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INDIRECT ORGANOGENESIS AND INDUCTION OF MORPHOGENIC CALLUS FOR IN VITRO PROPAGATION OF *Sansevieria masoniana*

Organogenesis Tidak Langsung dan Induksi Kalus Morphogenic pada Perbanyakan In Vitro *Sansevieria Masoniana*

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ABSRACT

Induction of morphogenic callus and meristemoid is essential to develop an effective in vitro propagation procedure and further mass propagation of economical valuable plant such as Sansevieria. In this study, callus induction on leaf explants was obtained using Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4 D). A massive production of *Sansevieria masoniana* organogenic callus was obtained on medium containing 0.35 mg/l 2.4 D and showed high shoot bud proliferation on a medium containing 6-benzyladenine (BA). No callus initiation and growth was observed on the MS medium supplemented with BA or on the medium without any plant regulator. Maximum regeneration frequency, the highest average number of shoots as well as number of leaves were achieved on a medium consisted of BA (2 mg/l) + 0.5 mg/L α-naphthaleneacetic acid (NAA) within 12 weeks of incubation. More roots produced when elongated shoots were transfered to free-hormone MS medium. Hence, micropropagation of *S. masoniana* was achieved successfully through indirect organogenesis pathway.

Keywords: Indirect organogenesis, morphogenic callus, sansevieria, in vitro propagation

ABSTRAK

Induksi morfogenik kalus dan meristemoid sangat penting dalam in vitro propagasi tanaman secara efektif dan selanjutnya untuk pembiakan secara cepat dan massal pada tanaman yang memiliki nilai ekonomis tinggi seperti Sansevieria. Dalam eksperimen ini, induksi kalus pada eksplan daun dilakukan pada media Murashige and Skoog (MS) yang dilengkapi dengan 2,4-dichlorophenoxyacetic acid (2,4 D). Organogenik kalus dari *Sansevieria masoniana* diproduksi secara massif pada MS media yang diperkaya dengan 2,4 D 0.35 mg/l dan menghasilkan formasi meristemoid setelah disubkultur pada media MS yang mengandung 6-benzyladenine (BA). Tidak ada inisiasi kalus

maupun pertumbuhan tunas yang diobservasi pada MS media yang dilengkapi dengan BA dan pada media tanpa hormon tumbuh. Maksimal regenerasi tunas, rata-rata jumlah tunas dan rata-rata jumlah daun plantula diperoleh pada medium yang dilengkapi dengan BA (2 mg/l) + 0.5 mg/L α-naphthaleneacetic acid (NAA). Hasil tersebut dicapai setelah inkubasi selama 14 minggu. Planlet sansevieria menghasilkan lebih banyak akar ketika dipindahkan ke media MS tanpa zat pengatur tumbuh. Berdasarkan hasil yang diperoleh dengan menggunakan metode in vitro seperti di atas, mikropropagasi tanaman *S. masoniana* diperoleh melalui organogenesis tidak langsung.

Kata kunci : Organogenesis tidak langsung, morfogenik kallus, sansevieria, kultur in vitro

Introduction

The genus of Sansievieria consists of more than 70 species that widely used as outdoor and indoor ornamentals due to their beautiful and various colours, shapes and patterns of the leaves. The plants are commanly propagated by vegetative leaf cutting and division. This conventional propagation is resulted in slow growth rate and inefficient for commercial productions. Therefore an efficient tissue culture technique is needed as an alternative method of producing large number of plantlets for further cultivation at commercial levels. By the means of traditional propagation, each 10 cm of leaf cutting usually produced not more than 3 propagules (Sarmast et al. 2009; Yusnita et al. 2011), but in vitro propagation, if explants taken in 1 cm pieces can mostly produce more than 100 plantlets (Sarmast et al. 2009).

In the tissue culture procedure, there are two ways of plant propogation, either by direct organogenesis or through indirect organogenesis. Direct or indirect organogenesis can be achieved in a plant species by manipulating the plant growth regulators and explant types (Martin and Madassery 2005; Ali et al., 2007; Adiyecha et al. 2013). Micropropagation of sansevieria species was successful through indirect organogenesis procedure by Shahzad et al., (2009). In their work, shoots regenerated from callus that previously induced on MS medium suplemented with IBA and from nodules raised on 2,4 D and 2,4 T containing medium. A

similar study by Sarmast et al. (2009) using *Sansevieria trisfasciata* leaves as explants was resulted in meristemoid production following callus induction, the meristemoid developed into shoots when cultured on the medium containing cytokinin. Another work by Yusnita et al. (2011) confirmed an indirect organogenesis pathway in the plant tissue culture of Sansevieria plants.

Indirect organogenesis could increase the possibility of producing large number of propagules and may lead to morphogenic variation in the resulting organs. However, minimizing the intervening unorganized callus growth is important factor to develop an effective plant propagation method. Therefore, the present experiment were aimed to induce callus cultures formation from leaf explants and regenerate of multiple shoots from morphogenic callus mass.

Material and Methods

The young fully expanded leaves of greenhouse grown *Sansevieria masoniana* were collected and washed with tap water for 10 min and transferred to airflow cabinet and surface sterilized with 70% ethanol for 5 min and then submerged in a commercial bleach solution (containing 5.25% sodium hypochlorite) for 7 min and were rinsed three times with sterile distilled water and cut into 1 cm pieces. Explants were cultured on MS medium (Murashige and Skoog 1962) containing 30 g/l sucrose and 8 g/l agar. Media's pH was adjusted to 5.7 (with HCl & NaOH 0.1 N) prior to being autoclaved for 20 min at 121^{o} C and 1.5 kg cm pressure. Explants were incubated on MS medium supplemented with 0.35 mg/l 2,4-D or BA and in the medium without growth regulator. Total number of explants for each treatment was 20. After 2 weeks of incubation, the calli were transferred to the medium containing 5 mg/l BA for shoot bud proliferation and followed by subculturing to MS medium supplemented with 2 or 5 mg/l BA + 0.5 mg/l NAA for shoot differentiated. After harvesting the regenerated shoots, the stock callus was subcultured onto fresh regeneration medium for shoot

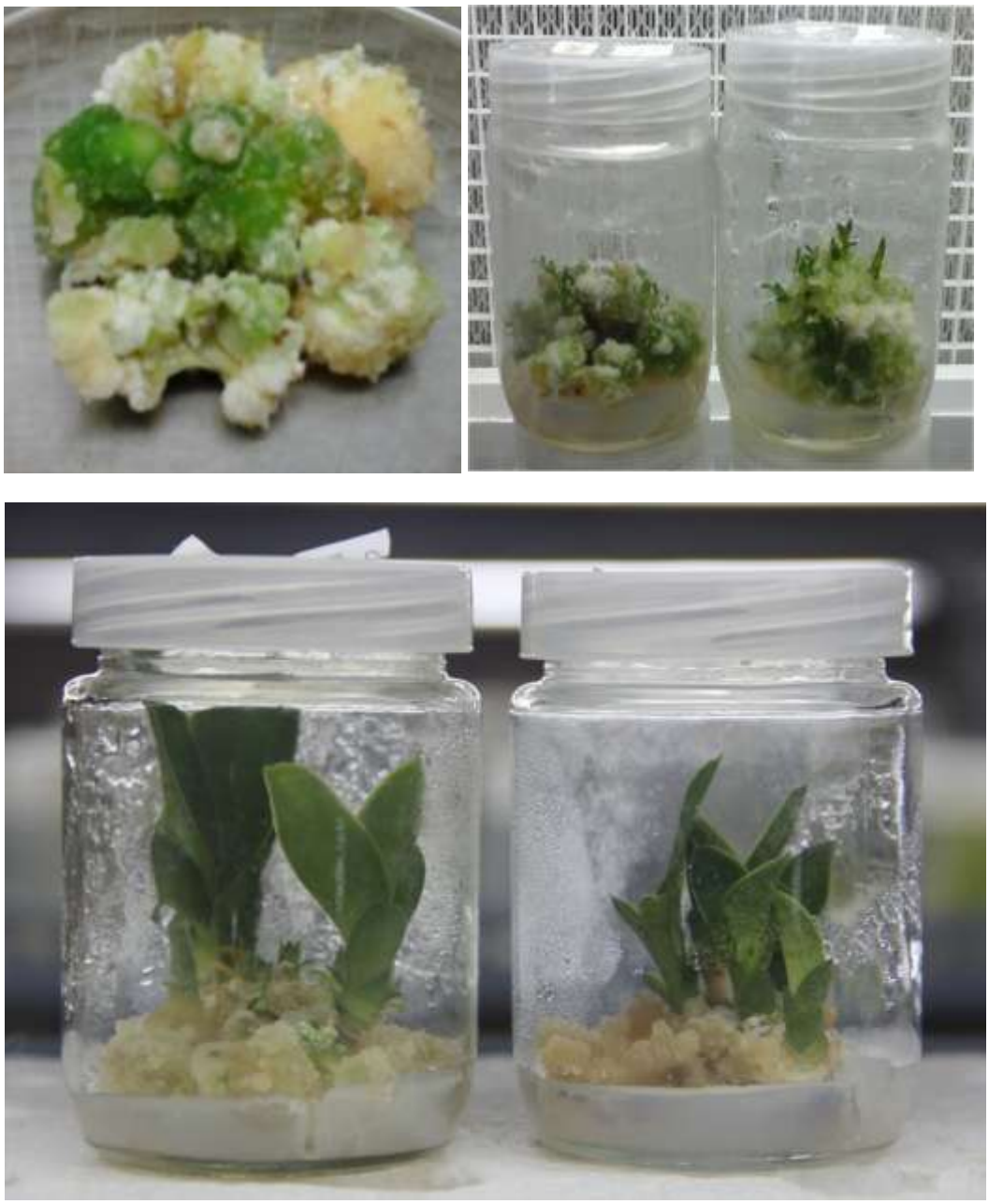
multiplication and elongation. Subcultures to the fresh media with the same treatments were conducted twice in 4 weeks intervals. All cultures were incubated in a culture room at 22^{o}C with cool-white fluorescent light at 2000 lux continuously.

The experiment was arranged in a completely randomized design with 5 replicates and each replicate consisted of 4 explants. Number of shoots and propagules (shoots + leaves) were recorded after 14 weeks. Data obtained were analyzed using one way analysis of variance (*ANOVA*) and means were compared using Least Significint Difference (LSD) test at $P = 0.05$.

Results

Leaf explants on MS basal medium and on the medium containing BA did not exhibit morphogenesis or callus formation. Inclusion of 2.4 D promoted induction of callus from all over the surface of explants, however, incorporation of a certain level of 2.4 D (0.35 mg/l) induced the formation of highly morphogenic callus. The callus was initially soft and completely off-white in colour but afterwards some areas on the surface of the callus appeared greenish in colour. When subcultured on basal MS medium containing BA, this callus produced meristemoid that developed into planlets upon subcultured on MS medium suplemented with BA and NAA (Picture 1). On hormone-free MS medium and BA containing medium, there was no any induced callus or growth was considerably observed.

Upon transfer of shoot buds (meristemoid) obtained on BA (5 mg/l) to MS medium supplemented with BA and NAA, shoot regenerated, elongated and produced leaves (Table 1). Elongated shoots were initially rooted when cultures were transfered to the freshly same medium. The individual shoots were separated from the regenerative callus mass and transferred to the full strength growth-regulator-free MS medium for more and vigorous roots formation.



Picture 1. Compact and greenish callus induced on MS + 0.35 mg/l 2.4 D **(A)** developed into meristemoid formation after being subcultured on MS + 5 mg/l BA **(B)**. Growth of regenerated shoot on MS + 2 mg/l BA + 0.5 mg/l NAA **(C)**.

Table 1. Effect of different different growth hormone on shoot regeneration rate and effects of cytokinin and auxins on growth of planlets from calli raised on 2,4-D (0.35 Mg/l)

Callus Induction Medium (MS)	Shoot regeneration Rate (%)	Growth Hormones (mg/l)	Number of shoots per explant (±SE)	Number of leaves per plantlets (±SE)
2.4 D (0.35 mg/l)	85	BA2 + NAA0.5	9.4 ± 0.60[a]	5.1 ± 0.32[a]
BA (2 mg/l)	-		5.2 ± 0.37[b]	3.9 ± 0.61[b]
Hormone free MS	-	BA5 + NAA0.5		

Means followed by a different letter in each column are significantly different at LSD test (*P = 0.05)*.

Discussion

In current experiment, callus and meristemoid induction occured when explants were cultured on MS medium supplemented with 0.35 mg/l 2.4-D (callus induction medium) for 2 weeks followed by transferring the morphogenic callus to MS medium fortified with BA 5 mg/l (proliferation medium) for 2 weeks. Organization of greenish smooth spots were observed from the surface of the callus after 10 days of culturing on the induction medium, which were modified into shoot buds with the appearance of flat leafy outgrowth on proliferation medium in the next 15 days.

The results showed that BA proved to be an efective cytokinin to induce the formation of adventitious buds which was then differentiated into multiple shoots on MS medium fortified with different concentrations of BA and NAA (regeneration medium). Further shoot regeneration was developed by repeating of organogenic cally on fresh regeneration medium. Shoots regeneration was observed on all media and the numbers of regenerants ranged from 5.2 to 9.4 per explant (Table 1). The shoot regeneration started after 2 weeks of incubation and the frequency of shoot regeneration was influenced by the combination concentrations of hormones. A best response of 2,4-D induced callus was observed on BA (2 mg/l) + NAA (0.5 mg/l) with an

average shoot number of 9.4 ± 0.60 as compared to 5.2 ± 0.57 on BA (5 mg/l) + NAA (0.5 mg/l). This result confirmed a previous study result (Shahzad et al. 2009) on micropropagation of *Sansevieria cylindrica* that the increase in BA concentration from 5 μM (1.1 mg/l) to 10 μM (2.25 mg/l) did not accelerate shoot regeneration. Their experiment result also showed a reduction in shoot number at reduced concentration of BA (1 μM). The advantageous effect of BA as well as BA and NAA combination is well documented in various studies conducted in sansevieria species (Anis and Shahzad 2005; Shahzad et al. 2009; Sarmast et al. 2009; Yusnita et al. 2009), and in other plants, *Momordica cymbalaria* Fenzl (Nikam et al. 2009), and *Curculigo Orchioides* Gaertn (Adiyecha et al. 2013).

The present study showed media supplemented with cytokinin (BA) was not effective on meristemoids formation before induced callus obtained on 2,4 containing medium, which was in consistence to the earlier report in *Sansevieria trifasciata* (Sarmast et al. 2009). Likely on BA containing medium, the MS medium devoid growth regulator (control) did not support any morphogenic response, hence callus formation is essential prior to meristemoids production in micropropagation of sansevieria. Callus formation occur profusely on the media enriched with auxin (2,4 D) and regenerated into shoots after being placed on the medium amended with cytokinin alone or cytokinin in combination with auxin. The earlier report of Sarmast et al. (2009), Shahzad et al. (2009), and Yusnita et al. (2011) are in aggrement with our results and proves the mode of in vitro propagation of sansevieria plants through indirect organogenesis pathway, and therefore further works are needed to study the direct organogenesis of in vitro propagation of sansevieria as well as to compare the quantity of direct and indirect plantlets formation.

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