

IN VITRO ORGANOGENESIS OF TWO SANSEVIERIA CULTIVARS ON DIFFERENT CONCENTRATIONS OF BENZYLADENINE (BA)

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ABSTRACT

In vitro regeneration of buds and shoots via organogenesis in two genotypes of *Sansevieria trifasciata* was established. Leaf segments (1 cm x 1 cm) of cv. Hahnii and cv. Lorentii were cultured on Murashige and Skoog (MS) basal medium containing 2,4-dichloro-phenoxyacetic acid (2,4-D) for 1 week, trans-ferred into MS medium without plant growth regulator (MS0) for 1 week, and then cultured to MS medium containing different concentrations of benzyladenine (BA:0; 0.1; 0.25; 0.5; 1 and 2 mg/l) for 3 consecutive passages with 4 week intervals. The results showed that BA proved to be an effective cytokinin to induce the formation of adventitious buds and shoots in two cultivars of *Sansevieria trifasciata*. The maximum of 12 propagules per explant of Lorentii and 9.3 propagules of Hahnii were obtained in medium with 2 mg/l BA after 14 weeks. Furthermore, regenerative capacity to form shoot buds and propagules was genotype dependent. The propagules number formed by cv. Lorentii was significantly higher than those formed by cv. Hahnii. The average shoot length formed by cv. Lorentii was also higher than those of cv. Hahnii. Shoots of both cultivars were successfully *ex vitro* rooted and acclimatized to the greenhouse with high survival rate (95.9 -100%).

Keywords: organogenesis, *Sansevieria trifasciata*, benzyladenine, genotypes

INTRODUCTION

Sansevieria (*Sansevieria trifasciata*) or snake plant is a genus of ornamental foliage suitable for either outdoor or indoor plants. Plants from this genus are widely used for their beautiful and various shapes, colors, and

patterns of their leaves. The color of its leaves varies from dark green, pale green, grayish green or a combination of green and white or yellow with a various snake skin pattern of the lamina. In addition to ornamental foliage, *sansevieria* is used as a plant that absorbs hazardous pollutant from the atmosphere. A research conducted by NASA found that snake plants were capable of absorbing carbon monoxide and lead from atmosphere. In Japan, it was reported that *sansevieria* could absorb many hazardous gases from atmospheres such as chloroform, formaldehyde, trichloroethylene, benzene and xylene in a significant amount (Suara Pembaharuan, 2005). People in Thailand believe that snake plants could be used as traditional medicinal plants for several diseases such as diabetes and cancer. Korean people believe that this genus can be used to overcome effect of various radiation, and some people of China think that planting *sansevieria* brings some good luck.

Since the year 2000, demand of *sansevieria* in Indonesia has dramatically increased and reached the highest in 2004, and remains high up to now. The cultivars of *sansevieria* mostly needed are cv. Lorentii or sword type and cv. Hahnii or the short type. Some of the local markets of *sansevieria* increase significantly due to the high demand for export. During the last five years, demand of *sansevieria*, especially from Korea and Japan, has been considerably high. For instance, since April 2005, 3500 plants of cv. Hahnii had been delivered for Malaysian buyers and one container of *sansevieria* was ordered by each of the buyers from Korea, Japan and Europe (Kontan, 2005).

The increase in *sansevieria* demand leads to an increase in both local and export market which in turn makes farmers grow this foliage extensively. However, extensive culture of

sansevieria could not fulfill the demand yet due to slow growing manner of plant propagation. Sansevieria is conventionally propagated by suckers and leaf cuttings. Both methods need plenty amount of plant materials and a long period of time to produce significant number of propagules. Generally, only 1-3 propagules are obtained from 1 leaf cutting for 2 months, and until 5 months the number of propagules remains constant without proliferation. Plant tissue culture could be an alternative to the conventional methods to produce a large number of propagules in a relatively short time. One pattern of *in vitro* regeneration of sansevieria is via organogenesis.

Blazich and Novitzky (1984) previously reported *in vitro* organogenesis of *Sansevieria trifasciata* using leaf segments as explants. In their report, explants were cultured on Murashige and Skoog (MS) (1962) medium supplemented with 0.25 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) for 2 weeks followed by cultures for 2 more weeks on MS without growth regulator (MS0) to induce callus formation. To induce adventitious shoot formation, explants were then transferred to MS + 0.3 mg/l kinetin. Wahyuningsih (2006) found that when 1x1 cm² leaf segments of *Sansevieria trifasciata* var. Lorentii were cultured on callus induction medium, followed by explant transfer to MS medium containing benzyladenine or kinetin, formation of adventitious shoots was observed. Responses of different cultivars to a certain growth regulator could be different as reported by Veltcheva and Svetleva (2005) in *in vitro* organogenesis of *Phaseolus vulgaris* L. This research was aimed to study the formation of adventitious shoots of two varieties of *Sansevieria trifasciata* (cv. Hahnii and cv. Lorentii) as affected by different concentrations of benzyladenine (BA).

MATERIALS AND METHODS

Plant Materials and Explant Preparation

This research was conducted in Plant Tissue Culture Laboratory, Department of Agronomy, College of Agriculture, The University of Lampung from March to August 2006. The plant materials were obtained from the local market in Lampung. The source plants of *Sansevieria trifasciata* cv. Hahnii and cv. Lorentii were maintained at green house with routine culture

procedures including watering, cleaning of the leaves and spraying with pesticide. Explants were taken from fully expanded young leaves cut at $\frac{3}{4}$ of the upper part for Hahnii and $\frac{1}{2}$ of the upper part for Lorentii. Explant surface-sterilization was started by washing the leaf cuttings using detergent under tap water for approximately 20 minutes. After that, leaf segments of 3 cm in size were rinsed in 5% of commercial bleach solution for 15 minutes followed by rinsing with tap water. The second sterilization was done in laminar air flow cabinet (LAFC, Bassaire, Hampshire, England). The leaf segments were soaked in 10% of Bayclin commercial bleach (Johnson, Jakarta, Indonesia, 5.25% NaOCl) solution for 15 minutes followed by three-time rinsing with sterile aquadest. One leaf blade of sansevieria cv. Hahnii was cut into ± 20 explants, whereas that of cv. Lorentii was cut into 40-50 explants.

All dissecting tools, ie., Petri dishes (Iwaki, Asahi Glass Co. Ltd. Thailand), forceps, scalpels (Maiden Stainless, Japan), bottles (Jakarta, Indonesia) and aquadest were sterilized by autoclaving (Tommy®, Tokyo, Japan) at 121°C and 1.5 kg/cm² for 30 min. After the surface was sterilized, explants were cut aseptically to approximately 1 cm x 1 cm and cultured on callus inducing medium which was MS basal medium supplemented with 30 g/l sucrose, vitamins and 0.25 mg/l 2,4-D (Merck KgaA, Darmstadt, Germany) for one week, followed by transferring to MS medium devoid of growth regulators (MS0). After being incubated for one week in MS0, all explants were transferred to shoot-inducing medium with different concentrations of benzyladenine (BA) (Merck KgaA, Darmstadt, Germany). 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 26±2°C provided by cool-white fluorescent light (Phillip, Indonesia) at 2000 lux continuously.

Organogenesis of Two Sansevieria Trivasciata Cultivars on Different Concentrations of BA

Treatments were arranged in factorial with 2 factors, i.e., first, *Sansevieria trifasciata* cultivars (Hahnii and Lorentii), and the second were concentrations of BA (0, 0.1, 0.25, 0.5, 1 and 2 mg/l). The experiment in this research was done in randomized block design with 5 replicates and each replicate consisted of 4

explants. Number of shoot buds (meristemoid structures < 0.5 cm), shoots (≥ 0.5 cm in length) and propagules (i.e., buds + shoots) and length of shoots were recorded after 14 weeks; in some treatments, it was after 18 weeks of cultures. In addition, cultures of *sansevieria* cv Lorentii with BA 0.5, 1.0 and 2.0 mg/l were subcultured three times, and the number of shoot buds and shoots were counted after 18 weeks. To measure all observed parameter, the plants were removed aseptically to a sterile Petri dish with millimeter block placed under the dish. Data was subjected to analysis of variance (ANOVA) using Minitab Statistical Software version 13.20. If there was any significant results of ANOVA, Least Significant Difference (LSD) method (Lentner and Bishop, 1986) at $P \leq 0.05$ was used to detect differences among treatments.

The basal medium formulation used for callus, meristemoid and shoot induction was macro and micro salts of Murashige and Skoog (1962) medium supplemented with 30 g/l sucrose, 0.1 mg/l thiamine-HCl, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl, 2 mg/l glycine and 100 mg/l myo-inositol (Merck KgaA, Darmstadt, Germany). The pH of medium was adjusted to 5.8 with 1N KOH or 1N HCl prior to addition of 7 g/l agar powder. The mixture was then boiled to make the agar soluble and a 30 ml- aliquots of medium were dispensed to 250 ml- culture bottles before autoclaving at 121°C and 1.5 kg/cm² for 20 min.

Ex vitro rooting of shoots (4-5 cm in length) was done by treating the base of the shoots with 0.2% (w/w) indolebutyric acid (IBA) (Merck KgaA, Darmstadt, Germany) and planting them in a 10 cm pot in diameter containing (1:1) mixture of sand and compost, then placed on benches in a shaded greenhouse. Irrigation with tap water was given once a day. After two months, survival of acclimatized plantlets was recorded.

RESULTS AND DISCUSSION

Results of this experiment showed that *in vitro* indirect organogenesis of two varieties of *Sansevieria trifasciata* can be done successfully from leaf segments as explants. Organogenesis

of *Sansevieria trifasciata* leaf explants was initiated by the formation of callus at the cutting edge of explants. All explants from the two cultivars tested swelled, and formed callus and greenish meristemoid during the first two weeks of cultures, and when explants were transferred to shoot-inducing medium containing various concentrations of BA, shoot bud and shoot initiation occurred.

In general, both BA concentrations and *sansevieria* genotypes affected organogenesis capacity of the explants tested. However, there was no interaction between BA concentrations and cultivars which affected variables recorded. The highest number of shoot buds and propagules in both cultivars was formed at medium with 2 mg/l BA. In addition, *Sansevieria trifasciata* cv. Lorentii showed higher organogenic capacity than that of cv. Hahnii (Table 1). The culture appearance of *Sansevieria trifasciata* cv. Hahnii is presented in Figure 1, while that of cv. Lorentii is in Figure 2. Those figures showed that regenerated shoots of cv. Hahnii were shorter than those of cv. Lorentii.

The mode of regeneration of two cultivars of *Sansevieria trifasciata* reported in this experiment was similar to the ones reported by Shahzad *et al* (2009) in *Sansevieria cylindrica*, i.e through indirect organogenesis. In these experiments, leaf explants were induced to form callus on IBA-containing medium or to form nodules on 2,4-D or 2,4,5-T containing medium, then the explants were induced to form shoots on BA+NAA containing medium. Their results showed that a maximum number of 17.6 shoots per culture was obtained from callus culture on medium with 5 μ M (1.1 mg/l) BA and 2 μ M (0.4 mg/l) NAA, whereas from nodule culture, a maximum of 25 shoots was obtained on medium with 5 μ M (1.1 mg/l) BA and 1 μ M (0.2 mg/l) NAA. Previously, Anis and Shahzad (2005) have reported direct shoot regeneration from leaf disc explants of *Sansevieria cylindrica*. In their experiment, the highest number of shoots per explants were obtained in MS medium supplemented with 10 μ M (2.25 mg/l) BA and 0.1 μ M NAA.

Table 1. Effects of cultivars and benzyladenine (BA) concentrations on number of shoot buds, number of shoots, number of propagules per explant and shoot length of two sansevieria cultivars after 14 weeks of cultures

Sansevieria cultivars	BA concentrations	Number of Shoot buds per explants	Number of Shoots per explants	Number of Propagules per explants	Shoot length (cm)
cv. Hahnii	0.00	2.0 f	1.5 c	3.5 d	0.7 cd
	0.10	4.0 e	2.5 ab	6.5 c	0.9 abc
	0.25	4.1 e	3.3 a	7.1 c	0.7 cd
	0.50	4.8 de	2.0 bc	6.8 c	0.6 cd
	1.00	5.4 d	1.8 bc	7.2 c	0.5 d
	2.00	7.0 b	2.3 bc	9.3 b	0.6 cd
cv. Lorentii	0.00	2.0 f	1.6 c	3.6 d	0.6 cd
	0.10	4.5 de	3.5 a	8.0 c	1.4 a
	0.25	5.0 d	2.6 ab	7.6 c	1.1 bcd
	0.50	6.1 c	2.4 bc	8.5 c	0.8 bcd
	1.00	7.6 b	2.6 ab	9.2 b	0.8 bcd
	2.00	8.6 a	3.4 a	12.0 a	0.9 bc

Significancy based on ANOVA (analysis of variance)

ns = non-significant	Main Effects :				
* P ≤ 0.05	Cultivars (C)	**	ns	**	**
** ≤ 00.01	BA (B)	**	**	**	*
	C x B	ns	ns	Ns	ns

Remarks= Mean values at the same column followed by the same letters indicate that there was no significant difference among treatment based on LSD at P≤ 0.05.

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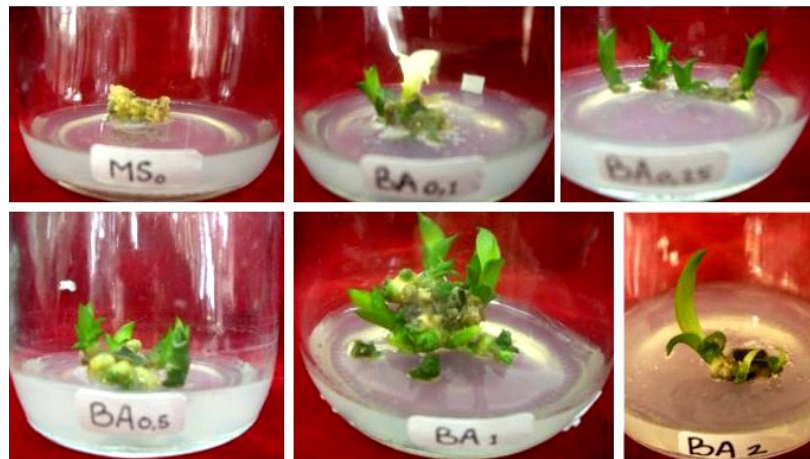


Figure 1. Appearance of *Sansevieria* cv. Hahnii cultures on MS medium with various concentrations of BA after 14 weeks

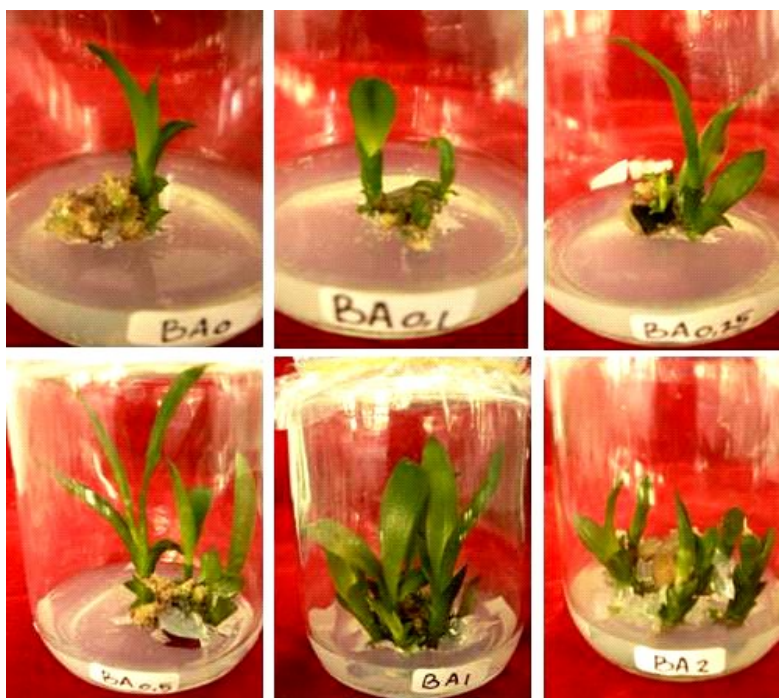


Figure 2. Appearance of cultures of *Sansevieria* cv. *Lorentii* on MS medium with various concentrations of BA after 14 weeks.

In this experiment, callus and meristemoid induction occurred when explants were cultured on MS medium supplemented with 0.25 mg/l 2.4-D for 1 week followed by transferring to MS medium without growth regulator (MS0) for 1 week. In previous reports (Blazich and Novitsky, 1984; Wahyuningsih, 2006), callus and meristemoid induction was done by culturing explants on MS+2.4-D for 2 weeks, followed by transferring to MS0 for 2 weeks, so meristemoid formation required 4 weeks.

Results of this experiment also revealed that genotypes significantly affected regeneration capacity of explant to form shoot buds or shoots. *Sansevieria trifasciata* cv. *Lorentii* showed higher organogenic capacity than cv. *Hahnii*, which was indicated by the higher number of shoot buds (8.6). The propagules (12.0) obtained in cv. *Lorentii* compared to those of cv. *Hahnii* (7.0 and 9.3, respectively). The mean length of regenerated shoots of cv. *Lorentii* was also higher than that of cv. *Hahnii*. The optimum concentration of cytokinin which resulted in the best multiplication rate of any micropropagation

could be species or even cultivar specific. Hodson de Jaramilo *et al.* (2008) found that cultivars affected shoot regeneration capacity in chrysanthemum (*Dendranthema grandiflora*) organogenesis. When subjected to the same BA+NAA containing medium, explants from the three chrysanthemum cultivars tested showed different responses. Genotype specificity for *in vitro* shoot regeneration was also reported by Hirimburegama and Gamage (1997) in tissue culture of banana and plantain. In *in vitro* organogenesis of sugar cane, Gandonou *et al.* (2005) and Ali *et al.* (2008) also reported that the best medium and growth regulators for callus and shoot induction depended upon the clone or the variety being cultured. The higher number of shoots per explant which was obtained in micropropagation of *Sansevieria cylindrica* reported by Shahzad *et al.* (2009) compared to the ones obtained in this experiment might be due to genotype dependent factor as well as the mode of regeneration and different growth regulators applied. Sriskandarajah *et al.* (2006) suggested that different regenerative capacity in

different species or genotypes was due to different endogenous phytohormones as well as different activities of several enzymes related to auxin and cytokinin metabolism, which in turn shifted the auxin and cytokinin pool.

Shoots of both cultivars were successfully *ex vitro* rooted and acclimatized in the greenhouse with 95.9-100% survival rate. Figure 3 shows the acclimatized plantlets of *Sansevieria trifasciata* cv. Hahnii (a) and cv. Lorentii (b) after 2 months in the greenhouse.

The propagation efficiency of the two cultivars of *Sansevieria trifasciata* by this micropropagation method was much higher than conventional propagation by leaf cuttings. Using leaf cuttings, we can get maximum of 2 leaf cuttings per blade of cv. Hahnii and maximum of 5 leaf cuttings per leaf blade of cv. Lorentii. In two months, if 2-3 plants are regenerated from each cutting, a maximum of 6 plants of cv. Hahnii and 15 plants of cv. Lorentii can be obtained, and since the shoots obtained by leaf cutting would not proliferate, the number of plants would not change until four or five months. Using this micropropagation method, we can get an average of 20 explants per leaf

blade from cv. Hahnii and 40 explants per leaf blade from cv. Lorentii. Considering an average 80 % success of sterilization and subculturing, we can obtain an average of 16 aseptic cultures of cv. Hahnii and 32 aseptic cultures of cv. Lorentii per leaf blade. Based on the data obtained in this experiment, after 4.5 months (18 weeks) we can get potentially $32 \times 11 = 352$ shoots of cv. Lorentii per leaf, provided that after the third subcultures (at 18 months) 11.1 shoots are obtained from cultures with 2 mg/l BA. The figures of cv Hahnii, on the other hand, based on the data available (from 14 weeks of cultures), we can get potentially 16×3 shoots = 48 shoots per leaf blade in 14 weeks (3.5 months). If one more subculture was conducted, we might expect at least doubled shoots per explant, and the figures with Hahnii might become 96 shoots obtained per leaf. With 95% survival rate of acclimatization, 91 plants of cv Hahnii and 334 plants of Lorentii would be obtained per leaf blade. Compared to propagation by leaf cuttings, micropropagation would potentially be about 15 times as efficient as leaf cuttings for *sansevieria* cv Hahnii, and about 22 times as efficient as leaf cuttings for cv. Lorentii.

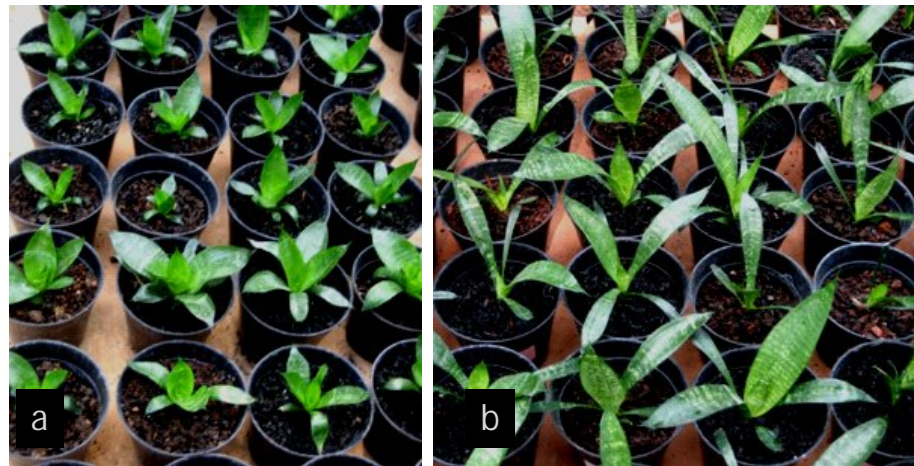


Figure 3. The acclimatized plantlets of *Sansevieria trifasciata* var Hahnii (a) and var. Lorentii (b) in the greenhouse (two-month)

CONCLUSIONS

Successful *in vitro* organogenesis of *Sansevieria trifasciata* var. Hahnii and Lorentii was performed. Results showed that both BA concentrations and *Sansevieria* cultivars affected organogenesis capacity of the explants. However, there was no interaction between BA concentrations and cultivars which affected variables recorded.

The highest number of shoot buds and propagules in both cultivars was formed at medium with 2 mg/l BA. *Sansevieria trifasciata* cv. Lorentii showed higher regeneration capacity than that of cv. Hahnii. Shoots of both cultivars were successfully *ex vitro* rooted and acclimatized in the green house with 95.9 % - 100% survival rate. Compared to propagation by leaf cuttings, this micropropagation method would be approximately 15 times as efficient as that for *sansevieria* cv Hahnii, and about 22 times as efficient as that for cv. Lorentii.

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