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Embryogenesis Regeneration Of Porang Seeds (Amorphophallus Mulleri Blume) With Various Concentrations Of Indole Butyryc Acid (IBA) And Benzyl Amino Purine (BAP) In Vitro

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Abstract
The experiment was carried out in the Laboratory of the Faculty of Agriculture, University of Garut which is located in the Lab of the Faculty of Agriculture, University of Garut. This experiment was carried out from August to November 2022. The method used in this study was a factorial complete randomized design (CRD) method consisting of 2 factors, namely the concentration of auxin Growth Regulatory Substance/IBA (A) and the concentration of Cytokinin Growth Regulatory Substance/BAP (B). factor was the growth regulator auxin (A), which consisted of three concentration levels: a1 = 0 mg L-1, a2 = 0.5 mg L-1, a3 = 1 mg L-1. The second factor was cytokinin growth regulators (B) with 3 concentration levels, namely b1 = 0 mg L-1, b2 = 3 mg L-1, b3 = 6 mg L-1. This study consisted of 9 treatment combinations and each treatment consisted of 3 replications, so there were 27 experimental units. Each experimental unit consisted of 3 bottles, each bottle consisted of 1 explant, so that the total explants required were 81 seed embryo explants. The day the roots appeared showed that the treatment factor A (IBA) at level b1 showed a1 was significantly different from a2 and a3. Treatment factor A at levels b2 and b3 showed that a1 was not significantly different from a2 and a3. Treatment factor B (BAP) at level a1 showed b1 was significantly different from b2 and b3, b2 was not significantly different from b3. Treatment factor B at levels a2 and a3 showed b1, b2 and b3 were not significantly different. Based on the results of statistical analysis of the number, height shows that treatment factor A (IBA) at level b1 shows no significant difference a1, a2 and a3. Treatment factor B (BAP) on number, height, diameter, fresh weight and dry weight of shoots at level a1 showed that b1 was not significantly different from b2 and b3, b2 was not significantly different from b3. Based on the results of statistical analysis of shoot diameter, it showed that treatment factor A (IBA) at level b2 showed that a1 was significantly different from a2 and a3. The best interaction for the parameter of shoot diameter occurred in the combination of treatments a1 and b2, namely IBA 0 mg L-1 (control) and BAP 3 mg L-1 (4.22 mm).

Keywords: Indole Butiryc Acid, Benzyl Amino Purine, number of days, number of shoots, shoot height, shoot diameter, shoot wet weight and shoot dry weight

Abstract
Penelitian ini dilaksanakan di Labolatorium Fakultas Pertanian Universitas Garut yang berada di Lab Fakultas Pertanian Universitas Garut. Percobaan ini dilaksanakan pada Agustus sampai November tahun 2022. Metode penelitian ini yaitu menggunakan rancangan acak lengkap (RAL) faktorial dengan 2 faktor yaitu konsentrasi zat Pengatur

tumbuh auksin/ IBA (A) dan konsentrasi Zat Pengatur Tumbuh sitokinin /BAP (B). Faktor pertama yaitu zat pengatur tumbuh degan hormon auksin (A) yang terdiri dari tiga taraf konsentrasi : a1 = 0 mg L-1, a2 = 0,5 mg L-1, a3 = 1 mg L-1. Faktor kedua yaitu zat pengatur tumbuh sitokinin (B) dengan 3 taraf konsentrasi yaitu b1 = 0mg L-1, b2 = 3mg L-1, b3 = 6 mg L-1. Penelitian terdiri dari 9 kombinasi perlakuan terdiri dari 3 ulangan, sehingga terdapat 27 unit percobaan. Setiap unit percobaan terdiri atas 3 botol, setiap botol terdiri dari 1 eksplan, sehingga total eksplan yang dibutuhkan yaitu 81 eksplan embrio biji porang. Hari munculnya akar bahwa faktor perlakuan A (IBA) pada taraf b1 menunjukan a1 berbeda nyata dengan a2 dan a3. Faktor perlakuan A pada taraf b2 dan b3 menunjukkan a1 tidak berbeda nyata dengan a2 dan a3. Faktor perlakuan B (BAP) pada taraf a1 menunjukkan b1 berbeda nyata dengan b2 dan b3, b2 tidak berbeda nyata dengan b3. Faktor perlakuan B pada taraf a2 dan a3 menunjukan b1, b2 dan b3 tidak berbeda nyata. Hasil analisis statistik pada penelitian jumlah, tinggi menunjukan, bahwa faktor perlakuan A (IBA) pada taraf b1 menunjukan tidak berbeda nyata a1, a2 dan a3. Faktor perlakuan B (BAP) pada jumlah, tinggi, diameter, berat basah dan berat kering tunas taraf a1 menunjukan b1 tidak berbeda nyata dengan b2 dan b3, b2 tidak berbeda nyata dengan b3. Hasil analisis pada diameter tunas menunjukan, bahwa faktor perlakuan A (IBA) pada taraf b2 menunjukan a1 berbeda nyata dengan a2 dan a3. Interaksi terbaik untuk parameter diameter tunas terjadi pada kombinasi perlakuan a1 dan b2 yaitu IBA 0 mg L-1 (kontrol) dan BAP 3 mg L-1 (4,22 mm).

Key words: Indole Butiryc Acid, Benzyl Amino Purine, number of days, number of shoots, shoot height, shoot diameter, shoot wet weight and shoot dry weight.

1. Introduction

Indonesia has many plants that can be used from generation to generation, one example is the porang plant. Porang is a species of the Amorphophallus genus, namely Amorphophallus muelleri Blume, which is included in the Araceae family with a distribution dominated by Asia and Africa (Anugerah et al. 2018). Porang belongs to the Araceae family and is a tuber type and can live in various soil conditions (Wati and Sunarti 2019).

Porang has a very large content of glucomannan compounds compared to the Amorphopallus plant. Glucomannan is a polysaccharide compound in the form of a gel including biomaterials such as glucose. Glucomannan in porang has many benefits in the health industry and as a food ingredient (Pasaribu 2019a). Porang is also used as an adhesive for paper, wool, cotton fabric, imitations and as a material for making paint (Sari et al. 2019). The health sector can treat tumors and diabetes as well as inflammation (Budiarti 2020). Agar and gelatin substitutes also come from the porang plant (Sari et al. 2019). Pasaribu (2019) stated that porang is used as a food source because it has a high fiber content, low carbohydrate and cholesterol content.

The many other benefits of the porang plant make this plant an export commodity. Based on data from the Indonesia Quarantine Full Automation System (IQFAST) or the quarantine agency, in 2021 porang exports reached 14.8 thousand tons, higher than in 2019, only 5.7 thousand tons (Science and Technology 2021).

The technique for getting lots of porang seeds quickly is using the in vitro tissue culture method. (Science and Technology 2021), explains that the culture method is a way of accelerating the seeds of a plant in a controlled growth medium, namely in a sterile culture bottle.

Culture using in vitro techniques, namely separating plant parts such as some cells, protoplasm, organs, tissues, and growing culture methods in a sterile environment (Yasin et al. 2021). The advantages of in vitro culture are that it can reproduce plants in a short time, is relatively uniform and can be .

Success with tissue culture modification requires the addition of hormones, growth regulators, to increase plant growth. According to Pasaribu (2019), PGR plays a role in plant growth and development and functions to influence and control plants from the vegetative phase to the generative phase.

A combination of the hormone auxin and the hormone cytokinin can be used as an ingredient to increase root growth (Science and Technology 2021). According to Nirmala et al. (2016), stated that high concentrations of auxin and cytokinin can make plantlet root growth faster, while the balance between these two hormones, endogenously and exogenously, greatly influences plant growth and development. This research will use porang seed explants which will be planted in MS media with a combination of various concentrations of IBA and BAP.

2. Materials and Methods

Experimental research was carried out in the Laboratory of the Faculty of Agriculture, Garut University, which is located in the Laboratory of the Faculty of Agriculture, Garut University. This experiment was carried out from August to September 2022. The materials for this research were explants from porang seed embryos, Murashige and Skoog basic media, auxin growth regulator (IBA), cytokinin growth regulator (BAP), water, 70 percent alcohol, disinfectant, Sodium Hydroxide (NaOH), Hydrochloric Acid (HCl), agar-agar, distilled water. The tools used in the research were culture bottles, scalpels, laminar air flow/enkas (LAF), tweezers, bunsen, culture rack, air conditioner (AC), plastic, rubber, aluminum foil, petridish, spray bottle, label paper, microscope, lighters, writing instruments, magnetic stirrers, beaker glasses, pH meters, Erlenmeyer glasses, measuring cups, analytical scales, autoclaves and cameras. Statistical analysis used the factorial Completely Randomized Design (CRD) method which consisted of 2 factors, namely the concentration of the Growth Regulator Substance auxin/IBA (A) and the concentration of the Growth Regulator Substance cytokinin/BAP (B).

3. Results and Discussion

Time of Shoot Emergence

The statistical results showed that there was no interaction between IBA and BAP concentrations on the emergence of shoots at planting age of 46 HST. The results of the analysis of the average time of shoot emergence can be seen in Table 1.

Table 1. Time of Shoot Emergence

Treatment	Average Time of Shoot Emergence (days)

IBA concentration	
a1 (Control)	1.61 a
a2 (0.5 mg L-1)	1.37 a
a3 (1 mg L-1)	1.30 a
BAP concentration	
b1 (Control)	1.57 a
b2 (3 mg L-1)	1.37 a
b3 (6 mg L-1)	1.34 a

Note: The average number followed by the same letter in each column is not significantly different according to Duncan's Multiple Range Test at the 5% level.

Based on the results of statistical analysis of the observed parameters of shoot emergence time, it shows that the concentrations of IBA and BAP independently show no significant differences from each other.

Number of Shoots

Statistical results showed that there was no interaction between IBA concentration and BAP on the number of shoots at planting age of 46 HST. The results of the analysis of the average number of shoots are in Table 2.

Table 2. Results of Statistical Analysis of Number of Shoots

Treatment	Average Number of Shoots
IBA concentration	
a1 (Control)	1.44 a
a2 (0.5 mg L-1)	1.22 a
a3 (1 mg L-1)	1.22 a
BAP concentration	
b1 (Control)	1.22 a
b2 (3 mg L-1)	1.56 a
b3 (6 mg L-1)	1.11 a

Note: Numbers followed by the same letter in each column are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Based on the results of statistical analysis, it shows that there are no significant differences between each other in the characteristics of the number of shoots.

Shoot Height

The statistical results for shoot height did not occur between the addition of various combinations of IBA and BAP on shoot height, namely planting age 46 HST. The results of the analysis of average shoot height can be seen in Table 3.

Table 3. ResultsStatistical Analysis of Shoot Height

Treatment	Average Shoot Height
IBA concentration	
a1 (Control)	0.69 a
a2 (0.5 mg L-1)	0.84 a
a3 (1 mg L-1)	0.86 a
BAP concentration	

b1 (Control)	0.73 a
b2 (3 mg L-1)	0.86 a
b3 (6 mg L-1)	0.80 a

Note: The average number is followed by the same letter in each column and is not significantly different according to Duncan's Multiple Range Test at the 5% level.

Based on the results of statistical analysis of the parameters for observing the number of shoots, the factors with the addition of IBA (A) at levels a1, a2 and a3 were not significantly different to all factors B. The results of the study showed that the administration of IBA growth regulators did not occur at treatment levels a1, a2 and treatment level a3.

Bud Diameter

The results of the analysis showed that there was an interaction between IBA and BAP concentrations on shoot diameter characteristics at 46 HST. The results of statistical analysis of the average days of shoot emergence are in Table 4.

Table 4. Analysis ResultsShoot Diameter Statistics

Treatment	Average Shoot Diameter BAP (B)		
IBA (A)	b1 (control)	b2 (3 mg L-1))	b3 (6 mg L-1))
a1 (control)	2.52 a A	4.22 b B	2.60 a A
a2 (0.5 mg L-1)	3.03 a A	3.25 a A	4.02 a B
a3 (1 mg L-1)	3.52 a A	2.90 a A	3.67 a A

Note: The average numbers followed by the same lowercase letter in the vertical direction and the same uppercase letter in the horizontal direction are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Table 4 shows that the a1b2 treatment provides better shoot diameter than the other treatments. The highest average of shoot diameter parameters occurred in the combination of treatments a1 and b2, namely IBA0 mg L-1 (control)and BAP3 mg L-1(4.22mm).

Shoot Wet Weight

The statistical results of shoot wet weight did not occur between the addition of various combinations of IBA and BAP on shoot wet weight at planting age of 46 HST. The results of data analysis on the average wet weight of shoots are in table 5.

Table 5. Results of Statistical Analysis of Shoot Wet Weight

Treatment	Average Shoot Wet Weight
IBA concentration	
a1 (Control)	180.61 a
a2 (0.5 mg L-1)	203.07 a
a3 (1 mg L-1)	189.44 a

BAP concentration	
b1 (Control)	180.70 a
b2 (3 mg L-1)	188.83 a
b3 (6 mg L-1)	203.59 a

Note: Numbers followed by the same letter in each column are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Based on the results of statistical analysis of the observation parameters of shoot wet weight, the results showed that the results were not significantly different from each other.

Shoot Dry Weight

The results of statistical analysis showed that there was no interaction between IBA and BAP concentrations on shoot fresh weight at planting age of 46 HST. Results of data analysis of average dry weight of shoots in Table 6.

Table 6. Results of Statistical Analysis of Shoot Dry Weight

Treatment	Average Shoot Dry Weight
IBA concentration	
a1 (Control)	20.69 a
a2 (0.5 mg L-1)	15.36 a
a3 (1 mg L-1)	13.63 a
BAP concentration	
b1 (Control)	9.89 a
b2 (3 mg L-1)	22.90 a
b3 (6 mg L-1)	16.89 a

Note: Numbers followed by the same letter in each column are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Based on statistical results, it shows that there are no significant differences between each other in the observed parameters of shoot dry weight($F_{count}<F_{0.05}$).

Discussion

The results of the analysis were that there was no interaction between IBA and BAP concentrations for all observed characters except for shoot diameter. The best shoot diameter characteristics were obtained in the a1b2 treatment (IBA concentration 0 mg L-1 and BAP mg L-1).

This interaction occurs due to the addition of PGR, as we can observe that a1b1 shows the highest value in the observation parameter for the time of shoot emergence (2.03 days) which means that media without treatment with the addition of PGR, either auxin or cytokinin, takes longer to produce shoots than the level other treatment. On the other hand, a3b3 proved the lowest value which proves that the addition of IBA and BAP zpt had an influence on the time of shoot emergence, namely 1.22 days.

Based on the DMRT test results, the addition of IBA and BAP concentrations gave the best results for the variable time of shoot emergence. This proves that the exogenous hormones in plantlets are able to meet the needs of plantlets to fulfill their growth. According to Ulya et al., (2020) the cytokinin hormone plays a role in stimulating plant development, for example the growth of shoots and leaves. As in Budiman's research (2021), BAP gives the best results to stimulate the emergence of stevia shoots with an average of 3.05 HST. Sridhar& Naidu (2011) also showed that BAP is better than other types of cytokinin for stimulating shoot emergence.

The interaction of the auxin hormone and the cytokinin hormone is a primary link for plant development, a higher concentration of auxin compared to cytokinin influences root development. The same concentration of cytokinin and auxin forms a lot of callus (Ulya et al., 2020). Not only does increasing the number of shoots the most, it can also increase cytokinin activity which will further increase the effectiveness of cell separation.

This happens because cytokinins have two important roles in in vitro propagation, namely triggering cell separation in tissue and triggering shoot development. The interaction between cytokinins and auxins, so that when used simultaneously, you must think about the appropriate content or ratio in the media (Samantha & Almalik, 2019).

The a1b2 level in the shoot diameter observation parameter is significantly different from the other treatment levels and is the highest average value, namely 4.22 millimeters. This happens because the added concentration of BAP causes the physiological processes of the explant to run efficiently in spurring the beginning of shoot development. This makes it possible for each plant to have a different hormone accumulation response for each concentration given. In accordance with Samantha& Almalik (2019) the resulting hormonal response is influenced by the concentration and type of plant. This is caused by the comparison of the concentration of endogenous hormones in the plant itself. So the speed of development that occurs in the explant is due to the appropriate interaction between endogenous hormones and exogenous hormones which causes the physiological processes in the explant to be efficient so that they can stimulate the start of shoot development.

The speed of development that occurs in shoots is due to the appropriate interaction between the media, endogenous hormones and the accumulation of exogenous growth regulators (PGRs). Balancing the concentration of BAP added to the media results in physiological processes in the explants being able to run efficiently in spurring the start of shoot development (Samsudin, 2020).

The IBA and BAP zpt addition treatment proved that there was no interaction and independent influence on the observed parameters of shoot number, shoot length, shoot fresh weight and shoot dry weight. This is suspected to contain endogenous growth regulators in the explants and their content is sufficient for the development of these parameters. Even though there was no interaction, it can be seen that on average in the treatment the number of b2 shoots (3 mg L-1) had an average value of 1.56, the height of b2 shoots (3 mg L-1) had an average of 0.86, The wet weight of B3 shoots (6 mg L-1) with an average value of 203.59 and the dry weight of B2 shoots (3 mg L-1) with an average value of 22.90 proves that the average value is greater than the other treatment levels. .

The results of the research above occur because PGR in tissue culture depends on the type of plant used and the purpose of the activity or the desired direction of plant development. The formation of shoots with tissue culture increases the success of producing fast and numerous seeds. The number of shoots formed is positively correlated with the seeds that can be produced by the tissue culture method so that it can stimulate the large shoot multiplication factor required for PGR accumulation.

There is a level of treatment that proves that no interaction occurs because IBA is a phytohormone from the auxin group which is one of the hormones that has the ability to induce the formation of callus, suppress the formation of callus or shoot morphogenesis, suppress the embryogenesis process and influence the genetic stability of plant cells (Suheriyanto & and Ruri Siti Ahlisari, 2012). IBA is also a synthetic auxin derivative of IAA which does not cause plants poisoning at large concentrations and is effective in helping plant roots (Budiman, 2021).

The role of cytokinins in plants is to control cell division, organ formation, cell and organ enlargement, preventing the destruction of chlorophyll, making chloroplasts, delaying senescence, opening and closing of stomata, and the growth of buds and shoots (Ans et al., nd). The accumulation of BAP at low concentrations of 2.5-5µM allows the growth of adventitious shoots on sweet oranges (Merigo, 2011). Saepudin et al (2022) report that plant metabolic capabilities are highly dependent on plant genetic capabilities (endogenous aspects). Samsudin (2020) reports that the interaction of auxin and cytokinin is a primary link in plant development, a high concentration of auxin from cytokinin forms more root development and a balanced auxin and cytokinin hormone tends to form callus. On the other hand, the greater the amount of cytokinin compared to auxin, plant development is more likely to produce shoots.

Saepudin et al (2022) reported that the natural cytokinin hormone contained in explants can trigger the media for shoot formation. A low ratio of the hormones auxin and cytokinin will create an imbalance in the explant and cause shoot production to be hampered.

SCytokinin has two important roles in in vitro propagation, namely stimulating shoot growth and stimulating cell division in tissue and. Cytokinin interacts with auxin, so concurrent use must consider the appropriate levels and ratios in the media (Wetherell, 1982). The results of this research are in accordance with research by Georgeo & Sherrington (1984) that ZPT with the right concentration can have an influence on the wet weight of the culture. Cytokinin combined with auxin can stimulate cell enlargement resulting in increased culture weight, especially by increasing water absorption by plant cells (Katuuk, 1989).

Rahayu et al (2003) reported that the large fresh weight of shoots was due to the large water content. Development and morphogenesis in vitro are influenced by the interaction and balance between PGRs added to the media and growth hormones produced endogenously by cultured cells (George and Sherrington, 1984). According to Ruswaningsih (2007), physiologically fresh weight consists of two contents, namely water and carbohydrates.

Not only the PGR accumulation aspect, different explant net weights can also be caused by the choice of explant used and contamination of the media or explants that occurs during the research process. Explants originating from porang seed embryos have different genetic conditions, so growth will not be uniform. This is the same as Gunawan's research (1987), that different plant cells and organ tissues can react differently to the culture media used.

According to Salisbury & Ross(1995) that ZPT given in the right concentration can affect the weight of the culture. The interaction of these developmental substances and ZPT will increase the wet weight of the culture (Wareing & Philips, 1981 in Butar-butar, 2006). The results of this research are in accordance with the opinion of Georgeo& Sherrington (1984) states that the ZPT given must be in the right concentration to be able to influence the wet weight of the culture. Another opinion is that if cytokinin is combined with auxin it can stimulate cell enlargement which is accompanied by an increase in culture weight, especially by increasing water absorption by the culture cells (Katuuk, 1989).

Salisbury& Ross (1995) reported that ZPT given in the right amount could have an influence on culture weight. The interaction of these developmental substances and PGR will increase the wet weight of the culture (Wareing & Philips, 1981 in Butar-butar, 2006).

4. Conclusion

Based on the experimental results and discussion, the following conclusions can be drawn: There is an interaction between IBA and BAP concentrations on shoot diameter parameters. IBA and BAP concentrations did not show any interaction with plantlet dry weight parameters, so there was no optimum IBA concentration at each BAP concentration that produced the highest plantlet dry weight for porang. To obtain the highest shoot diameter, it is recommended to use an IBA concentration of 0 mg L-1 and BAP of 3 mg L-1. Further research was carried out including adaptation and field tests from the results of porang seed embryo culture.

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