BIODIVERSITAS
Volume 25, Number 8, August 2024
Pages: 3496-2503

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250821

Polyploidization of three *Chrysanthemum* varieties in vitro at various levels of colchicine soaking length

FERANITA HARING[1], MUH. FARID[1,♥], IFAYANTI RIDWAN[1], MUHAMMAD FUAD ANSHORI[1], ANNASTYA NUR FADHILAH[1], KYLA BADZLIN HARTANTO[2]

[1]Department of Agronomy, Faculty of Agriculture, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km 10, Makassar 90245, South Sulawesi, Indonesia. Tel./fax.: +62-411-586014, ♥email: farid_deni@yahoo.co.id

[2]Program of Agrotechnology, Faculty of Agriculture, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km 10, Makassar 90245, South Sulawesi, Indonesia

Manuscript received: 25 June 2024. Revision accepted: 11 August 2024.

Abstract. *Haring F, Farid M, Ridwan I, Anshori MF, Fadhilah AN, Hartanto KB. 2024. Polyploidization of three* Chrysanthemum *varieties in vitro at various levels of colchicine soaking length. Biodiversitas 25: 3496-2503.* The demand for new types and varieties of chrysanthemums with unique characteristics, such as various colors, shapes, and sizes, continues to increase. One approach that can be taken to achieve this diversity is through mutation techniques using chemical mutagens such as colchicine to obtain polyploid plants. This research, which aims to determine the best varieties and soaking time for colchicine in forming polyploid plants in vitro, has the potential to impact the field of *Chrysanthemum* breeding significantly. The research was arranged in a split-plot design (SPD), with the main plot being the *Chrysanthemum* variety (v), consisting of the Pinka Pinky (v1), Lollipop (v2), and Maruta (v3) varieties. The subplot is the soaking time at a colchicine concentration of 0.05%, which consists of 0 hours (t0), 4 hours (t1), 8 hours (t2), and 12 hours (t3). The parameters observed were the number of shoots, number of leaves, number of roots, time to sprout, time to root, time to form plantlets, and polyploidy analysis. Chromosome doubling in chrysanthemum plants after 0.05% colchicine induction with different soaking times produced mixoploid plants or the highest chromosome doubling of 9.18% + 18.82%, namely in the Lollipop variety with a soaking time of 12 hours. Meanwhile, other treatments did not show chromosome doubling and remained diploid but had different levels of chromosome variation.

Keywords: *Chrysanthemum*, colchicine, in vitro, mutation, polyploidization

INTRODUCTION

Chrysanthemums are superior ornamental plants that are usually used as cut flowers or to be cultivated in pots with increasing popularity and are now the main focus in development (Mekapogu et al. 2022; Datta 2023). The need for chrysanthemums in Indonesia is relatively high, ranging from 1,000 to 1,500 pots for exhibitions and 5,000 for weddings. Apart from domestic needs, chrysanthemums are also exported to other countries, with export volume in 2021 reaching 131.4 tons, which increased by 67.8% to 220.6 tons in 2022, according to data from the BPS (2023). However, *Chrysanthemum* flower production in Indonesia in 2022 amounted to 323.61 million stalks, a decrease of 5.94% compared to the previous year, which reached 344.03 million stalks, even though demand for new types and varieties with unique characteristics continues to increase. Therefore, a solution is needed to produce new types and varieties of *Chrysanthemum* plants with distinctive traits, such as various colors, shapes, and sizes.

One approach that can be taken to achieve this diversity is through mutation techniques, which offer a very wide variety that can create a significant market due to the diversity of flower types and colors (Yoosumran et al. 2018; Mekapogu et al. 2020; Datta 2023). The polyploidization mutation technique can open new opportunities in developing *Chrysanthemum* varieties (Niazian and Nalousi 2020). Polyploidization is a change in which the entire set of chromosomes is duplicated through the process of mitosis or meiosis, which involves unreduced gametes and hybridization between species (Donne et al. 2020; Shariatpanahi et al. 2021). Artificial polyploidization generally uses chemicals, one of which is colchicine. The use of colchicine functions to prevent the formation of spindles in meristem cells in the mitosis stage so that the chromosomes divide into two, but in metaphase and anaphase, this does not occur and results in the number of chromosomes in the cell doubling (Forkosh et al. 2020; Khah et al. 2022). Colchicine functions as a polyploidy-inducing agent by inhibiting cell division at the mitosis stage (Roy et al. 2020; Touchell et al. 2020; Hailu et al. 2021). Thus, administering colchicine is a method that can be used to increase plant production by producing polyploid individuals in vitro (Widoretno et al. 2023; Hernandez et al. 2024). The resulting polyploid plants can potentially increase genetic strength and disease resistance and improve the quality of specific traits (Liu et al. 2022; Omere et al. 2022). Therefore, using colchicine for in vitro vegetative growth is an effective strategy for improving plant genetics to support plant resistance and production.

Polyploid organisms have cells with more than two sets of chromosomes (Fox et al. 2020; Mezzasalma et al. 2021). An increase generally follows chromosome doubling in plant morphological size, such as an increase in fruit,

flowers, stems, leaves, and roots (Miri 2020; Arindyaswari eta al. 2021; Hooghvorst and Nogues 2021). The size of the stomata in polyploid plants is generally larger, and the density of the stomata is reduced (Yao et al. 2023). Polyploidization does not always provide a positive response to plants. Success in producing polyploid cells depends on several factors, including the part of the plant being processed, the species, and the colchicine used (Touchell et al. 2020). Using colchicine on each plant will give a different response, depending on the concentration and length of soaking (Mastuti et al. 2022). Using colchicine in high concentrations and for a long time will cause plant growth to be hampered, so an effective concentration and the right soaking time are needed (Mangena 2021; Mastuti et al. 2022). Several studies have shown the effect of soaking time and colchicine concentration on the growth and characteristics of *Chrysanthemum* plants. Daryono and Rahmadani (2009) found that soaking for 12 and 24 hours with a concentration of 0.05% gave the best results on plant height, number of roots, leaves, and shoots. On the other hand, Nursalmin et al (2018) studied the Pasopati variety and found that a concentration of 0.04% colchicine in soaking for 1 hour had a better effect on the number of leaves, nodes, and roots, Sulistianingsih (2004) on hybrid dendrobium orchid plants showed that a concentration of 0.02% colchicine by soaking for 6 hours gave the best results on stem diameter and flower size. The results of the latest research by Sudirman (2022) on sapphira taro plants confirm that using colchicine at low concentrations and optimal soaking can effectively double chromosomes.

Previous research, including results from Heo et al. (2016), confirmed that careful soaking with low concentrations of colchicine can increase the efficiency of polyploidy production. Thus, applying this method provides a solid foundation for developing higher-quality *Chrysanthemum* varieties and opens the door to innovation in plant breeding. Based on the description above, research regarding the evaluation of long soaking treatments for several *Chrysanthemum* varieties in vitro is an interesting topic to develop. This research aims to identify the interaction of several *Chrysanthemum* varieties at various levels of colchicine soaking time and determine the varieties and soaking time that result in polyploidization in *Chrysanthemum* plants in vitro.

MATERIALS AND METHODS

Study area

The research was conducted at the Tissue Culture Laboratory, Department of Agricultural Cultivation, Faculty of Agriculture, Universitas Hasanudin, Makassar, South Sulawesi, Indonesia, from August to November 2023.

Procedures

Sterilization of bottles, planting tools, and laminar air flow cabinet (LAFC)

The bottles and tools are sterilized in an autoclave at a temperature of 121°C with a pressure of 17.5 psi (pounds per square inch) for one hour. Please turn on the blower and laminar lamp, spray the laminar with 70% alcohol, and dry it with tissue. *Laminar Air Flow Cabinet* (LAF) in UV for one hour.

Media preparation

Media preparation begins with making a stock solution with the composition of MS media, with the volume of each MS stock being 1,000 mL. Stock solution preparation is done by dissolving the chemical composition with sterile distilled water, then adjusting the pH to 5.8, placing it in a pan, and adding agar. Heat the media on an electric stove, then remove and pour 25 mL into each culture bottle after boiling. Next, the culture bottle was placed in culture wine for one week to determine the sterility of the media.

Colchicine solution preparation

Preparation of the colchicine solution begins with making a 0.6% colchicine stock solution (0.6 g colchicine/100 mL with a ratio of 50 mL DMSO and 50 mL sterile distilled water), which is modified by Sudirman (2022). Dilution of the colchicine solution for immersion treatment in 100 mL was carried out in laminar at a concentration of 0.05% by taking 8.3 mL of the colchicine stock solution and adding 91.7 mL of sterile distilled water, soaking the plantlets in colchicine solution and planting using in vitro plantlet shoots of *Chrysanthemum* plants, which are then used as explants for polyploidy induction material.

Maintenance

Maintenance was done by spraying 70% alcohol on the culture bottle to avoid contamination in each research treatment. This is carried out every day. If there is contamination, the culture bottle is immediately removed from the incubation room.

Data analysis

The research was arranged in a split-plot design (RPT), with the main plot being the *Chrysanthemum* variety (v), consisting of the Pinka Pinky (v1), Lolipop (v2) and Maruta (v3) varieties, as well as subplots is the soaking time at a colchicine concentration of 0.05%, consisting of 0 hours (t0), 4 hours (t1), 8 hours (t2) and 12 hours (t3). Each treatment was repeated three times so that there were 108 observation units. Data were analyzed using the F test to determine the interaction between varieties and soaking time. If the variance is real, continue with the least significant difference (LSD) further test at the 5% level and the orthogonal polynomial correlation test by calculating at the r-value; an r-value of 0 means there is no correlation between the two variables, 0-0.25 means very low correlation, 0.25-0.5 means medium correlation, 0.5-0.75 means high correlation, 0.75-0.99 means very high correlation and 1 means perfect correlation (Bargawa and Syahputra 2021) and calculate the R2 value to see how much an independent variable influences the dependent variable (Ozili 2023). The parameters observed were the number of shoots and leaves. Shoots were counted up to 12 WAP, excluding those at the base of the shoot. Leaves were counted based on the number that grew after soaking.

Other parameters included the maximum 12 WAP based on fully open leaves, the number of roots based on roots that have formed a maximum of 12 WAP, and the start of life when root nodules appear. The time to sprout (DAP) was determined by the day the first shoot appeared on the sample after soaking, the time to rooting (DAP) by the day the first roots appeared on the sample after soaking, and the time to forming plantlets (DAP) by the day the shoots and roots first formed with leaves. Polyploidy analysis was conducted using flow cytometry.

RESULTS AND DISCUSSION

Analysis of variance in the number of shoots, number of leaves, and number of roots

The results of variations in the characteristics of the number of shoots, leaves, and roots in Table 1 show that the variety treatment did not have a significant effect. On the other hand, the length of soaking and the interaction between the two greatly influence the number of *Chrysanthemum* shoots. Furthermore, the character's number of leaves and roots, variations in treatment, length of soaking, and course interactions influence these two characters. Regarding the number of shoots, the interaction of the Maruta variety without colchicine soaking (v3t0) gave the best number of shoots with an average value of 2.33. It was similar with soaking times of 4 hours (t1) and 12 hours (t3) and is very different from the soaking time, namely eight hours (t2). Regarding the number of leaves, the interaction of lollipop varieties without colchicine soaking (v2t0) gave the best number of leaves with an average value of 15.00. Significantly different from the other three levels of soaking time. For the character of the number of roots, the interaction between lollipop varieties with a soaking time of 8 hours (v2t2) gave the best number of roots with an average value of 7.67. Significantly different from the other three levels of soaking time.

Analysis of sprouting, rooting, and plantlet formation with the time variations

The results of the variation in characteristics of sprouting time, rooting time, and plantlet formation time in Table 2 not only demonstrate the significant impact of variations in treatment, soaking time, and their interaction on these three characters but also point to potential applications in agriculture and horticulture. For instance, the interaction of the pinka pinky variety without colchicine soaking (v1t0) produced the best rooting time, with an average value of 5.67 DAP, significantly different from the other three levels of soaking time. Similarly, the interaction of lollipop varieties without colchicine soaking (v2t0) led to the best sprouting time and form plantlets, with average values of 6.11 DAP and 6.22 DAP, respectively, and was significantly different from the other three immersion time levels.

Table 1. Characteristics of the number of shoots, number of leaves, and number of roots of several *Chrysanthemum* varieties at various levels of colchicine soaking time

Soaking length	Variety v1	Variety v2	Variety v3	CV (v) BNT 0.05
Number of shoots				
t0	1 $^{c}_{p}$	2 $^{b}_{p}$	2.33 $^{a}_{p}$	0.267
t1	1 $^{a}_{p}$	1 $^{a}_{q}$	1 $^{a}_{p}$	
t2	1 $^{a}_{p}$	1 $^{a}_{q}$	0 $^{b}_{r}$	
t3	1 $^{a}_{p}$	1 $^{a}_{q}$	1 $^{a}_{p}$	
Average	1	1.25	1.08	
NP(t) BNT 0.05	0.143			
Number of leaves				
t0	8.00 $^{c}_{q}$	15.00 $^{a}_{p}$	13.33 $^{b}_{p}$	0.741
t1	2.33 $^{c}_{r}$	4.83 $^{b}_{r}$	6.00 $^{a}_{r}$	
t2	11.67 $^{a}_{p}$	4.00 $^{b}_{s}$	0.00 $^{c}_{q}$	
t3	0.33 $^{c}_{s}$	8.00 $^{b}_{q}$	9.00 $^{a}_{s}$	
Average	5.58	7.96	7.08	
NP(t) BNT 0.05	0.481			
Number of roots				
t0	4.67 $^{b}_{p}$	5.83 $^{a}_{q}$	4.67 $^{b}_{p}$	0.591
t1	1.33 $^{b}_{r}$	1.67 $^{b}_{r}$	3.00 $^{a}_{q}$	
t2	4.00 $^{b}_{q}$	7.67 $^{a}_{p}$	0.00 $^{c}_{s}$	
t3	1.33 $^{b}_{r}$	1.00 $^{b}_{s}$	2.33 $^{a}_{r}$	
Average	2.83	4.04	2.50	
NP(t) BNT 0.05	0.370			

Notes: Numbers followed by the same letter in the same column (a, b, c) and row (p, q, r) mean that they are not significantly different in the BNT 0.05 follow-up test

Table 2. Characteristics of various characteristics of germination time, rooting time, and plantlet formation time for several *Chrysanthemum* varieties at various levels of colchicine soaking time

Soaking length	Variety v1	Variety v2	Variety v3	CV (v) BNT 0.05
Sprouting time				
t0	6.50 $^{a}_{s}$	6.11 $^{a}_{r}$	7.00 $^{a.m}$	1.834
t1	29.00 $^{c}_{r}$	40.67 $^{a}_{q}$	36.00 $^{b}_{q}$	
t2	33.78 $^{b}_{q}$	50.56 $^{a}_{p}$	0.00 $^{c}_{s}$	
t3	42.31 $^{c}_{p}$	50.89 $^{b}_{p}$	58.33 $^{a}_{p}$	
Average	27.90	37.06	25.33	
NP(t) BNT 0.05	1,353			
Root time				
t0	5.67 $^{a}_{s}$	5.72 $^{a}_{r}$	6.00 $^{a.m}$	1.834
t1	37.31 $^{a}_{r}$	38.56 $^{a}_{q}$	36.50 $^{a}_{q}$	
t2	83.25 $^{a}_{p}$	39.93 $^{b}_{q}$	0.00 $^{c}_{q}$	
t3	80.22 $^{b}_{q}$	82.98 $^{a}_{p}$	64.61 $^{c}_{p}$	
Average	51.61	41.80	26.78	
NP(t) BNT 0.05	1,353			
Shaping planlet time				
t0	6.50 $^{a}_{s}$	6.22 $^{a}_{s}$	7.00 $^{a.m}$	1.760
t1	37.31 $^{b}_{r}$	40.67 $^{a}_{r}$	38.50 $^{b}_{q}$	
t2	83.25 $^{a}_{p}$	50.56 $^{b}_{q}$	0.00 $^{c}_{s}$	
t3	80.22 $^{b}_{q}$	82.98 $^{a}_{p}$	64.61 $^{c}_{p}$	
Average	51.82	45.11	27.53	
NP(t) BNT 0.05	1,732			

Notes: Numbers followed by the same letter in the same column (a, b, c) and row (p, q, r) mean they are not significantly different in the BNT 0.05 follow-up test

Polynomial orthogonal correlation test

Number of shoots

The correlation value of the number of shoots (Figure 1) for the Pinka Pinky variety shows a value of (r)=0, meaning that there is no relationship between the length of colchicine soaking and the character of the number of shoots of the Pinka Pinky variety. In comparison, the correlation value for the Lollipop variety (r=0.96607) and the Maruta variety (r=0.97442). This means that there is a very close positive relationship between the length of soaking in colchicine and the characteristics of the number of shoots of the two varieties. The $R^{2\ value}$ for the Pinka Pinky variety shows a value of $R^{2=}$0%, meaning that there is no contribution from the long colchicine soaking treatment to the number of shoots of the Pinka Pinky variety. The R 2 value for the Lolipop and Maruta varieties is respectively R^2=0.9333 and R^2=0.9495, meaning that the contribution of the long-term colchicine soaking treatment to the number of shoots of the Lolipop variety was 93.3% and Maruta was 94.9%.

Number of leaves

The correlation value of number of leaves (Figure 2) for the Pinka Pinky variety (r=0.463141), meaning that there is a weak positive relationship between the length of colchicine soaking and the number of leaves for the Pinka Pinky variety, while the correlation value for the Lolipop varieties (r=0.993227) and Maruta (r=0.949052), meaning that there is There was a very close positive relationship between the length of colchicine soaking and the leaf number characteristics of the two varieties. The $R^{2\ values}$ for the Pinka Pinky, Lolipop, and Maruta varieties are respectively $R^{2=}$0.2145, $R^{2=}$0.9865, and $R^{2=}$0.9007, meaning that the contribution of the long soaking treatment of colchicine to the number of leaves of the Pinka Pinky variety is 21.5%, Lolipop is 98.7% and Maruta at 90.1%.

Number of roots

The correlation value of the number of roots (Figure 3) for the Pinka Pinky variety (r=0.551) and Lollipop (r=0.40694), meaning that there is a weak positive relationship between the length of soaking in colchicine and the number of roots for the two varieties, while the correlation value for the Maruta variety is (r=0), meaning there is no There is a relationship between the length of colchicine soaking and the number of roots of the Maruta variety. The $R^{2\ values}$ for the Pinka Pinky, Lolipop, and Maruta varieties are respectively $R^{2=}$0.3036, $R^{2=}$0.1656, and $R^{2=}$0.802, meaning that the contribution of the long soaking colchicine treatment to the number of roots of the Pinka Pinky variety is 30.4%, Lolipop is 16.6% and Maruta at 80.2%.

Sprouting time

The correlation value of sprouting time (Figure 4) for the Pinka Pinky varieties (r=0.983412), Lollipop (r=0.995741), and Maruta (0.646452), meaning that there is a very close positive relationship between the length of colchicine soaking and the germination time of these three varieties. The $R^{2\ values}$ for the Pinka Pinky, Lolipop, and Maruta varieties are respectively $R^{2=}$0.9671, $R^{2=}$0.9915, and $R^{2=}$0.4179, meaning that the contribution of the long soaking colchicine treatment to the germination time of the Pinka Pinky variety is 96.7%, Lolipop is 99.2% and Maruta at 41.8%.

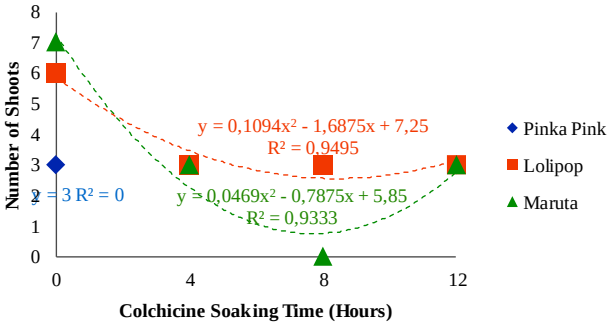

Figure 1. Orthogonal polynomial interaction graph of the influence of variety and length of colchicine soaking on the shoot number characteristics of *Chrysanthemum* plants in vitro

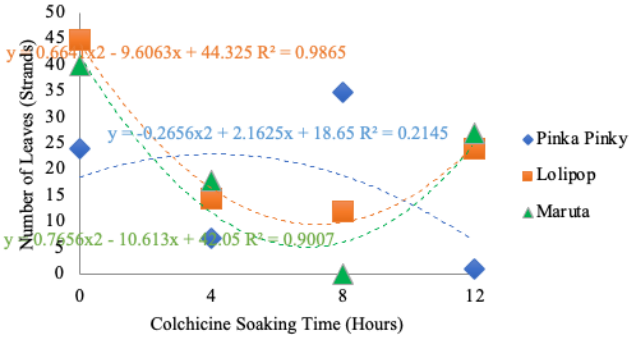

Figure 2. Orthogonal polynomial interaction graph of the influence of variety and length of colchicine soaking on the character of the number of leaves of *Chrysanthemum* plants in vitro

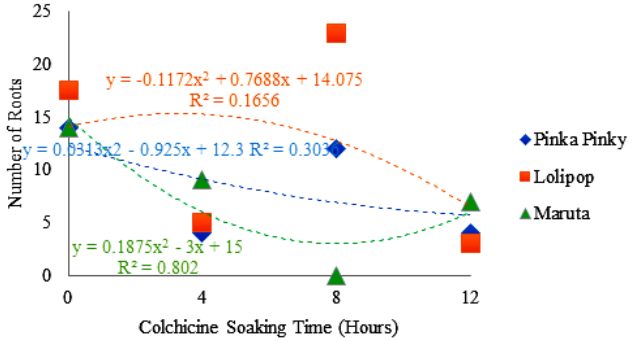

Figure 3. Orthogonal polynomial interaction graph of the influence of variety and length of colchicine soaking on the root number characteristics of *Chrysanthemum* plants in vitro

Time takes root

The correlation value of time takes root (Figure 5) for the varieties Pinka Pinky (r=0.9755), Lollipop (r=0.954568), and Maruta (0.686804), meaning that there is a very close positive relationship between the length of soaking in colchicine and the rooting time of these three varieties. The R^2 values for the Pinka Pinky, Lolipop, and Maruta varieties are respectively $R^{2=}$0.9516, $R^{2=}$0.9112, and $R^{2=}$0.4717, meaning that the contribution of the long colchicine soaking treatment to the rooting time of the Pinka Pinky variety is 95.2%, Lolipop is 91.1% and Maruta at 47.2%.

Time to form plantlets

The correlation value of time to form plantlets (Figure 6) for the Pinka Pinky varieties (r=0.974372), Lollipop (r=0.981326), and Maruta (0.663174), meaning that there is a very close positive relationship between the length of colchicine soaking and the rooting time of these three varieties. The R^2 values for the Pinka Pinky, Lolipop, and Maruta varieties are respectively $R^{2=}$0.9494, $R^{2=}$0.963, and $R^{2=}$0.4398, meaning that the contribution of the long soaking colchicine treatment to the rooting time of the Pinka Pinky variety is 94.9%, Lolipop is 96.3% and Maruta at 43.9%.

The differences in phenotype of various *Chrysanthemum* varieties are shown in Figure 7 and the differences in phenotype of chrysanthemums at various colchicine immersion times are shown in Figure 8.

Ploidy analysis of the three *Chrysanthemum* plant varieties induced by colchicine *in vitro*

Based on the results of ploidy analysis using the flow cytometry method in Table 3, it shows that chromosome doubling in chrysanthemums after induction of 0.05% colchicine with different soaking times produced mixoploid plants or the highest chromosome doubling of 9.18+18.82%, namely in the Lollipop variety with a soaking time of 12 hours (v2t3) (Table 3). Meanwhile, other treatments did not show chromosome doubling and remained diploid but had different levels of chromosome variation.

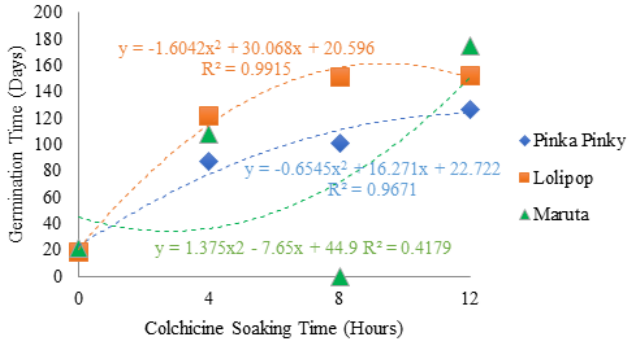

Figure 4. Orthogonal polynomial interaction graph of the influence of variety and length of colchicine soaking on the characteristics of *Chrysanthemum* germination time in vitro

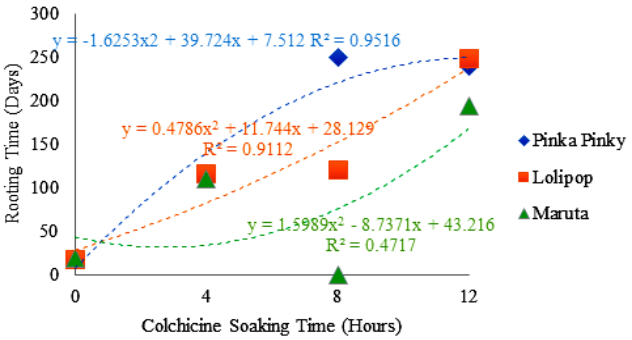

Figure 5. Orthogonal polynomial interaction graph of the influence of variety and length of colchicine soaking on the character of rooting time of *Chrysanthemum* plants in vitro

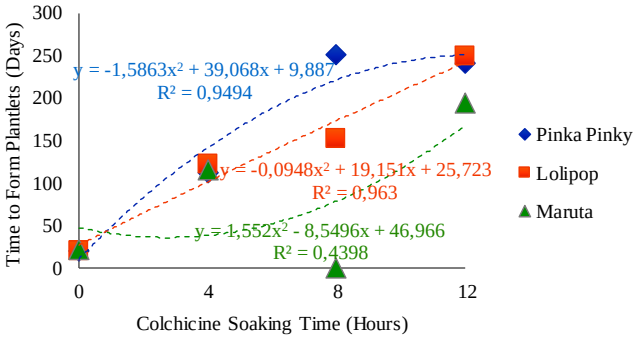

Figure 6. Orthogonal polynomial interaction graph of the influence of variety and length of colchicine soaking on the characteristics of *Chrysanthemum* plantlet formation time in vitro

Table 3. Results of ploidy analysis of the three *Chrysanthemum* varieties induced by colchicine in vitro

Treatment	Chromosomes detected	Mean-x	% Gated	Chromosomal dominance	Information
v1t2 (1)	2n	254.70	29.38	2n	Diploid
v1t2 (2)	2n	243.59	28.20	2n	Diploid
v1t2 (3)	2n	263.00	16.22	2n	Diploid
v2t3 (1)	2n-3n	264.17 352.16	9.18+18.82	3n	Mixoploid
v2t3 (2)	2n	219.40	39.39	2n	Diploid
v2t3 (3)	2n	206.38	14.89	2n	Diploid
v3t3 (1)	2n	206.37	33.78	2n	Diploid
v3t3 (2)	2n	219.13	38.57	2n	Diploid
v3t3 (3)	2n	219.66	24.30	2n	Diploid

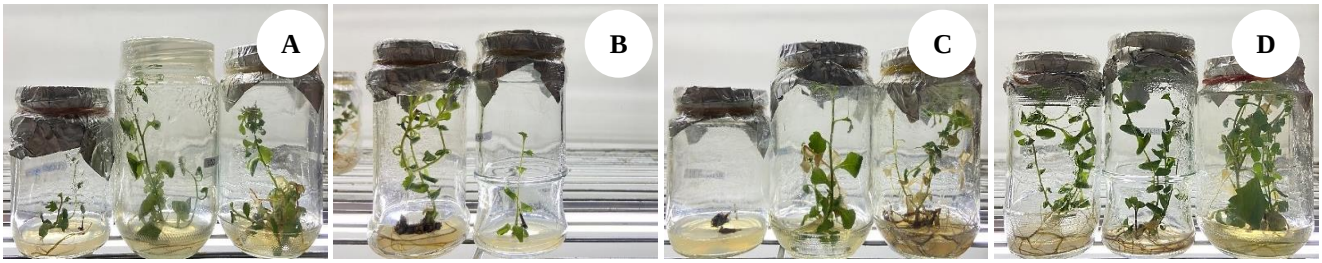

Figure 7. Growth of the three varieties (Pinka Pinky, Lollipop, and Maruta) of *Chrysanthemum* plants with a soaking time of A. 4 hours; B. A soaking time of 8 hours; C. A soaking time of 12 hours; D. The control

Figure 8. Growth of the A. Pinka Pinky variety; B. Lollipop variety; C. Maruta variety at 4 hours soaking time, 8 hours soaking time, 12 hours soaking time, and control

Discussion

The analysis of variance (Tables 1 and 2) shows that the treatment of *Chrysanthemum* varieties and soaking time and their interaction had a significant influence on almost all the observed characters, except for the observed character treatment of *Chrysanthemum* varieties on the number of shoots. Generally, a fairly large variance analysis can be an initial basis for selecting important characteristics in evaluating technology packages for growth and production (Fadhilah et al. 2022). This concept has been reported by Abduh et al. (2021) and Farid et al. (2022) in maize. Therefore, all characters can still be evaluated further with deeper analysis.

The effect of long-term colchicine soaking treatment with a contribution of more than 90% on the Lollipop and Maruta varieties shows that both varieties have susceptibility or sensitivity to colchicine soaking so that both show a quadratic response, in contrast to the Pinka Pinky variety, which does not show any response. This happens because long periods of soaking in colchicine can inhibit the growth of shoots or the morphology of a plant (Fathurrahman 2023). The Pinka Pinky variety is a Fiji pink-derived variety tolerant to 0.05% colchicine treatment. The effect of administering colchicine up to a specific dose can increase plant size, but an increase that exceeds the cell capacity to adapt to this mitosis-inhibiting agent can hurt cell growth, causing a decrease in shoot growth (Mo et al. 2020; Mangena and Mushadu 2023).

Observations of the characteristics of the number of leaves, sprouting time, rooting time, and plantlet formation time, which decreased with increasing soaking time, showed that long periods of soaking in plants caused plantlet growth to be hampered. On the other hand, soaking for a shorter duration or even no soaking treatment showed increased growth. This occurs due to longer soaking in colchicine, where polyploid induction often results in incompatibility in the induced plants. The finding that the Lollipop variety, with a soaking time of 8 hours, showed the highest number of roots, indicating its potential tolerance to the colchicine soaking period, is of significant importance. It underscores our research's impact on plant genetics and its potential to revolutionize plant breeding strategies.

Based on the phenotype of each *Chrysanthemum* variety shown in Figure 7, the Lollipop variety (v2) is a variety that is more tolerant and has better growth compared to the other two varieties in the overall soaking treatment given, namely, soaking time of 4 hours (A), soaking time of 8 hours (B), soaking time of 12 hours (C) and control (D). Likewise, Figure 8 shows the phenotypic performance of each variety, with the Lollipop (v2) (B) variety showing a more tolerant phenotypic appearance and better growth. The difference in response of the three varieties to colchicine administration was due to genetic differences in each variety. Plant sensitivity to colchicine is influenced by gene expression and different genetic regulations, where each variety has a unique gene expression pattern (Sun et al. 2020; Mangena and Mushadu 2023). Some varieties have a higher colchicine resistance or tolerance level than others (Siregar et al. 2022; Zeinullina et al. 2023; Hernandez et al. 2024). In addition, other factors can influence plant responses, such as differences in chromosome structure and polyploidization.

The results of polyploidization analysis using the flow cytometry method showed that the lollipop variety with a soaking time of 8 hours (v2t3) had the highest ploidy level with a mixoploid ploidy level of 9.18+18.82%. Mixoploid plants are organisms with a mixture of cells with various

ploidy levels in the same tissue or organ, the existence of which provides more complex internal genetic variation in producing different traits in an individual plant and can show more adaptability traits (Avila et al. 2020; Niazian and Nalousi 2020). Depending on the environment, however, the characteristics of mixoploid plants are often difficult to predict due to genetic differences in an organism (Julião et al. 2020; Goluch et al. 2021). Varieties that experience chromosome doubling are expected to be able to produce plants with larger flower sizes, rounder flower shapes, and deeper flower colors (Niazian and Nalousi 2020; Atoche et al. 2022). Sometimes, the results of polyploidization do not experience chromosome doubling (remaining diploid); several things cause this; according to Fomicheva et al. (2024), the selection and concentration of antimitotic substances and additional compounds, exposure times, and application methods should be tested on small populations of plants of interest or available plants, without doubling, increasing the concentration of antimitotic substances, exposure times, or different application methods can test.

The use of colchicine to induce chromosomal changes in the mitosis process does not always occur in every plant where it is applied. This is influenced by factors such as the type of plant, the dose of colchicine, and the time of application (Manzoor et al. 2019). This research indicates a significant interaction between different varieties of *Chrysanthemum* plants and the length of colchicine soaking, which causes polyploidization. Therefore, a combination of the lollipop variety with a soaking time of 8 hours (v2t3) is recommended for polyploidization of *Chrysanthemum.*

Based on research, the soaking time that produces polyploidy in chrysanthemums is the colchicine soaking time of 12 hours, and the *Chrysanthemum* variety that experiences polyploidy in in-vitro conditions is the lollipop variety. These findings provide insight into the influence of soaking duration and variety differences on the polyploidization process in chrysanthemum plants, which has important implications in plant breeding and tissue culture.

REFERENCES

Abduh ADM, Padjung R, Farid M. 2021. Interaction of genetic and cultivation technology in maize prolific and productivity increase. Pak J Biol Sci 24: 716-723. DOI: 10.3923/pjbs.2021.716.723.

Atoche JAV, Iiyama CM, Cardoso JC. 2022. Polyploidization in orchids: from cellular changes to breeding applications. Plants 11 (4): 469. DOI: 10.3390/plants11040469.

Arindyaswari A, Etikawati N, Suratman. 2021. Effect of colchicine on chromosome number, morphological character and ß-carotene production of *Amaranthus tricolor*'s red giti cultivar. Cell Biol Dev 5: 18-24. DOI: 10.13057/cellbioldev/v050103.

Avila AG, Fortea EG, Prohens J, Herraiz FJ. 2020. Development of a direct in vitro plant regeneration protocol from *Cannabis sativa* L. seedling explants: Developmental morphology of shoot regeneration and ploidy level of regenerated plants. Front Plant Sci 11: 534467. DOI: 10.3389/fpls.2020.00645.

Bargawa WS, Syahputra HH. 2021. Coal quality study using multivariate geostatistics. AIP Conf Proceed 2363 (1): 030012-11. DOI: 10.1063/5.0061103.

BPS [Badan Pusat Statistik]. 2023. Indonesian Horticulture Statistic. https://www.bps.go.id/publikasi.html.

Datta SK. 2023. *Chrysanthemum* in Role of Mutation Breeding in Floriculture Industry. Springer Nature Singapore, Singapore.

Daryono BS, Rahmadani WD. 2009. Karakter fenotipe tanaman krisan (*Dendranthema grandiflorum*) kultivar big yellow hasil perlakuan kolkisin. Jurnal Agrotropika 14 (1): 15-18. [Indonesian]

Donne R, Aïnama SM, Cordier P, Morizur CS, Desdouets C. 2020. Polyploidy in liver development, homeostasis and disease. Nat Rev Gastroenterol Hepatol 17: 391-405. DOI: 10.1038/s41575-020-0284-x.

Fadhilah AN, Farid M, Ridwan I, Anshori MF, Yassi A. 2022. Genetic parameters and selection index of high-yielding tomato F2 populations. Sabrao J Breed Genet 54 (5): 1026-1036. DOI: 10.54910/sabrao2022.54.5.6.

Farid M, Anshori MF, Ridwan I. 2022. Tomato F3 Lines development and its selection index based on narrow-sense heritability and factor analysis. Biodiversitas 23: 5790-5797. DOI: 10.13057/biodiv/d231132.

Fathurrahman F. 2023. Effect of colchicine mutagen on phenotype and genotype of *Vigna unguiculata* var. *sesquipedalis* the 7th generation. Biodiversitas 24: 1408-1415. DOI: 10.13057/biodiv/d240310.

Fomicheva M, Kulakov Y, Alyokhina K, Domblides E. 2024. Spontaneous and chemically induced genome doubling and polyploidization in vegetable crops. Horticulturae 10: 551. DOI: 10.3390/horticulturae10060551.

Forkosh E, Kenig A, Ilan Y. 2020. Introducing variability in targeting the microtubules: Review of current mechanisms and future directions in colchicine therapy. Pharmacol Res Perspect 8 (4): e00616. DOI: 10.1002/prp2.616.

Fox DT, Soltis DE, Soltis PS, Ashman TL, Van de Peer Y. 2020. Polyploidy: A biological force from cells to ecosystems. Trends Cell Biol 30 (9): 688-694. DOI: 10.1016/j.tcb.2020.06.006.

Goluch AT, Lipińska MK, Wielgusz K, Praczyk M. 2021. Polyploidy in industrial crops: Applications and perspectives in plant breeding. Agronomy 11 (12): 2574. DOI: 10.3390/agronomy11122574.

Hailu MG, Mawcha KT, Nshimiyimana S, Suharsono S. 2021. Garlic micro propagation and polyploidy induction in vitro by colchicine. Plant Breed Biotechnol 9 (1): 1-19. DOI: 10.9787/PBB.2021.9.1.1.

Heo JY, Jeong SH, Choi HR, Park SM. 2016. Polyploidy production in *Lilium leichtlinii* var. *maximowiczii* using colchicine. Anim Plant Sci 26: 1111-1116.

Hernández R, López A, Valenzuela B, Afonseca VD, Gomez A, Arencibia AD. 2024. Organogenesis of plant tissues in colchicine allows selecting in field trial blueberry (*Vaccinium* spp. cv Duke) clones with commercial potential. Horticulturae 10 (3): 283. DOI: 10.3390/horticulturae10030283.

Hooghvorst I, Nogués S. 2021. Chromosome doubling methods in doubled haploid and haploid inducer-mediated genome-editing systems in major crops. Plant Cell Reports 40 (2): 255-270. DOI: 10.1007/s00299-020-02605-0.

Julião SA, Ribeiro CDV, Lopes JML, Matos EMD, Reis AC, Peixoto PHP, Viccini LF. 2020. Induction of synthetic polyploids and assessment of genomic stability in *Lippia alba*. Front Plant Sci 11: 292. DOI: 10.3389/fpls.2020.00292.

Khah MA, Alshehri MA, Filimban FZ, Alam Q, Aloufi S. 2022. Influence of colchicine in causing severe chromosomal damage in microsporocytes of hard wheat (*Triticum durum* Desf.): Possible mechanisms and genotoxic relevance. Cytologia 87 (2): 137-143. DOI: 10.1508/cytologia.87.137.

Liu J, Yao Y, Xin M, Peng H, Ni Z, Sun Q. 2022. Shaping polyploid wheat for success: Origins, domestication, and the genetic improvement of agronomic traits. J Integr Plant Biol 64 (2): 536-563. DOI: 10.1111/jipb.13210.

Mangena P, Mushadu PN. 2023. Colchicine-induced polyploidy in leguminous crops enhances morpho-physiological characteristics for drought stress tolerance. Life 13: 1966. DOI: 10.3390/life13101966.

Mangena P. 2021. Effect of water stress on growth response of soybean plants pretreated with colchicine. ISHS Acta Horticult 1327: 121-128. DOI: 10.17660/ActaHortic.2021.1327.15.

Manzoor A, Ahmad T, Bashir MA, Hafiz IA, Silvestri C. 2019. Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants. Plants 8: 194. DOI: 10.3390/plants8070194.

Mastuti R, Munawarti A, Afiyanti M. 2022. The effect of colchicine on in vitro growth of ciplukan (*Physalis angulata*). IOP Conf Ser: Earth Environ Sci 1097: 012055. DOI: 10.1088/1755-1315/1097/1/012055.

Mekapogu M, Kwon OK, Song HY, Jung JA. 2022. Towards the improvement of ornamental attributes in *Chrysanthemum:* Recent progress in biotechnological advances. Intl J Mol Sci 23 (20): 12284. DOI: 10.3390/ijms232012284.

Mekapogu M, Vasamsetti BMK, Kwon OK, Ahn MS, Lim SH, Jung, JA. 2020. Anthocyanins in floral colors: Biosynthesis and regulation in chrysanthemum flowers. Intl J Mol Sci 21 (18): 6537. DOI: 10.3390/ijms21186537.

Mezzasalma M, Brunelli E, Odierna G, Guarino FM. 2023. Evolutionary and genomic diversity of true polyploidy in tetrapods. Animals 13 (6): 1033. DOI: 3390/ani13061033.

Miri SM. 2020. Artificial polyploidy in the improvement of horticultural crops. J Plant Physiol Breed 10 (1): 1-28.

Mo L, Chen J, Lou X, Xu Q, Dong R, Tong Z, Lin E. 2020. Colchicine-induced polyploidy in *Rhododendron fortunei* Lindl. Plants 9 (4): 424. DOI: 10.3390/plants9040424.

Niazian M, Nalousi AM. 2020. Artificial polyploidy induction for improvement of ornamental and medicinal plants. Plant Cell Tissue Organ Cult 142 (3): 447-469. DOI: 10.1007/s11240-020-01888-1.

Nursalmin A, Komariah A, Hidayat O. 2018. Pengaruh lama perendaman kolkisin terhadap pertumbuhan planlet (*Chrysanthemum morifolim* R) krisan varietas pasopati cara in vitro. Paspalum: Jurnal Ilmiah Pertanian 6 (2): 124-133. DOI: 10.4314/njb.v39i2.2.

Omere EA, Nwaoguala CNC, Emede TO. 2022. Polyploidy and its relevance in crop improvement. Niger J Biotechnol 39 (2): 9-19. DOI: 10.4314/njb.v39i2.2.

Ozili PK. 2023. The Acceptable R-Square In Empirical Modelling For Social Science Research. IGI Global, Hershey, Pennsylvania. DOI: 10.2139/ssrn.4128165.

Roy S, Kundu LM, Roy GC, Barman M, Chakraborty T, Ghosh P, Ray S. 2020. Deciphering colchicine-like actions of clerodin in terms of microtubule destabilization based mitotic abnormalities, G2/M-phase arrest, and plant polyploidy. bioRxiv 2020 (12): 1-35. DOI: 10.1101/2020.12.27.424481.

Shariatpanahi ME, Niazian M, Ahmadi B. 2021. Methods for chromosome doubling. Doubled Haploid Technol Vol 1: General Topics Alliaceae Cereals 1: 127-148. DOI: 10.1007/978-1-0716-1315-35.

Siregar M, Siregar LAM, Hanum C. 2022. Induction of mutation with colchicine in olimpus potato by in vitro culture. IOP Conf Ser: Earth Environ Sci 977: 012020. DOI: 10.1088/1755-1315/977/1/012020.

Sudirman. 2022. Induksi Mutasi Poliploidi Tanaman Talas Safira (*Colocasia esculenta* var. *antiquorum)* Pada Berbagai Konsentrasi dan Lama Perendaman Kolkisin Secara In Vitro. [Thesis]. Universitas Hasanuddin, Makassar, Indonesia. [Indonesian]

Sun FY, Liu L, Yu Y, Ruan XM, Wang CY, Hu QW, Sun G. 2020. MicroRNA-mediated responses to colchicine treatment in barley. Planta 251: 1-14. DOI: 10.1007/s00425-019-03326-9.

Touchell DH, Palmer IE, Ranney TG. 2020. In vitro ploidy manipulation for crop improvement. Front Plant Sci 11: 517580. DOI: 10.3389/fpls.2020.00722.

Widoretno W, Azriningsih R, Sukmadjaja D, Rosyidah M. 2023. In vitro induction and identification of polyploid *Amorphophallus muelleri* Blume plants by colchicine treatment. AGRIVITA J Agric Sci 45 (1): 87-97. DOI: 10.55927/ajcs.v2i10.6615.

Yao PQ, Chen JH, Ma PF, Xie LH, Cheng SP. 2023. Stomata variation in the process of polyploidization in Chinese chive (*Allium tuberosum*). BMC Plant Biol 23 (1): 595. DOI: 10.1186/s12870-023-04615-y.

Yoosumran V, Ruamrungsri S, Duangkongsan W, Kanjana S. 2018. Induced mutation of *Dendranthemum grandiflora* through tissue culture by ethyl methanesulphonate (EMS). Intl J Agric Technol 14 (1): 73-82.

Zeinullina A, Zargar M, Dyussibayeva E, Orazov A, Zhirnova I, Yessenbekova G, Hu YG. 2023. Agro-morphological traits and molecular diversity of proso millet (*Panicum miliaceum* L.) affected by various colchicine treatments. Agronomy 13 (12): 2973. DOI: 10.3390/agronomy13122973.