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King of Bitter (Andrographis paniculata Nees) Somatic Embryogeni Callus Culture on MS Medium With 2,4-D and BAP

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ABSTRACT. In this research we studied somatic embryogenesis derived on Sambiloto (*Andrographis paniculata*) callus culture by addition of BAP (benzylamino purine) and 2,4-D (2,4 diclorophenoxyacetic acid) on MS (Murashige and Skoog) Medium. King of Bitter seeds were germinated on MS medium for 3 weeks, then the second leaf that full opened used as explants. The explants were planted on medium with different concentration of growth regulator (BAP combined with 2,4-D) to callus induce. MS medium with 0.5 ppm BAP+ 0.5 ppm 2,4-D, 1 ppm 2,4-D, 1.5 ppm 2,4-D, and 2 ppm 2,4-D, six replications each treatment. The observation was using descriptive method for callus texture, somatic embryo derived from development phase after subcultured on MS medium with the same concrentration BAP and 2,4-D. The result showed that callus were formed on all of treatment (A, B,C and D), but callus with somatic embryogenesis was only found on C treatment, with the range of callus fresh weight (FW) were 0.6-1.0 g. After 3 weeks at second subcultured on MS medium with added 0.5 ppm BAP+ 1,5 ppm 2,4-D, somatic embryo development showed globular phase and heart shape phase.

Keywords: somatic embryogenesis, *Andrographis paniculata*, callus culture.

ABSTRAK. Pada penelitian ini telah dipelajari tentang embriogenesis somatik yang berasal dari kultur kalus sambiloto (Andrographis panikulata) dengan penambahan BAP (benzylamino purine) dan 2,4-D (2,4 diclorophenoxyacetic acid) pada Medium MS (Murashige dan Skoog). Benih King of Bitter dikecambahkan pada media MS selama 3 minggu, kemudian daun kedua yang terbuka penuh digunakan sebagai eksplan. Eksplan ditanam pada media yang diberi beberapa konsentrasi zat pengatur tumbuh (BAP dikombinasikan dengan 2,4-D) untuk menginduksi kalus. Medium MS dengan penambahan 0,5 ppm BAP+ 0,5 ppm 2,4-D (Perlakuan A), 0,5. ppm BAP + 1 ppm 2,4-D (perlakuan B), 0,5 ppm BAP + 1,5 ppm 2,4-D (perlakuan C), 0,5 ppm BAP + 2 ppm 2,4-D (perlakuan D), masing-masing perlakuan dengan 6 ulangan. Pengamatan menggunakan metode deskriptif terhadap tekstur kalus, embrio somatik yang diperoleh beserta fase perkembangannya setelah disubkultur pada media MS dengan konsentrasi BAP dan 2,4-D yang sama. Hasil penelitian menunjukkan bahwa kalus telah terbentuk pada semua perlakuan (A, B, C dan D), sedangkan kalus dengan embriogenesis somatik hanya pada perlakuan C, dengan kisaran berat segar kalus (FW) adalah 0,6-1,0 g. Setelah 3 minggu pada subkultur kedua pada media MS dengan penambahan 0,5 ppm BAP+ 1,5 ppm 2,4-D , perkembangan embrio somatik menunjukkan fase globular dan fase bentuk hati

Kata kunci: embriogenesis somatik, Andrographis panikulata, kultur kalus.

1. INTRODUCTION

Somatic embryogenesis is the developmental process by which bipolar structures that resemble zygotic embryos are developed from haploid or diploid somatic cell through an orderly embryological stage without gametes fusion. Two types of somatic embryogenesis are recognized: direct somatic embryogenesis (DSE), and indirect somatic embryogenesis (ISE). DSE is characterized by the induction of somatic embryos directly from pro-embryogenic cells from leaves, stem, micropores or protoplasts without the proliferation of calli, while in ISE somatic embryos are developed from friable embryogenic callus (Vega et al., 2009).

Somatic embryogenesis is unique process in plants and it is remarkable interest for biotechnological applications such as clonal propagation, artificial seeds and genetic engineering. Precisely, when somatic embryogenesis is integrated with conventional breeding programs and molecular and cell biological techniques, it provides a valuable tool to enhanced genetic improvement of crop species and phytomedicine (Vega et al., 2009; Habibah, 2009), especially for plant species with the highest potential medicine such as *Andrographis paniculata.*

Andrographis paniculata Nees (Family Acanthaceae), traditionally employed for centuries in Asia and Europe as a folklore remedy for wide spectrum of ailments, or n herbal supplement for health promotion, is nowadays incorporated into a number of herbal medicinal preparations. It is found in the Indian Pharmacopoeias and is a prominent component in at least 26 Ayurvedic formulas. In traditional Chinese medicine, it is an important “cold property” herb used to rid the body of heat, as in fevers and to dispel toxins from the body (Jarukamjorn, 2008). In Indonesia, *A. paniculata* is known as ‘sambiloto’, used as component of ‘jamu’ (traditional healthy drink). In Scandinavian countries, it is commonly used to prevent and treat the common cold. *A. paniculata* is one of the top 10 herbal medicines, which the Thai FDA has promoted as an alternative medicinal therapy for fever and inflammation.

Research on *A. paniculata* has revealed that the whole-plant extraction possesses many useful bioactivities, such as anti-inflammatory, antiviral, anticancer, and immune stimulatory activities. Furthermore, an improvement of bioactive compound through tissue, cell, callus culture has actively been investigating especially by adding elicitor treatments such as growth regulators (Habibah, 2009). However, callus culture of the somatic embryo is still rare. In some plants, the regeneration pathway stage can be obtained from the roots and leaves of young plants or mature embryos, while tissue culture via somatic embryos is very decisive and important stage for more advanced research purpose, for example on the *Phalaenopsis amabilis* (Sundari et al., 2023). Based on the description above, this research

was carried out for inducing *A. paniculata* embryogenic callus with growth regulators (BAP and 2,4-D) and to observe development of the somatic embryo phase.

2. METHODOLOGY

2.1 Plant material

Sambiloto (*A. paniculata*) seed capsules were surface-sterilized following procedure: briefly, the capsule that contain seeds were immersed in aqueous 25% (v/v) sunclean solution for 20 min and then rinsed 3 times in sterile distilled water. Then it was disinfected in 70% alcohol solution and rinsed in sterile distilled water. Seeds were collected with breaking capsule by using scalpel. Seeds were germinating on MS medium without growth regulator. The second leaf of 3 weeks old seedling was used as explants.

2.2 Embryogenic Callus Induction

Sambiloto leaves were cultured on MS medium with growth regulator (BAP combined with 2,4-D) to callus induce. MS medium with addition of 0.5 ppm BAP + 0.5 ppm 2,4-D (treatment A), 0.5 ppm BAP + 1 ppm 2,4-D (treatment B), 0.5 ppm BAP + 1.5 ppm 2,4-D (treatment C), 0.5 ppm BAP + 2 ppm 2,4-D (treatment D), each of treatment with 6 replications. And then explants cultured keep on the light condition at 26± 2^0C. And every 4 weeks callus was subcultured on MS media with the same growth regulator. After 4 weeks on the first subcultured, callus colour and texture were observed and callus fresh weight were calculated. Only embriogenic callus were subcultured again (the second subcultured) on MS media with the same growth regulator.

2.3 Development of Embryo somatic

After 3 weeks on second subculture, development phase of embryo somatic embryogenic were observed by using binocular microscope. Microscopic features were photographed using Olympus digital camera.

3. RESULT AND DISCUSSION

3.1 Embryogenic Callus Induction

Four weeks after explants planting on callus induction medium, growth respons of explants showed that callus were forming on each treatment. Whereas the colour, textures and range of fresh weight callus were very differed. Four weeks after the Sambiloto explants planted on callus induction medium, growth respons of explants showed that callus was forming on each treatment. Whereas the colours, textures and ranges of fresh weight callus were very different among by each other. From growth respon explants showed that growth regulator (BAP and 2,4-D) on MS medium had an effect fresh weight of callus. Whereas on the another study reported that the explant of *Catharantus roseus* leaves on MS medium with

0.75 mg/L 2,4-D or from adding 1 mg/L BA led to the formation of callus, which differentiated into branch-like growths by 11.6 - 12.0, respectively, 50 days after planting (Almemary, 2014) and formation callus *C. roseus* on Zenk medium with added by 10-5 untill 10-7 M BAP that combine with 0, 10 -5 untill 10-7 M NAA, on 28 days after planting(Prihatini et al., 2010) .

Figure1. Andrographis paniculata friable Callus on MS medium with added 0.5 ppm BAP + 1.5 ppm 2,4-D (C treatment)

From growth respon explants showed that growth regulator (BAP and 2,4-D) on MS medium at all of concentration can be used to callus induction. Whereas embryogenic callus showed only on C treatment (MS medium with added 0.5 ppm BAP + 1.5 ppm 2,4-D) (Fig. 1). Embryo somatic was derived on friable callus with white-yellowish colour and it showed a faster callus growing (Table 1), it was differed to treatment D (0.5 ppm BAP + 2 ppm 2,4-D) with callus FW were 0.6-1.0 g. Here, Auxin plays an important role in the induction of embryogenic callus and initiation of somatic embryo formation while cytokinins play a role in stimulating somatic embryo growth and development (Padua et al., 2020). Embryogenic calli can develop into somatic embryos on media containing a single cytokinin or added with auxins (JR Verma et al., 2018).

The embryos form either directly on the plant part or by the formation of callus, this process is carried out through three stages: the induction phase, the division stage, and the differentiation stage. Embryonic cells develop into three phases, the spherical, cardiac, and torpedo (Almemary, 2020).

Table 1. *A. paniculata* leaf explants growth respons on MS medium

Treatment	Callus		
	colour	Texture	Range of FW (g)
A (0.5 ppm BAP + 0.5 ppm 2,4-D)	white	Compact	0.1-0.2
B (0.5 ppm BAP + 1 ppm 2,4-D)	White	Compact	0.2-0.4
C (0.5 ppm BAP + 1.5 ppm 2,4-D	White-yellowish	Friable*	0.6-1.0
D (0.5 ppm BAP + 2 ppm 2,4-D	White-brownish	Friable, with nodular	0.2-0.4

**embriogenic callus*

Therefore, only callus on C treatment were subcultured on MS medium with the same concentration growth regulator (0.5 ppm BAP +1.5 ppm 2,4-D). On D treatment, 2,4-D increased on MS medium until 2 ppm caused callus growth inhibition, it showed by decreasing of FW of callus.

3.2 Development phase of Embryo somatic

Three weeks after the second subcultured of callus embryogenic, development phase embryosomatic were observed under binoculer microscope. It showed that embryo somatic of *A. paniculata* with globular fase development (Figure 2a.) and heart shape development phase (Figure 2b).

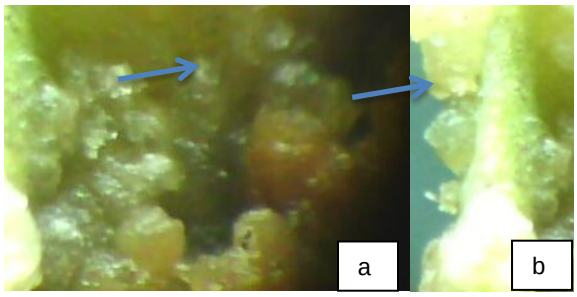

Figure 2. Embryo somatic of Andrgraphis paniculata with globular fase development (a) and heart shape fase development (b) 3 weeks old on the second subcultured (somatic embryo showed by arrow)

A complete development embryo phase were globular, torpedo, heart shaped and cotiledone phase. Whereas in researches of rice embryo somatic via tissue culture, embryo somatic development phase consist of globular (Vega et al., 2009; Mandal et al, 2003), torpedo and heart shape (Vega et al., 2009).

4. CONCLUSION

Sambiloto (*A. paniculata*) callus with somatic embryogenesis were formed only on MS medium with added by 0.5 ppm BAP +1.5 ppm 2,4-D (C treatment), with the range of callus fresh weight (FW) were 0.6-1.0 g. After 3 weeks at the second subcultured on MS medium with the same concentration growth regulator, somatic embryo development showed globular phase and heart shape phase.

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