

***In Vitro* Germplasm Conservation of
Garcinia mangostana L. and *Lansium domesticum* Corr.**

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ABSTRACT

The *in vitro* germplasm conservation of mangosteen (*Garcinia mangostana* L.) and longkong (*Lansium domesticum* Corr.) was investigated by suppression of shoot growth using growth retardants and increasing the osmotic potential of culture medium. Absciscic acid (ABA) at 2 mg/l affected to suppress shoot growth of mangosteen and longkong more than paclobutrazol. Although shoot growth of both plants was consequently inhibited for 12 months of conservation, the regrowth ability was successfully obtained when transferred to proliferation medium. Besides, increasing the concentration of agar up to 14 g/l was found to be more effective to suppress shoot growth of mangosteen and longkong by treatment of tissue desiccation. Severe succulence of mangosteen shoot was observed when shoots were cultured in a medium containing mannitol.

Keywords: Germplasm conservation, *Garcinia mangostana* L., *Lansium domesticum* Corr., growth retardants, tissue desiccation treatment

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) and longkong (*Lansium domesticum* Corr.) are the major fruit crops cultivated in the south of Thailand. Fruits of these species have superior gustatory qualities. Although, genetic variation of mangosteen and longkong are minimal and no cultivars have been reported [1-4], their germplasm should be conserved for the future breeding program.

At present, seed stores are the most popular germplasm conservation method, because it is easy for storage and management. However, there are large numbers of important tropical and sub-tropical fruits which produce recalcitrant seeds that quickly lose viability and cannot survive under desiccating conditions, hence conventional seed store strategies are not possible [5-7]. For this purpose, *in vitro* conservation would be the most convenient and reliable method compared to field conservation which requires much more space and labor for maintaining the collections that may experience damage due to natural disasters, pests and diseases [6,8,9]. Furthermore, tissue culture systems can propagate plant material with high multiplication rates in an aseptic environment without genetic variation and convenient for international exchange of germplasm [10]. *In vitro* conservation has already been applied to many species including tropical crops. Although several methods are employed, depending on the situation, the suppression of explant growth is essential for extending the duration of conservation. To suppress the growth rate of the plant *in vitro*, the addition of substances with osmotic properties such as mannitol can be used. As well as under field conditions, the growth of plant material could be reduced by the application of growth retardant under *in vitro* conditions [6-9,11].

This study was conducted to analyze the effects of growth retardants and tissue desiccation treatment on the growth suppression of mangosteen and longkong shoots to determine the appropriate conditions for *in vitro* conservation.

MATERIALS AND METHODS

Plant Material

Proliferation of mangosteen culture was established by aseptically cultured seeds on modified Murashige and Skoog (MMS) medium [12,13] supplemented with 5 mg/l 6-benzylamino purine (BA), 30 g/l sucrose and

8 g/l agar (pH 5.7). For longkong, seeds were sowed on MMS medium containing 5 mg/l BA, 1 mg/l indole-3-acetic acid (IAA), 30 g/l sucrose and 8 g/l agar (pH 5.7). Each seed was sowed in a 100 ml culture vessel containing 20 ml of culture medium, capped with a plastic cap. The proliferation cultures were incubated at 25 ± 2 °C and illuminated with 40 $\mu\text{mol}/\text{m}^2/\text{s}$ of cool white fluorescent tubes for 16 h/d of photoperiod.

Effects of ABA and Paclobutrazol on *In Vitro* Shoot Conservation of Mangosteen and Longkong

Shoot tips, 1 cm in length, were placed on MMS medium supplemented with 5 mg/l BA, 30 g/l sucrose and 8 g/l agar (pH 5.7). The culture medium was individually supplemented with abscisic acid (ABA) and β -[(4-chlorophenyl)methyl]- α -(1,1-dimethyl)-1H-1,2,4-triazole-1-ethanol (paclobutrazol) at 0, 0.5, 1.0, 1.5 and 2.0 mg/l. For the controls, shoot tips were placed on the proliferation medium. The shoot tips were cultured in a test tube (25 mm in inner diameter \times 150 mm in length) containing 15 ml of culture medium, capped with aluminum foil. One explant was cultured in each tube. The culture media were sterilized at 121 °C with pressure of 1.2 kg/cm² for 15 min. For medium containing ABA, the ABA was filter sterilized through 0.45 μm millipore filter before adding to the sterilized medium. All materials were cultured under the same conditions as those for the proliferation culture for 12 months. Each treatment contained 30 shoots. After 12 months of culture, the survival rate, shoot number, and growth characteristics of shoot were observed. The single shoot cuttings, 1 cm in length, were then excised from the conserved shoot clump and transferred onto a fresh proliferation medium depending on each species. Thereafter, the regrowth ability of each shoot was determined after a 2 months recovery period.

Effects of Agar Strength and Mannitol on *In Vitro* Shoot Conservation of Mangosteen and Longkong

Shoot tips, 1 cm long, were placed on MMS medium supplemented with 8, 10, 12, 14 g/l agar or 10, 20, 30, 40 g/l mannitol. In all the media, 5 mg/l BA and 30 g/l sucrose were supplemented. In the medium containing mannitol, 8 g/l agar was added. In the controls, the shoot tips were cultured on a proliferation medium for each species. The shoot tips were cultured in a test tube (25 mm in inner diameter \times 150 mm in length) containing 15 ml of culture medium, capped with aluminum foil. One explant was cultured in each tube. All culture media were

sterilized at 121 °C with pressure of 1.2 kg/cm² for 15 min. The materials were grown under the same conditions as those for the proliferation culture. Each treatment contained 30 shoots. After 12 months of culture, all explants from each treatment were evaluated for growth and recovery in the same manner as previously described.

RESULTS AND DISCUSSION

Effects of ABA and Paclobutrazol on *In Vitro* Shoot Conservation of Mangosteen and Longkong

The shoot growths of both species were suppressed by the application of ABA and paclobutrazol into the culture medium (**Tables 1 and 2**). In case of mangosteen, after 12 months of conservation, it was found that the controls had the highest viability at 99.33 %, while shoots cultured in the medium with ABA seemed to survive at higher rates than those cultured in the paclobutrazol medium (**Table 1**). Growth of shoots cultured in medium containing ABA and paclobutrazol was markedly suppressed when compared with those in controls (**Table 1 and Figure 1**). However, the new shoots produced during conservation in the treatment tested were not significantly different. The average shoot number ranged from 2.0 to 3.6 shoots (**Table 1**). After 2 months of culture, most of the shoots cultured in the ABA treatment swelled at the cut end of the shoot. The browning of the mangosteen shoot was observed in the shoots cultured with 2.0 mg/l paclobutrazol (**Figure 1**).

Table 1 Effects of ABA and paclobutrazol on *in vitro* growth of mangosteen (*Garcinia mangostana* L.) shoots.

treatment	concentration (mg/l)	after 12 months of conservation		after 2 months of recovery	
		viability (%)	average shoot number ^{1/}	viability (%)	average shoot number ^{1/}
control		99.33	5.7±3.20 b	100.00	7.4±6.07 ab
ABA	0.5	86.67	2.7±1.49 a	92.31	8.3±6.04 b
	1.0	71.43	2.3±1.70 a	62.50	2.0±2.35 a
	1.5	80.00	2.8±2.82 a	100.00	5.8±4.29 ab
	2.0	86.67	3.6±2.57 a	91.67	8.1±5.49 b
paclobutrazol	0.5	42.86	3.2±1.60 a	100.00	7.3±4.23 ab
	1.0	66.67	2.2±1.69 a	80.00	6.5±3.38 ab
	1.5	60.00	2.0±1.51 a	83.33	4.8±5.55 ab
	2.0	66.67	2.9±1.96 a	100.00	7.0±6.06 ab
F-test			*		*

^{1/}Data represented mean ± S.D. of shoot number (longer than 5 mm). The different letters within the same column showed significant difference at $p \leq 0.05$ analyzed by Duncan's Multiple Range Test (DMRT).

* = significant difference ($P < 0.05$)

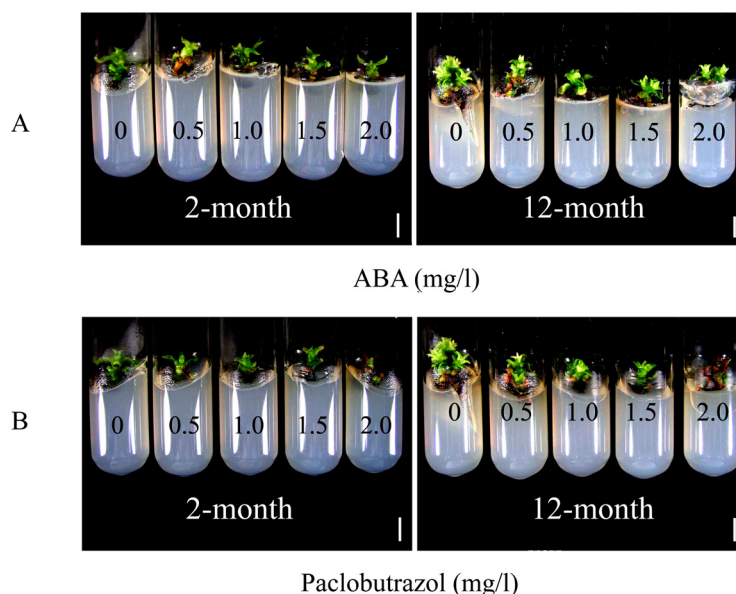


Figure 1 Characteristics of *in vitro* shoot growth of mangosteen (*Garcinia mangostana* L.) after 2 and 12 months of treatment in medium containing different concentrations of ABA (A) and paclobutrazol (B). (bar = 1 cm)

As for longkong, after conservation, most of the shoots survived at a higher rate than mangosteen (Tables 1 and 2). The results showed clearly that growth of shoots cultured with ABA and paclobutrazol was markedly suppressed when compared with controls. Although, there was no significant difference in suppressing shoot growth in all treatments tested, shoot growth was slightly reduced with the increasing of ABA and paclobutrazol concentrations. Most shoots swelled at the cut end and leaf fell after culture for 2 months (Table 2 and Figure 2).

Table 2 Effects of ABA and paclobutrazol on *in vitro* growth of longkong (*Lansium domesticum* Corr.) shoots.

treatment	concentration (mg/l)	after 12 months of conservation		after 2 months of recovery	
		viability (%)	average shoot number ^{1/}	viability (%)	average shoot number ^{1/}
control		100.00	2.4±0.51 b	100.00	2.2±0.60
ABA	0.5	100.00	1.4±0.74 a	100.00	2.2±0.80
	1.0	100.00	1.3±0.72 a	100.00	2.3±0.65
	1.5	100.00	1.2±0.56 a	85.71	2.0±1.04
	2.0	86.67	1.5±0.88 a	100.00	2.1±0.86
paclobutrazol	0.5	100.00	1.7±0.61 a	92.86	1.8±0.73
	1.0	85.71	1.5±0.80 a	80.00	2.0±0.93
	1.5	93.33	1.4±0.74 a	92.31	2.3±1.06
	2.0	100.00	1.1±0.52 a	87.50	1.6±0.79
F-test			*		ns

^{1/}Data represented mean ± S.D. of shoot number (longer than 5 mm). The different letters within the same column showed significant difference at $p \leq 0.05$ analyzed by Duncan's Multiple Range Test (DMRT).

ns = non-significant difference.

* = significant difference ($P < 0.05$)

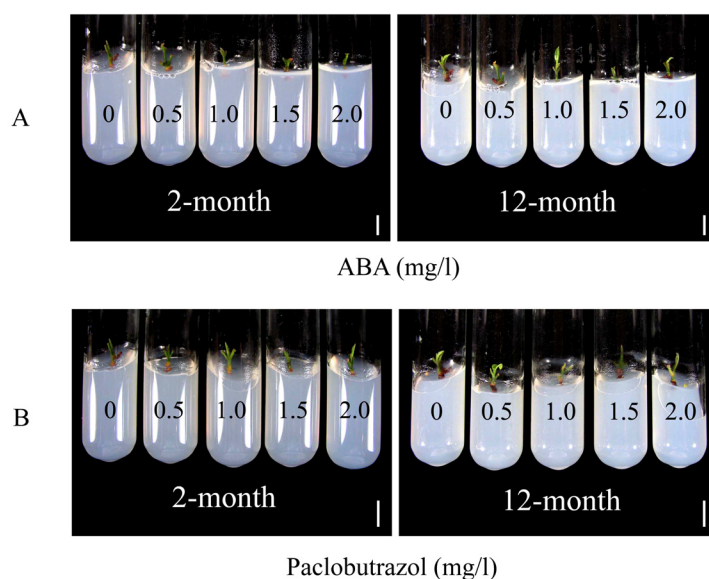


Figure 2 Characteristics of *in vitro* shoot growth of longkong (*Lansium domesticum* Corr.) after 2 and 12 months of treatment in medium containing different concentrations of ABA (A) and paclobutrazol (B). (bar = 1 cm)

After recovery growth for 2 months, although it was found that most mangosteen shoots could regrow and survive at high frequency rate ranging from 80 to 100 %, only shoots cultured in 1.0 mg/l ABA had the lowest survival rate at 62.50 % (**Table 1**). Large amounts of shoots were produced from shoots cultured in 0.5 and 2.0 mg/l ABA at 8.3 and 8.1 shoots, respectively (**Table 1**). New short multiple shoots with scaled leaves were observed in all treatments (**Figure 3**). On the other hand, longkong shoots cultured in control and ABA treatments had a higher survival rate in recovery growth than those cultured in paclobutrazol medium (**Table 2**). Although all treated shoots could produce on average 1.6 to 2.3 shoots, most shoots had fold leaves (**Table 2** and **Figure 4**).

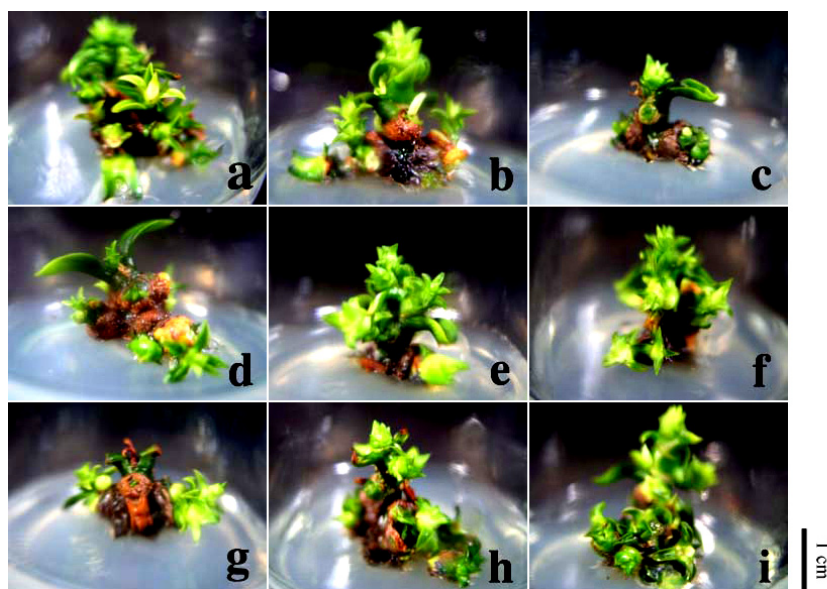


Figure 3 Growth characteristics of mangosteen (*Garcinia mangostana* L.) shoots after 2 months of recovery. The shoots were previously cultured on treatment of (a) control, (b) 0.5 mg/l ABA, (c) 1.0 mg/l ABA, (d) 1.5 mg/l ABA, (e) 2.0 mg/l ABA, (f) 0.5 mg/l paclobutrazol, (g) 1.0 mg/l paclobutrazol, (h) 1.5 mg/l paclobutrazol, and (i) 2.0 mg/l paclobutrazol.

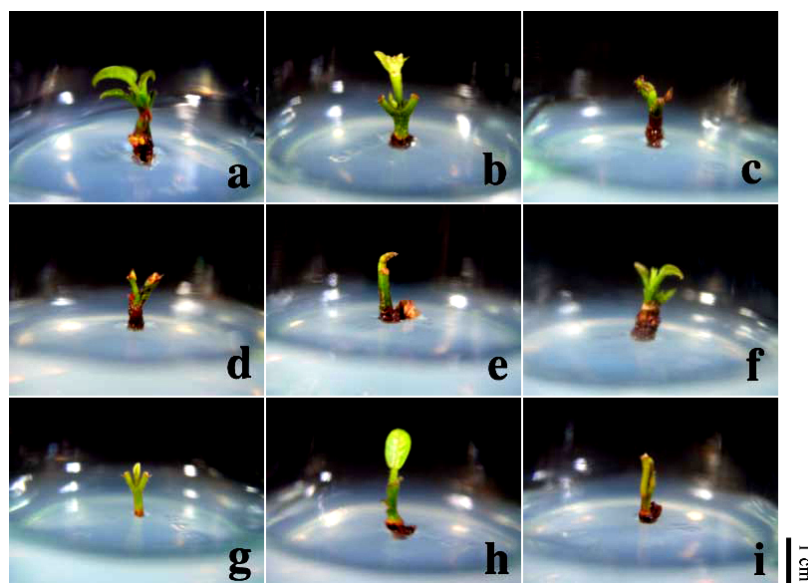


Figure 4 Growth characteristics of longkong (*Lansium domesticum* Corr.) shoots after 2 months of recovery. The shoots were previously cultured on treatment of (a) control, (b) 0.5 mg/l ABA, (c) 1.0 mg/l ABA, (d) 1.5 mg/l ABA, (e) 2.0 mg/l ABA, (f) 0.5 mg/l paclobutrazol, (g) 1.0 mg/l paclobutrazol, (h) 1.5 mg/l paclobutrazol, and (i) 2.0 mg/l paclobutrazol.

Effects of Agar Strength and Mannitol on *In Vitro* Shoot Conservation of Mangosteen and Longkong

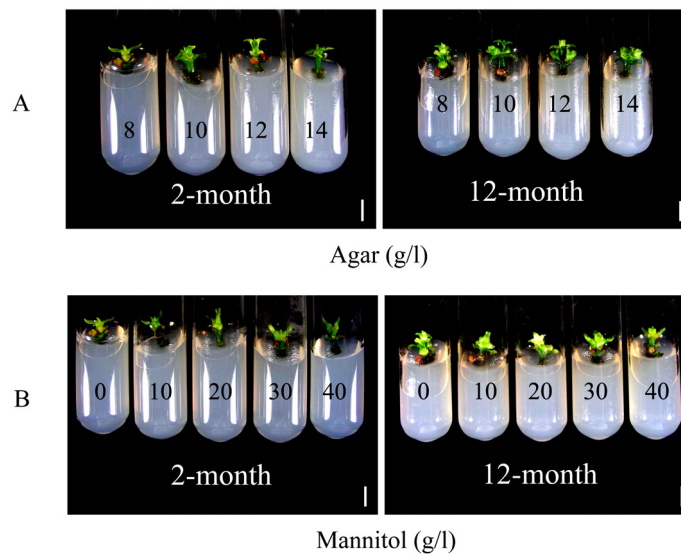
Increasing the concentration of mannitol was more effective for suppressing shoot growth of mangosteen than agar (**Table 3**). After 12 months of conservation, the shoots cultured on medium containing high concentration of agar were still deep green and swelling occurred at the cut end of the shoots. Although new shoots with only a few scaled leaves emerged, some leaves turned yellow and fell (**Figure 5**). The shoots had lowest viability at 40 % when cultured on medium containing 30 g/l mannitol (**Table 3**). Furthermore, severe succulence of tissues occurred in the shoots cultured on the medium containing mannitol (**Figure 5**).

Table 3 Effects of agar strength and mannitol on *in vitro* growth of mangosteen (*Garcinia mangostana* L.) shoots.

treatment	concentration (g/l)	after 12 months of conservation		after 2 months of recovery	
		viability (%)	average shoot number ^{1/}	viability (%)	average shoot number ^{1/}
agar	8	86.67	3.3±1.60 d	92.86	4.4±3.10 b
	10	93.33	1.6±0.93 ab	58.33	2.6±1.99 ab
	12	100.00	2.7±0.91 cd	100.00	4.1±1.87 ab
	14	100.00	1.5±0.64 ab	100.00	3.8±3.26 ab
mannitol	10	100.00	2.2±0.86 bc	91.67	3.6±1.69 ab
	20	80.00	1.7±0.78 ab	66.67	2.6±0.52 ab
	30	40.00	1.2±0.41 a	83.33	1.6±0.89 a
	40	86.67	1.1±0.28 a	40.00	1.5±1.00 a
F-test			*		*

^{1/}Data represented mean ± S.D. of shoot number (longer than 5 mm). The different letters within the same column showed significant difference at $p \leq 0.05$ analyzed by Duncan's Multiple Range Test (DMRT).

* = significant difference ($P < 0.05$)

**Figure 5** Characteristics of *in vitro* shoot growth of mangosteen (*Garcinia mangostana* L.) after 2 and 12 months of treatment in medium containing different concentrations of agar (A) and mannitol (B). (bar = 1 cm)

After 2 months of conservation, longkong shoots in medium containing high concentration of agar and mannitol swelled at the cut end and leaves fell were observed (**Figure 6**). Mannitol was more effective in growth suppression of longkong shoots than agar, even after 12 months of conservation (**Table 4** and **Figure 6**).

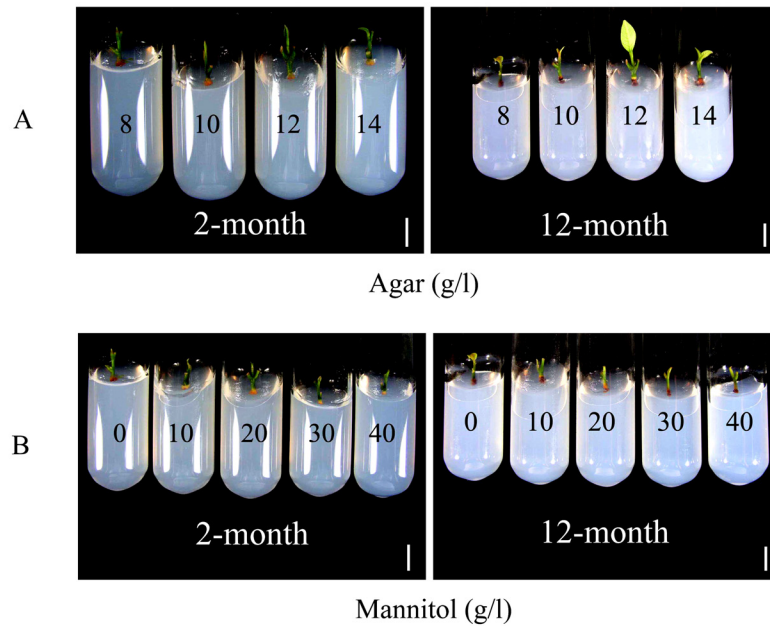


Figure 6 Characteristics of *in vitro* shoot growth of longkong (*Lansium domesticum* Corr.) after 2 and 12 months of treatment in medium containing different concentrations of agar (A) and mannitol (B). (bar = 1 cm)

Table 4 Effects of agar strength and mannitol on *in vitro* growth of longkong (*Lansium domesticum* Corr.) shoots.

treatment	concentration (g/l)	after 12 months of conservation		after 2 months of recovery	
		viability (%)	average shoot number ^{1/}	viability (%)	average shoot number ^{1/}
agar	8	100.00	1.3±0.46 ab	85.71	2.4±0.79 b
	10	85.71	1.6±1.16 bc	100.00	2.3±0.85 b
	12	100.00	1.6±0.65 bc	93.33	2.0±0.78 b
	14	100.00	1.9±0.64 c	93.33	2.1±0.66 b
mannitol	10	100.00	1.1±0.36 ab	100.00	1.3±0.47 a
	20	100.00	1.0±0.00 a	93.33	1.1±0.27 a
	30	100.00	1.1±0.35 ab	40.00	1.0±0.00 a
	40	100.00	1.0±0.00 a	64.29	1.1±0.33 a
F-test			*		*

^{1/}Data represented mean ± S.D. of shoot number (longer than 5 mm). The different letters within the column showed significant difference at $p \leq 0.05$ analyzed by Duncan's Multiple Range Test (DMRT).

* = significant difference ($P < 0.05$)

The regrowth of mangosteen shoots cultured on medium with agar and mannitol at concentrations lower than 30 g/l was as vigorous as that of the controls (**Table 3** and **Figure 7**). As for longkong, the results from recovery growth showed clearly that shoots cultured on medium containing high concentrations of agar had more growth with some small folded leaves than those cultured on medium with mannitol (**Table 4** and **Figure 8**).

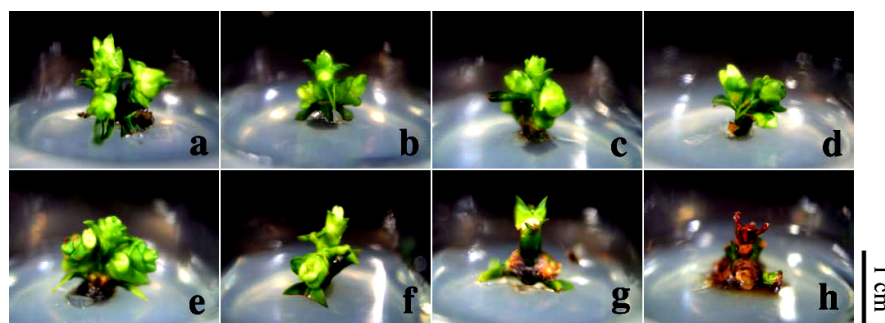


Figure 7 Growth characteristics of mangosteen (*Garcinia mangostana* L.) shoots after 2 months of recovery. The shoots were previously cultured on treatment of (a) 8 g/l agar, (b) 10 g/l agar, (c) 12 g/l agar, (d) 14 g/l agar, (e) 10 g/l mannitol, (f) 20 g/l mannitol, (g) 30 g/l mannitol, and (h) 40 g/l mannitol.

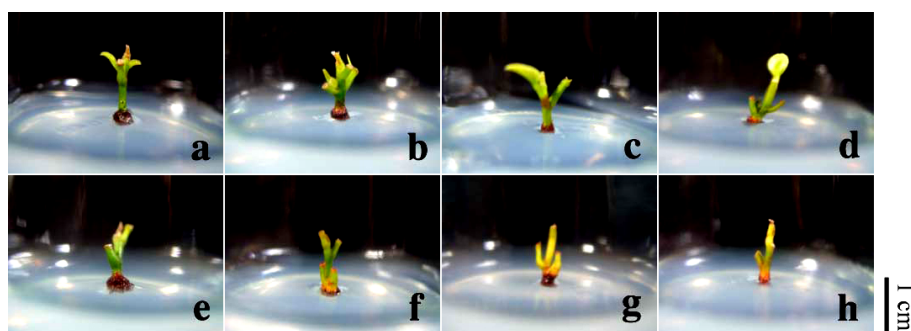


Figure 8. Growth characteristics of longkong (*Lansium domesticum* Corr.) shoots after 2 months of recovery. The shoots were previously cultured on treatment of (a) 8 g/l agar, (b) 10 g/l agar, (c) 12 g/l agar, (d) 14 g/l agar, (e) 10 g/l mannitol, (f) 20 g/l mannitol, (g) 30 g/l mannitol, and (h) 40 g/l mannitol.

Generally, ABA acts as an endogenous growth retardant. The important role of ABA is to inhibit auxins, cytokinins, and gibberellins. These hormones enhance growth, cell division and cell elongation in plants [14,15]. Some researchers have used it for growth reduction *in vitro*. Gopal et al [16] succeeded in conserving the nodal segments of potato (*Solanum tuberosum* L.) for over 18 months by the addition of 2.11 mg/l of ABA. Moreover, ABA is also involved in several other physiological processes of plants such as stomatal closure, embryo morphogenesis, development of seeds, synthesis of storage proteins and lipids, seed germination, and leaf senescence. Especially stomatal closure

causes the low metabolism energy used because stomatal closure influences the diffusion rate of CO₂ into stomata and water loss [17,18]. During culturing shoots with ABA, plants still engage in photosynthesis that causes the synthesis of storage proteins and lipids. These give rise to the best growth after recovery [19].

Paclobutrazol has been known as one of the most effective growth retardants for fruit crops in the field. It is assumed to retard the growth by interfering with gibberellic acid (GA) biosynthesis [20]. It suppresses growth by blocking three steps in the terpenoid pathway for production of GA by binding with and inhibiting the enzyme that catalyzes the metabolic reactions. When GA production is inhibited, cell division still occurs, but the new cells do not elongate. It results in compressing into a shorter length of shoot and internodes [21]. Paclobutrazol also induces morphological modifications of leaves such as smaller stomatal pores, thicker leaves and increased number and size of surface appendages on leaves [21]. Pateli et al [22] reported that the effect of increasing paclobutrazol concentration on *Epidendrum radicans* caused a small increase in leaf thickness by 17-37 %. At higher concentrations of paclobutrazol, it caused negative effects such as burned leaves and browning.

For the inhibition of growth by tissue desiccation treatment, sugar alcohols such as mannitol was added to the medium or supplemented medium with a high concentration of agar. Mannitol and high concentrations of agar increased the osmotic pressure in the culture medium that affected the movement of nutrients from the medium into the plant. Osmotic pressure in the plant is related to the diffusion of molecules through a permeable membrane from a region of higher solute concentration to a region of lower solute concentration. Fluid will enter the cell via osmosis until the osmotic potential is balanced. Adding osmoticum such as agar and mannitol into the medium causes an increase in osmotic pressure by decreasing the water potential in the medium. This causes water to move into the plant slowly because the medium has low water quantity for diffusion [23]. Reduction of the water potential in the cell causes a reduction of chemical activity of water and thereby modifies the structure of water in cells. It can cause a change in the structure of the sheath of hydration around proteins and thereby reduce their efficacy. This causes slow growth of the cultured shoot when osmoticums are added [24]. After the shoot recovery, shoots can grow and develop but the swelling of the shoot can be observed since stomas are closed.

CONCLUSIONS

The appropriate conditions for growth reduction of tissue-cultured shoots of mangosteen and longkong to prolong the conservation period were found. Supplements of ABA as a growth retardant to the medium, and increasing the osmotic potential by adding agar at high concentration were most effective for growth suppression and retention of the ability of regrowth after 12 months of conservation.

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บทคัดย่อ

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การเก็บรักษาเชื้อพันธุกรรมของมังคุด (*Garcinia mangostana* L.) และลองกอง (*Lansium domesticum* Corr.) ในสภาพปลอดเชื้อ

งานวิจัยนี้ทำการศึกษาวิธีการที่เหมาะสมในการเก็บรักษาเชื้อพันธุกรรมมังคุด (*Garcinia mangostana* L.) และลองกอง (*Lansium domesticum* Corr.) ในสภาพปลอดเชื้อด้วยการใช้สารชะลอการเจริญเติบโตและการเพิ่มแรงดันออสโมซิสของอาหาร กรดแอบไซซิก (ABA) มีประสิทธิภาพในการชะลอการเจริญเติบโตของยอดมังคุดและลองกองมากกว่าสารพาโคลบิวทราโซล หลังการเก็บรักษาปลายยอดของพืชทั้งสองชนิดในอาหารที่เติม ABA เข้มข้น 2.0 มิลลิกรัมต่อลิตรเป็นเวลานาน 12 เดือน พบว่าการเจริญของปลายยอดถูกยับยั้ง แต่การเจริญที่เกิดขึ้นใหม่หลังการเก็บรักษายังคงอยู่ในระดับที่น่าพอใจ นอกจากนี้ การเพิ่มความเข้มข้นของวุ้นสูงถึง 14 กรัมต่อลิตรสามารถลดการเจริญของปลายยอดมังคุดและลองกองได้อย่างมีประสิทธิภาพ โดยพบอาการผิดปกติของปลายยอดมังคุดในลักษณะบวมน้ำเมื่อทำการเก็บรักษาปลายยอดในอาหารที่เติมแมนนิทอล

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