In-vitro Callus Induction of Durian (Durio zibethinus Murr.) Leaves Using Kinetin and 2,4-D (Dichlorophenoxyacetic acid)

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RESEARCH ARTICLE

In-vitro Callus Induction of Durian *(Durio zibethinus* Murr.*)* Leaves Using Kinetin and 2,4-D *(Dichlorophenoxyacetic acid)*

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ABSTRACT

Durian (*Durio zibethinus* Murr.) is a tropical fruit grown in Southeast Asia and it has high nutritional and economic values and highly appreciated by consumers in Indonesia. Unfortunately, there are several obstacles in planting durian in Indonesia, specifically in Aceh. One of the abstacles is the scarcity of superior durian plants. Generally, plant conservations done conventionally through vegetative propagation. However, this technique has disadvantage such as harming the initial plants. Therefore, plant culture tissue has been used as a modern technique to develop durian plants. The objective of this research was to evaluate the effect of kinetin and 2,4 D on development of durian leaf explants through in-vitro bioassay. The research was conducted at Tissue Culture Laboratory, Faculty of Agriculture, Malikussaleh University from January to April 2019 using Completely Randomized Design (CRD) Factorial with 2 factors observed. The first factor was Kinetin (K): 0.0, 0.1 and 0.5 ppm. The second factor was auxin 2,4 D: 0.0, 0.5 and 1.0 ppm. The results showed that the in-vitro application of kinetin affected the induction of leaf callus. The application of 0.5 ppm of kinetin gave the best result compared to others. The in-vitro application of 2,4 D 0.5 ppm also possessed the best result compared to others. There was no interaction beween in-vitro application of kinetin and 2,4 D on the growth of durian leaf callus at all parameters observed.

1. INTRODUCTION

Durian (*Durio zibethinus* Murr.) is one of horticultural commodities which possess high nutritional and economic values and it plays an important role in increasing national economy in Indonesia. Durian is known as *The King of Fruits*, originated from Southeast Asia (Feng *et al.,* 2016). This fruit is considered as a prospective commodity not only for Indonesian people but also for export activity. Aceh is a province in Indonesia which its cities has durian production centers. Those cities are North Aceh, Pidie and Bireuen (Deptan, 2012). The production centers in North Aceh are located in Sawang, Buloh and Cot Girek.

Durian plants produced in North Aceh possess genetic diversity which can be seen from its vegetative morphology and also its quality. This diversity offers the uniqueness and it has enriched the genetic diversity of Aceh durians. This plant has unique flavor and strong odour. Until today, researches about Aceh durian are still scanty. Limited information about this plant in Aceh is causing weak protection of local genetic resources in Aceh which resulted in extinction of Aceh

durians or led to emblezzement of genetic resources by other parties.

One obstacle in durian cultivation in Aceh is the extinction of initial plants. Generally, the preservation is done conventionally through vegetative propagation. However, this technique has disadvantages such as harming the initial plants. To prevent the extinction of this genetic resource, the modern technique is needed. One of them is through in-vitro bioassay. This method is believed to produce better seeds and identical to its parental plants (true to type) (Yunus *et al.,* 2010).

There are many factors affecting the success of in-vitro bioassay, for example the application of growth regulators (Sugiono dan Hasbianto, 2014) and the source of explants. There are 2 types of growth regulator used in in-vitro bioassay, such as cytokinin and auxin. The use of auxin together with cytokinin enables us to decide what type of morphogenesis we want to have. 2,4-D (type of auxin) dan kinetin (type of cytokinin) are synthetic growth regulator which generally used in tissue culture researches. Plant researches about propagative technique in-vitro in plants have been done by many researchers, for instance in mangosteens (Handayani et al., 2013), orchid *Phalaenopsis* (Anjani, 2011), sugarcanes (Mekonnen *et al*., 2013) and durians (Sugiyarto dan Kuswandi, 2013; Handayani *et al*., 2018).

The application of combination of growth regulators 2,4 D and kinetin to culture media can stimulate the callus formation in cinnamon in various concentrations of 2,4 D and kinetin. The application of I mg/L of 2,4 D and 0,1 mg/L demonstrated the fastest formation of callus. The application of 2 mg/L 2,4 D and 1.0 mg/L possessed highest weight of callus in cinnamon explants (Teguh, 2017). The best direct somatic embryo induction in Arabica coffee variety Kartika-1 from young leaf explants obtained from MS media treated with 4 mg/L 2,4 D combined with 0.1 mg/L kinetin (Riyadi dan Tirtoboma, 2004). The application of 0.5 mg/L of 2,4 D and 1 mg/L of kinetin gave the best result on rodent tubers (*Typhonium flagelliforme* (L.) BI) (Sitinjak *et al,* 2015).

Different parts of plants are used as explants for micropropagation such as shoot tip, nodal and intermodal segments, shoot meristem, leaf disks and floral parts (Yusnita, 2003; Chugh *et al.,* 2009), seeds and micro shoots (Handayani et al., 2013). Plant part used as explants to produce callus in durian plants is its leaves. The young leaves are most preferred as regeneration is often obtained from young or actively growing organ.

2. MATERIALS AND METHODS

2.1 Place and duration

This research was conducted in Tissue Culture Laboratory, Faculty of Agriculture, Malikussaleh University, North Aceh from March to June 2019.

2.2 Research methods

The materials used in this research were young leaves obtained from durian seedbeds in North Aceh, alcohol, bleaching liquid, sugar, fungicides, bactericides, spiritus, agar, distilled water, 2,4 D, kinetin, MS media, and $HgCl_2$. Tools used in this research were *Laminar Air Flow Cabinet* (LAFC), *autoclave*, *hotplate*, dan planting tools.

This research used Completely Randomized Design (CRD) Factorial with 2 factors replicated 10 times. The first factor was different concentrations of kinetin (0.0, 0.1 and 0.5 mg/L) and the second factor was different concentrations of 2,4 D (0.0, 0.5, dan 1 mg/L).

Durian seeds were sown in sand seedbeds (polybags) vertically with position of rootlets facing the ground. 2-month durian seedlings were applied as explants. Durian leaf explants have been washed with distilled water for 15 minutes and been soaked in detergent liquid 15 minutes, then continued with soaking each of them with fungicides/bactericides 8 g/L for 20 minutes and then rewashed them with distilled water. Explants then being soaked into alcohol liquid 70% for 3 minutes and been washed with distilled water. Explants then have been soaked into $HgCl_2$ (concentration of 0.05%) for 20 minutes and been washed using distilled water and been put onto sterilized papers.

Sterilized explants have been cut with removing its both end tips. Middle part of leaves has been cut sized 1x1 cm, and then planted into prepared growing media. Durian leaf disks was planted into growing media with position of lower leaves facing the growing media

(abaxial). The observation was done to evaluate the callus growth, growth time, percentage of developing callus and callus colour.

3. DATA ANALYSIS

Data obtained from this research was analysed using F test and if the means were significant at probability level 5%, the analysis continued using Duncan's Multiple Range Test (DMRT) at probability level 5%. The data analysis has been assigned using software SAS v9.12.

4. RESULTS AND DISCUSSIONS

The results showed that there was no interaction between concentrations of kinetin and 2.4 D to all parameters observed. However, the application of kinetin and 2,4 D gave an effect to callus growth. The results of percentage of callus growth have been decribed in Table 1.

Table 1. The percentage of callus growth on durian leaf explants treated with kinetin and 2,4 D in vitro

Treatments	Callus growth (%)				
	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP
Concentrations of kinetin (K) :					
0 mg/L (K0)	32 (0.88 a)	46 (0.95 a)	57 (1.00 a)	57 (1.00 a)	67 (1.06 a)
0.1 mg/L (K1)	11 (0.74 b)	14 (0.78 b)	35 (0.90 a)	53 (0.99 a)	67 (1.07 a)
0.5 mg/L (K2)	10 (0.76 ab)	17 (0.80 ab)	32 (0.88 a)	53 (0.99 a)	57 (1.01 a)
Concentrations of 2,4-D (D) :					
0 mg/L (D0)	0 (0.70 a)	0 (0.70 b)	6 (0.74 b)	6 (0.74 c)	6 (0.74 b)
0.5 mg/L (D1)	20 (0.82 a)	44 (0.94 a)	61 (0.03 a)	82 (1.14 a)	88 (1.17 a)
1 mg/L (D2)	20 (0.81 a)	20 (0.81 ab)	38 (0.91 a)	50 (0.97 b)	67 (1.07 a)

Mean values followed by the same letters in the same columns did not differ significantly as determined by DMRT at probability level 5%. Values in parenthesis were results of transformation using formula $\sqrt[2]{(x+0,5)}$ WAP = Weeks After Planting

Table 1 illustrated that the application of kinetin has increased the callus growth at 3 and 4 weeks after planting. The application of kinetin 0 mg/L (K0) and 0.1 mg/L (K1) demonstrated highest percentage of callus growth compared to application of kinetin 0.5 mg/L (K2).

Also, the application of 2.4 D statistically improved the callus growth. The application of 2,4 D 0.5 mg/L (D1) and 1 mg/L (D2) possessed the highest percentage of callus growth compared to 2,4 D 0 mg/L (D0). Generally, explants will have different reactions towards their developments on each observation. The results were presented in Figure 1.

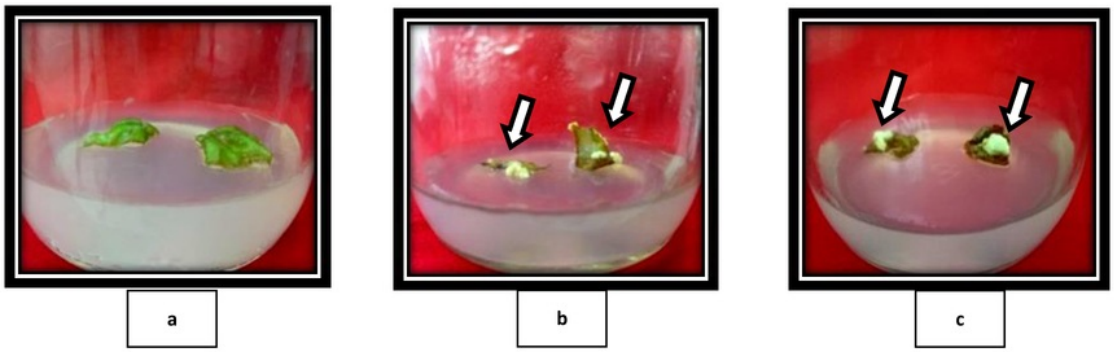

Figure 1. The growth of durian leaf explants. Initiated explants (a); Callus appeared on explants (b); explants with developing callus on them (c).

Figure 1a showed a planted explant. This explant did not show sign of growth at the earlier observation. Figure 1b showed induced callus explant. Generally, explants induced callus at 4-7 weeks after application. Figure 1c showed an explant at the end of observation which had bigger callus and its color dried on the leaf tip. Most of leaf explants appeared to be swollen at first before forming the callus.

The growth of callus on explants is one of indicators of successful application in plant culture. Callus is a growing mass of unorganized cells (Indah dan Ermavitalini, 2013). Callus goes through 3 (three) stages in its development such as induction, cell division and differentiation (Zulkarnain dan Lizawati, 2011). Growing callus needs growth regulator such as auxin to induce the callus. Auxin is a plant growth regulator which contributed to development of plant cells, phototropism, geotropism, apical dominance, root growth, parthenocarpy, abscission, callus formation and respiration. 2,4-D (2,4-Dichlorophenoxyacetic Acid) is an auxin often used to stimulate the formation of callus. The application of 2,4 D affect the growth of callus (percentage of callus growth). Auxin increases the cell growth, cell division and development of adventive roots. Auxin is needed in culture media to improve somatic embryogenesis on cell suspension cultures. High concentration of auxin will stimulate callus formation and suppress morphogenesis (Marlin *et* al., 2012).

The results revealed that the application of kinetin and 2,4 D both alone and combination did not differ significantly on callus growth period. The results were given in Table 2.

Table 2. Time length of callus growth on durian leaf explants treted with kinetin and 2,4 D in vitro

Treatments	Callus growth period (DAP)
Concentrations of Kinetin (K) :	
0 mg/L (K0)	26.96 (5.09 a)
0.1 mg/L (K1)	34.41 (5.86 a)
0.5 mg/L (K2)	29.86 (5.43 a)
Concentrations of 2,4-D (D) :	
0 mg/L (D0)	30.66 (5.52 a)
0.5 mg/L (D1)	27.67 (5.23 a)
1 mg/L (D2)	32.73 (5.63 a)

Mean values followed by the same letters in the same columns did not differ significantly as determined by DMRT at probability level 5%. Values in parenthesis were results of transformation using formula $\sqrt[2]{(x+0,5)}$ DAP = Days After Planting

Table 2 illustrated that the application of growth regulator kinetin did not affect callus growth period. Also, the application of 2.4 D did not influence the period of callus growth. Time length for callus to grow on leaf explants of durian depends on the explants. Each explant has different reaction when absorbing nutrients and also it depends on how much growth regulator given to explant. This statement is in line with Mandang (2013) *in* Mayasari (2017) that the application of growth regulators in small amount can help or inhibit the growth of explants.

The results of F test revealed that concentrations of kinetin and 2,4 D have improved the callus development. The results of DMRT at probability 5% were presented in Table 3.

Table 3. Percentage of callus development on durian leaf explants treated with kinetin and 2,4 D in vitro.

Treatments	Percentage of callus development (%)				
	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP
Concentrations of Kinetin (K) :					
0 mg/L (K0)	(0.72 a)	3 (073 a)	6 (0.75 a)	9 (0.76 a)	12 (0.78 a)
0.1 mg/L (K1)	0 (0.70 b)	0 (0.70 b)	1 (0.71 b)	3 (0.73 b)	8 (0.76 b)
0.5 mg/L (K2)	0 (0.70 b)	0 (0.71 b)	3 (0.73 ab)	8 (0.76 ab)	13 (0.79 a)
Concentrations of 2,4-D (D) :					
0 mg/L (D0)	0 (0.70 a)	0 (0.70 a)	0 (0.70 a)	0 (0.70 b)	0 (0.70 b)
0,5 mg/L (D1)	1 (0.71 a)	1 (0.72 a)	5 (0.74 a)	10 (0.77 a)	16 (0.81 a)
1 mg/L (D2)	1 (0.71 a)	2 (0.72 a)	4 (0.73 ab)	7 (0.75 a)	11 (0.78 a)

Mean values followed by the same letters in the same columns did not differ significantly as determined by DMRT at probability level 5%. Values in parenthesis were results of transformation using formula $\sqrt[2]{(x+0,5)}$ WAP = Weeks After Planting

Table 3 showed that the application of kinetin has generated the callus development. The application of kinetin 0.5 mg/L (K2) demonstrated higher percentage of callus development compared to other treatments. The application of kinetin 0.5 mg/L in this research gave higher callus growth compared to other concentrations. The application of kinetin in a culture media promotes callus growth. Also, the application of cytokinin plays an important role in stimulating the growth and development of explants. Limited amounts given to a culture media could inhibit the cell division of cultured tissue (Zulkarnain, 2009).

The application of 2,4 D possessed an effect to development of callus. Concentration 0.5 mg/L of 2.4 D (D1) contributed better callus growth compared to other concentrations. It indicated that the application of 0.5 mg/L (D1) has induced callus on durian leaf explants, while the lowest concentration of 2,4 D (D1) demonstrated the lowest result.

This result corroborates with a statement that application of auxin to an MS media has induced callus growth on durian leaf explants (Sugiyarto dan Kuswandi, 2013). High concentration of auxin needed to stimulate callus formation (Marlin *et al.*, 2012). This high concentration of auxin will stimulate callus formation and inhibit morphogenesis. It also increases the activity of auxin helping the explants to induce callus in this research. The color of the callus will generally change if certain reactions occur. Reactions that can cause callus discoloration, for example, the loss of chlorophyll compounds from the callus that causes the color of the callus to turn greenish white or other colors. The colors of callus were shown in Figure 3.

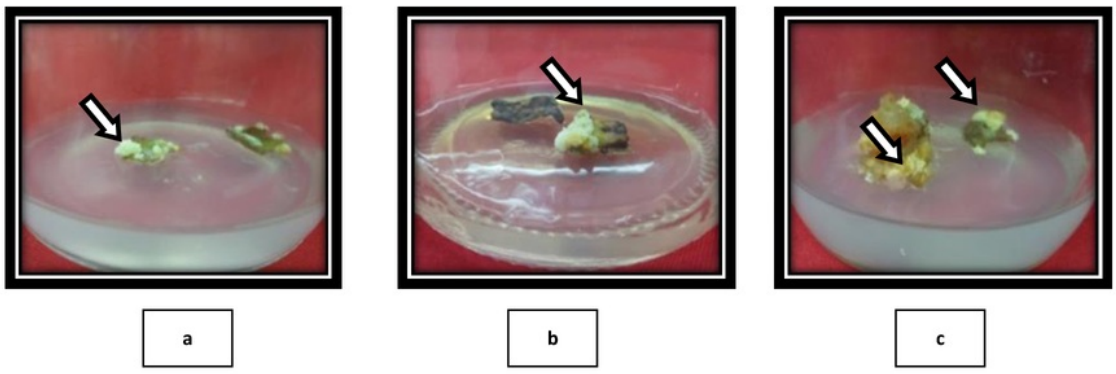

Figure 3. Callus colors. white (a); cream (b); brown and green yellowish (c).

Figure 3 revealed that each explant has induced callus with different color and shape. These differences influenced by active compounds contained in the explants or media.

Callus growth on durian leaf explants started in white color. However, this colour of callus changed with time, associated with several reactions occurred in explants or planting media of explants. The colour changes were classified into the following categories: white, milky white, brown and green yellowish. These categories of colour indicated that each treatment demonstrated different respons shown in different colors.

The color of the callus is dominated by white, which indicates that the callus is an embryonic tissue that does not yet contain chlorophyll, but has starch content in the form of stored polysaccharides in plants (Muliati *et al.,* 2017; Ariati *et al.*, 2012). Greenish white callus was probably the brightest callus with lesser amount of chlorophyll compounds (Widyawati, 2010).

The green color in callus was linked to cytokinin activity in chlorophyll formation (Rahayu dan Mardini, 2015). Callus color indicates the availability of chlorophyll compound in plants. The formation of green callus indicating the presence of chlorophyll in high amounts (Fatmawati, 2008). The changes in callus color from yellow to greenish yellow, followed by the formation of spots or green nodules, that was a sign of shoot formation (Lestari dan Yunita, 2008). If callus color turns into brown or dark colour, it indicates that callus has problem called browning callus, which caused by accumulation and and oxidation of phenolic compound formed in explants or callus. Callus browning suppressed explant growth and inhibited callus formation

In this research, not all explants has grown well, some of them has turned into yellow color and eventually died due to inappropriate selection of type of explants, concentration of growth regulators and improper balance of disinfectant used in sterilization stage. It influenced the success of callus formation. (Santoso dan Fatimah, 2014). Therefore, explants will have difficulties in inducing callus if there is unbalanced application of growth regulators. Explants will not be able to grow and die.

CONCLUSION

The application of kinetin has influenced the callus induction on durian leaf explants in vitro. The application of kinetin 0.5 mg / L (K2) demonstrated the best effect compared to other concentrations. The application of 2,4-D also gave a positive effect to callus induction on durian leaf explants through in vitro bioassay. The provision of 2,4-D 0.5 mg/L (D1) revealed the best concentration compared to others. There was no interaction between kinetin and 2,4-D applications to the callus growth on durian leaf explants in vitro in all variables observed.

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