CALLUS INDUCTION AND PROLIFERATION OF *Artemisia cina* Berg ex Poljakov

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ABSTRACT

This study was conducted to evaluate the effect of light and dark condition on callus induction and callus proliferation from callus of Artemisia cina. Sterilized explants were cultured on MS medium containing 1 mg/l 2.4-D and placed under light and dark condition.The result showed that Callus induction were more effective on dark condition, however the callus proliferation were more effective on light condition. The best stage to regenerate is 48 days after transferred from induction medium.

Key words: Callus, induction, proliferation, Artemisia cina, Berg ex.

INTRODUCTION

Herbs and natural ingredients are generally not a new thing since those are needed for drugs in order to solve the health problems. Plants and microbes are the main source of secondary metabolites (Hayashi *et al*., 1997; Armaka *et al*., 1999; Lin *et al*., 1999; Baso *et al*., 2005), and to be the main source of new drugs (Harvey, 2000), both such as phenol compounds, alkaloids, terpenoids, as well as non-protein amino acids (Smith, 1976). *Artemisia cina* included in the genus Artemisia (Asteraceae) is one of the plants that can be used as a medicinal plant. The phytopharmacological evaluation of aerial parts of this genus showed the presence of anti-inflammatory (Ahmad *et al*., 1992), antibacterial (Kristianti *et al.,* 2009; Kristianti *et al*.,2010; Kovats *et al*., 2010), anthelmintic (Tariq *et al*., 2009) and antimalarial activities (Cubukcu *et al*., 1990).

Plant tissue culture is an innovative technique for enhanced production of valuable drugs from medicinal plants (Thorpe, 1990). Callus culture is one technique of tissue culture that can be used in plant propagation. Plants regenerated from callus culture are genetically identical to the source material and free from pathogens, and it is possible to produce a huge number of plantlets in a very short period of time (Shirin *et al*., 2007).The production of embryogenic calli and its subsequent regeneration are the basic prerequisites for the potential use of these techniques. Successful in embryogenic calli induction is depended on many factors such as plant genotype, explants type, culture medium, plant growth regulator and culture environment (Khana *et al*., 1998).Light is known to influence the rates of cell division (Fraser *et al*., 1967; George and Sherrington, 1984) and ethylene evolution (Huxter *et al*., 1981), which, in turn, may influence caulogenesis and rhizogenesis (Cornejo-Martin *et al*., 1979). Therefore, the duration and timing of light application play an important role in explants morphogenesis (Noth and Abel, 1971). High quality of callus was obtained on Thai Rice Aromatic "KDML 105" when it was grown on the MS medium containing 3% sucrose at 25°C under dark condition (Summart *et al*., 2008). Dark condition was the best treatment for callus induction in seed and root explants of *Cynodon dactylon* (Salehi *et al*., 2008). The purpose of this study was to determine the effect of the condition of the environment light/dark on callus growth of A. cina.

MATERIAL AND METHOD

Plant Material

Explants of *Artemisia cina* were collected from The Medicinal Plants Research Center (BBPTO) Tawangmangu Indonesia.

Callus Induction

In order to promote callus induction, sterile explants of leaves were aseptically placed on Murashige and Skoog medium (MS) supplemented 1 ppm 2.4-D containing 30 g/l (w/v) of sucrose and 8 g/l (w/v) agar. The explants were incubated at 25^{o}C under dark and light condition (1200 Lux). The percentage of explants producing callus was recorded every 4 days by scoring for 20 days. The Observations were scored based on the time of callus formation and number of root, as followed:

Callus Proliferation

The callus formed from induction were weighing and transferred to a MS medium supplemented 1 mg/l, 2.4-D containing 30 g/l (w/v) of sucrose and 8 g/l (w/v) agar. The explants were incubated at 25^{o}C under dark and light condition (1.200 lux). Callus growth was observed for 12 weeks. Sub-cultures were conducted every 16 days. In every sub culture the callus were weighted and observed to classify into three classes namely: callus friable;. callus not friable and callus senescence.

RESULT AND DISCUSSION

Callus Induction of *Artemisia cina*

In order to establish the most suitable condition for callus induction we placed the explant under dark and light condition. Callusing was initiated on the eighth days after induction both in the dark and light condition. The phenomenon that occurs in callus induction of *A. cina* is that root emergence on the surface of leaves and callus appears at the bottom of the leaf (Figure 1).

However the score of callus formed and number of root were higher when the explants placed in the dark condition compare to the presence of light (Table 2 and Figure 2). Dark condition was also found to be preferable for the growth of *Lycopersicum esculentum*, *Daucus carota,* and

Table 1. Scoring of callus formation and number of the root.

Score	Details
1	Callus and roots do not appear
2	Callus appeared less than or equal to 5% to 10% of the leaf surface
3	Callus appeared less than or equal to 5% to 10% of the leaf surface area and the number of roots is less than or equal to 5 to 10
4	Callus appeared greater than 10% to 30%, the number of roots greater than 10 to 20
5	Callus appeared greater than 10% to 30%, the number of roots is less than or equal to 5 to 10
6	Callus appeared greater than 30% to 50%, the number of roots greater than 10 to 20
7	Callus appeared greater than 30% to 50%, the number of roots is less than or equal to 5 to 10
8	Callus appeared greater than 50%, the number of roots greater than 10 to 20
9	Callus appeared greater than 50%, the number of roots is less than or equal to 5 to 10

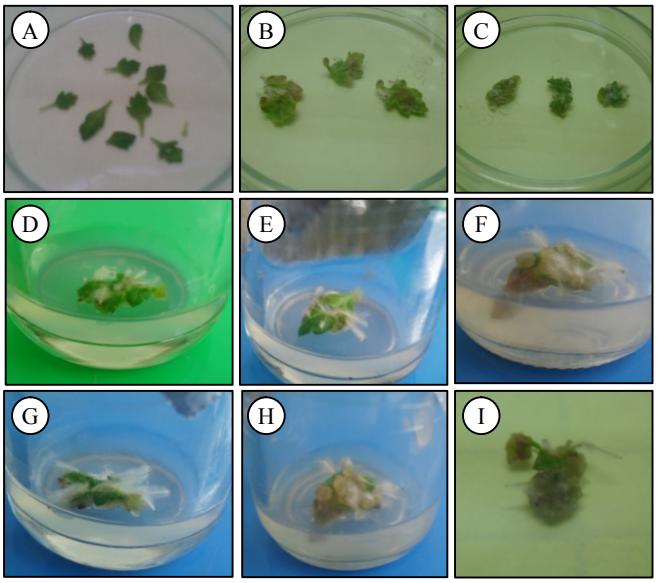

Figure 1. A = Initial callus induction material; B = Callus age 8 days after induction in light conditions; C = Callus age 8 days after induction in dark conditions; D = Callus than 12 days after induction in light conditions; E = callus age of 12 days in dark conditions; F = callus age of 16 days in dark conditions; G = callus than 16 days in light conditions; H = age of 20 days after callus induction in dark conditions; I = age of 20 days after callus induction in light conditions.

Table 2. Scoring callus formed and the number of roots in dark or light conditions.

Day	Light	Dark	Number of root (Light)	Number of root (Dark)
4	1.25	1.31	0.00	0.00
8	2.12	2.06	0.56	0.93
12	5.75	6.64	4.93	5.43
16	7.25	7.35	6.68	7.21
20	7.80	8.50	6.81	8.28

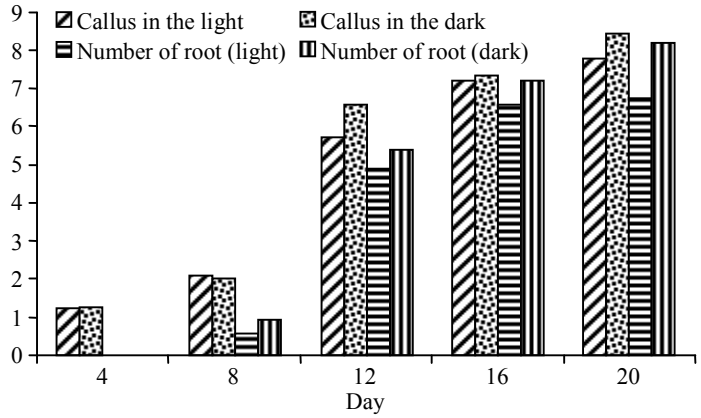

Figure 2. Callus growth on callus induction stage.

Arabidopsis thaliana callus (Hangarter and Stasinopoulos, 1991). The reduced growth caused by light can be primarily to photochemical alteration. The result showed that light-induced growth reduction was primarily due to photo degradation of component in medium than auxin.

Callus Proliferation

Callus proliferation phase aimed is to obtain callus of somatic embryos in higher number to be regenerated. The proliferation observation carried out for 12 weeks or 5 stages of proliferation that consists of initial proliferation (P0) to P5 (Figure 3). In The other hand observations of proliferation could be used to determine the exact age of callus to regeneration. The best stage to regenerate from proliferation callus was 48 days after transferred from callus induction medium (P2).The result indicated that light appeared to have an effect on growth of callus at proliferation stage. It was observed that the fresh weight of callus by the end of incubating period in the light was higher than fresh weight of callus incubated in the dark condition(Table 3 and Figure 4).

Similar result on the effect of light on callus growth was observed in the culture of *Cistanche deserticola* and *Eustoma grandiflorium*, where the presence of light was found to increase the production of callus (Ouyong *et al*., 2003; Mousavi *et al*., 2012). The increase of callus growth in the light condition might be related to the rate of nutrient up take which was found higher than those

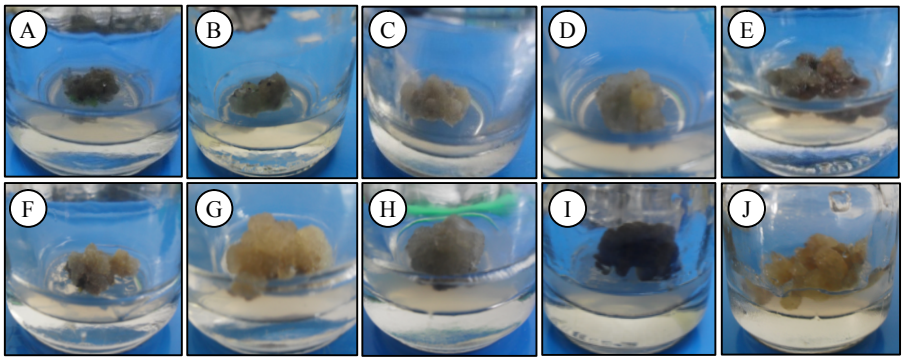

Figure 3. Callus Proliferation Stage. A = Early proliferation (P0) dark conditions, 16 days after transferred; B = Early proliferation (P0) light conditions, 16 days after transferred; C = Proliferation 1 (P1) dark conditions, 32 days after transferred; D = Proliferation 1 (P1) light conditions, 32 days after transferred; E = Proliferation 2 (P2) dark conditions, 48 days after transferred; F = Proliferation 2 (P2) light c onditions, 48 days after transferred; G = Proliferation 3 (P3) dark conditions, 64 days after transferred; H = Proliferation 3 (P3) light conditions, 64 days after transferred; I = Proliferation 4 (P4) under dark conditions, 80 days after transferred; J = Proliferation 4 (P4) conditions, 80 days after transferred.

Table 3. The average weight of callus proliferation.

Treatment	P0	P1	P2	P3	P4
Light	0.547	0.828	1.542	1.04	1.627
Dark	0.510	0.772	1.343	1.215	0.218

Table 4. Conditions callus proliferation stage.

Proliferasi Stage	Friable callus on light condition	Friable callus on dark condition	Not friable callus on light condition	Not friable callus on dark condition	Senescence callus on light condition	Senescence callus on dark condition
P0	100%	100%	0%	0%	0%	0%
P1	100%	100%	0%	0%	0%	0%
P2	50%	18%	50%	82%	0%	0%
P3	66%	71%	34%	29%	0%	0%
P4	53%	71%	47%	29%	0%	0%
P5	12.5%	3.60%	40.6%	53.60%	46.9%	42.90%

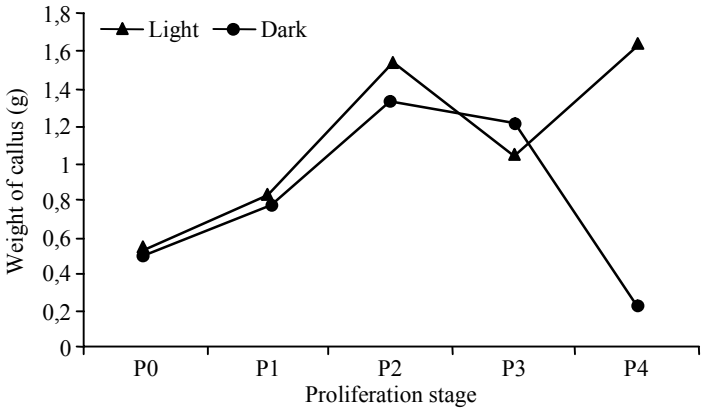

Figure 4. The Growth of callus proliferation stage.

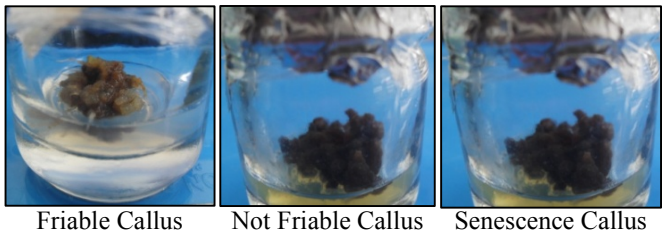

Figure 5. Callus induction.

in dark condition (Muse, 1989). The growth of callus decreased on both dark and light condition at P3 stage of callus proliferation (64 days after transferred from induction medium) (Figure 3). In contrast the percentage of callus friable was greater in P3 both in the dark and light conditions. Reduction in the growth of callus on P3 suspected in these conditions to form callus develops friable (Table 4).

CONCLUSION

Research conducted showed that callus induction affected by the dark while callus proliferation is affected by light condition. The best stage to regenerate is 48 days after transferred from induction medium.

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