RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
58 (2) 731-741, 2026
http://doi.org/10.54910/sabrao2026.58.2.23
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

RESPONSE OF CHRYSANTHEMUM M1 MUTANTS TO BENZYL AMINO PURINE (BAP) WITH IN VITRO PROPAGATION

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SUMMARY

Chrysanthemum (*Chrysanthemum morifolium* L.) is an economically important ornamental crop whose improvement increasingly relies on mutation breeding and efficient in vitro propagation. The successful mutant propagation highly comes from influences of plant growth regulators, particularly benzyl amino purine (BAP), which plays a key role in shoot induction. This study aimed to determine the response of 15 M1 chrysanthemum mutants to different BAP concentrations in shoot proliferation under in vitro conditions. The experiment used a split-plot design, with BAP concentrations (0, 0.5, 1.0, 1.5, and 2.0 ppm) as main plots and 15 M1 mutants as subplots. Observed parameters included plantlet height, internode length, and the number of roots, internodes, leaves, and shoots. Data analysis used ANOVA (analysis of variance), correlation, and orthogonal polynomial regression. Flow cytometry (BD Accuri C6+) evaluated ploidy status. Results identified the number of shoots as the primary trait, with significant genotype × BAP interaction. The M4m mutant treated with 1.0 ppm BAP produced the highest average number of shoots, indicating superior responsiveness. Supporting traits also positively correlated with shoot proliferation. Ploidy analysis revealed chromosome doubling in several mutants, particularly m2p. Overall, 1.0 ppm BAP emerged as recommended for efficient in vitro propagation of chrysanthemum M1 mutants, with M4m as the most responsive genotype.

Keywords: Chrysanthemum (*C. morifolium* L.), mutants, BAP, plantlet height, internode length, number of shoots and leaves

Communicating Editor: Dr. Gwen Iris Descalsota-Empleo

Manuscript received: March 5, 2025; Accepted: January 8, 2026.

Citation: Haring F, Farid M, Dungga NE, Musa Y, Ridwan I, Adnan, Fadhilah AN (2026). Response of chrysanthemum M1 mutants to Benzyl Amino Purine (BAP) with in vitro propagation. *SABRAO J. Breed. Genet. 58 (2) 731-741. http://doi.org/10.54910/sabrao2026.58.2.23*.

Key findings: The chrysanthemum (*C. morifolium* L.) mutant plants in interaction with BAP concentrations produced the highest number of shoots in in vitro mutant propagation. Based on the results, the BAP concentrations were great recommendations for better propagation of chrysanthemum M1 mutant plants.

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* L.) is an ornamental plant in the form of cut and potted flowers with high economic value and is famous in Indonesia (Lintang *et al.*, 2020; Udayana *et al.,* 2023). According to the Central Statistics Agency (2024), the chrysanthemum plant production reached 465,359,950 stalks in 2019, then decreased in 2020 to 2022, with an average output of 373,999,738 stalks, and in 2023, it again increased (464,604,008 stalks). Therefore, the chrysanthemum plant needs thorough attention to considerably enhance its production. However, due to low export volume and quality, the chrysanthemum plants do not meet the quality standards desired by the consumer countries. The quality standards of chrysanthemum plants desired by consumer countries include large flowers, upright stems with a plant height of ±70 cm, clean flowers with no spots, and sturdy crowns that do not fall off easily (Mekapogu *et al.*, 2022; Abhishek and Bala, 2023). Therefore, in Indonesia, various efforts are essential to improve consumer quality standards for better international market prices.

Quality improvement can prevail through plant breeding with in vitro mutation induction. Hence, mutation breeding is one of the effective ways to increase the genetic variation in ornamental plants. Mutation breeding causes variations in the gene and chromosome structures, thus creating genetic diversity (Swarup *et al.*, 2021; Yali and Mitiku, 2022). In ornamental plants, mutation induction has reached wide development, aiming to produce genotypes with superior characteristics. Several varieties of chrysanthemum were derivatives of mutation breeding, with unique and commercially valuable characteristics (Haspolat, 2023; Tutuncu *et al.*, 2023). These past findings were greatly analogous to the establishment of in vitro techniques that play a vital role in accelerating the regeneration of mutants. Colchicine is one of the important mutagens often used in chromosome duplication. Colchicine, obtained from *Colchicum autumnale* seed extracts, can induce mutation and acquire polyploids at the right concentration and time (Hailu *et al.*, 2021; Murthy and Ramesh, 2021).

Their results showed colchicine concentrations (0.075% and 0.1%) resulted in polyploidization in chrysanthemum plants in vitro in the chrysanthemum variety Pinka Pinky, compared with the control and other lower concentrations. BAP is one type of ZPT, or plant growth regulator (PGR), that is often applicable in mutant evaluation in subculture activities. BAP is a group of active cytokinins, and by adding it to the media, it can encourage shoot proliferation with more shoots (Patmasari and Amarullah, 2020). However, BAP with a specific dose can better work as a growth hormone in shoot propagation and plantlet production. BAPs play a crucial physiological role by promoting cell division, stimulating meristematic activity, and breaking apical dominance; they help enhance shoot induction in in vitro culture. Previous studies also demonstrated the optimal BAP concentrations (0.5–1.5 ppm) significantly increased shoot multiplication in various ornamental and horticultural species, while excessively high levels may inhibit rooting or induce abnormal morphology.

The M1 mutants used in this study originated from colchicine-induced mutagenesis in several chrysanthemum varieties, resulting in genetically diverse mutant lines with potentially different physiological responses to plant growth regulators. The mutant codes—p, l, and m—represent different explant sources used during mutagenesis, which may contribute to variation in regeneration performance. Given these genetic differences, each mutant line

could exhibit distinct cytokinin sensitivity, making the evaluation of BAP concentrations essential for optimizing propagation outcomes.

The researchers hypothesized that different M1 chrysanthemum mutants would exhibit varied responses to BAP concentrations, and determining the optimal cytokinin level could significantly enhance shoot proliferation, the key trait for successful mutant propagation. Therefore, understanding the interaction between genotypes and BAP concentrations is crucial for establishing an effective micropropagation protocol and accelerating the selection of superior chrysanthemum mutants. The presented research aimed to enhance the production and improve the quality of chrysanthemum plants through mutation breeding with colchicine at various BAP concentrations in vitro, as well as obtain the chrysanthemum plant varieties with superior properties.

MATERIALS AND METHODS

Laboratory-scale research commenced in 2024 at the Plant Tissue Culture Laboratory, Department of Agricultural Cultivation, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. The study employed a split-plot design, with the main plots being the BAP concentrations, consisting of 0 ppm (k0), 0.5 ppm (k1), 1.0 ppm (k2), 1.5 ppm (k3), and 2.0 ppm (k4). The subplots were the M1 chrysanthemum mutants comprising M1p (m1), M2p (m2), M3p (m3), M4p (m4), M5p (m5), M1l (m6), M2l (m7), M3l (m8), M4l (m9), M5l (m10), M1m (m11), M2m (m12), M3m (m13), M4m (m14), and M5m (m15). The genotype groups' p, l, and m indicate the varietal origins of the mutants, with p derived from the variety Pinka Pinky, l from the variety Lollipop, and m from the variety Maruta. These mutants resulted from colchicine-induced mutagenesis involving different explant sources, resulting in three distinct genetic backgrounds. Treatment combinations totaled 75, with each treatment repeated three times, with the number of units for each treatment in each replication being two units; hence, 450 experimental units in all.

The implementation of the study comprised sterilization of the room, bottles, and planting tools; preparation of the culture media (1000 ml of MS medium); and planting (subculture) on MS medium equipped with ZPT-BAP according to the treatment, with each culture bottle consisting of two explants. The MS medium contained 30 g L^{-1} sucrose and 7 g L^{-1} agar, with the pH adjusted to 5.8 before autoclaving it at 121 °C for 15 min. After filter sterilizing the BAP stock solutions, they entailed mixing into the cooled medium (approximately 45 °C temperature). The study used explants consisting of nodal segments (±1–1.5 cm), which underwent surface sterilization before culture. Maintaining cultures at 25 °C ± 2 °C was under a 16-h photoperiod with a light intensity of approximately 1,000–1,500 lux.

In chrysanthemum explants, the parameters observed were plantlet height (cm), internode length (cm), and the number of roots, internodes, leaves (blades), and shoots. Observations took place six weeks after subculture**.** The obtained data underwent the analysis of variance (ANOVA) according to the split-plot design. The significant means reached further comparison and separation with the least significant difference ($LSD_{0.05}$) test. Correlation and regression analyses continued using Statistical Tool for Agricultural Research (STAR 2.0.01 software) and Microsoft Office Excel.

The conduct of ploidy analysis used flow cytometry (BD Accuri C6+, USA). Young leaf samples (30–50 mg) entailed the following process: chopped in Galbraith's buffer, filtered through a 50-µm nylon mesh, stained with propidium iodide (50 µg mL^{-1}), and analyzed following the procedures described by Doležel *et al.* A minimum of 5,000 nuclei events succeeded in their recording for each sample to determine relative DNA content.

RESULTS

Analysis of variance

The analysis of variance showed significant effects of BAP concentrations, genotypes, and

their interaction on shoot number, plantlet height, leaf number, and internode number (Table 1). The mutant codes p, l, and m correspond to their varietal origins, with p = Pinka Pinky, l = Lollipop, and m = Maruta. This coding system represents the three genetic backgrounds evaluated in this study. The M3p at a BAP concentration of 0.05 ppm gave the best plantlet height (19.33 cm) and was significantly different from other M1 chrysanthemum mutants. The M5p at a BAP concentration of 0.05 ppm showed the highest internode length (1.67 cm) and was nonsignificantly different from the M5l (m10) but substantially different from other M1 chrysanthemum mutants. The M2p at a BAP concentration of 1.0 ppm revealed the most roots (26.33) and emerged significantly different from other M1 chrysanthemum mutants.

The analysis of variance for the number of internodes, leaves, and shoots showed BAP concentrations, M1 chrysanthemum mutants, and their interactions have a highly significant effect on these parameters (Table 2). The M3p at a BAP concentration of 0.5 ppm exhibited the highest number of internodes (17.00), declaring it as significantly different from other M1 chrysanthemum mutants. The M4l at a BAP concentration of 1.0 ppm expressed the ultimate number of leaves (27.00), which was nonsignificantly different from the M2p but substantially different from the other three M1 chrysanthemum mutants. The M4m at the BAP concentration of 1.0 ppm enunciated the maximum number of shoots (1.58), nonsignificantly different from the M4m and substantially different from the other three M1 chrysanthemum mutants.

Correlation analysis

According to the correlation analysis, the number of shoots attained the designation as the primary trait in chrysanthemums. The characters that revealed a significant positive correlation with the main character were the plantlet height (0.44**), the number of leaves (0.58**), the number of internodes (0.58**), and the number of roots (0.47**). However, the internode length showed a nonsignificant correlation with the primary character in chrysanthemums.

Regression analysis

Based on the orthogonal polynomial regression analysis, the plantlet height showed a correlation value of r = 0.68, displaying a significant positive relationship between BAP concentrations and the increased plantlet height in chrysanthemums (Figure 1a). The regression analysis further revealed that internode length showed a correlation value of r = 0.90, signifying a considerable positive relationship between BAP concentrations and increased internode length (Figure 1b). The regression analysis indicated that the number of shoots exhibited a correlation value of r = 0.13, and the number of leaves showed a correlation value of r = 0.1. Both values revealed a weak relationship between BAP concentrations and the increased number of shoots and leaves in chrysanthemums (Figure 1c). The internodes disclosed a correlation value of r = 0.65, while the number of roots showed a correlation value of r = 0.56, and both values enunciated a significant positive relationship existed between BAP concentrations and increased number of internodes and roots in chrysanthemums (Figure 1d).

Ploidy analysis

From the ploidy analysis using the flow cytometry method, the chromosome doubling was evident in chrysanthemum plants after colchicine induction (0.01% and 0.05%), and different BAP concentrations produced mixoploid plants with the highest chromosome doubling (5.65+2.61%) in the M1 chrysanthemum mutants M2p (m2) (Table 3). However, other treatments showed chromosome doubling to diploid with varying levels of chromosomal variations in the chrysanthemums.

Table 1. Plantlet height, internode length, and number of roots in chrysanthemum M1 mutants with various BAP concentrations.

Plantlet height						
Mutants	Concentrations					CV (G)
	K0	K1	K2	K3	K4	LSD $_{0.05}$
M1	6.67	5.00	8.67	3.67	5.67	1.68
M2	16.75	16.50	14.33	12.50	21.58	
M3	17.58	19.33	14.00	14.83	4.83	
M4	10.42	8.50	8.00	8.30	8.33	
M5	6.85	9.17	7.00	9.17	7.17	
M6	10.58	10.58	8.42	13.15	12.83	
M7	2.50	2.50	3.83	4.67	4.33	
M8	12.83	11.75	12.00	15.17	15.00	
M9	9.08	11.2	10.17	8.50	12.28	
M10	15.92	17.25	14.50	11.58	16.08	
M11	16.50	16.58	14.83	12.17	14.17	
M12	1.33	4.50	4.83	3.50	6.53	
M13	4.30	4.50	5.25	6.50	6.83	
M14	15.58	13.83	11.67	15.50	11.83	
M15	10.92	9.67	10.17	6.33[b]	10.00	
CV (K) LSD$_{0.05}$	1.64					
Internode length						
M1	1.12	1.20	1.41	1.01	1.10	
M2	1.29	1.33	1.15	1.09	1.56	
M3	1.04	1.14	1.09	1.05	1.08	
M4	0.85	0.93	1.02	1.26	1.59	
M5	1.03	1.67	1.21	1.03	1.24	
M6	1.33	1.01	0.92	1.31	1.26	
M7	0.701	0.68	1.18	1.22	1.62	
M8	1.40	1.16	1.00	1.09	0.97	0.31
M9	1.15	0.98	0.85	1.11	0.80	
M10	1.22	1.41	1.19	1.21	1.42	
M11	1.37	1.20	1.02	1.28	1.62	
M12	0.23	1.15	1.10	1.94	1.41	
M13	0.95	1.15	1.27	1.20	1.41	
M14	0.97	1.23	1.17	1.05	0.79	
M15	1.04[a]	1.10	1.27	0.60	0.95	
CV (K) LSD$_{0.05}$	0.31					
Number of roots						
M1	4.5	4.83	12.67	2.33	3.83	
M2	8.67	9.5b	26.33	10.83	10.67	
M3	13.5	8.67	19.50	4.83	3.83	
M4	9.33	8.00	3.83	6.00	2.67	
M5	4.00	3.50	3.33	3.50	3.83	1.50
M6	5.17	9.50	12.50	8.67	4.67	
M7	2.17	1.33	3.33	3.33	2.33	
M8	6.50	8.00	8.33	5.83	4.67	
M9	9.17	12.50	16.50	7.33	16.83	
M10	12.33	13.67	5.33	8.50	9.67	
M11	5.83	6.17	13.50	10.50	10.17	
M12	1.50	0.67	2.00a	1.33	2.00	
M13	3.67	4.17	3.67	5.17	5.33	
M14	10.00	7.83	10.33	14.00	14.17	
M15	8.83	4.67	1.00	5.67	5.33	
CV (K) LSD$_{0.05}$	1.52					

Table 2. The number of internodes, number of leaves, and number of shoots in chrysanthemum M1 mutants with various BAP concentrations.

	Number of internodes					
Mutants	Concentrations					CV (G)
	K0	K1	K2	K3	K4	$LSD_{0.05}$
M1	6.00	4.17	6.17	3.67	5.17	1.52
M2	13.00	12.50	12.50	11.50	13.83	
M3	13.00	17.00	12.83	14.17	4.50	
M4	16.83	9.17	8.00	6.83	5.50	
M5	12.33	5.50	5.83	9.00	6.00	
M6	6.67	10.67[a]	9.17	10.17	10.17	
M7	8.00	4.00	3.33	4.00	2.83	
M8	3.50	10.17	12.00	14.00	15.50	
M9	9.17	1.50	12.00	7.67	15.33	
M10	13.17	12.33	12.17	9.67	11.33	
M11	12.17	13.83	14.50	9.50	8.83	
M12	5.67[a]	4.00	4.50	1.83	4.67	
M13	4. 67	4.00	4.17	5.50	5.00	
M14	16.17	11.33	10.00	14.84	15.00	
M15	10.50	8.83	8.17	10.33	10.50	
CV (K) $LSD_{0.05}$	1.50					
	Number of leaves					
M1	9.67	1.17	17.50	7.00	12.50	
M2	20.17	20.17	26.83	22.50	20.00	
M3	23.67	21.33	20.83	17.83	15.67	
M4	16.33	4.17	8.67	10.00	9.17	
M5	9.67	2.33	8.67	10.83	8.50	
M6	12.33	14.33	25.33	13.17[b]	9.00	
M7	4.17	4.50	6.00	5.67	3.83	
M8	7.17	9.83	15.00	15.00	12.33	1.95
M9	12.17	17.00	27.00	10.33	20.50	
M10	17.17	15.50	23.50	12.83	13.67	
M11	13.50	15.17	8.33[c]	13.00	15.00	
M12	4.67	4.17	8.50	4.50	5.67	
M13	9.83	9.67	7.67	9.17	8.33	
M14	22.00	12.67	14.33	19.17	20.33	
M15	19.50	10.8	9.33	13.17	13.17	
CV (K) $LSD_{0.05}$	1.94					
	Number of shoots					
M1	1.07	0.80	1.47	0.71	1.15	
M2	0.98	0.90	0.90	0.9	1.00	
M3	1.29	1.15	1.00	1.07	0.71	
M4	1.22	0.80	1.00	0.9	1.00	
M5	0.98	0.71	0.80	1.15	1.00	
M6	1.00	1.00	1.00	0.90	1.00	
M7	0.71	0.80	0.90	1.00	0.71	
M8	0.80	0.90	1.35	1.07	1.07	0.20
M9	0.90	1.21	1.29	0.90	1.35	
M10	1.21	1.14	1.14	0.90	1.00	
M11	1.07	1.15	1.00	1.15	1.15	
M12	0.90	0.90	1.00	0.90	0.71	
M13	0.90	0.90	1.00	1.00	1.07	
M14	1.47	1.00	1.58	1.35	1.35	
M15	1.22	1.07	1.00	1.15	1.00	
CV (K) $LSD_{0.05}$		0.20				

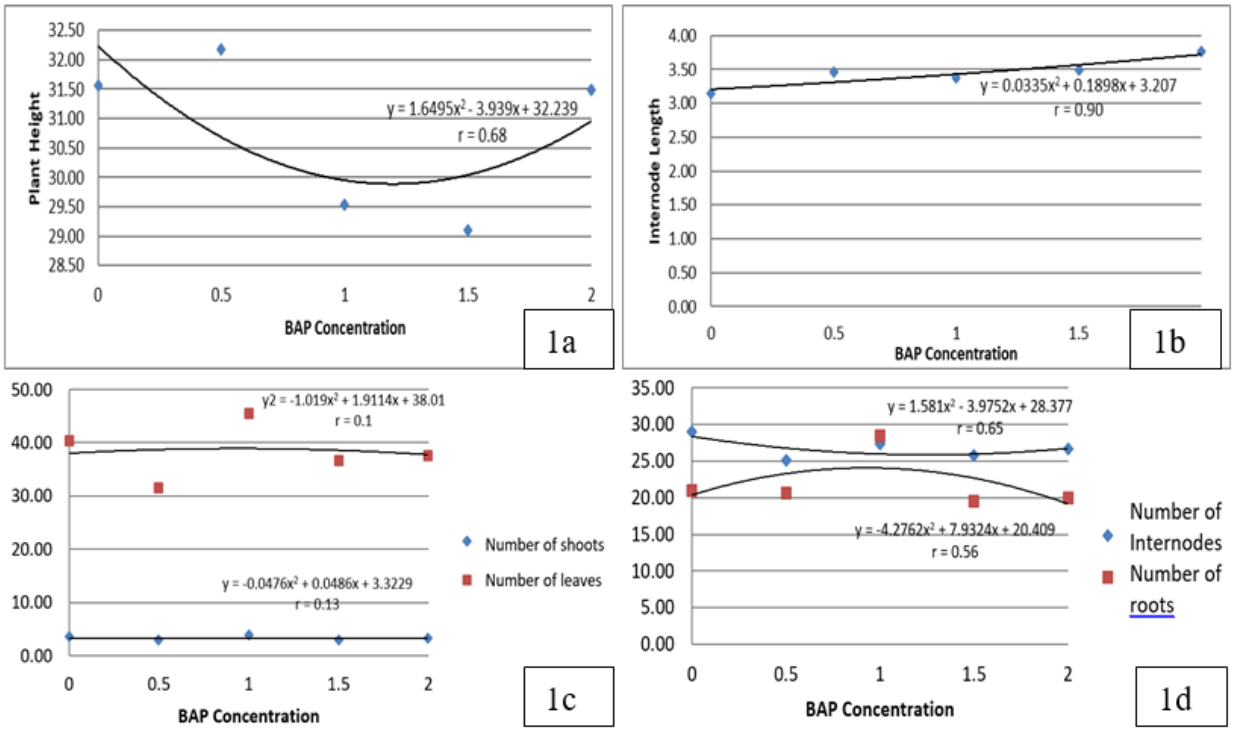

Figure 1. Polynomial orthogonal regression analysis of plantlet height (1a), internode length (1b), the number of shoots and leaves (1c), and the number of internodes and roots (1d) of chrysanthemum M1 mutants.

Table 3. Ploidy analysis of three varieties of chrysanthemum plants induced by colchicine in vitro.

Treatment	Chromosome Detected	Mean-x	Gated population (%)	Chromosome dominant
G1 (Triploid)	3n	300.79	15.94	3n
G2 (Mixoploid)	2n-3n	201.96-298.74	5.65+2.61	2n
Diploids				
G3	2n	227.28	13.52	2n
G4	2n	215.05	44.04	2n
G5	3n	280.54	9.63	3n
G6	2n	243.96	17.91	2n
G7	3n	298.49	31.33	3n
G8	2n	193.12	18.57	2n
G9	2n	218.04	50.20	2n
G10	2n	202.05	41.25	2n
G11	2n	247.40	24.85	2n
G12	2n	246.60	19.37	2n
G13	2n	233.56	24.87	2n
G14	2n	207.59	64.10	2n
G15	2n	211.00	60.70	2n

DISCUSSION

The M1 chrysanthemum mutant M4m (m14) with BAP concentration (1.00 ppm) succeeded in recording the highest number of shoots (1.58), being a primary trait. Novitasari *et al.* (2023) reported the BAP concentration of 1.00 ppm had a significant effect on the growth of citrus shoots, with a better percentage of shoot induction, faster shoot speed time, and better explants growth morphology than other BAP concentrations (1.5, 2.00, and 2.5 ppm). Additionally, Hakim and Dalimunthe's (2022) findings showed a better effect of the BAP concentration (1.00 ppm) compared with the control on the number of appearing shoots. According to Zainal *et al.* (2023), the BAP at the right concentration proved very effective in stimulating shoot multiplication because the addition of BAP in in vitro propagation media plays a better role in organogenesis. The growth regulator BAP is one of the cytokinin groups that can stimulate and induce shoots; however, its type and concentration depend on the plant type. The superior performance at 1.00 ppm BAP suggests that moderate cytokinin levels create a more balanced hormonal environment, particularly with endogenous auxin, allowing optimal meristem activation. At higher BAP concentrations, excessive cytokinin may disrupt auxin transport and hormonal crosstalk, leading to reduced shoot initiation or abnormal tissue development. This is consistent with the principle that an optimal cytokinin–auxin ratio is essential for sustained shoot organogenesis.

Other supporting characteristics of the main character also showed the best results at BAP concentrations from 0.5 to 1.00 ppm, compared with higher BAP concentrations. The plantlet height, internode length, and number of internodes reached a record of increased values with the BAP concentration of 0.5 ppm. However, the number of roots and leaves was visible, with maximum values at the BAP concentration of 1 ppm. This indicates that lower BAP concentrations show better results and emerged as the proper concentrations for growth in chrysanthemum plantlets. The BAP is a group of cytokinins that can stimulate meristem cells in explants to divide and develop into shoots and produce leaves (Oliveira *et al.*, 2022; Hakim and Dalimunthe, 2022; Mosoh *et al.*, 2024). The growth regulator triggers plant growth at a low concentration, and the higher concentration could cause contradictory function because hormones work better at a low and most optimal concentration. These results also indicate that lower BAP concentrations support more physiological stability, allowing explants to maintain balanced resource allocation between shoot formation, leaf development, and root initiation. When cytokinin levels remain within the optimal range, photosynthetic tissues develop more effectively, supporting higher carbohydrate supply to the meristem, which enhances shoot multiplication. Excess cytokinin tends to suppress root growth and disrupt growth vigor, explaining the decline at higher concentrations.

The 15 M1 chrysanthemum mutants showed varied results for character. The enhanced plantlet height resulted in the M1 M3p (m3) mutant; the maximum internode length appeared with the M1 M5p (m5); and the most roots occurred in the M1 M2p (m2). Furthermore, the maximum number of internodes emerged with the M1 M3p (m3), with the maximum number of leaves shown by the M1 M4l (m9) mutant. The superior number of shoots surfaced from the M1 M4m (m14) mutant in the chrysanthemum. The differences shown by M1 chrysanthemum mutants may refer to the diverse genetic makeup of mutants. According to Jung *et al.* (2021) and Bidabadi and Jain (2020), the morphological appearance of the explants received influences from the development level of the explants by using meristematic and genetic factors in the plants. The differences in the M1 chrysanthemum mutants, even though belonging to the same parent, also resulted from the previous mutations, thus affecting the genetic structure of the produced mutants. The mutation induction with different mutagens will cause variations in the genetic structure of plants in the form of point mutations, changes in base sequences, deletions, duplications, and chromosome translocations, as well as disruption of the mitosis and meiosis processes (Kumar, 2024; Ali and Suryakant, 2024).

The interactions between the BAP concentrations and the M1 chrysanthemum mutants were highly significant, continuing the data for correlation and regression analyses to determine the relationship among the traits (Fadhilah *et al.*, 2022). Based on the correlation analysis, the plantlet height and the number of roots, internodes, and leaves had a very significant positive correlation with the number of shoots being a primary character. This indicates a close relationship existed between these supporting characters, with their respective roles in increasing the number of shoots. According to Baak *et al.* (2020) and Mohajan (2020), a robust relationship among the characters was evident through a real correlation value, not only because of the opportunity but also because of the relationship between the two variables. Physiologically, these strong correlations reflect coordinated vegetative growth, where increased leaf number enhances photosynthetic capacity and carbohydrate supply to developing shoots, while longer internodes indicate higher meristematic activity and cellular elongation that support shoot emergence. Root development, although less dominant, contributes through improved water and nutrient acquisition. Together, these relationships show genotypes with stronger vegetative vigor allocate resources more efficiently toward shoot formation, explaining their superior regeneration performance.

Plants' vegetative growth in vitro with colchicine administration can increase plant production by producing polyploid individuals (Miri, 2020; Widoretno *et al.*, 2023). Mutant propagation materialized by adding plant growth regulators to accelerate the organogenesis. The growth regulator is a natural, non-nutritional compound that stimulates growth and development and appears to be active with certain concentrations. The BAP can also be useful in mutant evaluation in subculture activities (Manohar *et al.*, 2021). Therefore, the right concentration of BAP can be an effective strategy to improve the plant genetic makeup and support plants in resistance and productivity.

Polyploidization analysis depended on the principle of immunofluorescence examination of a suspension of waiting cells. The analysis tended to be fast and effective to analyze the number of chromosomes in individual cells (Manohar *et al.*, 2021; Sliwinska *et al.*, 2022). Based on the flow cytometry analysis, the highest mixoploid ploidy level (5.65% + 2.61%) was in the M1 chrysanthemum M2p (M2) mutant. Mixoploid plants are organisms that have a mixture of cells with various ploidy levels in the same tissue of plants (Shariatpanahi *et al.*, 2021). Mutants that experience chromosome duplication should considerably be able to produce plants with larger flower sizes, rounder flower shapes, and more intense flower colors.

CONCLUSIONS

Lower BAP concentrations (0.5–1.0 ppm) were more effective in promoting vegetative growth and shoot multiplication of M1 chrysanthemum mutants. The M4m mutant treated with 1.0 ppm BAP produced the highest number of shoots while supporting traits such as plantlet height, internode length, and the number of internodes, roots, and leaves, which also performed best. Correlation and regression analyses indicated strong positive relationships between shoot number and these supporting traits. Flow cytometry revealed mixoploidy in the M2p mutant, suggesting that in vitro propagation after mutation can generate chromosomal variation. Overall, these findings provide practical insights for optimizing BAP levels in in vitro propagation and guiding future mutant selection.

ACKNOWLEDGMENTS

The research work received support from the Hasanuddin University, Makassar, Indonesia, through the scheme of Penelitian Fundamental Kolaboratif UNHAS (Collaborative Fundamental Research), with grant number 00309/UN4.22/PT.01.03/2024.

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