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Effects of Chitosan as the Growth Stimulator for *Grammatophyllum speciosum* in Vitro Culture

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***Abstract*—**The effects of chitosan, a biodegradable polymer, were studied in *Grammatophyllum speciosum* protocorm-like bodies (PLBs) in vitro culture. The chitosan concentration of 0, 5, 10, 15, 20, 25, 50 or 100 mg/l were supplemented in half-strength Murashige and Skoog (1/2 MS) liquid or on agar media containing 2% (w/v) sucrose. The results showed that liquid medium supplemented with 15 mg/l chitosan showed the highest relative growth rate (7-fold increase) of PLBs. On 1/2 MS agar medium supplemented with 25 mg/l chitosan gave the highest relative growth rate (4-fold increase). The relative growth rate of G. speciosum PLBs on agar medium was significantly lower than that in liquid medium. Moreover, chitosan, supplemented to agar medium promoted shoot formation but not rooting. However, supplementation at too high a level, such as 100 mg/l can inhibit growth and kill PLBs.

***Keywords*—**Chitosan, *Grammatophyllum speciosum*, Growth stimulator

I. Introduction

GRAMMATOPHYLLUM speciosum, a rare Thai orchid, is the only member of genus Grammatophyllum that is native to Thailand [1]. This epiphytic orchid is also considered to be the largest member among the orchid family. It can be found from South-East Asia to New Guinea. Apart from its irregular sizes, another out standing characteristics is that the blooming can remain for up to 2 months. In Thailand, this orchid is also used to prepare an herbal elixir against inflammation.

Due to the traditional asexual propagation of orchids whcih is extremely slow; tissue culture is a useful technique for rapid propagation of orchids. Tissue culture was first introduced and applied to Cymbidium orchids by Morel in 1960 [2]. After that, tissue culture methods, culture media and various explants for orchid tissue culture were introduced and developed by several workers. A biodegradable polymer, chitosan, is a deacetylated form of chitin which is present in various organisms, such as fungi, crustaceans, insects and some algae. Chitosan has been reported to act as a plant growth stimulator in some plant species including orchids. Nge et al. [3] reported that the application of chitosan enhanced the growth of *Dendrobium phalaenopsis* PLBs in vitro. The supplement of 1.75% (v/v) chitosan solution in culture medium enhanced root and shoot biomass of grapevine plantlets in vitro [4].

In this work, we studied the effects of chitosan on an in vitro propagation of *G. specisosum* to investigate whether it can be used as an alternative candidate for growth stimulation.

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II. Materials and Methods

A. PLBs induction from G. speciosum shoot tips

G. speciosum PLB induction was done following the procedure of Sopalun et al. [5]. In brief, shoot tips (about 2.0 mm) from in vitro seedlings (8-10 cm long) were excised using a stereo microscope under sterile conditions and cultured in 1/2 MS liquid medium containing 2% (w/v) sucrose without any plant growth regulators. Cultures were maintained under standard conditions at 25 ± 2 ^{o}C under white fluorescent light at intensity of 37 µmol m^{-2} sec^{-1} for 16 h per d with shaking at about 110 rpm for 24 h.

B. Effects of chitosan on G. speciosum PLBs growth

To study the effects of chitosan on G. speciosum PLBs growth, 1.0 g G. speciosum PLBs (0.1-0.2 cm in diameter) were cultured in 1/2 MS liquid medium supplemented with 2% (w/v) of sucrose and various concentrations of chitosan (0, 5, 10, 15, 20 ,25 30, 50 or 100 mg/l) at pH 5.7. After 1-month culture, the fresh weight of PLBs was recorded and expressed as the relative growth rate.

C. Relative growth rate measurement

Fresh weight of PLBs was recorded after 1-month culture and expressed as relative growth rate calculated based on the fresh weight of cultured tissue as follows:

$$\text{Relative Growth Rate } (\%) = \left(\frac{Wf - Wi}{Wi} \right) x100$$

Wf = final weight
Wi = initial weightIf

D. Statistical analysis

All experiments were arranged by completely randomized

design (CRD). Data from in vitro cultures were subjected to analysis of variance (ANOVA) and means were compared using the least significant difference (LSD) test.

III. Results

A. Effects of chitosan on PLB in vitro propagations

The relative growth rate of G. specioum PLB increased significantly after 1-month culture when chitosan was supplemented in 1/2 MS liquid medium containing 2% (w/v) sucrose (Fig. 1). The highest relative growth rate (756%) was obtained with 15 mg/l chitosan. However, the relative growth rate was significantly reduced when chitosan concentrations were more than 15 mg/l. Moreover, supplementation with 100 mg/l chitosan caused PLB necrosis and releases some browning compounds into the medium (Fig. 2A).

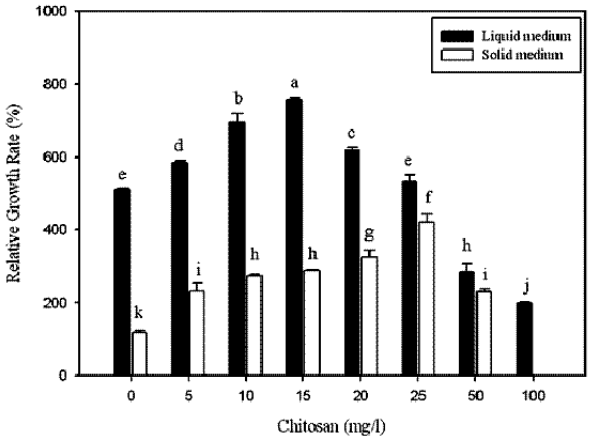

Fig. 1 Effects of chitosan on relative growth rate of *G. speciosum* PLBs cultured for 1 month in 1/2 MS liquid and on 1/2 MS agar media containing 2% (w/v) of sucrose, supplemented with various concentrations of chitosan. Column and bar represent mean ± standard error (SE), SE was calculated from five independent experiments by one-way ANOVA. Bars with different letter differs significantly ($p < 0.05$).

When chitosan was supplemented in 1/2 MS to solid medium, 25 mg/l chitosan gave the highest relative growth rate after 1-month culture (Fig. 1). PLBs did not survive on 1/2 MS solid medium supplemented with 100 mg/l of chitosan (Fig. 2B). Overall, the relative growth rate of G. speciosum PLBs on solid media was lower than those obtained using liquid media at the same concentration of chitosan.

B. Effects of chitosan on PLB development

Varying the concentrations of chitosan on 1/2 MS solid medium led to differences in the development of G. speciosum PLBs (Table I). At 5, 10, and 15 mg/l chitosan promoted leaf development and the highest number of leaves per explant was obtained using 15 mg/l of chitosan (1.8 leaves per explant). Morever, supplementation with 5, 10 and 15 mg/l chitosan increased the number of new PLBs and the number of shoots. However, chitosan supplementation did not promote rooting.

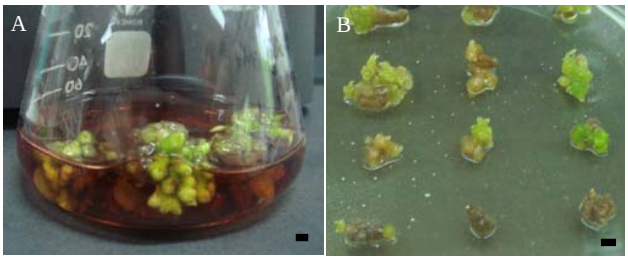

Fig. 2 Effects of Chitosan on *G. speciosum* culture; A: G. speciosum PLBs cultured in 1/2 MS liquid medium supplemented with 100 mg/l chitosan, B: Some PLBs did not survive when culture on 1/2 MS solid medium supplemented with 100 mg/l chitosan. Bar = 1 mm.

IV. Discussion

As previously reported, we confimed with *G. speciosum* micropropagatiion that chitosan can function as a plant growth stimulator for orchid production or tissue culture [5]. The optimal amount of 15 mg/l of chitosan supplementation for the highest PLB growth rate and 5 to 15 mg/l supplementation to promote leaf and shoot formation was similar to the results of Nge et al. [3] who reported that the optimal concentration of chitosan for promoting growth of Dendrobium orchid tissue

TABLE I
EFFECTS OF CHITOSAN ON GROWTH OF *G. SPECIOSUM* CULTURED ON 1/2 MS AGAR MEDIUM FOR 1-MONTH

Chitosan concentration (mg/l)	PLB Diameter (mm)	No. of new PLBs per explant	No. of shoots per explant	No. of roots per explant	No. of leaves per explant
0	54 ± 2 b	9.2 ± 0.7 b	0.8 ± 0.4 b	0 a	0 a
5.0	62 ± 4 ab	11.2 ± 1.0 a	3.0 ± 1.0 a	0.1 ± 0.1 a	1.4 ± 0.8 a
10.0	61 ± 3 ab	10.5 ± 0.6 ab	3.1 ± 0.8 a	0.1 ± 0.1 a	0.7 ± 0.3 ab
15.0	69 ± 4 a	11.2 ± 0.7 a	3.1 ± 0.6 a	0 a	1.8 ± 0.6 a
20.0	58 ± 7 ab	9.6 ± 1.1 ab	1.1 ± 0.6 b	0 a	0 a
25.0	58 ± 4 ab	10.7 ± 0.5 ab	0.9 ± 0.3 b	0 a	0 a
50.0	56 ± 4 ab	8.4 ± 0.8 b	0.6 ± 0.3 b	0 a	0 a

Values are presented as means ± SE. Different letters within a column indicate significant differences at $p < 0.05$ according to analysis of variance (ANOVA).

was also 15 mg/l. Barka et al [4] reported that 1.75% (v/v) chitosan solution in culture medium enhanced root and shoot biomass of grapevine plantlets in vitro. However, in this study we found that chitosan supplement to solid medium promoted shoot formation but not rooting. Chitosan may play a role in enhancing growth and development by some signaling pathway to auxin biosynthesis via a tryptophan-independent pathway [6], but as seen here, supplementation at too high a level such as 100 mg/l can inhibit growth and kill PLBs.

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