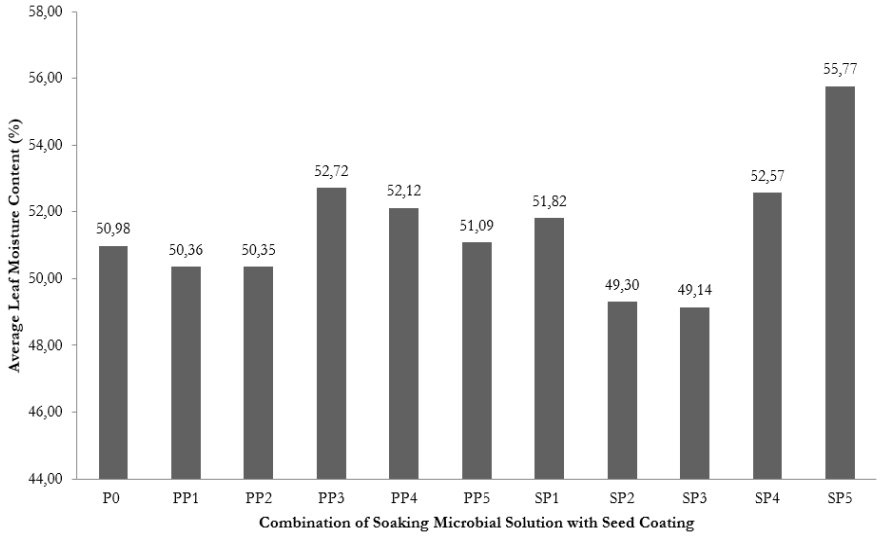


Figure 4. Diagram of leaf moisture content of ebony seedling treated by microbial solution treatment with seed coating. See Table 1 for treatment information

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.

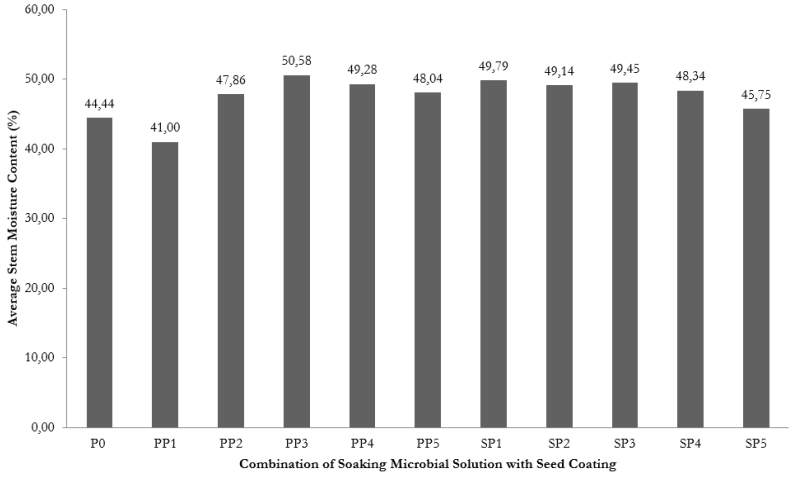


Figure 5. Diagram of the stem moisture content of ebony seedlings treated by the treatment of microbial solutions with and without seed coating. See Table 1 for treatment information

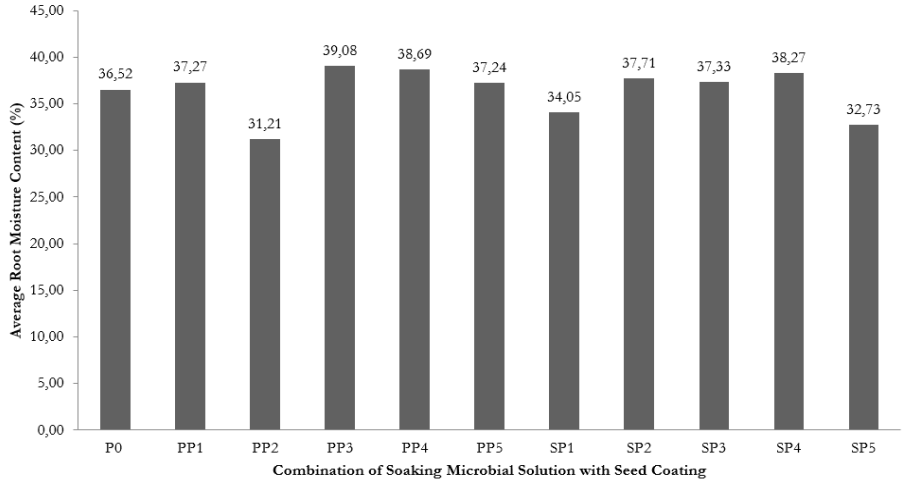


Figure 6. Diagram of root moisture content of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

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

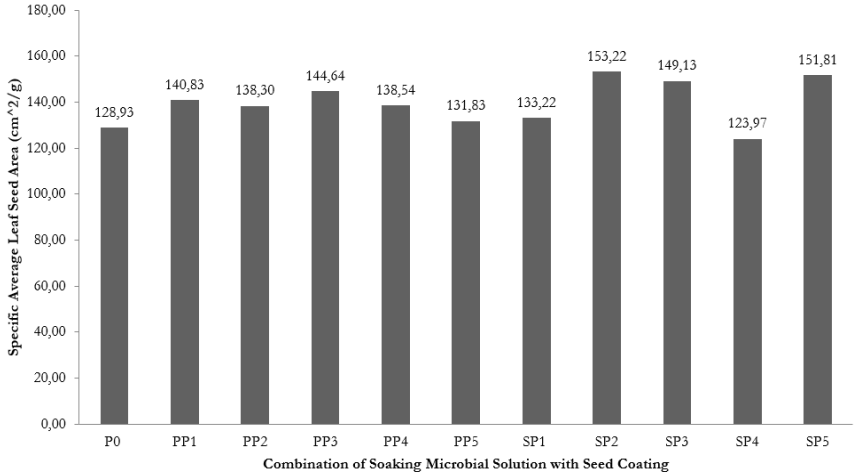


Figure 7. Specific leaf area of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

**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).

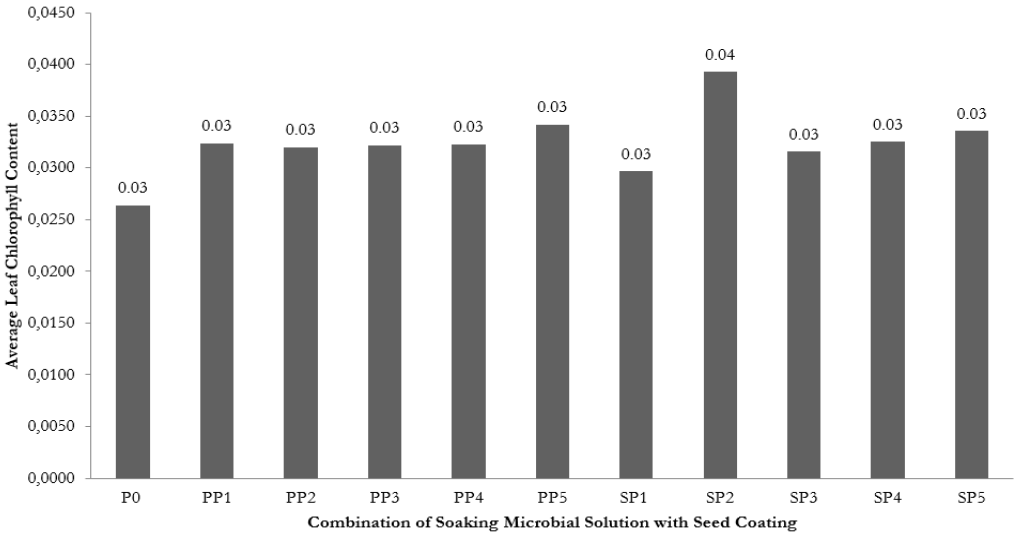


Figure 8. Diagram of leaf chlorophyll content of ebony seedlings treated by microbial solution treatment with and without seed coating. See Table 1 for treatment information

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.

## ACKNOWLEDGEMENT

The author would like to thank RISTEKDIKTI and Hasanuddin University, who have supported this research through Master Thesis Research Scheme 2022 with number contract 090/E5/PG.02.00.PT/2022.

## REFERENCES

Adinugraha, H. A. (2012). Pengaruh cara penyemaian dan pemupukan NPK terhadap pertumbuhan bibit mahoni daun lebar di pesemaian. *Jurnal Pemuliaan Tanaman Hutan*, *6*(1), 1–10. doi://10.20886/jpth.2012.6.1.1-10.

Altaf, M. (2021). Functional diversity of nitrogen-fixing plant growth-promoting Rhizobacteria: The story so far. In *Soil Nitrogen Ecology*. Springer. pp. 327–348. doi://10.1007/978-3-030-71206-8_16.

Danu, & Kurniaty, R. (2013). Effect of Media and Shade on Gerunggang Seedling Growth (*Cratoxylom arborescens* (Vahl) Blume). *Jurnal Perbenihan Tanaman Hutan*, *1*(1), 43–50. doi://10.20886/bptpth.2013.1.1.37.

Darwo, & Sugiarti. (2008). Pengaruh dosis serbuk spora cendawan Scleroderma citrinum Persoon dan komposisi media terhadap pertumbuhan tusam di persemaian. *Jurnal Penelitian Hutan dan Konservasi Alam*, *5*(5), 461–472. doi://10.20886/jphka.2008.5.5.461-472.

Djamhuri, E., Yuniarti N., and Purwani H. D. (2012). Viability and Initial Growth of Akasia Krasikarpa (*Acacia crassicarpa* A. Cunn. Ex Benth.) Seedling from five seed source in Indonesia. *Silvikultur Tropika*, *03*(03), 187–195. doi://10.29244/j-siltrop.3.3.%25p.

Ekawati, M. (2007). Pengaruh media multiplikasi terhadap pembentukan akar dari tunas in vitro nenas (*Ananas comosus* (L.)) Merr. cv. smooth

cayenne pada media pengakaran. Skripsi. Bogor: Institut Pertanian Bogor.

Fabre, D., Dingkuhn, M., Yin, X., Clément-Vidal, A., Roques, S., Soutiras, A., & Luquet, D. (2020). Genotypic variation in source and sink traits affects the response of photosynthesis and growth to elevated atmospheric $CO_2$. *Plant Cell and Environment*, *43*(3), 579–593. doi://10.1111/pce.13693.

Gardner, F. P., R. B. & Pearce, R. L. M. (1992). *Fisiologi Tanaman Budidaya*. Jakarta: Universitas Indonesia Press.

Garinas, W. (2012). Karakteristik bahan baku kaolin untuk bahan pembuatan badan isolator listrik keramik porselen fuse cut out (Fco). *Jurnal Sains dan Teknologi Indonesia*, *11*(2), 120–125. doi://10.29122/jsti.v11i2.829.

Gusmayanti, E., & Sholahuddin. (2015). Specific leaf area and leaf area index of sago plants in Sungai Ambangah village, *West Kalimantan. SEMIRATA*, *5*(1), 184-192.

Gusmiaty, Restu, M., Larekeng, S.H., & Setiawan, E. (2020). The optimization of in vitro micropropagation of betung bamboo (Dendrocalamus asper backer) by medium concentrations and plant growth regulators. *IOP Conf. Series: Earth and Environmental Science*, *575*, 012024. doi://10.1088/1755-1315/575/1/012024.

Haase, D. L. (2008). Understanding forest seedling quality: measurements and interpretation. *Tree Planters' Note*s, *52*(2), 24–30.

Haggag, L. F., Merwad, M. A., Shahin, M. F. M., & Fouad, A. A. (2014). Effect of NPK and bio-fertilizers as soil application on promoting growth of " Toffahi " olive seedlings under greenhouse condition. *Journal of Agricultural Technology*, *10*(6), 1607–1617. http://www.ijat-aatsea.com.

Herpinawati; Zulkifli, D. S. (2010). Growth Rate and Biomass of Rhizopora Apiculata Seedlings in Delta Upang Banyuasin Waters, South Sumatra. *Maspari*, *01*, 30–34. doi://10.56064/maspari.v1i1.1116.

Ikrarwati, I., Ilyas, S., & Yukti, A. M. (2015). Keefektifan pelapisan benih terhadap peningkatan mutu benih padi selama penyimpanan. *Jurnal Penelitian Pertanian Tanaman Pangan*, *34*(2), 145. doi://10.21082/jpptp.v34n2.2015.p145-152.

Jumadi, O. L., & Hartono. (2015). Production of growth regulator IAA (Indole Acetic Acid) and phosphate solubilization ability in nitrogen fixing bacteria isolates from Takalar Regency. *Jurnal Bionature*, *16*(1), 43–48. doi://10.35580/bionature.v16i1.1568.

Kalay, A. M., Kesaulya, H., Talahaturuson, A., Rehatta, H., & Hindersah, R. (2020a). Aplikasi pupuk hayati konsorsium strain Bacillus sp. dengan berbeda konsentrasi dan cara pemberian terhadap pertumbuhan bibit pala (*Myristica fragrans* Houtt). *Agrologia*, *9*(1), 30–38. doi://10.30598/a.v9i1.1060.

Koryati, T., Purba, D. W., Surjaningsih, D. R., Herawati, J., Sagala, D., Purba, S. R., Khairani, M., Amartani, K., Sutrisno, E., & Panggabean, N. H. (2021). *Fisiologi Tumbuhan*. Yayasan Kita Menulis.

Kurniaty, R., & Budiman, B. (2010). Pengaruh media dan naungan terhadap mutu bibit suren (*Toona sureni* Merr.). *Jurnal Penelitian Hutan Tanaman*, *7*(2), 77–83. doi://10.20886/jpht.2010.7.2.77-83.

Trivana, L., Pradhana, A. Y., Manambangtua, A. (2017). Time optimization of the composting of organic fertilizer based on goat manure and coconut coir dust using EM4 bio-activator. *Jurnal Sains &Teknologi Lingkungan*, *9*, 16–24. doi://10.20885/jstl.vol9.iss1.art2.

Malau, S. (2005). *Perancangan percobaan*. Medan: Universitas HKBP Nommensen.

Masniawaty, Mustari, K., Astuti, Gusmiaty, Larekeng, H., Yani, A., & Rahim, I. (2019). Exploration of bacteria associated with chili peppers' rhizosphere and their capacity to absorb and produce gibberellin hormone. *IOP Conference Series: Earth and Environmental Science*, *343*, 012059. doi://10.1088/1755-1315/343/1/012059.

Mayasari, A., Kinho, J., & Suryawan, A. (2012). Association of ebony (*Diospyros* spp.) with dominant tree species in Tangkoko Nature Reserve, North Sulawesi. *Info BPK Manado*, *2*(1), 55-72.

Meliala, T. A. W. (2018). Pengaruh cara aplikasi pupuk ganggang coklat (*Sargassum polycystum* L.) dan pupuk anorganik terhadap ketersediaan N, P, K tanah inceptisol dan pertumbuhan bawang Dayak (*Eleutherine americana* Merr.). Skripsi. Fakultas Pertanian, Universitas Sumatra Utara.

Musyarofah, N., Susanto, S., Aziz, S., & Kartosoewarno, S. (2007). Response of pegagan (*Centella asiatica* L. Urban) to natural fertilizer application under shade. *Jurnal Agronomi Indonesia*, *224*(35), 217–224. doi://10.24831/jai.v35i3.1335.

Nurhasybi, Sudrajat, D. J., & Suita, E. (2020). *Kriteria Bibit Tanaman Hutan Siap Tanam: untuk*

*pembangunan hutan dan rehabilitasi lahan*. Bogor: IPB Press.

Paembonan, S. A., Larekeng, S. H., Millang, S., & Meinardus. (2021). The dynamics of physiological properties of ebony (*Diospyros celebica* Bakh) based on crown position and altitude. *IOP Conference Series: Earth and Environmental Science*, *807*(032016), 1–9. doi://10.1088/1755-1315/807/3/032016.

Pamungkas, T., & Rini, P. (2013). Variation in growth characteristics of jabon seedlings from two different provenances. *Jurnal Penelitian Hutan Tanaman*, *10*(1), 7–15. doi://10.20886/jpht.2013.10.1.7-16.

Prananda, R., & Riniarti, M. (2014). Growth response of jabon (Anthocephalus cadamba) seedlings with cow dung compost on weaning media. *Jurnal Sylva Lestari*, *2*(3), 29–38. doi://10.23960/jsl3229-38.

Prihastanti, E. (2011). Perkecambahan biji dan pertumbuhan semai kakao (Theobroma cacao L.) asal Sulawesi Tengah yang dibudidayakan di Kabupaten Banyumas Jawa Tengah. *Anatomi Fisologi*, *XIX*(1), 8–15. doi://10.14710/baf.v19i1.2579

Purwaningsih, S. (2007). *Kemampuan serapan karbondioksida pada tanaman hutan kota di kebun raya Bogor*. Skripsi. Bogor: Institut Pertanian Bogor.

Qiu, Y., Amirkhani, M., Mayton, H., Chen, Z., & Taylor, A. G. (2020). Biostimulant seed coating treatments to improve cover crop germination and seedling growth. *Agronomy*, *1*0(2), 154. doi://10.3390/agronomy10020154.

Restu, M., Bachtiar, B., Gusmiaty, & Larekeng, S. H. (2019). Gibberellin and IAA production by rhizobacteria from various private forest. *IOP Conference Series: Earth and Environmental Science*, *270*(1). doi://10.1088/1755-1315/270/1/012018.

Rohmanah, S. (2016). *Pengaruh Variasi Dosis dan Frekuensi Pupuk hayati (Biofertilizer) Terhadap Pertumbuhan Tanaman Kacang Hijau* (*Vigna radiata* L.). Skripsi. Surabaya: Universitas Airlangga.

Santi, L. P. (2016). Pengaruh asam humat terhadap pertumbuhan bibit kakao (Theobroma cacao) dan populasi mikroorganisme di dalam tanah humic dystrudept. *Jurnal Tanah dan Iklim*, *40*(2), 87–94. doi://10.2017/jti.v40i2.5542.

Santoso, B., & Chairil A., S. N. (2002). Cultivation of ebony trees (Diospyros celebica Bakh.). *Berita Biologi*, *6*(2), 277–282. doi://10.14203/beritabiologi.v6i2.1494.

Saraswati, R. (2012). Teknologi pupuk hayati untuk efisiensi pemupukan dan keberlanjutan sistem produksi pertanian. *Seminar Nasional Pemupukan dan Pemulihan Lahan*, 727–738.

Scharwies, J. D., & Dinneny, J. R. (2019). Water transport, perception, and response in plants. *Journal of Plant Research*, *132*(3), 311–324. doi://10.1007/s10265-019-01089-8.

Sembiring, E. P. B. (2020). *Pengaruh Media Semai True Shallot Seed (TSS) terhadap Pertumbuhan Vegetatif Bawang Merah*(*Allium ascalonicum* L.). Skripsi. Tasikmalaya: Universitas Siliwangi.

Setiawati, T., & Syamsi, I. F. (2019). Stomatal characteristics based on time estimation and differences in light intensity in leaves of Hibiscus tiliaceus Linn. in Pangandaran, West Java. *Jurnal Pro-Life*, *6*(2), 148–159. doi://10.33541/jpvo16Iss2pp102.

Siregar, H.M., & Utami, N.W. (2002). Effectiveness of organic and N fertilizers on ebony (*Diospyros celebica* Bakh.) seedling growth. *Berita Biologi*, *6*(2), 283-287. doi://10.14203/beritabiologi.v6i2.1495.

Sudrajat, & Siagian N. (2014). Effect of Phosphorus and Potassium Fertilization on the Growth of Oil Palm (*Elaeis guineensis* Jacq.) in the Main Nursery. *Agrovigor*, 7(2), 105-116. doi://10.21107/agrovigor.v7i2.1445.

Sudrajat D., Yulianti, Danu, Rustam E., & Suwandhi I. (2019). Genetic diversity in the Growth of White Jabon (*Neolamarckia cadamba*) Provenance-Progeny test: Comparing Studi in the Nursery and Field. *Biodiversitas*, *20*(5), 1325-1332. doi://10.13057/biodiv/d200512.

Sumenda, L. (2011). Analisis Kandungan Klorofil Daun Mangga (*Mangifera indica* L.) pada Tingkat Perkembangan Daun yang Berbeda. *Jurnal Bios Logos*, *1*(1). doi://10.35799/jbl.1.1.2011.372.

Sumiasri, N., & Setyowati, N. (2006). Effect of several media on the growth of ebony (Diospyros celebica Bakh) seedlings through seed propagation. *Biodiversitas*, *7*(3), 260–263. doi://10.13057/biodiv/d070313.

Suryatmana, P., & Sobardini, D. (2016b). Effect of seed coating application on degraded seed viability and soybean plant growth. *Jurnal Kultivasi*. *15*(2), 107–113. doi://10.24198/kultivasi.v15i2.11894

Sutopo, L. (1988). *Teknologi Benih*. Jakarta: CV Rajawali.

Syafrida, M., Darmanti, S., & Izzati, M. (2018). Effect of drying temperature on moisture content, flavonoid content and antioxidant

activity of leaves and tubers of teki grass (*Cyperus rotundus* L.). *Jurnal Bioma*, *20*(1), 1410–8801. doi://10.14710/bioma.20.1.44-50.

Tavakol, E., & Pakniyat, H. (2007). Evaluation of some drought resistance criteria at seedling stage in wheat (*Triticum aestivum* L.) cultivars. *Pakistan Journal of Biological Sciences*, 10(7), 1113–1117. doi://10.3923/pjbs.2007.1113.1117.

Torey, P. C., Nio, S. A., Siahaan, P., & Mambu, S. M. (2014). Karakter morfologi akar sebagai indikator kekurangan air pada padi lokal superwin. *Jurnal Bios Logos*, *3*(2). doi://10.35799/jbl.3.2.2013.4431.

Wiyono, S. (2012). Formulation of Biofungicide Flour with Dual Active Ingredients Pseudomonas Fluorescens PG 01 and Bacillus Polymixa BG 25. *Jurnal Ilmu Pertanian Indonesia. 17*(3), 180–185. ISSN: 0853-4217.

Zhang, J., Kopparapu, N. K., Yan, Q., Yang, S., & Jiang, Z. (2013). Purification and characterisation of a novel chitinase from persimmon (Diospyros kaki) with antifungal activity. *Food Chemistry*, *138*(2–3), 1225–1232. doi://10.1016/j.foodchem.2012.11.067.

Zhao, T., Deng, X., Xiao, Q., Han, Y., Zhu, S., & Chen, J. (2020b). IAA priming improves the germination and seedling growth in cotton (*Gossypium hirsutum* L.) via regulating the endogenous phytohormones and enhancing the sucrose metabolism. *Industrial Crops and Products*, *155*. doi://10.1016/j.indcrop.2020.112788.

Zheng, H., Ouyang, Z., Xu, W., Wang, X., Miao, H., Li, X., & Tian, Y. (2008). Variation of carbon storage by different reforestation types in the hilly red soil region of southern China. Forest Ecology and Management, 255(3–4), 1113–1121. doi://10.1016/j.foreco.2007.10.015.