



The Effects of IBA (Indole butyric acid) Hormone on the Growth of *Leucaena leucocephala* Acid Tolerant Mutant in Tissue Culture Technique

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ABSTRAK

Leucaena leucocephala merupakan tanaman pakan ternak jenis leguminosa yang memiliki nutrisi baik untuk ternak terutama kandungan protein. Pembibitan tanaman hijauan merupakan salah satu strategi untuk meningkatkan pasokan pakan dari segi kualitas, kuantitas dan kontinuitas. Kultur jaringan merupakan teknik untuk memperoleh tanaman pakan yang seragam dalam waktunya relatif cepat dibandingkan cara konvensional. Benih yang dihasilkan dapat beradaptasi dengan berbagai cekaman lingkungan seperti kondisi tanah masam dan kering serta tingkat salinitas yang tinggi untuk menghasilkan benih mutan yang unggul. IBA merupakan salah satu jenis auksin yang dapat menginduksi perakaran pada tanaman. Penelitian ini bertujuan untuk mengetahui efektivitas hormon IBA pada tanaman pakan ternak *Leucaena leucocephala* dengan menggunakan teknik kultur jaringan. Rancangan yang digunakan dalam penelitian ini adalah rancangan acak lengkap (RAL) dengan eksplan tanaman lamtoro sebanyak 11 galur mutan yaitu galur M1-M11 (mutan+MS+1ppm IBA) dan 2 lamtoro indukan tanpa penyinaran gamma yaitu K0 (lamtoro indukan+MS+0ppm IBA), K1 (lamtoro indukan+MS+1 ppm IBA), masing masing 15 ulangan, perlakuan yang berpengaruh nyata dilanjutkan dengan uji tukey. Variabel yang diamati adalah pertambahan panjang akar, tinggi vertikal tanaman, jumlah tunas, persentase tanaman berakar. Hasilnya adalah konsentrasi hormon IBA pada 1 ppm memberikan hasil yang optimal untuk pertumbuhan mutan. Peningkatan panjang akar dan peningkatan tinggi vertikal tanaman menunjukkan hasil terbaik pada strain mutan M9, jumlah tunas menunjukkan hasil terbaik pada strain mutan M9 dan M11 dan persentase tanaman berakar tertinggi pada strain mutan M3 dan M11.

Kata kunci: IBA, kultur jaringan, *Leucaena leucocephala*, mutan.

ABSTRACT

Leucaena leucocephala is a leguminous animal feed plant that has good nutrition for livestock, especially protein. Breeding forage plants is one strategy to increase feed supply in terms of quality, quantity and continuity. Tissue culture is a technique to obtain uniform feed plants. The time is relatively fast. The resulting seeds can adapt to various environmental stresses such as acidic soil conditions and dry and high salinity levels to produce superior mutant seeds. IBA is a type of auxin that can induce rooting in plants. This study aims to determine the effectiveness of the IBA hormone in animal feed plants *Leucaena leucocephala* using tissue culture techniques. The design used a completely randomized design (CRD) with lamtoro plant explants as many as 11 mutant lines. Namely, the M1-M11 line (mutant+MS+1ppm IBA) and 2 broodstock lamtoro without gamma irradiation, namely K0 (lamtoro broodstock +MS+0ppm IBA), K1 (lamtoro broodstock + MS + 1 ppm IBA), every 15 replicates, the treatment which has a significant effect are followed by the Tukey test. Variables observed were an increase in root length, plant vertical height, number of shoots, percentage of rooted plants. The results were that the concentration of IBA hormone at 1 ppm gave optimal results for the growth of mutants. An increase in root length and increase in plant vertical height showed the best results on the M9 strain, the number of shoots showed the best results on the M9 and M11 strains and the highest percentage of rooted plants on M3 and M11 strains.

Keywords: IBA, *Leucaena leucocephala*, mutant, tissue culture.

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Pendahuluan

The nutritional content of feed plants has its proportions and advantages. Among the animal feed groups, namely gramineae and legumes, the legume group has a fairly high nutritional content. Based on its nutritional content, this plant is a source of protein, fibre, and good mineral supplementation for livestock productivity. One of the tree legume varieties is *Leucaena leucocephala*, with a high protein quality of around 15% to up to 38% (Zayed *et al.*, 2014).

The development of plant biotechnology for *Leucaena leucocephala* with tissue culture techniques (in vitro propagation) can maximize the supply of forage seeds that are uniform and have high productivity compared to conventional plant cultivation (Wattimena *et al.*, 2011). Tissue culture techniques can produce plant seeds in large quantities under controlled conditions, and the time required is relatively fast (Manapaki, 2016). Adding ZPT (*Growth regulating substances*) in tissue culture media can provide more optimal growth results. IBA (*Indole butyric acid*) is a growth regulator. IBA is a type of auxin hormone with a high ability to initiate rooting. This hormone can also synthesize the amino acid tryptophan by positively reacting to callus stimulation, cell growth, and root formation (Shofiana *et al.*, 2013).

A mutant candidate for the *Leucaena leucocephala* plant has been produced through the selection process due to 400 grey gamma-ray irradiation adapted to acid media pH 3.4 collection of the Agrostology Laboratory of the Faculty of Animal Science, IPB University. Based on this background, it is necessary to conduct further research at the multiplication stage with the addition of IBA to obtain the best mutant growth in each plant strain of *Leucaena leucocephala*.

Metode Penelitian

Research Material

The materials used as explants in this study were *Leucaena leucocephala* is adapted to acid pH 3.4. Materials obtained from the collection of the Plant Tissue Culture Laboratory, Plant and Pasture Science and Technology section, Faculty of Animal Science, Bogor Agricultural University, spirits, 70% alcohol, aquades, jelly, sugar, 2% KOH, MS (Murashige Skoog), carbon charcoal active, growth regulator IBA type (*Indole butyric acid*) is taken with a concentration of 1 ppm.

The tools used are culture bottles, aluminium foil, laminar air flow, spatula, spoon, ohr pipette, bulb, scalpel, tweezers, beaker, callipers, magnetic stirrer, pH meter, autoclave, heat-resistant plastic, petri dish, timer, bottles, bunsen, analytical scales, weighing containers, thread, scissors, tissue, refrigerated room, and stationery.

Research Procedure

Space preparation

The room temperature and the lighting of the tissue culture room are set automatically with the tool settings. The room is regulated using air conditioning at a temperature of 16°C, and lighting is regulated using fluorescent lamps for 16 hours a day which functions to carry out the photosynthesis process in plants.

Tool sterilization

Tools in the form of culture bottles, spatula, scalpel, tweezers, Petri dishes, scissors and bottles were washed with soap and then rinsed clean. After that, the tool is dried and put in a heat-resistant plastic for sterilization. The device was sterilized using an autoclave at a temperature of 121°C and a pressure of 17.5 psi for 15 minutes, then stored in a tissue culture room. Before the subculture process is carried out, it is necessary to sterilize by heating the planting tool with tweezers and scalpel on a Bunsen fire until the tool turns reddish.

Mutant preparation

The *Leucaena leucocephala* plant was obtained from the collection of the Agrostology Field Laboratory of the Faculty of Animal Science, IPB University. *Leucaena Leucocephala* mutant plants were acid-tolerant pH 3.4 and control *Leucaena leucocephala* plants with strain numbers M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11 (*Leucaena leucocephala* mutant) and K0, K1 (*Leucaena leucocephala* control).

Media creation

The media used in this study were basal MS media as control medium (K0) and MS media with 1 ppm IBA (K1, M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11). Preparation of control media consisted of MS 4.43 g.L⁻¹ and sugar 30 g.L⁻¹; after that, it was put into a beaker given 1 L of aquadest and homogenized using a magnetic stirrer. After the homogeneous solution, the pH is measured using a pH meter and conditioned at pH 5.7. If it is less than that pH, is added KOH, and if it is more than that pH, is added HCl. Next, jelly is added as much as 7 g.L⁻¹.

1 and 1 g.L-1 activated carbon charcoal into the solution; after that, it was heated to boiling using a hot plate magnetic stirrer at a speed of 250 ppm and a temperature of 380°C. Then the growth regulator IBA type was added according to the treatment. The media was put into culture bottles of 10 ml each in 195 bottles and covered with aluminium foil. Furthermore, the media was sterilized using an autoclave at a temperature of 121°C and a pressure of 17.5 psi for 15 minutes. The sterile media is stored in the tissue culture room and observed for a week; if contaminated media is not used as a planting medium.

Planting room preparation

The Laminar airflow work area is sterilized using 70% alcohol then wiped with a tissue; after that, ultraviolet (UV) light is turned on for 15-20 minutes for the sterilization process, then the blower and lights are turned on during the work process.

Multiplication

The main media used MS media with the addition of IBA type growth regulator with a level of 1 ppm in the *Leucaena leucocephala* mutant. *Tarramba* variety was tolerant to acid pH 3.4 (M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11) and control *Leucaena leucocephala* (K0 and K1), with 15 replicates for each strain. Plant explants in shoots and stems were then transferred to the treatment medium through the subculture technique in laminar airflow; each bottle consisted of 1 explant, so the total sample was 195.

Research Variable

1. Increase in Plant Root Length (mm)

The increase in root length was measured every 7 days for 5 WAP (Week After Planting). The increase was obtained by calculating the average increase in root length using a calliper.

2. Plant Vertical Height Gain (mm)

Plant height was measured using a calliper with a measurement time every 7 days for 5 WAP. Height gain was obtained by calculating the average difference in plant height in the final week with the initial week.

3. Growth of shoots (unit)

The number of shoots was measured every 7 days for 5 WAP by counting the number of shoots for each plant line.

4. Number of Rooted Plants (%)

The number of rooted plants was calculated based on the percentage divided by the total number of samples in each treatment.

Statistical Analysis

The design used in this study was a completely randomized design (CRD) with lamtoro plant explants as many as 11 mutant lines. Namely, the M1-M11 line (mutant+MS+1ppm IBA) and 2 broodstock lamtoro without gamma irradiation, namely K0 (lamtoro broodstock +MS+0ppm IBA), K1 (lamtoro broodstock + MS + 1 ppm IBA), every 15 replicates.

The data obtained from the observations were analyzed using ANOVA. If there is a significant difference between treatments, further tests are carried out using the Tukey test; data analysis is carried out using the SPSS application.

RESULTS AND DISCUSSION

Increase in Plant Root Length

Roots are one of the important factors for plant growth, and this is because roots function to absorb nutrients in the media. The sign of a rooted *Leucaena leucocephala* plant is small fibres in the media at the bottom. The increase in the length of plant roots given the hormone IBA in *Leucaena leucocephala* variety of *Tarramba* Acid tolerant is listed in Table 1.

Table 1. Increase in root length of *Leucaena leucocephala* by giving 1 ppm IBA hormone at the age of 1-5 WAP (Week After Planting).

Strain	Age (WAP)				
	1	2	3	4	5
	mm				
K0	0,00±0,00	0,00±0,00	3,70±1,60 ^{bc}	0,42±0,47 [*]	1,07±1,19 ^c
K1	0,00±0,00	0,00±0,00	4,38±2,23 ^{cd}	3,21±2,91 ^{cd}	3,02±2,82 ^{bc}
M1	0,00±0,00	0,00±0,00	5,24±1,98 ^{ab}	4,48±2,00 ^{bc}	4,20±1,99 ^{ab}
M2	0,00±0,00	0,00±0,00	2,35±1,36 ^{bc}	2,85±1,43 ^{cd}	3,20±1,53 ^{bc}
M3	0,00±0,00	0,00±0,00	3,63±2,46 ^{bc}	6,60±2,36 ^{ab}	5,37±3,42 ^{ab}
M4	0,00±0,00	0,00±0,00	5,22±2,36 ^{ab}	4,22±2,07 ^{bc}	4,63±2,39 ^{ab}
M5	0,00±0,00	0,00±0,00	3,88±2,53 ^{bc}	4,43±1,79 ^{bc}	3,68±2,37 ^{bc}
M6	0,00±0,00	0,00±0,00	1,69±2,64 ^c	1,63±1,52 ^{de}	4,72±3,13 ^{ab}
M7	0,00±0,00	0,00±0,00	3,63±3,06 ^{bc}	3,05±1,71 ^{cd}	2,99±2,88 ^{bc}
M8	0,00±0,00	0,00±0,00	3,62±4,07 ^{bc}	3,74±3,25 ^{cd}	3,02±3,11 ^{bc}
M9	0,00±0,00	0,00±0,00	7,54±2,94 [*]	7,99±2,94 [*]	5,98±1,94 ^{ab}
M10	0,00±0,00	0,00±0,00	3,76±2,54 ^{bc}	4,17±2,50 ^{bc}	7,07±4,29 ^a
M11	0,00±0,00	0,00±0,00	1,41±2,53 ^c	2,28±2,82 ^{cd}	4,24±2,12 ^{ab}

Description: IBA=Indole Butyric Acid. K0=control+MS+IBA 0 ppm, K1=control+MS+IBA 1 ppm, M1- M11= Mutant+MS+IBA 1 ppm. Different superscripts in the same column showed a significant effect (p<0.05) based on the Tukey test.

The analysis of variance in the increase in plant root length showed a significant interaction (P<0.05). In the first and second weeks, there was no root growth in all plant strains; roots began to form in the third week with the highest increase in the M9 strain with an increase of 7,54 mm and the highest in the fourth week with an increase of 7.99 mm, the fifth week the highest was in the M10 strain with an increase of 7,07 mm, this indicates that the root length

increase of the selected mutant plant was higher than that of K0. The M9 mutant line had the best root length gain with the highest average compared to other plant lines. Breeding of the M9 strain with the provision of IBA can significantly increase root growth. The M9 mutant strain is a superior plant mutant obtained from genetic engineering and is acid-tolerant at pH 3.4. According to Marga (2020) research, engineered *Leucaena Leucocephala* has a high level of genetic diversity, so the possibility of obtaining superior mutants is also higher. Giving the IBA type of auxin hormone with a concentration of 1 ppm in the growing media can stimulate root growth and increase the number and quality of roots. Following 2014 *et al.* (2013) and Zulastr *et al.* (2020) statements, IBA has activity as a rooting hormone and the fastest root formation time occurs at 1 ppm IBA treatment. The effectiveness of auxin in influencing root length is to expand cell volume by slowing down calcium pectin compounds, causing cell walls to become elastic (Nurbaeti *et al.*, 2020). The expansion of the cell volume results in the exchange of K⁺ and H⁺ ions within the cell wall, and this is done to maintain ion balance when the apical meristem elongates. When the elongation has been completed, the hormone auxin will stop its role in inhibiting the calcium pectin compound [1].

Plant Vertical Height Increase

Height gain is one of the variables that describe plant growth to see the response of plant morphology to the treatment given. The results of vertical height gain treated with IBA on *Leucaena leucocephala* Tarramba variety acid-tolerant is listed in Table 2.

Table 2. Increase in vertical height of *Leucaena leucocephala* plants with 1 ppm IBA hormone administration at 1-5 WAP.

Strain	Age (WAP)				
	1	2	3	4	5
	mm				
K0	0,61±0,60 ^b	1,18±1,53 ^{de}	0,91±1,08 ^b	1,16±1,71 ^{cd}	1,95±4,25 ^c
K1	0,57±1,06 ^b	4,61±4,49 ^{cd}	3,39±3,51 ^{ab}	2,22±2,27 ^{cd}	3,66±3,07 ^{bc}
M1	0,66±1,22 ^b	4,99±3,67 ^{ab}	3,19±4,08 ^{ab}	2,96±3,04 ^{cd}	6,52±4,62 ^{ab}
M2	1,06±1,60 ^b	5,39±4,39 ^a	1,93±2,73 ^b	1,74±2,05 ^{cd}	5,05±3,64 ^{bc}
M3	0,78±1,39 ^b	4,73±2,40 ^{bc}	6,56±3,22 ^a	5,37±2,17 ^{ab}	2,75±2,35 ^{bc}
M4	0,00±0,00 ^b	3,97±2,61 ^{de}	1,57±2,97 ^b	3,42±3,29 ^{cd}	6,28±4,71 ^{ab}
M5	0,65±1,30 ^b	5,55±3,51 ^a	4,31±2,46 ^{ab}	4,16±2,53 ^{bc}	2,87±2,45 ^{bc}
M6	1,78±2,85 ^b	4,49±2,88 ^{de}	3,29±3,53 ^{ab}	2,25±3,00 ^{cd}	1,69±2,64 ^c
M7	0,57±1,06 ^b	4,61±4,49 ^{cd}	3,39±3,51 ^{ab}	2,68±2,58 ^{cd}	3,63±3,06 ^{bc}
M8	0,80±1,71 ^b	1,43±1,99 ^{de}	3,52±3,20 ^{ab}	3,95±3,80 ^{bc}	3,62±4,07 ^{bc}
M9	0,50±0,82 ^b	0,91±1,08 ^{de}	1,16±1,71 ^b	8,22±4,74 ^a	7,54±2,94 ^a
M10	0,28±0,51 ^b	0,81±1,05 ^a	6,16±5,98 ^a	4,00±2,81 ^{bc}	3,76±2,54 ^{bc}
M11	5,17±5,90 ^a	4,00±2,81 ^{de}	3,76±2,54 ^{ab}	0,21±0,48 ^d	1,41±2,53 ^c

Description: IBA=Indole Butyric Acid. K0=control+MS+IBA 0 ppm, K1=control+MS+IBA 1 ppm, M1- M11= Mutant+MS+IBA 1 ppm. Different superscripts in the same column showed a significant effect (p<0.05) based on the Tukey test.

The analysis of variance in the vertical height increase of plants showed a significant interaction (P<0.05). In the first week, the highest increase was in the M11 mutant line with an increase of 5.17 mm, the second week the highest increase was in the M5 mutant strain with an increase of 5.55 mm, the third-highest week was the M3 mutant strain with an increase of 6.56 mm, the fourth week and the fifth week were the highest. The M9 mutant strain with the highest increase reaching 8.22 mm indicates that the vertical height increase of the selected mutant plant was higher than K0. The M9 mutant strain had the best vertical height gain and had the highest mean compared to other plant strains. The effectiveness of the hormone IBA in the growth of plant vertical height had a good effect on the growth of mutant plants of *Leucaena Leucocephala*. This research conducted by Firmansyah *et al.* (2014) states that the hormone can stimulate the formation of the apical meristem, thereby increasing the increase in plant height. According to Supriyanto *et al.* (2011) and Zayed *et al.* (2014), root growth will increase plant height, where nutrients to support plant growth are sufficient, and plants can grow optimally vertically and horizontally.

Number of Shoots

Shoots are one of the new plant organs that grow on each plant. The number of shoots is calculated based on the number of new branches that appear on the plant. The growth of shoots treated with IBA on acid-adapted *Leucaena leucocephala* plants is shown in Table 3.

Table 3. The number of *Leucaena leucocephala* plants with the administration of 1 ppm IBA hormone at 1-5 WAP.

Strain	Age (WAP)				
	1	2	3	4	5
	unit				
K0	1,00±0,00 ^c	1,87±0,35 ^c	2,93±0,26 ^c	3,00±0,00 ^a	3,20±0,41 ^c
K1	1,40±0,51 ^c	2,93±0,26 ^b	2,93±0,26 ^c	3,20±0,41 ^{de}	3,87±0,74 ^{bc}
M1	1,00±0,00 ^c	2,20±0,41 ^c	2,87±0,52 ^c	4,27±0,80 ^c	4,53±0,64 ^{ab}
M2	1,20±0,41 ^c	1,87±0,35 ^c	2,93±0,26 ^c	3,80±0,41 ^{cd}	3,87±0,35 ^{bc}
M3	1,00±0,00 ^c	1,87±0,35 ^c	2,93±0,26 ^c	3,87±0,35 ^{bc}	3,87±0,35 ^{bc}
M4	1,40±0,51 ^c	2,93±0,26 ^b	2,93±0,26 ^c	3,87±0,35 ^{bc}	3,87±0,35 ^{bc}
M5	1,40±0,51 ^c	2,27±0,46 ^c	2,93±0,26 ^c	4,07±0,59 ^b	4,07±0,59 ^b
M6	1,87±0,35 ^b	2,93±0,26 ^b	2,93±0,26 ^c	3,87±0,35 ^{bc}	3,87±0,35 ^{bc}
M7	1,87±0,35 ^b	2,93±0,26 ^b	3,87±0,35 ^b	3,87±0,35 ^{bc}	4,07±0,46 ^b
M8	1,87±0,35 ^b	2,93±0,26 ^b	3,80±0,41 ^{ab}	3,87±0,35 ^{bc}	4,00±0,53 ^b
M9	2,27±0,46 ^b	2,93±0,26 ^b	3,87±0,35 ^{ab}	5,00±0,53 ^a	5,27±0,88 ^a
M10	2,93±0,26 ^a	3,87±0,35 ^a	3,87±0,35 ^{ab}	4,47±0,74 ^{ab}	5,13±0,92 ^a
M11	2,93±0,15 ^a	3,87±0,15 ^a	4,27±0,59 ^a	5,00±0,93 ^a	5,27±0,80 ^a

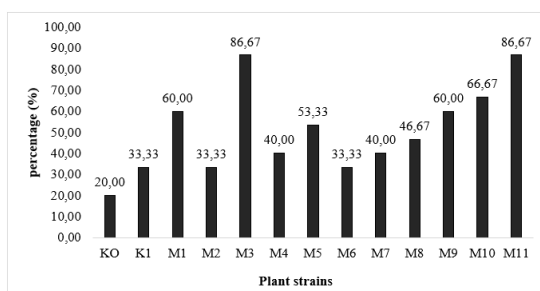
Description: IBA=Indole Butyric Acid. K0=control+MS+IBA 0 ppm, K1=control+MS+IBA 1 ppm, M1- M11= Mutant+MS+IBA 1 ppm. Different superscripts in the same column showed a significant effect (p<0.05) based on the Tukey test.

The analysis of variance in the number of plant shoots showed a significant interaction ($P < 0.05$). In the first week and second week, the highest number was in the M10 and M11 mutant strains with an average number of 2.93 units and 3.87 shoot units; the third week, the highest number was in the M11 mutant strain with an average number of 4.27 units, the fourth week and the fifth-highest was in the M9 and M11 mutant strains with the highest average number of around 5.27 units. The number of shoots of the selected mutant plants was higher than K0. Mutant strains M9 and M11 had the best number of shoots and had the highest average compared to other plant strains. The formation of shoots begins to appear in the first week after planting because the rooting hormone begins to work and is well absorbed by the plant. Based on Lestari (2011) research, the time required for explants to grow shoots ranged from 4 to 11 DAP (Days After Planting). The effectiveness of the IBA hormone in the formation of plant shoots had a good effect on *Leucaena leucocephala* plant mutants. The hormone auxin's function plays a role in regulating plant growth and development, including growth in shoots (Clan, 2020). The IBA type of auxin hormone can encourage cell extension and division, differentiation of xylem and phloem tissues (Nugroho *et al.*, 2019). The administration of IBA will encourage optimal shoot formation and root initiation (Karti *et al.*, 2019).

Percentage of Rooted Plants

The number of rooted plants was calculated based on replications that could last up to 5 weeks. The number of rooted plants can indicate the response of plants in receiving growth regulators according to their function. The results showed that the highest number of rooted plants was in the M3 and M11 mutant strains of 86.67%, while the lowest was in the K0 strain of 20.00%. Graph 1 shows the percentage yield of rooted plants that were treated with the growth effect of IBA on *Leucaena leucocephala* cv. Tarramba is acid tolerant.

Graph 1. Percentage of *Leucaena leucocephala* rooted plants with 1 ppm IBA hormone given at 5 WAP (Week After Plant).



Plant number affects plant growth so that it affects the percentage of rooted plants. Mutant plants *Leucaena leucocephala* showed different interactions in each plant strain because these interactions were influenced by varying plant responses to adaptation and interactions between genetics and hormones. This statement is in line with research conducted by (Harahap, 2012), which states that a combination of genetic and environmental factors will display plant characters. The addition of the hormone IBA concentration of 1 ppm provides optimal root growth because it follows the levels of auxin required by the *Leucaena leucocephala* plant. This research was conducted by Rinaldy (2019), which stated that the addition of 1 ppm IBA hormone in *Leucaena leucocephala* plants showed the highest percentage of rooted plants. Each plant has a different response to hormones; this is influenced by the concentration given. If the concentration is too low, the hormone will not work effectively. Meanwhile, if the concentration is too high, then the hormone will be inhibiting. Adding auxin to plants as a growth regulator can increase plant development by affecting membrane proteins that can accelerate protein and nucleic acid synthesis (Izudin, 2013); adding auxin also affects new roots formation (Firmansyah *et al.*, 2014).

Kesimpulan

Based on the research that has been done, it can be concluded that the IBA hormone concentration of 1 ppm in the planting medium of the *Leucaena leucocephala* variety Tarramba mutant gave optimal results on the growth of mutants. The increase in root length showed the best results on the M9 strain, the increase in vertical plant height showed the best results on the M9 strain, the number of shoots showed the best results on the M9 and M11 strains and the highest percentage of rooted plants on M3 and M11.

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