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SELECTION ON EXPLANT OF RED DURIAN FOR THE FORMATION OF CALLUS INVITRO

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ABSTRACT

Tissue culture is an alternative to propagate the seeds of Red Durian uniformly and in great quantity. Type of explants isone of important factors that affects on callus induction. The callus will be used to form somatic embryogenesis. Objective of the research was to find out the explants type, which is able to form callus. This research used single factor, the explants. Explants used in this research include leaf, stem, and seed. The explants is grown in MS medium by adding ZPT 2,4 D 200 ppm, Picloram 0.5 mg/L. Results of the research showed that the highest contamination level was on explants, which derived from stem 100%, and leaf 70% with fungi as the contaminant. Explants which are able to produce shoot, are derived from seed explants. The age of the callus is 25 HSK, yellowish white with a solid callus structure.

Keywords: Explants selection, red durian, callus, in vitro.

1. INTRODUCTION

In Banyuwangi, red durian has been well-known as excellent fruit because it has high economic value. This fruit costs from Rp 150,000 to million rupiahs per fruit. Red durian is spread over several sub-districts that include Glagah, Songgon, Licin, and Kalipuro.

In general, red durian is proliferated vegetatively and generatively. Vegetatively, this plant is propagated by minigrafting technique. And generatively, it can be propagated by seeds. In order to obtain seeds, which are identical with the parents, the farmers are used to apply minigrafting, but such propagation takes a long time and less effective to produce more seeds, and it may cause some damages on plants that will make the plants to be unproductive.

Tissue culture is one of alternatives to propagate red durian seeds in great quantity and uniformly. In tissue culture, selection of appropriate explants is required to obtain a part of plant, which has active cells to divide. And then, the cells will divide and form callus. Then, this callus can be used for somatic embryogenesis formation, the development of complete embryo from vegetative cells or somatic cells, which were derived from various sources of explants (Zulkarnain, 2009).

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According to Mastuti (2017), the best parts of the plant, either cells or tissues which will be grown, are so-called explants that are derived from the whole parts of the plant, such as organ (root, stem, leaf, seed) and tissues, as well as specific cells (pollen, endosperm, mesophyll, cotyledon, and hypocotyls. But, the success level for each explant is different. Furthermore, Mastuti (2017) also reported that some tissues showed better response than the other ones.

Type of explants is one of important factors that affects on callus induction. This research applied several types of explants, such as leaf, stem, and seed. Objective of the research was to determine type of explants, which is able to form callus.

2. MATERIAL AND METHOD

Location and Time of the Research

The research was conducted at Laboratory for Tissue Culture, Faculty of Agriculture and Animal Husbandry, University of 17 Agustus 1945, Banyuwangi. The research was conducted from February to July 2019.

Source of Explants

Genotype of red durian used in this research was derived from Songgon Sub-district, Banyuwangi Regency in East Java. Explants used in this research were leaf tips, which are completely developed, young stem that was taken 10 cm from the tip, and seeds.

Medium

Explants were grown in MS standard medium by adding growth regulator substance (ZPT) 2.4 D 200 ppm, Picloram 0.5 mg/L. The medium was sterilized using autoclave at 121 °C for 30 minutes.

Sterilize the Explants

Explants that derived from the leaves were sterilized using detergent and soaked for 10 minutes, and then rinsed under running water thoroughly. After that, they were soaked in alcohol 70% for a minute. In LAF, the explants were soaked in clorox 20% for 10 minutes, clorox 10% for 5 minutes, and then soaked in ppm 0.5 ml/100 ml sterile aquades for 10 minutes. Each explant was rinsed using sterile aquades three times.

Sterilization on seed explants was performed in several steps as followed: explants were washed using detergent for 10 minutes, and then rinsed under running water thoroughly. After that, in LAF, the explants were soaked in ppm 1 ml/100 ml sterile aquades. Furthermore, the explants were rinsed using sterile aquades three times.

Transfer Explants to In Vitro Medium

The sterilized explants were transferred to in vitro medium and incubated in a chamber at 20-25 °C and light intensity 1000 – 2000 lux.

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Parameter of Observation

Percentage of the contaminated explants

Explants may be contaminated by fungi or bacteria. Fungal contamination is characterized by the emergence of fine-white threads and bacterial contamination is characterized by the emergence of slimy spots on the medium or the explants. Equation to determine percentage of the contaminated explants is as follows :

$$\%\ contaminated\ explants = \frac{Amount\ of\ the\ contaminated\ explants}{Total\ explants}\ x\ 100$$

Percentage of explants, which produce callus (%)

Explants, which produce callus, are fresh explants and responsive to the emergence of callus. Percentage of explants, which produce callus, can be calculated using the equation below:

$$Emergence\ of\ callus = \frac{\text{Amount of explants with the emergence of callus}}{\text{Total explants}}\ \text{x } 100\%$$

Age of the callus emergence

The explants age that produce callus were calculated from initial planting of the explants to the emergence of callus on the explants, which was counted based on days after culture (DAC).

3. RESULTS AND DISCUSSIONS

Results of the research showed that the highest contamination level was found on explants, which were derived from stem 100%, leaf 70%, and the lowest contamination level was found on explants that derived from seeds 30% (Figure 1.)

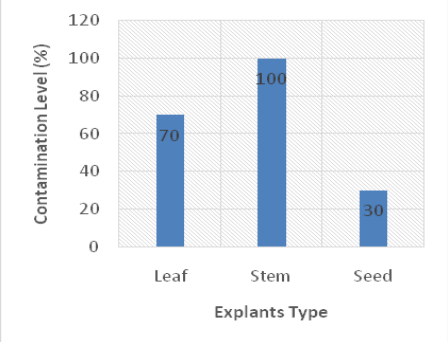

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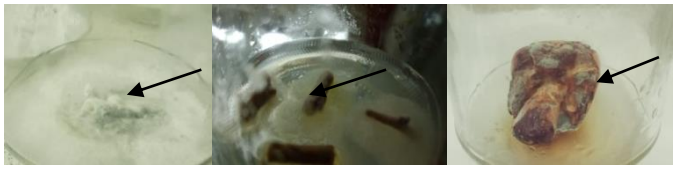
Figure 1. Contamination Level on Several Explants (%)

Pandiangan (2003) reported that contamination may occurs on explants, both external and internal, microorganisms that enter into the medium, unsterilized bottle for culture or less sterile planting tools, dirty workroom and culture (contain spores in the atmosphere of the laboratory) and carelessness during the implementation. In order to create an aseptic condition, it could apply autoclave heating, disinfectant or ultraviolet light, so that the disturbing microbes could be exterminated. Zulkarnain (2009) also reported that several sources of contaminant on tissue culture may be caused by medium as a result of imperfect sterilization process, improper and less accurate planting implementation and work environment, types of explants (internally, the contaminant is carried in the tissues; externally, the contaminant exists on the explants surface as a result of incomplete sterilization procedures).

The highest contaminant is fungi, as being observed, the contaminated explants show symptoms such as found on leaf explants, in which white contamination spread over the explants surface evenly; on stem explants, the contamination shows blackish brown; and on seed explants, the contamination shows white spots (Figure 2). Age of the fungal contaminant emergence on leaf is when the explants in the medium in vitro at 14 DAC (days after culture), stem at 7 DAC, in intact seed at 45 DAC (Table 1).

Table 1.Types of Contaminant on Diverse Explants

Explants	Fungi (%)	Bacteria (%)	Age of the Contaminant Emergence (Days After Culture)
Leaf	100	0	14
Stem	80	20	7
Seed	100	0	45



a. Fungal contamination on leaf explants

b. Fungal contamination on stem explants

c. Fungal contamination on seed explants

Figure 2. Fungal Contamination on Diverse Explants

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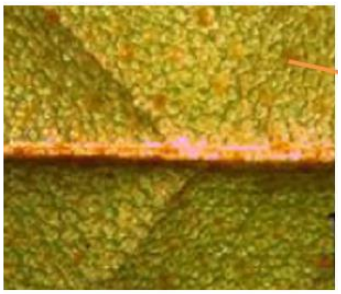
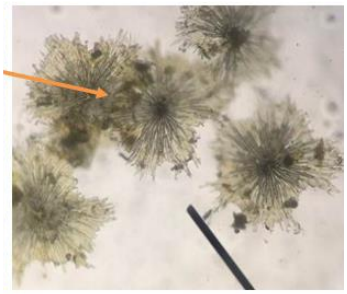
High contamination on explants, which derived from leaf and stem, was caused by the existence of trichome on surface of the young stem and leaf of the red durian. Trichome is hairs, which grown from epidermis cells. Fahn (1990) reported that trichome is epidermal stretching. Nugroho (2006) suggested that trichome on leaf usually functions to (1) reduce evaporation, (2) pass on the stimulation, (3) reduce animal disorders, (4) assist seeds distribution, (5)assit the pollinating, and (6) absorb water and mineral salts in the soil. Trichome structures are varied, in which trichome on durian has flat, scaly hairs, and many cells.

On red durian, trichome emerges on external surface of vegetative organs, such as leaf and stem. According to Mulyani (2006), trichome type is divided into two types namely non-glandular trichome (non-glandular hairs) and glandular trichome (glandular hairs). Based on results of the observation, durian has non-glandular trichome in thorny stem form (Figure 3.b).



a. Red durian leaf layer of the lower epidermis (abaxial), source : Widiastuti, et all, 2018



b. on-glandular trichome (thorny star shaped), source : Rohmana, 2015

Figure 3. Appearance of trichome on Abaxial Part of Leaf Explants

Non-glandular trichome on young leaf of red durian is golden yellow that covers the whole surface of abaxial leaf (Figure 3.a). Fahn (1979) suggested that non-glandular trichome functions as barrier the entry of pathogen through stomata. So that the pathogen will not be able to enter through stomata, but it settles in trichome, which cause the durian leaf is difficult to be sterilized and easily contaminated during propagation via tissue culture. Moreover, it causes sterilization on leaf and stem become less effective.

During the application of leaf explants in the in vitro culture, fungal hyphas emerge on the abaxial part of the leaf at 14 DAC (days after culture). After that, the hyphas form mycelium and cover the entire surface of leaf explants (Figure 2.a). On stem explants, fungal hyphas grow on the whole surface of the stem, so that the fungal mycelium grow quickly and cover the entire surface of the stem (Figure 2.b). Fungal contaminant on leaf and stem explants of red durian is

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an exogene contaminant microbial, which is difficult to be sterilized due to the fungus attaches on the trichome.

During sterilization on leaf explants, trichome on the abaxial surface of the leaf is cleaned up by scraping the surface of abaxial leaf using scalpel, and then sterilized using PPPM 10% for 15 minutes and followed with in vitro culture. 20 DAC leaf explants could not develop due to chlorophyll degradation, and finally disturb the explants metabolism. Such chlorophyll degradation on leaf explants deteriorates the physiological process of the explants, which finally causes the leaf dead (Figure 4).

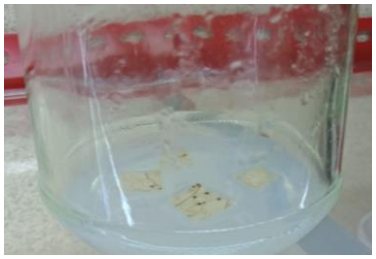

Figure 4. Condition of leaf explants at 20 days after culture (DAC)

Explants, which are able to produce callus, derived from seeds. Callus emerges on cotyledon surface. The callus cells emerge for the firt time at 25 days after culture (DAC). The emerging callus on the red durian seed has yellowish white color and solid structure (Figure 5).

Figure 5 Formation process of callus on Durian seed.

4. CONCLUSION

Explants, which is able to proliferate and form callus, are derived from the seeds of red durian that having low contamination level, 30%.

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