Sterilization Method For In Vitro Propagation Explant Embryo Of Durio Kutejensis (Hassk.) & Becc From Kalimantan

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Abstract: Durio kutejensis the local durian forest result Kalimantan that has not been widely known outside of the island and not much cultivated. One of the obstacles of plant propagation is seedling superior quality are not available. Propagation through the technique of in vitro (Tissue culture) in plants Durio kutejensis (Hassk) & Becc not been widely tested, because the woody plants have grown a great degree of difficulty. Commonly cultivated plants through in vivo techniques. Sterilization is an important part of the culture in vitro. Sterilization of models have been tried, to overcome the initial barriers of culture. The development of in vitro embryo culture D. kutejensis through embryo explants using Murashige and Skoog (MS) with the addition of growth regulators GA_3 (gibberellic acid) and BAP (Benzilaminopurine) produces elongation of hypocotyl added shoot growth and root elongation. Callus formed from embryonic explants green and yellowish white with compact texture on the addition of growth regulators BAP and 2.4 D 1gr/l. Callus formation has not been followed by the development of embryogenesis and not yet differentiation into shoots and roots.

Key words: Durio kutejensis (Hassk) & Becc, Sterilization Method, Propagation in vitro, Embryo explant

I. Introduction

Durio kutejensis (Hassk.) Becc or local durian, Bombacaceae family. Lai is endemic in the forests of Borneo origin. Fruit flavor is sweet and sticky and flavorful soft fruit [3]. Appearance approached Durio zibethinus (durian). The difference between the two seen in the flesh and skin color. D. kutejensis yellowish fruit, soft fruit leather spines, fluffier pieces of meat in a bright yellow to orange, while the durian has a white to yellowish flesh and skin sharp thorns. Light yellow to dark red color on the fruit, which is a carotenoid compound carotene and xantofil. Carotenoids are a source of immune response that act as antioxidants [7]. Antioxidants play an important role for the defense of the body and the side effects caused by free radical antioxidant compounds derived from plants such as vitamin C, vitamin E, carotenoids, phenolic acids, polyphenols, and flavonoids have a very important role in health [23;14]. Antioxidants also defend themselves from predators, competitors and to support the process of reproduction [12]. [1] Reported the antioxidant properties of the fruit pulp extract D. kutejensis has antioxidant properties and potential to treat From the village Batuah Kutai regency in East Kalimantan, has released three varieties of Hyperpigmentation and can be used as a skin lightening agent. D. kutejensis seeded fruit like national: lai Batuah, lai Kutai and Lai Mahakam. Each variety has superior properties are different from each other [19].

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Aspects of the potential of plants as a source of antioxidants and efforts D. kutejensis breeding. Excess of three varieties of the sweet taste, thicker meat and meat color yellow to bright orange [24]. D. kutejensis fruit is a seasonal fruit with fruit once a year, the fruit produced also varies. The result is still limited, tree crops are still not widely known, especially outside the island of Borneo. Plant D. kutejensis not widely cultivated and durian. Fruit production so far are not sufficient to meet market demand, both locally and for the fulfillment of production to other islands. The quality of the fruit in the market is still very diverse, but the fruit traits of seed production of superior plants [21]. The diversity of germplasm is the basis for breeding and cultivation. The main problem in the development potential of plants D. kutejensis is the availability of quality seeds is still limited. Seed varieties and clones of the parent tree crops available is very limited. Knowledge of how to plant propagation D. kutejensis very limited, and growing is still a problem, they are the result of heredity or natural plant still grows wild and only a small portion that has been cultivated in the form of garden plants [27]. Time to bear fruit after planting takes a long time, the nature of the Derivative will be Known after fruiting plants Factors also be the cause of the disease, why farmer are reluctant to grow this fruit. Fruit seeds can not be stored longer and easily grow into new plants. The downside of this plant is the result of the derivative is not necessarily the same as the parent plant or may not be consistent with the desired seed [5]. Germplasm required for the preservation of plants. Breeding plants through tissue culture is less, because of the high degree of difficulty [10]. Explants woody plants often secrete phenol compound causes browning [30].

2. Materials and Methods

2.1. Plant Material

Plants from the village Durio kutejensis Batuah Loa Janan, Kutai Regency, East Kalimantan, Indonesia.

2.2 sterilization Eksplan

Sterilization through two stages of treatment, sterilization outside using detergent for 30 minutes, soaking explants with the fungicide and bactericide for 60 minutes and was followed by washing with running water for 30 minutes. Stages sterilization resume in laminar air flow by soaking the explants in 70% alcohol for 3 minutes, dettol 20% 10 'and dettol 10% for 5' adds tween 3 drops and soaking in Betadine 10 minutes (Table 1) The test results are then used to process sterilization of explants in this study. Dettol liquid used is the active ingredient kloroksilenol antiseptic (C8H9ClO) 48% w / v acts as an antiseptic to address the problem of infection at the incision. Dettol used also serve to accelerate the wound healing process occurs and helps reduce plant stress level. After the sterilization process continued with the planting of explants on MS medium without the addition of PGR and embryo cultures were incubated in a room without lights for 14 days, to reduce the trigger explants from browning.

2.3. Planting Eksplan

Explants derived embryos, grown in culture medium MS 0 and incubated in the dark without a light culture room to prevent browning of the culture or the culture media. After going through a pre- condition of 10-14 days after planting , healthy media without browning and uncontaminated immediately transferred to a culture with lighting. ± 1000-2000 lux light intensity, temperature of 20°-25°C . Observations explants sterilization parameters are calculated using the following formula :a. Fresh Percentage (%): observed explants were fresh, not experience. Changes both in the media and on explants

$$\text{Fresh Percent (\%)} = \frac{\text{Number of fresh explants}}{\text{Total number of explants}} \times 100$$

b. The percentage of contamination (%): Do the observation and calculation of the Percentage of contaminated explants (CP)

$$\text{CP (\%)} = \frac{\text{Number of contaminated explants}}{\text{Total number of explants}} \times 100$$

c. Percentage of browning (%): Do the observation and calculation of the percentage of browning explant. Experience that changes both in the media and on the explants.

$$\text{Browning Percent (\%)} = \frac{\text{Number of explant browning}}{\text{Total number of explants}} \times 100$$

2.4. Embryo Initiation, Elongation Elongation of shoots and roots.

The development of the embryo explants cultured D. kutejensis to MS medium agar, which contains growth regulators BAP (0 ; 1 ; 2 ; 3) mg / l and growth regulators GA3 (0 ; 1 mg / l) and observed every week for 12 MAP , which consists of long observation hypocotyl elongation of embryos , shoot height and number of shoots that form in the embryo culture , root growth and root elongation.

2.5. callus formation .

Hipokotyl and shoot tips initiation used as explants and ditransper to MS in order to increase the concentration of growth regulators BAP (0 ; 1 ; 2 ; 3) mg / l and PGR 2,4 D (0 ; 1 mg / l) to form callus . Observations were made on the texture and color of callus formation of callus.

2.6. Statistic analysis

Test data propagation in vitro is determined by the growth parameters were analyzed using analysis of variance (ANOVA) using SPSS 22 (SPSS, Inc, USA). When a significant impact on the treatment continued with Duncan test with a rate of 95 %.

3. Results

3.1. Sterilization

Sterilization is an important part of the tissue culture. A common problem is the culture of fungi and bacteria contamination, brownish or explants were stagnant. Observations on the sterilization method used, in accordance with the explant to be used and the contamination and browning. Sterilization through two stages of treatment, sterilization outside using detergent for 30 minutes, soaking explants with the fungicide and bactericide for 60 minutes and was followed by washing with running water for 30 minutes. Sterilization stages proceed in a laminar air flow with explant immersion in 70% alcohol for 3 minutes, dettol 20 % 10 ' and dettol 10 % for 5 ' tween added 3 drops and soaking in Betadine 10 minutes. Sterilization of explants meant to clean the surface of the explants.

Table 1. *Response embryo explants D. kutejensis on five test sterilization treatment. Sterilization*

No	Method of sterilization	Response Exsplants (%)					
		Code	Time (Minute)	the number of explants	Fresh	Browning	Contamination
1	Control	P1					
2	Detergent, dithane M45 & agrept- alcohol 70%	P2	30 60- 3	40	0	10	90
3	Detergent, dithane M45 & agrept alcohol 70%, betadine	P3	30 60 3 10	40	10	77,5	12,5
4	Detergent dithane M45 & agrept alcohol 70%, bayclin20-10%+tween 3 drops, Betadine.	P4	30 60 3 10-5 10	40	0	95	5
5	Detergent dithane M45 & agrept alcohol 70% dettol 20-10%+ tween 3 drops, Betadine.	P5	30 60 3 10-5 10	40	96	4	0

Observations on the sterilization method used, in accordance with the explant to be used and the least contamination and browning . Sterilization through two stages of treatment , sterilization outside using detergent for 30 minutes , soaking explants with the fungicide and bactericide for 60 minutes and followed by washing with running water for 30 minutes . Stages sterilization proceed in a laminar air flow by soaking the explants in 70% alcohol for 3 minutes , dettol 20 % 10 ' and dettol 10 % for 5 ' added tween 3 drops and soaking in Betadine 10 minutes test results are then used to process sterilization of explants in this study

Figure 1: (a) Fruit Durio kutejensis **(b)** Seeds as a source of explants

It was observed plant growth regulator that is supplied with different concentration gave different explants growth response. BAP Award significant effect on the average elongation of embryos produced (Table 1), while GA3 no effect on hypocotyl elongation. The resulting embryo elongation varies on each explant . The highest yield of 6.2 cm of lengthening hypocotyl explants is indicated by the addition of GA3 PGR 1 ppm + BAP 3 ppm after 12 weeks, while the lowest value of 3.80 cm is shown in embryonic explants on MS medium without PGR.

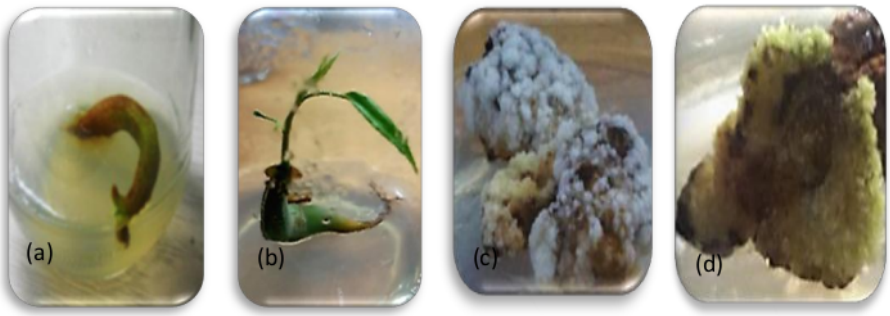

Figure 2: (a) *Elongation embryo* ***(b)*** *Culture of embryos* ***(c)*** *Induksi callus on embryos* ***(d)*** *Callus formation on hipokotyl*

Table 2. *Average length of hypocotyls which appears on medium the addition of PGR given different BAP and GA_3 on Embryo Culture after12 weeks.*

Concentration PGR (mg/l)	Mean ±Standard error (SE)
GA0BA0	3,800±0,094 [a]
GA0BA1	4,580±0,103 [b]
GA0BA2	4,730±0,067 [b]
GA0BA3	5,780±0,415 [d]
GA1BA0	4,680±0,078 [b]
GA1BA1	4,680±0,078 [b]
GA1BA2	5,360±0,150 [c]
GA1BA3	6,200±0,200 [e]

Note: *Figures followed by the same letter in columns and rows the same is not significantly different based DMRT with Level 95%.*

Explants showed a response in organogenesis can be directly grown to form shoots and roots, without going through callus formation. The treatment of variation PGR GA_3 and BAP to the length of shoots explant showed significant effects on shoots produced.

Table 3. *Average length shoots appearing on MediaThe addition of PGR given different BAP and GA_3 after 12 weeks:*

Concentration PGR (ppm)	Mean ±Standard error (SE)
GA0BA0	2,310±0,074 [a]
GA0BA1	2,470±0,067 [b]
GA0BA2	2,580±0,079 [c]
GA0BA3	3,030±0,095 [e]
GA1BA0	2,460±0,097 [b]
GA1BA1	2,530±0,067 [bc]
GA1BA2	2,780±0,123 [d]
GA1BA3	3,340±0,108 [f]

Note: *Figures followed by the same letter in columns and rows the same is not significantly different based DMRT with level 95%.*

The longest root elongation for 12 weeks, contained on PGR addition of 1 ppm GA_3 and BAP 2 ppm with an average length of 2.5 cm and at the root of the provision of PGR BAP 2 ppm without GA_3 produce root elongation of 2.46 cm. The treatment with the addition of BAP can help the process division and cell elongation and Stimulate root formation was GA_3 has not shown the ability to stimulate root growth of the embryo D. kutejensis Organ showed a response indirectly when explants grow through callus which later differentiate into shoots and roots. According to [31] the addition of PGR BAP treatment can increase the number of shoots in the cultivation in vitro. BAP included in a group of cytokines that play an active role in the proliferation of shoots.

Table 4. *Value Average Root Length (cm) On Various Treatments ZPT GA_BAP*

Concentration ZPT(ppm)	Mean ±Standard error (SE)
GA0BA0	0,880±0,078
GA0BA1	1,070±0,176
GA0BA2	2,460±0,556
GA0BA3	0,740±0,117
GA1BA0	0,670±0,095
GA1BA1	1,570±0,453
GA1BA2	2,500±0,860
GA1BA3	0,720±0,103
BA1	0,775±0,137[a]
BA2	1,320±0,421 [b]
BA3	2,480±0,705 [c]

Note: *Figures followed by the same letter in columns and rows the same is not significantly different Based DMRT with level 95%.*

4. Discussion

Sterilization is an important part of the tissue culture . A common problem is fungal and bacterial contamination, browning or explant stagnating. [26] stated obstacles that arise in the tissue culture caused by several factors: human factors, environmental and explants. Environmental and human culture is a source of contaminants, in addition to media contaminants. The explants are carriers of bacterial contaminants, fungi and spores. Sterilization of explants is intended to clean the surface of the explants. The problem of browning becomes an important problem in culturing embryonic explant D. kutejensis. Shortly after the embryo explants D. kutejensis in isolation, some embryos begin to experience browning. The emergence of brown or black on explant D. kutejensis cause stunted growth and development of explants and cause death in explant. [13] argued in oxidative conditions due to wounding , enzymes are naturally synthesized by plants as a defense . Browning generally occurs due to the effect of the accumulation of phenolic compounds are oxidized due to the mechanical

stress on the wounding of explants, such as cutting, stripping, bruising and disease or the aging process in the explants. The phenol compound is eksidase and tyrosinase enzyme polyphenol. According [6] when a cell is damaged, the contents of the cytoplasm and the vacuole becomes mixed, then oxidized phenolic compounds inhibit the enzyme activity. Phenol compounds excessive toxic and will damage the tissue explant tissue explants and then die. Browning or blacking out of the explants characterized by discharge of material sterilan an oxidation process of phenol (phenol oxidase) that the copper content such as polyphenol oxidase and tyrosinase, which is released or synthesized when the injured tissue [16]. Mucus on explants after explant are grown can also phenolic compounds, are compounds that are generally owned by woody plants. Browning showed a high degree of difficulty in the development of woody plants in several treatment by tissue culture techniques, so it is with culture D. kutejensis. [16] reported a phenol compound often connoted as inhibitors in in vitro culture. [10] reported experiencing explant browning if left unchecked will cause death in plants. Growth regulator supplied with different concentration gave different explants growth response. BAP Award significant effect on the average elongation of embryos produced, while GA_3 no effect on hypocotyl elongation. Bud formation from embryonic explant source D. kutejensis shows the results of growth varies, depending on the compatibility with the addition of growth regulators on the media. Elongation and bud formation culture derived from hypocotyl elongation without callus formation. The treatment with the addition of 2 ppm BAP able to assist the process of cell division and elongation and stimulate root formation. Award 1 mg/L GA_3 and 2 mg/L BAP stimulates fine root growth compared embryonic D. kutejensis BAP treatment by administering PGR without GA_3. According to [4] one of the functions of GA_3 and cytokinin that affect root growth and differentiation. Concentration is too high or low will affect the performance of the cell metabolism. In a culture that is a piece of organ seed embryo explants with the addition of 1 ppm 2,4D callus formed solid. Callus color seen on some treatments are white, yellow and golden yellow. The ability of embryo culture in generating compact callus yellowish white, and there is also a callus which has a shiny white color indicates that it is still active callus differentiated. At the plant embryo culture D. kutejensis, plant age and the nature of the embryo seed recalcitran also affects the ability of the explants to grow and regenerate into a callus, but auxin levels were high as in award above 2 ppm in embryos D. kutejensis can also be inhibiting the growth of culture . [29] argued that good quality callus has a green color , is the effect of cytokines in the formation of chlorophyll. BAP is a group of growth regulators of cytokines that contribute to the formation of buds. MS medium, adding a combination of cytokines such PGR (BAP) and auxin (2,4 - D) is to form a callus derived from the seed embryo explants D. kutejensis. The results showed that in the third week explant culture shoots D. kutejensis looks swell on each explant. Swelling in explants showed that plant growth regulators 2,4- D and BAP were able to be absorbed by the tissue explants embryos. [11] suggested that the concentration of endogenous cytokines at the end of the twigs of plants is very high, so the addition of exogenous cytokines at low concentrations has been able to induce the formation of callus .

5. Acknowledgement

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