THE INCREASE IN *IN VITRO* SHOOT MULTIPLICATION RATE OF *Dendrocalamus asper* (Schult. f.) Back. ex Heyne

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ABSTRACT: A method for micropropagation was developed for *Dendrocalamus asper*, an economically and environmentally important bamboo. Disinfected seeds were cultured in flasks containing 20 ml of Murashige and Skoog's medium (MS) supplemented with BA (1.0-7.0 mg l⁻¹) or kinetin (1.0-7.0 mg l⁻¹). Multiple shoots (6.53) were formed on MS medium supplemented with 3.0 mg l⁻¹ BA and reached 1.49 cm in length. Continuous shoot proliferation was achieved on a MS medium supplemented with BA (1.0-7.0 mg l⁻¹). The multiplication rate of 3.30 fold was achieved on MS medium supplemented with 3.0 mg l⁻¹ BA. Propagules were excised from multiple shoots and transferred to rooting medium. After 4 weeks, high *in vitro* rooting was achieved on MS supplemented with 7.0 mg l⁻¹ IBA. 3.70 cm in length root system developed 8.0-9.0 roots in 28 days. A high rate of plant survival (85%) was obtained within 2 weeks.

Keywords: *Dendrocalamus asper*, BA, IBA, kinetin, micropropagation, NAA.

INTRODUCTION

The industrial revolution from 1800s to 1900s not only developed the global economics, but also emitted 850 billion tons of CO₂ into environment through combustion of fossil fuels, oil, coal and gas... Besides, changes in land use and deforestation added 370 billion tons of CO₂. Human activities not only produce a huge amount of CO₂, but they also damage the forests-carbon sinks of the planet. There are difficulties for human to make a balance between economical development and environmental protection.

Bamboo tree absorbs CO₂ through photosynthesis and generates up to 35% more oxygen than an equivalent stand of tree. After 3 to 5 years, each hectare of mature bamboo sequesters 62 tons of CO₂ per year [18]. Bamboo is well-developed, expand rapidly and is a multipurpose tropical clumping bamboo with high economic value. The important fact is that bamboo can be harvested without the destruction.

Alexander and Rao (1968) [1] described the first research on bamboo embryo culture. The technique of release of protoplast from bambusa leaf tissue has been reported [17]. Mehta et al. (1982) [8] were successful in regeneration of bamboo plantlets via somatic embryogenesis.

Micropropagation of *D. hamiltonii* has been reported [16] on MS medium [10] with 2.5

mg l⁻¹ BA. Godbole et al. (2002) [6] used nodal segments to regenerate *D. hamiltonii* via somatic embryogenesis on MS medium with BA (2.5 mg l⁻¹) and 2,4-D (1.0 mg l⁻¹). Lin et al. (2004) [7] reported the role of TDZ in the induction of somatic embryogenesis of *Bambusa edulis*. High germination rate of somatic embryogenesis (80%) was achieved on medium supplemented with 0.455 μM TDZ.

D. asper plays an important role in daily life, thus it becomes one of important cultivated crops in Vietnam and several countries of the Asia-Pacific region. The mature culms are utilized in construction, decoration, and they are suitable for pulp, paper, matting and rayon. Moreover, *D. asper* is cultivated at highland, bare hill, coastal regions... to against soil erosion and it is also an important source for handicraft villages. Tender shoot of *D. asper* is not only a high quality food, but also an important export commodity.

For some problems, the traditional methods for propagation of *D. asper* are time-consuming and difficult. Vegetative propagation such as cutting and rhizomes are bulky, tricky to handle, transport and very slow to grow. Thus, the plant cell culture protocols of *D. asper* were described. Singh et al. (2003) [15] reported a simple method for large-scale propagation of *D. asper* via culm and culm-branch. Two steps method for accelerated mass propagation of *D. asper* via nodal segments was described [3].

Nodal segments were cultured on semisolid medium with 5 mg l⁻¹ BAP, then *in vitro* generated axillary shoots were cultured on liquid MS medium supplemented with 5 mg l⁻¹ BAP and 40 mg l⁻¹ adenine sulphate. 93.33 % High rooting potential of shoots (93.33%) was achieved when shoots were cultured on liquid medium supplemented with 1.0 mg l⁻¹ IBA.

The present paper described a method to increase *in vitro* shoot multiplication rate of *D. asper*.

MATERIALS AND METHODS

Materials

Explant source of the present study is *D. asper* seeds which was brought from Thailand.

Methods

Seed germination and shoots formation of D. asper

Seeds of *D. asper* were stored at 4°C for 3 months. They were dehusked and surface-sterilized with javel-Viso (50%) for 20 min. and rinsed with sterile distilled water for 3 times. Disinfected seed were germinated in 100 ml flasks (1 seed per flask) containing 20 ml of germination medium [MS medium supplemented with 30 mg l⁻¹ sucrose, 8 g l⁻¹ agar and BA (1.0, 3.0, 5.0 and 7.0 mg l⁻¹) or kinetin (1.0, 3.0, 5.0 and 7.0 mg l⁻¹)].

Effect of BA on shoot proliferation of D. asper

Clumps developed from the seeds were excised and transfered to medium for further multiplication. MS medium supplemented with 30 mg l⁻¹ sucrose, 8 g l⁻¹ agar and BA (1.0, 3.0, 5.0 and 7.0 mg l⁻¹).

Effect of auxin (IBA or NAA) on rooting potential of D. asper propagules

Two shoot propagules excised from multiple shoots and transfered to rooting medium containing 30 mg l⁻¹ sucrose, 8 g l⁻¹ agar and IBA (1.0, 3.0, 5.0 and 7.0 mg l⁻¹) or NAA (1.0, 3.0, 5.0 and 7.0 mg l⁻¹).

Acclimatization

Four-week-old plantlets with well developed root systems were transfered to

chamber using natural light within 20 days and the plants eventually were established in soil in open nursery.

Cultural conditions

All media were autoclaved (121°C at 1 atm for 20 min.) after adjustment of the pH 5.7-5.8.

All growth stages of this study were incubated under conditions: 25 ± 2°C, 60 ± 5 % RH and a 12-h photoperiod under a photosynthetic photon flux density of 45 µmol m⁻² s⁻¹.

Statistical analysis

We observed shoot formation, leaf formation, root formation and the number of shoots, leaves or roots were recorded by visual counting. Data were collected after 28 days of culture.

Data were test by Duncan's multiple range test [5] at 5% level using SPSS (version 16.0) software package.

RESULTS AND DISCUSSION

Seed germination and shoot multiplication

Miller et al. (1955) [9] reported cytokinin influence on shoot formation via protein-synthesis. The concentration gradient of plant growth regulators would be changed and set up new gradient via supplement of exogenous cytokinin in medium. The establishment of new gradient affect to break dormancy of seed and stimulate shoots formation.

Seeds cultured on MS medium germinated within 3-5 days (table 1). The number of shoots per seed was greatest on medium with 3.0 mg l⁻¹ BA (6.53 shoots/seed) (figure 1b2, b3). Seed inoculated on medium containing 1.0 mg l⁻¹ BA or without BA developed 1-2 shoots within 28 days. Present result is different from result of Ayra et al. (1998) [2]. Ayra et al. (1998) [2] reported *D. asper* seed inoculated on medium containing 5.0 mg l⁻¹ BA developed 10-15 shoots within 6 weeks. At increased BA levels (7-10 mg l⁻¹) shoot proliferation increased to 25-30 shoots per seed. BA induced direct shoot regeneration form seedling has also been reported in *Alnus glutinosa* [13]. Seed germination and shoots multiplication were not

affected by kinetin. Barejee et al. (2011) [3] also reported effect of BA on shoots formation of *D. asper* was better than effect of kinetin. Kinetin did not result in shoot proliferation of *Bambusa nutans* when added alone at

concentrations ranging from 2.32 to 6.79 μM [11]. Negi et al. (2011) [12] reported shoots formation of *Bambusa balcooa* remained dormant on medium containing kinetin alone and ultimately died.

Table 1. Effect of BA and kinetin on seed germination and shoot formation of *D. asper* after 28 days

BA (mg l^{-1})	KIN (mg l^{-1})	Average germination time (days)	Number of shoots/seed (shoots)
0.0	-	4.13a	0.73c
1.0	-	3.87a	2.47b
3.0	-	3.53a	6.53a
5.0	-	3.20a	2.40b
7.0	-	2.80a	2.40b
-	1.0	2.40a	1.07b
-	3.0	2.40a	1.07b
-	5.0	2.67a	1.60b
-	7.0	2.93a	1.80b

*Means in the same column that are followed by different letters are significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test.

Figure 1. Effect of BA on shoot formation from seed of *D. asper* after 28 days
a. 1.0 mg l^{-1} BA; b1, b2, b3. 3.0 mg l^{-1} BA; c. 5.0 mg l^{-1} BA; d. 7.0 mg l^{-1} BA.

Figure 2. Effect of kinetin on shoot formation from seed of *D. asper* after 28 days
a. 1.0 mg l^{-1} KIN; b1, b2. 3.0 mg l^{-1} KIN; c. 5.0 mg l^{-1} KIN; d. 7.0 mg l^{-1} KIN; e. Dead shoot.

Table 2. Effect of BA and kinetin on shoot development of *D. asper* after 28 days

BA (mg l^{-1})	KIN (mg l^{-1})	Shoot length (cm)	Number of leaves/shoot (leaves)	Leaf square (cm^2)
-	-	5.54a	1.73ab	0.22b
1.0	-	2.13ab	1.87a	0.58a
3.0	-	1.49b	1.13abc	0.21b
5.0	-	1.39b	1.00abc	0.05b
7.0	-	1.37b	0.93bc	0.00b
-	1.0	3.63a	0.80c	0.09b
-	3.0	1.61b	1.40abc	0.31ab
-	5.0	2.20ab	1.00abc	0.14b
-	7.0	2.39ab	1.13abc	0.09b

*Means in the same column that are followed by different letters are significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test.

On PGR-free medium, shoots regenerated with mean length of 5.54 cm were obtained (table 2). This result might be due to seeds cultured on this medium gave less number of shoots (1-2 shoots) than media with BA or KIN thus the nutritional competition was not happened strongly (table 1, 2). Shoot formation on medium supplemented 1.0 mg l⁻¹ BA got 2.13 cm in length, gave the best number of leaves (1.87) and leaf square (0.58 cm²) (table 2). When shoot on medium containing kinetin got more

than 5 cm in length, axillary shoot formation was happened. Cytokinin is capable of inducing axillary shoot formation. The first hypothesis was reported that cytokinin could reduce IAA oxidase of axillary shoots thus it leads to the increase in axillary shoots elongation via the increase in endogenous auxin. The second hypothesis was reported cytokinin stimulated axillary shoots formation via the transportation of nutrients and vitamins. Shoots rooted on MS medium with kinetin.

Figure 3. Effect of BA on shoot proliferation of *D. asper* after 28 days

a. 0.0 mg l⁻¹ BA; b. 1.0 mg l⁻¹ BA; c1, c2. 3.0 mg l⁻¹ BA; d. 5.0 mg l⁻¹ BA; e. 7.0 mg l⁻¹ BA.

Table 3. Effect of BA on shoot proliferation of *D. asper* after 28 days

BA (mg l ⁻¹)	Multiplication rate	Number of shoots/explant (shoots)	Shoot length (cm)	Number of leaves (leaves)	Leaf square (cm ²)
-	1.20b	1.50b	5.93a	3.40a	0.78a
1.0	2.61a	4.90ab	5.01a	2.00b	0.54ab
3.0	3.30a	7.45a	3.48ab	1.93b	0.96a
5.0	2.46a	5.23ab	4.00ab	1.18b	0.32b
7.0	2.27a	5.60a	2.81b	1.18b	0.40b

*Means in the same column that are followed by different letters are significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test.

Table 4. Effect of NAA and IBA on rooting ability of *D. asper* after 28 days

NAA (mg l ⁻¹)	IBA (mg l ⁻¹)	Average rooting time (days)	Average number of roots (roots)	Root length (cm)	Shoot length (cm)	Number of leaves (leaves)
1.0	-	18.67ab	1.33c	1.20cd	6.93ab	4.00ab
3.0	-	19.67ab	3.33bc	2.37bc	4.90b	2.01ab
5.0	-	20.00a	5.00b	0.33d	6.83ab	3.64ab
7.0	-	17.67ab	3.67bc	0.77d	4.67b	1.00b
-	1.0	16.67b	3.00bc	0.73d	8.53ab	2.00ab
-	3.0	20.00a	2.33c	3.07ab	8.20ab	6.12a
-	5.0	19.67ab	3.33bc	2.93ab	7.73ab	3.67ab
-	7.0	17.33ab	8.67a	3.70a	10.70a	6.67a

*Means in the same column that are followed by different letters are significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test.

Shoots on MS medium supplemented with 3.0 mg l⁻¹ BA gave the best number of shoots per explant (7.45 shoots) (figure 3) and the best shoot multiplication rate (3.30) (table 3). When PGR-free medium was used, the cultured shoot propagules increased in length but the least shoot multiplication rate (1.20). Shoot propagules on shoot multiplication medium (MS supplemented with 1.0, 5.0 and 7.0 mg l⁻¹ BA) remained develop and ultimately died for some time (figure 3b, 3e). Chang et al. (2003) [4] reported effect of BA on shoot tip proliferation was better than those of TDZ, kinetin and 2iP in micropropagation of *Zantedeschia albomaculata*. However, shoot multiplication rate, number of shoots, shoot

length increased with the increased BA concentration (2.22-8.78 μ M) in the MS medium. Micropropagation of *Thymus piperella* [14] was reported BA stimulated shoot proliferation of explants. With the increase in BA level (0.0-1.5 mg l⁻¹), the number of shoots increased.

Rooting of shoots and acclimatization

The micropropagation of *D. asper* could not complete without rooting potential of shoots. Rooting potential of *D. asper* effected on survival plants when plants were transferred to soil. Auxin was main factor which stimulated rooting of *D. asper*.

Figure 4. Effect of NAA and IBA on root ability of *D. asper* after 28 days

a. 1.0 mg l⁻¹ NAA; b. 3.0 mg l⁻¹ NAA; c. 5.0 mg l⁻¹ NAA; d. 7.0 mg l⁻¹ NAA;
e. 1.0 mg l⁻¹ IBA; f. 3.0 mg l⁻¹ IBA; g. 5.0 mg l⁻¹ IBA; h. 7.0 mg l⁻¹ IBA.

The best rooting potential of shoots was achieved when shoots were cultured on medium supplemented with 1.0 mg l⁻¹ IBA after 16.67

days (table 4, figure 4h). Present result was different from result of Arya et al. [2] (1998). Propagules were transferred to rooting medium

supplemented with IBA, NAA rooted readily within 8-12 days. Propagules on medium containing 7.0 mg l^{-1} IBA developed the best number of roots (6.67), root length (3.70 cm), shoot length (10.70 cm) (table 4, figure 4). Arya et al. [2] (1998) also reported the root systems

of propagules increased from 4.3 to 26.2 roots per propagule with the increased IBA concentration ($0.5\text{-}10.0 \text{ mg l}^{-1}$) in MS medium.

The plants were established in soil in nursery and a high rate of plant survival (85%) was obtained within 2 weeks.

Figure 5. Micropropagated *D. asper* plants

CONCLUSION

Shoots formation from seed was stimulated on MS medium supplemented with BA (3.0 mg l^{-1}).

The multiplication rate of 3.30 fold was achieved on MS medium supplemented with 3.0 mg l^{-1} BA.

High efficiency of *in vitro* rooting was achieved on MS supplemented with 7.0 mg l^{-1} IBA.

A high plant survival rate (85%) was obtained within 2 weeks.

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REFERENCES

1. Alexander M. P., Rao T. C., 1968. *In vitro* culture of bamboo embryo. Curr. Sci., 37: 415.
2. Arya S., Shamar S., Kaur I. R., Dev Arya I., 1998. Micropropagation of *Dendrocalamus asper* by shoot proliferation using seed. Plant Cell Rep., 18: 879-882.
3. Banerjee M., Gantait S. and Pramanik B. R. 2011. A two step method accelerated mass propagation of *Dendrocalamus asper* and their evaluation in field. Physiol. Mol. Biol. Plants, 17(4): 387-393.
4. Chang H. S., Chakrabarty D., Hahn E. J. and Paek K. Y., 2003. Micropropagation of calla lily (*Zantedeschia albomaculata*) via *in vitro* shoot tip proliferation. In vitro Cell Dev-Pl, 39: 129-134.
5. Duncan D. B., 1955. Multiple range and multiple *F*-tests. Biometrics, 11: 1-42.
6. Godbole S., Sood A., Thakur R., Sharma M. and Ahuja P. S., 2002. Somatic embryogenesis and its conversion into plantlets in a multipurpose bamboo *Dendrocalamus hamiltonii* Nees et Arn. ex Munro. Curr. Sci., 83: 885-889.

7. Lin C. S., Lin C. C., Chang W. C., 2004. Effect of thidiazuron on vegetative tissue-derived somatic embryogenesis and flowering of bamboo *Bambusa edulis*. Plant Cell Tiss Org., 76: 75-83.
8. Mehta U., Rao I. V., Mohan Ram H. Y., 1982. Somatic embryogenesis in bamboo. In Proc. 5th Inter Cong Plant Tiss Cell Cult., Tokyo, Japan.
9. Miller C. O., Skoog F., von Saltza H. M., Okumura F. S., Strong F. M., 1955. Kinetin: Structure and synthesis of kinetin. J. Am. Chem. Soc., 77: 2662-2663.
10. Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.
11. Negi D., Saxena S., 2010. *In vitro* propagation of *Bambusa nutans* Wall. ex Munro through axillary shoot proliferation. Plant Cell Rep, 5: 35-43.
12. Negi D., Saxena S., 2011. Micropropagation of *Bambusa balcooa* Roxb. through axillary shoot proliferation. In vitro Cell Dev-Pl, 47(5): 604-610.
13. Perinet P., Lanlonde K., 1983. *In vitro* propagation and nodulation of the actinorhizal host plant, *Alnus glutinosa* L.. Plant Sci. Lett., 29: 9-17.
14. Sáez F., Sánchez P., Piqueras A., 1994. Micropropagation of *Thymus piperella*. Plant Cell Tiss Org., 39: 269-272.
15. Singh S., Kumar P., Ansari S. A., 2003. A simple method for the large-scale preparation of *Dendrocalamus asper*. Sci. Horti-Amsterdam, 100: 251-255.
16. Sood A., Ahuja P. S., Sharma M., Sharma O. P., Godbole S., 2001. *In vitro* protocols and field performance of elites of an important bamboo *Dendrocalamus hamiltonii* Nees et Arn. ex Munro. Plant Cell Tiss Org., 71: 55-63.
17. Tseng T. C., Lin D. F., Shaio S. Y., 1975. Isolation of protoplast from crop plant. Bot. Bull. Acad. Sinica., 16: 55-60.
18. Wikipedia, 2012. Bamboo textiles. From Wikiperdia, the free encyclopedia http://en.wikipedia.org/wiki/Bamboo_textiles. Reference time 10/02/2012.

NGHIÊN CỨU TĂNG HỆ SỐ NHÂN NHANH CHỒI CỦA CÂY TRE MẠNH TÔNG (*Dendrocalamus asper* (Schult. f.) Back. ex Heyne) NUÔI CÂY IN VITRO

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TÓM TẮT

Phương pháp nhân giống đã được áp dụng trên tre mạnh tông (*Dendrocalamus asper*) là một trong những loại tre có giá trị cao về kinh tế và môi trường. Hạt tre mạnh tông sau khi khử trùng được chuyển vào môi trường MS (20 ml/bình nuôi cấy) bổ sung BA (1,0; 3,0; 5,0; 7,0 mg/l) hoặc kinetin (1,0; 3,0; 5,0; 7,0 mg/l). Cụm chồi (6,53) xuất hiện trên môi trường MS bổ sung 3,0 mg/l BA đạt chiều cao 1,49 cm. Giai đoạn nhân nhanh chồi được tiến hành trên môi trường MS bổ sung BA (1,0; 3,0; 5,0; 7,0 mg/l). Hệ số nhân nhanh chồi 3,30 được ghi nhận trên môi trường MS bổ sung 3,0 mg/l BA. Các cụm chồi được chuyển từ môi trường nhân nhanh sang môi trường ra rễ. Sau 4 tuần, quá trình tạo rễ *in vitro* tốt được ghi nhận trên môi trường MS bổ sung 7,0 mg/l IBA. Hệ thống rễ từ 8-9 rễ đạt chiều dài 3,70 cm sau 28 ngày. Tỷ lệ sống sót sau khi đưa cây ra vườn ươm là 85% sau 2 tuần.

Từ khóa: *Dendrocalamus asper*, BA, IBA, kinetin, nhân giống, NAA.

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