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Callogenesis of *Durio zibethinus* using Flower Bud Explant

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Abstract. Propagation of superior durian (*Durio zibethinus*) are constrained due to the limited number of the tree. Grafting and budding are commonly used in durian propagation, however these techniques give destructive effect to mother plant. Alternatively, in vitro technique can be chosen for propagation of superior durian. The research aims to study the possibility of durian flower bud as an explant for micropropagation because the part is relatively more sterile than other organs. Half strength Murashige dan Skoog media were used to grow explant with addition of 1 ppm 2,4-dichlorophenoxyacetic acid (2,4-D) and various combinations of sucrose concentration (30 gr/L, 50 gr/L and 70 gr/L), and Thidiazuron (TDZ, 0.2 ppm, 0.4 ppm and 0.8 ppm). Totally, there were nine treatment combinations with six replications, respectively. Parameters observed were number of explants-producing callus, colour and callus morphology. The result indicated that flower bud of durian had a potential as an explant for micropropagation due to their ability to induce callogenesis (69.2%). Combination of 45 gr/L sucrose and 0.8 ppm TDZ gave the best result in inducing callus growth. Most of the callus showed white colour and friable callus.

INTRODUCTION

Indonesia has some superior cultivar of durian such as Petruk, Bawor and Bokor. Number of superior durian trees are very limited, causing limited superior durian production, as a result the market demands are not being met. Propagation of fruit tree seedlings can be done by several techniques, namely budding, grafting and cutting [1]. However, the above techniques tend to damage the mother plant and potentially disrupt the production of the fruit. Generative propagation through seeds has a disadvantage which resulted in seedlings showing different properties from the parent and the long harvest. Therefore, propagation of superior durian using in vitro technique should be chosen. There are various factors that determine the success of in vitro cultures, such as explant sources, sugar sources and plant growth regulators (PGR). Micropropagation of durian has been done employing various explant such as apical and lateral buds [2], immature leaves [2-5], zygotic embryo [6], or nodes [7-8]. All these researchers were able to produce callus and embryonic callus, but had no success in explant regeneration, thus an alternative is needed to look for other explant sources. Previous study reported that phenolic compound production during in vitro culture of durian was the main problem. Explants of woody plants often secreted phenolic compound that causes browning of the explant [9-10]. Addition of a suitable carbon source was required to avoid excessive phenolic secretion in culture medium [10]. At higher sucrose concentrations in the culture media, phenolic concentration and tissue necrosis increased, causing sharp decreased in shoot regeneration of the three sugar beet lines [11].

Contamination is still the main problem in multiplication of durian in vitro. It is necessary to study the flower bud as explants considering that this organ is still closed so that it is relatively more sterile than other organs as a source of explants. Durian flower bud are abundant, producing the same genotype and phenotype as the parent, not damaging the mother plant and minimum contamination. PGR is an important factor in the success of in vitro culture. Thidiazuron (TDZ) is a growth regulating agent from the cytokinin group which is widely used to induce callus (callogenesis) or induction of somatic embryogenesis. TDZ is a powerful and potent synthetic growth regulator, leading to a wide array of in vitro and in vivo applications in plants including prevention of leaf yellowing, enhanced photosynthetic activity, breaking of bud dormancy, fruit ripening, as well as proliferation of adventitious shoots, callus production, and induction of somatic embryogenesis [12]. Another important factor in

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durian plant tissue culture is the media. Namhomchan [2] used woody plant med (WPM) supplemented with cytokinin (BA, 2ip or kinetin), Lizawati *et al.* [4] used Murashige-Skoog (MS) added with 2,4-D and BAP to induce callus proliferation, Zulkarnain *et al*. [5] used WPM to induce callus proliferation in Durian cv. Selat. and Hermayani, *et al*. [8] used WPM, MS and B5-Gamborg with addition of 2,4-D and TDZ. The research aimed to describe the response of flower bud explants to various sucrose and TDZ concentration and the potency in durian micropropagation.

METHOD

Plant Material

Flower bud explants were obtained from local cultivar durian at Semarang Regency, Central Java, Indonesia. The explants were flower bud of 8 years mother plant. The abundant numbers and sterility condition were the consideration of explant choice.

Surface Sterilization

Flower buds were rinsed with tap water and rubbed up with hand and cotton bud. Later they were smeared by 5.25% Clorox bleach until the scales peeled slightly, then the remnant removed by cotton bud, and finally left for 30-50 minutes. The explants were washed with tap water for 1 hour. The explants were then put into laminar air flow (LAF) and dipped in 96% alcohol, flamed over bunsen burner until the outer skin dries. The dried skin was cut and discarded, while the rest of the skin were cultured in the media. Observation of all parameter was done (every week) for six weeks.

Media Preparation

Basal media used in this experiment was half strength MS media. The disinfected explants were germinated aseptically in basal media with addition of TDZ and sucrose according to treatment combination. The media was solidified with 0.8% agar. The pH of the medium was adjusted to 5.8 and autoclaved at 120ºC for 20 min. Growth regulators were added to the media before autoclaving. About 30 ml of MS media was poured into each of 650 mL wide mouth jar where the plant tissue was cultured in the glass container with screw plastic lid.

Culture Condition

Surface sterilized explants were cultured into media and embedded to increase surface contact with media. Each bottle as planted with one explant, incubated under cool white fluorescent light in incubation room at 25±2ºC, at 16/8 h light/dark photoperiod with light supplied by cool white fluorescent lamps ± 1000 lux. After 6 weeks of culturing, the callus induction percentage was recorded to observe the colour and morphology of callus. The calluses were subcultured every three weeks to avoid browning.

Research Design and Data Analysis

The experiment was performed on a completely randomized design RD) with two factors, i.e. TDZ concentration and sugar concentration. There we three TDZ concentration, i.e. 0.2 ppm, 0.4 ppm and 0.8 ppm. There were three sugar concentration trialed, i.e. 30 mg/L, 45 mg/L and 60 mg/L. In total, there were 9 treatments combination and each combination was replicated six times, with each replicate containing three expl s. Mean values percentage of explants forming callus were analysed using a two-way ANOVA. Significant differences among treatments were analysed using Duncan's multiple range test (DMRT). The colour and callus morphology were analysed descriptively.

RESULTS AND DISCUSSION

Callus induction

Based on the callus formation, the response of flower bud explant to various concentration of TDZ and sucrose were shown in Table 1. All the combination treatments resulted in >50% callus formation. This data revealed that durian flower bud haD a potential as an explant for in vitro proliferation. The highest response was achieved in combination of 0.8 ppm TDZ and 45 gr/L sucrose which reach in 100% of the explant formed the callus. Separately, the highest callus formation (76,85%) were reached in 0.8 ppm TDZ compared to 0.2 and 0.4 ppm. Amount of 45 gr/L sucrose resulted in highest callus formation of the explant (75.66%). Higher and lower concentration of sugar decreased the callus formation.

Optimum callusing was observed on MS+0.5 mg/L TDZ+1.0 mg/L NAA in both leaf and petiole explants of *Aconitum balfourii* [13]. Yaguinuma *et al*. [14] reported the highest percentage of explants with callus induction and with shoot clusters were observed in *Urochloa*cv. Marandu using 4 mg/L. For all genotypes *of Urochloa*, the best plant regeneration was recorded with 4 mg/L. Moradi *et al*. [15] reported all the combinations containing TDZ and NAA gave 100% callus induction of *Crocus sativus*. The highest amount of crocin was detected in style originated callus grown on the medium containing 3 mg/L NAA + 1 mg/L TDZ. All the data indicated that TDZ had a potential to induce callus although it depends on the concentration and source of explants.

TABLE1. Callus formation percentage of flower bud explant to various TDZ concentration and sucrose

TDZ concentration	Sucrose concentration gr/L		
	30	45	60
0.2 ppm	67,67%[b]	55,56%[a]	57,14%[a]
0.4 ppm	83,33%[c]	71,43%[b]	57,14%[a]
0.8 ppm	55,56%[a]	100%[c]	75,00%[b]

Mean values followed by the same letter(s) within a column are not significantly different ($P = 0.01$).

Generally, 3% sucrose had been used to promote the growth and development of callusin the culture medium. Sucrose as carbon source had been used to promote the percentage of callus production. Srichuay *et al*. [16] reported that the highest callus induction frequency (87.5%) was obtained from clone 1-em on callus induction media (CIM) with 8% sucrosein *Hevea* anther culture. Meanwhile, Chaudhary *et al*. [17] recorded that the highest mean callusing of broccoli was obtained in 12% concentration of sucrose and was found tobe significantly superior to 14% and 10% sucrose concentrations. The highest sucrose concentration (40 g/l), which was added into MS medium, could induce the maximum callus wet-weight [18]. All the data showed the importance effect of sucrose concentration in induce callus formation. Nevertheless, some researcher reported the negative effect of high concentration of sucrose. Sari and Kusum [19] mentioned that the highest wet weight callus was found on medium which were treated with 30 g/L of sucrose, compared to the higher or lower concentration of sucrose.

Colour and Morphology of Callus

Colour of durian callus and morphology (texture) after the treatment are summarized in Table 2. The data showed that most of durian callus were friable and white.

TABLE 2. Colour and morphology of callus durian in various TDZ and sucrose concentration after six weeks

Treatment	Callus colour	Callus morphology
0,2 ppm TDZ, 30 gr/L sucrose (MA2)	White-brownish	Friable
0,2 ppm TDZ, 45 gr/L sucrose (MA4)	White-greenish	Compact
0,2 ppm TDZ, 60 gr/L sucrose (MA8)	White-greenish	Compact
0,4 ppm TDZ, 30 gr/L sucrose (MB2)	White	Friable
0,4 ppm TDZ, 45 gr/L sucrose (MB4)	White	Friable
0,4 ppm TDZ, 60 gr/L sucrose (MB8)	White	Friable
0,8 ppm TDZ, 30 gr/L sucrose (MC2)	White	Friable
0,8 ppm TDZ, 45 gr/L sucrose (MC4)	White-brownish	Friable
0,8 ppm TDZ, 60 gr/L sucrose (MC8)	white	Friable

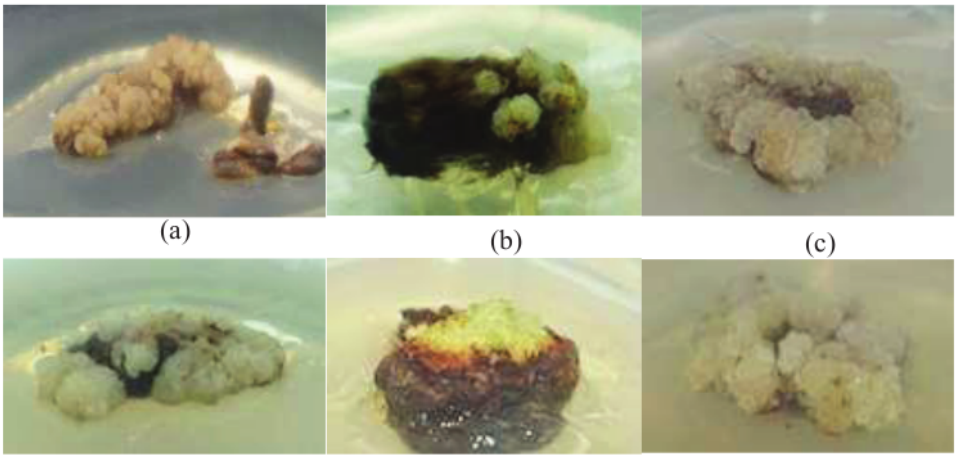

FIGURE 1. Callus morphology from some concentrationcombinationof sucrose and TDZ.
a. MA4, b. MB4, c. MB8, d. MC2, e. MC4, f. MC8

Callus colour of durian varied from white, white-greenish, to white-brownish and most of them were whitish (Table 2 and Figure 1). This result is in accordance with Supriati *et al*. [20] who reported white callus colour in mango and Zulkarnain *et al*. [5] in durian. Nevertheless, both researchers reported the most of callus were friable. Sari and Kusuma [19] reported that callus *M*17*ecodiatuberosa* treated with any sucrose concentrations had crumb textured and green to yellowish green color. Callus formed from embryonic explants green and yellowish white with compact texture in *Duriokutejensis* [21]. He *et al*. [22] obtained yellowish and mostly friable in structure callus of Chinese kale. The reported data indicates that the colour and texture of the callus were highly variable depending on the source of the explants. The colour of explants depended on genotype, plant regulator, source of carbon etc. After six weeks of culture and twice subcultured, callus growth decreased proliferative ability. Further research is needed to find the right callus proliferation media. The research showed the potential of durian flower bud as an alternative explant for callus production.

CONCLUSION

Our result indicated that flower bud of durian had a potential as an explant for micropropagation due to their ability to induce callogenesis (69.2%). Combination of 45 gr/L sucrose and 0.8 ppm TDZ gave the best result in inducing callus and embryogenic callus. Most of the callus showed white colour and friable callus. Friable callus had higher ability to form buds than the compact ones.

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