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In vitro growth response on three provenances of Jabon Merah based on auxin and cytokinin combinations

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Abstract. This study aimed to determine the combination of plant growth regulators (PGR) that affects the growth of three provenances of Jabon merah through in vitro culture. This research was conducted in December 2017 to March 2018 at Biotechnology and Tree Breeding Laboratory, Hasanuddin University, Makassar. Research step consisted of sterilization, stock solutions and culture media preparation, and planting. The observation parameters were plant height, time of leaf formation, number of leaf, leaf length, number of root, root length, and percentage of dead explant. The results obtained from this observation were (1) the provenance significantly affected the time of leaf formation, number of root, root length, and percentage of explant explant, (2) the combination of PGR significantly affected the number of roots and root length, (3) the interaction between PGR and provenance was of a significant effect on the root length, (4) The combination of 1 ppm IBA and 0.5 ppm TDZ showed the best PGR in increasing the root length, (5) combination of 3 ppm IBA and 0.5 ppm TDZ increased the number of root, and (6) Sidrap Provenance was the best PGR based on the number of roots, root length and low mortality rate.

1. Introduction

Jabon Merah is a fast-growing type of local plants which starting to attract the timber industry, especially for industrial plantation forest. The wood type is a softwood with low to medium density with reddish white color and smooth texture characteristics. It is suitable for pulp and paper industry, furniture industry, fruit crates, children's toys, matches, shoe pads, boards, and other wood products [1]. In order to fulfill market needs, production of jabon Merah wood is highly needed on a large scale. Procurement of in-vitro superior seeds or tissue culture is the best alternative at this time. According to Yusnita (2003), tissue culture is a technique to develop plant parts in form of cells, tissues or organs in an aseptic state as in-vitro, use of artificial media containing complete nutrition, Plant Growth Regulator (PGR), and conditions of controlled culture, temperature and lighting [2].

Some breeding research about Jabon Merah [3], [4], [5] had reported. Research tissue culture already conducted by Putriana (2016) which showed 7 ppm of kinetin concentration was the best amount to induce leaves and roots in the cultivation of in-vitro Jabon Merah [6]. Marzuki, et al.,



(2016) has reported a combination of 4 mg / l BAP + 0.3 / l IAA was the best concentration to form jabon merah leaves [7].

Information about types of PGR to support the development of Jabon Merah through tissue culture with cytokinin (kinetin and BA), and auxin (IAA) are still limited, therefore this study was conducted to obtain an in-vitro breeding method for jabon merah with a combination of auxin (IBA) and cytokinin (BAP and TDZ).

2. Research Materials and Method

Research was conducted from December 2017 to March 2018 at Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Universitas Hasanuddin, Makassar.

2.1. Tools and plant material

The tools and materials used in this study were graph paper, tally sheet, autoclave, analytical scale, hot plate and magnetic stirrer, laminar air flow cabinet (LAFB), erlenmeyer, microwave, culture bottle, 10 ml and 100 ml measuring cup, trophy cups, petri dishes, spatulas, micro pipettes, tweezers, scalpels, Bunsen, alcohol bottles, sprayers, lighters, aluminum foil, scissors, plastic wrap, tissue, masks, handsoons, and cameras. The explants used in this study were in-vitro node seeds from three provenances obtained from the 2nd Regional Seed/Seedling Forest Office (BPTH). Then media material used were Murashige and Skoog (1962) consisting of A stock solution, stock B, stock C, stock D, Stock E, Stock F, myo inositol, vitamins stock, sucrose, and compacting material

2.2. Method

2.2.1. Sterilization. The tools were sterilized with two stages. Stage I was done by inserting wrapped petri dish, scalpel, tweezers, and scissor to oven for 2 hours. Then, a bottle containing aquades and a culture bottle with lid was inserted into autoclave for 20 minutes at 121oC temperature. Stage II was carried out in a laminar air flow cabinet after the media was done. A culture bottle containing media was given a UV in LAFB for an hour. Tools such as tweezers, scissor and scalpel, were dipped in 96% alcohol and burned with Bunsen before planting.

2.2.2. Media Creation. The types of media used were Murashige and Skoog [8]. The media was made by with 30 g sucrose dissolved with sterile distilled water in the Erlenmeyer and homogenized with a hot plate and magnetic stirrer. Then stocks A to F, myo-inositol, and vitamins were added. The pH was measured with a standard of 5.7. If a media solution showed result less than 5.7 then NaOH should be added, and if its more, then HCl should be added. Next, 8 g of compactor material was inserted and distilled water was added to 1 liter. The solution putted into the microwave boiled and homogenized again. PGR was mixed into medium with concentration according to treatment. Then, putted into 22 bottles for ½ liter of media. Bottles were inserted to autoclave, sterilized at 121oC with pressure amount of 15-17 psi for 15 minutes. Planting was started after bottles were cooling down.

2.2.3. Planting. Planting was performed inside a sterilized LAFB. Steps for planting tissue culture were as follows :

- a. Explants cutted into several parts based on number of nodes from the explants.
- b. A piece of explant was planted in one culture bottle closed to Bunsen, and wrapped with plastic wrap.
- c. The culture bottles were labeled with media type and date of planting.
- d. Bottles were placed on the incubation rack and the data was collected and observed.

Observations were conducted every week during six weeks of duration (6 DAP). However, leaf formation was observed every 2 days until the end of the observation. Parameters observed including :

- a. Plant height (cm), measured from base of stem to end of stem
- b. When leaves were formed (DAP), calculated from the day after planting
- c. Number of leaves, calculated from the number of leaves
- d. Leaf length (cm), measured from the base to the tip on the longest leaf of each explant
- e. Number of roots, calculated from number of roots formed
- f. Root length (cm), measured from base to end on the longest root of each explant
- g. Percentage of Failed Explant (%) formula is as follows :

$$\frac{\text{Amount of Failed Explant}}{\text{Total Numbers of Planted Explant}} \times 100\%$$

- h. Percentage of Contaminated Explant (%) formula is as follows:

$$\frac{\text{Amount of Contaminated Explant}}{\text{Total Numbers of Planted Explant}} \times 100\%$$

2.2.4. *Research Design.* The research design used was a two-factor factorial design in Completely Randomized Design (CRD).

1. First Factor within Famili (f), namely :

- f1 = Sidrap Jabon merah
- f2 = Buton Jabon merah
- f3 = Banggai Jabon merah

2. Second Factor was MS media with a combination of growth regulator (m), namely:

- m1 = control
- m2 = BAP 1 ppm + IBA 0,5 ppm
- m3 = BAP 2 ppm + IBA 0,5 ppm
- m4 = BAP 3 ppm + IBA 0,5 ppm
- m5 = TDZ 1 ppm + IBA 0,5 ppm
- m6 = TDZ 2 ppm + IBA 0,5 ppm
- m7 = TDZ 3 ppm + IBA 0,5 ppm
- m8 = BAP 2 ppm + TDZ 2 ppm + IBA 0,5 ppm

The combination of the two factors above obtained from 24 combinations of treatments and each combination was repeated for four times. Thus, the total number of experimental units was 96 units

Data analysis was using linear models as follows :

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

Note :

- Y_{ijk} = Observed value for the k-th measure of Provenance treatment (i) and PGR treatment (j)
- μ = the overall population mean (grand mean)
- α_i = additive main effect of level i from provenance
- β_j = additive main effect of level j from PGR
- $(\alpha\beta)_{ij}$ = non-additive interaction effect of treatment (i,j) from both factors
- ε_{ij} = Trial error

Observation data analyzed by using analysis of variance (ANOVA), if there were significant results, then the 5% Tukey significance test should be conducted with R statistical software (R Core team). Data did not fulfill assumption of a normal distribution was carried out by BoxCox transformation with R statistic. Number of roots and root lengths parameters were not found, thus The Aligned Rank Transform of non-parametric with R statistic was used to analyzed.

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3. Results and Discussion

3.1. Plant Height

The results of analysis of variance showed that the combination treatment of PGR, provenance and combination of both did not significantly influence plant height. The average plant height (Figure 1) showed that highest height was 1.62 cm from 1 ppm TDZ + IBA 0.5 ppm (m5) and the lowest height was from BAP 1 ppm + IBA 0.5 ppm (m2) for 0.86 cm. However, this study result was contrast with study by June (2014). The previous study highlights that addition of BAP 1 mg/l was best concentration to produce highest bud height for jабon merah, and it only a single cytokinin, while this study used a combination of auxin and cytokinin.

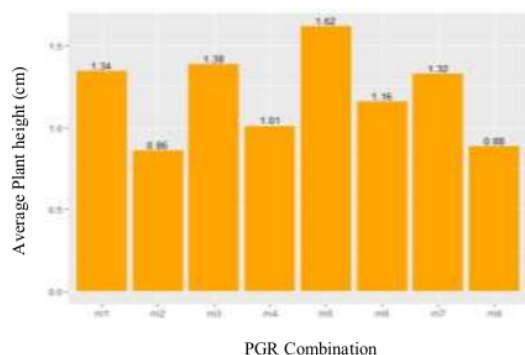


Figure 1. Average plant height with PGR treatment.

The average plant height of provenance treatment (Figure 2) showed that the highest number was from Sidrap provenance which was 1.31 cm, and Banggai and Buton provenances showed the same average plant height, 1.14 cm. High number on Sidrap provenance was indicated due to young age of the explants than Banggai and Buton provenances. During explants selection, only morphological form was considered.

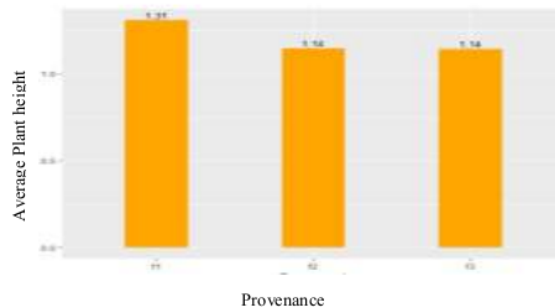


Figure 2. Average plant height with provenance treatment.

The highest average value from the combination of PGR and provenance treatment (Figure 3) from f1m5 (Sidrap provenance with 1 ppm + IBA 0.5 ppm) was 1.88 cm. While the lowest was 0.22 cm from f2m8 (Buton provenance with BAP2 ppm + 2ppm TDZ + IBA 0.5 ppm). High average number from a combination of f2m8 due to a balance between two combinations of PGR. Isnaeni's (2008) found that TDZ media had significant effect on height growth, and high amount of TDZ concentration will reduce height growth [9].

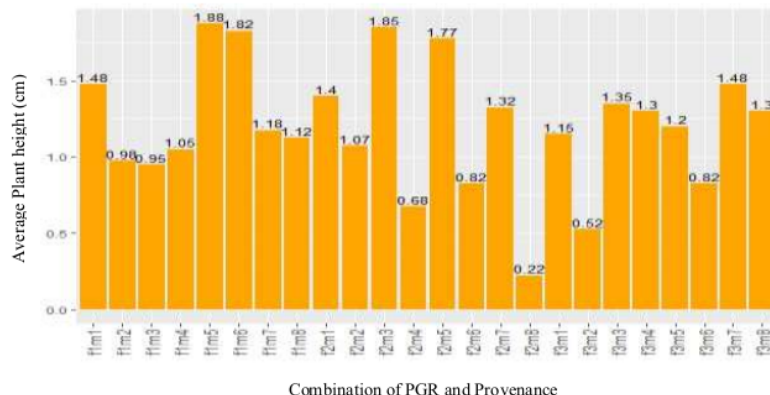


Figure 3. Average plant height with combination of PGR and provenance treatment.

3.2. Time of Leaf Appearance

The result of analysis of variance showed that provenance had a significant effect on the time of leaf appearance, thus Tukey test was carried out to obtain real difference values. Its presumably due to source of tree seeds. According to Cahyono and Rayan (2012), differences between trees caused by growing environment, genetic differences between trees, and interactions between genetic, environmental, and seed sources [10].

Table 1. Results of tukey test on effect of provenance on time of leaf appearance.

Treatment	Average (days)	Note
f1	14,40625	A
f2	9,84375	Ab
f3	8.87500	B

Note : If there are same letters in different columns, then it is not significantly different. f1: Sidrap provenance, f2: Buton provenance, and f3: Banggai provenance.

Tukey test results (Table 1) showed that Sidrap Provenance was significantly different from Banggai provenance, but not with Buton provenance. Buton provenance was not significantly different with Banggai provenance based on time of leaf appearance. Furthermore, it can be concluded that Banggai Provenance gives the best average value based on time of leaf appearance, which was 8 days. The average time of leaf appearance from PGR treatment (Figure 4) showed PGR 6 (TDZ2 ppm + IBA0.5 ppm) had the fastest leaf appearance which was at day 7 (7 DAP) because TDZ cytokinins made explants leaf growth better While PGR m2 (BAP1 ppm + IBA 0.5 ppm) leaf was started to appeared at 9 DAP. Previous study has reported buds and leaves tended to grow when planted on TDZ addition media, while the addition of BAP caused growth approached stationary [11].

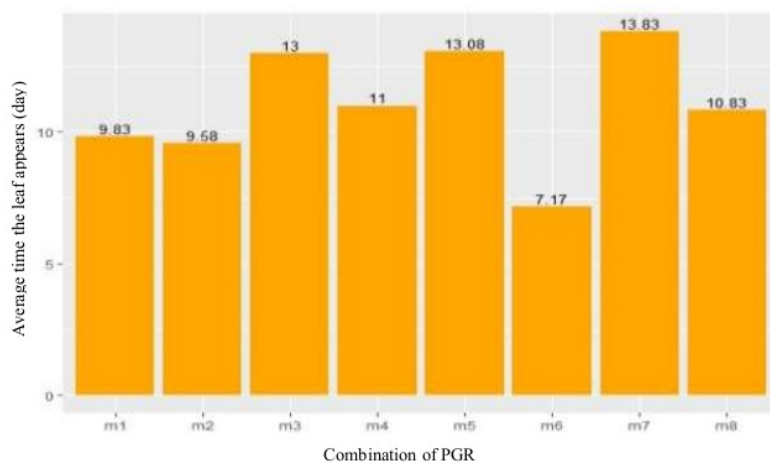


Figure 4. Average time of leaf appearance with PGR treatment.

Figure 5 showed the average of leaves appearance was 3 DAP from a combination of f2m6 (Buton provenance with 2 ppm + IBA 0.5 ppm) and f3m6 (Banggai provenance with 2 ppm TDZ + IBA 0.5 ppm). While the combination of f1m2 (Sidrap Provenance with 2 ppm + IBA 0.5 ppm PGR BAP) appeared at 18 DAP. These results were different because of the provenance factor. Karjadi (2008) reported differences in explant origin and plant species would influence the effectiveness of PGR. This difference because Sidrap provenance already contains enough endogenous auxin hormones thus additional auxin actually made development of the explants inhibited.

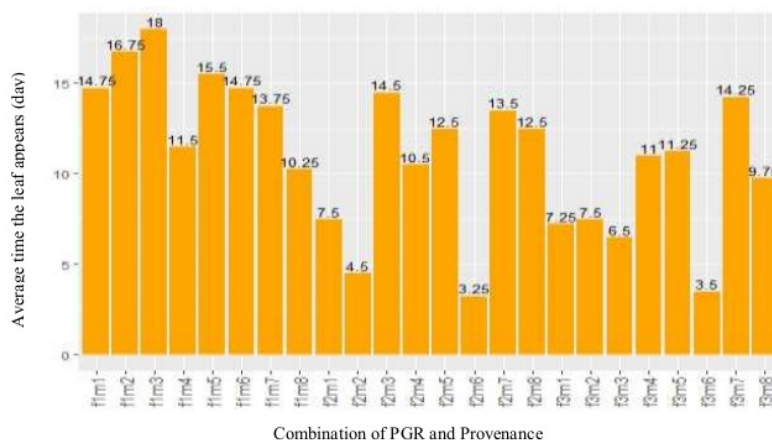


Figure 5. Average time of leaf appearance with combination of PGR and provenance treatment.

3.3. Amount of Leaf

Analysis of variance results obtained between treatment of PGR, provenance, and combination of both had no significant effect on the number of leaves. The average number of leaves from PGR treatment (Figure 6) showed the m2 (1 ppm ZAP BAP + 0.5 ppm IBA) gave the highest average number of leaves which was 5.83 leaves while the lowest average was from m6 (TDZ 2 ppm + IBA 0.5 ppm) and m1 (without PGR). High number of m2 was due to main function of BAP as a cytokinin hormone to stimulate leaf growth and elongation of leaf growth points. While IBA is an auxin hormone that is useful for cell division and bud multiplication. According to Yusnita (2003), BAP able to increase chlorophyll production, and made process of photosynthesis faster which formed carbohydrate compounds for the process of leaf growth [2].

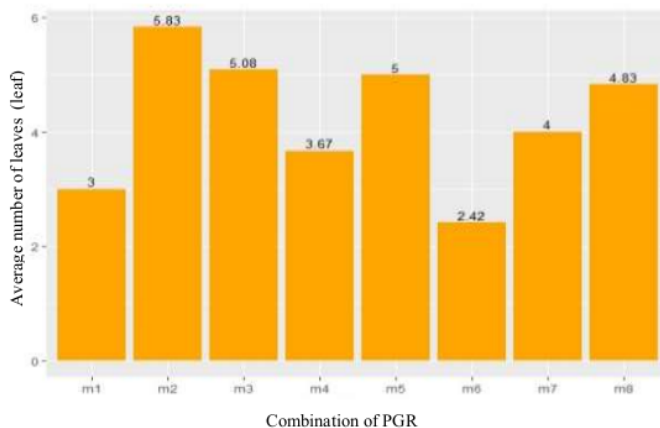


Figure 6. Average number of leaves with PGR treatment.

The average number of leaves with provenance treatment (Figure 7) showed Sidrap provenance had the highest average number and Buton provenance had the lowest because of the influence of cytokinin was responded well by Sidrap provenance. High cytokinin content of auxin would stimulate leaf growth and buds, while on the other side low amount stimulate root growth [12].

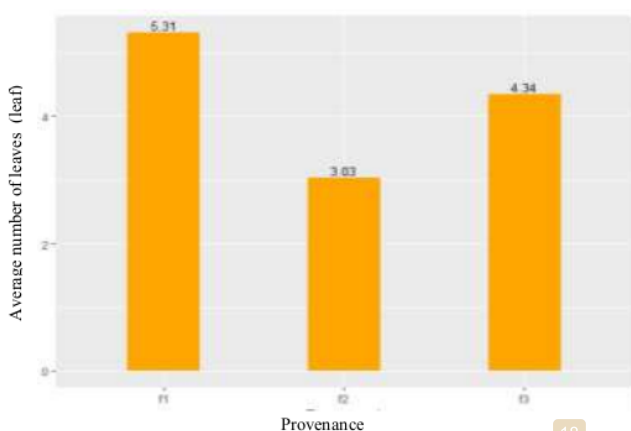


Figure 7. Average amount of leaves with provenance treatment.

The average number of leaves from the combination of PGR and provenance treatment (Figure 8) showed f3m2 (Banggai Provenance with 1 ppm + IBA 0.5 ppm PGR BAP) gave the highest average of 10.75 amount of leaves, whereas f2m6 (Buton Provenance with 2 ppm + IBA 0.5 ppm PGR TDZ) gave the lowest average of 0.75 amount of leaves. Thidiazuron cytokinins should be used in low concentration [13]

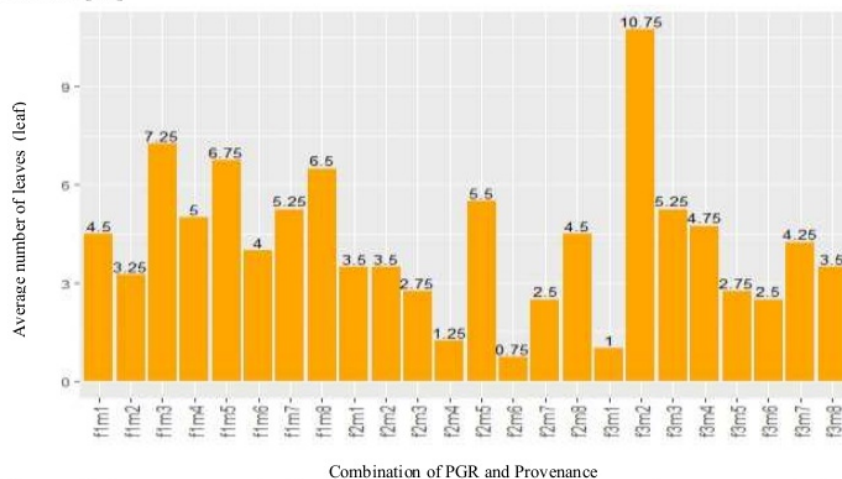


Figure 8. Average amount of leaves with combination of PGR and provenance treatment.

3.4. Leaf Length

PGR, provenance, and combination of both treatments had no significant effect on the length of leaf explants of jabon merah. The longest average number of leaves from PGR treatment (Figure 9) was m3 (BAP 2 ppm + IBA 0.5 ppm) with a value of 0.66 cm. While treatment with no PGR had 0.62 cm length. This insignificant difference between BAP 2 ppm + IBA 0.5 ppm and control media because endogenous auxin in explants already sufficient, however the addition of exogenous auxin will have no effect on leaf length growth. The lowest average value was from 2 ppm TDZ + IBA 0.5 ppm.

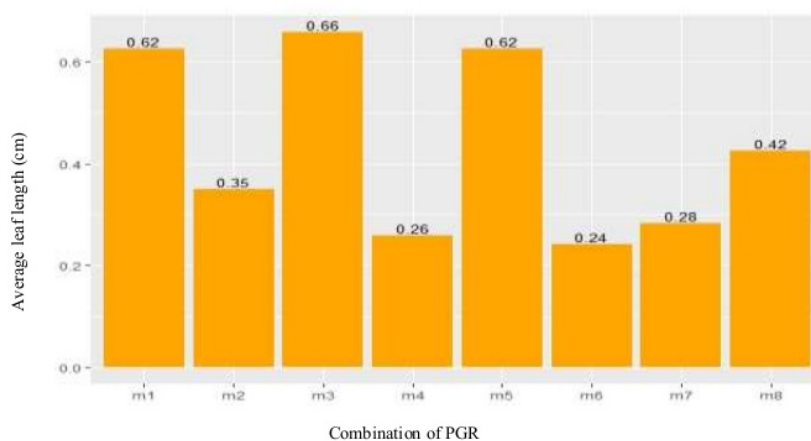


Figure 9. Average leaves length with PGR treatment.

Results of the average leaf length of provenance treatment (Figure 10) showed the highest average was Banggai provenance, and the lowest was Buton provenance. The difference between leaves length of these three provenances were not too significant because the provenances were relatively the same. Regia (2017) also reported that provenance of jabon merah from Piru, Wakal, Wakasiu, Obi and Buton did not significantly affect the morphology of jabon merah which include on leaf length and number of venation [14].

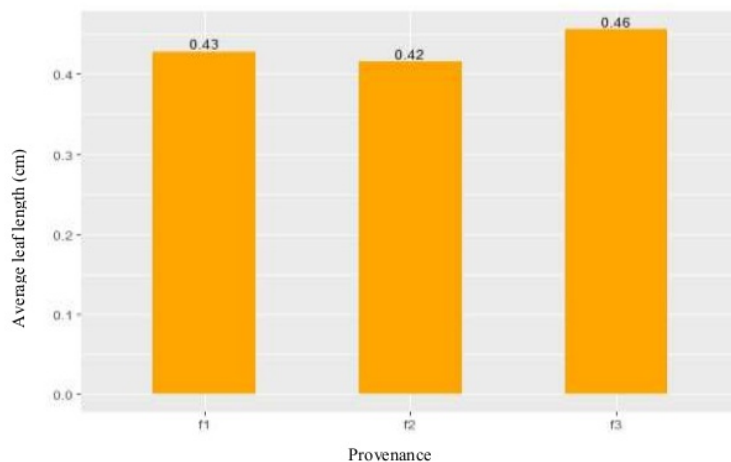


Figure 10. Average leaves length with provenance treatment.

The average leaves length from the combination of PGR and provenance (Figure 11) showed the highest average length was 1.02 cm from f3m3 (Banggai Provenance with 1 ppm + IBA 0.5 ppm PGR BAP). While the lowest was 0.05 cm from a combination of f2m6 (Buton Provenance with 2 ppm + IBA 0.5 ppm PGR TDZ). The low number of leaf length in f2m6 doubtedly due to high exogenous cytokinin, and this theory in line with study by Wattimena in 1992 stated that too high amount of exogenous cytokinin will make explants become saturated and tend to have no response.

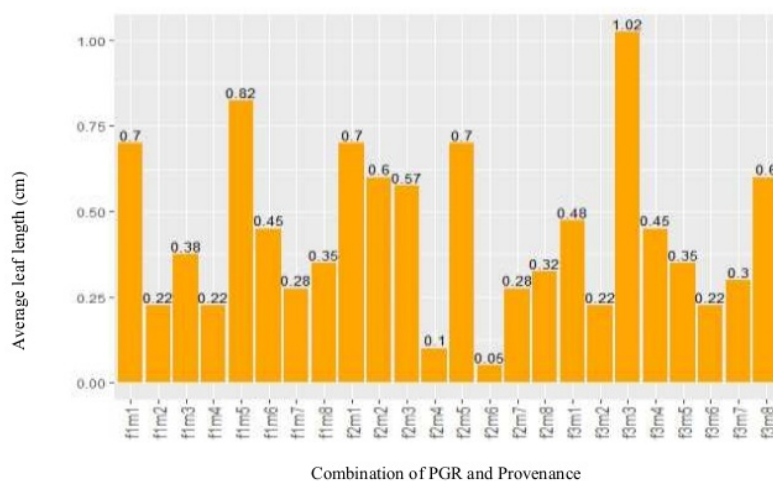


Figure 11. Average leaves length with combination of PGR and provenance treatment.

3.5. Number of Roots

Average number of roots showed different results from various treatments. Although variable analysis showed that the treatment of PGR and provenance had significant effect on the number of roots, whereas the interaction of both influences was not real.

Table 2. Result of post-hoc contrast test with provenance treatment.

Treatment	Average (cm)	Note
f1	13.7825	A
f2	11.59375	B
f3	11.5975	B

Note : If there are same letters in different columns, then it is not significantly different. f1: Sidrap provenance, f2: Buton provenance, and f3: Banggai provenance.

The results of the post-hoc contrast test in Table 2 showed that the number of roots of Sidrap provenance had significant different with Buton and Banggai provenances, and Buton provenance with Banggai was not significantly different. The low average number of roots in Buton and Banggai provenance was presumably due to low content of endogenous auxin. Widiastoety (2014) explained that root formation had relation with the content of auxin and endogenous cytokinins in plant tissues [15].

Table 3. Result of post-hoc test of PGR treatment.

Treatment	Average Number	Note
m7	34.3334	B
m6	33.9167	B
m5	29.334	B
m1	0.4167	A
m2	0.3334	A
m3	0.25	A
m4	0	A
m8	0	A

Note : If there are same letters in different columns, then it is not significantly different. m1 (control), m2 (BAP 1 ppm + IBA 0.5 ppm), m3 (BAP 2 ppm + IBA 0.5 ppm), m4 (BAP 3 ppm + IBA 0.5 ppm), m5 (TDZ 1 ppm + IBA 0.5 ppm), m6 (TDZ 2 ppm + IBA 0.5 ppm), m7 (TDZ 3 ppm + IBA 0.5 ppm), m8 (BAP 2 ppm + TDZ 2 ppm + IBA 0.5 ppm).

Table 3 showed post-hoc contrast test on the effect of PGR treatment with number of roots. Addition of PGR on m5, m6, and m7 gave significantly different result rather than PGR on m1, m3, m4, and m8. These result had indication that m2, m3, m4, and m8 media were containing BAP which inhibit root growth with the highest average number of roots was 0.4167. However, this result was in line with study by Rainiyati et al. (2007) that BAP hormone can inhibit roots formation, block roots growth, and inhibit influence of auxin on root initiation in tissue culture [16].

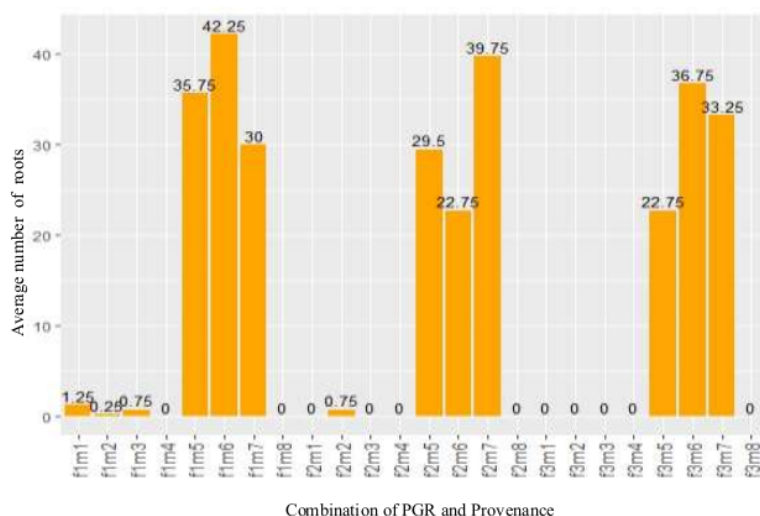


Figure 12. Average number of roots with combination of PGR and provenance treatment.

3.6. Length of Roots

The results of analysis of variance showed that the treatment of PGR, provenance and both combination had a very significant effect on root length.

Table 4. Result of post-hoc contrast test of root with provenance treatment .

Treatment	Average (cm)	Note
f1	0.434375	A
f2	0.287500	Ab
f3	0.193750	B

Note : If there are same letters in different columns, then it is not significantly different. f1: Sidrap provenance, f2: Buton provenance, and f3: Banggai provenance.

The results of post-hoc tests between Sidrap and Buton, and Buton and Sanggai provenances were not significantly different, while Sidrap provenance was significantly different from Banggai provenance. In the Sidrap provenance average length was 0.43 cm, then Buton 0.28 cm and last was Banggai 0.19 cm. Low root length for Banggai provenance due to low auxin content. Sufficient amount of endogenous auxin content can caused better growth for root length. Sulichantini (2016) had also argues that an increase in auxin can increase root length [17]. Study by Sari et al. (2015) explained that higher concentration of auxin than cytokinin would stimulate root growth [12].

Table 5. Result of post-hoc contrast test of root with PGR treatment.

Treatment	Average (cm)	Note
m5	0.816	C
m7	0.650	C
m6	0.575	C
m1	0.200	B
m2	0.183	B
m3	0.016	AB
m4	0	A
m8	0	A

Note: If there are same letters in different columns, then it is not significantly different. m1 (control), m2 (BAP 1 ppm + IBA 0.5 ppm), m3 (BAP 2 ppm + IBA 0.5 ppm), m4 (BAP 3 ppm IBA 0.5 ppm), m5 (TDZ 1 ppm + IBA 0.5 ppm), m6 (TDZ 2 ppm + IBA 0.5 ppm), m7 (TDZ 3 ppm + IBA 0.5 ppm), m8 (BAP 2 ppm + TDZ 2 ppm +I BA 0.5 ppm).

m5 was the highest average in number of roots, allegedly there was sufficient balance in roots of explants. Furthermore, bud formation generally requires cytokinin while root formation requires auxin (Lestari, 2010). However, it is often necessary to combine both depending on the ratio of auxin to cytokines or vice versa

Table 6. Average length of roots with combination of PGR and provenance treatment.

PGR Combination (ppm)	Provenance	Average
m5	f1	1.12
m5	f2	1.07
m7	f2	0.87
m6	f1	0.82
m6	f3	0.67
m7	f3	0.62
m1	f1	0.6
m7	f1	0.45
m2	f1	0.42
m5	f3	0.25
m6	f2	0.22
m2	f2	0.12
m3	f1	0.05
m8	f3	0
m2	f3	0
m3	f3	0
m4	f3	0
m1	f3	0
m3	f2	0
m4	f2	0
m1	f2	0
m8	f2	0
m8	f1	0
m4	f1	0

Note: m1(control), m2 (BAP 1 ppm + IBA 0.5 ppm), m3 (BAP 2 ppm + IBA 0.5 ppm), m4 (BAP 3 ppm IBA 0.5 ppm), m5 (TDZ 1 ppm + IBA 0.5 ppm), m6 (TDZ 2 ppm + IBA 0.5 ppm), m7 (TDZ 3 ppm + IBA 0.5 ppm), m8 (BAP 2 ppm + TDZ 2 ppm +I BA 0.5 ppm). f1 (Sidrap provenance), f2 (Buton provenance), and f3 (Banggai provenance).

Table 10 showed the combination of m5f1 (1 ppm + IBA 0.5 ppm TDZ with Sidrap provenance) became the longest root due to a balance of endogenous auxin hormones from Sidrap provenance with a combination of PGR auxin and exogenous cytokinin. In line with the opinion from Lestari (2010), a combination of auxin and cytokinin was needed to grow roots. Addition of PGR IBA can inhibit root growth. Rainiyati, et al., (2017) explained that the addition of BAP also able to inhibit root formation and inhibit auxin for root initiation in tissue culture [16].

3.7. Failed Explant

The results analysis of variance showed that the combination treatment of PGR, provenance and both combination had no significant effect on the percentage of failed explants. Average percentage of

failed explants (Figure 13) was 25% at PGR m2 (BAP 1 ppm + IBA 0.5 ppm), m4 (TDZ 1 ppm + IBA 0.5 ppm), and m8 (BAP 2 ppm + 2 ppm TDZ + IBA 0.5 ppm). While PGR combinations containing m3 (BAP 2 ppm + IBA 0.5 ppm), m5 (1 ppm TDZ + IBA 0.5 ppm), and m7 (3 ppm TDZ + IBA 0.5 ppm) were not failed. Failed explants in some treatments was due to contamination from bacteria and fungi. Study by Ardiansyah, et al., (2014) stated that initial explants would be resistant to bacteria, but in the end explants will died.

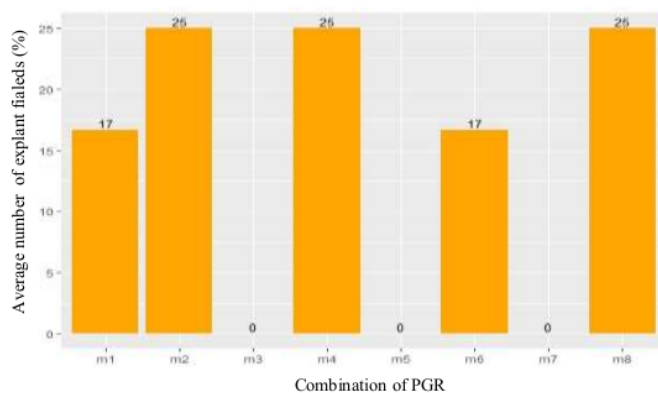


Figure 13. Average percentage of failed explant with PGR treatment.

Sidrap provenance had the lowest percentage of failed explant which was 6% and Buton had highest percentage, 19% and secondly, Banggai provenance with average percentage of 16%. From these results, Sidrap provenance might become the best choice. Average percentage of failed explants from provenance treatment is shown in Figure 14.

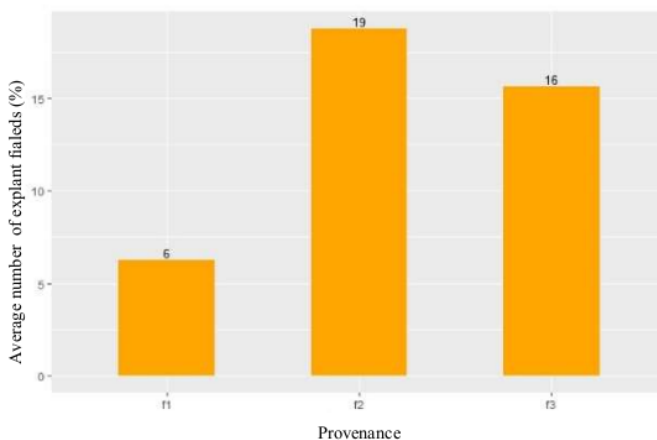


Figure 14. Average percentage of failed explant with provenance treatment.

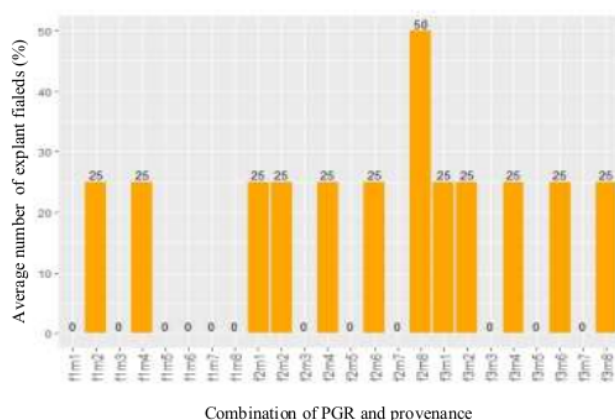


Figure 15. Average percentage of failed explant with combination of PGR and provenance treatment.

Highest percentage for failed explants was 50% in Buton provenance with a combination of 2 ppm BAP + 2 ppm TDZ + 0.5 ppm IBA. Furthermore, combinations which were not failed until the end of observations were f1m1, f1m3, f1m5, f1m6, f1m7, f1m8, f2m3, f2m5, f2m7, f3m3, f3m5, and f3m7, while another 11 combinations failed percentage were 25%. High accumulation of phenolic substances in explants changed explant color to browned and died. Hutami (2008) concluded that the occurrence of brown discoloration due to a collection of polyphenol oxidase released by tissue when cell was injured in an oxidized state [18]. Average percentage of failed explant with PGR and provenance treatment showed below in Figure 15.

This study concluded that explants planted on media with no PGR were almost the same as explants planted in media containing growth regulators, except for root growth. It means that plant can indeed grow without exogenous PGR because it contains endogenous PGR, although the certain amount is still unknown. Successful *in vitro* regeneration of the plant material depends on numerous aspects such as genetic makeup, explant type, media composition, PGRs as well as the culture conditions. Study in organogenesis of *Acacia* via *in vitro*; which would encouragingly be worthwhile for researchers to exploit this perennial woody legume genus with enormous multidimensional value, with more innovative approaches, in order to promote the cause for its improvement [19]. The procedure for *Philodendron* reported can assist in the large-scale multiplication of elite self-heading cultivars of *Philodendron* in the future [20].

4. Conclusion

1. Interaction between Sidrap provenance and combination of 1 ppm TDZ + 0,5 ppm IBA formed the highest average in plant height and root length.
2. Interaction between Buton provenance and combination of 2 ppm TDZ + 0,5 ppm IBA gave the fastest average of leaf formation.
3. Interaction between Banggai provenance and combination of 1 ppm BAP + 0,5 ppm IBA induced the highest average of number of leaf.
4. Interaction between Banggai provenance and combination of 2 ppm BAP + 0,5 ppm IBA performed the best average of leaf length.
5. Interaction between Sidrap provenance and combination of 2 ppm TDZ + 0,5 ppm IBA produced the highest average of number of root.
6. Interaction between Buton provenance and combination of 2 ppm BAP + 2 ppm TDZ + 0,5 ppm IBA obtained the highest average of percentage of death explant.

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