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Micropopagation of *Acacia mangium* M1 Generation Obtained by Mutation Breeding Technique

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ABSTRACT

Acacia tree is one of the fastest growing forest species and provides advantages such as good wood quality, tolerance for various types of soil and environment. The wood of Acacia can be used as the main raw material for paper pulp. Acacia is one of the trees that is used as greenery in urban areas. The production of Acacia wood can be done through agricultural extensification efforts which can be carried out by expanding the Acacia plantation area on saline land. Currently, there are stands of *Acacia mangium* generation M1 which is mutated from 13 years old *A. mangium* mutations planted in the SEAMEO BIOTROP experimental garden. Seed yields from the M1 generation plants need to be tested for germination and sterility as the M2 generation. However, the M1 and M2 generation trees must also be propagated vegetatively. Vegetative propagation for the M1 and M2 *A. mangium* generations has never been done. Therefore, this study was aimed at seeking the right technology for vegetative propagation of the M1 and M2 generations of *A. mangium*. Results of direct observation showed that around the planting area of *A. mangium* M1 generation there were no growth of *A. mangium* saplings. Consequently, the seeds were taken from the M1 generation of *A. mangium*, which is called the M2 generation of *A. mangium*. To prove the existence of M2 generation, it is necessary to carry out seed germination as a test for seed sterility. Germination is an important activity from dormant seeds to growing seedlings depending on seed viability, suitable environment and in some plants depending on the seeds' efforts to break dormancy. Therefore, it is necessary to conduct seed testing to determine the seeds' viability or the ability of seeds to grow into seedlings under optimum environmental conditions. This study was conducted to prove the infertility of the M1 generation seeds of *A. mangium* obtained from mutation breeding techniques. This study is the first step in the conservation effort for *A. mangium* which aimed to prove the initial hypothesis that *A. mangium* seeds are sterile. The objective of this research were to: (i). determine the sterility of the M2 generation seeds of *A. mangium*; (ii) propagate *A. mangium* by means of shoot cuttings and grafts using materials from mature trees (M1) and from germinating M2 seeds, and (iii) obtain sterilization technique and the approriate media composition for micropropagation of *A. mangium.* Five treatments were applied to germinate the seeds of *A. mangium* Willd, namely*:* (A) without treatment (control); (B) cutting the cotyledons of the seeds; (C) sanding the seeds in the hylum section; (D) soaking in water for 24 hours in plain water (room temperature); (E) immersion treatment for 30 seconds in 90 ^{o}C water. This study provided information on preliminary treatment for *A. mangium* seeds to be used by the general public in terms of cost effeciency, time and easiness for field application. Four methods of sterilization were used to sterilize the explant of *A. mangium* i.e., treatment 1: 10% concentration of NaOCl for 10 minutes; treatment 2: 10% concentration of NaOCl for 15 minutes; treatment 3: 15% concentration of NaOCl for 10 minutes; and treatment 4: 15% concentration of NaOCl for 15 minutes. The explant was planted on modified MS medium. Experimental study on micropropagation technique was conducted using a Factorial Completely Randomized Design (CRD) with two factors and three replicates. The first factor was BAP (0 mg/L, 1 mg/L, 2 mg/L, and 3 mg/L), the second factor was NAA (0 mg/L, 0.1 mg/L, 0,2 mg L). , so there are 12 treatment.

The results of this study showed that each treatment provided different results on the percentage of *A. mangium* seed germination, i.e., treatment A with a percentage value of 6%,

treatment B with a percentage value of 91%, treatment C with a percentage value of 88%, treatment D with percentage value of 4%, and treatment E with a percentage value of 94%. Overall, the best treatment for germinating *A. mangium* seeds was treatments B, C and E. Germination of *A. mangium* seeds in treatments B, C and E was above 80%. This is categorized as high seed germination, thus proving that *A. mangium* seeds are not sterile. Meanwhile, the smallest percentage of seed germination occurred in treatments A (control) and D (soaking the seeds in water for 24 hours). The highest germination rate of *A. mangium* seeds with treatment E was 2.27% / day. Germination growth of *A. mangium* seeds carried out in each treatment reached a growth point of 0 on the 15th to the 20th days. The highest germination value was produced by seeds with treatment E, which was 0.64% / day, while the lowest was in treatment D, which was 0.0047% / day. The characteristic of Acacia seeds is having hard outer skin surface, so the seeds require pre-treatment to increase their germination. However, these Acasia seeds are not sterile.

The best and most effective sterilization method was explants immersion with treatment 3 (5.25% NaOCl solution 15% concentration for 10 minutes) capable of producing 84.52% sterile explants. The results of the micropropagation study showed that the best medium for propagating *A. mangium* explants were by tissue culture in MS medium with the addition of 1 mg/L BAP and 0.1 mg/L NAA. Micropropagation is an effort to produce considerable amounts of *A. mangium* explants. The multiplication rate varied from 1 to 7 depending on the growth regulator used. Cytoxines and auxin in plant are very closely related to the process of cell division and morphogenesis that direcly influence the formation of shoots and bud extention. The optimal condition of media for maximum shoot production will differ according to the physiological age of the plant. The 0.1 mg/L NAA and 1 mg/L BAP concentrations at 8 WAP provided 16 mm plant height.

Propagation of shoot cuttings using materials from old trees (M1) and from germinated seeds (M2) can produce cuttings that are capable of rooting. Cuttings from M2 germinated seedlings showed signs of root growth. Meanwhile, adult tree cuttings experienced rooting difficulty because the cells have aged so that the percentage of rooting on the cuttings decreases with the age of the tree.

1. INTRODUCTION

1.1 Background

Acacia mangium is a type of plant native to eastern Indonesia, namely Maluku islands in the Eastern Region and is classified as fast-growing species. This species has several advantaged, including short fiber and able to grow on critical land. The wood species of *A. mangium* has various uses, including for pulp, paper, particle board, and wood chips (Krisnawati *et. al.* 2011), so this species is very pontential and is widely developed in plantation forests to supply raw materials, especially for industry pulp and paper (Bachega *et al.* 2016).

Ecologically, the regeneration process of *A. mangium s*pecies is very fast and is capable of producing large amounts of seeds, so that with its seeds this species is able to spread and dominate outside its natural habitat. *A. mangium* was also listed invasive in several countries, including Afrika, Western Australia, Malaysia (Sabah), Bangladesh, and Brazil. The species *A. mangium* has also predominated in several areas in Indonesia, so there is concern among plantation forest business actors that this species will change its status to invasive.

Many research activities to increase the growth *A. mangium* species have been done, but research to suppress excessive natural spread and improve wood chemistry, especially cellulose, has not been widely carried out. Gijarto (2008) carried out mutation breeding activities in *A. mangium* using a physical mutagen, namely gamma ^{137}Cs. Based on research, it was shown that the *A. mangium* seeds produced by gamma ray irradiation result (M1 generation) had a faster growth rate than the M0 generation. The use of mutation technology is one of technique to bring up new characters. The advantages of *A. mangium* generation M1 as a result of gamma ray radiation ^{137}Cs have longer and better fiber length than *A. mangium* that was irradiated, the density of the bark grooves with high cellulose content and lower levels of extractives and lignin in the M1 generation, trees this superior also has a branch-free height and good natural pruning ability, the brach diameter is quite small and the branch angle is good, the stem shape tends to be straight and cylindrical (Yunus *et al.* 2017). The results of direct observations showed that in planting area of *A. mangium* generation M1 did not find any *A. mangium* saplings growing. This indicates that the M1 generation M1 of *A. mangium* has the potential to be developed.

Currently, there are stands of *A. mangium* generation M1 mutated from mutations planted in the SEAMEO BIOTROP experimental garden, aged 13 years. Seed yields from

the M1 generation plants need to be tested for germination and sterility as the M2 generation. However, the M1 and M2 generation trees also need to be propagated vegetatively. Vegetative propagation for *A. mangium* generations M1 and M2 has never been done, so this research is looking for the right technology for vegetative propagation of M1 and M2 generations. To supply it to the pulp and paper industry, plants that have cellulose with long fibers are needed. This can be obtained through a breeding program. One technique in breeding is to use the mutation technique. The avaibility of *A mangium* seeds which have pontential high cellulose in nature is limited. Currently there is *A. mangium* generation M1 in the SEAMEO BIOTROP experimental garden that needs to be researched to find out and confirm whether the superior tree *A. mangium* generation M1 is a candidate for superior plant breeder that have the characteristics of fast growth, steril seeds, and high cellulose content, so that it has potential to be developed in plantation forests. The vegetative propagation technique is an effective technique in order to produce superior seed that are uniform and have the same characteristic as the parent. In this study, two vegetative techniques were used, namely by shoot cuttings and tissue culture. Elbasheer and Osman (2017) have conducted research on the effectiveness and efficiency of various types and concentrations of antimicrobial compounds in tissue culture sterilization for Acacia species. Research for the in vitro propagation of *A. mangium* has been carried out by several researcher, including Gantait *et al.* (2018), Chauhan and Jha (2018). In the technique developed by Chauhan and Jha (2018), sterilization of shoot explants of *A. mangium* was carried out by immersing the explants in 10% Tween 20, which was then followed by immersion in absolute alcohol for 1 minute, and ended by immersing in 0.1% $HgCl_2$ for 6 minutes. In another technique by Ismail *et al* (2016), sterilization of root nodules of *A. mangium* using 5.25% NaOCl for 4 minutes and followed by 95% alcohol for 3 minutes. Girijashankar (2011) successfully sterilized *Acacia auriculiformis* explants by immersing 1% NaOCl for 15 minutes which had previously been soaked in 70% alcohol for 1 minute. Various sterilization techniques need to consider the environmental conditions of the explants and the effect of the sterilization technique on the environment. The example of the sterilization technique above shows the need for optimization of the sterilization technique on the existing explants. This shows that with its various advantages, tissue culture has the potential to be developed as a plant propagation technique, especially for the purposes of uniform and quality plants.

This research is important for superior acacia. The research is also related to the Programme Thrust SEAMEO BIOTROP for 10th Five Year Development Plan (2018 –

2023), i.e. Topic Under Sustainable Management of Intensively Used Ecosystem and Landscape Program. It consists among others use of biodiversity for enhance and sustained production.

1.2 Objective

The objective of this study were:

1. To determine the sterility of the M2 generation seeds of *A. mangium*.
2. To propagate *A. mangium* by means of shoot cuttings and grafts using materials from mature trees (M1) and from germinating M2 seeds
3. To obtain sterilization technique and the approriate media composition for micropropagation of *A. mangium.*

1.3 Expected Output

The expected results of this study are to obtain superior seeds, sterile in vitro explants and induction techniques for *A. mangium* propagation. The benefits and importance of conducting this research because *A. mangium* has the potential to be developed and its products can be used for companies and the wider community. The advantage of *A. mangium* is that it has high nitrogen fixation ability and in other species *A. mangium* to survive in a very dry environment (Paula *et al.* 2018). Besides, *A. mangium* can grow quickly because it has an intensive root system and can also grow in soils with low fertility, so that it can be utilized for soil rehabilitation. *A. mangium* also has a high economic value because the wood produced is very heavy, hard, very strong, sturdy, not easily deformed and is not easily cracked (Sharma *et al.* 2011). Also, wood from *A. mangium* can be used as furniture, doors, and frames.

In this study obtained a sterilization technique protocol and induction technique of *A. mangium* seed

2. State of the art of the research

The success of tree breeding depends on many factors, including genetic diversity, as well as heritability of expected traits and potential genetic gains. Seedlings grown from seeds that collected from different sources will differ in their growth. Optimum growth is ensured by site quality in accordance with the growth requirements, and it is also influenced by the seed quality. Quality seed is an expression used to describe the ability of a seed to adapt and grow after planting. This can be improved through tree breeding

program by establishing a progeny test from selected parental trees having good genetic characters, which later is converted to a seed orchard, as sources of seeds for high quality plantation by conducting sterility and fiber quality tests for the selection of superior clones. Identification of parental trees as genetic resources and selection of traits that have economic value, which can be used as selection criteria would result in the success of breeding programs. In addition, studies on the sterilization and shoot induction techniques of *A. mangium* support forestry plant breeding programs. Increasing Acasia production can be done by improving planting material sources through plant breeding programs. Selection is one of the important factors that determine the success of a plant breeding program. Vegetative propagation is an effective technique by means of shoot cuttings and tissue culture in order to produce superior seeds that are uniform and have the same characteristics as the parental trees, can be made continuously easily so that a large number of seedlings can be obtained. With the implementation of this research, the future opportunities for the availability of *A. mangium* seeds which have high cellulose potential which are limited in nature can be overcome. Vegetative nurseries are very useful for plant breeding programs, including for the development of clone banks (genetic conservation), clone seed gardens, propagation of plants which are important results of controlled crosses, for example hybrids or steryl hybrids that cannot reproduce sexually, mass propagation of selected plants (Masson A & Monteuuis 2017).

3. MATERIALS AND METHODS

3.1. Sterility test of Acacia superior seed M 1 generation

Materials

The materials used are the seeds of *A. mangium* M1 generation in the SEAMEO BIOTROP field, filter paper, plastic bags, and distilled water. The tools used are stationery, labels, poles, tray, ladders, machetes, buckets, mines, and *waterbath*.

Methods

***A. mangium* Seed Collection**

Tree *A. mangium* generation M1 which is included in the superior category. The tree was observed for flowers and fruit. If it is fruitful and the fruit is ripe, then the samples were collected. *A. mangium* seeds generation M1 were put into a plastic bag. The sorted seeds were stored in a refrigerator at -4 ^{o}C, after undergoing a drying process using

sunlight for one day. This condition is classified as a safe moisture content condition for storage of acacia seeds.

Seed germination of *A. mangium*

Seeds were colleted from *A. mangium* tree og the M1 generation were dried and put into containers. Furthermore, the character of *A. mangium* seeds was observed. Breaking dormancy of *A. mangium* seeds were carried out by 5 treatments, namely: (A) without treatment (control), (B) cut the cotyledon of the seed, (C) sanding the seeds in the hylum section, (D) immersion in water for 24 hours in plain water (room temperature), (E) immersion in water at ±90 ºC for 30 seconds. Then, each treatment was soaked in water for 24 hours. The seeds were drained, then arranged on a petridish lined with three sheets of filter paper that had been given water to maintain moisture. The seeds were germinated for each treatment. Germination evaluation was carried out every day for 1 month.

The calculation formula of the observed parameters is:

a. Seed germination

Germination was calculated in percen with the following formula:

$$DK = \frac{n_1 + n_2 + n_3 + \dots + n_i}{N} \times 100\% = \sum ni \times 100\%$$

ni = Number of germinated seeds at observation i

N = Number of seeds tested

b. Germination speed

Germination speed was calculated in days with the following formula:

$$KB = \frac{n_1 h_1 + n_2 h_2 + n_3 h_3 + \dots + n_i h_i}{n_1 + n_2 + n_3 + \dots + n_i}$$

ni= Number of seeds that germinated on day i (grains)

hi= The number of days required to reach the number of sprouts to ni

c. Germination value

According Sutopo (1985), the parameters that include the rate and percentage of germination are referred to as germination values. The germination value is the peak

value times the average daily germination value which can be calculated by the following formulas:

Peak value

$$PV = \frac{\text{\% germination at T}}{\text{Days needed to achieve it}}$$

PV= Peak germination value

T = The point at which the germination rate begins to decrease

Mean Daily Germination

$$MDG = \frac{\text{\% germination at G}}{\text{Total number of test days}}$$

Germination value (NP) = PV x MDG

PV = Peak germination value

MDG = Average daily germination value

3.2. Propagation of *A. mangium* Generation M1 with Vegetative Techniques

3.2.1 Propagation by tissue culture technique

Materials

The material of this research is *A. mangium* seeds on mutated M1 generation. Chemicals for sterilization using three non-metal antimicrobial compounds, namely detergents, ditiocarbamate with active ingredients mankozeb (compound A), compounds made from active NaOCl 5.25% reacted with sodium hypochlorite and hydrogen oxide and Tween20 solution (90%) as a protective solution of tissue explants (compound B), and 70% alcohol (compound C). MS media with 6-BAP (Benzylaminopurine) enrichment of 1 mg / L; NAA (Naphthalene acetic acid) 0.5 mg / L; 100 mg of myo inositol; pyridoxine-HCl 0.5 mg / l; thiamine-HCl 0.1 mg / L; nicotinic-acid 0.5 mg /L; glysine 2 mg / L; 30 g /L sucrose and 8 g / L agar; with a pH adjustment of 5.7 using NaOH and / or HCl. Media sterilization using an autoclave at 121 ^{0}C; 1.2 kg / cm^{2} for 20 minutes.

Method

Preparation of *A.mangium* seeds

The source of explants used for in vitro propagation of *A. mangium* is explants derived from *A. mangium* with clear types, species and varieties and must be healthy and free from pests and diseases. The explants used are seeds.

Seed Sterilization

A. mangium seeds were sterilized by two stages. The first stage have done outside of LAFC with 3 kinds of immersion in sequentially, that are immersion in 100 mL distilled water plus 3 drops of tween 20 for 30 minutes, soaking in a fungicide solution (benomil 50.4%) 2 g / L plus 3 drops of tween 20 for 60 minutes, and soaking in a bactericidal solution (20% streptomycin sulfate) 2 g / L plus 3 drops of tween 20 for 60 minutes. Each step of immersion is followed by flushing with distilled water 3 times. The second step were soaked by disinfectant in the form of sodium hypochlorite solution (5.25% NaOCl) in LAFC. Immersion of seeds with 5.25% NaOCl were conducted with 4 kinds of immersion methods as presented in Table 1.

Tabel 1 Comparison of sterilization method procedures for *A. mangium*

Methodes	NaOCl 5.25* % concentrations	Soaking times (minutes)
1	15	15
2	15	10
3	10	15
4	10	10

* The 5.25% NaOCl solution in mixtured with 3 drops tween 20.

Seed have been sterilized and then were planted in MS medium. Observation on effectiveness of sterilization methods were conducted for 1 month with observation time every weeks. The variabels observed including contaminated explant, dead and sterile. Each variabel were calculated by :

$$\text{Percentage of contamination explants} = \frac{\Sigma \text{ explants contaminated} \times 100\%}{\Sigma \text{ explants planting}}$$

$$\text{Percentage of mortality explants} = \frac{\Sigma \text{ explants dead} \times 100\%}{\Sigma \text{ explants planting}}$$

Percentage of sterile explants = 100% - (% contaminated + % mortality)

Shoots Induction

Media preparation

Making of media initiation have done by preparing ± 500 mL of distilled water put into a 1000 mL measuring flask. The macro, micro, and vitamin nutrient feedstock (Appendix 1) were put into a measuring flask using a pipette according to the determined concentration. Sucrose was added as much as 30 g to the solution, then the aquades were added until the solution reaches 1000 mL. During media preparation, the solution were stirred on a magnetic stirrer by inserting magnetic stirring bars into the measuring flask so that the solution was homogeneous. After that the pH was set to 5.7. If the solution does not show the pH value, then add a few drops of 0.1 M NaOH to raise pH or 0.1 M HCl to decrease the pH. After the pH is appropriate, then put of agar as a media compactor. The media was heated to boil. MS media solution was put in bottle the culture as much as ±20 mL. The culture bottles containing the media were covered by plastic. The culture bottles were sterilized in an autoclave at 121 ^{0}C for 20 minutes. In preparation of treatment media are the same as preparation of initiation media, but were added ZPT appropriate with combination in table 2.

The media used in the first initiation activity *A.mangium* seeds were MS media that were added sitokinin (BAP 0.5 mg/L). After 1 month, it were put into media 0. Then the treatment media used were MS media plus ZPT as cytokinins (BAP) and auxins (NAA). The treatment media consisted of 12 combinations of the two ZPT concentrations, so that are obtained 12 treatment media with each different concentration can be seen in Table 2.

Tabel 2. The combination of the concentration of two kinds ZPT in the treatment media in MS base media.

Treatment media	Concentration of growth regulators (mg/L)	
	6-benzylaminopurine (BAP)	NAA
A	0.0	0.0
B	1.0	0.0
C	2.0	0.0
D	3.0	0.0
E	0.0	0.1
F	1.0	0.1
G	2.0	0.1
H	3.0	0.1
I	0.0	0.2
J	1.0	0.2
K	2.0	0.2
L	3.0	0.2

Sterile explants have been initiated in MS media with BAP 0.5 mg/L, then were induce in the treatment media. The experiment used factorial completely randomized design (CRD), with 2 factors, namely the type of ZPT and ZPT concentration. Kinds of ZPT consist of 2 levels that are BAP and NAA.

Maintenance

The culture bottles were stored in a rack in the culture room with a temperature of 22−25 ^{0}C and were placed under a fluorescent lamp 36−40 watt. The lighting duration of the lamps were set to 16 hours on and 8 hours lights out.

Growth of *A.mangium* shoots

Growth and development of explants will be observed for 8 weeks after planting (MST). The measured variables include:

a. Time of appearance of shoots. Observations were conducted daily by looking at the development of shoots on explant.
b. The number of shoots. The number of shoots were calculated at the end of observation at 8 MST by counting the number of shoots that formed.
c. The high of shoots. The shoots height were measured from the the difference in height of the shoot at the end of the observation with the shoot height when it entered the treatment medium. Measurements will be made using millimeter paper in placed under a petri dish.

3.2.2 Seedling growth of *A. mangium* generation M2

Materials and Tools

The materials and tools used in testing the growth of *A. mangium* seedlings of M2 generation were as follows: *A. mangium* seedling of M2 generation, polybags, growth media, lid, watering can, stationery, ruler, calliper, camera,

Research procedure

Seedlings resulting from the germination of *A. mangium* seeds of the M2 generation were planted in polybags containing growth media in the form of soil, cocopeat, rice husk in a ratio of 1:1:1 (v/v/v). Then the polybag containing the seedlings is put into a plastic lid measuring 2 m x 1 m x 1 m, for 30 days, then the lid is opened to adjust to the humidity and temperature of the surrounding environment for 2 weeks. Watering is carried out every 2 days because the humidity of the growing media is maintained by the cocopeat. Then the

seedlings were separated into two groups, namely in the greenhouse (100 seedlings) and in the field/open area (200 seedlings). For growth analysis (height, diameter, number of leaves) 20 replicates were randomly selected.

3.2.3 Seedling Cutting Technique *A. mangium* generation M2

Materials

The cutting media used were husk charcoal, cockpit, a mixture of 50% husk charcoal: 50% cocopeat, and a 70% husk charcoal mixture: 30% cocopeat. Other materials are ZPT Root up, cuttings from *A. mangium* seedlings aged 4 months from field locations and greenhouses. The equipment needed is a plastic cup, scissors, cuttings, labels, markers, plastic lids, rubber bands

Research procedure

The planting media used were husk charcoal, cocopeat, a mixture of 50% husk charcoal: 50% cocopeat, and a 70% husk charcoal mixture: 30% cocopeat. Previously, the mixture of each media was sterilized first using an autoclave. The sterile media was then put into a glass pot and poured with water and fungicides and pesticides a few days before planting the cuttings.

The cutting material was prepared by selecting orthotropic branches/twigs with a diameter of 2-5 mm on *A. mangium* generation M2 seedlings from two locations, namely the Greenhouse and the field. Store the cut branches in a plastic bucket filled with water. Cut the cutting material to leave 1 internode with 2 stem nodes (nodes). Furthermore, the leaves are reduced to leave 1/3 of the leaves so that transportation on the leaves is reduced. The base of the cutting material 6. The base of the cutting material that has been cut is then smeared with a Root Up paste. Cuttings material that has been smeared is then positioned upside down for +/- 5 minutes.

Planting cuttings begins with making a planting hole first. Plant the cuttings that have been dipped Root up into the hole and compact the planting medium using your fingers. The ready tub is then given a plastic lid on the pot. Watering was done once a week

3.2.4 Technique for shoot cuttings from old trees

The mother tree used in this study is the result of irradiation by Gijarto (2008), and the results were planted in 2008 in the SEAMEO BIOTROP experimental garden so that the mother tree will be 13 years old in 2021. The results of Yunus (2016) study show the mother tree of *A. mangium* M1.13 has the best height and diameter growth. This tree needs to be propagated by vegetative propagation through shoot cuttings.

To stimulate the growth of the cutting material, branch pruning is carried out. The emerging shoots are used as cutting material. This is to rejuvenate the parent tree for easy propagation. Pruning is done on the lowest branch using a saw. This can trigger the growth of new shoots in areas close to the former pruning, with an orthotropic and younger growth direction. The tree performance of *A. mangium* No. M1.13 can be seen in Figure 1. The growth of new shoots is juvenile, with an orthotropic direction of growth, and the leaves are light green, sometimes even true leaves appear from *A. mangium* which indicates that the new shoots are juvenile.

The criteria for the cutting material used in this study were orthotropic, juvenile growth, with a diameter of 2 -5 mm, healthy, not too old. The length of each cutting material ranges from 6-12 cm, consists of 2-3 segments and is left with 1-2 leaves that have been cut and left 1/3 of them. Cuttings are put in a bucket of clean water to reduce dehydration.

The hormones used in this study were Root up with the active ingredients 1-Naphthalene acetamide (NAD) 0.20%, 2-methyl 1-Naphthalene acetamide (m-NAD) 0.003%, Indole 3-Butyric Acid (IBA) 0.06%. and 4% thiram. In addition, in other experiments, pure auxin hormone in the form of IBA which has been dissolved using NaOH with a concentration of 1,000 ppm was also used. The use of IBA was reported to be successful in forming various root primordia on various forestry plant cuttings.

There were 4 types of growing media used during the cutting experiment from old tree *A. mangium*, namely: cocopeat: husk charcoal: compost 1:1:1 (v/v/v); cocopeat: husk charcoal 1:1 (v/v); cocopeat 100%; and 100% cocopeat with zeolite base.

Before planting the base of the cuttings, ZPT Root Up was added to the cuttings in the form of a paste. The base that has been spiked with paste is then turned over for -/+ 15 minutes, then planted. In cuttings using IBA solution, shoot cuttings were soaked for 10 minutes. ZPT IBA was previously dissolved using 1% NaOH to obtain a solution with a concentration of 1000 ppm.

Covering the cuttings using UV plastic which is directly attached to the tub and tied with rubber and using a transparent container/jar with a lid. In addition, tubs and containers are also placed in large hoods and are also provided with 60% paranet shade.

3.2.5. Graft technique

Materials

The materials and tools used are sharp knives, ZPT root up, water, growing media (soil: cocopeat: compost), plastic wrap, and raffia rope.

Procedure

Transplantation was done by selecting the appropriate stem with a diameter of 0.5 cm – 3 cm. The branches were slashed 5 cm long using a knife to remove the bark, then followed by scraping the cambium and left for a few minutes. The cut part is then applied with ZPT root-up paste which has been diluted to form a paste on the slashed stem segments, especially at the upper internode border (close to the leaf). Growing media in the form of: soil, coccipit, compost. Cover the stem of the incision with growth medium and tie it using raffia rope. Leave the graft for a few weeks to see root growth. Planting of grafts is done by preparing media in large polybags for planting the grafts. the growing media package was opened carefully so as not to damage the roots, then placed into polybags. If there are more leaves than the existing roots, reduce branches and/or leaves to avoid wilting seedlings. The results of grafts in polybags are watered sufficiently and place the seeds in the shade.

4. RESULTS AND DISCUSSION

4.1. Characteristics of *A. mangium* Seeds

Table 3. Observation of seed character of *A. mangium* generation M2

No.	Characterization	Information
1	Seed shape	Longitudinal, elliptical, oval to oblong
2	Seed color	black
3	Seed surface	Smooth, slippery
4	Seed coat type	Shiny
5	Embryo color	White
6	Seed size	3-4 mm × 2 mm
7	Seed coat	Layered
8	Special features	Placenta (hylum) is orange
9	Seed Character	Ortodoks
10	Germination type	Epigeal
11	Average weight of 1000 seeds	8.9491 gram

The seed is part of the plant that is used for propagation. Quality seeds include physical, physiological and genetic qualities. Physical quality includes size, weight and visual appearance of seeds. Physiological quality describes the ability to germinate and seed vigor, while genetic quality reflects superior traits inherited by the parent plant related to growth and appearance of stands in the field. Genetic quality is largely determined by the condition of the seed source. From the conditions of selected or tested seed sources, it is possible to obtain genetic advances that will affect stand productivity at the end of the cycle. The seeds of *A. mangium* observed were seeds derived from the mutation of the M1 generation. Table 3. showed that the seeds of *A. mangium* are shiny black in color with shapes varying from longitudinal, elliptical, and oval to oval, small in size 3-4 mm × 2 mm, the surface of the seeds is smooth and slippery, the seed coat is layered, the color of the embryo is white, the placenta (hylum) is orange. Seed size has an impact on germination and survival under stressful conditions. Plants from larger seeds, compared to smaller seeds, show competitive advantages in germination and stress tolerance of seedlings (Mao *et. al*. 2019). *A. mangium* seeds are one of the orthodox seeds, so these seeds can be stored and survive for a long time because orthodox seeds are seeds that undergo a drying process while on the tree. Orthodox seeds are seeds that can be stored for a long time, the moisture content can be lowered to below 10%, and can be stored at low temperature and humidity. Its viability can be extended by lowering the humidity and storage temperature (Murrinie *et. al*. 2017). Seeds require an optimum moisture content for storage, most seeds have an optimum moisture content of 6-11% storage. Seeds require

an optimum moisture content for storage, most seeds have an optimum moisture content of 6-11% storage. According to Purba *et al.* (2013) stated that seed deterioration during storage was caused by higher seed moisture content. This causes the respiration rate to be faster so that more CO_2 and heat are produced. This physiological activity can be suppressed through an ideal storage water content so that the germination of the seeds can still be maintained until the time for the seeds to germinate. Seed storage aims to maintain the viability of the seeds in order to remain high until the seeds are planted. The seed storage container is a plastic bottle because it is airtight so it can maintain the moisture content of the seeds during the storage period. Furthermore, the incubation of seeds for germination test in a dark room, laboratory room conditions have an average temperature of 27.8 ^oC with a relative humidity of 70.8%.

Based on the observation that the seeds of *A. mangium* had an epigeal germination type and the average weight of 1000 seeds was 8.9491 grams (Table 3). The calculation of the weight of 1000 seeds of *A. mangium* generation M2 was used to design seed requirements for a seedling and planting program. In general, large seeds will produce seeds that have good vigor. The results of calculations from the National Research Council (1983), one kilogram of clean A. mangium seeds contains an average of 80,000–110,000 seeds. According to Yuniarti (2016), there are four classifications of the average weight of 1000 seeds of *A. mangium* from class I (weight; > 12,185 g), class II (moderate weight; 9,485-12,185 g), class III (moderate; 9,020- 9.485 g), and class IV (mild; 7.373-9.020 g). This weight indicates that the seeds of *A. mangium* generation M2 from the SEAMEO-BIOTROP experimental garden are classified as light seeds because they are in the weight range of 7.373-9.020 grams per 1000 seeds or small in size. The weight of *A. mangium* seeds per 1000 fruit obtained from the acacia tree (M1) whose parents had been given gamma mutation treatment had a lighter weight of 8,949 compared to the *Acacia crassicarpa* species that had been bred or not. *A. crassicarpa* seeds when compared with *A. mangium* seeds with a weight ratio of 1000 fruit weighs 18.53 to 22.08 grams (Yuniarti 2013). One type of plant can have varying seed sizes. Larger seeds have higher vigor than smaller seeds. Large and heavy seeds contain more food reserves and larger embryos than small seeds. According to Wulandari *et al.* (2015) that for certain types, seeds that have a larger weight and size have better physical and physiological quality than seeds that have a smaller weight and size, resulting in seed viability and seed vigor higher, percent germination and better seedling growth compared to smaller seeds. Based on the movement of the cotyledons upward, *A. mangium* germination is an epigeal germination

type. In plant growth and development epigeal germination occurs when the hypocotyl (embryo axis or ovule under the cotyledon) extends upward, the elongation of the hypocotyl pushes the cotyledons above it, so that the cotyledons come out and are on the surface.

4.2. General Condition of Seeds and Initial Viability of Seeds of *A. mangium*

Seeds were selected based on their condition that is not wrinkle, oval, and large. This is based on the characteristics of Acacia seeds that can grow. The seeds of *A. mangium* are hard seeds, so treatment is needed so that the seeds can germinate. Each seed has a different ability to germinate, even though the genetic and physiological conditions are the same. This is caused by environmental conditions that can determine a sprout. By giving different treatments to the same type of seed, the growth ability of each seed will be known. The ability of the seed is expressed by germination and the speed at which germination can be active.

Physiological qualities describe the ability to germinate and seed vigor. Based on the results of the study, the average percent germination of *A. mangium* seeds in treatment A was 6%, treatment B was 91%, treatment C was 90%, treatment D was 4%, and treatment E was 94% (Table 4 and Figure 1). The germination of *A. mangium* seeds in treatments B, C and E was above 80%. This is categorized as high seed germination, thus proving that *A. mangium* seeds are not sterile. Of all the variables observed, the control treatment and 24-hour water immersion showed the lowest results, namely 6% and 4%, respectively. *A. mangium* seeds in control and without scarification yields were lower because the seeds were still experiencing dormancy which made it difficult for water to get into the seeds so that imbibition as the beginning of seed germination could not occur.

Germination of *A. mangium* seeds was very high 94% in treatment E, namely immersion for 30 seconds in boiling water (at 90°C). Treatment with boiling water to increase germination of hard seed species. The basic histological structures of the permeable and impermeable tests are identical. Therefore, impermeability is not a consequence of a particular cell layer, but the result of a particular chemical composition of the cells that are part of the seed coat of that species (Yousif *et. al.* 2019). The seeds of *Crassocephalum crepidioides* and *Conyza canadensis* can survive after being given a heating treatment for 30 minutes at 55˚C and it is known that *Ageratum conyzoides* seeds

can survive more than 60˚C to 90˚C. This is conducted to see the tolerance of seeds to high temperature treatment (Yuan & Wen 2018).

Mechanical scarification by wounding nail clippers allows the seed coat to be injured so that water can pass through and the imbibition process occurs. Scarification is able to provide an impermeable condition to the seed coat (which was initially impermeable) so that the seeds can absorb water. Water enters the seed causing enzyme activation, reshuffle of food reserves, molecular transport, increased respiration and assimilation, initiation of cell division and enlargement, and elongation of radicle cells followed by the emergence of radicles from the seed coat. The water accelerates the emergence of radicles and increases the growth of other parts of the embryo so that the normal sprouts that are formed are also high. The high germination rate is the result of the rapid process of seed germination metabolism and sufficient food reserves are available in the seeds. Sufficient food reserves are needed as a substrate for respiration to produce energy in increasing germination metabolism. This is indicated by the high germination yield of Acasia with nail clipper injury. The same study on saga seeds (*Abrus precatorius* [L.]) showed that mechanical scarification was effective in breaking seed dormancy by wounding with nail clippers in the cotyledons (Nurmiaty *et. al.* 2014). Injury with nail clippers in the cotyledons showed a higher yield. This is presumably because the wound area of the nail clipper is larger than the sanding at the hilum, so that water and gas enter the seeds more easily. In the research of Elfianis *et al.* (2019) showed that wounding of the seed coat was able to increase germination and the sanding treatment showed high speed and germination of acacia seeds. Physical dormancy in seeds is caused by water-resistant seeds or fruit skins.

Observations on the 2nd day showed the structure of the sprouts that appeared only radicles. On the 14th day, all normal sprout structures, starting from the radicle, epicotyl, hypocotyl and plumule have appeared. However, the size of all normal sprout structures was too high. In acacia plants that have physical dormancy, the impermeability of the seed coat has a characteristic in the form of a lignin epidermis. Physical dormancy breaking methods are needed in order to facilitate the imbibition process.

Tabel 4. The results of the calculation of the average percentage of germination

Treatment	Average of germation (%)
A	6
B	91
C	90
D	4
E	94

Note:
Treatment A: No Treatment (Control)
Treatment B: Cut the cotyledon of the seed
Treatment C: Sanding the seeds in the hylum
Treatment D: Soaking 24 hours with plain water (room temperature)
Treatment E: Immersion for 30 seconds in boiling water (at 90°C)

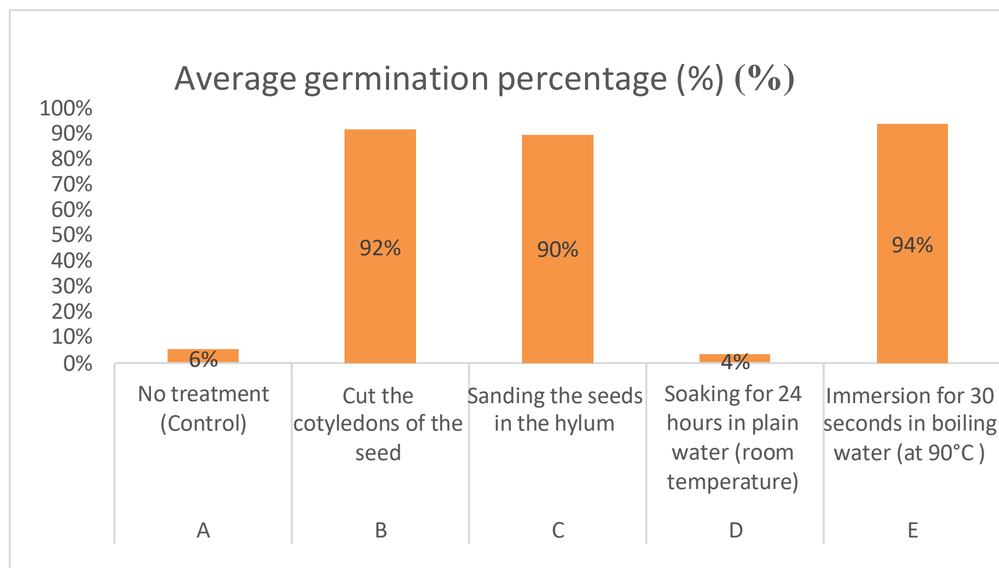

Figure 1. Average germination of *A. mangium* seeds in the graph

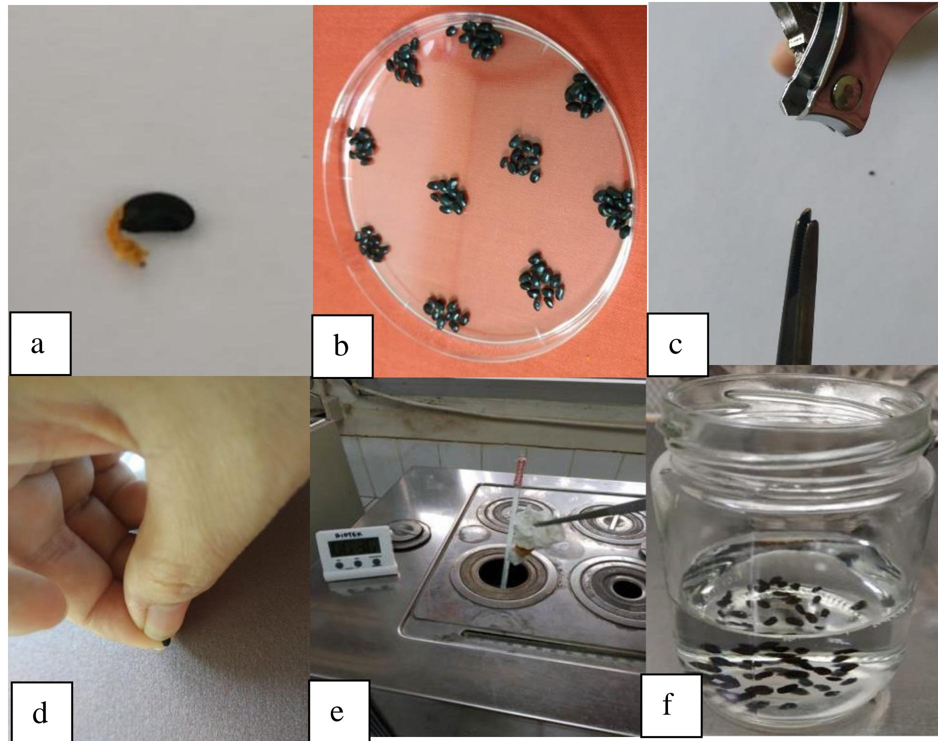

Figure 2. Treatment of A. mangium seeds a) *A. mangium* seeds with hylum, b) *A. mangium* seeds without treatment (control), c) cutting the cotyledons on *A. mangium* seeds, d) Sanding at the hylum on *A. mangium* seeds, e) immersion for 30 seconds in water at 90 ºC, f) 24 hours immersion in plain water (room temperature).

The daily germination percentage is shown in Figure 3. The graph shows that the daily germination percentage of germination reached a growing point of 0, starting from the 15th to the 20th day. The highest daily germination was in treatment E.

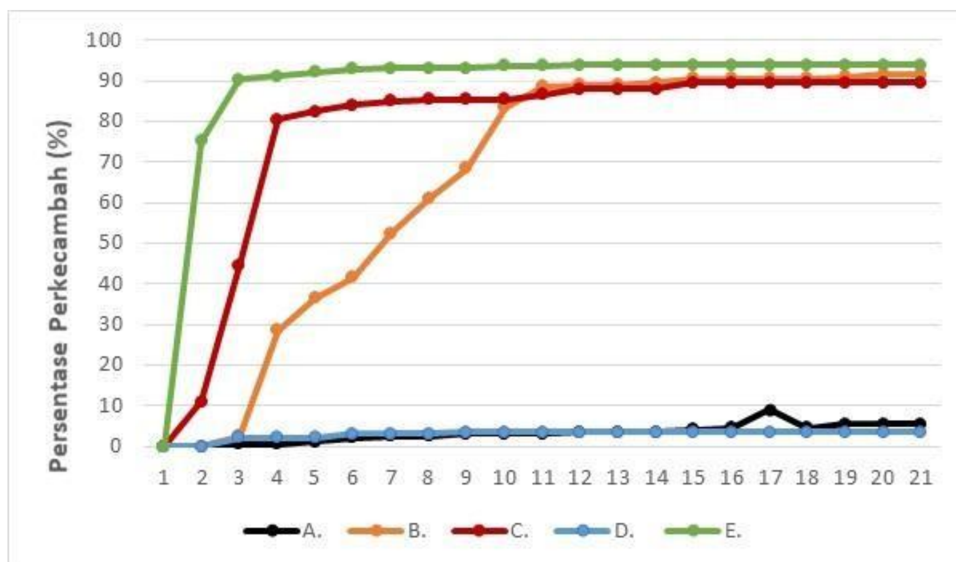

Figure 3. Graph of Daily Germination Percentage of *A. mangium* seeds

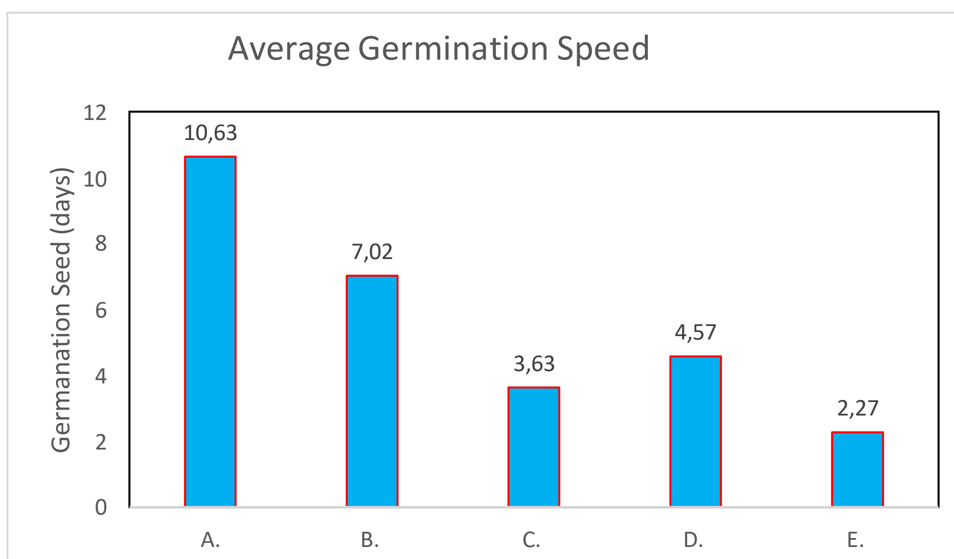

Figure 4. Average Germination Speed of *A. mangium* seeds

Seed germination speed showed that *A. mangium* seeds germinated faster in treatment E (immersion for 30 seconds in hot water at 90°C) of 2.27/days (Figure 4). The speed of germination showed that treatment with boiling water caused the seed coat to be softer and helped the process of imbibition and the exchange of O_2 either through the skin wall, micropyle or hilum of seeds that had the potential for seed germination. According to Kusuma *et. al.* (2019), that boiling water can widen the size of the seed coat pores through tension which facilitates the process of water absorption into the seeds. Fast germination also occurred in treatment C (sanding of seeds in the hylum) of 3.63/day. The speed of germination growth on sanding was caused by the thinning of the skin on the seeds of A. mangium, causing faster germination compared to the control treatment and soaking for 24 hours. This indicates that the imbibition or absorption of water by *A. mangium* seeds becomes faster so that germination growth is faster and more common (Yousif *et. al.* 2019).

Tabel 5. Recapitulation of average PV, MDG and NP for each treatment

Perlakuan	PV (% hari)	MDG (% hari)	NP (% hari)
A	0,95	0,02	0,019
B	0,76	0,41	0,31
C	0,76	0,39	0,39
D	0,47	0,01	0,0047
E	0,61	1,05	0,64

Note: NP (germination value), PV (peak germination value), MDG = average daily germination value.

The germination value means the percentage of seeds that germinate per day, so it has a relationship with the germination rate. If the germination rate only shows the average number of days to germinate, then the germination value shows the number of seeds germinating in percent per day until the end of the test which is a reflection of growth strength under non-optimal conditions (Kusuma et.al. 2019). Based on Table 5 shows that the best germination value is the germination value produced by seeds with treatment E, while the lowest is seeds without treatment A (control) which is 0.0047% / day.

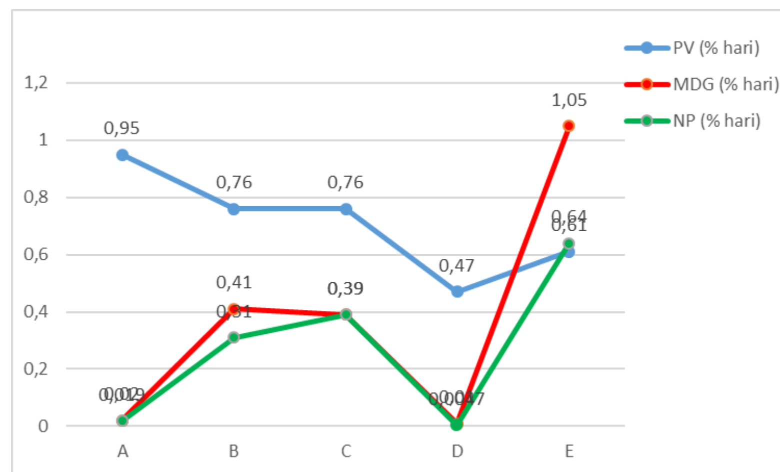

Figure 5. Germination value of *A. mangium*

The percentage value of daily germination of seeds soaked in room temperature water for 24 hours is smaller than without treatment because seeds soaked in room temperature water may not get enough water supply to stimulate the germination process, while seeds using treatment germinate faster (Figure 5). Scarification is one of the initial treatments for seeds aimed at breaking dormancy and accelerating seed germination (Dharma *et. al.* 2015).

The difference in the value of seed germination without treatment with seeds treated with soaking for 24 hours in ordinary water resulted in lower values because imbibition could occur but the radicle could not split or penetrate the cover. Seeds that used pre-treatment were faster and germinated by immersing them for 30 seconds in boiling water (at 90°C). According to Kusuma *et. al.* (2019) that in order to increase the rate and uniformity of seed germination, pre-germination treatment is necessary which is determined by seed quality or vigor and ability to germinate, treatment for breaking dormancy and germination conditions such as water, temperature, media, light and free from pests and diseases.

Table 6. Explant sterilization results at 4 MST

Observation parameters	Treatment (%)			
	1	2	3	4
Contamination	60.71	40.48	5.95	4.76
Bacteri	47.62	34.52	4.76	4.76
Fungi	13.10	5.95	1.19	0.00
Dead	17.86	32.14	9.52	33.33
Sterile	21.43	27.38	84.52	61.91

The results of the comparison of sterilization methods for *A. mangium* explants are presented in Figure 6. The results of the sterilization of seed explants in 4 treatments showed different results for each treatment.

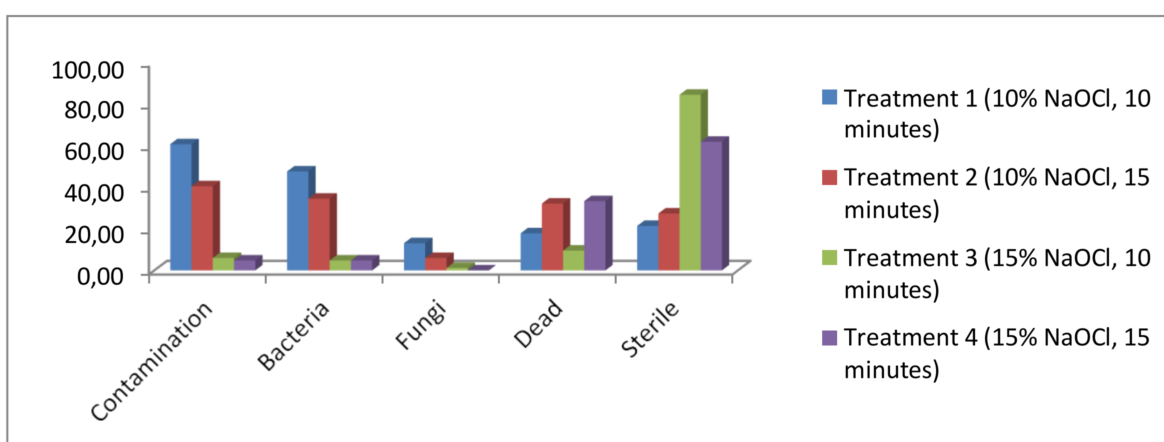

Figure 6. Effect of sterilization method on sterile *A. mangium* explants produced at 4 WAP.

The explant immersion method with 5.25% NaOCl solution at 15% concentration for 10 and 15 minutes was able to produce high sterile explants, but there were sterile explants that died. The percentage of sterile explants obtained ranged from 21.43% to 84.52% (Table 6). The explant immersion method with 5.25% NaOCl solution at 15% concentration for 10 minutes was able to produce 84.52% sterile explants (Figure 6). Therefore, the effectiveness of the best sterilization method chosen was immersion of the explants in a 5.25% NaOCl solution at a concentration of 15% for 10 minutes. The highest contamination in treatment 1 was 60.71%, the lowest contamination in treatment 4 was 4.76% but the explants died of 33.33%. In principle, explant sterilization is to sterilize from microorganism contamination, but without killing the explants. The presence of contamination in explants was caused by microorganisms such as fungi and bacteria. The time of appearance of contaminants in explants varies, but generally

occurs 2-3 weeks after planting. The highest contamination in this study was caused by bacteria by 47.62%, while contamination by fungi occurred by 13.10% (Figure 6).

Activities in tissue culture begin with the explant sterilization stage, the explant sterilization process is carried out to remove unwanted sources of contaminants with minimal disturbance to the explants. Based on the concentration and time of immersion of explants in 5.25% NaOCl solution, the method of immersing explants in 5.25% NaOCl solution at 15% concentration for 10 minutes (treatment 3) was better because it was able to produce sterile explants that were stable at 4 WAP and low explant mortality (9.52 %) and contamination (5.95%). These conditions indicate that the right concentration of sterile material and the selection of the length of time the explant is exposed to the sterilant can minimize the damage to the explant and maintain the chance of survival of the explant. The presence of contaminants in the sterilized explants was thought to be caused by a less than optimal sterilization process. In cultured explants of *A. mangium,* contamination still occurred with the highest percentage of contamination caused by bacteria of 47.62%. Contamination consists of two types, namely internal contamination and external contamination. Sources of internal contamination in explants can be caused by contaminants originating from within the plant tissue so that it cannot be removed only by surface sterilization, such as the presence of endophytic bacteria in plant tissue. Internal contamination is more difficult to treat because the contaminants are resistant to the sterilizing agents commonly used in external sterilization. Endophytic microbes that cause internal contamination can inhibit and even kill explants (Sinha *et al*. 2016). These endophytic microbes are able to survive several cycles of in vitro culture without showing symptoms or signs that can be seen in the growth media. According to Kekuda (2016), in the genus Acacia there are several species of endophytic bacteria Actinomycetes, namely *Streptomyces, Actinoallomurus, Amycolatopsis, Kribbella* and *Microbispora*. Wolf research (2007) showed that the bacteria that often appear in in vitro culture are *Agrobacterium, Bacillus, Corynebacterium, Enterobacter, Lactobacillus, Pseudomonas*, *Staphylococcus*, and *Xanthomonas*. The absorption of nutrients from the media by explants will encourage endophytic bacteria in plant tissues to be carried out and develop in the media. In vitro environmental conditions with large amounts of nutrients and sucrose, high humidity, and warm temperatures can encourage the growth of microorganisms quickly, so that it will interfere with the growth and development of explants and cause death.

According to Old *et al*. (1997) there are 11 types of fungi that commonly attack *A. mangium* in the field. The eleven fungi cause black spots on the leaves and also grow at the base of the tree. The eleven fungi were *Altenaria alternata, Atelocauda digitata, Cercospora sp., Colletotrichum gloeosporoides, Curvularia geniculata, Ganoderma shalceu, Glomerella cingulata, Nigrospora sphaerica, Oidium sp., Pestaloptiosis sp., and Phomopsis sp*. According to Cassells *et al.* (2003), fungal contamination in in vitro cultures of woody plants came from several species, namely *Penicillium, Aspergillus,* and *Fusarium.* According to Damajanti *et al*. (2015), fungi that attack in vitro cultures are *Mucor* and *Rhizopus* species with morphological characteristics in the form of hyphae shaped like white to black-gray threads, with sporangium visible on the explants. *A. mangium* is a plant that is prone to pests and diseases, especially those caused by fungi. One example of an attack caused by Ganoderma is in the second generation of Industrial Plantation Forests (HTI) (Sulendra *et al*. 2017).

A. mangium explants induced into the treatment medium showed a response in the form of shoot emergence (Table 7). Based on observations, almost all treatments were able to produce sprouted explants. The fastest shoot emergence started at 7 days, and the longest shoot emergence occurred at 18 days. Table 4 showed that the number of shoots produced, the highest number of shoots of 7 shoots occurred on MS media with treatment F (BAP 1 mg/L and IAA 0.1 mg/L).

Table 7. Effect of BAP and NAA on induction medium (MS) on *A. mangium* explants at 8 WAP.

Treatment	Span of budding time (DAP)	Range of shoots	Increase in shoot height (mm)
A	16-18	1	4,3
B	7-10	1-2	6
C	7-10	2-3	5
D	7-10	3-6	12
E	12-18	1	6,7
F	7-14	2-7	16
G	7-14	3-5	7,3
H	7-14	3	13,7
I	12-14	1	4
J	7-14	2-6	12,3
K	7-14	2-3	7
L	7-10	3-4	5,6

The tissue culture propagation technique applied in this study was carried out through seed culture of *A. mangium*. The media and the type of explant used, the addition of PGR also affected the success of tissue culture. The transfer of sterile explants from the initiation to the induction medium with the addition of PGR was aimed at multiplying or doubling the shoots. Explants of A. mangium in control MS medium and treatment with 0.1 mg/L and 0.2 mg/L NAA produced shoots. This condition shows that physiologically the endogenous cytokinin content in explants is very high. The provision of higher BAP than NAA can encourage shoot formation. According to Hendaryono and Wijayani (2012) stated that shoot formation will occur when the concentration of cytokinins is higher than auxin. The balance between auxin and cytokinin is very important in inducing shoots because each of these substances has a role in inducing shoots. Media with NAA (0.1−0.2 mg/L) produced almost the same percentage of shoots and number of shoots. This condition can occur because the balance of BAP and NAA concentrations has not been achieved which is added to the media to produce shoots in A. mangium explants. In addition to the emergence of shoots, the increase in shoot height in cultured explants can show the potential for morphogenesis and the adaptability of plants to their environment, as well as the growth capacity of explants (Prihartini 2004). Auxins have a role in cell elongation, while cytokinins play a role in cell division. In this study, the combination of cytokinins (BAP 0, 1, 2, 3 mg/L) and auxin (NAA 0, 0.1−0.2 mg/L) used resulted in fluctuating height growth. In MS media with F treatment which was able to produce the best increase in shoot height and a wider range of shoots. When the BAP concentration was increased to 1 mg/L, there was a decrease in shoot height. In *Gyrinops versteegii* micropropagation, the same condition occurred because the incubation period was too long in media containing cytokinins, causing inhibition of shoot elongation (Akbar *et. al*. 2017). Induction of mangosteen seeds resulted in short shoot growth with the use of too high BAP of 10 mg/L (Harahap *et. al.* 2014). In addition, in some types of slow growing plants, the nature of plant growth can affect the success of in vitro culture (Yelnitis and Joni 2015).

The results of the analysis of the number of shoots of *A. mangium* at the age of 8 WAP showed that the PGR type, PGR concentration and their interaction had a significant effect on the increase in explant height. Figure 7 shows that treatment of 0 mg/L MS BAP media with NAA at various concentrations (0, 0.1, 0.2 mg/L) resulted in an increase in shoot height. The best treatment in producing an increase in shoot height occurred in treatment F (BAP 1 mg/L and NAA 0.2 mg/L) with a value of 16 mm. Treatment of MS BAP 0 mg/L media combined with NAA at various concentrations (0, 0.1, 0.2 mg/L)

resulted in an increase in shoot height which decreased with the addition of NAA concentration. Plantlet *A. mangium* tends to decrease with increasing concentration of NAA, because Auxin in low concentrations will stimulate cell enlargement and elongation after cell division stimulated by cytokinins. But if the concentration of auxin used is too high, it will cause inhibition of cell elongation. The higher the concentration of auxin, the higher the concentration of ethylene produced, this will cause inhibition of auxin activity in cell elongation, but will increase cell dilation. A. superior mangium needs to be carried out tree breeding activities which are usually carried out at the age of 10 years or more. However, as the age of the parent tree increases, the success of explant induction will decrease.

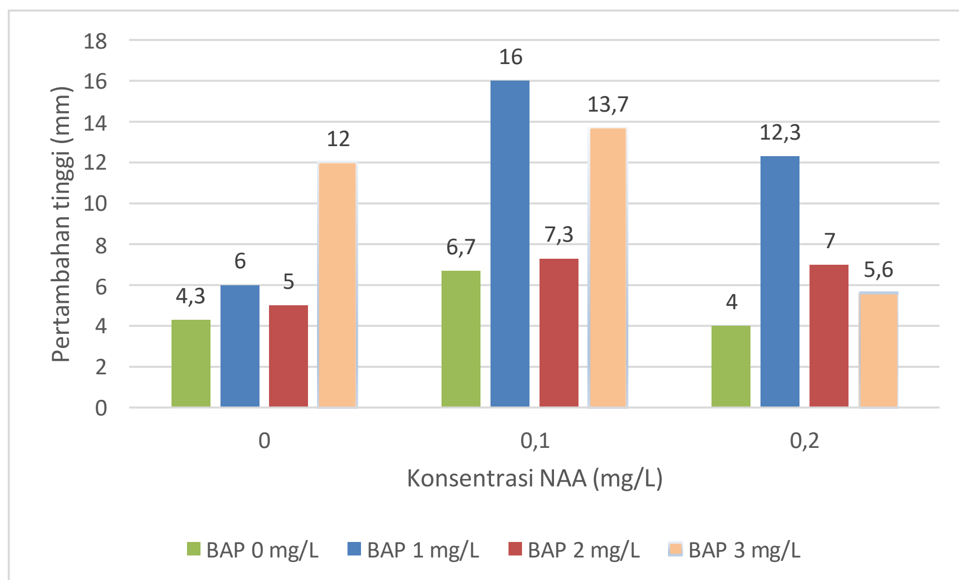

Figure 7. Increase in height of *A. mangium* explants in MS medium with a combination of BAP (0, 1, 2, 3 mg/L) and NAA (0, 0.1, 0.2 mg/L) at 8 WAP.

4.4. Seedling growth of *A. mangium* generation M2

Table 8. Growth of seedlings of *A. mangium* generation M2 in the greenhouse and the field at the age of 4 months

Location	Height (cm)	Diameter(mm)	Number of leaves (fruit)
Field	23.5 a	2.3 a	10 a
Greenhouse	19.8 b	1.7 b	10 a

Note: the numbers followed by the same letter in the same column show no significant difference at the 95% confidence level

Table 8 shows the growth in height and diameter of *A. mangium* M2 generation in the field was better than in the Greenhouse. This indicates that the growth of *A. mangium* generation M2 requires a higher intensity of sunlight for its photosynthetic needs, even though the number of leaves is the same. These seedlings must be maintained to be ready for use for propagation through shoot cuttings to produce clones of *A. mangium* generation M2 from trees.M1.13. *A. mangium* seedlings are usually kept in the nursery for 12 weeks or until they reach a height of 25–40 cm to be ready for planting in the field (Krisnawati et al. 2011). Srivastava (1993) recommends two pruning of roots and hardening of seedlings (adaptation of seedlings to full sun) before field planting.

In this study, the M2 generation *A. mangium* seedlings were maintained by weeding, fertilizing, and watering. The fertilizer used is NPK 16:16:16 given 1 gram per seed. Fertilizer is dissolved in clean water and applied by spraying 2 times a month, until the age of 11 weeks, then given NPK 16:16:16 granular fertilizer. Seedlings are maintained until materials can be obtained for the needs of shoot cuttings from seedlings. At the age of 15 weeks, the cutting material has begun to be used for shoot cuttings.

4.5. Seedling Cutting Technique *A. mangium* generation M2

Observation The percentage of survival of cuttings propagation using seed material from the greenhouse was carried out in October 2021. Interim observations showed, at 2 WAP (Sunday after planting) primordia appeared which would later grow into root cells. Assessment of root growth can be done perfectly at 8 WAP, so the assessment can't be done thoroughly. However, temporary observations at 4 WAP have shown signs of root growth, further presented in Table 9.

Table 9. Number of live shoot cuttings, callused and rooted Age 4 MST material from green-house.

Type of planting media	Number of cuttings planted	Live cuttings	Callus cuttings	Rooted cuttings
HC (100%)	3	1	1	0
C (100%)	3	3	3	0
HC (50%) + C (50%)	3	3	1	1
HC (70%) : C (30%)	3	1	0	0

Note: HC = husk charcoal, C = cocopeat

Propagation of shoot cuttings using seed material from the field can be observed as temporary growth at 3 WAP. At this time there is no visible sign of root growth such as callus/root primordia (Tabel 10).

Table 10. Number of live shoot cuttings, callused and rooted Age 3 WAP the material came from field

Type of planting media	Number of cuttings planted	Live cuttings	Callus cuttings	Rooted cuttings
HC (100%)	4	3	0	0
C (100%)	8	7	0	0
HC (50%) + C (50%)	7	7	0	0
HC (70%) : C (30%)	6	6	0	0

Note: HC = husk charcoal, C = cocopeat

4.6. Technique for shoot cuttings from old trees

Planting of shoot cuttings from old *A. mangium* tree material was carried out during the period from September to November 2021. The limited number of cuttings in the form of juvenile shoots made it impossible to plant in large numbers, so the planting was carried out in stages and each stage was evaluated from the previous planting. The evaluation was carried out regarding the use of growth media and hormone applications.

Table 11. Effect of growing media on the percentage of live cuttings and rooted cuttings

experiment	Type of planting media	Number of cuttings planted	End of Observation	Live percentage (%)	Cutting percentage
1	C: HC: Co 1:1:1	28	9 MST	0 (0%)	0 (0%)
2	C : HC 1:1	30	12 MST	3 (10%)	1 (3%)
3	C 100% A	26	10 MST	7 (27%)	1 (4%)
4	C 100% B	43	9 MST	8 (19%)	2 (5%)
5	C 100% + Z	40	4 MST	24 (60%)	0 (0%)

Note: C= cocopeat, HC = husk charcoal, Co = compost, Z= Zeolite

At the end of the observations at each stage of planting, the survival rate of cuttings looks very low. Cuttings were judged to be rotten/dead after blackened stems and phyllodia/false leaves fell. Many cuttings material rotted before rooting. Planting 210 cuttings during the experiment, only 2% of cuttings (3 pieces) were successfully rooted. Rooting occurred at 7, 8, and 9 MST. The type of cutting material that comes from old

trees is suspected to be the failure of the cuttings to take root. According to Lyon *et al.* (1997) *A. mangium* from the age of 1-3 years old, shoot cuttings are generally rooted at 6 WAP, 3 WAP, up to 6 WAP. After 6 MST the cuttings will die or take root. This shows that the rooted cuttings in this study were rooted too late.

The cutting material used in the study came from a 13-year-old mother tree, this is also suspected to be the cause of the low percentage of rooted cuttings. According to Husen (2006) in teak, the percentage of rooting on cuttings decreases with the increasing age of the parent tree from 2 months, 15 years to 30 years.

Figure 8. Shoot cuttings of M1 generation *A.mangium* from old trees that have successfully rooted

4.7. Graft technique

The failure of the cutting technique of *A. mangium* generation M1.13 from old trees needs to be solved by developing an air layering technique to provide plant propagation material both by cuttings and tissue culture techniques. Another advantage of this grafting technique is that it can produce superior clones that are more juvenile. Plant propagation by vegetative means has the advantage of being able to fully inherit the growth characteristics of its parents. The grafting technique can be used to obtain superior saplings from selected plus trees to build a clone bank. In this study, the grafting technique used is still exploratory, with the target of looking for opportunities to get rooted grafts first as consideration for the development of further grafting techniques. The trees used for the graft study had diameters calculated at breast height, namely 35 cm (tree 1), 31.4 cm (tree 2), and 4.6 cm (tree 3). The grafting medium used was a mixture of soil, cocopeat, and compost.

Figure 9. Successful rooted *A. mangium* from graft

The results of grafting of *A. mangium* generation M1 on three trees can be seen in table 12. Table 12 shows that only tree number 3 produced rooted grafts, while trees No.1 and No.2 did not produce roots. It is presumed that trees No.3 is seedling of *A. mangium* generation M1 or as *A. mangium* generation M2. The grafts from trees No.1 and No.2 were all failed to rooting and died. The diameters of the trunks of parent trees 1 and 2 were much larger and the trees were quite old than trees 3 which had successfully taken root. The diameter of the tree is too large and the age of the tree which is much older than tree 3 is thought to have caused the failure to take root in trees 1 and 2.

Table 12 Graft results of *A. mangium* generation M1

Number	Rod diameter(cm)	Skin thickness(mm)	Number of roots (fruit)
1.1	1.6	0.6	0
1.2	1.4	0.8	0
1.3	1.8	1.1	0
Average	1.6	0.8	0
2.1	0.6	0.9	0
2.2	1.1	1.0	0
2.3	1.2	0.6	0
Average	1.0	0.8	0
3.1	0.7	0.4	0
3.2	1.2	0.8	8
3.3	1.2	1.0	4
3.4	1.5	1.1	2
3.5	1.2	0.8	1
3.6	2.5	1.1	9
3.7	1.3	1.0	2
Average	1.7	1.0	4

Figure 10: M1 generation *A. mangium* graft for cutting material source

The grafts from old trees are currently being bred by planting them on a fertile growing medium so that they can be used as pruning gardens for cuttings or tissue culture materials. It is important for the rejuvenation of plant material for the following reasons:

1. Old plant materials are generally able to bring out their genetic potential, namely growing fast, and having a high volume, for example in tree number M1.13, but it is very difficult to propagate by cuttings.
2. If the cutting material can be rejuvenated, it will be easier to take plant material for cuttings or tissue culture because there is no need to climb
3. Maintenance of old tree grafts that have been rejuvenated will be easier than maintaining old trees to obtain cutting material.

5. CONCLUSION

Characteristics of *A. mangium* seeds that have a hard outer skin surface, so they require pre-treatment to increase their germination. This is evidenced by the seed germination test. Of all the treatments given to *A. mangium* seeds, it showed that the treatment by immersion in hot water 90 ^{0}C for 30 seconds, scissors, and sandpaper was a very efficient treatment for acacia seed germination, with an average germination value of 94%, 92%, and 90%. Germination of *A. mangium* seeds in treatments B, C and E was above 80%. This is categorized as high seed germination, thus proving that *A. mangium* seeds are not sterile. Germination growth of *A. mangium* seeds carried out in each treatment reached a growth point of 0 on the 15th to the 20th day. The highest germination rate was in treatments E and C. The highest germination value was the germination value produced by seeds with treatment E (0.64%/day), while the lowest was in treatment D (0.0047%/day). The effectiveness of the best sterilization method was explants immersion with treatment 3 (5.25% NaOCl solution 15% concentration for 10 minutes) capable of producing 84.52% sterile explants. The results of the micropropagation study showed that the best medium for *A. mangium* explants were propagated by tissue culture in MS medium with the addition of 1 mg/L BAP and 0.1 mg/L NAA (treatment F), because this treatment was able to produce the best explants, shoot number, and shoot height increase.
Propagation of shoot cuttings using material from old trees (M1) and from germinated seeds (M2) can produce cuttings that are capable of rooting, cuttings from seedlings germinated M2 show signs of root growth. Meanwhile, adult tree cuttings have difficulty because the cells have aged so that the percentage of rooting on the cuttings decreases with the age of the tree.

6. PERSONAL INVESTIGATOR AND RESEARCHER

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3. Aditya Pratama (Member)

7. REFERENCES

Adinugraha HA, Pudjiono S, Herawan T. 2007. Teknik Perbanyakan Vegetatif Jenim444s Tanaman *Acacia mangium.* Info Teknis. Vol. 5(2).

Akshatha KR, Chandrashekar HM, Somashekarappa J. Souframanien. 2013. Effect of gamma irradiation on germination, growth, and biochemical parameters of *Terminalia arjuna* Roxb. Radiation Protection and Environment Radiation Protection and Environment. Vol 36 (1):38-44.

Akbar A, Faridah E, Indrioko S, Herawan T. 2017. Induksi Tunas, Multiplikasi dan Perakaran *Gyrinops versteegii* (Gilg.) Domke Secara *In Vitro*. J. Pemul. Tan. Hut. 11(1): 155 - 168.

Agustiani El. 2016. Respon Pertumbuhan Tunas Saninten (*Castanopsis Argentea* (Blume) A.Dc.) Terhadap Pemberian Zat Pengatur Tumbuh BAP dan IAA Secara *In Vitro* [Skripsi]. Bogor (ID): Institut Pertanian Bogor.

Bachega LR., Bouillet JP, Piccolo DC, Saint-André L, Bouvet JM., Nouvellon Y, Gonçalves, José L. de Moraes, Robin, Agnès, Laclau JP. 2016. Decomposition of Eucalyptus grandis and Acacia mangium leaves and fine roots in tropical conditions did not meet the Home Field Advantage hypothesis. *Forest Ecology and Management, 359, 33–43.*

Browning B. 1967. Methods of Wood Chymestry Vol.1. New York (UK): Interscience Publ.

Chauhan R, Jha SK. 2018. Genetic Stable Mass Propagation of *Acacia mangium* Wild. from Mature Plus Tree. *Indian Journal of Biotechnology,* 17: 128-133.

Elbasheer YHA, Osman EE. 2017. Effective and economical explants surface sterilization protocol for microbial contamination of field grown explants in vitro cultures of some forest trees; Acacia senegal as a model. *Basic Research Journal of Microbiology*, *4*(2), 12–17. Retrieved fromhttp://basicresearchjournals.org/microbiology/abstract/Elbadri and Yahia.html.

Dharma IPES, Samudin S, Adrianton. 2015. Perkecambahan benih pala (Myrista fragrans Houtt) dengan metode skarifikasi dan perendaman ZPT alami. e-Jurnal Agrotekbis. 3(2): 158-167.

Elfarisna E, Niaga H, Puspitasari RT. 2016. Toleransi Tanaman Akasia (Acacia Mangium Wild.) terhadap Tingkat Salinitas di Pembibitan. *Daun: J. Ilmiah Pertanian dan*

Kehutanan. *3*(2), 54-62.

Elfianis R, Hartina S, Permanasari I, Handoko J. 2019. Pengaruh skarifikasi dan hormon giberelin (GA3) terhadap daya kecambah dan pertumbuhan bibit palem putri (Veitchia merillii). *J. Agrotek. 10*(1), 41-48.

Elfiani E, Jakoni J. 2015. Pengujian daya berkecambah benih dan evaluasi struktur kecambah benih. *Dinamika Pertanian*, *30*(1), 45-52.

Gantait S, Kundu S, Das PK. 2018. Acacia: An exclusive survey on in vitro propagation. *Journal of the Saudi Society of Agricultural Sciences*, 17 (2): 163-177.

Girijashankar V. 2011. Micropropagation of multipurpose medicinal tree *Acacia auriculiformis*. *J. Med. Plant Res*. 5: 462–466.

Gijarto SI. 2008. Pertumbuhan Bibit *Acacia mangium* Dart Biji Yang Diradiasi Dengan Sinar Gamma. [Skripsi]. Bogor (ID): Institut Pertanian Bogor.

Harahap F, Poerwanto R, Suharsono, Suriani C, Rahayu S. 2014. *In vitro* and rooting of mangosteen (*Garcinia mangostana* L.) on medium with different concentrations of plant growth regulator. *Hayati J Bioscience* 21(4):151-158.

Ismail, Syafikah F, Malahubban, Masnindah., Sajili, Mohammad Hailmi, Aziz A, Fitri Z. 2016. Plant Growth-promoting Properties of Cultivable Endophytic Root Nodule Bacterial Isolates from *Acacia mangium* Willd. *Research in Plant Biology*, 6:14-18.

[ISTA] International Seed Testing Association. 2014. International Rules for Seed Testing.Switzerland (CH): ISTA.

Kekuda TRP. 2016. Isolation, Characterization and Antimicrobial Potential of Endophytic Actinomycetes. International Journal of Current Microbiology Applied Sciences 5(7):100-16.

Krisnawati H, Kallio M, Kanninen M. 2011. *Acacia mangium* Willd. Ekologi, Silvikultur dan Produktivitas. CIFOR, Bogor, Indonesia.

Kusuma M, Payung D, Rahmawati N. 2019. Uji Daya Kecambah Benih Akasia (Acacia Mangium Willd) Di Desa Teluk Kepayang Kecamatan Kusan Hulu Kabupaten Tanah Bumbukalimantan Selatan. J Sylva Scienteae 02:1.

Le DK, Ha HT. 2017. Research and development of acacia hybrids for commercial planting in Vietnam. *Vietnam Journal of Science,Technology and Engineering*, 59 (1) : 36-42.

Mao P, Guo L, Gao Y, Qi L, Cao B. 2019. Effects of seed size and sand burial on germination and early growth of seedlings for coastal Pinus thunbergii Parl. in the

Northern Shandong Peninsula, China. Forests, 10(3).https://doi.org/10.3390/f10030281

Murrinie ED, Yudono P, Purwantoro A, Sulistyaningsih E. 2017. Dentifikasi Sifat Benih Kawista (*Feronia Limonia* (L.) Swingle) Untuk Tujuan Penyimpanan. Prosiding SNATIF. 502-516.

Masson A, Monteuuis O. 2017. Rubber tree clonal plantations: grafted vs self-rooted plant material. Bois Et Forest Des Tropiques. 332(2).

Nurhasbi, Sudrajat DJ, Aisyah PS. 2008. Penentuan Kriteria Kecambah Normal Yang Berkorelasi Dengan Vigor Bibit Tusam (*Pinus merkusii* Jungh et de Vriese). Jurnal Penelitian Hasil Hutan. Vol.5(1):1-11.

Nurmiaty Y, Ermawati, Purnamasari VW. 2014. Pengaruh Cara Skarifikasi Dalam Pematahan Dormansi Pada Viabilitas Benih Saga Manis (*Abrus precatorius* [L.]). J. Agrotek Tropika. Vol. 2(1): 73 – 77.

Old KM, See SS, Sharma JK. 1997. Diseases of Tropical Acacias. Jakarta (ID): CIFOR.

Paula RR, Bouillet JP, de M. Gonçalves JL, Trivelin PCO, de C Balieiro F, Nouvellon Y, Oliveira, Julianne de C, Júnior, José C. de Deus., Bordron, Bruno., Laclau, Jean-Paul. 2018. Nitrogen fixation rate of *Acacia mangium* Wild at mid rotation in Brazilis higher in mixed plantations with *Eucalyptus grandis* Hill ex Maidenthan in monocultures. *Forest Science,* 75(1): 1-14.

Purba HWS, Sitepu FE., Haryati. 2013. Viabilitas benih rosella (*Hibiscus sabdarifa* L.) pada berbagai kadar air awal dan kemasan benih. J Online Agrotek1(2): 318-326.

Rulliaty S. 2008. Karakteristik Kayu Muda Pada Mangium (*Acacia Mangium* Willd.) dan Kualitas Pengeringannya. The Center for Forest Products Research and Development Center, Bogor.

Saikia M, Shrivastava K, Sureshkumar SS. 2012. An Efficient Protocol for Callus Induction in *Aquilaria malaccensis* Lam. Using Leaf Explants at Varied Concentrations of Sucrose. International Journal of Plant Research 2(6): 188-194. DOI: 10.5923/j.plant.20120206.03.

Sharma SK, Kumar P, Rao RV, Sujatha M, Shukla, SR. 2011. Rational utilization of plantation grown Acacia mangium Willd. *Journal of the Indian Academy of Wood Science*, 8 (2): 97–99.

Shu QY, Forster BP, Nakagawa H. 2012. Plant Mutation Breeding and Biotechnology. FAO. Guitenberg Press Ltd.

Sinha K, Deka AC. 2016. Effect of Osmotoc Stress on *In Vitro* Propagation of *Musa* sp. (Malbhog variety). African Journal of Biotechnology 15(12):465-71.

Sunarti S, Putri AI. 2009. Inisiasi Tunas Acacia Hibrid (*Acacia mangium* & *A. auriculiformis*) Secara *In Vitro* Jurnal Pemuliaan hutan 3(1): 11-21.

Sugiyarto L, Kuswandi PC. *2013*. Eksplorasi Metode Sterilisasi dan Macam Media Untuk Perbanyakan Durian (*Durio zibethinus*, L.) Secara *In Vitro.* J. Sains Dasar 2(1) 20 – 24.

Sulendra S, *Suryantini R, Wulandari*. 2017. Ketahanan Semai Akasia (*Acacia mangium*) Pada Variasi Umur Terhadap Infeksi Ganoderma Spp. J. Hut. Les. Vol. 5(3): 653 – 658.

[TAPPI] Technical Association of Pulp and Paper Industry. 1996. TAPPI Test Methods. Atlanta (US): TAPPI Press.

Wolf JB. 2015. Tissue Culture Methods. Baltimore (US): University of Maryland.

Yousif MAI, Wang YR, Hu XW. 2019. Seed dormancy and dormancy breaking of selected Acacia species from Sub-Saharan Africa. Seed Sci. and Tech. 47(2):131-144. https://doi.org/10.15258/sst.2019.47.2.02.

Yuan X, Wen B. 2018. Seed Germination Response to High Temperature and Water Stress in Three Invasive Asteraceae Weeds from Xishuangbanna, SW China. J. Plos One. https://doi.org/10.1371/journal.pone.0191710

Yunus EP, Supriyanto, Siregar IZ, Tjirosemito S, Mawardi I. 2017. Keragaan Pertumbuhan dan Keragaman Genetik *Acacia Mangium* Willd. Umur 7 Tahun Hasil Iradiasi Sinar Gamma (Generasi M1). Jurnal Silvikultur Tropika 08(2): 88-95.

Yuniarti N. 2013. Pengaruh Metode Ekstraksi dan Ukuran Benih Terhadap Mutu Fisik - Fisiologi Benih Acacia Crassicarpa. *Jurnal Penelitian Hutan Tanaman* , 129 - 137.

APPENDIX