Ilmu Pertanian (Agricultural Science)
http://journal.ugm.ac.id/jip
Vol. 9 No. 2 August, 2024: 68–76 | DOI: doi.org/10.22146/ipas.95898

Effects of explants and culture medium compositions on quality of chrysanthemum 'Jayanti Agrihorti' rooted cuttings

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Article Info

Received : 3rd May 2024
Revised : 27th May 2024
Accepted: 3rd July 2024

Keywords:
Benzyl amino purine (BAP), explants, gibberellic acid (GA_3), growth variables

Abstract

'Jayanti Agrihorti' is a superior chrysanthemum therefore rooted cuttings quality is required. In vitro propagation increases the rate of propagation and produces true-to-type plants. The research to obtain the best explants and culture medium composition that is capable of producing high-quality plants. The research was arranged in a randomized complete block design consisting of two factors. The first factor was the explant, including the apical shoot of 0.5 cm, 1.0 cm, and the nodal segment. The second factor was in vitro culture medium composition (CP), consisting of CP1 (Murashige and Skoog (MS) medium for initiation, followed by MS + 2.5 $mg.L^{-1}$ gibberellic acid (GA_3) for subculture), CP2 (MS + 0.25 $mg.L^{-1}$ benzyl amino purine (BAP) for initiation, followed by MS for subculture), CP3 (MS + 0.25 $mg.L^{-1}$ BAP for initiation, followed by MS + 2.5 $mg.L^{-1}$ GA_3 for subculture), and CP4 (MS + 0.5 $mg.L^{-1}$ BAP for initiation, followed by MS + 0.25 $mg.L^{-1}$ BAP for subculture). Acclimatization was performed after third subculture. The apical shoot size of 0.5−1.0 cm is optimum for producing chrysanthemum-rooted cuttings. Meanwhile, MS medium for initiation stage, followed by MS + 2.5 $mg.L^{-1}$ GA_3 for subculture is the best culture medium composition for in vitro propagation. This explant and culture medium composition produced higher chlorophyll a, b, and a+b content, thereby resulting in higher plant, more leaves, larger stem diameter, and longer root length. This are recommended for chrysanthemum propagation, particularly in 'Jayanti Agrihorti'.

INTRODUCTION

The chrysanthemum cut flower is placed in the second position in the world's top trade of cut flowers (International, 2022). The chrysanthemum 'Jayanti Agrihorti' is the standard type with a white color, making this variety an alternative for chrysanthemum growers both in Indonesia and around the world. The superiority of this variety can be recognized from the stem structure, which has thick, sturdy, and short flower stalks so that the flower buds are not easily broken (Sanjaya, 2021). This variety has a lot of potential to be developed. Therefore, it is necessary to provide high-quality rooted cuttings. Applying in vitro propagation techniques can enhance propagation rates and get true-to-type plants with good quality (Eisa et al., 2022). Explant sources, such as the nodal segments (Jevremovic et al., 2012; Pant et al. 2015) and shoot tip (Jahan et al., 2021), provide an efficient method of rapid clonal production of genetically stable and true-to-type progeny. According to Zalewska et al. (2010), the highest growth rate is at the tip of the

How to cite: Shintiavira, H., Pramanik, D., Daniyanti, R.D., Pertiwi, M.D., and Rachmawati, F. (2024). Effects of explants and culture medium compositions on quality of chrysanthemum 'Jayanti Agrihorti' rooted cuttings. *Ilmu Pertanian (Agricultural Science)*, 9(2), pp. 68–76.

ISSN 0126-4214 (print) ISSN 2527-7162 (online)

shoot compared to the base. Meanwhile, shoot growth is higher in meristems with larger sizes (Wang et al., 2014). In fact, shoot tip explants that are too small are difficult to isolate and have a chance of developing callus. The potential of apical shoots for organogenesis and their true type makes them relatively easy to work with on a commercial scale. Therefore, in this research, the apical shoots with a larger size than the shoot tips were used. However, the effect of apical shoot size on plantlet growth and the quality of rooted cuttings produced has never been studied. In addition, successful in vitro propagation is influenced by the combination of explant source, type, and concentration of plant growth regulators (Eisa et al., 2022).

Some studies about in vitro propagation of chrysanthemum by the combination of explant sources, types, and concentrations of plant growth regulators have been reported using apical shoot with a size of about 0.5 cm (Waseem et al., 2009), nodal segment (Yesmin et al., 2014), and meristem (Brailko et al., 2018) cultured on Murashige and Skoog's (MS) medium supplemented with cytokinin and auxin (Waseem et al. 2009; Yesmin et al. 2014; Tymoszuk and Miler, 2019) and on MS medium supplemented with indole 3-butyric acid (IBA) for rooting (Waseem et al., 2009; Yesmin et al., 2014; Brailko et al., 2018; Jahan et al., 2021). Some PGRs also influence pigment metabolism, which functions for plantlet growth during in vitro culture (Mihovilović et al., 2020). Based on previous studies, all the authors used the combination of cytokinin and auxin for initiation and subculture medium and auxin for rooting medium. In contrast, Imtiaz et al. (2019) and Jahan et al. (2021) used MS medium supplemented with 44.39 µM BAP. These were in line with Winarto (2017) in which the shoot tips sized 1–1.5 mm long were cultured on MS + 0.5 mg.L^{-1} BAP, followed by MS+0.25 mg.L^{-1} BAP, and MS medium for subculture until rooting was used for plant rejuvenation. However, the use of BAP for shoot initiation resulted in a relatively large number of shoots, shorter internodes and relatively longer growth, while increased 6-BA concentration from 22.20 µM to 44.39 µM resulted in gradual decline (Imtiaz et al., 2019). This influenced the slow multiplication of chrysanthemums. Therefore, a combination of cultural medium composition from initiation to acclimatization was used in this research by modifying the use of chrysanthemum culture medium compositions, including MS-supplemented BAP at initiation, followed by MS-supplemented GA_3 at the next subculture for rapid elongation shoot and rapid propagation. The GA_3 was able to accelerate the increase in plant height through internode elongation (Miao et al., 2020). Then, in this culture medium compositions, there was no medium for rooting plantlets. This research was conducted because it is very important to provide rooted cuttings of the chrysanthemum 'Jayanti Agrihorti'. The aim of the research was to obtain the best apical shoot size as a source of explants and obtain an in vitro culture medium compositions that is capable of producing quality plants.

MATERIALS AND METHODS

Preparation of plant materials and in vitro culture media

The research work was conducted at the Tissue Culture Laboratory of the Indonesian Ornamental Crop Institute. The standard type of Chrysanthemum, 'Jayanti Agrihorti' was used as plant material. The in vitro propagation was started by cuttings the axillary shoot of the chrysanthemum's mother plants. The mother plant used were 4–6 months old. The leaves of the axillary shoot were sterilized following the method of Shintiavira et al. (2014). The culture medium was based on the MS medium (Murashige and Skoog, 1962). The MS medium supplemented with 7 g.L^{-1} agar (Duchefa Biochemie, Netherlands) and 3% sugar (Himedia, India) was used as the standard basic medium in this experiment. Then, the medium was supplemented with plant growth regulators such as benzoyl amino purine (BAP) and gibberellic acids (GA_3) according to the treatments (Table 1). The pH of the medium was adjusted to 5.8 and autoclaved at 121°C for 20 minutes. After being removed from the autoclave, the medium could be used after 3 days.

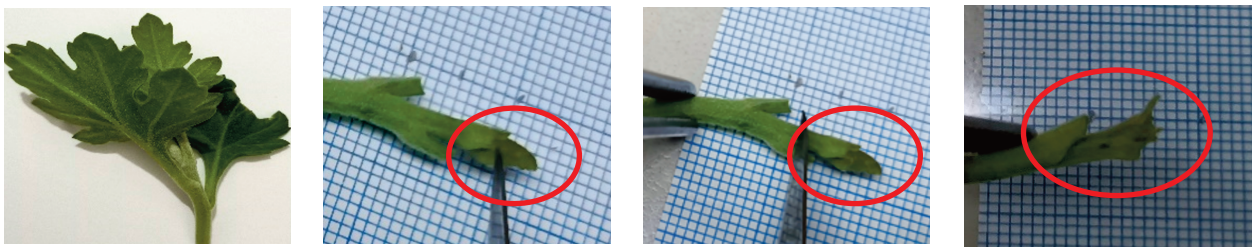
Research design

The research was arranged in a randomized complete block design with two factors. The first factor was the explants source, which consisted of the apical shoot sized 0.5 cm, the apical shoot sized 1.0 cm, and the nodal segment of chrysanthemum's axillary shoot (Figure 1). The second factor was in vitro culture package of propagation, consisting of four treatments (Table 1). The culture results were

Table 1. In vitro culture medium composition of chrysanthemum propagation

Code	In vitro culture media composition of chrysanthemum in each stage			
	Initiation	Subculture-1	Subculture-2	Subculture-3
CP1	MS	MS + 2.5 mg.L^{-1} GA_3	MS + 2.5 mg.L^{-1} GA_3	MS + 2.5 mg.L^{-1} GA_3
CP2	MS + 0.25 mg.L^{-1} BAP	MS	MS	MS
CP3	MS + 0.25 mg.L^{-1} BAP	MS + 2.5 mg.L^{-1} GA_3	MS + 2.5 mg.L^{-1} GA_3	MS + 2.5 mg.L^{-1} GA_3
CP4	MS + 0.5 mg.L^{-1} BAP	MS + 0.25 mg.L^{-1} BAP	MS + 0.25 mg.L^{-1} BAP	MS + 0.25 mg.L^{-1} BAP

Remarks: CP = culture medium composition; MS = Murashige and Skoog medium.



(a) (b) (c) (d)

Figure 1. The axillary shoot of the chrysanthemum's mother plants (a); apical shoot sized 0.5 cm (b); apical shoot sized 1.0 cm (c); and nodal segment (d)

maintained at a temperature of 21–24°C and exposed to 16 hours of light and 8 hours of darkness using Philips 18 W LED lamps. Acclimatization was carried out after the third subculture. The acclimatization was done by cutting plantlet at about four nodes from the tip. Then, root stimulants and 2% fungicides were applied to the base of the plantlet. The plantlet was grown on rice husk charcoal for four weeks.

Variable of observation

The initiation stage was observed six weeks after culture. The variables observed in the initiation stage were: (1) the shoot height (cm); (2) the number of leaves; (3) the internodes length (cm) (calculated by dividing the length of the shoot by the leaves number); and (4) the percentage of contamination. The subcultures were done three times at intervals of eight weeks, and similar growth variables as in initiation stages were observed in addition to the pigment variable. The first sub-culture was done six weeks after initiation. The leaf pigment analyzes such things as chlorophyll a, chlorophyll b, and chlorophyll a+b content based on Khaleghi et al. (2013) and the carotenoid content based on Miler and Zalewska (2014). After acclimatization, the observed variables included the shoot height (cm), the number of leaves, the internode length (cm), the stem diameter (cm), and the root length (cm) at four weeks after planting (WAP).

Statistical analysis

The data were analyzed using analysis of variance (ANOVA). If a significant effect existed, further testing would be needed to determine the difference between treatments using DMRT with alpha 5%.

RESULTS AND DISCUSSION

Initiation-subculture

There was no interaction between the explants and the culture medium composition on the growth of the chrysanthemum 'Jayanti Agrihorti' at the initiation stage. Using the apical shoots sized 0.5 cm and 1.0 cm as explants could reduce the contamination compared to the nodal segment explants. The apical shoots sized 0.5 cm accelerated the time of initiation. This influenced the height of the shoot, number of leaves, and length of the internodes. The initiation medium using MS without PGR (CP1) had positive implications in increasing the shoot height and length of internodes but had negative impacts on the number of leaves compared to the other media (CP2–CP4, which were MS+0.25-0.5 mgL^{-1} BAP) (Table 2). After three times of subculture, there was no interaction between the explant sources and the composition of the medium on the growth of the chrysanthemum 'Jayanti Agrihorti' in the variables of the number of leaves, the height of the shoot, and the length of

the internode. The apical shoot explants sized 0.5 cm and 1.0 cm produced a higher shoot height and a more significant number of leaves than the nodal segment did at the first to third subculture. Meanwhile, the CP1 medium resulted in the highest shoot height but the lowest number of leaves in the first to third subculture compared to the others. The CP4 produced the lowest height of shoot and the highest number of leaves in the first to third subculture compared to the others, and it had the longest internode (Table 2). There was a significant interaction between the explant sources and culture medium compositions in the variable of leaf pigment at the first to third subculture. Both the apical shoots sized 0.5 cm and 1.0 cm cultured on the CP1–CP3 media had the highest chlorophyll-a content compared to those cultured on the CP4 medium. The nodal segment cultured on the CP4 medium had the lowest chlorophyll-a content in the first to third subcultures (Figure 2a). A similar pattern was found between chlorophyll a and total chlorophyll content in the apical shoots sized 0.5 cm, 1 cm, and nodal segments (Figure 2c). The chlorophyll-b content was generally lower than chlorophyll-a in the first and second subculture. Both the apical shoots sized 0.5 cm and 1.0 cm cultured on the CP1 medium had the lowest chlorophyll-b content, but when they were cultured on the CP4 medium, they had the highest chlorophyll-b content. Meanwhile, the nodal segment cultured on both CP1 and CP4 media had the lowest chlorophyll-b (Figure 2b). Furthermore, the carotenoid content showed no significant difference in both the apical shoot sized 0.5 cm and 1.0 cm cultured on the CP1–CP4 media. In contrast, the nodal segment cultured on the CP4 medium had the lowest carotenoid content (Figure 2d).

Based on the results of the study, the apical shoots sized 0.5 cm could regenerate faster than others. Many PGRs are accumulated in apical shoots and are actively involved in cell division. In addition, the shoot tip had a low contamination rate. According to Zhang et al. (2015), callose deposits in the apical shoot meristem inhibits virus movement from cell to cell through plasmodesmata. Meanwhile, the nodal segment had slower growth than the shoot tip. According to Dierck et al. (2016), auxins are transported basipetal and indirectly inhibit the axillary bud outgrowth by restricting auxin export from the axillary buds to the stem, limiting auxin export from the axillary buds to the stem, or increasing cytokinin levels. Then the auxin stimulates cytokinin in the root area so that acropetal cytokinin is transported

Table 2. Plantlet growth at the initiation and subculture stages of various chrysanthemum explants in different culture package

Treatments	Initiation stage				Subculture stages								
	Contaminations (%)	Height of shoots (cm)	Number of leaves	Length of internodes (cm)	Height of shoots (cm)			Number of leaves			Size of internodes (cm)		
					SC-1	SC-2	SC-3	SC-1	SC-2	SC-3	SC-1	SC-2	SC-3
Explants													
E1	14.00 b	3.03 a	17.56 a	0.17 a	3.28 a	2.37 a	2.74 a	16.74 a	15.76 a	14.63 a	0.20 a	0.16 a	0.18 a
E2	15.00 b	2.52 b	16.68 a	0.15 b	3.02 a	2.26 a	2.59 a	17.00 a	14.04 a	12.75 b	0.19 ab	0.17 a	0.20 a
E3	17.00 a	2.34 b	17.04 a	0.14 b	2.50 b	2.03 b	2.49 a	14.36 b	11.90 b	12.44 b	0.17 b	0.22 a	0.20 a
Culture medium compositions													
CP1	14.81 a	2.90 a	15.61 b	0.18 a	3.33 a	2.22 a	2.61 ab	14.18 b	11.14 b	11.98 b	0.23 a	0.26 a	0.21 a
CP2	13.70 a	2.80 a	17.72 a	0.15 b	3.19 a	2.28 a	2.58 ab	14.14 b	15.04 a	13.51 ab	0.22 ab	0.15 b	0.19 a
CP3	11.11 a	2.54 ab	17.23 a	0.14 bc	3.24 a	2.23 a	2.94 a	16.21 b	14.68 a	13.94 a	0.19 b	0.16 ab	0.21 a
CP4	11.11 a	2.25 b	17.80 a	0.13 c	1.96 b	2.05 b	2.30 b	19.28 a	14.72 a	13.64 ab	0.10 c	0.14 b	0.16 b
	-	-	-	-	-	-	-	-	-	-	-	-	-
CV (%)	20.40	16.67	9.33	13.95	16.50	11.21	13.39	15.39	17.12	13.39	13.42	16.40	15.57

Remarks: SC = subculture. CV is the coefficient of variation. E1 = apical shoot sized 0.5 cm, E2 = apical shoot sized 1.0 cm, E3 = nodal segment. CP1 = MS for initiation stage, followed by MS+2.5 mg.L^{-1} GA_3 for subculture.; CP2 = MS+0.25 mg.L^{-1} BAP for initiation stage, followed by MS for subculture; CP3 = MS+0.25 mg.L^{-1} BAP for initiation stage, followed by MS+2.5 mg.L^{-1} GA_3 for subculture; CP4 = MS+0.5 mg.L^{-1} BAP for initiation stage, followed by MS+0.25 mg.L^{-1} BAP for subculture. Means followed by the same letters in the same column show no significant difference based on the Duncan Multiple Range Test with an alpha of 0.05. Sign (-) shows no interaction between explants and culture medium compositions.

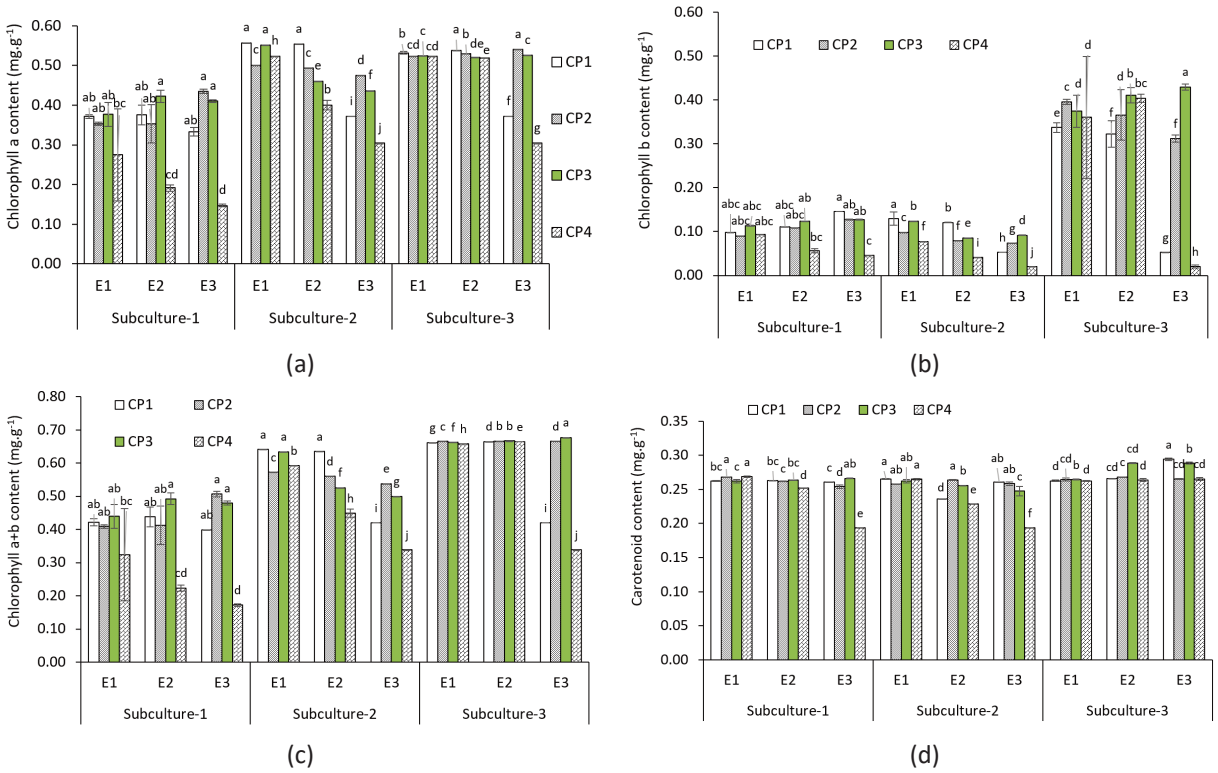


Figure 2. The effects of culture package and type of explants on the content of Chlorophyll a (a), b (b), a+b (c), and carotenoid (d) content (mg.g^{-1}) in plantlet leaf segments of chrysanthemum

Remarks: E1 = apical shoot sized 0.5 cm, E2 = apical shoot sized 1.0 cm, E3 = nodal segment. CP = Culture package. CP1 = MS for initiation stage, followed by MS+2.5 mg.L^{-1} GA_3 for subculture; CP2 = MS+0.25 mg.L^{-1} BAP for initiation stage, followed by MS without PGR for subculture; CP3 = MS+0.25 mg.L^{-1} BAP for initiation stage, followed by MS+2.5 mg.L^{-1} GA_3 for subculture; CP4 = MS+0.5 mg.L^{-1} BAP for initiation stage, followed by MS+0.25 mg.L^{-1} BAP for subculture. Means followed by the same letters in the same column show no significant difference based on the Duncan Multiple Range Test with an alpha of 0.05.

to the axillary part to function for the growth of axillary buds (Dierck et al., 2018). The application of different medium culture compositions in vitro influenced the performance of plantlets. The initiation medium using MS without PGR increased the height of the shoot. It showed that initiating chrysanthemum in vitro propagation did not need PGR. The MS medium without PGR has supported an expansion of the leaf cells (Arumugam et al., 2020).

The explant sources and PGR also affected the accumulation of metabolites in plant culture, including chlorophylls and carotenoids, and impacted the growth of plant culture (Kulus et al., 2020). The apical shoots sized 0.5 cm and 1.0 cm produced higher chlorophyll a, b, and a+b content than the nodal segment did. This result is in line with study reported by Lazare et al. (2021), stating that naturally, hormones and other secondary metabolites are accumulated in the apical shoot (Lazare et al., 2021). The MS medium supplemented with 2.5 mg.L^{-1} GA_3 or 0.25 mg.L^{-1} BAP for subculture produced higher chlorophyll content. Meziani et al. (2019) indicated that GA_3 increased shoot and root lengths compared to medium without PGR. The highest chlorophyll content was also caused by the medium supplemented with BAP used for proliferation rate and shoot production (Mihovilović et al., 2020). However, the composition of the CP4 medium (MS+0.5 mg.L^{-1} BAP followed by MS+0.25 mg.L^{-1} BAP) affected the decrease in chlorophyll a, b, and a+b in plantlets in the second and third subcultures. Using BAP for initiation followed by subculture increased the concentration of BAP in plants, which plays a role in cell division rather than cell expansion (Wu et al., 2017). In addition, using higher concentrations of BAP causes abnormalities in plantlets (Arumugam et al., 2020). Furthermore, the ability to regenerate chrysanthemum plantlets from the first up to the third subculture affects the quality of acclimatization results.

Table 3. The length of roots (cm) in the acclimatization stage

Explants	Culture medium compositions			
	CP1	CP2	CP3	CP4
Apical shoot sized 0.5 cm	4.33 ab	3.50 ab	4.00 ab	3.00 bc
Apical shoot sized 1.0 cm	4.75 a	3.75 ab	4.12 ab	3.58 ab
Nodal segment	3.03 bc	1.94 cd	4.46 ab	1.50 d
CV = 13.61%				

Remarks: CV is the coefficient of variation. CP1 = MS for initiation stage, followed by MS+2.5 mg.L^{-1} GA_3 for subculture; CP2 = MS+0.25 mg.L^{-1} BAP for initiation stage, followed by MS without PGR for subculture; CP3 = MS+0.25 mg.L^{-1} BAP for initiation stage, followed by MS+2.5 mg.L^{-1} GA_3 for subculture; CP4 = MS+0.5 mg.L^{-1} BAP for initiation stage, followed by MS+0.25 mg.L^{-1} BAP for subculture. Means followed by the same letters in the same column show no significant difference based on the Duncan Multiple Range Test with an alpha of 0.05.

Table 4. Plant growth in the acclimatization from in vitro propagation

Treatment	Variables			
	Height of plants (cm)	Number of leaves	Length of internodes (cm)	Diameter of stems (mm)
Explants				
Apical shoot sized 0.5 cm	4.56 a	8.25 a	0.61 a	1.61 a
Apical shoot sized 1.0 cm	4.21 a	7.64 ab	0.58 a	1.56 a
Nodal segment	4.04 a	6.23 b	0.70 a	1.44 a
Culture medium compositions				
CP1	4.58 a	9.44 a	0.48 c	1.82 a
CP2	4.24 ab	7.37 b	0.57 b	1.92 a
CP3	4.54 a	7.86 ab	0.58 b	1.19 b
CP4	3.74 b	4.77 c	0.88 a	1.22 b
	-	-	-	-
CV (%)	13.95	22.46	21.53	13.61

Remarks: SC = subculture. CV is the coefficient of variation. CP1 = MS for initiation stage, followed by MS+2.5 mg.L^{-1} GA_3 for subculture; CP2 = MS+0.25 mg.L^{-1} BAP for initiation stage, followed by MS for subculture; CP3 = MS+0.25 mg.L^{-1} BAP for initiation stage, followed by MS+2.5 mg.L^{-1} GA_3 for subculture; CP4 = MS+0.5 mg.L^{-1} BAP for initiation stage, followed by MS+0.25 mg.L^{-1} BAP for subculture. Means followed by the same letters in the same column show no significant difference based on the Duncan Multiple Range Test with an alpha of 0.05. Sign (-) shows no interaction between explants and culture medium compositions.

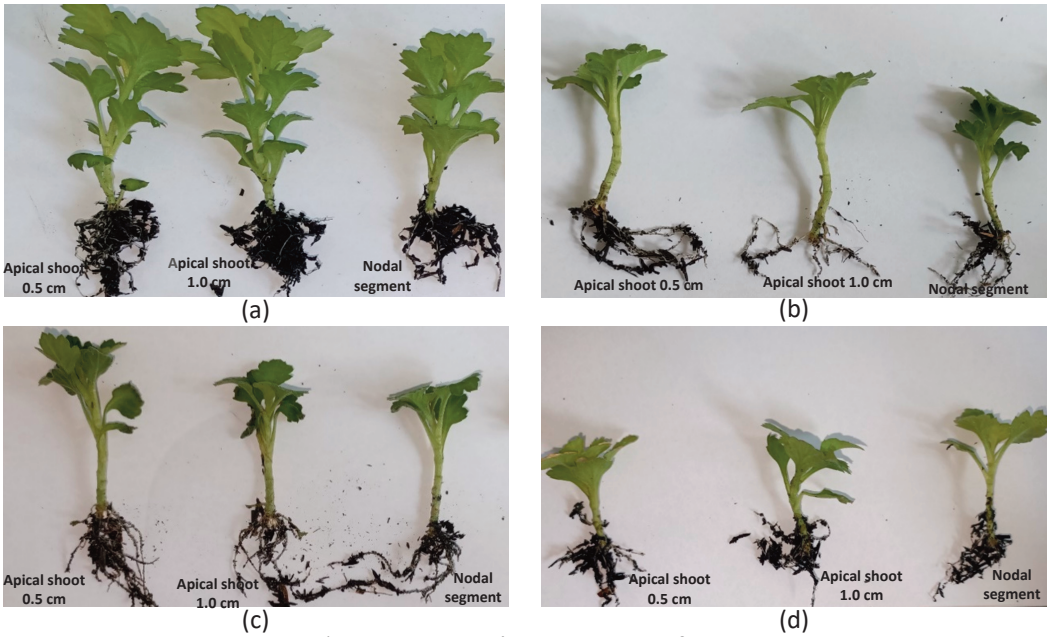


Figure 3. The chrysanthemum 'Jayanti Agrihorti' rooted cuttings from in vitro propagation in various culture package

Remarks: (a) CP1=MS for initiation stage, followed by MS+2.5 mgL^{-1} GA_3 for subculture, (b) CP2= MS+0.25 mgL^{-1} BAP for initiation stage, followed by MS without PGR for subculture, (c) CP3= MS+0.25 mgL^{-1} BAP for initiation stage, followed by MS+2.5 mgL^{-1} GA_3 for subculture, (d) CP4 = MS+0.5 mgL^{-1} BAP for initiation stage, followed by MS+0.25 mgL^{-1} BAP for subculture.

Acclimatization

There was no interaction between the explant sources and the culture package in plant height, leaf number, internode length, and shoot diameter variables at acclimatization. The apical shoots ranging in size from 0.5 to 1.0 cm produced a higher plant height, larger number of leaves, and a larger stem diameter than the nodal segment did. The CP1 medium provided the best performance. It produced plants with a height of 4.83 cm, a number of leaves of 9.44, a stem diameter of 1.82 mm, and a root length of 4.33 cm. Meanwhile, plantlets cultured on the CP4 medium had lower plant height, number of leaves, and diameter of the stem than plantlets cultured on other media (Table 2). Meanwhile, there was an interaction between the type of explant and culture package in the root length variable. The chrysanthemum rooted cuttings from the CP1 medium had the longest roots compared to those from the CP2 and CP4 media. The apical shoots sized 0.5 cm had the longest roots planted on the CP1–CP4 media. The nodal segment cultured on the CP4 medium produced the shortest roots (Table 3).

The apical shoots sized 0.5 and 1.0 cm produced a higher plant height, larger number of leaves, and bigger stem diameter than the nodal segment did (Table 4). This because shoot tips are the best explants for in vitro propagation because of their higher growth frequencies and disease-free plantlets (Arumugam et al., 2020). Then, the MS medium without PGR for initiation stage, followed by MS medium supplemented with 2.5 $mg.L^{-1}$ GA_3 for subculture stage produced the highest plant height, largest number of leaves, largest stem diameter, and longest root. The GA_3 regulates root growth by controlling cell elongation (Ubeda-Tomas et al., 2020). The root growth supports the growth of plant height, number of leaves and stem diameter. Meanwhile, plantlets cultured on MS+0.5 $mg.L^{-1}$ BAP for initiation stage, followed by MS+0.25 $mg.L^{-1}$ BAP for subculture produced shorter plant height, smaller number of leaves, and smaller stem diameter. The accumulation of BAP from initiation and first to third subculture caused the plants to be tuned in the plant of height, leaf number, and diameter of the stem. Also, culturing the nodal segment on medium supplemented with BAP from initiation to subculture stage inhibited the root growth. The BAP in in vitro culture interfered with the root length when planted in the acclimatization stage because BAP inhibited the mitotic index in root meristems (Polanco and Ruiz, 1997). Laplaze et al. (2007) mentioned that cytokinins could inhibit lateral root initiation directly in xylem pole pericycle cells.

This study showed that the apical shoots sized 0.5 cm were the best explants. Then, MS medium without PGR for initiation stage, followed by MS medium supplemented with 2.5 $mg.L^{-1}$ GA_3 for subculture was the best culture package to produce chrysanthemum rooted cuttings. However, the result of the culture package was on the contrary to the hypotheses. The performance of the plants could be seen in Figure 3.

CONCLUSIONS

The study concluded that apical shoots with the size of 0.5–1.0 cm were the optimum explants for producing chrysanthemum-rooted cuttings. Meanwhile, MS medium without PGR for initiation stage, followed by MS+2.5 $mg.L^{-1}$ GA_3 for subculture was the best culture medium composition in vitro propagation compared to the others. This explant and culture media composition produced higher chlorophyll a, b, and a+b content.

ACKNOWLEDGEMENTS

The authors are grateful to the Indonesian Ministry of Agriculture for funding this research through the Riset Pengembangan Inovatif dan Kolaboratif (RPIK) projects.

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