Effect of BA and 2iP on Shoot Proliferation and Somaclonal Variation of *Gardenia jasminoides* Ellis *in vitro* Culture

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ABSTRACT Shoot tips of *Gardenia jasminoides* Ellis were successfully propagated in B_5 agar medium with BA (0, 2.5, 5, 7.5 and 10 mg.l^{-1}) and 2iP (0, 2.5, 5, 7.5 and 10 mg.l^{-1}) concentrations. The number of shoots in medium with 10 mg.l^{-1} BA was 7 times greater than those in medium without BA. The BA induced plantlets had 100% survival rate and normal growth rate *ex-vitro*. The number of shoots in medium with 7.5 mg.l^{-1} 2iP was 4 times greater than in 2iP free medium. All explants receiving 2iP gave chimeric plants showing different degrees of white leaf tissue. This is the first report of somaclonal variation induced by 2iP in *G. jasminoides*. Chimeric plants had 70% survival rate and showed slow growth *ex-vitro*.
Abbreviations: BA - 6-Benzylaminopurine, 2iP - 6-(γ,γ-dimethylallyamino) purine, B_5 - B_5 medium, MS - Murashige and Skoog medium

KEYWORDS: *Gardenia jasminoides* Ellis, *in vitro* culture, benzylaminopurine, 6-(γ,γ-dimethylallyamino) purine, somaclonal variation.

INTRODUCTION

Gardenia jasminoides Ellis, family Rubiaceae, is an ornamental woody plant. It has white flowers with sweet fragrance.[1] It is used as a cut flower and a garden shrub in Thailand. It is a popular pot plant in the US and many European countries.[2] In conventional propagation, terminal cutting of *G. jasminoides* results in a low proliferation rate. Micropropagation of *G. jasminoides* via *in vitro* organogenesis using modified Murashige and Skoog medium (MS) offers higher proliferation rate per each starting plant.[3] However, such a modified MS medium consists of complex chemical mixture. The use of such a modified basal medium is inconvenient for routine propagation.

Cytokinins are plant growth regulators used for stimulating cell division, as well as for the formation and growth of axillary and adventitious shoots. Two kinds of cytokinins are available. One is the naturally occuring cytokinins, which include zeatin, 6-(γ,γ-dimethylallyamino) purine (2iP), and adenine. Another type is synthetic cytokinins. This group consists of substituted purines, ie, 6-benzylaminopurine (BA) and 6-furfurylaminopurine (kinetin), and phenylureas such as thiadiazuron.[4]

This investigation was to evaluate the use of standard medium B_5 in *G. jasminoides* tissue culture. The effects of two different cytokinins, 2iP and BA, on shoot proliferation and somaclonal variation of *G. jasminoides* were also presented.

MATERIALS AND METHODS

Young shoots of *Gardenia jasminoides* Ellis, 5 cm in length, were obtained from 5 plants grown in natural condition, one from Suan Luang Rama IX Botanic Garden, Bangkok, Thailand, the others from Faculty of Science, Mahidol University, Bangkok, Thailand. Shoot tips collected from the same mother plant were divided for two different cytokinin treatments each time cultures were initiated. After leaf removal, selected shoots were washed with mild detergent and rinsed in tap water for 10 min. The shoots were then soaked in 15% (v.v^{-1}) Clorox in 0.25% (v.v^{-1}) Tween-20 solution. After 10 min, the shoots were soaked for 10 min in 10% (v.v^{-1}) Clorox in 0.25% (v.v^{-1}) Tween-20 solution, followed by five 5-min rinses in sterile distilled water. Excised shoot tips, 0.5-1.0 cm in length, were used as explants.

Surface sterilized explants were cultured on 0.7% agar B_5 medium[5], half in medium with 0, 2.5, 5.0, 7.5 and 10.0 mg.l^{-1} BA (Sigma Co, Ltd.) while the other half was in medium with 0, 2.5, 5.0, 7.5 and 10.0 mg.l^{-1} 2iP (Sigma Co, Ltd.). All explants were maintained at 25±2°C under 16-h photoperiod provided by Gro-lux fluorescent lamp (37 mmol. m^{-2}.s^{-1}). All were subcultured at 2 weeks interval.

At 45 days, explants receiving BA were all swollen but showed no shoot elongation. Decision was then made to have all subcultured on BA-free B_5 agar medium to facilitate shoot growth. All were then subcultured to BA-free B_5 medium every 2 weeks. All cultures formerly exposed to 2iP were evaluated after 90 days. At 90 days, shoot length from explants formerly exposed to BA was unmeasurable due to their swollen nature. They were then evaluated at 120 days when shoot tips finally lengthened. Experiments were repeated 5 times, each treatment with 5 explants.

Root induction was achieved by transferring new shoots to B_5 agar medium with no plant growth regulator. Plantlets with roots were transplanted to damp vermiculite in containers with clear lids under the same environment as those in cultures. Plantlets were acclimatized to normal environment by replacing the container lids with colorless transparent plastic sheets with 1 mm pinholes for 1 month. Plantlets were then potted and kept in greenhouse under 50% natural light. All were transferred outdoors after 2 months.

Results and Discussion

Explants receiving BA and 2iP showed different developments of new shoots. By day 45 explants propagated in the medium with BA yielded shoot tips with multiple microshoots ranging from 1-4 mm. However, apical shoot tips from BA treated explants showed basal swelling (Fig 1). The subculturing of explants on BA free B_5 agar medium was necessary in order to increase shoot length. Prolonged culturing in initiation medium resulted in stout shoots. At 120 days, new shoots increased in length and opened up their leaves. Explants previously cultured in 2.5 mg.l^{-1} BA and 10 mg.l^{-1} BA B_5 medium were the first and the last to open up their leaves, respectively. However, the latter gave the highest number of new shoots (7.3 shoots per explant) with the average length of 0.9 cm. The control gave only 1 shoot with average length of 2.8 cm (Table 1).

The 2iP treatment gave multiple shoots with no swellings. Explants in 7.5 mg.l^{-1} 2iP in B_5 medium gave the highest number of shoots (4 shoots per explant) with average length of 1.6 cm. Explants in 2iP free medium gave only one 1.2 cm shoot. The longest new shoots came from explants receiving in 10.0 mg.l^{-1} 2iP (Table 2).

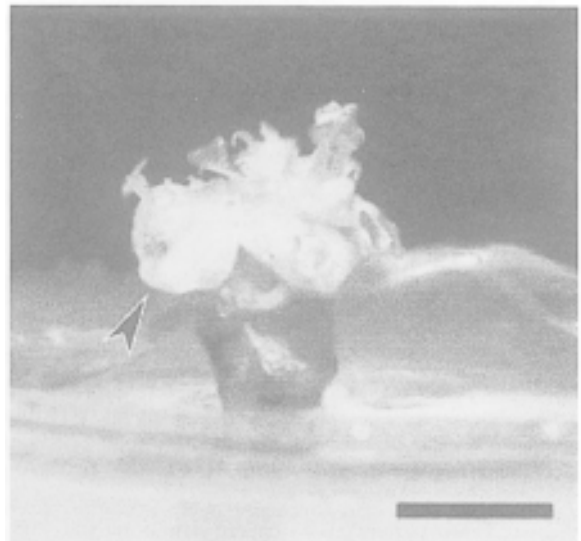

Fig 1. The swollen shoot apex of *Gardenia jasminoides* Ellis (arrowhead) from shoot tip cultured in B_5 medium with 10.0 mg.l^{-1} BA at 45 days. (Bar = 1 cm).

Table 1. Effect of BA concentrations on the number of new shoots per explant and shoot length of *Gardenia jasminoides* Ellis new shoots *in vitro* at 120 days.

BA (mg.l^{-1})	Number of new shoots per explant	Shoot length (cm)
0	1.0 a	2.8c
2.5	5.3b	1.6b
5.0	4.3b	1.1a
7.5	5.7b	0.9a
10.0	7.3c	0.9a

Means followed by different letters in same column are significantly different.

Table 2. Effect of 2iP concentrations on number of new shoot per explant and shoot length of *Gardenia jasminoides* Ellis new shoots *in vitro* at 90 days.

2iP (mg.l^{-1})	Number of new shoots per explant	Shoot length (cm)
0	1.0 a	1.2a
2.5	1.0a	2.1b
5.0	2.0a	1.4a
7.5	4.0b	1.6a
10.0	1.0a	4.7c

Means followed by different letters in same column are significantly different.

In this investigation, BA was superior to 2iP in giving more shoots per explant when same concentrations of the two plant growth regulators were compared. This holds true for all concentrations of plant growth regulators used throughout the investigation. The result obtained here is similar to the results from Economou and Spanoudaki.[6]

By day 120, shoots obtained from cultures with different BA concentrations showed slightly different shoot morphology (Fig 2). Explants receiving 2.5 $mg.l^{-1}$ BA gave distinctively long shoots. The shoot length inversely decreased with increasing BA concentration (Table 1). The dense clump of new shoots was obtained from 10 $mg.l^{-1}$ BA treatment. Explants formerly treated with 10 $mg.l^{-1}$ BA gave 7.3 shoots significantly different from the number obtained from explants receiving 2.5, 5.0 and 7.0 $mg.l^{-1}$ BA, respectively.

After day 120, new shoots obtained from BA treated explants were individually separated and placed on BA-free B_5 agar medium. All shoots showed self-rooting in BA-free medium within 30 days. All plantlets originated in BA medium showed dark green leaves like the mother plant. Survival rate of plants obtained from BA medium was 100%. *G. jasminoides* from BA culture gave normal flowers at the age of 18 months. Our study resulted in higher number of shoots compared to those received by using modified MS medium.[6] As a result, the use of normal basal medium B_5 with BA is an improved method for *G. jasminoides* micropropagation *in vitro*.

On the contrary, all new shoots obtained from 2iP treated medium showed somaclonal variation in leaf color (Fig 3). All shoots had leaves with white streaks of different degrees. Hand-free cross section of leaves from the mother plants and variegated leaves from 2iP cultures were made. Cross sections of leaves from mother plants showed a lot of green chloroplasts in parenchymatous tissue. Green portion of variegated leaves showed lots of chloroplasts in each parenchymatous cell while the white portion showed only 1-5 chloroplasts per parenchymatous cell (Fig 4 a,b). However, there was no report of chimeric plants in any new shoots obtained in the study using modified MS medium.[6]

Chimeric shoots from 2iP cultures could easily be rooted in 2iP-free B_5 medium. Plantlets obtained from *in vitro* nodal culture of these chimeric plants retained chimeric characters. However, the growth rate of these chimeric plants was 50% less than the rate of plants obtained from BA medium. The survival rate of chimeric plants in the nursery is 70%. Plantlets with more than 80% white tissue died during acclimatization period. None of variegated *G. jasminoides* from 2iP culture grown under normal condition had flowered at the age of 2 years.

Mutations in plants can be found both at chromosomal level as well as in the extra-nuclear

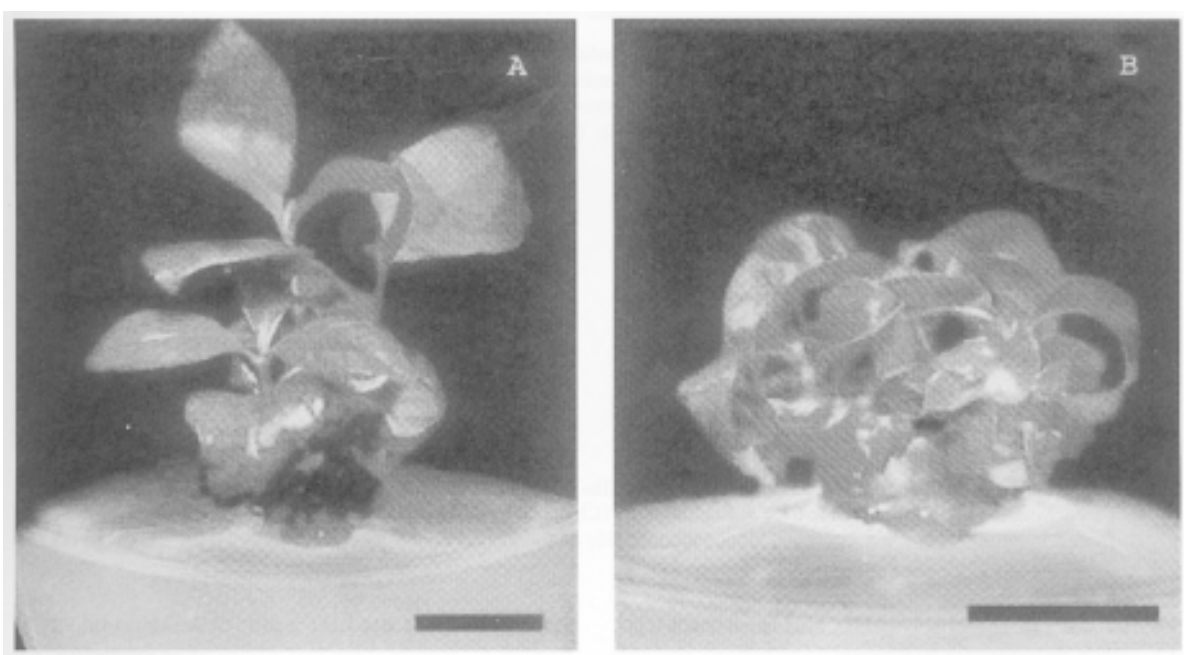

Fig 2. The shoot tips of *Gardenia jasminoides* Ellis on BA free B_5 medium at 120 days. (A) Shoot tips previously cultured in 2.5 $mg.l^{-1}$ BA medium gave distinctively long shoots with normal dark green leaves. (B) Shoot tips previously cultured in 10.0 $mg.l^{-1}$ BA medium gave dense clump of new shoots. Plantlets had compact form with cresent form with cresent shape leaves. (Bar = 1 cm).

Fig 3. New shoots of *Gardenia jasminoides* Ellis cultured on B_5 medium with different concentrations of 2iP at 90 days. (A) New shoot cultured in B_5 medium with no 2iP showed dark green leaves. (B), (C) New shoots cultured in B_5 medium with 7.5 and 10.0 $mg.l^{-1}$ 2iP respectively. (B) and (C) showed variegated leaves with different degrees of white streaks. Notice B_5 medium with 7.5 $mg.l^{-1}$ 2iP gave multiple new shoots (arrowheads in B). (Bar = 1 cm).

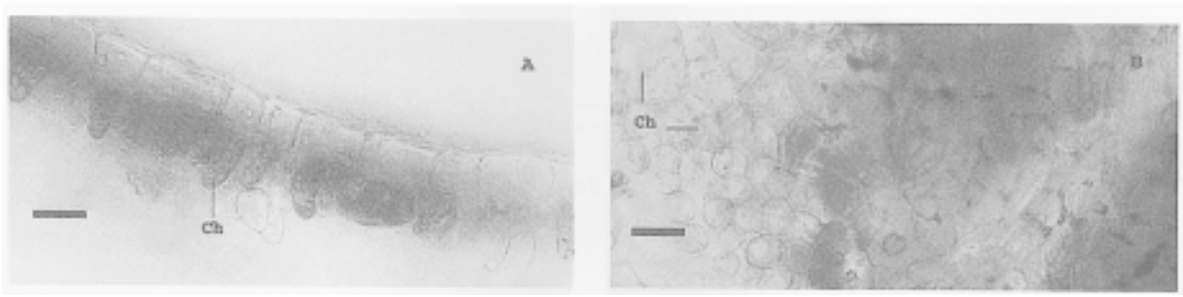

Fig 4. (A) Cross sections of variegated *Gardenia jasminoides* Ellis leaf from 2iP culture. Lower epidermis was removed. Green parenchymatous cells are shown with lots of chloroplasts (Ch) in green portion of the leaf. (B) Dorsal view of a variegated leaf from 2iP culture with lower epidermis removed. Left side of the picture shows parenchymatous cells containing very few chloroplasts from white portion of the leaf.

DNA found in the chloroplasts and mitochondria[7]. Mutation frequencies in natural populations are very low and varied greatly in different plants. However, these frequencies can be significantly increased by the use of chemical or physical mutagenic substances. This is the first report of somaclonal variation in *G. jasminoides* leaf tissue after receiving 2iP *in vitro.*

Cytokinin is a class of plant growth regulator. It is arbitrarily defined in terms of its capacity to

promote *in vitro* cell division and growth of callus tissues[8]. There was no report of 2iP as a mutagenic agent to date. The reduction of the number of green chloroplasts in *G. jasminoides* as a result of exposure to 2iP implied an inhibition of chloroplast production. Further investigation of reduced chloroplast production by 2iP is suggested.

Conclusions

Gardenia jasminoides Ellis was successfully propagated *in vitro* using standard B_5 agar medium. *G. jasminoides* culture in B_5 agar medium with 10 $mg.l^{-1}$ BA gave up to 7 true to form shoots per explant within 120 days. These plants have 100% survival with normal growth rate. Treatment with 2iP gave chimeric new shoots in 90 days. This is the first report of somaclonal variation in *G. jasminoides* leaf tissue from explants receiving 2iP. Treatment with 2iP yielded 100% chimeric plants. They maintained their chimeric character with further subculturing but exhibited slow growth.

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