CALLUS INDUCTION AND PROLIFERATION OF ARTEMISIA CINA BERG EX POLJAKOV

INDUKSI KALUS DAN PROLIFERASI ARTEMISIA CINA BERG EX POLJAKOV

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ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi efek kondisi terang dan gelap pada induksi dan proliferasi kalus dari kalus *Artemisia cina*. Eksplan steril akan dikulturkan pada media MS dengan kandungan 2,4-D 1 mg/L dan diletakkan pada kondisi terang dan gelap. Hasil penelitian menunjukkan bahwa induksi kalus akan lebih efektif pada kondisi gelap, sedangkan proliferasi kalus lebih efektif pada kondisi terang. Tahap terbaik untuk proses regenerasi adalah hari ke-48 setelah transfer dari media induksi.

Kata kunci: kondisi gelap, kondisi terang, proliferasi kalus, induksi kalus, *Artemisia cina*

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 (Kristiani et al., 2009; Kristiani 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

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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 (Comejo-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 percent 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 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°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:

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

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°C under dark and light condition (1200 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

eight 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 (Fig.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 condition (Table 2). Dark condition was also found to be preferable for the growth of *Lycopersicum esculentum*, *Daucus carota* and *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.

Fig.1. A. Initial callus induction material; B. The 8 days-old callus after induction in light conditions; C. The 8 days-old callus after induction in dark conditions; D. the 12 days-old callus in light conditions; E. The 12 days-old callus in dark conditions; F. The 16 days-old callus in dark conditions; G. The 16 days-old callus in light conditions; H. The 20 days-old callus after callus induction in dark conditions; I. The 20 days-old callus after induction in light conditions.

Table 2. Scoring Callus Formed and The Number of Roots In Dark/ Light Conditions

Time of observation (Day)	Score callus formed in light condition	Score callus formed in dark condition	Number of root (light condition)	Number of root (Dark condition)
4	1.25	1.31	0.0	0.0
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
21	7.80	8.50	6.81	8.28

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 (fig.2).

At the stage of P0 and P1 cells of callus on friable condition both the dark and light conditions, the stage of P2 -P4 cells of callus on friable and not friable condition on both dark and light conditions, the stage of P5, cells of callus on friable, not friable and senescence conditions in the dark and light conditions (fig.2).

Fig. 2. 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 conditions, 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) under light conditions, 80 days after transferred.

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 (d.a.t) 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 at the end of incubating period in the light was higher than fresh weight of callus incubated in the dark condition (Table 3).

Similar result on the effect of light on callus growth was observed in the culture of *cistanche deserticola* and *Eustoma grandiflorum*, 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 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) (Fig.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)

Table 3. The average weight of callus proliferation

Treatment	P0	P1	P2	P3	P4
Light condition	0.547	0.828	1.542	1.04	1.627
Dark condition	0.51	0.772	1.343	1.215	0.218

Table 4. Conditions Callus Proliferation Stage

Tahap proliferasi	Percentage of friable callus	Percentage of friable callus	Percentage of not friable callus	Percentage of not friable callus	Percentage of senescence callus	Percentage of senescence callus
	Light condition	Dark condition	Light condition	Dark condition	Light condition	Dark condition
P0	100%	100%	0.0%	0.0%	0.0%	0.0%
P1	100%	100%	0.0%	0.0%	0.0%	0.0%
P2	50%	18%	50%	82%	0.0%	0.0%
P3	66%	71%	34%	29%	0.0%	0.0%
P4	53%	71%	47%	29%	0.0%	0.0%
P5	12.5%	3.6%	40.6%	53.6%	46.9%	42.9%

A. Friable Callus

B. Not Friable Callus

C. Senescence Callus

Fig.3. Callus Condition

Conclusion

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

Knowledge

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