# *IN VITRO* REGENERATION OF PUMMELO CV. CIKONENG FROM COTYLEDON AND EPICOTYL

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## ABSTRAK

Penelitian ini dilakukan untuk mendapatkan protokol regenerasi Pomelo yang optimum dari eksplan kotiledon dan epikotil. Percobaan disusun secara faktorial dengan dua faktor dan diulang 20 kali. Faktor pertama adalah tipe eksplan, yaitu potongan kotiledon dan epikotil pamelo cv. Cikoneng, sedangkan faktor kedua adalah formulasi media. Media yang digunakan: (1). MS + 1,0 mg BAP/l + 0,5 mg K/l + 0,5 mg NAA/l; (2). MS + 2,0 mg BAP/l + 0,5 mg K/l + 0,5 mg NAA/l; (3). MS + 1,0 mg BAP/l + 1,0 mg K/l + 0,5 mg NAA/l; (4). MS + 2,0 mg BAP/l + 0,5 mg K/l + 1,0 mg NAA/l; (5). MS + 2,0 mg BAP/l + 1,0 mg K/l + 1,0 mg NAA/l. Empat kotiledon (1,0-1,5 cm) akan diletakkan secara abaksial dan 5 potongan epikotil (1,0 cm) diletakkan horizontal di media. Pengamatan dilakukan terhadap inisiasi tunas (hari), banyaknya eksplan yang membentuk tunas, tinggi tunas, banyaknya tunas, daun dan akar. Hasil menunjukkan bahwa perlakuan kotiledon dan media 1 atau 3 memberikan jumlah tunas, daun dan akar yang tertinggi. Kombinasi perlakuan tersebut merupakan perlakuan yang paling cepat memberikan respon untuk inisiasi tunas. Dianjurkan untuk perbanyakan pamelo dilakukan dengan eksplan kotiledon dan media 1.

Kata Kunci: regenerasi, kotiledon, epikotil, pamelo, *Citrus maxima*

## *ABSTRACT*

*The objective of the present research was to obtain an efficient and reproducible regeneration protocol from cotyledon and epicotyl. Experiment was arranged in a factorial design with two factors, replicated 20 times. The first factor was type of explant, i.e. cotyledon and epicotyl segments of Pummelo cv. Cikoneng, while the second factor was the media. The media were as follows: (1). MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l; (2). MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l; (3). MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l; (4). MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l; (5). MS + 2.0 mg BAP/l + 1.0 mg K/l + 1.0 mg NAA/l. Four cotyledons (1.0-1.5 cm) were placed abaxially and 5 epicotyl segments (1.0 cm) were placed horizontally on the medium in each culture jar. Observation was made on number of days to induce shoot, number of explant forming shoots, shoot height, number of shoots, leaves, and roots. Result of the study showed that treatment of cotyledon explant culture in media 1 or media 3 gave the highest number of adventive shoots, number of leaves, and number of roots. Those combinations of treatment were the fastest in shoot initiation. It is suggested that cotyledon and media 1 is used for pummelo propagation.*

*Keywords: regeneration, cotyledon, epicotyl, pummelo, Citrus maxima*

## INTRODUCTION

Plant genetic resources are rapidly vanishing due to deprivation of habitat or selective reduction, leading to genetic vulnerability endangering the plant industry and future needs. Among the fruit crops, the genus Citrus and its relatives are important. Pummelo, the largest fruit size among citrus, is an under-utilized fruit with a potential for commercialization. The pummelo originated most likely from Indonesia where it grew wild (Niyomdham, 2003). It is also presumed to have been grown by the Chinese for thousands of years (Min, 1997). However, most believed that its primary centre of diversity is Southeast Asia (Malaysia, Thailand, Indonesia, the Philippine) from where it spreads to China, the Indian subcontinent and to Iran (Paudyal and Haq, 2008).

In Indonesia, most of the pummelo are grown in homestead gardens or farmer field and only several cultivars are conserved ex-situ in the field gene bank such as in Botanical Garden and Research Institute for Citrus and Other Tropical Fruits (Balitjestro), Malang. Such collections are vulnerable to biotic and abiotic hazards. For example three commercial pummelos, i.e. cultivar Nambangan from Magetan, Bali Jingga from Pati and Cikoneng from Sumedang were almost extinct in 1980s, because of the outbreak of CVPD (Citrus Vein Phloem Degeneration) and Botryodiplodia theobromae (Source: Kompas, Mei 19, 2003). In the end of 1990s, cultivar Cikoneng could be restored from two healthy plants grown in the forest, while cultivar Bali Jingga could be restored from the only old plant left in the garden. Therefore, there is an urgent need to seek for other alternative ex-situ conservation of the pummelo germplasm. So far, effort of conservation ex-situ is also hampered by the availability of infrastructure, human resource and funding.

Germplasm conservation can cover a range of work including exploration, collection, characterization, maintenance, and rejuvenation of particular group of both cultivated and wild relative of plants. It is a very important basis of breeding material for specific objective. Therefore, it must be one of the main focuses in supporting agriculture development as well as maintaining the existence of genetic variability for future needs.

Tissue culture can be of great interest for conservation and multiplication of recalcitrant and vegetatively propagated species such as pummelo. It presents advantages as follow: (1) very high multiplication rates; (2) aseptic system: free from fungi, bacteria, viruses (after thermotherapy and indexing) and insect pests, production of pathogen-free stocks; (3) reduction of space requirements; (4) reduced genetic erosion to zero under optimal maintenance; (5) reduction of the expenses in labour costs. Moreover, tissue culture systems greatly facilitate exchange of germplasm, because the size of the sample is reduced and the germplasm is shipped in sterile conditions (Withers, 1989). *In vitro* conservation and cryopreservation offer alternative strategies for medium and long-term storage of germplasm. However, *in vitro* conservation requires high effiency in micropropagation, preservation of genetic information, maximum survival rate, and reduction of subculturing frequency (Malaurie et al., 1998). Increasing the subculture period to every 6 or 12 months saves money and reduces the contamination rate and the accidental mutation of genotypes. To support *in vitro* conservation, technique to propagate pummelo *in vitro* is needed. The objective of the present study was to obtain an efficient and reproducible regeneration protocol from cotyledon and epicotyl.

## MATERIALS AND METHODS

This research was conducted at ICABIOGRAD. Seeds of pummelo cv. Cikoneng were obtained from Orchard of H. Soom in Sumedang, West Java. Experiment was arranged in a factorial design with two factors, replicated 20 times. The first factor was type of explant, i.e. cotyledon and epicotyl segments (1.5 to 2.0 mm thick) of Pummelo cv. Cikoneng, and the second factor was the media. The media were as follows: (1). MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l; (2). MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l; (3). MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l; (4). MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l; (5). MS + 2.0 mg BAP/l + 1.0 mg

K/l + 1.0 mg NAA/l. Four cotyledons (1.0-1.5 cm) were placed abaxially and 5 epicotyl segments (1.0 cm) were placed horizontally (Begum *et al.*, 2003; Garcia-Luis *et al.*, 1999) on the medium in each culture jar. The culture medium was based on the inorganic salts of Murashige and Skoog (1962) supplemented with myo-inositol 100 mg/l, thiamine-HCl 10 mg/l, 10 mg/l pyridoxine, nicotinic acid 1 mg/l and sucrose 30 g/l . The pH of the medium was adjusted to 5.8 before the addition of 8 g/l agar, and the mixture was then autoclaved at 18-20 Psi at 120 $^{O}$C for 20 minutes. The medium was dispensed as 40 ml aliquots into culture jars (baby jar). All cultures were incubated in the dark at 22±2 $^{O}$C until the explant showed any formation of nodules as an indication of shoot initiation. The culture then was incubated under a 16 hours day length, with an irradiance provided by cool white fluorescent tubes at a temperature of 26±2 °C. Shoots with leaf were transferred to full MS media and if necessary it would be moved to MS + malt extract 500 mg/l + NAA 10 mg/l + 100 mg/l activated charcoal to root.

Observation was made on number of days to induce shoot, number of explant forming shoots, number of shoots, shoot height, and number of leaves, and number of roots.

## RESULTS AND DISCUSSION

### Adventive Shoot Formation

During shoot initiation, change of color and size occured on cotyledon of pummelo cv. Cikoneng in all media (Figure 1). According to Hopkins (2004) each cell has different differentiation potential. Cotyledon enlarged and its color changed from yellow to green 1 week after planting (WAP). Salisbury and Ross (1995) stated that process of cotyledon enlargement was caused by water uptake eventhough dry weight did not increase. Growth of cotyledon was promoted by cytokinin and later the cotyledon became photosynthetic organ and changed its color to green.

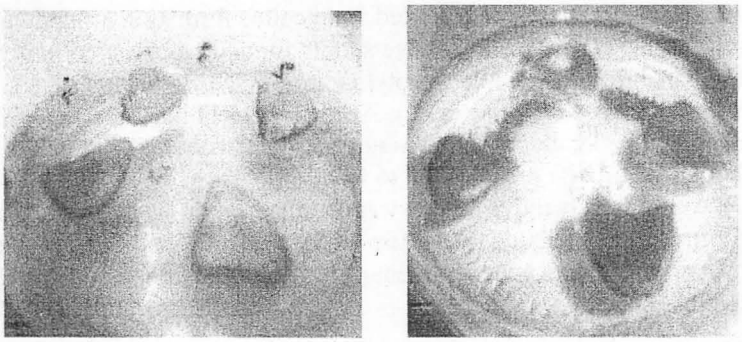
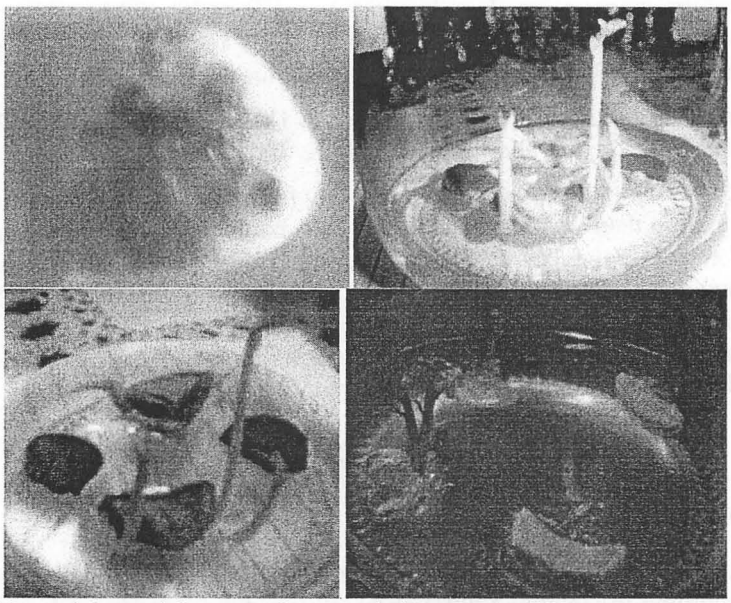


Figure 1. Size and color change of pummelo cv. Cikoneng cotyledon

Adventive shoot initiation appeared as nodules or protrution in greening cotyledons which later became shoots (Figure 2, upper left). The time needed to form adventive shoots ranged between 4-5 WAP in all media. Adventive shoots grew from cotyledon-cut side (Figure 2, upper right and bottom). Cotyledon position also influenced growth direction or shoot polarity. In previous study, when inner side of the cotyledon was positioned in contanct with media (adaxial position), shoot appeared toward the media and later grew upward. The earliest shoot was formed in media 1, i.e. MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l and media 3, i.e MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l. First leaf grew between 6-8 MST. In every leaf's growth, it was followed by height increase of the plantlet. In this experiment, we also observed cotyledon which changed its

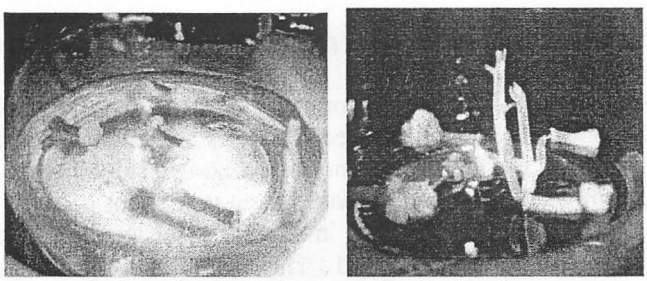
color to pale yellow (Figure 2, lower right). Later, the cotyledon changed to brown, became smaller and dead.



Upper left= nodules grew from cotyledon, Upper right= shoot formed, Bottom left= shoot formed, leaf started to green, Bottom right= Cotyledon with multiple shoots or without shoot (yellowing).

Figure 2. Adventive shoot formation from cotyledon explants of Cikoneng pummelo.

Adventive shoot initiation on epicotyl started from callus formation around cutting position of epicotyl segments (Figure 3). Then, the adventive shoots formed from the callus. Similar pathway was reported by Saini dan Jaiwal (2002) in epicotyl culture of *Vigna mungo* where adventive shoot was formed from callus (*indirect organogenesis*). According to Filho (2001) callus type formed at the epicotyl segments was type I callus which was not friable and could differentiate directly to form special organ like shoot or leaf. The time needed to form shoot ranged between 5-8 WAP. Similar to cotyledon explants, the earliest adventive shoots were formed in media 1 and media 3. Appearance of adventive shoots formed from epicotyl was different from that formed from cotyledon (Figure 2). Adventive shoots from epicotyls looked transparent or vitrous and their growth was slower than that obtained from cotyledons (Figure 3).



Left= callus formed in two ends of epicotyl segments
Right= shoots showing transparent appearance

Figure 3. Formation of adventive shoots from epicotyl of pummelo cv. Cikoneng

Not all cultured explants yielded adventive shoots. The number of adventive shoots obtained from each segment of cotyledon and epicotyl ranged between 1-5 shoots. Percentage of explants forming adventive shoots at 7 WAP in all media is presented in Table 1.

Table 1. Percentage of explant forming adventive shoots of pummelo cv. Cikoneng at 7 WAP

| Treatment | Explant forming adventive shoots (%) | |
|---|---|---|
| Cotyledon + media 1 | 38.75 | Note: |
| Cotyledon + media 2 | 8.75 | 1= MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l |
| Cotyledon + media 3 | 36.25 | 2= MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l |
| Cotyledon + media 4 | 7.50 | 3= MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l |
| Cotyledon + media 5 | 8.75 | 4= MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l |
| Epicotyl + media 1 | 7.00 | 5= MS + 2.0 mg BAP/l + 1.0 mg K/l + 1.0 mg NAA/l |
| Epicotyl + media 2 | 8.00 | |
| Epicotyl + media 3 | 11.00 | |
| Epicotyl + media 4 | 4.00 | |
| Epicotyl + media 5 | 4.00 | |

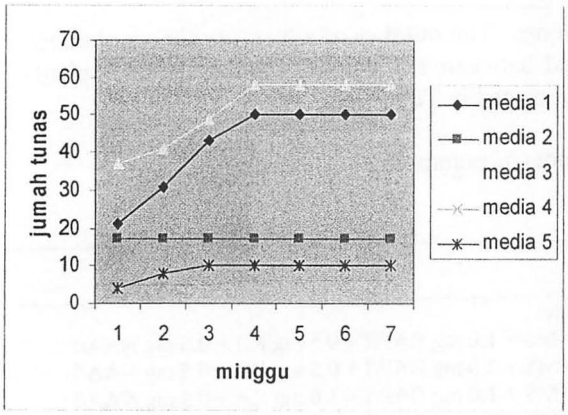
The highest percentage of explant forming adventive shoots was reached by cotyledon in media 1 (38.75%), eventhough it was similar to cotyledon in media 3 (Table 1). The lowest percentage of explant forming adventive shoots was reached by epicotyl in media 4 and media 5 (4.00%).

Percentage of adventive shoot to number of explant is presented in Table 2. This showed the efficiency of shoot formation per explant cultured. The number of shoot was counted from both normal and abnormal shoots at 7 WAP. The highest efficiency was obtained on cotyledon cultured in media 3 (72.50%), while the lowest was obtained on epicotyl in media 5 (5.00%).

Adventive shoot initiation occured in the first 4 weeks (Figure 4). After 4 WAP, cotyledon did not form shoot. Up to 7 WAP observations, there was some shoot growth, however they were abnormal. The shoot stopped growing and finally dried out. In epicotyl explant, the number of shoot formed was lower than that in cotyledon (Figure 5). The shoots also grew slower. The highest percentage of shoot to number of explant in epicotyl explant was obtained in media 3, i.e. 15% (Table 2). This might be due to the explant size. The larger the explant size gave more energy source for and faster adventive shoot initiation.

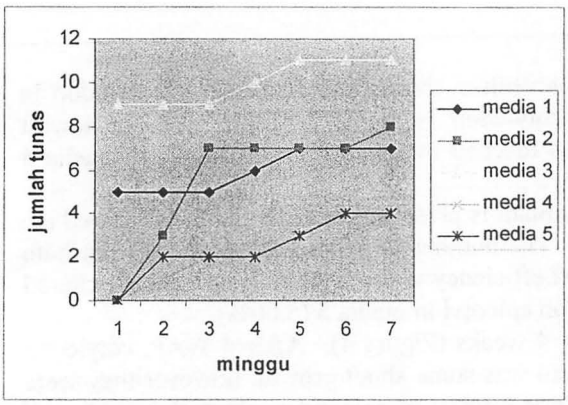
Table 2. Percentage of adventive shoot formed to number of explant at 7 WAP

| Treatment | Percentage of adventive shoot to number of explant (%) | |
|---|---|---|
| Cotyledon + media 1 | 62.50 | Note: |
| Cotyledon + media 2 | 21.25 | 1= MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l |
| Cotyledon + media 3 | 72.50 | 2= MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l |
| Cotyledon + media 4 | 7.50 | 3= MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l |
| Cotyledon + media 5 | 12.50 | 4= MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l |
| Epicotyl + media 1 | 8.00 | 5= MS + 2.0 mg BAP/l + 1.0 mg K/l + 1.0 mg NAA/l |
| Epicotyl + media 2 | 9.00 | |
| Epicotyl + media 3 | 15.00 | |
| Epicotyl + media 4 | 7.00 | |
| Epicotyl + media 5 | 5.00 | |




Note:
1= MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l
2= MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l
3= MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l
4= MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l
5= MS + 2.0 mg BAP/l + 1.0 mg K/l + 1.0 mg NAA/l

Figure 4. Number of adventive shoots obtained from cotyledon of pummelo cv. Cikoneng




Note:
1= MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l
2= MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l
3= MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l
4= MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l
5= MS + 2.0 mg BAP/l + 1.0 mg K/l + 1.0 mg NAA/l

Figure 5. Number of adventive shoots obtained from epicotyl of pummelo cv. Cikoneng

A summary of variance analysis is presented in Table 3. Treatment of media, type of explants and their interactions significantly influenced shoot height and leaf number in all stage of observation. On root number, treatment of media, type of explants and their interactions significantly influenced on 5 to 12 WAP, except the interaction on 9 WAP was not significant.

**Adventive Shoot Height**

Interaction effect of explant type and media on adventive shoot height is presented in Table 4. In general, the height of shoot obtain from cotyledon was higher than those obtained from epicotyl. Growth of shoots obtained from epicotyl was slower than that obtained from cotyledon. At 2 WAP, shoot from cotyledon reached height of 1.3 cm while the one from epicotyl was only 0.8 cm.

Table 4 showed that treatment media 1 + cotyledon achieved the highest shoot height, however, it was not significant to treatment media 3 + cotyledon at 2, 3, dan 4 WAP. At 1 WAP, media 3 + cotyledon was not significant to media 2, 4, 5 + cotyledon while at 2-4 WAP it was significant. At 2-4 WAP, combination of media 1 + cotyledon and media 3 + cotyledon were significant to other treatments

Table 3. Summary of analysis of variance on media and explant treatments and their interactions in adventive shoot formation

| Variable | WAP | Treatment | | |
|---|---|---|---|---|
| | | Media | Explant | Media x Explant |
| Shoot height[a] | 1 | ** | ** | ** |
| | 2 | ** | ** | ** |
| | 3 | ** | ** | ** |
| | 4 | ** | ** | ** |
| Leaf number[b] | 1 | ** | ** | ** |
| | 2 | ** | ** | ** |
| | 3 | ** | ** | ** |
| | 4 | ** | ** | ** |
| | 5 | ** | ** | ** |
| | 6 | ** | ** | ** |
| | 7 | ** | ** | ** |
| | 8 | ** | ** | ** |
| | 9 | ** | ** | ** |
| | 10 | ** | ** | ** |
| | 11 | ** | ** | ** |
| | 12 | ** | ** | ** |
| Plantlet height[a] | 13 | ** | ** | ** |
| Root number[a] | 1 | - | - | - |
| | 2 | - | - | - |
| | 3 | ns | ns | ns |
| | 4 | * | ** | * |
| | 5 | ** | ** | ** |
| | 6 | ** | ** | ** |
| | 7 | ** | ** | ** |
| | 8 | ** | ** | * |
| | 9 | ** | ** | ns |
| | 10 | ** | ** | ** |
| | 11 | ** | ** | ** |
| | 12 | ** | ** | ** |

WAP= week after planting, ** = highly significant ($\alpha = 1$ %). * = significant ($\alpha = 5$ %), ns = not significant, a = Data was transformed with $\sqrt{(x+0.5)}$, b = Data were transformed with $\sqrt{(x+1)}$

## Number of Leaf

Observation on leaf number of shoot obatined from cotyledon and epicotyl was conducted 1 week after the shoots were subcultured in rooting media. Figure 6 and 7 showed the average number of leaf up to 12 WAP. In general, number of leaf was between 1-2. Leaf growth was slow. In 12 weeks, the highest number of leaf was achieved by shoots obtained from cotyledon cultured in media 1 and media 3 (Figure 7). The leaf number can be used as indicator for the number of internode. The low number of leaf influenced plant height. The lesser the leaf number the lower the shoot height. On treatment combination of epicotyl + media 1, increase of leaf number occured after week 5 and no further increase until 12 WAP. On treatment combination of epicotyl + media 2, 3, 4, 5 did not show any increase of leaf number (Figure 7).

## Root Number

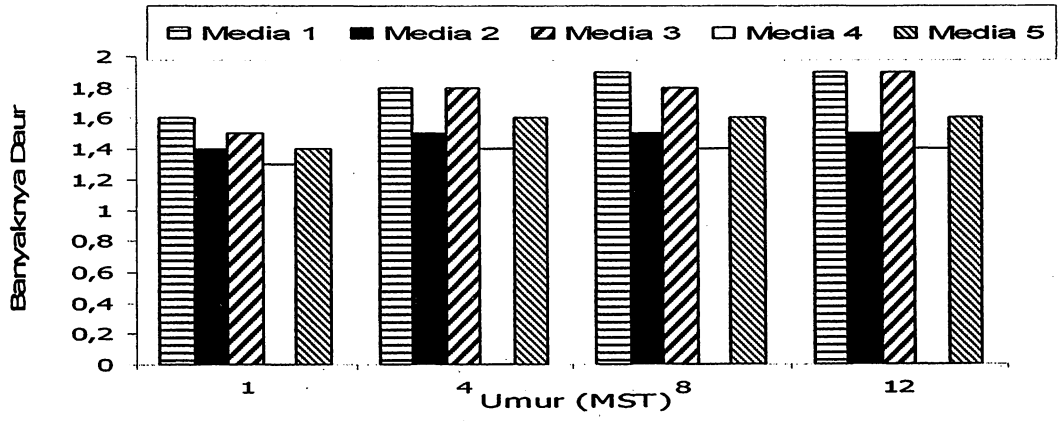
Number of root was observed 1 week after subculturing. Up to 2 WAP no root was appeared. The earliest root formed on shoot obtained from cotyledon planted in media 1 3 weeks after planting

shoot in rooting media (Figure 8). Vitrous shoot obtained from epicotyl could not form root. Shoots obtained from cotyledon cultured in media 1 or media 3 gave the highest number of root (Figure 9).

Table 4. Interaction effect of explant type and media on adventive shoot height of pummelo cv. Cikoneng

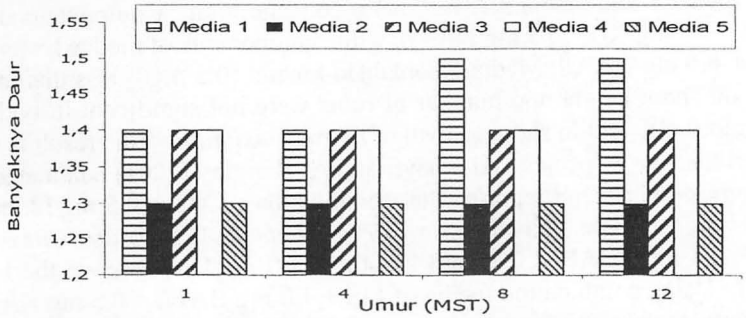
| WAP | Explant Type | Media Formulation | | | | |
|---|---|---|---|---|---|---|
| | | Media 1 | Media 2 | Media 3 | Media 4 | Media 5 |
| | | ..... cm ..... | | | | |
| 1 | Cotyledon | 1.1a | 1.0 ab | 1.0 ab | 0.8bc | 0.8bc |
| | Epicotyl | 0.8bc | 0.7c | 1.0 ab | 0.7c | 0.7c |
| 2 | Cotyledon | 1.6a | 1.1b | 1.4a | 0.8cd | 1.0 bc |
| | Epicotyl | 0.8cd | 0.8cd | 1.0bc | 0.8cd | 0.7d |
| 3 | Cotyledon | 1.8a | 1.3b | 1.6a | 0.9c | 1.0 c |
| | Epicotyl | 0.8c | 0.85c | 1.1bc | 0.8c | 0.8c |
| 4 | Cotyledon | 2.0a | 1.3b | 1.7a | 0.9c | 1.1bc |
| | Epicotyl | 0.8c | 0.8c | 1.0bc | 0.8c | 0.8d |

Value followed by the same letter is not significant according to Honestly Significant Difference Test. Media 1= MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l, Media 2= MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l, Media 3= MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l, Media 4= MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l, Media 5= MS + 2.0 mg BAP/l + 1.0 mg K/l + 1.0 mg NAA/l




1= MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l, 2= MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l, 3= MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l, 4= MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l, 5= MS + 2.0 mg BAP/l + 1.0 mg K/l + 1.0 mg NAA/l

Figure 6. Interaction of explant type and media on leaf number of pummelo cv. Cikoneng




1= MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l, 2= MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l, 3= MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l, 4= MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l, 5= MS + 2.0 mg BAP/l + 1.0 mg K/l + 1.0 mg NAA/l

Figure 7. Effect of epicotyl and media on leaf number of pummelo cv. Cikoneng

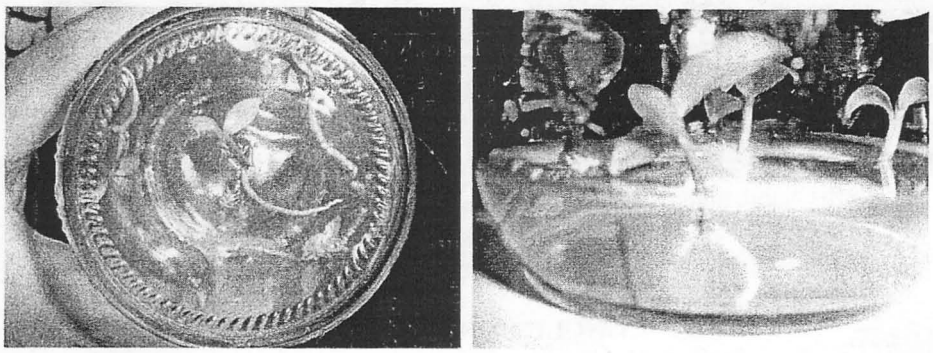
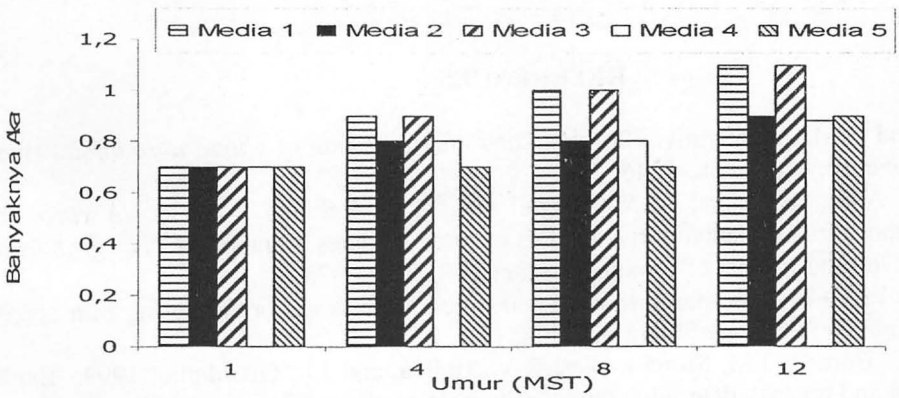


Figure 8. Growth of pummelo root in rooting media containing active charcoal.




1= MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l, 2= MS + 2.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l, 3= MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l, 4= MS + 2.0 mg BAP/l + 0.5 mg K/l + 1.0 mg NAA/l, 5= MS + 2.0 mg BAP/l + 1.0 mg K/l + 1.0 mg NAA/l

Figure 9. Effect of cotyledon and media on root number of pummelo cv. Cikoneng

The difference of media 1 and media 3 is in kinetin concentration. Components of media 1 was MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l, while components of media 3 was MS + 1.0 mg BAP/l + 1.0 mg K/l + 0.5 mg NAA/l. Media 1 contained kinetin (0.5 mg/l) less than media 3 (1 mg /l). Since the effect on shoot height and number of roots were not significant in both media 1 dan 3, use of media was more efficient in shoot growth of *Citrus maxima* L. This result is supported by previous reports in various Citrus species. Al-Khayri and Al-Bahrany (2001) conducted research on propagation of *Citrus aurantifolia* and reported that combination of MS + 0.5 mg/l kinetin + 1.0 mg/l BAP gave the best shoot growth. However, in *Citrus reticulata* Blanco dan *Citrus jambhiri* Lush justru media MS + 2.0 mg/l BAP + 0.5 mg/l kinetin + 1.0 mg/l NAA gave the best result (Ramkrishna, *et al.* 2005). Using combination media of MS + 1.0 mg BAP/l + 0.5 mg K/l + 0.5 mg NAA/l we could propagate three cultivars of pummelo Nambangan, Srinyonya, and Cikoneng (Figure 10).

Left= Nambangan, Middle= Cikoneng, Right= Srinyonya

Figure 10. *In vitro* propagation of several pummelo cultivars (C. *maxima*)

## CONCLUSION

In conclusion, treatment of cotyledon explant cultured in media 1 or media 3 gave the highest number of adventive shoots, leaf number, root number. Those combination of treatments was the fastest in shoot initiation. It is suggested that cotyledon and media 1 is used for pummelo propagation.

## REFERENCES

Al-Khayri, J.M and A.M. Al-Bahrany. 2001. *In Vitro* micropagation of *Citrus aurantifolia* (lime). Current Science. 81(9):1242-1246.

Begum F., M.N. Amin, S. Islam, M.A.K. Azad and M.M. Rehman. 2003. *In vitro* plant regeneration from cotyledon-derived callus of three varieties pummelo (*Citrus grandis* (L.) Osbeck). On Line Journal of Biological Sciences 3 (8): 751-759.

Filho, J. C. 2001. In vitro adventitious shoot regeneration from sweet orange using thin epicotyl section. Brazilian Society of Plant Breeding. (1)1: 27-34.

Garcia-Luis, A., Y. Bordoń, J.M. Moreira-Dias, R.V. Molina, and J.L. Guardiola. 1999. Explant orientation and polarity determine the morphogenic response of epicotyl segments of troyer citrange. Annals of Botany 84: 715-723.

Malaurie B., M-F. Trouslot, J. Berthaud, M. Bousalem, A. Pinel and J. Dubern. 1998. Medium-term and long-term in vitro conservation and safe international exchange of yam (*Dioscorea*

spp.) germplasm. Electronic Journal of Biotechnology (EJB) Vol.1 No.3, Issue of December 15, 1998. Accessed: February 04, 2009.

Min, Y.Y. 1997. Study on diverse centre of origin of pummelo germplasm. China Citrus 26(1): 3-5.

Niyomdham, C., 2003. *Citrus maxima* (Burm.) Merr. *In* Edible Fruits and Nuts, p.128-131. E.W.M. Verheij and R.E. Coronel (*Eds.*). PROSEA (Plant Resources of South-East Asia) Foundation, Bogor, Indonesia. http://www.proseanet.org. Accessed: May 19, 2004.

Murashige, T. and F. Skoog. 1962. A rapid medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15:473-497.

Paudyal, K.P. and N. Haq. 2008. Variation of pomelo (*Citrus grandis* (L.) Osbeck) in Nepal and participatory selection of strains for further improvement. Agroforest Syst. 72:195–204.

Ramkrishna, N. Khawale and S.K. Singh. 2005. *In vitro* adventitive embryony in citrus: A technique for citrus germplasm exchange. Curr. Sci., 88(8):1309-1311.

Saini, R. and P.K. Jaiwal. 2002. Age, position in mother seedling, orientation, and polarity of the epicotyl segments of blackgram (*Vigna mungo* L. Hepper) determines its morphogenic response. Plant Science 163:101-109.

Salisbury, F. B dan C.W. Ross. 1995. Fisiologi Tumbuhan Dasar. Penterjemah: D.R. Lukman dan Sumaryono. ITB Press. Bandung. 346 p.

Withers, L.A. 1989. *In vitro* conservation and germplasm utilisation. p. 309-334. *In* The Use of Plant Genetic Resources. D.H. Brown, D.R. Marshall, O.H. Frankel & J.T. Williams (*Eds*). Cambridge University Press. IBPGR, Cambridge.