Morphological Characteristics Selection of Acid-tolerant *Leucaena leucocephala* Mutant to Addition of IBA Hormone (Indole Butyric Acid) in Tissue Culture

Karti PDMH[2]*, Muhklisani[1], Prihantoro I[2]

[1]Department of Feed Nutrition Science, Faculty of Animal Science, IPB University, Dramaga Campus of IPB, Bogor 16689, Indonesia
[2]Feed Nutrition Study Program, Faculty of Animal Science, Bogor Agricultural University Postgraduate School. Indonesia
**E-mail: pancadewi@apps.ipb.ac.id*

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ABSTRAK

Karti PDMH, Muhklisani, Prihantoro I. Seleksi Karakteristik morfologi mutan *Leucaena leucocephala* toleran asam terhadap penambahan hormon IBA (*Indole butyric acid*) pada kultur jaringan. JITV 27(4):170-176. DOI: http://dx.doi.org/10.14334/jitv v27i4.2960.

Leucaena leucocephala merupakan tanaman pakan jenis leguminosa yang memiliki kandungan protein yang tinggi. Kultur jaringan merupakan salah satu teknik yang dapat digunakan untuk menseleksi mutan tanaman pakan ternak secara in vitro. IBA (*Indole butyric acid*) merupakan salah satu jenis auksin yang dapat menginduksi perakaran dan pertumbuhan pada tanaman. Penelitian ini bertujuan untuk seleksi karakteristik morfologi mutan *Leucaena leucocephala* toleran asam terhadap penambahan hormon pengakaran IBA pada kultur jaringan. Rancangan yang digunakan dalam penelitian ini adalah rancangan acak lengap (RAL) dengan eksplan tanaman lamtoro sebanyak 11 galur mutan toleran asam pH 3,4 hasil irradiasi sinar gamma 400 gy yang telah di berikan perlakuan pada media asam Al^{3+} 300 ppm yaitu galur K1-K11 (mutan+MS+1ppm IBA) dan 2 lamtoro indukan tanpa penyinaran gamma yaitu P0 (lamtoro indukan+MS+0 ppm IBA), P1 (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. Peningkatan panjang akar dan peningkatan tinggi vertikal tanaman menunjukkan hasil terbaik pada strain mutan K10, jumlah tunas menunjukkan hasil terbaik pada strain mutan K9 dan K11 dan persentase tanaman berakar tertinggi pada strain mutan K3 dan K11. Penambahan IBA dapat meningkatkan karateritik morfologi mutan *Leucaena leucocephala*.

Kata Kunci: IBA, Leucaena leucocephala, mutan, kultur jaringan

ABSTRACT

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Leucaena leucocephala is a legume forage plant that has a high protein content. Tissue culture is a technique that can be used to select mutants for forage plants in vitro. IBA (*Indole butyric acid*) is one type of auxin that can induce rooting and growth in plants. This study aimed to select the morphological characteristics of the acid-tolerant *Leucaena leucocephala* mutant to the addition of the hormone IBA in tissue culture. The design used in this study was a completely randomized design (CRD) with lamtoro plant explants as many as 11 acid-tolerant mutant lines pH 3.4 resulting from 400 gy irradiation which had been treated on 300 ppm Al^{3+} acid media, namely the K1-K11 strain (mutant+MS+1ppm IBA) and 2 parent trees *Leucaena leucocephala* without gamma irradiation, namely P0 as *Leucaena leucocephala* parent+MS+0ppm IBA, P1 as *Leucaena leucocephala* parent + MS + 1 ppm IBA with 15 replicates. The treatment which had a significant effect was continued with the test Tukey. Variables observed were an increase in root length, plant vertical height, number of shoots, and percentage of rooted plants. The increase in root length and increase in plant vertical height showed the best results on the K10 mutant strain, the number of shoots showed the best results on the K9 and K11 mutant strains, and the highest percentage of rooted plants on the K3 and K11 mutant strains. The addition of IBA can increase the morphological characteristics of the *Leucaena leucocephala* mutant.

Key Words: IBA, *Leucaena leucocephala*, Mutant, Tissue Culture

INTRODUCTION

Forage crops are needed by livestock with a fairly high portion. In ruminants, ration consumption covers about 40-80% of the total dry matter of the ration or about 1.5% to 3% of the live weight of livestock (Abdullah et al. 2005). The nutritional content of fodder crops has its proportions and advantages (Geng et al. 2020). 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, fiber, 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; De Angelis et al. 2021).

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 after the plant already has been selected. Tissue culture techniques can produce plant seeds in large quantities under controlled conditions, and the time required is relatively fast (Loyola-Vargas & Ochoa-Alejo 2018). Lamtoro (*L. leucocephala* cv. Tarramba) embryogenic callus showed an optimal response at a concentration of 2,4-D ZPT 1,5 mg/L, this research is initial research in the assembly of lamtoro to acid soil tolerance (Manpaki et al. 2018).

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 (Frick & Strader 2018). This hormone can also synthesize the amino acid tryptophan by positively reacting to callus stimulation, cell growth, and root formation (Wattimena et al. 2011). This research was conducted by Nurbaeti et al. (2020), which stated that the addition of 1 ppm IBA hormone in *Leucaena leucocephala* plants showed the highest percentage of rooted plants.

A mutant candidate from the *Leucaena leucocephala* plant has been produced through the selection process due to 400 gy (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 root stage with the addition of IBA to see the characteristics and selection of root growth from the mutants.

MATERIALS AND METHODS

Research material

Materials used as explants in this study were *Leucaena leucocephala* Teramba variety is adapted to Al^{3+} 300 ppm 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, aqua dest, 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.

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

Research procedure

Space Preparation

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 16ºC, 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 121ºC 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 a 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 resulting from 400 gy which had been treated on 300 ppm Al^{3+} acid media as strain numbers K1, K2, K3, K4, K5, K6, K7, K8, K9, K10, K11 (*Leucaena leucocephala* mutant) and control P0, P1 are *Leucaena leucocephala* parent (plants are not irradiated with gamma ray)

Media creation

The media used in this study were basal MS media as control medium (P0) and MS media with 1 ppm IBA (P1, K1, K2, K3, K4, K5, K6, K7, K8, K9, K10, K11). 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 aqua dest and homogenized using a magnetic stirrer. Next, jelly is added to as much as 7 g.L-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^{0}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 aluminum foil. Furthermore, the media was sterilized using an autoclave at a temperature of 121ºC 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 and 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 (K1, K2, K3, K4, K5, K6, K7, K8, K9, K10, K11) and control *Leucaena leucocephala* is namely with P0 (without added with IBA), and P1 (with added with IBA). 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 bottles.

Statistical design and analysis

The design used a completely randomized design (CRD) with *Leucaena leucocephala* explants as many as 11 mutant lines were acid-tolerant pH 3.4 resulting from 400 gy irradiation which had been treated on 300 ppm $Al3^+$ acid media, Namely, the K1-K11 lines were added with1 ppm IBA (mutant+MS+1ppm IBA) and 2 *Leucaena leucocephala* parent trees without gamma irradiation, namely P0 without added with IBA (*Leucaena leucocephala* parent+MS+0 ppm IBA), P1 was added with 1 ppm IBA (*Leucaena leucocephala* parent + MS + 1 ppm IBA), each with15 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

Root extension

Roots are important for plant growth because they are needed to absorb nutrients in the media. The sign of root length in *Leucaena leucocephala* is root growth

Table 1. Root length of *Leucaena leucocephala* until 5 weeks after planting

Strain	Week after planting				
	1	2	3	4	5
	……………mm……………				
P0	0.00±0.00	0.00±0.00	3.70±1.60 ^{bc}	0.42±0.47 ^{e}	1.07±1.19 ^{c}
P1	0.00±0.00	0.00±0.00	4.38±2.23 ^{bc}	3.21±2.91 ^{cd}	3.02±2.82 ^{bc}
K1	0.00±0.00	0.00±0.00	5.24±1.98 ^{ab}	4.48±2.00 ^{bc}	4.20±1.99 ^{ab}
K2	0.00±0.00	0.00±0.00	2.35±1.36 ^{bc}	2.85±1.43 ^{cd}	3.20±1.53 ^{bc}
K3	0.00±0.00	0.00±0.00	3.63±2.46 ^{bc}	6.60±2.36 ^{ab}	5.37±3.42 ^{ab}
K4	0.00±0.00	0.00±0.00	5.22±2.36 ^{ab}	4.22±2.07 ^{bc}	4.63±2.39 ^{ab}
K5	0.00±0.00	0.00±0.00	3.88±2.53 ^{bc}	4.43±1.79 ^{bc}	3.68±2.37 ^{bc}
K6	0.00±0.00	0.00±0.00	1.69±2.64 ^{c}	1.63±1.52 ^{de}	4.72±3.13 ^{ab}
K7	0.00±0.00	0.00±0.00	3.63±3.06 ^{bc}	3.05±1.71 ^{cd}	2.99±2.88 ^{bc}
K8	0.00±0.00	0.00±0.00	3.62±4.07 ^{bc}	3.74±3.25 ^{cd}	3.02±3.11 ^{bc}
K9	0.00±0.00	0.00±0.00	7.54±2.94 ^{a}	7.99±2.94 ^{a}	5.98±1.94 ^{ab}
K10	0.00±0.00	0.00±0.00	3.76±2.54 ^{bc}	4.17±2.50 ^{bc}	7.07±4.29 ^{a}
K11	0.00±0.00	0.00±0.00	1.41±2.53 ^{c}	2.28±2.82 ^{cd}	4.24±2.12 ^{ab}

P0=control+MS+IBA 0 ppm, P1=control+MS+IBA 1 ppm, K1- K11= Mutant+MS+IBA 1 ppm. Different superscripts in the same column showed a significant effect (P<0.05) based on the Tukey test which can be seen at the bottom of the culture bottle. The increase in the root length that gives the hormone IBA to acid-tolerant *Leucaena leucocephala* is listed in Table 1.

The analysis of variance in the increase of plant root length showed a significant difference (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 K9 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 K10 strain with an increase of 7.07 mm, this indicates that the root length increase of the selected mutant plant was higher than P1or P0. The K9 mutant line had the best root length gain with the highest average compared to other plant lines. K9 strain with the addition of IBA can significantly increase root growth. The K10 mutant strain is a response to an increase in root length at 5 weeks after planting. According to Marga et al. (2020) and Manpaki et al. (2017), 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 Arlianti et al. (2013) and Zulastri 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 auxin hormone will stop its role in inhibiting the calcium pectin (Apriliani et al. 2015). Rafique et al. (2012) said that a significantly high root length (0.96 cm) was found on MS medium supplemented with 1 mM IBA followed by 1.5 mM IBA (0.63 cm).

Plant height

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 plant height as treated with IBA on *Leucaena leucocephala* acid-tolerant are listed in Table 2.

In the first week, the highest increase was in the K11 mutant line with an increase of 5.17 mm, the second week the highest increase was in the K5 mutant strain with an increase of 5.55 mm, the third week the highest increase was recorded in K3 mutant strain with an increase of 6.56 mm, while in the fourth and fifth weeks K9 had the highest increase. The K9 mutant strain with the highest increase reaching 8.22 mm indicates that the plant height increase of the selected mutant plant was higher than P1 and P0. The K9 mutant strain had the best vertical height gain and had

Table 2. Plant height of *Leucaena leucocephala* plants until 5 weeks after planting

Strain	Week after planting				
	1	2	3	4	5
	……………….mm……………….				
P0	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}
P1	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}
K1	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}
K2	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}
K3	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}
K4	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}
K5	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}
K6	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}
K7	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}
K8	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}
K9	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}
K10	0.28±0.51 ^{b}	0.81±1.05 ^{e}	6.16±5.98 ^{a}	4.00±2.81 ^{bc}	3.76±2.54 ^{bc}
K11	5.17±0.90 ^{a}	4.00±2.81 ^{de}	3.76±2.54 ^{ab}	0.21±0.48 ^{d}	1.41±2.53 ^{c}

P0=control+MS+IBA 0 ppm, P1=control+MS+IBA 1 ppm, K1- K11= Mutant+MS+IBA1 ppm. Different superscripts in the same column showed a significant effect (P<0.05) based on the Tukey test the highest mean compared to other plant strains. The effectiveness of the hormone IBA in the growth of the plant vertical height had a good effect on the growth of mutant plants of *Leucaena Leucocephala*. Research conducted by Firmansyah et al. (2014) states that the hormone can stimulate the formation of the apical meristem, thereby increasing plant height. According to Supriyanto & Prakasa (2011) and Satbhai et al. (2015), 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.

The analysis of variance in the number of plant shoots showed a significant effect (P<0.05). In the first week and second weeks, the highest shoot number was in the K10 and K11 mutant strains with an average number of 2.93 units and 3.87 shoot units; in the third week, the highest number was in the K11 mutant strain with an average number of 4.27 units, while in the fourth and fifth weeks, the highest was recorded in the K9 and K11 mutant strains with the highest average number of around 5.27 units. The number of shoots of the selected mutant plants was higher than P1 and P0. Mutant strains K9, K10, and K11 had the best number of shoots at 5 weeks after planting, and had the highest average compared to other plant strains. Based on Harahap et al. (2012) 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 (Marga et al. 2020; Zhang et al. 2022). The IBA type of auxin hormone can encourage cell extension and division (Wang & Ruan 2013). The addition of IBA will encourage optimal shoot formation and root initiation (Karti et al. 2019).

Percentage of rooted plants

The percentage of rooted plants from *Leucaena leucocephala* mutant showed different effects in each plant strain were influenced by varying plant responses to adaptation 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 such as hormones will display plant characteristics. The addition of the hormone IBA concentration of 1 ppm provides optimal root growth because it follows the levels of auxin

Table 3. The number of *Leucaena leucocephala* plants shoots until 5 weeks after planting

Strain	Week after planting				
	1	2	3	4	5
	………………mm………………				
P0	1.00±0.00 [c]	1.87±0.35 [c]	2.93±0.26 [c]	3.00±0.00 [e]	3.20±0.41 [c]
P1	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]
K1	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]
K2	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]
K3	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]
K4	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]
K5	1.40±0.51 [c]	2.27±0.46 [c]	2.93±0.26 [c]	4.07±0.59 [bc]	4.07±0.59 [b]
K6	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]
K7	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]
K8	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]
K9	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]
K10	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]
K11	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]

P0=control+MS+IBA 0 ppm, P1=control+MS+IBA 1 ppm, K1- K11= Mutant + MS + IBA1 ppm. Different superscripts in the same column showed a significant effect (P<0.05) based on the Tukey test.

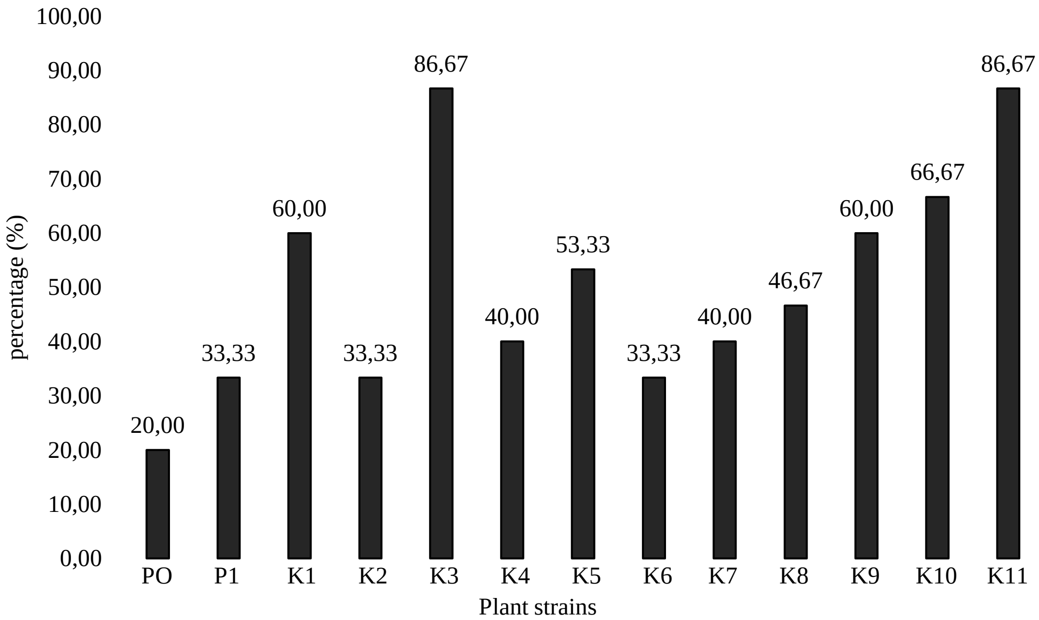

Figure 1. Percentage of *Leucaena leucocephala* rooted plants at 5 Weeks After Plant.

required by the *Leucaena leucocephala* plant (Nurbaeti et al. 2020; Rinaldy et al. 2019). The highest percentage of *Leucaena leucocephala* rooted plants at 5 weeks after the plant is K3 and K11. This research showed that the addition of 1 ppm IBA hormone in the *Leucaena leucocephala* mutant gave a high percentage of rooted plants compared with P0 (without adding IBA). It is different from what (Wijaya & Sudrajad 2019) said that a concentration of 2 ppm IBA showed better root growth compared to others. Concentrations 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 inhibited. Adding auxin to plants as a growth regulator can increase plant development by affecting membrane proteins that can accelerate protein and nucleic acid synthesis (Saini et al. 2013), and adding auxin also affects new root formation (Firmansyah et al. 2014). The rooting percentage of the seedling-derived shoots from the Olive plant was 76% for 'Arbequina' and 'Gordal Sevillana' cultivars and 100% for 'Manzanilla de Sevilla' cultivar, whereas, with the electro-pulse method, the rooting percentages were 68, 64 and 88%, respectively (Padilla et al. 2009).

CONCLUSION

Based on the research that has been done, it can be concluded that the increase in root length showed the best results on the K10 strain, the increase in vertical plant height showed the best results on the K9 strain, and the number of shoots showed the best results on K9, and K11 strains. The highest percentage of rooted plants on K3, and K11. Root length, vertical height, shoot number, and the rooted percentage were higher on *Leucaena leucocephala* mutant with added IBA 1 ppm compared with *Leucaena leucocephala* without added IBA.

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