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Effect of BA NAA and 2,4-D on Micropropagation of Jiaogulan (*Gynostemma pentaphyllum* Makino)

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ABSTRACT

Shoots tips and axillary buds of *Gynostemma pentaphyllum* Makino were used as explants and cultured on MS medium supplemented with 0.05, 0.1 and 1.0 mg/l BA. After 12 weeks, new shoots came out and the MS medium contained with 1.0 mg/l BA gave the highest shoots (7.28 shoots) and their average height was 2.22 cm. Young leaves were used as explants for callus induction. Explants were cultured on MS supplemented with vary concentration of 2,4-D (0.1,0.5 and 1.0 mg/l). After 12 weeks, explants on MS medium supplemented with 1.0 mg/l 2,4-D gave the biggest callus which their average diameter were 0.9375 cm. When cultured explants of *Gynostemma pentaphyllum* Makino on MS medium supplemented with combination of (0.05, 0.1, 1.0 and 2.0 mg/l) BA and (0.05, 0.1 and 1.0 mg/l) NAA for 12 weeks. The highest average new shoots were induced from MS medium supplemented with 1.0 mg/l BA and 0.1 mg/l NAA which was 6.8 shoots, and MS medium supplemented with 2.0 mg/l BA and 0.05 mg/l NAA gave the lowest average new shoots (2.7 shoots), and the average root length (1.8 cm). Plantlets were complete and ready for transplanting to in vivo.

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1. Introduction

Gynostemma Pentaphyllum, is an herbaceous vine. This therapeutic vine belongs to the cucumber as the Cucurbitaceae family. It was found that it has disease-prevention and therapeutic features. It contains numerous saponins (more than ginseng), trace materials, amino acids, vitamins and proteins. It is famous for its anti-oxidant and adaptogenic effects. Gynostemma as an adaptogen increases the body's resistance to stress, trauma, and anxiety. It has the unique ability of restoring homeostasis (balance and equilibrium) to all five body systems, i.e., the cardiovascular, digestive, immune, nervous and reproductive systems. The plant tissue culture technique plays an important role in the preservation and propagation of germplasm (Iankova *et al.*, 2001, Bhatia *et al*., 2002). This work reports the feasibility of the utilization of tissue culture techniques to establish a protocol for micropropagation of *Gynostemma pentaphyllum*, which can be a source for medicinal production.

2. Materials and Methods

Young shoot tips and axillary buds of *G. pentaphyllum* Mokino. were used as explants. These explants were surface sterilized with commercial bleach 15 % (V/V) (5.25 % sodium hypochlorite) with a few drops of Teepol (surfactant) for 10 min. and 5% commercial bleach for 10 min and soaked 3 times with sterilized distilled water for 2 min. each. After sterilization, explants were cultured in MS medium (1962) supplemented with 0, 0.5, 1.0, 2.0 and 3.0 mg/l BA, 2% sucrose, 0.25% gelrite.

2.1 Callus Induction

Sections of young leaves of about 0.5 cm^2 were used as explants. These explants were cultured in MS medium (1962) supplemented with 0.1, 0.5 and 1.0 mg/l 2,4-D, 2 % sucrose, 0.25 gelrite. Each treatment was tested, with 25 replications.

2.2 Shoot and Root Induction

New shoot tips and axillary buds were used as explants and cultured in MS medium (1962) supplemented with a combination of (0.05, 0.1, 1.0, and 2.0 mg/l) BA and (0, 05,0.1 and 1.0 mg/l) NAA, 2% sucrose, 0.25% gelrite.

All media in this experiment were adjusted to a pH of 5.6 before autoclaving at 121 º C and 1.1 kg cm^{-1}, cool white fluorescent lights (60 µmol m^{-2} S^{-1}) on 16/8 –h photoperiod, and

incubated at 25±1ºC. All cultures were subcultured every 3 weeks in the same media.

For statistical analysis, the data were subjected to one-way analysis of variance (ANOVA) to assess treatment difference and interaction using the SPSS 11.3 statistical package for windows. Significant means were tested by Turkey's Test ($p \leq 0.05$).

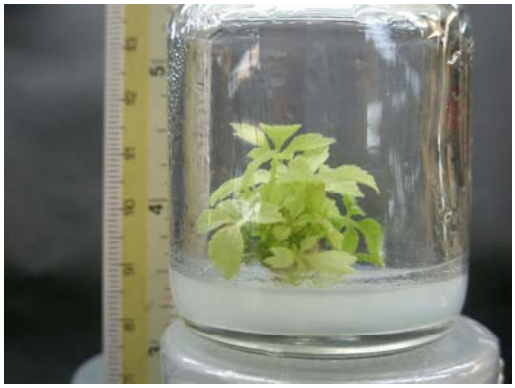

Figure 1: The explants were producing new shoots after culturing them for 80 days.

Table 1: Average number of shoots and plant height of *Gynostemma pentaphyllum* on MS medium supplemented with varying concentration of BA and cultured for 80 days.

MS medium with	No. of shoots*	Plant height(cm)*
BA 0 mg/l	3.285a	1.375a
BA 0.05 mg/l	5.167a	1.75a
BA 0.1 mg/l	5.400a	5.56b
BA 1.0 mg/l	7.285b	2.222a

*Average mean are significant different ($p<0.05$)
a, b in the same row Tukey's test at $p<0.05$

3. Results

Shoots tip and axillary buds of *Gynostemma pentaphyllum* were used as explants and had quickly micropropagation response *in vitro* by the diverse type of explants. These explants were cultured on MS medium supplemented with varying concentrations of (0, 0.05, 0.1, 1.0 mg/l) BA. The explants were producing new plants that can be easily acclimated after culturing them for 80 days (Figure 1). Explants expanded and formed new buds in the fifth week and their buds grew up and formed new shoots within the seventh week and some callus were also promptly induced. After 80 days, buds which grew at the base of explants were differentiated and formed new shoots as shown in Table 1. MS medium supplemented with 1.0 mg/l BA

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gave the highest average number of new shoots (7.285 shoots), but MS medium supplemented with 0.1 mg/l gave the highest average plant height of 5.56 cm.

Test of growth regulators - The best results were obtained using shoot tip and axillary bud for the micropropagation of *Gynostemma pentaphyllum.* The explants response to BA and NAA, individually and in combination, were evaluated for 80 days after inoculation. Explants were cultured in MS medium with different combination of (0, 0.05, 0.1, 1.0 mg/l) BA and (0, 0.05, 0.1, 1.0mg/l) NAA. After culturing for 80 days, explants were formed new shoots roots and callus as in Table 2. Explants were cultured with BA and NAA and formed callus when their hormone auxin (0.05 and 0. 1 mg/l NAA) and cytokinin (0.05 mg/l BA) was not balance. In MS medium supplemented with 0.05 or 0.1 mg/l NAA and 2.0 mg/l BA produced the longest root lengths which were 1.8and 1.6 cm, respectively. In this experiment, MS medium supplemented with 1.0 mg/l BA and 0.1 mg/l NAA gave the highest number of new shoots (6.8 shoots).

Table 2: Average diameter of callus, root length and No. of new shoots after cultured for 80 days.

MS medium		Diameter of callus (cm)*	Root length (cm)*	No. of new shoot(shoot)*
BA(mg/l)	NAA(mg/l)			
0	0	0.30 b	1.05 b	0.60a
0.05	0	0.125ab	0a	3.00bc
0.05	0.05	0.425b	0.25a	3.00bc
0.05	0.1	0.400b	0.35a	3.00bc
0.05	1	0.170ab	4.047c	0.566a
0.1	0	0.150ab	0a	0.95ab
0.1	0.05	0.200ab	0.400ab	0.667a
0.1	0.1	0.350b	0.95ab	0.60a
0.1	1.0	0.220ab	0.25a	0.250a
1.0	0	0a	0a	1.10ab
1.0	0.05	0. 111a	0a	0.967ab
1.0	0.1	0.111a	0a	6.80d
1.0	1.0	0.100a	0a	4.150c
2.0	0	0.100a	0a	0.600ab
2.0	0.05	0.222ab	1.80b	2.70bc
2.0	0.1	0.267ab	1.60b	1.50b
2.0	1.0	0.3ab	0.50ab	1.2b

*Average mean are significant different ($p<0.05$)
a b c in the same row are not significant different by Tukey's test at $p<0.05$

3.1 Callus induction

After cultured section of young leaves on MS medium supplemented with 0.1, 0.5 and 1.0 mg/l 2,4-D for 80 days. These explants formed callus in each treatment and their callus were green. The measured diameter of these callus are presented in Table 3. Where Ms medium supplemented with 1.0 mg/l 2,4-D gave the highest diameter of 0.93 cm. These callus occurred at the edge of the explants and expanded to the whole explants.

Table 3: Average diameter of callus cultured in MS medium with very concentration of 2,4-D after culturing for 80 days.

MS medium with	Average diameter of callus (cm)
2,4-D 0 mg/l	0.267c
2,4-D 0.1 mg/l	0.550b
2,4-D 0.5 mg/l	0.667ab
2,4-D 1.0 mg/l	0.937a

*Average means are significantly different ($p<0.05$)
a b c in the same row are not significantly different by Tukey's test at $p<0.05$

4. Discussion

4.1 Induction of multiple shoots via organogenesis

Induction of multiple shoots through shoot tip and axillary bud which used as explants were cultured on MS medium supplemented with either alone and various concentrations of BA. There are a few growth and organogenetic responses observed from explants after culturing for 12 weeks on PGR-free MS medium. Thus growth of shoot tip and axillary bud will less for multiplication in medium without PGR as reported earlier (Rout *et al*, 2000). All treatments which incorporated with BA will able to induce multiple shoots and plant height. Number of new shoots in this study was agree with the previous study from Pranom Detviitkul and Narumon Monkolchaipakdee (1991) cultured *Gynostemma pentaphyllum* and get the highest average shoots in MS medium supplemented with 1.0 mg/l kinetin. In this experiment the result showed that MS medium supplemented with 1.0 mg/l BA gave the highest average new shoot where as 7.285 shoots. This result was the same as Jala (2011) did with young shoot tip of Wishbone Flower which cultured on MS medium supplemented with 1.0 mg/l BA and 0.1 mg/l NAA.

When cultured shoot tip and axillary buds on MS medium supplemented with combination

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of BA and NAA. All treatment corporate with PGR was able to induce callus multiple shoots and spontaneous rooting after cultured for 12 weeks. Such type of callusing has been reported earlier that no callus in the same family of Cucurbitaceae (Ratree, 1993). In this experiment, the highest average diameter of callus was obtained in MS medium supplemented with 0.5 mg/l BA and 0.05 or 0.1 mg/l NAA. With this formation of medium, a few callus and root length were occurred also.

The highest multiplication rate of 6.8 shoots per plant was observed in MS medium supplemented with 1.0 mg/l BA and 1.0 mg/l NAA. With this formulation of medium no root and a little callus were formed. Statistical test showed that number of shoot induced in BA at 1.0 mg/l either alone or incorporated with NAA. Explants which added with NAA produced more roots and rooting occurred during the second month of initiation culture. This result were the same as Jala (2012) in the study of young shoot tip of Curcuma longa L.which cultured on MS medium supplemented with 2mg/l BA and 0.5 mg/l NAA gave the highest growth rate.

According to Kyung-Min *et al*. (2009) cultured cucumber which are the same family Cucurbitaceae. They cultured them in MS medium supplemented with 0.1 mg/l NAA to induce complete plantlets.

Concentrations of 2.4-D did not show any positive response for callus induction in any of the tested types of explants when evaluation was carried out 50 days after inoculation. On the other hand, treatment of diverse tissues of *Gynostemma pentaphyllum* with different NAA concentrations produced variable callus induction responses. Occurrence of relevant callogenesis was inversely proportional to NAA concentration. Complete absence of light was a requirement for best callus induction. The calli were yellowish and nodular with morphogenic aspects. Plant can be regenerated from these calli by indirect organogenesis (Mello *et al*., 2001). According to these authors, histological analysis revealed that callus was formed from hypertrophied cortical parenchyma cells of the explants. Some of these cells underwent division while the surrounding cells accumulated starch. Callus was capable of shoot bud regeneration after 70 days.

5. Conclusion

Shoot tip and axillary bud of *G. pentaphyllum* Makino. cultured on MS medium supplemented with 1.0 mg/l BA for 12 weeks. New shoots occurred in MS medium supplemented with 1.0 mg/l BA and gave the highest average new shoots and height, 7.28 shoots and 2.22 cm, respectively. MS medium without BA (control) gave the lowest average new shoots (3.28 shoots) and height (1.375 cm).

When cultured tissues of *G. pentaphyllum* Mikino about 0.5 cm on MS medium supplemented with the combination of 2.0 mg/l BA 0.05 or 0.1 mg/l NAA for 80 days and gave the highest number of new shoots and roots, their average new shoots, 2.7 and 1.5 shoots, respectively. These concentrations gave the highest average root length, 1.8 and 1.6 cm, respectively. In MS medium supplemented with 1.0 mg/l BA and 0.1 mg/l NAA gave the highest average new shoots, 6.8 shoots. This concentration gave only new shoot but no root.

When cultured section of young leaves on MS medium supplemented with 1.0 mg/l 2,4-D for 12 weeks. This concentration gave the biggest diameter of callus which is 0.937 cm. and their callus are green.

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