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Preservation and development of Giao Co Lam (Gynostemma pentaphyllum) in the hot tropics of Vietnam

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***Abstract*—The internode segments of Giao Co Lam (Gynostemma pentaphyllum) sterilized with diluted solution of javel (50%) for 20 minutes reached to 73.33% sterile explants. In vitro cutting stem segments of Giao Co Lam cultured on MS medium supplemented with 1.0 mg/L BA and 0.1 mg/L NAA gave the highest shoot induction (6.8 shoots/ explant). The regenerated shoots cultured on MS ½ medium were suitable for growth of shoots with 5.2 cm in height and 4.0 leaves per plantlet. For root induction, the MS ½ medium supplemented with 0.25 mg/L IBA was appropriate and the root length could be in 7.6 cm in this medium. The rate of survival plantlets is 85% in garden.**

***Keywords*—Gynostemma pentaphyllum, multiplication of Giao Co Lam, medicinal plant, shoot proliferation**

I. Introduction

Gynostemma pentaphyllum known as medicinal herb is famous for the anti-stress properties, restoring the body's balance and improving the memory. It contains more than 100 kinds of dammarane-type triterpenoid saponins. The number of saponins in Giao Co Lam is more than 3 - 4 times of those of ginseng. Besides, It contains many vitamins, minerals, trace elements, amino acids and proteins (Huang et al., 2008a; Razmovski-Naumovski et al., 2005). According to Kiem et al. (2009), Giao Co Lam (GCL) enhances the lipid metabolism led to stabilize cholesterol levels in the blood and reduce the fat effectively; balance the blood pressure; prevent the thrombosis, the cardiovascular and brain complications, anti-aging; increase the appetite and good sleep. In addition, Giao Co Lam increases the immunity and inhibits the growth of tumors significantly (Blumert & Liu, 1999).

In the research of the scientists in Vietnam National Institute of Medicinal Materials and Swedish Karolinska Institute on Giao Co Lam, a new substance had been found and named as Phanoside. This substance has the strong hypoglycemic effect, stimulates the insulin secretion of pancreas and increases the sensitivity of target cells to insulin (Norberg et al., 2004).

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Furthermore, Phan Van Kiem et al. (2009) in Korea had extracted the active ingredients of gypenosides in Giao Co Lam Vietnam and tested on the tumors in lung, colon, breast, uterus, prostate with very good results. The new bioactive is capable of inhibiting and killing the aforementioned cancer cells and improving the immune system of the body.

With many pharmaceutical effects above, Giao Co Lam has been using in the production of drugs and functional foods. Therefore, the source of wild GCL has been overexploited. There have been some studies of GCL breeding applying traditional techniques such as cuttings, sowings, etc. however the propagation coefficient and uniformity of plant is low. Plant tissue culture techniques play an important role in the conservation and breeding of crops, especially valuable medicinal plants. This study aims to determine the feasibility of internode culture techniques for establishing the technical process of GCL micropropagation (*Gynostemma pentaphyllum*), a source of medicinal materials in the production of pharmaceuticals.

II. Materials and methods

A. *Materials*

The 1-year-old internodes of GCL originated from Lang Son was acclimatized and grown in Cu Chi district, Ho Chi Minh City, Viet Nam.

The experimental media included micro and macro minerals of MS medium (Murashige, Skoog, 1962), vitamin, sucrose, agar, BA (benzyl aminopurin), NAA (naphthalenacetic acid) and IBA (indolbutyric acid). The pH of media was adjusted to 5.8 before autoclaving at 121 ^{o}C, 1 atm for 20 minutes.

Experimental conditions: The experiments were maintained under 16 hours/day in lighting period, 2,000 lux in light intensity, 24 ± 2 ^{o}C in temperature; 75-80% in average humidity.

B. *Methods*

The experiments were arranged in a completely randomized design (CRD) with 3 replications. Each treatment was cultured 15 explants. Data was recorded after 6 weeks of culture and subjected to analysis of variance with the MSTATC statistical software package.

C. *Experimental design*

Experiment 1. The examination of the concentrations of Javel solution (NaOCl 5%) and treatment time on the rate of sterile explants: The 3cm-long internodes have been moved

into the laminar flow bench, washed with the bleach solution in 10 minutes, and then rinsed with sterile distilled water. Subsequently, those were disinfected with ethanol 70% in 1 minute, followed by the different concentrations of Javel solution (50% and 100%) added with several drops of Tween-80 in different periods (10, 15, and 20 minutes) and rinsed with sterile distilled water. After sterilization of the explants, the necrotic portions of internodes were removed and cultured on MS medium without plant growth regulators. The treatments were arranged in a completely randomized design (CRD) with 3 replications. This experiment consisted of six treatments and each treatment was cultured 15 explants. Observation and record of results was carried out after 2 weeks of culture.

Experiment 2. The effects of BA and NAA concentrations on the shoot multiplication: The in vitro internodes with approximately 1.5 to 2 cm in size were cultured on MS media added with sucrose 20 g/L, agar 8 g/L, BA (0.1, 0.5, 1.0, 1, 5, 2.0 mg/L) in combination with NAA (0, 0.1, 0.2, 0.3 mg/L). The experiment was arranged in a completely randomized design (CRD) with 3 replications. This experiment consisted of 24 treatments and each treatment was cultured 15 explants. Observation and record of results was carried out after 6 weeks of culture.

Experiment 3. The effects of mineral media on the growth of shoots: The in vitro 2cm-long shoots were cultured on different mineral media: MS, MS ½ (half of macro minerals), ½ MS (half of macro and micro minerals), and KC (Knudson C) added with sucrose 20 g/L, agar 8 g/L. The experiment was arranged in a completely randomized design (CRD) with 3 replications. This experiment consisted of four treatments and each treatment was cultured 15 explants. Observation and record of results was carried out after 6 weeks of culture.

Experiment 4. The effects of IBA concentrations on the root formation: The in vitro 2cm-long shoots were cultured on optimal mineral media (determined in content 3) added with sucrose 20 g/L, agar 8 g/L, and IBA (0, 0.25, 0.5, 1.0 mg/L). The experiment was arranged in a completely randomized design (CRD) with 3 replications. This experiment consisted of four treatments and each treatment was cultured 15 explants. Observation and record of results was carried out after 6 weeks of culture.

III. Results and discussion

A. *The effect of the Javel concentrations and treatment time on the sterilization of explants*

The sterilization of explants with Javel solution (NaOCl 0.5%) in 2 concentrations including 50% (diluted with sterile distilled water) and 100% in 10 minutes, 15 minutes and 20 minutes showed that the rate of sterile explants gained from 0 - 73.33%. The explants sterilized with Javel solution at a concentration of 50% during 20 minutes gained the highest rate of sterile explants (73.33%) compared to the other treatments (Table 1). However, this result is statistically insignificantly different compared with that of the treatment disinfected with a javel solution at a concentration of 50% during 10 minutes with the rate of sterile explants reached 60.00%. Therefore, the commercial Javel solution (Hypochlorite-Na 5%) is suitable for disinfection of fungi and bacteria penetrating on the surface and tissue of the explants. The sterilization of explants at a concentration of 50% javel in 15 to 20 minutes is appropriate for internodes of Giao Co Lam.

TABLE 1. EFFECT OF JAVEL CONCENTRATIONS AND TREATMENT TIME ON RATE OF ASEPTIC EXPLANTS

Concentration of javel	Treatment time (minutes)	Rate of aseptic explants (%)
Javel (50%)	10	20.00^{bc}
	15	60.00^{ab}
	20	**73.33^{a}**
Javel (100%)	10	20.00^{bc}
	15	6.67^{c}
	20	0.00^{c}
CV (%)		7.536

* Means followed by same letters within a column are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$ level.

B. *The effect of BA and NAA concentrations on shoot multiplication*

The *in vitro* internodes cultured on MS medium supplemented with BA and NAA in different concentrations showed that: The explants in all treatments induced the shoot formation with callus and roots after culturing in 6 weeks. On the MS medium without growth regulators, some explants also formed shoot induction and growth after 6 weeks of culture. However, the treatment of growth regulators with 1.0 mg/L BA and 0.1 mg/L NAA is optimal for shoot proliferation (6.8 shoots/explant) (Table 2) (Figure 1-2). In this treatment, the shoots did not generate roots and formed little callus.

TABLE 2. EFFECT OF BA AND NAA ON SHOOT PROLIFERATION OF GIAO CO LAM

NAA	BA	Rate of shoot-formed explants (%)	Shoot number (shoots/explants)
0	0.1	49.7^{fg}	1.0^{ij}
0	0.5	86.3^{bc}	4.2^{cd}
0	1.0	80.3^{d}	3.0^{e}
0	1.5	53.3^{f}	2.7^{e}
0	2.0	41.7^{hi}	0.6^{ij}
0.1	0.1	39.7^{ij}	0.6^{ij}
0.1	0.5	96.3^{a}	4.4^{c}
0.1	**1.0**	**100.0^{a}**	**6.8^{a}**
0.1	1.5	78.7^{d}	3.0^{e}
0.1	2.0	51.3^{fg}	1.5^{gh}
0.2	0.1	35.7^{j}	0.5^{j}
0.2	0.5	76.3^{d}	3.0^{e}
0.2	1.0	80.0^{d}	3.8^{d}
0.2	1.5	88.7^{b}	5.2^{b}
0.2	2.0	49.3^{fg}	1.8^{fg}
0.3	0.1	45.7^{gh}	1.1^{hi}
0.3	0.5	46.3^{gh}	2.7^{e}
0.3	1.0	78.0^{d}	3.2^{e}
0.3	1.5	80.7^{cd}	5.4^{b}
0.3	2.0	59.3^{e}	2.2^{f}
CV (%)		3.95	7.48

* Means followed by same letters within a column are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$ level.

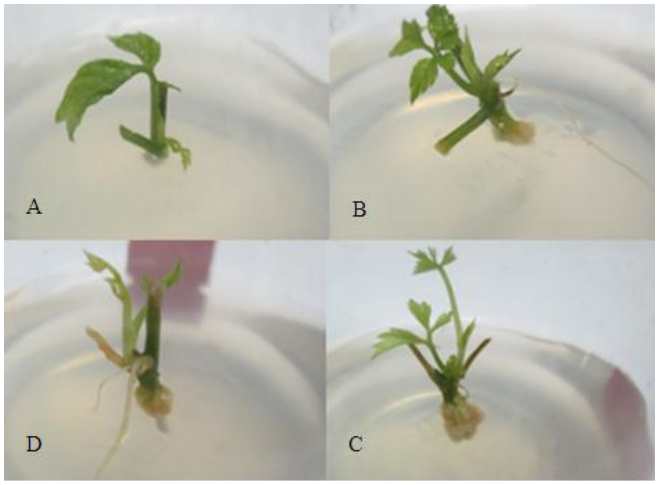

Figure 1. The shoot induction of Giao Co Lam internodes on the media supplemented with NAA and BA in different concentrations after 2 weeks of culture. (A) Control, (B) BA 1 mg/L + NAA 0.1 mg/L, (C) BA 1 mg/L + NAA 0.3 mg/L (D) BA 2 mg/L + NAA 0.1 mg/L

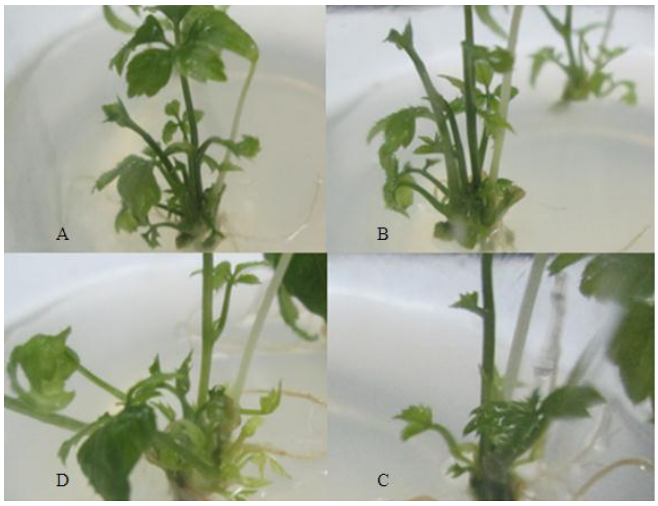

Figure 2. The shoot induction of internodes on the media supplemented with NAA and BA in different concentrations after 6 weeks of culture. (A) BA 1 mg/L + NAA 0.1 mg/L, (B) BA 1.5 mg/L + NAA 0.1 mg/L, (C) BA 1.5 mg/L + NAA 0.2 mg/L, (D) BA 2 mg/L + NAA 0.3 mg/L.

The result of this study is similar with that of others (Zhang et al., 1989). The node segments of Giao Co Lam induced shoot multiplication best on MS medium supplemented with BA 1.0 mg/L and IAA 0.05 mg/L. Another study of Giao Co Lam multiplication (*Gynostemma pentaphyllum*) on MS medium supplemented with BA (1.0 mg/L) and IAA (0.5 mg/L) is best (Shi et al., 2007). According to Lam et al. (2015), *in vitro* propagation of Giao Co Lam is best on MS medium supplemented with Kinetine (0.4 mg/L) and BA (0.5 mg/L) and the coefficient of shoot multiplication reached 4.36 times (small buds, dark green) lower than that of this study.

C. *Effect of mineral media on the growth of in vitro shoots*

After the shoot proliferation is the growth phase, thus the medium component is an important factor to ensure the standard and quality of plantlets which easily adapt to external conditions in the period of post-tissue culture. For each different species of plant, the different demand of mineral compositions determined is essential. The experiment was conducted by selecting 2 cm-long shoots and culturing in the experimental media after 6 weeks of culture. The collected results are shown in Table 3:

TABLE 3. EFFECT OF MINERAL MEDIA ON THE GROWTH OF *IN VITRO* SHOOTS.

Treatments	Mineral media	Height of shoots (cm)	Leaf Number
M0	MS	3.4^{b}	2.8^{b}
M1	**MS 1/2**	**5.2^{a}**	**4.0^{a}**
M2	1/2 MS	4.8^{ab}	3.3^{ab}
M3	KC	4.0^{b}	2.7^{b}
CV (%)		4.20	4.54

* Means followed by same letters within a column are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$ level.

In the M1 treatment, the average height and leaf number of shoots cultured on MS ½ is highest with 5.2 cm and 4 leaves per shoot, respectively, next to the ½ MS and KC medium. The height and leaf number of shoots was lowest on M0 treatments with MS mineral medium and the shoots grew weak and generated callus. A study of melon showed that the optimal rooting medium is the ½ MS medium without plant growth regulators with the survival percentage of plantlets up to 100% (Huijun et al., 2011; Wei et al., 2005). However, this investigation shows that the growth of shoots is suitable with the MS ½ medium.

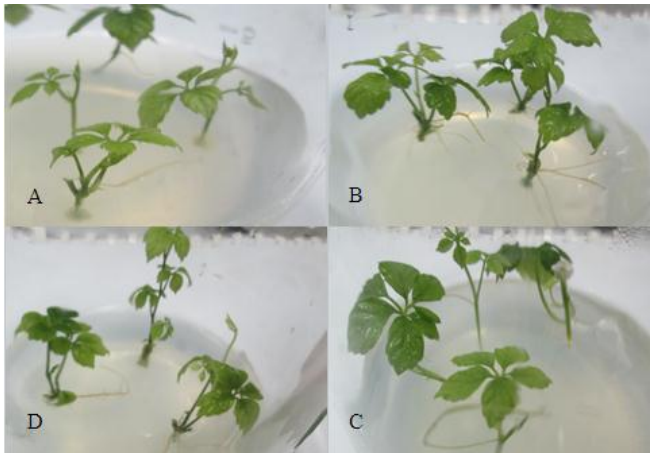

Figure 3. The growth of the Giao Co Lam shoots on different mineral medium. (A) MS, (B) MS ½, (C) ½ MS, (D) KC.

D. *The effect of the concentrations of IBA on the rooting of Giao Co Lam*

The experiment was conducted by selecting the 2cm-long shoots and cultured in the medium supplemented with different concentrations of IBA after 6 weeks of incubation. The results in Table 4 shows the highest root length and number of roots in treatment R1 with 7.6 cm and 6.4 roots per plantlet, respectively. In this study, the control treatment without IBA shows the number of roots and root length lower than that treatment with the concentrations of 0.25 mg/L and 0.5 mg/L. At the higher concentration of IBA (1.0 mg / L), the number of roots and root length is lower than that of the control treatment. Therefore, the optimal concentration of growth regulators for each specific objective needs to be determined for root formation.

TABLE 4. EFFECT OF IBA CONCENTRATIONS ON ROOT FORMATION OF SHOOT

Treatment	Concentration of IBA (mg/L)	Root length (cm)	Root Number
R0	0.00	4.5^{c}	3.2^{c}
R1	**0.25**	**7.6^{a}**	**6.4^{a}**
R2	0.50	5.4^{ab}	5.2^{ab}
R3	1.00	4.2^{c}	2.8^{c}
CV (%)		6.61	10.07

* Means followed by same letters within a column are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$ level.

According to Zhang et al. (1989) and Wang et al. (1992), the shoots of Giao Co Lam cultured on ½ MS medium supplemented with IBA (1mg/L) were suitable for the formation of roots. Another study showed that MS medium supplemented with IBA (0.1 mg/L) is appropriate for rooting of Giao Co Lam shoots (100%) and the number of roots per shoot reached 4.16 (Lam et al., 2015). The studies showed that there are different in the concentration of different growth regulators affecting the root formation of of Giao Co Lam. In this study, the number of roots and root length formed on MS ½ medium added with 0.25 mg/L IBA is higher than that of previous reports.

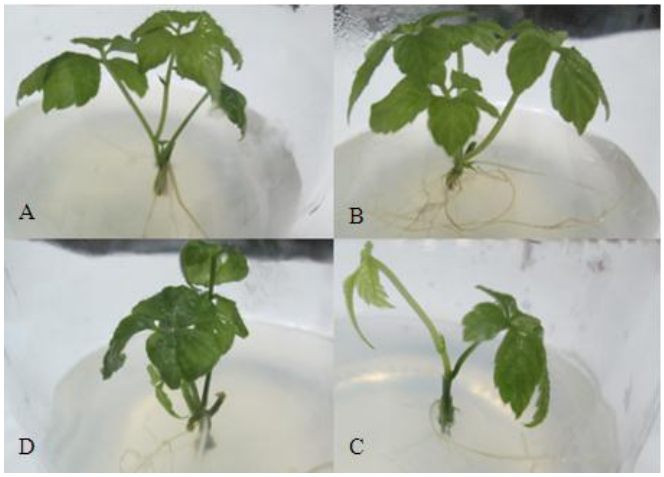

Figure 4. The rooting induction of the Giao Co Lam shoots on media supplemented with IBA at different concentrations. (A) Control, (B) 0.25 mg/L, (C) 0.5mg/L, (D) 1.0 mg/L.

Plantlets fully developed with stems, leaves and roots will be transferred to the nursery. This is the most difficult stage in the *in vitro* propagation techniques, especially for herbaceous plants. The shoots transplanted into the rooting medium for 6 weeks will be transferred to natural conditions from 1-2 weeks for acclimation. The plantlets were removed from the bottle, washed and planted with high density on the foam tray (58x36x12.5 cm) containing clean soil (Tribat) covered with plastic from 7-10 days. And then, the plantlets were removed from the tray and planted on beds with black mesh cover. The rate of survival plantlets after 2 months is 85%.

Figure 5. Giao Co Lam were transferred the garden after 2 months.

IV. Conclusion

Based on the investigation micropropagation of *Gynostemma pentaphyllum*, we determined that concentration of javel solution (NaOCl 5%) and aseptic time suitable for sterilizing the explants to establish the *in vitro* culture were 50% and 20 minutes, respectively; the appropriate concentration of plant growth regulators for the generation and multiplication of shoots is BA (1.0 mg/L) and NAA (0.1 mg/L), the suitable mineral medium for the growth of shoots is the MS ½; the suitable concentration of IBA for the rooting of *in vitro* shoots is MS ½ medium supplemented with 0.25 mg/L.

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