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Seedling Propagation of Kulo Chrysanthemum by Tissue Culture

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Abstract: Aims of this research were to get the right dosage of Plant Growth Regulkator (PGR) and Coconut Water (CW) for shoot multiplication of Kulo chrysanthemums. The study was conducted using a factorial design of three factors namely IAA (0.0; 0.5 and 1.0 ppm), BAP (0, 0.5 and 1.0 ppm) and coconut water (0.0 and 30%). The explants used were clean micro cuttings and were cultured on Murashige and Skoog (MS) media. The media was added IAA, BAP and coconut water. The result showed that the best shoot multiplication of Kulo chrysanthemums was the MS media using combination of 0.5 ppm and 1.0 of IAA, 0.5 ppm of BAP and 30% of coconut water. Good rooting was revealed by the combination of IAA 0.5 and 1 ppm with 30% coconut water and BAP 1 ppm and 30% coconut water.

Keywords: Kulo Chrysanthemum, Multiplication, BAP, IAA, Coconut Water

Introduction

Flower of Kulo Chrysanthemums (Lokon white) is white chrysanthemum that have been designated as chrysanthemum varieties from Tomohonby decree ofI Indonesian Minister of Agriculture in 2012 number 2276 / kpts / SL-120 / VIII / 2012 (Antaranews.com Manado, Manado Express, 2015). The flower of Kulo Chrysanthemums has advantages such as high durability and large flower size (Fig. 1). Description of the flower is attached (Attachment 1).

Propagation of Kulo Chrysanthemums by farmers in Tomohonis by planting shoots which were grown from tip shoot and lateral shoot. This propagation has encountered problem such as difficulty in producing large number of shoot in short time as well as probability to produce plant carrying diseases if the maternal plant was infected by disease. Tissue culture is an alternative method for plant propagation to avoid seedling contaminated by disease.

Plant tissue culture is propagation using small explants which are grown on media aseptic and work aseptically. The use of tissue culture technology has advantages such as (George dan and de Klark, 2008): (1) The plant material used is very small; (2) small room is needed to reproduce and maintain the plant; (3) it can produce seeds from plants that slow and difficult vegetative propagated; (4) the production of seedling can be continuous throughout the year and are not dependent on climate change; (5) vegetative plant material can be stored in a long period and (6) lack of energy and space for the purpose of propagation and maintenance of plant stock.Therefore, the seed multiplication through tissue culture is to be more cost effective than traditional methods of propagation. Propagation through tissue culture is determined by many factors such as genetic factors and media including Plant Growth Regulator (PGR). Plant genetic factoris the most influence to growth and development in tissue culture. Therefore, Kulo chrysanthemum propagation through tissue culture needs to be explored using Plant Growth Regulator (PGR) which can increase shoot multiplication, the growth of shoots and plantlet until acclimatization process.

Fig. 1: Flower of KuloChrysanthemums from Tomohon

Astutik (2007) reported that BAP 1 mg/l resulted large number of shoots. Mandang (1993) revealed her research on Bogor chrysanthemum that the use of BAP 0.5 mg/l produced shoots of 6.72 more than those without the use of BAP. On the other hand, Mariska and Lestari (1988) *in* Lestari (2011) suggested that kinetin 10 mg/l was good for shoot chrysanthemum. Nalini (2012) showed different result that tissue culture of chrysanthemum was good on MS treated by kinetin 3 mg/l and IAA 2.0 mg/l resulting 67.8% of success.

Application of combination of Auxin and cytokinin at for varieties of chrysanthemum was used by Basri (2008) resulting Yellow Fuji and Elen Tawn Talk varieties were growing good using media MS treated by IBA 0.25 ppm and BAP 1.50 ppm. Whereas, white Fuji variety was also good by using combination of NAA 0.50 ppm and BAP 1.50 ppm.

Hence, it is clear that the addition of PGR is very varied with different types of chrysanthemum used. The differences above are related to genetic differences of chrysanthemum used in propagation through tissue culture.

Therefore, it is necessary to study the use of PGR in propagation through tissue culture techniques to obtain large number of shoots and better growth.

The use of coconut water in tissue culture has been shown to promote plant growth. The composition of coconut water of tenga variety was reported by Mandang (1993) suggesting that the composition of coconut water was almost similar to the components of media MS (Attachment 2). Therefore, coconut water is recommended to be used for substitution of media MS to encourage the chrysanthemum growth in *in vitro*. Mandang *et al*. (1997) showed that the use of coconut water on media MS was increasing orchid protocorm. Research Mandang *et al*. (1997) resulted that partly substituted MS media with coconut water was able to increase shoot dry weight chrysanthemum and chlorohill content. Therefore the use of coconut water is important for tissue culture.

AIM

1. To find a precise combination of dose IAA , BAP and coconut water for shoot multiplication
2. To determine a precise combination of dose IAA and BAP and coconut water concentration for root growing of Kulo chrysanthemum

Materials and Methods

Material

Used Material were clean Kulo chrysanthemum which was cultured to media MS0 (MS without PGR), Indole Acetic Acid (IAA), Benzyl Amino Purine (BAP), coconut water and MS basic media.

Method

The research was conducted using a factorial design of three factors namely IAA (0.0, 0.5 and 1.0 ppm), BAP (0.0, 0.5 and 1.0 ppm) and coconut water (0.0 and 30%). The explants used were clean micro cuttings and were cultured on Murashige and Skoog (MS) media.

MS Media stock at a dose of 4 g/l was dissolved and was added IAA, BAP and coconut water according to treatment.

Three micro cuttings leaf explants were cultured on culture bottle in Laminar Air Flow Cabinet (LAFC) and covered with aluminium foil. The bottles were placed in the culture rack mounted, lighting with TL lamp, with intensity around 2000 lux. Photoperiode was controlled 16-20 hours. Room temperature was 24-26°C.

Observation was made at age of ten weeks of plant after culture. The characters observed were: (1) plant height; (2) number of shoots; (3) wet weight of plant; (4) The length of root and (5) root number.

Result

Plant Height

Plant height of Kulo chrysanthemum cultured on MS media added with IAA, BAP and coconut water was showed in Table 1.

Table 1: Plant height of Kulo chrysanthemum cultured on MS media added IAA, BAP and coconut water

Treatment					
		IAA (ppm)			
BAP (ppm)	Coconut water (%)	0		0.5	1.0
0.0	0	5.58^{abcd}		7.22^{bcdef}	7.82^{cdef}
	30	9.37^{ef}		7.73^{bcdef}	7.95^{cdef}
	0.5	0	4.73^{abc}	5.10^{abcd}	5.52^{abcd}
	30	6.03^{abcde}		7.50^{bcdef}	8.32^{def}
	1.0	0	2.98^{a}	2.75^{a}	2.90^{a}
		30	9.82^{f}	5.85^{abcde}	4.13^{ab}

BNJ 0,05: 3.52

Number followed by similar alphabet was not significant difference based on honestly significant difference test at level of 0.05

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Table 1 showed that BAP treatment resulted the shortest Kulo chrysanthemum *in vitro* at all of IAA treatments. While BAP combined with coconut water was resulting tall plant of Kulo chrysanthemum which was not different from all the coconut water treatment of 30% (Fig. 2 and 3).

This result supports Mandang (1993) that the use of IAA or coconut water increasing plant height of chrysanthemum in contrast with BAP treatment.

Number of Shoot

Effect of IAA, IBA and coconut water on number of shoot of kulo chrysanthemum was shown on Table 2 and Fig. 4 and 5.

Table 2 showed that treatment given BAP without IAA treatment produced more shoot compared to without BAP. It happened also to treatment which was given IAA 0.5 and 1.0 g/l.

Fig. 2: Kulo chrysanthemum at BAP media (above) and coconut water (below)

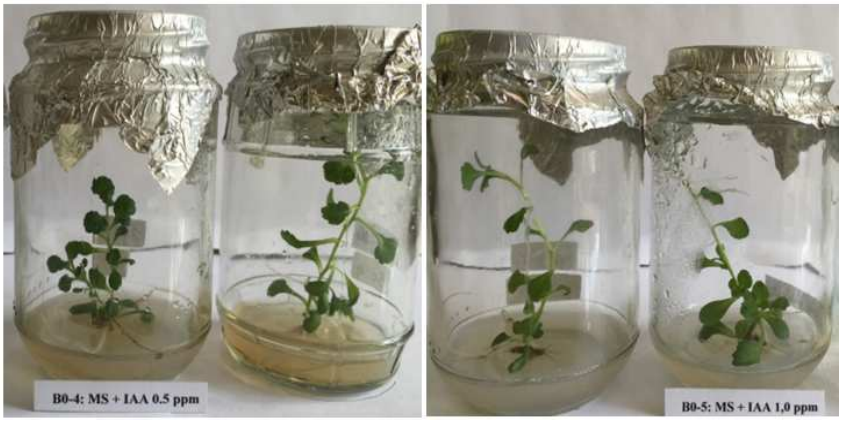

Fig. 3: Growth of Kulo chrysanthemum at IAA media 0.5 ppm (left) and IAA media 1 ppm (right)

Fig. 4: Chrisanthemum growth on media without PGR and coconut water (left) and with PGR BAP and coconut water (rigth)

Table 2: Number of shoot of Kulo chrysanthemum on media added with IAA, BAP and coconut water

Treatment		IAA (ppm)			
BAP (ppm)	Coconut water (%)	0		0.5	1.0
0.0	0	1.50[a]		1.50[abc]	1.67[abc]
	30	1.50[a]		2.33[abc]	2.17[abc]
	0.5	0	6.50[def]	5.67[cde]	6.83[efg]
	30	5.00[abcde]		6.83[efg]	10.00[fg]
	1.0	0	5.67[cde]	3.33[abcde]	5.50[bcde]
		30	2.67[abcd]	10.67[g]	3.30[abcde]

BNJ 0,05: 3.88

Number followed by similar alphabet was not significant difference based on honestly significant difference test at level of 0.05

Table 3: Shoot wet weight of Kulo chrysanthemum (g) on media added IAA, BAP and coconut water

Treatment		IAA (ppm)			
BAP (ppm)	Coconut water (%)	0		0.5	1.0
0.0	0	0.41[a]		0.47[a]	0.62[a]
	30	0.97[abc]		0.78[ab]	0.79[ab]
	0.5	0	1.78[bc]	1.34[abc]	2.17[cd]
	30	1.73[bc]		4.63[f]	3.23[de]
	1.0	0	1.19[abc]	0.93[ab]	1.05[abc]
		30	0.97[abc]	3.39e	0.97[abc]

BNJ 0,05: 1.2

Number followed by similar alphabet was not significant difference based on honestly significant difference test at level of 0.05

Figures 4 and 5 revealed that the chrysanthemum cultured on BAP 0.5 media which was added IAA 0.5 ppm and IAA 1.0 ppm and also was given coconut water producing more shoots than without BAP. It can be explained that the BAP is the cytokinins that increase plant cell division and ultimately increase the number of shoots.

Shoot Total Weight per Explants

The shoot total wet weight of Kulochrysanthemum on the media added with IAA, BAP and Coconut Water was showed in Table 3.

Table 3 showed that the treatment BAP 0.5 ppm whether they were given and were not given coconut water resulted higher shoot total weight than without BAP. Alike the treatment BAP 0.5 ppm at the BAP 1.0 given coconut water and IAA 0.5 ppm produced higher wet weight than without BAP. This is caused by the number of shoot on that treatment more than without BAP, IAA and coconut water (Fig. 4 and 5).

Root Length

Root length of Kulo chrysanthemum on media added with IAA, BAP and coconut water was showed at Table 4.

Table 4 showed that additional IAA and coconut water without BAP was increasing root length compared to treatment BAP 0.5 and 1.0 ppm except for combination of BAP 1.0 ppm and coconut water. The use of cytokinin is to inhibit root growth. These results support the finding that high of cytokinin (0.5-10 mg/l) generally suppresses the formation of root (Schraudolf and Reinert, 1959; George and Sherrington, 1984; Harris and Hart, 1964). For lateral root initiation is affected by cytokinin.

Fig. 5: Kulo chrysanthemum on BAP media 0.5 g/l added with 30% of coconut water and IAA 1.0 g/l

Table 4: Root length of Kulo chrysanthemum on media added with IAA, BAP and coconut water

Treatment		IAA (ppm)			
BAP (ppm)	Coconut water (%)	0.0		0.5	1.0
0.0	0	1.68^{ab}		2.37^{bc}	2.59^{bc}
	30	2.47^{bc}		2.89^{c}	3.12^{c}
	0.5	0	0.71^{a}	0.98^{a}	1.00^{a}
	30	0.86^{a}		1.13^{a}	1.26^{a}
	1.0	0	0.71^{a}	0.71^{a}	0.71^{a}
		30	3.24^{c}	0.92^{a}	0.87^{a}

BNJ 0,05: 1.00

Number followed by similar alphabet was not significant difference based on honestly significant difference test at level of 0.05

Table 5: Effect of coconut water, IAA, BAP on root number of Kulo chrysanthemum

Treatment		IAA (ppm)			
BAP (ppm)	Coconut water (%)	0		0,5	1,0
0.0	0	1.79^{ab}		2.99^{bc}	3.00^{bc}
	30	3.77^{c}		4.07^{c}	3.58^{c}
	0.5	0	0.79^{a}	0.79^{a}	1.05^{a}
	30	0.90^{a}		1.90^{ab}	1.38^{a}
	1.0	0	0.71^{a}	0.71^{a}	0.71^{a}
		30	4.10^{c}	1.02^{a}	0.90^{a}

BNJ 0,05 : 1.49

Number followed by similar alphabet was not significant difference based on honestly significant difference test at level of 0.05

Root Number

Root number of Kulo chrysanthemum on the media added with IAA, BAP and Coconut Water was showed at Table 5.

Table 5 showed that the most root number was detected on coconut water combined with BAP 1 ppm treatment as well as treatment combination of IAA 0.5 and 1.0 ppm. In addition, it was found at 30% of coconut water combined with IAA 0 ppm and BAP 0 ppm. This pattern was identical with root length.

Discussion

The results of this study showed that the height of chrysanthemum kulo increased by coconut water treatment. This is because coconut water consists of 1,3 difenyl urea (5.8 mg/l), a type of growth promotor (Shant and Steward, 1955; Tulecke *et al*., 1961). Paris and Duhamet (1953) in Tulecke *et al*. (1961) also found auxin's concentration 0.07 mg/l in coconut water. IAA is an auxin stimulating shoot elongation by stimulating

wall loosening factors such as elastin to loosen cell wall. Mandang (1995) found that coconut water can stabilize the media, a media's buffer. It was found that MS media without coconut water, the media pH changed 1.3 unit (plus 1 cc NaOH 1N) and MS media substitute 40% with coconut water, media pH only changed 0.20 unit. Mandang *et al*. (1997) also found on banana tissue culture with MS media added 30% coconut water, at 12 weeks after culture media pH only changed 0.8 unit compared to media without coconut water the change was 2.8 unit. This is because coconut water (Tenga cultivar) consists of malic acid, succinic acid and citric acid (Mandang, 1993). This result supports the study done by Norstog and Smith (1963) in Thorpe *et al*. (2008) which found that 100 mg/l malic acid acted as an effective buffering agent in their medium for barley embryo culture and also appeared to enhance growth in the presence of glutamine and alanine.

Number of shoots increased on BAP treatment with and without IAA. In combination BAP, coconut water and IAA produced the highest shoot number. It is showed that BAP as cytokinin played significant role in increasing the number of shoots of chrysanthemum kulo. This result was in line with the finding of the study done by Ngmuo, Mneney and Ndakidemi (2013). The study found that the use of BAP increased shoot number of Musa sp yangambi variety. The similar result also found by Ashraf *et al*. (2014) in tissue culture of Chlorophytum borivilianum Sant. The study concluded that BAP was significantly effective on shoot multiplication. Cytokinin activates RNA synthesis, stimulates protein synthesis and activities of some enzymes (Kulaeva, 1980 in van Standen, Zazimmalova and George, 2008).

Shoot wet weight alike shoot number. The treatment of BAP 0.5 and 1.0 ppm plus IAA 0.5 ppm and coconut water showed the highest shoot wet weight. Media added coconut water and IAA resulted in higher root length and root number compared to treatment added BAP. Agampodi and Jayawardena (2008) recorded treatment of coconut water extract contains 143 uM IAA resulting the best root induction and development.

Physiological changes in auxin concentration and high endogenous auxin concentration are normally associated with a high rooting rate at beginning rooting process (Caboni *et al*., 1997; Agampodi and Jawarden, 2008).

Conclusion

1. MS media should be combined with IAA 1.0 ppm, BAP 0.5 ppm and 30% of coconut water or combined with IAA 0.5 ppm, BAP 0.5 ppm and 30% of coconut water for highest shoot multiplication of kulo chrysanthemum
2. Combination of IAA and 30% coconut water and combination of BAP 1 ppm and 30% of coconut water produces good rooting

Acknowledgement

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Ethics

This article presents is an original and valid work also contains unpublished material.

References

Agampodi, V.A. and B. Jayawardena, 2008. Effect of coconut (*Cocos nucifera* L.) water extracts on adventious root development in vegetative propagation of *Dracaena purplecompacta* L. Acta Physiologiae Plantarum, 31: 279-284.

Ashraf, M.F., M.A. Aziz, N. Kemat and I. Ismail, 2014. Effect of Cytokinin types, concentrations and their interactions on *in vitro* shoot regeneration of Chlorophytum borivilianum Sant. Fernandez. Electronic J. Biotechnology, 17: 275-279.

Astutik, 2007. Kajian Zat Pengatur Tumbuh dalam Perkembangan Kultur Jaringan Krisan. Buana Sains, 7: 113-121.

Basri, Z., 2008. Multiplikasi empat varietas krisan melalui teknik kultur Jaringan. J. Agroland, 15: 271-277.

George dan, E.F. and G.J. de Klark, 2008 Micropropagation: Uses and Methodes in Plant Propagation by Tissue Culture, George, E.F., Michael, A. Hall dan and G.J. de Klark (Eds.). Springer, Netherlands.

George, E.F. and Paul D. Sherrington. 1984. Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories Exegetick Limited. pp: 709.

Lestari, G.E., 2011. Peranan zat pengatur tumbuh dalam perbanyakan tanaman melalui kultur Jaringan. J. AgroBiogen, 7: 63-68.
DOI: 10.21082/jbio.v7n1.2011.p63-68

Manado Express. 2015. Krisan Kulo "Jawara" di Festival Hortikultura Mataram. www.manadoexpress.co.

Mandang, J.P., 1993. The Role of Coconut Water in Chrysanthemum (*Chrysanthemum morifolium* RAMAT) Tissue Culture. Unpublised dissertation in partial fulfilment of the requirements for degree of Doctor, Bogor Agricutural University. Bogor.

Mandang, J.P., 1995. Air Kelapa sebagai Bahan Substitusi Media MS pada Kultur Jaringan Krisan. Media Publikasi Ilmiah Eugenia, 1: 1-11.

Mandang, J.P., W. Tilaar, L.B. Sumaiku dan and J. Salindeho, 1997. Penggunaan air kelapa dan bahan pengganti agar murni pada kultur *in vitro* Pisang Barangan. Media Publikasi Ilmiah Eugenia, 3: 193-197.

Mandang, J.P., W. Tilaar, D.M.F. Sumampow and Suhari, 1997. Pertumbuhan Krisan yang mengalami Vitrivikasi Pada media MS yang sebagian di subst dengan air Kelapa. Media Publikasi Ilmiah Eugenia.

Mariska, I. Dan E.G. Lestari. 1988. Perbanyakan Tanaman krisan melalui teknik kultur jaringan. Buletin Peragi, 2: 19-25.

Nalini, R., 2012. Micropropagation of chrisanthemum (Chrisanthemum morifolium) using shoot tip as explants. Int. J. Food Agriculture Vet. Sci., 2: 62-66.

Ngomuo, M., E. Mneney and P. Ndakidemi, 2013. The Effect of Auxins and Cytokinin on Growth and Development of (*Musa sp.*) Var. "Yangambi" Explants in Tissue Culture. Am. J. Plant Sci., 4: 2174-2180.

Thorpe, T., C. Stasolla, E.C. Yeung, G.J. de Klerk, A. Roberts and E.F. George, 2008. The Components of Plant Tissue Culture Media ll: Organic Additions, Osmotic and pH Effects and support systems. In: Plant Propagation by Tissue Culture. George, E.F., Michael A. Hall dan G.J. de Klark (Eds). Springer, Netherlands.

Tulecke Walter, Leonard H Weinstein, Allan Rutner, Henry J. Laurencot Jr. 1961. The biochemical composition of coconut water (coconut milk) as related to its use plant tissue culture. Contr. Boyce Thomson Inst., 21: 115-128.

Van Standen J, E. Zazimalave, E.F. George. 2008. Plant Regulators II: Cytokinin, their Analogues and Antagonists. In: Plant Propagation by Tissue Culture. George, E.F., Michael A. Hall dan G.J. de Klark (Eds). Springer, Netherlands.

Attachment 1. Kulo Chrysanthemum plant description

Plant height: 110 – 120 cm
Time to flowering: 60 – 70 days after planting
Flower type: standard
Flower shape: decorative
Amount of flower: 1 flower/stem
Flower diameter: 17 – 20 cm
Duration freshness: 7-14 days after cutting
Adaptation to high land 750-1200 m sea level
Breeder: Jemmy A. Matindas, Karel F Lala, Budi Marwoto, M. PramaYufdi, Jemmy Palendeng, B.H. Maliangkay, Deby V.Y. Tumilaar, Rita Kock, Yanny Lasut.
Decree of Indonesian Minister of Agriculture:
No. 2776/Kpts/SL.120/8/2012.

Attachment 2: The Composition of Mature Tenga Coconut Water (Mandang, 1993)

No.	Components	Content
1	Sucrose	0.961%
2	Glucose	0.517%
3	Fructose	0.575%
4	Sorbitol	0.794%
5	Nitrogen	19.00 mg/100 ml
6	Phosphorus	0.569 mg/100 ml
7	Potassium	460.750 ppm
8	Calcium	394.700 ppm
9	Magnesium	135.225 ppm
10	Malic acid	0.455%
11	Succinic acid	0.58%
12	Cytric acid	0.006%
13	Aspartic	0.0066% (0.0056%)*
14	Glutamic	0.0272% (0.0234%)
15	Serine	0.0081% (0.0070%)
16	Histidine	0.0024% (0.0022%)
17	Glysine	0.0015% (0.0016%)
18	Tyrosine	0.0036% (0.0027%)
19	Treonine	0.0020% (0.0017%)
20	Arginine	0.0423% (0.0372%)
21	Alanine	0.0015% (0.0011%)
22	Methionine	0.0016% (0.0009%)
23	Valine	0.0030% (0.0025%)
24	Phenylalanine	0.0024% (0.0020%)
25	Isoleusine	0.0017% (0.0016%)
26	Leusine	0.0027% (0.0024%)
27	Lisine	0.0040% (0.0043%)

*After Autoklaved