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The effect of types and concentrations of auxins on callus induction of *Centella asiatica*

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Abstract. *Rahayu S, Ika Roostika I, Bermawie N. 2016. The effect of types and concentrations of auxins on callus induction of Centella asiatica. Nusantara Bioscience 8: 283-287.* Centella (*Centella asiatica* L.) is one of medicinal crops that have various utility as healing wounds, inflammation, rheumatic, hemorrhoids, tuberculosis, dysentery, leprosy, and fever and also as appetizer. In recent years the demand for raw materials of medicinal from Centella has increased. Until now, Centella has been harvested directly from nature without replanting so that this plant was worried to be extinct gradually. In vitro culture is one of the techniques for plant propagation. This technique has proved to be able to propagate a plant in large numbers, short time and uniform. Callus induction is initial stages of plant propagation by indirect organogenesis and somatic embryogenesis. The objectives of this research were to find out the most suitable type of auxins and its concentrations to induce callus formation of Centella. Explants used in this research were leaves and petioles taken from sterile culture of Centella var. Castina 1. Three kinds of auxins were used to this research, i.e. 2,4-D, Picloram and Dicamba at the levels of 2, 4 and 6 mgL^{-1}. The result indicated that the most suitable type of auxin for inducing callus formation was 2,4-D or Dicamba at concentration of 4 mgL^{-1} which gave the highest percentage of callus formation, and the highest fresh weight as well.

Keywords: Auxins, callus, induction *Centella asiatica*, in vitro

INTRODUCTION

Centella (*Centella asiatica* L.) is usually used as raw materials for medicinal industry, cosmetics, and aromatherapy. The utilization of the plant is either in the form of fresh, dry or mixed material as processed products like jamoe. Along with the more expensive price of medicine in the market, this situation encourages the public more interest in using natural product for medicines. Unfortunately, the increase of demand has not been accompanied by the availability of raw materials in a considerable number in the field because the plant is conventionally propagated through stem cutting and seed. On the other hand, the availability of the plant in nature is decreasing because of overexploitation without replanting. The great demand, especially from pharmaceutical industries, cannot be fulfilled (Martin 2004; Nath dan Buragohain 2005).

In vitro culture is an alternative method for large scale propagation in order to support commercial industry. One of the in vitro steps is callus formation. Callus consists of undifferentiated and unorganized cell mass. The callus formation will undergo three stages of development process, namely induction, differentiation and cell development, respectively (George 2008). Callus is widely used either for basic research or for the application in world of industry (Ikeuchi et al. 2013). Auxin is needed for callus induction to form explants. Auxin is very effective to initiate the cell division, cell elongation, development and growth (Gaspar et al. 1996; George 2008). The response of cell to auxin will enter the dedifferentiation stage, and then cells start to divide again (George 2008). The use of 2,4-D for callus induction was widely used in various plants compared with the other auxins like Dicamba and Picloram, either in single or in combination. The objectives of this research are to find out the most suitable type and concentration of auxins to induce callus formation of Centella.

MATERIALS AND METHODS

The research was conducted at the Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (ICABIOGRAD), Indonesian Agency for Agricultural Research and Development (IAARD), from August to October 2015. Materials used for the culture were stolon of Centella variety Castina 1 which was planted in greenhouse. Stolons were disinfected by 0.5 gL^{-1} benomyl and 20 gL^{-1} streptomycin sulfate for 1 hour, respectively. Subsequently, they were sterilized with 70% alcohol for 5 min, 15.75% sodium hypochlorite for 7 min and 10.5 % sodium hypochlorite for 9 min. They were rinsed with sterile aquadest three times. The apical shoots of the stolon were isolated. They were planted on MS (Murashige dan Skoog 1962) medium enriched with 0.5 mgL^{-1} BAP and then incubated for 4 weeks. For callus induction, leaves (± 0.5 cm) and petioles (± 1 cm) were

isolated from sterile cultures and planted on MS medium enriched with three types of auxins, namely 2,4-D (2,4-dichlorophenoxyacetic acid), Picloram (4-amino-2,5,6-trichloro-2-pyridinecarboxylic acid) and Dicamba (3,6-dichloro-2-methoxy benzoic acid) at the concentration of 2, 4, and 6 mgL^{-1}. pH media was adjusted to 5.8. All media were added with 30 gL^{-1} sugar and solidified with 2.5 gL^{-1} phytagels. The cultures were incubated in the culture room with photoperiodicity for 16 h, temperature at 22ºC and light intensity of 300 lux. Every treatment consisted of 4 replications (bottle). Each bottle contained 4 explants.

Parameters observed were percentage of callus induction, fresh weight of callus, rate of callus growth, color of callus and texture of callus. The observations were conducted for 6 weeks. Data collected was analyzed with arithmetic and discussed descriptively.

RESULTS AND DISCUSSIONS

Results

The use of auxin is important for callus induction. At 6 weeks in culture, calli were formed on all tested media. However, the rate of callus growth varied because the types and concentrations of auxins tested affected on all parameters observed either on leaf or petiole explants. In all treatments of auxins, the percentage of callus formation and fresh weight tended to increase at the concentrations of 2 to 4 mgL^{-1} but decreased at the concentration of 6 mgL^{-1}. These occurred at all types of auxin used (2,4-D, Picloram dan Dicamba) either on leaf or petiole explants (Figure 1 and 2; Table 1 and 2). Dicamba was effective enough for callus induction but still rarely to be used in Centella. Interestingly, the use of Dicamba as a single plant growth regulator in this research was effective enough as shown in the percentage of callus induction, the mean of callus fresh weight and the rate of callus growth.

On leaf explants until the sixth weeks of culture, the percentage of callus induction was 100% at the concentrations of 2 and 4 mgL^{-1} 2,4-D and then decreased become 81,3%. On the other side, all concentrations of Dicamba yielded 100% callus formation. In this current study, the highest mean of fresh weight of callus was obtained from 4 mgL^{-1} Dicamba i.e. 0.27 g (Table 1).

Table 1 showed that a good rate of callus growth (+++) on leaf explants were observed from 2,4-D (2 dan 4 mgL^{-1}) and 2 mgL^{-1} Dicamba. The fresh weight callus produced from 4 mgL^{-1} Dicamba was better than 2 mgL^{-1} Dicamba, however, the rate of callus growth from 2 mgL^{-1} Dicamba (+++) was better than that from 4 mgL^{-1} Dicamba (++) because a part of callus texture of 4 mgL^{-1} Dicamba was thick and showed as cotton like structures (Fig. 1). When petiole was used as the explants, the 100% callus formation was produced from all concentrations of 2,4-D until the sixth week of culture. However, the highest fresh weight and callus growth were obtained by 4 mgL^{-1} 2,4-D and Dicamba (Table 2).

Color and texture of Centella callus obtained from both explants either leaves or petioles were very influenced by types and concentrations of auxins used (Table 1 and 2) as well as the in vitro culture period. On leaf explants from the beginning of callus induction until the fourth week of culture, the color of callus was cream, but after that time they turned to brown in 2,4-D treatment. Meanwhile, in Picloram and Dicamba treatment, the color of the callus was still cream. Similarly their textures, at the beginning of callus induction until the fourth week of culture, were still solid but after that time the texture callus started to become friable, except callus in 2,4-D treatment that was still compact (Table 1). Visual appearance of callus morphology on petiole explants from concentration of 2 mgL^{-1} either 2,4-D (Fig. 2A) or Picloram (Figure 2D) was similar to 4 mgL^{-1} Dicamba (Fig. 2H), whereas all of callus structures were globular and embryogenic. These structures were known as regenerable calli becoming shoots. Most of calli were globular structures and glossy especially on petiole explants from Dicamba treatments at the sixth week of culture (Fig. 2 G-I).

Table 1. The effect of types and concentrations of auxins on leaf explants at the sixth week in culture

Treatment (mgL^{-1})	Callus induction (%)	Fresh weight of callus (g)	Rate of callus growth	Color and texture of callus
2,4-D 2	100	0.24 ± 0.13	+++	Brown, compact
2,4-D 4	100	0.25 ± 0.14	+++	Brown, compact
2,4-D 6	81.3	0.09 ± 0.04	++	Brown, compact
Picloram 2	25	0.04 ± 0.03	++	Cream, friable
Picloram 4	50	0.04 ± 0.02	+	Cream, friable
Picloram 6	25	0.04 ± 0.03	+	Cream, friable
Dicamba 2	100	0.25 ± 0.15	+++	Cream, friable
Dicamba 4	100	0.27 ± 0.17	++	Cream, friable
Dicamba 6	100	0.21 ± 0.14	++	Cream, friable

Notes: Data are mean from 4 replications, **= No growth, + = Bad, ++ = Enough, +++ = Good

Table 2. The effect of types and concentrations of auxins on petiole explants at the sixth week in culture

Treatment (mgL^{-1})	Callus induction (%)	Fresh weight of callus (g)	Rate of callus growth	Color and texture of callus
2,4-D 2	100	0.21 ± 0.04	+++	Cream, friable
2,4-D 4	100	0.29 ± 0.02	+++	Cream, friable
2,4-D 6	100	0.23 ± 0.04	++	Cream, friable
Picloram 2	50	0.29 ± 0.25	++	Cream, friable
Picloram 4	50	0.04 ± 0.01	+	Cream, friable
Picloram 6	25	0.15 ± 0.05	+	Cream, friable
Dicamba 2	50	0.06 ± 0.00	++	Cream, friable
Dicamba 4	83.3	0.34 ± 0.14	+++	Cream, friable
Dicamba 6	50	0.08 ± 0.01	+	Cream, friable

Notes: Data are mean from 4 replications, **= No growth, + = Bad, ++ = Enough, +++ = Good

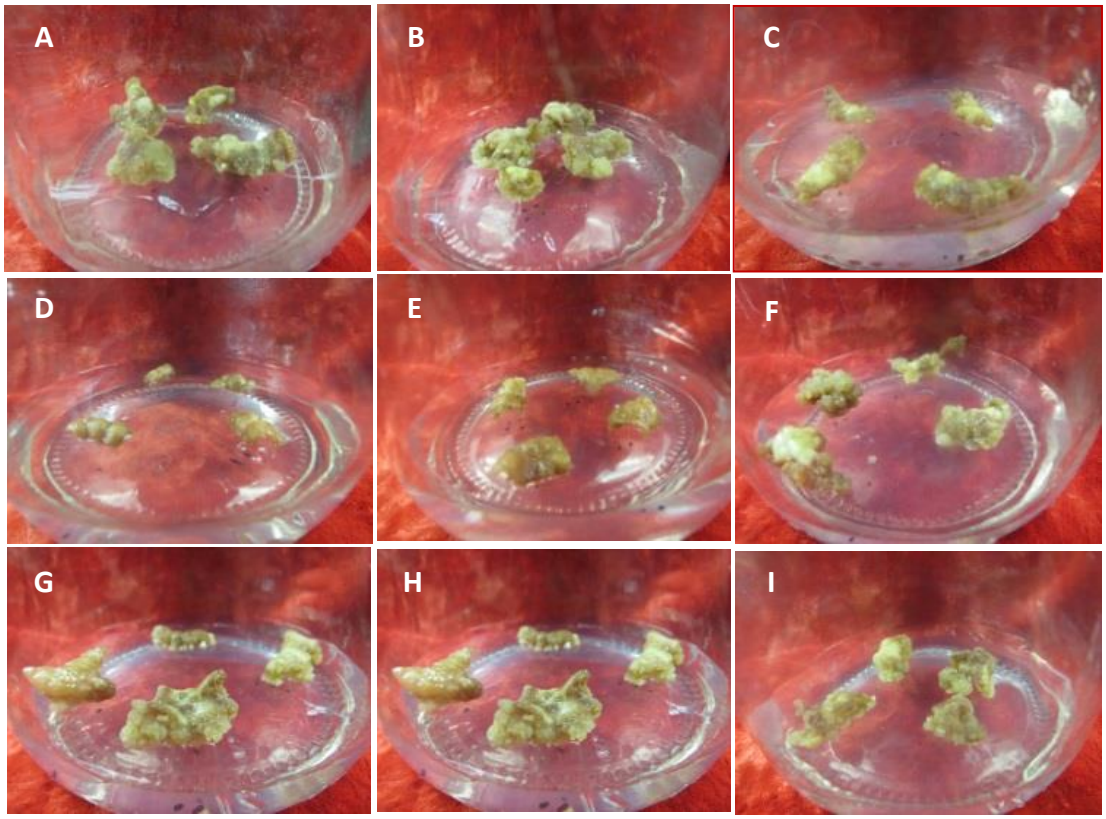

Figure 1. The visual appearance of callus formation induced from different types and concentrations of auxins by using leaf explant six weeks of culture. A-C: auxin 2,4-D (2, 4 and 6 mgL^{-1}), D-F: Picloram (2, 4 and 6 mgL^{-1}), and G-I: Dicamba (2, 4 and 6 mgL^{-1})

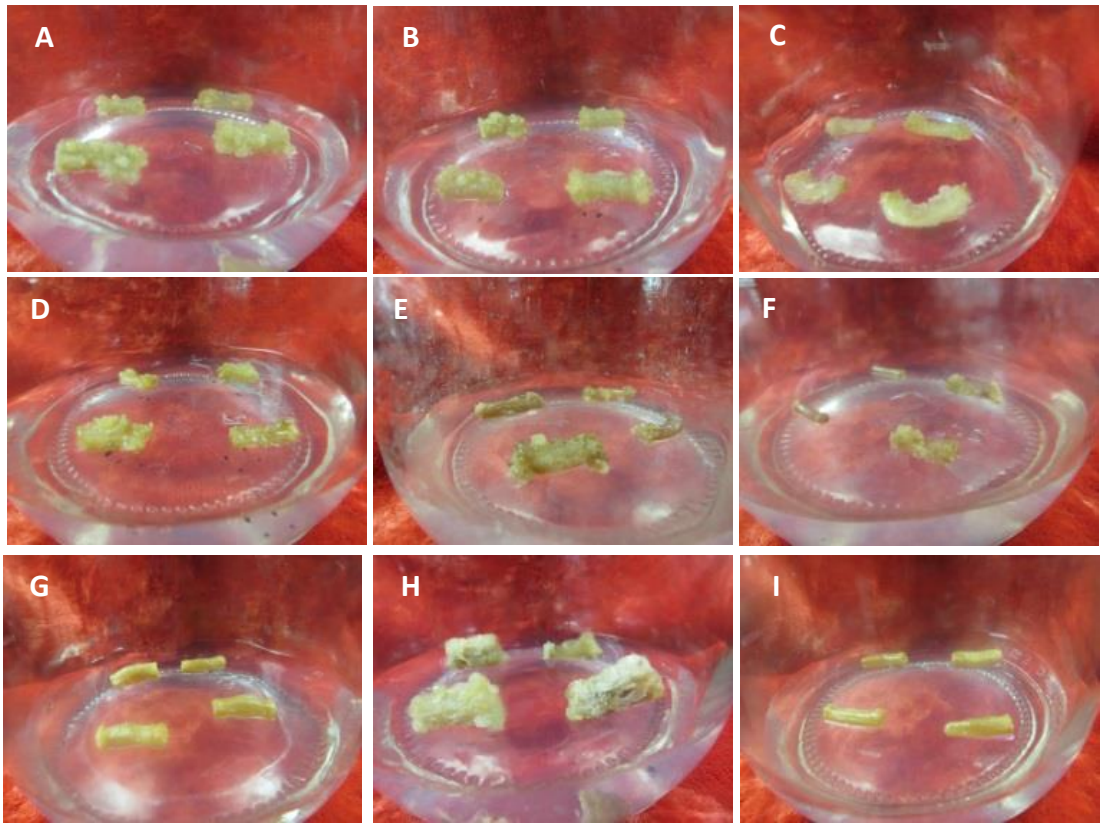

Figure 2. The visual appearance of callus formation induced from different types and concentrations of auxins by using petiole explant six weeks of culture. A-C: auxin 2,4-D (2, 4 and 6 mgL^{-1}), D-F: Picloram (2, 4 and 6 mgL^{-1}), and G-I: Dicamba (2, 4 and 6 mgL^{-1})

Discussion

Based on this research, it was known that the response of the explants was different depending upon the types of auxins, the morphology of various callus. It means that the different types of explants produced different callus morphology if they were cultured on auxins types. This result was confirmed by of Zia et al. (2007) that callus morphology and induction response much depended on explant source, explant size, medium content, and plant growth regulator in media and culture condition as well.

The percentage of callus induction and fresh weight of callus increased with the auxin concentration of 2 to 4 mgL^{-1} followed by decreasing rate at concentration of 6 mgL^{-1}. Nevertheless, 4 mgL^{-1} was the most suitable concentration. In other words, Centella explant either leaf or petiole gave the best response to all auxin types tested at 4 mgL^{-1} concentration. The effective auxin types for induction and fresh weight of Centella callus were 2,4-D, Dicamba, and Picloram, respectively. A bit difference of previous result had been reported by Tan et al. (2010) that 2,4-D was the best auxin type for callus induction on Centella followed by Picloram and Dicamba. The use of Dicamba on Centella culture is still rarely; however, concentration of more than 2 mgL^{-1} Dicamba could give a better result to induce callus. Ren et al. (2010) proved that 4 mgL^{-1} Dicamba was apparently the best callus induction of wheat. Lee et al. (2012) reported that concentration of 3 mg/L Dicamba yielded the best callus induction on wildrye grass plant, and likewise Can et al. (2004) reported that the concentration of 5 mgL^{-1} Dicamba was the best concentration to induce callus on ryegrass plant.

The rate callus induction in this research was mainly at 2 mg/L 2,4-D (++) treatment which showed similar rate (+++) result of Panathula et al. (2014) and Tan et al. (2010) in the same plant. While the use of other auxins, in this case, Dicamba and Picloram on Centella plant were still very rare.

Color and texture of Centella callus gained from both explants either leaves or petioles were very influenced by the use of types and concentrations of auxins. The similar result reported by Martin (2004) and Elaleem et al. (2009) that texture, color, and structure of callus were influenced by medium formulation and also concentrations and types of plant growth regulator used. Auxin has an important role in callus induction, but it depends on the presence of types and concentration of auxins in culture medium, because different types of auxin may give different effects (Baskaran et al. 2006; Ren et al. 2010). Leaf explants produced brown callus and compact texture at all concentrations of 2,4-D. The same result reported by Panathula et al. (2014) showed that the use of 1.5 dan 2 mgL^{-1} 2,4-D produced callus brown in color and compact in texture while lower concentrations (0.5 dan 1 mgL^{-1}) yielded yellowish green and friable callus. Joshi et al. (2013) stated that the use of leaf and petiole explants on 2,4-D (0.5 mgL^{-1}) containing medium yielded cream callus meanwhile on 2,4-D (1 mgL^{-1}) yielded green callus. The result of this research was different from the result reported by Tan et al. (2010) that the use of 0.5-2.5 mgL^{-1} 2,4-D yielded whitish green and friable callus and the result reported by Reddy et al. (2013) that the use of 2 mgL^{-1} 2,4-D yielded whitish and compact callus.

The calli from Picloram and Dicamba treatments were stable as cream and friable, similar to the research reported by Tan et al. (2010) in which the calli from both types of auxins were whitish and friable. In this research, the brown color was also observed from 2,4-D treatment. It might be caused by the accumulation of phenolic compounds that would gather in cytoplasm cell, then underwent oxidation and polymerization so that the oxidated products showed brown or black in color (Lukas et al. 2000).

Light intensity in the culture room may also be able to influence the change of callus color (Arumugam et al. 2011). The presence of light intensity in the culture room at 300 lux would increase the secretion of phenolic compound that caused browning. Thereby, the change of color and texture of callus was not only affected by different types of explant but also influenced by the concentrations and types of auxins added in MS basal medium and the condition of room culture. Besides those factors, the success of callus induction depended on genotype of the plant used as explant (Hussaini et al. 2015).

In general, the result of this research could be concluded that 4 mgL^{-1} 2,4-D or Dicamba was the best auxin types causing the best response for induction of Centella callus derived from leaf and petiole explants.

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