Callus Formation and Its Characteristics of Mountain Papaya *in Vitro* Endosperm Culture

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Abstract. Endosperm as a result of double fertilization in Angiospermae shows high level chromosomes and polyploidy. It is also considered as dead tissue that is unable to be generated to form plantlet. The aim of this research is to determine the effect of kinetin and 2.4-dichlorophenoxyacetic acid (2.4-D) in induction of callus formation of mountain papaya. This research used factorial randomized block design with eighteen groups, one fruit was used for one experimental group. Culture using Murashige and Skog (MS) media with combination of three level of kinetin [(0, 1, 3) mg L^{-1}] and six level of [2.4-D (0, 1, 2, 3, 4, 5) mg L^{-1}]. Concentration of 2 mg L^{-1} 2.4-D was the best treatment in stimulating callus growth by producing the highest percentage of callus, 24.44 %. The combination of 1 mg L^{-1} kinetin and 3 mg L^{-1} 2.4-D was the best treatment in accelerating callus induction of the endosperm was 19.84 d after planting. In this study shows that induction callus of endosperm of mountain papaya could be stimulates by kinetin and 2.4-D.

Keywords: Genetic improvement, micropropagation, plant tissue culture, polyploidy, *Vasconcellea pubescens* A. DC.

1 Introduction

Mountain papaya (*Vasconcellea pubescens* A. DC) is a species of Angiosperm belonging to the Caricaceae family. It originates from the Andes Mountains in South America. The distribution the plant covers several regions, including Mexico, Chile, Ecuador, and the Andes Mountains, as well as Colombia [1]. Its fruit has a unique taste and distinctive aroma, so this commodity is often processed into various foods such as syrup and jam. Mountain papaya fruit contains vitamin C [2], minerals, and carotenoids [3], but low in sodium, fat, and calories and is used to treat skin and digestive problems [4]. Based on the results of phytochemical tests, mountain papaya leaves contain flavonoids, tannins, saponins [5], alkaloids [2], papain [6], and antioxidants [7], where these compounds have potential as

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antimicrobials [8], and as ingredients for the manufacture of drugs, cosmetics, and the food industry [9].

Compared to its tropical relative, *Carica papaya* L., mountain papaya has several disadvantages, such as smal fruit with numerous seeds results in smaller edible portions. Hence, the plants are not largely cultivated. Improvement of genetic quality can be done with endosperm culture technique which can produce triploid plants with seedless fruits. Triploid plants are generally sterile or fail to form seeds due to chromosomal imbalances during the meiotic phase [10]. This property is important to improve the value of the fruits. Another advantage of using endosperm as an explant in tissue culture is that it has superior vegetative growth and resistance to disease [11].

The regeneration of mountain papaya plants through endosperm culture techniques has never been done before. Therefore, a micropropagation technique with tissue culture techniques are needed for endosperm callus induction which later can also be the foundation for further research.

2 Methods

The research design used was a factorial randomized block design with Plant Growth Regulator (PGR) as factors. The first factor is kinetin consisting of three levels [(0, 1, 3) mg L^{-1}], and the second factor is 2,4-Dichlorophenoxyacetic acid consisting of six levels [(0, 1, 2, 3, 4, 5) mg L^{-1}]. Thus, eighteen treatment combinations were obtained. The single research unit was a culture bottle containing five endosperm mountain papaya explants. The total explants used in this study were 1 620 explants.

The material used was an endosperm mountain papaya explant derived from mountain papaya fruit taken from the Dieng Plateau, Central Java, Indonesia. Mountain papaya fruit washed using running water. The seeds are taken using tweezer and then stored in a sterile beaker. Sterilization was carried out in a Laminar Air Flow Cabinet with 2.6 % sodium hypochlorite (NaClO) solution mixed with ten drops of tween 20, rinsed three times using sterile distilled water. Furthermore, sterilization was continued using 70 % alcohol and shaken for 5 min. Then rinse three times using sterile distilled water.

Observations were made based on several aspects, first, day of callus formation. The data was collected on a daily basis to observe callus formation on explant. Second, the percentage of callus formation and browning was observed at the end of the research which is day 56. Third, callus color and texture were remarked using munsell colour for plant tissue and pinching with tweezer, respectively. Data were analyzed using non-parametric statistical analysis Kruskal-Wallis H and Scheirer Ray Hare test with SPSS 26.

3 Results and discussion

The explant had different responses to variation of plant growth regulator but it definitely at least one PGR had to exist to induce callus formation. Treatment K0D0 (without Kinetin and 2,4-D) failed to promote callus emergence (Table 1). Based on Kruskal-Wallis H non-parametric statistical test (Table 2), it was found that the parameters of the day of callus formation and the percentage of callus formation in all single treatments were significant or had a significant effect, while the interaction between the two was not significant (Table 3). Meanwhile, in the percentage of browning explants, only treatment K (kinetin) was found which was significant, while treatment D (2,4-Dichlorophenoxyacetic) and the combination of K and D were not significant.

Callus induction and differentiation in various plants have been widely carried out. This process utilizes the role of a combination of two phytohormones, auxin and cytokinin. Its availability, either endogenously or added to the growth medium, has been well documented in the plant regeneration process.

Table 1. Response of mountain papaya endosperma to combination of kinetin and 2.4-D.

Treatment combination	Callus formation (d)	Callus formation (%)	Callus texture
K0D0	0.00	0.00	-
K0D1	30.14	15.56	Friable, compact
K0D2	31.85	24.44	Friable
K0D3	25.00	8.89	Friable
K0D4	33.75	11.11	Friable
K0D5	31.63	7.78	Friable
K1D0	30.83	4.44	Friable
K1D1	33.36	22.22	Friable, compact
K1D2	26.20	21.11	Friable, compact
K1D3	19.84	13.33	Friable
K1D4	23.13	17.78	Friable
K1D5	30.24	21.11	Friable
K3D0	32.83	6.67	Friable
K3D1	37.30	6.67	Friable
K3D2	21.67	3.33	Friable, compact
K3D3	22.50	6.67	Friable
K3D4	26.00	16.67	Friable
K3D5	22.00	2.22	Friable

Notes: K: Kinetin, D: 2.4-D, number indicates PGR concentrations in mg L^{-1}.

Table 2. Summary of Kruskal-Wallis H test on Kinetin and 2,4-D treatment.

Treatment	Significance value		
	Day of callus formation	Percentage of callus formation	Percentage of browning explants
K (Kinetin)	0.02	0.00	0.00
D (2.4-D)	0.01	0.01	0.58

Notes: Significance value below 0.05 means significant.

Table 3. Summary of Scheirer Ray Hare test (SRH test) on Kinetin and 2,4-D combination treatment.

Observation	H	*P* value
Day of callus formation	10.424	0.404
Percentage of callus formation	10.698	0.382
Percentage of browning explants	12.445	0.256

Notes: *P* value below 0.05 means significant.

In general, a balanced percentage of auxin and cytokinin increases callus formation, while higher levels of auxin versus cytokinin or vice versa stimulate root and shoot renewal, respectively [12]. The use of cytokinins is believed to influence callus formation, besides that the cell wall lignification process was detected to decrease, thereby increasing callus initiation in vitro. It has been found that callus growth usually begins in the wound area of the explant cut and continues to cover the entire explant [13]. Several studies have shown that using cytokinin and auxin together is better than using auxin or cytokinin alone [14].

The best treatment to produce a relatively higher percentage of callus, 24.44 %, is a combination of treatments kinetin 0 mg L^{-1} and 2 mg L^{-1} of 2.4-D. Dalila's worked on callus induction of *Barringtonia racemosa* (L.) Spreng. endosperm explants in different types of basal media showed that concentration of 2 mg L^{-1} of 2.4-D promoted 100 % of callus formation [15]. Kinetin when its applied solely fail to produce callus [15] in this research also showed that single use of kinetin impact to minimal callus biomass production. In a nutshell of 2.4-D is necessary for inducing callus formation. Tabel 1 also showed that callogenesis failed to start when exogenous auxin and sitokinin were not added to medium. Kinetin in certain or low concentration accelerated callus growth when 2,4-D present, 19.84 d after planting.

Table 1 shows that almost all calluses formed had a friable texture and several calluses with a compact texture. Of the 181 total calluses formed, there were only five calluses (K0D1, K1D1, K1D2, K3D2) which had a compact texture, while calluses with friable texture were spread in all treatments. Based on the results of the above study, the combination of kinetin and 2.4-D was given form a callus that is dominantly crumbly compared to a compact texture.

Based on the results in Figure 1 regarding the percentage of explants that experienced browning, the highest percentage results were found in the K3D2 treatment with 30.00 % and the lowest 2.22 % in the K0D2 treatment. The average percentage of browning in the K3 treatment was 17.78 %, in the K1 treatment it was 10.10 % and in K0 it was 6.44 %. This shows that in the treatment with higher kinetin levels, many explants were experienced browning, compared to the treatment with low kinetin levels (K1) or no kinetin (K0) was given.

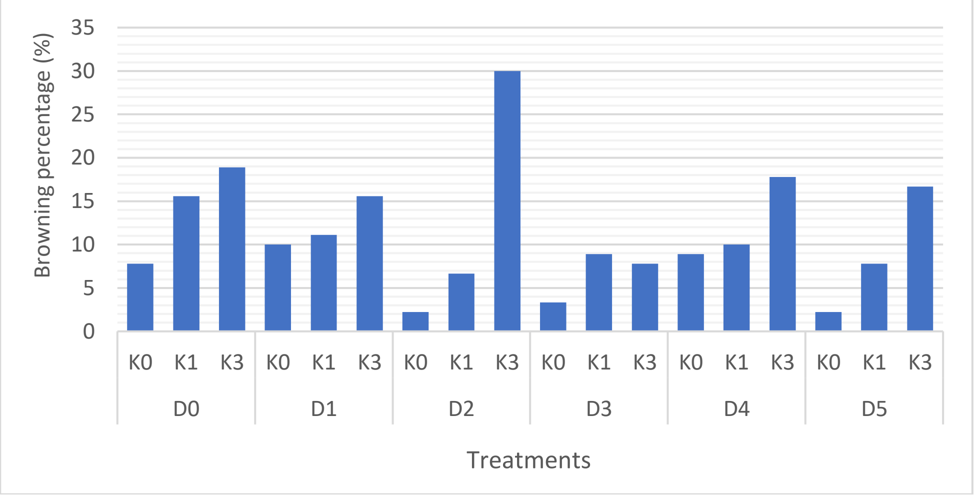

Fig. 1. Browning percentage on explant.

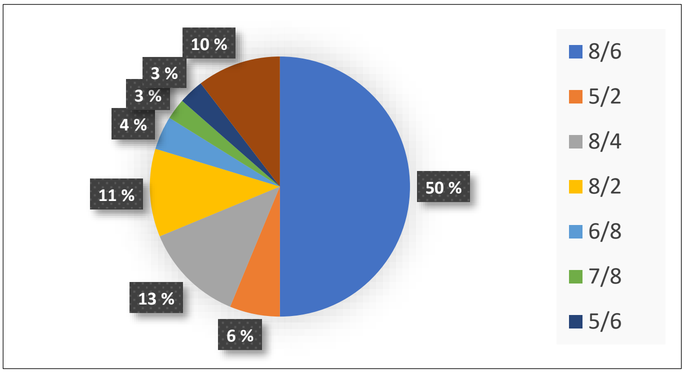

Fig. 2. Callus colours in response of kinetin and 2.4-D treatments (munsell colour for plant tissue).

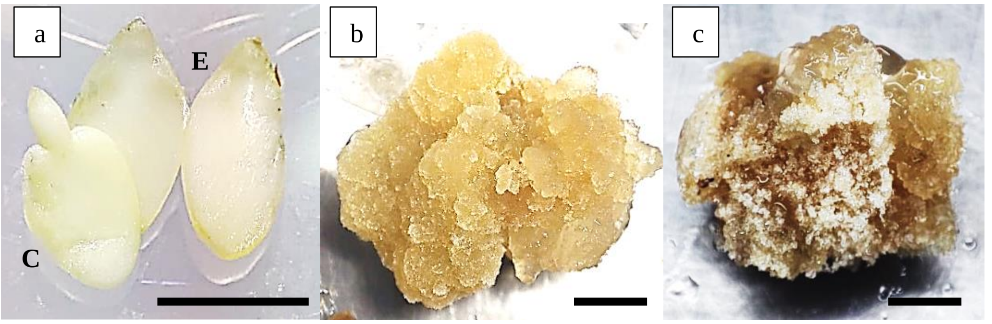

Fig. 3. Callus textures after 56 d after planting. a) endosperm with cotyledone, C: cotyledone, E: endosperm, b) friable callus, c) compact callus, bar: 1 cm.

Polyphenols release from cell's vacuoles resulted in explant browing, it is a natural mechanism for plant reaction against wounding. The enzymes polyphenoloxidase and peroxidases catalyze the reaction between different phenolic compounds and molecular oxygen, producing quinones which are highly reactive and non-specifically polymerized proteins and produce dark pigments, melanin [16].

Callus color can also be used as an indicator of the quality of the callus [17]. Light color of plant tissue culture impact to its growth rate. In this research, after 56 d, 50 % of all calluses had color yellow brown with value/chroma, 8/6. (Figure 2). Callus texture is one of the parameters used to determine callus quality, in this study the callus formed was dominant with friable structure (Figure 3). The texture that was formed was thought to be due to the administration of the hormone auxin in the form of 2.4-D on the explant growth medium. 2.4-D is better for producing calluses with friable texture compared to other auxins. Callus structure that is crumbly or easily broken is considered better, because friable are easy to separate into several parts or into single cells, this causes oxygen aeration between cells to increase.

4 Conclusion

Callus induction using mountain papaya endosperm explants has been successfully carried out using various combinations of concentrations of kinetin and 2.4-D with the best treatment in stimulating callus formation, namely 2 mg L^{-1} 2.4-D, with friable texture and yellowish white colour, treatment. The best in accelerating callus formation were 1 mg L^{-1} kinetin and 3 mg L^{-1} 2.4-D. In addition, in this study it was found that the higher the kinetin, the higher the potential for browning.

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