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Study on Type and Concentration of Plant Growth Regulator on Shoot Development of Pummelo [*Citrus maxima* (Burm.) Merr.] cv. Taptimsiam

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Pummelo [*Citrus maxima* (Burm.) Merr.] cv. Taptimsiam, economic fruit crops, is geographical indication of Nakorn Sri Thammarat province. It is most favorite pummelo because it's red pulp, the taste so sweet and soft. They are export and the premium for souvenir from Nakorn Sri Thammarat province. However, they had problem in Citrus tristeza virus and Greening. The present study was to produce the new plant that without this disease by apical meristematic tissue. Shoot explant were excised and cultured on solid Murashige and Skoog (MS) medium supplemented with 0-1 mg/l NAA (α-naphthaleneacetic acid) and 0-2 mg/l BA (N_6-benzyladenine). The cultures were placed under light conditions at 14 h photoperiod, 27±1 ^{o}C to initiate callus induction and plant regeneration for 3 months. The result revealed that solid MS medium supplemented with 1.5 mg/l BA gave the average number of shoot at 7.83/explant better than another culture media. Solid MS medium supplemented with 1 mg/l BA gave the highest average shoot length at 5.2 centimeter, significant different with another culture media. This technique can sole the farmer in the further.

Key words: Citrus maxima (Burm.) Merr, plant growth regulator, shoot

Introduction

Pomelo is a fruit tree. It can be grown all over Thailand that a fruit with good taste, high yield, and high nutritional value. Especially pomelo cv. Tubtimsiam is a grapefruit with dark red color, soft and sweet. They are large fruit, diameter about 16-22 inches, the head of the skin, the soft, soft hairs and delicious. The colors are unique that it's a proud symbol of Pak Phanang district and people in Nakhon Si Thammarat. The sale price of 300 baht per fruit (fresh weight about 1.7 kg), the farmers over there interesting attention to the planting them. In addition, relevant government agencies have been promoting the knowledge to develop, grow, and harvest the produce in good quality, as well as to develop the product to a unique area

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and to enter into quality standards such as GAP certification under the plant food safety program. The selection is OTOP products 5 stars until the copyright. GI (geographical indication) in the name of "Pomelo cv. Tubtimsaim in Pak Phanang district will bloom around February to March and it will take about eight months (about August to September) will provide mature fruit and ready to sell. It can be stored for a long time because of the thick shell. Farmers can grow for consumption or trade, but most are grown for trade because of the good price and can be purchased as a deposit. The peel of grapefruit can also be processed into peel grapefruit and peel of grapefruit as well. Based on the crop data in 2014, there are 1,430 rais of cultivated land, yield about 595,870 fruit as well 199 million. Grapefruit propagation can be done by seed, but will somaclonal variation and slow yield. The farmers turned to propagate by layering, whereas the propagation by layering is not sure that the stem will come from the good plant because the trees are high yields and good quality is don't sell their branches because they want to sell their produce. Most importantly, branches of layering are often affected by disease such as tristeza and greening disease (Pongpattanabut and Sdoodee, 2012; Teixeira *et al*., 2008). The disease can be transmitted by budding and layering propagation (Jagoueix *et al*., 1994; Shokrollah *et al*., 2011), which causes damage of the citrus family. If the farmers have propagation with the diseases branches that is the wrong way to many areas. They are made lower yields and lower quality (Roistacher, 1991). Therefore, to solve this problem we make the new clone of pomelo cv. Tubtimsaim without diseases. Plant propagation with biotechnology by tissue culture is a way to solve the problem of producing disease-free plants (Tegen and Mohammed, 2016). The focus is on the use of biotechnology for the propagation of pomelo using by apical meristem.

Materials and methods

Shoot tips of Pummelo [*Citrus maxima* (Burm.) Merr.] cv. Taptimsiam were washed under running tap water for 10 minutes and they were rinsed with 70% alcohol for 30 sec, followed by 20% (w/v) sodium hypochlorite together with 1-2 drops of Tween-20 for further 20 min, 10% (w/v) sodium hypochlorite together with 1-2 drops of Tween-20 for further 5 min. The final step of sterilization was carried out in a laminar air flow chamber by rinsing the plant material 3 times in sterile distilled water. Shoot explants (consisted of apical meristimatic tissue) were excised with sterile scalpel blade, and then they were inoculated on 15 types of culture media for callus and shoot induction. The media were consisted of solid MS medium supplemented with 0-2 mg/l BA and NAA, 3% (w/v) sucrose. The pH was adjusted to 5.7 with 1 N NaOH or HCl and autoclaved at 121 ◦C with 15 p.s.i. (1.04 kg cmi^2) pressure for 15 min. The cultures were

maintained at 27±1°C under a 14-h photoperiod of 50 µmol m^{-2} s^{-1} irradiance provided by cool white fluorescent. Completely randomized design with 10 replicates (each replicate consists of 1 apical meristimatic tissue) was performed. Callus induction and shoot induction were recorded every month. Data were analysed by ANOVA. Means were separated with Duncan's multiple range tests (DMRT) at the 0.01 of probability. The F-test showed significant differences among means.

Results and Discussion

Different culture media gave the different response on the number of cultures producing shoot and shoot lenght. Shoot tips were developed to callus initiation after culture on shoot induction medium for 1 week (Figure 1). However, some of Shoot tips did not respond to calluses but developed to shoot after culture for 2 weeks (Fig. 1). Solid MS medium supplemented with 1.5 mg/l BA gave the highest average of shoot 7.83 shoot/explant, followed by MS medium supplemented with 0.5 mg/l NAA and 1.5 mg/l BA and MS medium supplemented with 1.0 mg/l NAA and 1.5 mg/l BA gave the average of shoot at 6.43 and 6.03 shoot/explant respectively, a difference that was statistically significant ($p \leq 0.01$) with other culture media. Whereas MS medium without plant growth regulator, MS medium supplemented with 0.5 mg/l NAA and MS medium supplemented with 1.0 mg/l NAA have least of shoot than another culture media. Considering explant in different month found that solid MS medium supplemented with 1.5 mg/l BA gave the highest number of shoot all month at 4.5, 6.5 and 12.5 shoots, respectively (Table 1, Figure 2). The difference was statistically significantly greater ($p \leq 0.01$) with another concentration of plant growth regulator.

In case of shoot length were culturing on different culture media for 3 months. The result showed that solid MS medium supplemented with BA 1.0 mg/l gave the highest average of shoot length was 5.2 cm (Table 2), followed by solid MS medium supplemented with 0.5 mg/l NAA with BA concentrations of 0.5 and 2 mg/l gave the average shoot length was 5.0 cm significantly different ($p \leq 0.01$) with the other culture media (Table 2, Fig 3).

Cytokinins and auxins are the major phytohormones regulating plant growth and development. Cytokinin is a classic phytohormone involved in cell division, growth, and organogenesis. Auxin, like cytokinin, is involved in a myriad of developmental and environmental processes; embryo patterning, cell division and elongation, vascular differentiation, lateral root initiation, gravitropism, and phototropism (Tanaka *et al.*, 2006; Berleth and Sachs, 2001). However, auxin and cytokinin ratio have the affect effecting on development of plant tissue that developed to new plantlet. The optimal ratio will make the tissue developing to perfect plantlet. Singh *et al*. (1994) reported that *Citrus reticulata* Blanco and *Citrus limon* Burm.f. from a 2-3 mm shoot tip and placed on MS supplemented with 1.0 mg/l BAP and each of kinetin and NAA at 0.50 mg/l.

Sweet orang gave the highest number of shoot at 6.1 shoots/explant, shoot length 2.6 cm and a number of leaves 2.4 leaf/explant and lemon gave yielding a crest of 6.7 shoots/explant, shoot length 2.5 cm and leaf number of 2.6 leaf/explant. Different genotype gave the different responses on development of citrus family.

Table 1 Effect of MS medium supplemented with different concentration of NAA and BA on Shoot number of Pummelo [*Citrus maxima* (Burm.) Merr.] cv. Taptimsiam after culturing for 3 months.

Culture medium	**No. of shoots/plant**			**average**
	1 month	2 month	3 month	
1. MS free	1.40^{g}	1.40^{d}	1.40^{d}	1.40^{c}
2. MS + BA 0.5 mg/l	2.60de	3.00^{c}	4.80^{g}	3.47^{abc}
3. MS + BA 1 mg/l	3.40^{bc}	4.30^{b}	7.00^{dE}	4.90^{abc}
4. MS + BA 1.5 mg/l	4.50^{a}	6.50^{a}	12.50^{a}	7.83^{a}
5. MS + BA 2 mg/l	2.90^{cd}	4.60^{b}	9.00^{c}	5.50^{abc}
6. MS + NAA 0.5 mg/l	1.50^{g}	1.50^{d}	2.00^{h}	1.67^{bc}
7. MS + NAA 0.5 mg/l + BA 0.5 mg/l	2.70^{de}	4.30^{b}	7.80^{d}	4.93^{abc}
8. MS + NAA 0.5 mg/l + BA 1 mg/l	3.50^{bc}	4.40^{b}	6.30^{ef}	4.73^{abc}
9. MS + NAA 0.5 mg/l + BA 1.5 mg/l	3.90^{b}	4.70^{b}	10.70^{b}	6.43^{ab}
10. MS + NAA 0.5 mg/l + BA 2 mg/l	3.70^{b}	4.30^{b}	6.30^{gf}	4.77^{abc}
11. MS + NAA 1 mg/l	1.70^{fg}	1.70^{d}	1.70^{h}	1.70^{bc}
12. MS + NAA 1 mg/l + BA 0.5 mg/l	2.20^{ef}	3.40^{c}	6.00^{f}	3.87^{abc}
13. MS + NAA 1 mg/l + BA 1 mg/l	2.50^{de}	3.50^{c}	6.10^{ef}	4.03^{abc}
14. MS + NAA 1 mg/l + BA 1.5 mg/l	2.70^{de}	4.20^{b}	11.20^{b}	6.03^{abc}
15. MS + NAA 1 mg/l + BA 2 mg/l	2.70^{de}	3.60^{c}	7.60^{d}	4.63^{abc}
F-test	**	**	**	**
C.V. (%)	17.72	13.50	11.31	16.17

** Significant differences at $P \leq 0.01$

Means values followed by the same letter within each column are not significantly different according to DMRT.

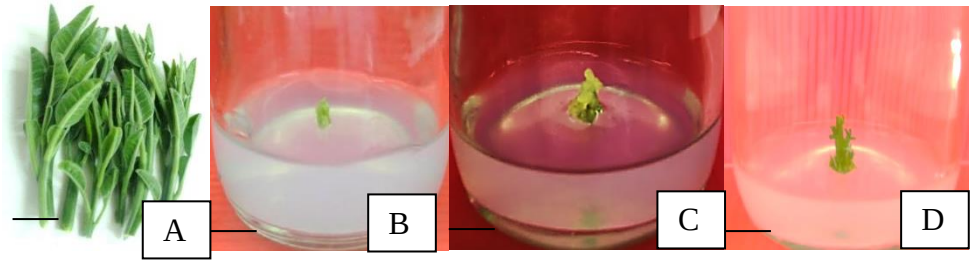

Figure 1 Shoot explant of Pummelo [*Citrus maxima* (Burm.) Merr.] cv. Taptimsiam (A) were surface sterilize after cultured on culture media for one week (B) and three months became to calluses (C) and shoot (D) (bar = 1.0 cm).

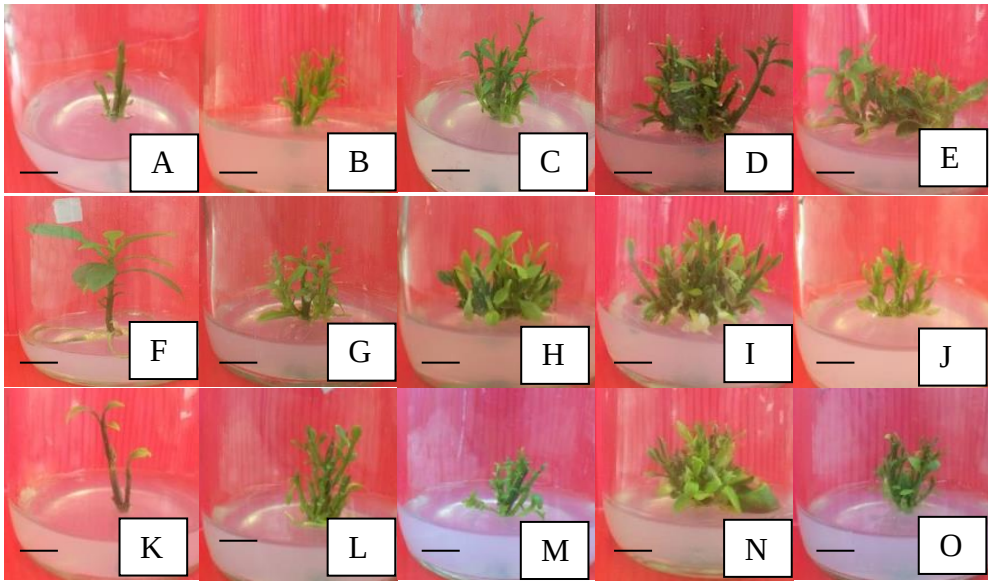

Figure 2 Shoot characteristics of Pummelo [*Citrus maxima* (Burm.) Merr.] cv. Taptimsiam after culturing on MS medium supplemented with different plant growth regulator for 3 months (bar = 0.5 cm).

A : MS free.
B : MS medium supplemented with 0.5 mg/l BA.
C : MS medium supplemented with 1 mg/l BA.
D : MS medium supplemented with 1.5 mg/l BA.
E : MS medium supplemented with 2 mg/l BA.
F : MS medium supplemented with 0.5 mg/l NAA.
G : MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA.
H : MS medium supplemented with 0.5 mg/l NAA and 1 mg/l BA.
I : MS medium supplemented with 0.5 mg/l NAA and 1.5 mg/l BA.
J : MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA.
K : MS medium supplemented with 1 mg/l NAA.
L : MS medium supplemented with 1 mg/l NAA and 0.5 mg/l BA.
M : MS medium supplemented with 1 mg/l NAA and 1 mg/l BA.
N: MS medium supplemented with 1 mg/l NAA and 1.5 mg/l BA.
O : MS medium supplemented with 1 mg/l NAA and 2 mg/l BA.

Table 2 Effect of MS medium supplemented with different concentration of NAA and BA on Shoot length of Pummelo [*Citrus maxima* (Burm.) Merr.] cv. Taptimsiam after culturing for 3 months.

Culture medium	Shoot length (cm.)
1. MS free	4.8[d]
2. MS + BA 0.5 mg/l	3.8[j]
3. MS + BA 1 mg/l	5.2[a]
4. MS + BA 1.5 mg/l	4.9[c]
5. MS + BA 2 mg/l	4.0[h]
6. MS + NAA 0.5 mg/l	4.1[g]
7. MS + NAA 0.5 mg/l + BA 0.5 mg/l	5.0[b]
8. MS + NAA 0.5 mg/l + BA 1 mg/l	3.9[i]
9. MS + NAA 0.5 mg/l + BA 1.5 mg/l	4.4[f]
10. MS + NAA 0.5 mg/l + BA 2 mg/l	5.0[b]
11. MS + NAA 1 mg/l	3.7[k]
12. MS + NAA 1 mg/l + BA 0.5 mg/l	4.8[d]
13. MS + NAA 1 mg/l + BA 1 mg/l	3.2[l]
14. MS + NAA 1 mg/l + BA 1.5 mg/l	4.5[e]
15. MS + NAA 1 mg/l + BA 2 mg/l	3.0[m]
F-test	**
C.V. (%)	5.67

** Significant differences at $P \leq 0.01$

Means values followed by the same letter within each column are not significantly different according to DMRT.

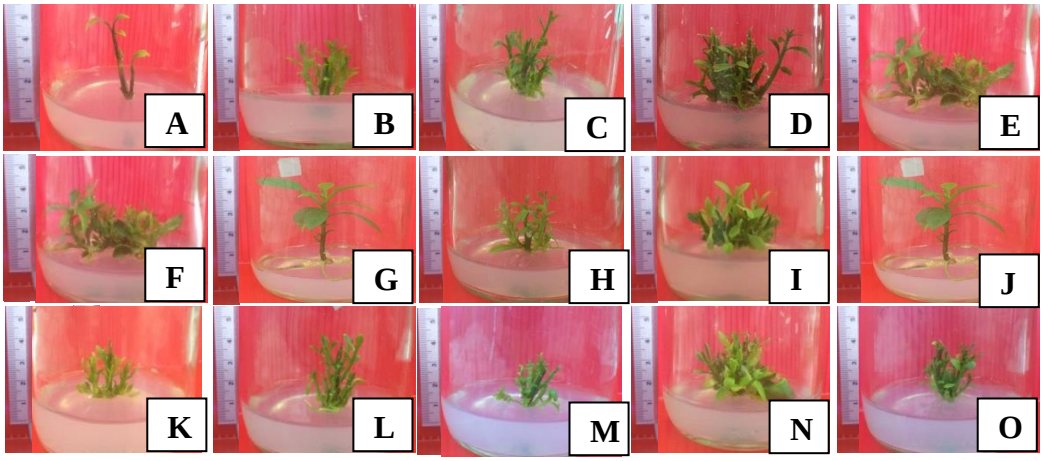

Figure 3 Shoot length of Pummelo [*Citrus maxima* (Burm.) Merr.] cv. Taptimsiam after culturing on MS medium supplemented with different plant growth regulator for 3 months.

A : MS free.

B : MS medium supplemented with 0.5 mg/l BA.

C : MS medium supplemented with 1 mg/l BA.

D : MS medium supplemented with 1.5 mg/l BA.

E : MS medium supplemented with 2 mg/l BA.

F : MS medium supplemented with 0.5 mg/l NAA.

G : MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA.

H : MS medium supplemented with 0.5 mg/l NAA and 1 mg/l BA.
I : MS medium supplemented with 0.5 mg/l NAA and 1.5 mg/l BA.
J : MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA.
K : MS medium supplemented with 1 mg/l NAA.
L : MS medium supplemented with 1 mg/l NAA and 0.5 mg/l BA.
M : MS medium supplemented with 1 mg/l NAA and 1 mg/l BA.
N: MS medium supplemented with 1 mg/l NAA and 1.5 mg/l BA.
O : MS medium supplemented with 1 mg/l NAA and 2 mg/l BA.

Conclusion

Shoot tip were cultured on solid MS medium supplemented with 1.5 mg/l BA gave the average number of shoot at 7.83/explant and Solid MS medium supplemented with 1 mg/l BA gave the highest average shoot length at 5.2 centimeter.

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