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การชักนำให้เกิดยอดทวีคูณในส้มโอสายพันธุ์ทองดี*

Multiple shoot induction of pomelo [*Citrus maxima* (Burm.) Merr.] 'Thongdee'

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บทคัดย่อ

ส้มโอพันธุ์ทองดีเป็นหนึ่งในผลไม้เขตกึ่งร้อนที่มีความสำคัญเป็นอันดับต้นๆในพื้นที่จังหวัดนครปฐม แต่จากปัญหาอุทกภัยครั้งใหญ่ที่ผ่านมาทำให้ต้นส้มโอได้รับความเสียหายและตายไปเป็นจำนวนมาก งานวิจัยนี้จึงศึกษาวิธีชักนำให้เกิดยอดทวีคูณจากชิ้นส่วนเนื้อเยื่อปลายยอดของต้นส้มโอพันธุ์ทองดี โดยนำเมล็ดส้มโอมาทำความสะอาดโดยการฟอกฆ่าเชื้อและนำมาเพาะเลี้ยงในอาหารสูตร MS เป็นเวลา 8 สัปดาห์ ผลการทดลองพบว่าเมล็ดที่ไม่พบการปนเปื้อนอยู่ระหว่าง 60.3 – 80.23% จากนั้นนำเนื้อเยื่อปลายยอด และชิ้นส่วนข้อจากต้นอ่อนที่ได้ไปเลี้ยงในอาหารสูตร MS ร่วมกับ BA ความเข้มข้น 2.22 – 8.87 ไมโครโมลาร์ หรือ เลี้ยงในอาหารสูตร MS ร่วมกับ BA ความเข้มข้น 2.22 – 8.87 ไมโครโมลาร์ ร่วมกับ IBA1.23 ไมโครโมลาร์ ผลการทดลองพบว่าอาหารสูตร MS ร่วมกับ BA4.44 ไมโครโมลาร์ และ IBA1.23 ไมโครโมลาร์ ชักนำให้เกิดยอดทวีคูณได้มากที่สุดเท่ากับ 5.0 ยอด และมีใบ 29.3 ใบจากชิ้นส่วนเนื้อเยื่อปลายยอดอย่างไรก็ตามพบว่าอาหารสูตร MS ร่วมกับ BA4.44 ไมโครโมลาร์ เพียงอย่างเดียวชักนำให้เนื้อเยื่อส่วนข้อเกิดยอดทวีคูณได้มากที่สุดเท่ากับ 5.6 ยอด และมีใบ 32.2 ใบ

* our experiment was aimed to investigate the appropriated *in vitro* propagation techniques in order to obtain the multiple shoot of the Siamese pomelos 'Thongdee' for propagation

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Abstract

Pomelo (*Citrus maxima*) cv. 'Thongdee' was one of the important tropical fruit in NakhonPathom province. Multiple shoot induction of pomelo was carried out for micropropagation and germplasm preservation to reduce the loss due to flooding. Seeds were collected and surface-disinfected, then cultured on MS medium for 8 weeks. It was found 60.3 – 80.23% sterile condition from surface sterilization of seeds. Then, shoots and nodes from seedlings were cultured on MS medium supplemented with 2.22 – 8.87 µM BA alone or in combinations with 1.23 µM IBA. MS medium supplemented with 4.44 µM BA and 1.23 µM IBA was suitable for multiple shoot induction from a shoot explant obtaining 5.0 shoots with 29.3 leaves. However, MS medium supplemented with 4.44 µM BA was appropriate for a node explant providing 5.6 shoots with 33.2 leaves.

INTRODUCTION

Citrus maxima (Burm.) Merr. (pomelo as a common name), a member of the family Rutaceae, is an attractive tropical fruit, native to South and Southeast Asia. The Siamese pomelo or som-o was well known in Thailand long time ago because its taste likes a sweet, mild grapefruit with little of the grapefruit's bitterness. There are several varieties of Siamese pomelo such as 'Thongdee' and 'Kao Nampueng' for Nakhon Pathom Province and 'Kao Tang Kwa' for Chai Nat Province (Mäkynen et al., 2013). In 2011, due to severe flooding in Thailand, various varieties were damaged in consequence. Thus, our experiment was aimed to investigate the appropriated *in vitro* propagation techniques in order to obtain the multiple shoot of the Siamese pomelos 'Thongdee' for propagation and germplasm preservation to reduce the loss due to flooding.

MATERIALS AND METHODS

Plant Material

Seeds of *Citrus maxima* 'Thongdee' were surface-sterilization with 15% Clorox (6% NaHClO) for 10 min followed by 10% Clorox for 5 min, then rinsed three times with sterile distilled water. Sterilized seeds were cultured on MS medium for 8 weeks, then 2 explant types, an apical shoot (about 1 cm long with 2 – 3 leaves) and a node(about 1 cm long) were used as explants for multiple shoot induction.

Media and Culture Conditions

A medium used for multiple shoot induction was Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 3.0 g/L sucrose and 5.5 g/L agar (Hardy Diagnostics

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Criterion™) supplemented with 2.22, 4.44, 6.66 and 8.87μM BA alone or in combinations with 1.23μM IBA. The pH of all media was adjusted to 5.6 prior to adding agar and autoclaving. Explants were cultured in 240-ml glass jars containing 50 ml of culture medium for 12 weeks. All cultures were incubated at 24±1°C and under a 16-h photoperiod at 35 to 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by cool-white fluorescent lamps.

Statistical Analysis

Whole fresh weight (FW), Number of shoots, roots and leaves, shoot height and root length were recorded after 8 weeks of culturing. Data were analyzed by Duncan's New Multiple Range Test at p = 0.05 (Duncan, 1955).

RESULTS

Surface-sterilization and germination of pomelo 'Thongdee' seeds

Seeds were surface-sterilization providing 80.23% sterile condition with 61.92% germination. Seeds germinated 30 – 55 days after culturing (Table 1; Figure 1).

Table 1 Surface-sterilization and germination of pomelo "Thongdee' seeds cultured on MS medium for 12 weeks

Seed set	Seed number	Contamination (%)	Germination	
			Days	%
1	48	10.42	44-61	62.50
2	207	26.08	24-42	60.31
3	89	10.11	21-63	74.16
Total	344	19.77	30-55	61.92

Figure 1. Germination of pomelo 'Thongdee' seeds cultured on MS medium for 8 weeks

Effects of BA and IBA on multiple shoot induction frompomelo 'Thongdee' apical shoots

After 12 weeks of culturing, apical shoots of pomelo 'Thongdee' on MS medium supplemented with BA alone (2.22, 4.44, 6.66 and 8.87 μM) or BA in combinations with IBA (1.23μM), either BA alone or BA in combination with IBA provided significantly more multiple shoots (2.6 – 5.0 shoots) than control with no growth regulation (1.1 shoots) did (Table 2). However, BA at 4.44 μM in combination of IBA at 1.23 μM providing 5.0 shoots, average shoot height 0.8 cm per shoot, with 29.3 leaves and 0.248 g whole fresh weight (Table 2; Figure 2).Moreover, the number of shoots decreased with exceeded concentrations of BA over 4.44 μM in combinations with IBA (1.23 μM).

Table 2 Effects of BA and IBA on multiple shoot induction of pomelo 'Thongdee' from apical shoots cultured on MS medium for 12 weeks

PGRs (μM)		Growth of shoots from an apical shoot[1/]					
BA	IBA	Shoot No.	Shoot height (cm)	Root No.	Root length (cm)	Leaf No.	Whole FW (g)
0.0	0.0	1.1±0.10c	0.7±0.10ab	0.9	8.85	06.4±0.60c	0.289±0.0385a
2.22	0.0	3.4±0.69b	0.6±0.10b	0.0	0	12.2±3.59bc	0.157±0.0296ab
2.22	1.23	2.6±0.64b	0.6±0.09ab	0.0	0	10.0±1.88bc	0.196±0.0337ab
4.44	0.0	2.8±0.33b	0.7±0.13ab	0.0	0	12.5±2.04bc	0.252±0.0768ab
4.44	1.23	5.0±0.77a	0.8±0.09ab	0.0	0	29.3±5.83a	0.248±0.0444ab
6.66	0.0	3.8±0.44ab	0.9±0.14a	0.0	0	20.1±3.00b	0.224±0.0283ab
6.66	1.23	2.6±0.22b	0.5±0.08b	0.0	0	11.7±1.50bc	0.123±0.0166ab
8.87	0.0	3.1±0.50b	0.6±0.11ab	0.0	0	15.1±3.99bc	0.201±0.0345ab
8.87	1.23	3.1±0.43b	0.7±0.12ab	0.0	0	14.1±2.97bc	0.186±0.0427ab

[1/] n = 20, Values (mean ± S.E.) in the same column are not significantly different by Duncan's new multiple range test at p = 0.05

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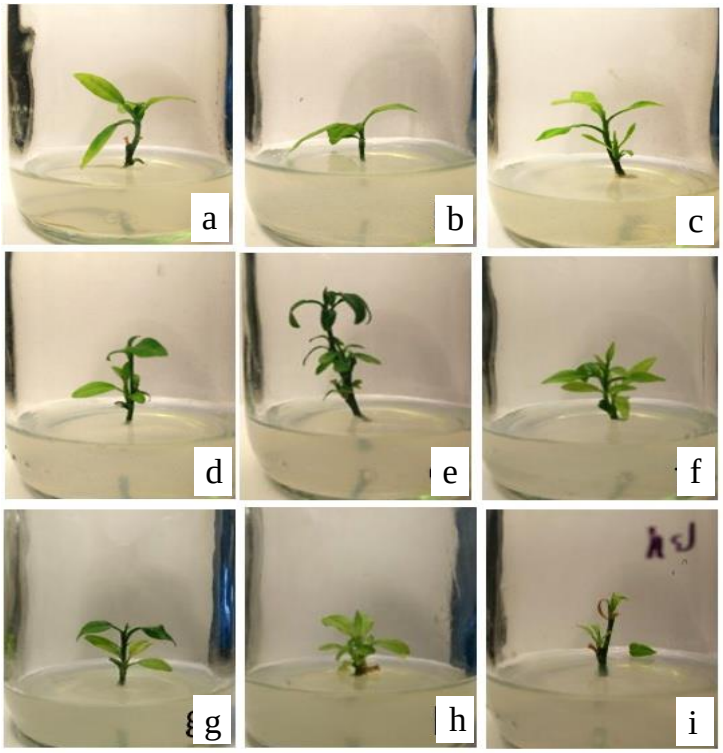

Figure 2. Multiple shoot induction of pomelo 'Thongdee' from a shoot cultured on MS medium supplemented with BA and IBA at different concentrations for 12 weeks a) MS; b) MS + 2.22 µM BA; c) MS + 2.22 µM + 1.23 µM IBA; d) MS + 4.44 µM BA; e) MS + 4.44 µM + 1.23 µM IBA; f) MS + 6.66 µM BA; g) MS + 6.66 µM + 1.23 µM IBA; h) MS + 8.87 µM BA; i) MS + 8.87 µM + 1.23 µM IBA

Effects of BA and IBA on multiple shoot induction from pomelo 'Thongdee' nodes

After 12 weeks of culturing, nodes (axillary buds) of pomelo 'Thongdee' on MS medium supplemented with BA alone (2.22, 4.44, 6.66 and 8.87 µM) or BA in combinations with IBA (1.23 µM) provided the similar results found from the apical shoot culture. Either BA alone or BA in combination with IBA provided significant more multiple shoots (3.4 – 5.6 shoots) than control with no growth regulation (1.8 shoots) did (Table 3).

Table 3 Effects of BA and IBA on multiple shoot induction of pomelo "Thongdee' from nodes (axillary buds) cultured on MS medium for 12 weeks

PGRs (μM)		Growth of shoots from axillary buds from a node[1/]					
BA	IBA	Shoot No.	Shoot height (cm)	Root No.	Root length (cm)	Leaf No.	Whole FW (g)
0.0	0.0	1.8±0.20 b	0.7±0.10 ab	0.7	7.37	10.5±1.65 b	0.319±0.036 a
2.22	0.0	3.4±0.43 ab	0.8±0.07 ab	0.0	0	23.3±5.53 ab	0.218±0.032 a
2.22	1.23	3.9±0.96 ab	0.8±0.15 ab	0.0	0	20.6±6.10 ab	0.337±0.098 a
4.44	0.0	5.6±1.26 a	0.9±0.12 ab	0.0	0	33.2±6.64 a	0.352±0.053 a
4.44	1.23	4.4±0.72 ab	0.7±0.07 b	0.0	0	23.8±3.90 ab	0.263±0.045 a
6.66	0.0	3.7±0.62 ab	0.7±0.06 ab	0.0	0	21.3±2.82 ab	0.271±0.048 a
6.66	1.23	4.5±0.58 ab	1.0±0.12 a	0.0	0	23.9±2.99 ab	0.318±0.041 a
8.87	0.0	4.7±0.67 ab	0.6±0.08 b	0.0	0	23.8±4.83 ab	0.235±0.047 a
8.87	1.23	4.5±0.54 ab	0.8±0.07 ab	0.0	0	21.0±4.17 ab	0.278±0.054 a

[1/] n = 20, Values (mean ± S.E.) in the same column are not significantly different by Duncan's new multiple range test at p = 0.05

DISCUSSION

The low seed germination rate in our experiment might due to its natural mechanical barriers such as wax cuticle, suberine, palisade tissues, and macrosclereid layers that block the uptake of water and oxygen diffusion (Vleeshouwers et al., 1995). The studies on effects of BA and IBA on multiple shoot induction from pomelo 'Thongdee' apical shoots and nodes showed similar results with the work of Paudyal and Haq (2000) on *Citrus grandis* L. Osbeck. They found that 1.8 mM BA alone induced the greatest number of shoots per explant (5.2 shoots) after cultured for 6 weeks but our results suggested that higher concentrations of BA was not decreased the number of shoots per explants when applied alone without IBA. The best cytokinin for direct shoot regeneration in citrus was BA (Germana et al., 2008, Tallon et al., 2013). However, our results did not show the shoot length decreased with an increase in BA concentration. These findings are in contrast with those of Molina et al. (2007).

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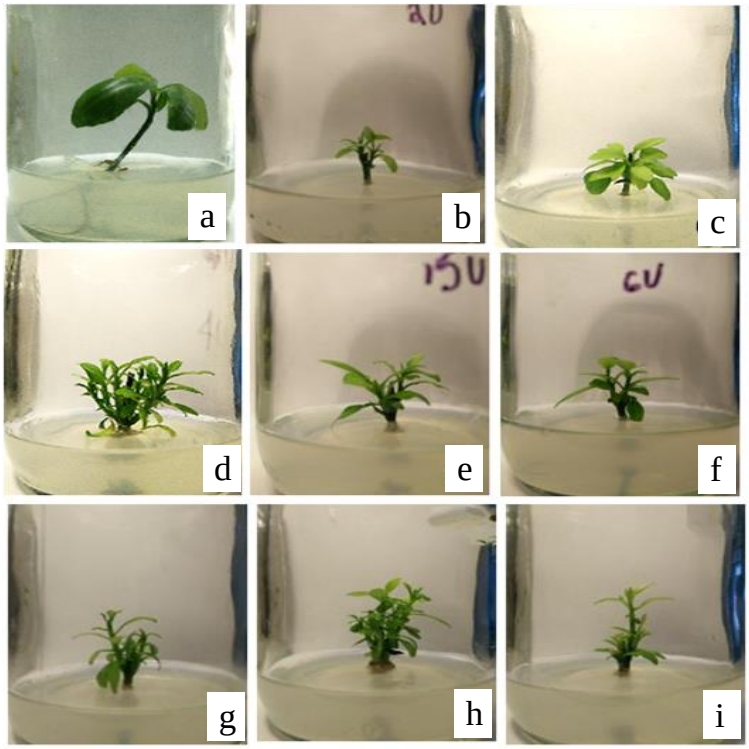

Figure 3. Multiple shoot induction of pomelo 'Thongdee' from a node cultured on MS medium supplemented with BA and IBA at different concentrations for 12 weeks
a) MS; b) MS + 2.22 µM BA; c) MS + 2.22 µM + 1.23 µM IBA; d) MS + 4.44 µM BA; e) MS + 4.44 µM + 1.23 µM IBA; f) MS + 6.66 µM BA; g) MS + 6.66 µM + 1.23 µM IBA; h) MS + 8.87 µM BA; i) MS + 8.87 µM + 1.23 µM IBA

CONCLUSIONS

From the study, it was demonstrated that the most effective media for multiple shoot induction from a shoot explant and a node explant of *Citrus maxima* 'Thongdee' were MS medium supplemented with 4.44 µM BA in combination with 1.23 µM IBA and MS medium supplemented with 4.44 µM BA, respectively. The development of appropriate technique for *in vitro* multiple shoot induction is necessary for mass propagation, germplasm collections and breeding program.

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References

Duncan, D.B. (1955). "Multiple Range and Multiple F Tests." Biometrics 11 (1). [Wiley, International Biometric Society]: 1–42. doi:10.2307/3001478.

Germana, M.A., Macaluso, L., Patricolo, G., Chiancone, B. (2008): "Morphogenic response *in vitro* of epicotyl segments of *Citrus macrophylla"*. Plant Biosystems 142: 661-664.

Mäkynen, K., Jitsaardkul S., Tachasamran P, Sakai N., Puranachoti S., Nirojsinlapachai N., Chattapat V, Caengprasath V., Ngamukote S., and Adisakwattana S. (2013). "Cultivar variations in antioxidant and antihyperlipidemic properties of pomelo pulp (*Citrus grandis* [L.] Osbeck) in Thailand". Food Chemistry. 139: 735–743.

Molina, R.V., Castello, S., Garcia-Luis, A., Guardiola, J.L. (2007). "Light cytokinin interactions in shoot formation in epicotyl cuttings of Troyer citrange cultured *in vitro"*. Plant Cell Tissue and Organ Culture 89: 131-140.

Murashige, T. and Skoog, F. (1962). "A revised medium for rapid growth and bio-assays with tobacco tissue cultures." Physiologia Plantarum. 15: 473–497

Paudyal, K.P. and Haq N. (2000). "*In vitro* propagatipon of Pummelo (*Citrus grandis*L. Qsbeck)."*In Vitro Cell. Dev. Biol. Plant* 36: 511–516.

Silva, R.P., Almeida W.A.B., Souza E.S. and Filho F.A.A.M. (2006). "*In vitro* organogenesis from adult tissue of 'Bahia' sweet orange (*Citrus sinensis*)."*Fruits* 61: 367–371.

Tallon, C.I., Porras, I., Perez-Tornero, O. (2013). "High efficiency *in vitro* organogenesis from mature tissue explants of *Citrus macrophylla* and *C. Aurantium"*. *In Vitro* Cellular and Developmental Biology – Plant. 49: 145-155.

Vleeshouwers, L.M.; Bouwmeester, H.J. and Karssen, C.M. (1995). "Redefining seed dormancy: an attempt to integrate physiology and ecology." Journal of Ecology. 83: 1031-1037.