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EXPLORING IN VITRO POLYPLOIDIZATION IN CHRYSANTHEMUM CULTIVARS: EFFECTS OF COLCHICINE CONCENTRATIONS ON MORPHOLOGICAL AND PLOIDY VARIATIONS

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SUMMARY

The promising research aims to determine the response of three chrysanthemums (*Chrysanthemum morifolium* R.) cultivars to the concentration of colchicine on polyploidization of plantlets. Node cuttings of three cultivars of chrysanthemum, Pinka Pinky, Lolipop, and Maruta, received four different concentrations of colchicine (0.025%, 0.05%, 0.075%, and 0.1%), for four hours before being sub-cultured to MS media. Observations on the plantlets for morphological and ploidy levels ensued in comparison with the control treatment. Results showed significant variations among chrysanthemum cultivars in response to the colchicine concentrations. Triploids (3n) and Tetraploids (4n) were evident on plantlets of cultivar Pinka Pinky at colchicine concentrations (0.025%, 0.075%, and 0.1%) compared with other cultivars resulting in diploids and mixoploids. In the cultivar Pinka Pinky, the morphological variations emerged from the plantlets of the triploids (3n) and tetraploids (4n) in leaves and root numbers, plant height, stem segment number, and length. This recent study put a baseline for further study on the polyploidization of chrysanthemums using different sources of explant.

Keywords: Chrysanthemum (*C. morifolium* R.), cultivars, colchicine concentrations, in vitro, polyploidy, morphological variations

Key findings: In chrysanthemum (*C. morifolium* R.) cultivars, responses to different colchicine concentration varied and resulted in various triploids and tetraploid plants. Induced polyploidization due to colchicine also developed morphological variations for the leaves and root numbers, plant height, and stems segment parameters of the chrysanthemum cultivars.

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INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* R.) is the second-largest member of the cut flowers after roses traded globally (Patel *et al.*, 2021). It is an ornamental plant with high economic value and has become very popular in Indonesia for the last two decades. The improvement of chrysanthemum plants' quantity and quality can use plant breeding techniques in the form of in vitro mutation induction. Mutation breeding results in variations in the genetic structure that occur in the DNA, causing genetic diversity.

In vitro mutation can proceed by mutagen treatment, and colchicine ($C_{22}H_{25}O_6N$) can cause polyploidy in different plants (Hadi, 2019; Herlinda *et al.,* 2022). Colchicine functions as a chemical mutagen to produce polyploid plants with multiple sets of the chromosomes (Yulia *et al.,* 2022). Colchicine works by preventing the formation of microtubules and doubling the number of chromosomes to enhance the mutation and variations in a short period of time in crop plants (Aravind and Dhanavel, 2021).

Generally, colchicine works effectively at a concentration of 0.01%–1.00%; however, it also works efficiently at a concentration of 0.001%–1.00%, with a treatment time ranging from three to 24 hours (Shinta and Minarno, 2018). The concentration and duration of colchicine immersion can cause chromosome doubling. Colchicine concentrations (0.01% and 0.05%), with a soaking time of 12 and 24 hours, had the best effects on morphological observations in various chrysanthemum cultivars, viz., plant height, number of roots, number of leaves, and number of shoots (Daryono and Rahmadani, 2009).

Colchicine concentration of 0.04%, with a one-hour soaking time on the growth of Pasopati cultivar plantlets, had a better effect on morphological observations, such as, number of leaves, nodes, and roots (Nursalmin *et al.,* 2018). In hybrid dendrobium orchid plants, the colchicine with a concentration of 0.02%, at a soaking time of six hours, showed a significant impact, with best results on stem diameter and flower size (Sulistianingsih *et al.*, 2004).

Mutation induction through tissue culture propagation is a considered very effective accelerator of the desired in vitro selection, increasing plant diversity in a short time commercially, without affecting the basic characteristics of the original cultivar (Maluszynski *et al*., 1995). Similarly, past studies have reported in vitro mutagenesis in chrysanthemums (Haspolat, 2023). The colchicine recommended use can produce tetraploids, aneuploids, octoploids, and still diploid plants; however, the offspring will produce different characteristics. In mutation, the chemical compounds can easily break down into free radicals, and can react with amino acids, which can cause variations in the different properties.

Colchicine affects actively dividing cells by inhibiting the spindle thread mechanism starting from the prophase stage. This mechanism can happen through inhibition of the cell division process after doubling the DNA and chromosomes and developing an imbalance in chromosome migration during the mitosis process (Damayanti and Mariska, 2003). Based on the above description, it was necessary to carry out research on the polyploidization of three cultivars of chrysanthemum (*Chrysanthemum morifolium* R.) with various concentrations of colchicine through in vitro mutagenesis.

MATERIALS AND METHODS

The recent study conducted at the Plant Tissue Culture Laboratory, Hasanuddin University, Indonesia. The research work proceeded from May to August 2023. The generous provision of explants of the chrysanthemum came from the Bonto-Bonto Tissue Culture Laboratory, District Bontomarannu, Gowa Regency, Indonesia.

Experimental design

Node cutting of three chrysanthemum cultivars, Pinka Pinky, Lolipop, and Maruta received four colchicine concentration treatments (0.025%, 0.05%, 0.075%, and 0.1%) for four hours before being sub-cultured to MS media. A control treatment setup for

comparison had the cuttings treated with distilled water only. The treatments had three replications, with three culture bottles used for each treatment combination, resulting in a total of 135 bottles as experimental units. All the treatments used a split-plot design, with cultivars as the main plots and colchicine concentrations as the subplots.

Sterilization and colchicine treatments

Before planting, all the bottles and planting tools used underwent sterilization in an autoclave at 121 °C for 30 minutes. With the blower and lamp of the Laminar Air Flow Cabinet (LAF) switched on, spraying it with 96% alcohol continued, followed by drying with a tissue. Then, sterilizing the LAF employed the Ultraviolet for one hour. The colchicine treatment preparation initially made a 0.6% colchicine stock solution. The colchicine solution preparation continued in the laminar airflow. Weighing as much as 0.6 g colchicine proceeded placing into an Erlenmeyer added with 100 ml solution, consisting of 50 ml dimethyl sulfoxide solution and 50 ml sterile distilled water homogenized. The Erlenmeyer containing the colchicine solution remained in the refrigerator before use. Dilution of colchicine concentration in 100 ml transpired in the laminar using the dilution equation of M1.V1 = M2.V2. Making a liter of the colchicine treatment of 0.025%, 0.05%, 0.075%, and 0.1% continued by diluting the colchicine stock solution of 4.1, 8.3, 12.5, and 16.7 ml, respectively, in one liter of sterile distilled water.

Plantlet treatments and planting

The plant part used as polyploidy induction material was the stem of the chrysanthemum plantlet. The plant stems had their branches and leaves removed, with the stems with buds cut up to 1–3 cm in size. Before soaking, the stems sustained rinsing with sterile distilled water four times. Soaking ensued in a colchicine solution of 0% concentration (control: sterile distilled water), 0.025%, 0.05%, 0.075%, and 0.1%, with a soaking time of four hours. The stems' immersion in a culture bottle contained 10 ml of colchicine solution and shaken at 100 rpm on a shaker. After soaking, the explants' rinsing with sterile distilled water occurred four times, and then, planted in the MS culture media. Chrysanthemum plantlets' incubation continued for 12 weeks at 23 °C.

Morphological observations and polyploidy analysis

The morphological characteristics observed were the parameters of number of shoots, leaves and roots, plant height, and number and length of internodes. Polyploidy level analysis proceeded using flow cytometry (BD Accuri C6+, USA). Samples came from leaf pieces measuring approximately 0.5 cm × 0.5, placed on a Petri dish, then dripped with 250 µl of Nuclei Extraction Buffer and a little polyvidone, before chopping until fine with a razor blade. The filtered chopped leaves used a 30 µm Millipore sieve. The filtrate bore placing in a cuvette tube, and then, added with 350 µl of staining solution, propidium iodide, and RNase for analysis (Normasiwi *et al.,* 2021).

Data analysis

The data obtained from the different observations underwent assessment according to the analysis of variance (ANOVA), following the split-plot design in a randomized block design (RBD). After getting the significant differences among the treatments, the means' further comparison and separation employed the least significant difference ($LSD_{0.05}$) test. Correlation and regression analyses also proceeded to measure the relationship between two or more recorded research variables. The STAR (Statistical Tool for Agricultural Research) program and Microsoft Office Excel were tools used for the data analysis. Utilizing for ploidy analysis the flow cytometry tool (BD Accuri C6+, USA).

RESULTS

Genotypes and colchicine concentration treatments caused varying effects on the

Table 1. Percentage of plantlet life of three chrysanthemum cultivars from the second to the 12th week after in vitro colchicine induction.

Cultivars	Colchicine (%)	Percent of Survived Plantlets (%) at Observation Age					
		2	4	6	8	10	12
Pinka Pinky	0	100	100	100	100	100	100
	0.025	100	100	100	100	100	100
	0.05	100	94.44	94.44	94.44	94.44	94.44
	0.075	88.89	77.78	77.78	77.78	77.78	77.78
	0.1	94.44	88.89	88.89	88.89	88.89	88.89
Lolipop	0	100	100	100	100	100	100
	0.025	94.44	83.33	83.33	83.33	83.33	83.33
	0.05	94.44	94.44	94.44	94.44	94.44	94.44
	0.075	88.89	77.78	77.78	77.78	77.78	77.78
	0.1	83.33	61.11	61.11	61.11	61.11	61.11
Maruta	0	100	100	100	100	100	100
	0.025	100	94.44	94.44	94.44	94.44	94.44
	0.05	94.44	77.78	77.78	77.78	77.78	77.78
	0.075	88.89	72.22	72.22	72.22	72.22	72.22
	0.1	77.78	44.44	44.44	44.44	44.44	44.44

Note: Plantlets contaminated with bacteria and fungi are not included as data on the percentage of plantlet death.

percentage of survival rate of chrysanthemum plantlets (Table 1). Most shoot deaths incurred characteristics of browning due to high concentrations of the colchicine mutagen treatment. A lethal dose of 50 occurred in the cultivar Maruta, with a colchicine concentration of 0.1%. Control treatment with a colchicine concentration of 0% on the plants of three cultivars of chrysanthemum showed no plantlet death until the 12th week. However, the colchicine concentrations (0.025%, 0.05%, 0.075%, and 0.1%) indicated the higher colchicine concentration giving a lower percentage of plantlet survival in all the chrysanthemum cultivars.

Based on the ploidy analysis using flow cytometry (BD Accuri C6+, USA), the results displayed the doubling of chromosomes after colchicine induction produces diploid (2n), triploid (3n), tetraploid (4n), and mixoploid (diploid + triploid chromosome sets) in the chrysanthemum plants (Table 2, Figure 1). In polyploids, the best treatment was evident in the cultivar Pinka Pinky, with colchicine concentration of 0.075% producing a tetraploid chromosome set (4n), with a coefficient of variation value of 4.79%. However, it was not much different from the treatment with 0.1% concentration, which also produced a tetraploid chromosome set, with a coefficient of variation value of 6.10%.

Triploid chromosome sets appeared in the cultivars Pinka Pinky and Maruta treated with colchicine concentrations of 0.025% and 0.075%, respectively. Coefficient of variation values calculated for both treatments were 6.05% and 7.88%, respectively. However, the cultivar Lolipop, with a colchicine treatment of 0.1%, produced a mixoploid chromosome set (diploid + triploid). In this treatment, the triploid chromosome set was more stable, with a coefficient of variation value (6.47%) compared with their diploid counterpart (10.31%). The control treatments resulted in diploid (2n) plants in all cultivars of the chrysanthemum.

The regression analysis presented in Figure 2, indicated the cultivar Pinka Pinky has the linear equation of $y = 9017.9x + 650.42$, $r = 0.8137$, and R2 = 0.6621. The correlation value ($r = 0.8137$) means a significant positive relationship occurred between the colchicine concentration treatment and an increase in the formation of the ploidy index of chrysanthemum cultivar Pinka Pinky plants. The R2 value (0.6621/66.21%) means the effect of colchicine concentration during 12 weeks of incubation of the cultivar Pinka Pinky was 66.21% in the chrysanthemum.

Table 2. Results of polyploidy analysis of the three chrysanthemum cultivars resulting from in vitro colchicine induction.

Cultivar	Colchicine (%)	Mean PI	CV (%)	Chromosome dominant	
Pinka Pinky	0	719.277	10.41	2n	Diploid
	0.25	953.717	6.05	3n	Triploid
	0.05	700.123	9.54	2n	Diploid
	0.075	1.620.170	4.79	4n	Tetraploid
	0.1	1.513.290	6.10	4n	Tetraploid
Lolipop	0	743.364	5.88	2n	Diploid
	0.25	720.752	13,31	2n	Diploid
	0.05	773.180	8.12	2n	Diploid
	0.075	782.348	6.87	2n	Diploid
	0.1	778.613	10,31	2n	Mixoploid
		948.559	6,47	3n	(Diploid + Triploid)
Maruta	0	774.386	5,32	2n	Diploid
	0.25	778.353	13,16	2n	Diploid
	0.05	789.595	6.37	2n	Diploid
	0.075	1.146.457	7,88	3n	Triploid
	0.1	710.061	6.52	2n	Diploid

Mean PI (Ploidy Index), CV% (coefficient of variation).

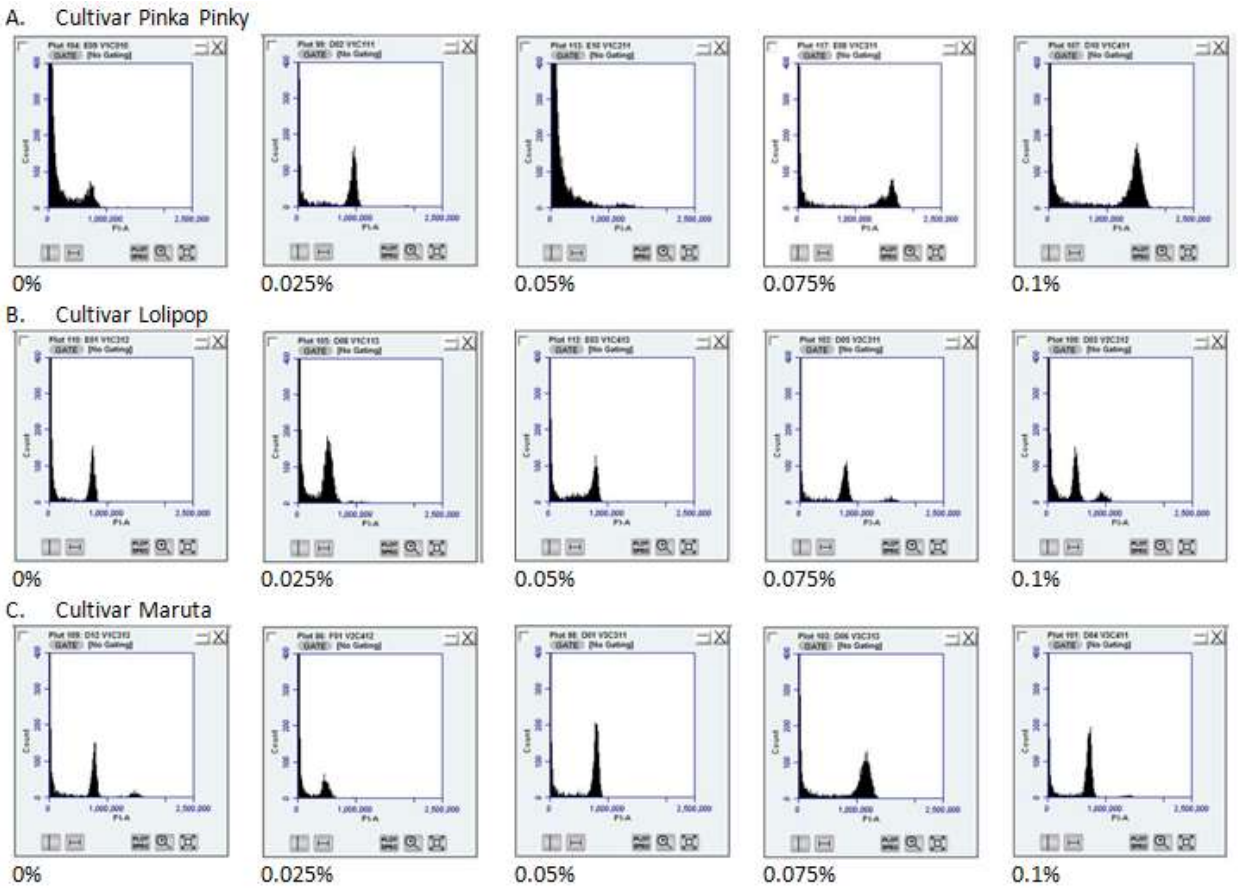

Figure 1. Results of polyploidy analysis using flow cytometry on three cultivars of chrysanthemum induced by colchicine in vitro.

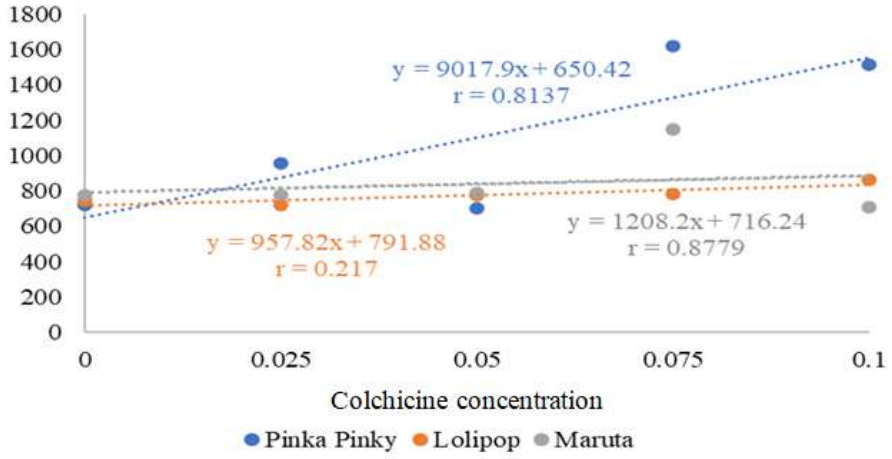

Figure 2. Results of regression analysis between the colchicine concentrations and in vitro chrysanthemum plant ploidy index values.

Table 3. Morphological characters of triploids (3n) and tetraploid (4n) plantlets of Chrysanthemum at 12 WAP.

Characters	Triploids (3n) Pinka Pinky + 0.025%	Tetraploids (4n) Pinka Pinky + 0.075%	Tetraploids (4n) Pinka Pinky + 0.1%	Control (Pinka Pinky + 0%)
Number of Leaves (leaves)	8.83	4.00	2.00	7.92
Number of Roots (roots)	7.00	1.00	1.00	4.67
Plant Height (cm)	11.75	5.00	2.50	4.78
Number of Segments	8.50	3.67	1.00	6.17
Segment Length (cm)	1.40	0.50	0.25	0.77

Cultivar Lollipop emerged with the linear equation of y = 957.82x + 791.88, r = 0.217, and R2 = 0.0472. The correlation value (r = 0.217) means the least relationship existed between the colchicine concentration treatment and an increase in the formation of the ploidy index in the chrysanthemum cultivar Lollipop plants. The R2 value (0.047/4.7%) implies the effect of colchicine concentration during 12 weeks of incubation of cultivar Lollipop plants was only 4.7%. Meanwhile, the linear equation for the cultivar Maruta was y = 1208.2x + 716.24, r = 87.795, and R2 = 0.770. The correlation (r = 0.87) revealed a significant positive relationship between the colchicine concentration treatment and an increase in the formation of the ploidy index of the cultivar Maruta plants. The R2 value (0.77/77%) enunciated the colchicine concentration effect during 12 weeks of incubation of cultivar Maruta plants was 77%.

Overall, the colchicine treatments significantly affected the morphological characters of chrysanthemums planted in vitro. Compared with the control, all the tetraploid plantlets showed a decline in the values of morphological characters (Table 3). However, the triploid plantlets exhibited an enhancement in leaf and root numbers, plant height, segment number, and length in the chrysanthemum.

DISCUSSION

Polyploidization process comprised alterations in the number of chromosomes in the cells of an individual to more than the normal number of chromosomes. In crop plants,

polyploidization can be applicable using colchicine, a chemical compound that can duplicate the chromosomes in cells. This compound functions by inhibiting cell division in plants, resulting in cells with double the number of chromosomes than the normal number. Colchicine is vital in prohibiting the activity of the chromosome-binding threads (spindle), hence, the chromosomes which divided do not separate during anaphase in cell division (Nst *et al*., 2018).

The results revealed flow cytometry analysis of chrysanthemum plant chromosome doubling reached tetraploid (4n). Apart from that, the analysis also provided the diploid, triploid, and mixoploid chromosome sets. This indicates chrysanthemum plants can become polyploidy by treating the cultivars with colchicine at different concentrations. The best treatment, cultivar Pinka Pinky with colchicine concentration (0.075%), produced a tetraploid chromosome set (4n), with a coefficient of variation of 4.79%. Colchicine is a known compound that can inhibit microtubules in metaphase during mitosis, and the concentration required for this inhibition is usually relatively high (Zhang *et al.,* 2020). Although, the same concentration was not significantly different from the colchicine treatment 0.1%, which also produced the tetraploid chromosome set, the higher concentration gave a better coefficient of variation value of 6.10%. In the presented study, the mean ploidy index value of the control plants, namely 700,000, served as a reference; thus, one can say the mean PI value was 700,000 – 900,000 (2n), the mean PI value was 900,000 – 1,200,000 (3n), 1,200,000 – 1,500,000 (4n), and so on. Therefore, to determine the ploidy level, one must know the mean PI value of the control plants. The coefficient of variation (CV) value helped determine whether the resulting peak is desirable or not; however, a better CV must be small.

Cultivar Pinka Pinky easily undergoes polyploidization compared with the two other cultivars of chrysanthemum, Lollipop and Maruta. The ploidy analysis showed the colchicine concentration of 0.025% produced 3n, while 0.075% and 0.1% produced the tetraploid plants, and the others only produced up to 3n chromosomes in chrysanthemum. Administration of colchicine can produce tetraploid, aneuploid, octoploid, and still diploid plants; however, the offspring will produce different morphological characteristics (Damayanti and Mariska, 2003).

Mixoploid plants have diploid and triploid/tetraploid nuclei, also assessed based on the relative number of nuclei (Cimen, 2020). In polyploidy induction, a high percentage of mixoploid yields are generally recognizable as a drawback of the procedure because the unstable polyploidy state often reverts partially or completely to the diploid state after successive cell division cycles (Esfahani *et al.,* 2020). In the mixoploid shoot, the development of two different cells causes competition during its growth.

The ploidy analysis revealed chromosome doubling occurs randomly, giving a non-uniform effect on each cell in an individual. Giving colchicine to plants in vitro can grow callus because colchicine is a chemical compound that can inhibit cell division by interfering with spindle formation during mitosis. This causes plant cells to not divide normally and produces undifferentiated cells or calluses. The callus formed can then be induced to form new shoots (Kwun *et al.*, 2013).

The colchicine 0% concentration (control) displayed the best morphology because the colchicine is toxic and causes stress to the plants, thus affecting the growth of plant morphology (Figure 3). Therefore, in vitro mutation research needs to be sub-cultured again to see further differences between the 0% concentration and other concentrations of colchicine (0.025%, 0.05%, 0.075%, and 0.1%). However, the plants are no longer stressed due to the colchicine mutagen treatment. Colchicine is highly toxic to plants; hence, its low doses are quite reliable to reduce its toxic effects (Sajjad *et al.,* 2013; Mohsin *et al*., 2023; Marir, 2024). The optimal dose of colchicine used in polyploid production varies widely, with concentrations ranging from 0.01% (Thao *et al.,* 2003) to 1.0% (Demtsu *et al.,* 2013).

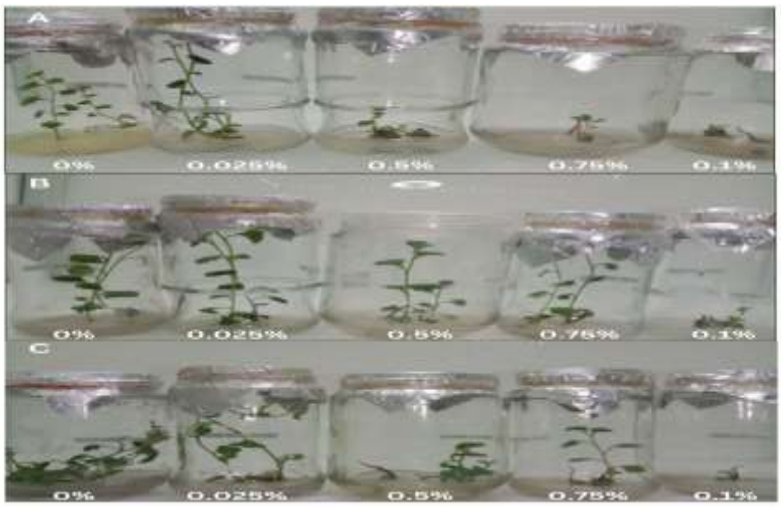

Figure 3. Morphological appearance of chrysanthemum cultivars 12 weeks after in vitro colchicine induction in the control, (A) Pinka Pinky, (B) Lolipop, and (C) Maruta.

Regression analysis states the relationship between the independent and dependent variables. The regression analysis disclosed the multiple r value (0.76) was significant, which means a considerable positive association exists between the colchicine concentrations and the ploidy index value in chrysanthemum cultivars. The coefficient of determination value was 0.447 (44.7%), which means x explains y by 44.7%, with the rest influenced by other factors.

CONCLUSIONS

A varied response to colchicine concentration in inducing tetraploids in chrysanthemum cultivars was evident. Producing tetraploid (4n) plants in the cultivar Pinka Pinky, the colchicine concentrations were 0.075% and 0.1%. Nevertheless, inducing polyploidization in chrysanthemum can occur with a lower colchicine concentration (0.025%). Colchicine-induced tetraploid had some morphological variations, such as, leaves and root numbers, plant height, and stem segment characters. The higher the concentration of colchicine can decrease the morphological parameter values in chrysanthemum plantlets.

REFERENCES

Aravind S, Dhanavel D (2021). Induced physical and chemical mutagenesis on marigold (*Tagetes erecta* L.) to determine the lethality, germination and seedling survivability. *Int. J. Bot. Stud.* 6: 235-237.

Cimen B (2020). Induction of polyploidy in C35 Citrange through in vitro colchicine treatments of seed-derived explants. *Int. J. Fruit Sci.* 20: 1929-1941.

Damayanti FI, Mariska I (2003). Induction of polyploidy with colchicine in F1 hybrids resulting from crosses between species on vanilla plants from Ciamis. *Ber. Biol.* 6: 589-594.

Daryono BS, Rahmadani WD (2009). Phenotypic characteristics of chrysanthemum plants (*Dendranthema grandiflorum*) big yellow cultivar result of colchicine treatment. *J. Trop. Agron*. 14: 15-18.

Demtsu B, Taychasinpitak T, Wongchaochant S, Manochai B (2013). Induced mutation by colchicine treatment of somatic embryos in 'namwa' banana (*Musa* sp. ABB). *Int. Trans. J. Eng. Manag*. 4: 311-320.

Esfahani ST, Karimzadeh G, Naghavi MR (2020). In vitro polyploidy induction in Persian poppy (*Papaver bracteatum* Lindl.). *Caryologia* 73: 133-144.

Hadi RA (2019). Application of concentration of liquid complementary fertilizer (PPC) on the growth of several polyploid chrysanthemum genotypes. *Agro. Wiralodra* 2: 52-59.

Haspolat G (2023). Induction of mutagenesis on Chrysanthemums. *Ornam. Hortic*. 28: 431-441.

Herlinda TO, Sianturi RUD, Triastinurmiatiningsih T (2022). Inducing chromosomal mutations using colchicine against tembesu plantlets (*Fragraea fragrans*. Roxb) in-vitro. *J. Perbenihan Tanam. Hutan.* 10: 131-148.

Kwun HJ, Shuda M, Feng H, Camacho CJ, Moore PS, Chang Y (2013). Merkel cell polyomavirus small T antigen controls viral replication and oncoprotein expression by targeting the cellular ubiquitin ligase SCFFbw7. *Cell Host Microbe* 14: 125-135.

Maluszynski M, Ahloowalia BS, Sigurbjomsson B (1995). Application in vivo and in vitro mutation techniques for crop improvement. *Euphytica* 85: 303-315.

Marir EMA (2024). Effect of carbon sources and tyrosine on the accumulation of bioactive compound (pyrethrins) in *Chrysanthemum hortorum*. *SABRAO J. Breed. Genet.* 56(2): 846-857. http://doi.org/10.54910/sabrao2024.56.2.35.

Mohsin RM, Abd Asal KN, Kamaluddin AA, Zaky AA (2023). Genotypes and storage duration effects on the quality of cut flower - gerbera (*Gerbera jamesonii* Hook). *SABRAO J. Breed. Genet*. 55(1): 260-267. http://doi.org/10.54910/sabrao2023.55.1.24.

Normasiwi S, Efendi M, Rahman W, Hafiizh EA, Ermayanti TM, Lelono RA, Yunarto N (2021). Growth, stomata and trichome characteristics of diploid and tetraploid *Artemisia annua* L. plants. *IOP Conf. Ser. Earth Environ. Sci.* 762: 012022.

Nst MWA, Setiado H, Damanik RIM (2018). The effect of colchicine on the genotypic and phenotypic diversity of the aglaonema plant (*Aglaonema cochinense* Schott Var. Lady Valentine). *J. Agroteknologi* 6: 599-608.

Nursalmin A, Komariah A, Hidayat O (2018). Effect of colchicine soaking time on Chrysanthemums plantlet growth (*Chrysanthemum morifolium* R.) of Pasopati variety in vitro method. *Paspalum: J. Ilm. Pertan.* 6: 124-133.

Patel D, Suthar R, Solanki HA (2021). Chemical preservation for increasing shelf life of *Chrysanthemum indicum* L. cut flower. *Res. Rev: J. Environ. Sci.* 3: 1-7.

Sajjad Y, Jaskani MJ, Mehmood A, Ahmad I, Abbas H (2013). Effect of colchicine on in vitro polyploidy induction in African marigold (*Tagetes erecta*). *Pak. J. Bot.* 45: 1255-1258.

Shinta S, Minarno EB (2018). Phenotypic characteristics of black rice plants (*Oryza sativa* L.) wojalaka variety result of induction with colchicine. *Prosiding Seminar Nasional Sains dan Teknologi Terapan*. 1: 43-52.

Sulistianingsih R, Suyanto N, Anggia (2004). Improving the quality of hybrid dendrobium orchids by giving colchicine. *Ilmu Pertanian* 11: 13-21.

Thao NTP, Ureshino K, Miyajima I, Ozaki Y, Okubo H (2003). Induction of tetraploids in ornamental alocasia through colchicine and oryzalin treatments. *Plant Cell, Tissue and Organ Culture* 72: 19-25.

Yulia N, Prihantoro I, Karti PDMH (2022). Optimizing the use of colchicine mutagen to increase productivity of stylo plants (*Stylosanthes guianensis* [Aubl.] Sw.). *J. Ilmu Nutrisi dan Teknol. Pakan* 20: 19-24.

Zhang YS, Chen JJ, Cao YM, Duan JX, Cai XD (2020). Induction of tetraploids in 'Red Flash' caladium using colchicine and oryzalin: Morphological, cytological, photosynthetic and chilling tolerance analysis. *Scientia Hortic.* 272: 109524.