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RESEARCH ARTICLE

The Morpho-Somatic and Chromosomal Changes in Colchicine Polyploidy Induction *Colocasia esculenta* var. Antiquorium

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ABSTRACT Increasing the productivity of *Colocasia esculenta* var. Antiquorum is very necessary to enhance the mass of tubers. This can be achieved through polyploid mutation method using colchicine to improve tuber size by increasing the number of chromosomes. Therefore, this study aimed to determine the effect of colchicine on morphology and polyploid changes of *Colocasia esculenta* var. Antiquorum at various concentrations and duration of immersion *In vitro*. The analysis was carried out as a completely randomized factorial design, with the first factor being colchicine concentration, consisting of 0.0%, 0.05%, 0.075%, and 0.1%. The second factor was the immersion time which consisted of 8 and 16 hours. The results showed that the best combination of concentration and immersion time on somatic morphology was 0.075% colchicine with 8 hours and 16 hours of immersion. Meanwhile, the best combination of chromosomal changes was 0.05% colchicine with 16 hours of immersion. These results indicated that the treatment of 0.05% colchicine concentration with a soaking time of 16 hours can be recommended for induction of polyploid mutations in *Colocasia esculenta*.

Keywords *Colocasia esculenta*, Polyploid mutation, Colchicine, Diploid, Tetraploid, *In vitro*

INTRODUCTION

Colocasia esculenta var. Antiquorum is a type of taro with a small tuber size (small corm taro), also known as Japanese taro (Maretta *et al*. 2016). The plant exhibits a high level of resistance to weather changes, which allows it to adapt to different seasons. Based on the data from the Food Security Service of South Sulawesi Province, the productivity of *Colocasia esculenta* var. Antiquorum in 2020 was 7.214 tons. However, the exports of *Colocasia esculenta* L. tubers only amount to 300 tons/ha due to the low productivity of the plant (Nagano *et al*. 2016).

Increasing the productivity of *Colocasia esculenta* var. Antiquorum can be done through plant breeding by induction of polyploid mutations. Polyploid induction aims to increase crop productivity because polyploid (tetraploid) plants have a more significant figure, fruit size, tuber, and flower than diploid plants (Suryo 2007) and are also resistant to specific environmental stresses (Deng *et al*. 2012).

Polyploid can be carried out by giving chemical mutagens such as colchicine to plant meristem tissue. Colchicine changes the number of plant chromosomes and causes gene mutations on the scale of seeds and vegetatively propagated plants. It also induces polyploid by inhibiting the formation of spindle fibers during cell division. However, the number of chromosomes increases, but cell division does not occur, thereby producing polyploid cells with a doubled number of chromosomes (Manzoor *et al*. 2018, 2019; Ade *et al*. 2010).

Colchicine is an important mutagen that prevents micro-

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tubule formation and doubles the number of chromosomes. It is commonly used to develop polyploid plants and functions as a mitotic toxin, which produces multiple plant mutagenic effects (El-Nashar *et al*. 2015). Colchicine not only helps in doubling chromosomes but also causes plant mutations, resulting in the formation of Colchis-mutants (Ari *et al*. 2015). The concentration of colchicine for seed treatment usually ranges from 0.1% to 0.8%, but high doses cause malformations and reduce the production of tetraploid plants. Therefore, it is recommended to use colchicine with the lowest possible concentration (Pirkoohi *et al*. 2011).

The success of polyploid induction depends on the explant, plant species, colchicine concentration, and duration of exposure. However, induction must be carried out carefully by plant breeders as excessively high concentrations cause problems in the form of abnormalities in developing seedlings (Manzoor *et al*. 2018, 2019). Several studies on polyploid induction using colchicine have been successful with different concentrations, such as Papaver bracteatum L. (Esfahani *et al*. 2020), Allium Sativum L. (Ayu *et al*. 2019), Colchicum chalcedonicum Azn. (Karlik *et al*. 2020), Neolamarckia cadamba (Eng *et al*. 2021), Dionaea muscipula Ellis (Jala 2014), Nigella sativa (Gupta *et al*. 2021), and Rhododendron fortunei L. (Mo *et al*. 2020). This shows that polyploid induction can be used to create or select better offspring for further use.

Based on the description above, it is necessary to investigate induction of polyploid mutations in *Colocasia esculenta* var. Antiquorum. Therefore, this study aims to determine the effect of colchicine on morphology and chromosome polyploid of *Colocasia esculenta* var. Antiquorum plantlets on the interaction of concentration and immersion time *In vitro*. The novelty of this study is that it is the first to explore induction of polyploid mutations in *Colocasia esculenta* var. Antiquorum.

MATERIALS AND METHODS

Experimental design

This study was performed at the Tissue Culture Laboratory, Department of Agricultural Cultivation, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, 90245, Indonesia. The analysis employed a 2-factor factorial (F2F) experiment with a Completely Randomized Design (CRD) as the environmental design, with three replications in each of the eight treatments. The combination of treatment between colchicine concentration c0 = 0%, c1 = 0.05%, c2 = 0.075%, c3 = 0.1%) and immersion time (t1 = 8 hours; t2 = 16 hours). Each replication consisted of three shoots, resulting in a total of 82 individual shoots, as the experimental unit observed.

Experimental conditional

The plantlets used as material for polyploid induction were eight weeks old *Colocasia esculenta* var. Antiquorum shoots, which were selected as explants. The *in vitro* shoots were removed and the petioles were cut to a size of 1-3 cm. The immersion was carried out in laminar and the equipment used was sterile. The shoots were immersed in different concentrations of colchicine solution, namely 0% (sterile distilled water) (c0), 0.05% (c1), 0.075% (c2), and 0.1% (c3). Each shoot was immersed in a culture bottle containing 15 mL of colchicine solution and shaken at 100 rpm on a shaker for 8 hours (c1) and 16 hours (c2). After immersion, the explants were washed with sterile distilled water 3-4 times and planted into shoot propagation medium, namely MS media with 2 mg L^{-1} BAP and 1 mg L^{-1}IBA. The medium contained sugar (30 g L^{-1}), with the pH being adjusted to 5.8, and compacted with 7 g L^{-1} agar. The shoots were kept in an incubation room at a temperature of 25-26℃ with 16 hours/day irradiation and an irradiation intensity of ± 1,000-2,000 lux (Modification of Dwiyani 2015). Subsequently, the shoots were incubated for 16 Weeks After Planting (WAP) and observations were made every week from outside the culture bottle for 16 WAP.

Observation of parameters

Several parameters observed from shoot explants were the percentage of living shoots (%), the number of living leaves, the number of roots and the number of compound shoots.

$$\text{Percentage of living explants} = \frac{\Sigma \text{explants planted} - \text{explants dead}}{\Sigma \text{explants planted}} \times 100\%$$

Chromosome analysis with flow cytometry

Polyploid level analysis was performed using flow cytometry. For each treatment, samples from the best plantlet vigor were collected from the leaves of the treated plants, measuring ± 25 mm^2. The samples were ground using a razor blade in a petri dish with 0.2 mL of extraction buffer (solution A from Partec Kit) and incubated for 10 minutes at room temperature. Subsequently, the supernatants from the samples were filtered using nylon kesh measuring 30 m and placed in a cuvette, which was added to 1 mL of DAPI solution. The samples were incubated again for 1 minute and inserted into the Flow cytometry tool. The tool read the light intensity of the measured sample through the graph displayed to show the number of ploidy changes that occurred (Escobedo-Gracia-Medrano *et al*. 2018).

Data analysis

The data analysis was carried out using the F test to determine the interaction between colchicine concentration and immersion time. When the variance obtained had a significant effect, further analysis was conducted using the Duncan's Multiple Range Test (DMRT) at a 5% significance level to determine the effect of differences between treatments. Data processing was carried out using the Statistical Tool for Agricultural Research (STAR) program and Microsoft Office Excel 2019. The data were analyzed by correlation analysis to determine the relationship between the parameters (Anshori *et al*. 2022).

RESULTS

Percentage of living shoots of *Colocasia esculenta* var Antiuorum *in vitro*

The results of the living shoots in Table 1 and Fig. 1 showed that the control shoots had a very high percentage of survival compared to the combination of concentration and immersion time. In this study, there were differences in the number of shoots planted due to contamination of the media before treatment. Therefore, out of the 18 propagated shoots, there was a variation in the number of shoots that grew before colchicine immersion treatment. However, this can be corrected through the percentage of living shoots, which were analyzed via graphs.

Based on the graph of living shoot percentage, this study showed a negative graph with an increase in colchicine concentration and time of immersion. The graph formed a linear regression with the formula Y = 7.2751x + 82.378, and the coefficient of determination (R2) was 0.3131, as presented in Fig. 2. Based on this formula, the lethal 50 was in 0.1% colchicine concentration with 8 hours of immersion. The relationship between the percentage of surviving shoot explants was inversely proportional to the concentration and duration of colchicine immersion. As the concentration

Table 1. Percentage of living shoots on each treatment of colchicine concentration and time of immersion.

Treatments	Shoot planted	Living shoots	Dead shoots	Living percentage (%)
c0t1	13	13	0	100.00
c1t1	9	4	5	44.44
c2t1	9	4	5	44.44
c3t1	12	4	8	33.33
c0t2	13	13	0	100.00
c1t2	18	5	13	27.78
c2t2	18	5	13	27.78
c3t2	18	4	12	22.22
Total	110	52	56	47.27

c0t1 (0% colchicine, immersion 8 hours); c1t1 (colchicine 0.05%, immersion 8 hours); c2t1 (colchicine 0.075%, immersion 8 hours); c3t1 (colchicine 0.1%, immersion 8 hours); c0t2 (0% colchicine, immersion 16 hours); c1t2 (colchicine 0.05%, immersion 16 hours); c2t2 (colchicine 0.075%, immersion 16 hours); c3t2 (colchicine 0.1%, immersion 16 hours).

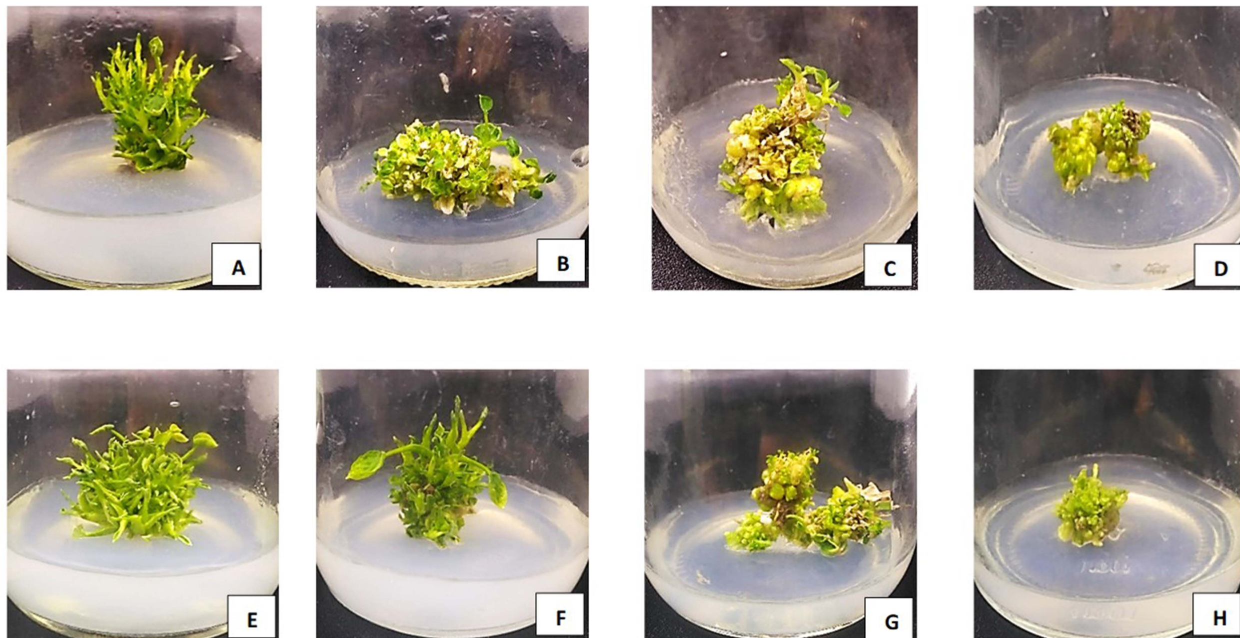

Fig. 1. *Colocasia esculenta* var. antiquorum plants with several concentrations of colchicine and *In vitro* immersion time after 16 weeks. (A) c0t1 (0% colchicine, 8 hours immersion); (B) c1t1 (colchicine 0.05%, immersion 8 hours); (C) c2t1 (colchicine 0.075%, immersion 8 hours); (D) c3t1 (colchicine 0.1%, immersion 8 hours); (E) c0t2 (0% colchicine, immersion 16 hours); (F) c1t2 (colchicine 0.05%, immersion 16 hours); (G) c2t2 (colchicine 0.075%, immersion 16 hours); (H) c3t2 (colchicine 0.1%, immersion 16 hours).

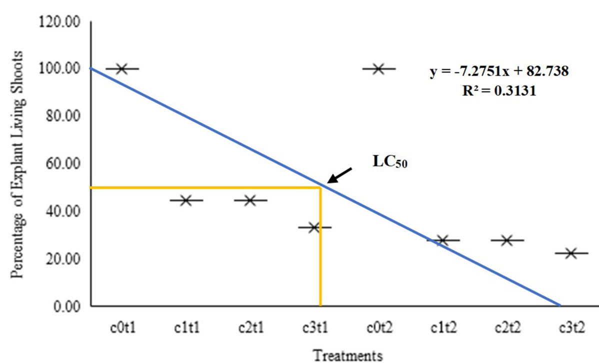

Fig. 2. Graph letal concentrasion of living shooting percentage of *Colocasia esculenta* var. antiquorum from first week to sixteenth week after *In vitro* induction. c0t1 (0% colchicine, immersion 8 hours); c1t1 (colchicine 0.05%, immersion 8 hours); c2t1 (colchicine 0.075%, immersion 8 hours); c3t1 (colchicine 0.1%, immersion 8 hours); c0t2 (0% colchicine, immersion 16 hours); c1t2 (colchicine 0.05%, immersion 16 hours); c2t2 (colchicine 0.075%, immersion 16 hours); c3t2 (colchicine 0.1%, immersion 16 hours).

and duration of immersion of colchicine increased, the number of explants that survived decreased. However, the survival plantlet in the chemical mutants had unique trait changes, making it necessary to achieve a balance between the plantlet regeneration and optimum diversity in chemical mutation induction. Therefore, the lethal 50 served as a practical point to meet both of these objectives.

The analysis of the mean square of variance in polyploid mutation induction was presented in Table 2. The results showed that the treatment duration of colchicine immersion in *Colocasia esculenta* var. Antiquorum and concentration levels significantly affected all observation parameters. The interaction effect between concentration level (c) and immersion time (t) also significantly affected the number of leaves, roots, and shoots, as well as the coefficient of diversity in the medium category.

Number of living leaves

The results of the DMRT test ($\alpha = 0.05$) in Fig. 3 showed the number of living leaves of *Colocasia esculenta* var. Antiquorum in different treatments. The best treatment was

Table 2. Mean square analysis of variance on several observation parameters in *In vitro* polyploid induction experiments of *Colocasia esculenta* var. Antiquorum plants.

Source	DF	Mean square in *annalysis of variance* (ANNOVA)		
		Number of living leaves	Number of roots	Number of compound shoots
Time immersion (T)	1	234.375**	54.000**	15.042[ns]
Colchicine concentration (C)	3	349.708**	67.667**	494.153**
Interaction of T × C	3	70.708**	18.111**	86.708**
Error	16	4.333	0.542	12.458
CV (%)		16.82%	19.44%	18.40%

**very significant ($P \leq 0.01$), *significant ($P \leq 0.05$).
ns: no significant, DF: degree of free, CV: coefficient of variance.

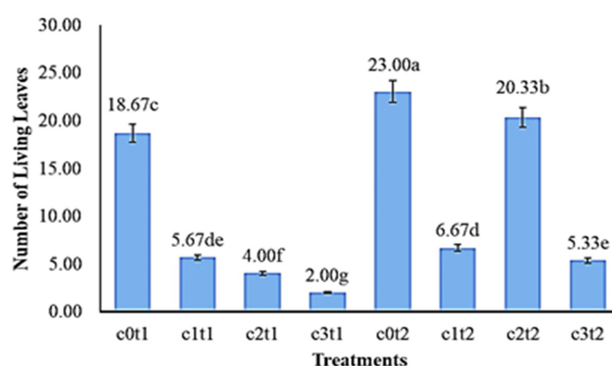

Fig. 3. The effect of colchicine concentration and immersion time on the number of living leaves of *Colocasia esculenta* var antiquorum plants *In vitro*. Numbers followed by the same letter (a-g) mean that they are not significantly different in the DMRT test with a level of = 0.05.

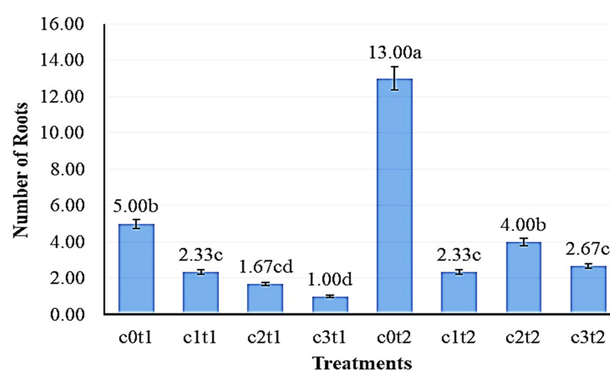

Fig. 4. Effect of colchicine concentration and immersion time on root number of *Colocasia esculenta* var. antiquorum *In vitro*. Numbers followed by the same letter (a-d) mean that they are not significantly different in the DMRT test with a level of = 0.05.

c0t2 with 23.00 leaves, compared to c2t2, c1t2, and c3t2 with 20.33, 6.67, and 5.33 leaves, respectively. The lowest mean number of living leaves of *Colocasia esculenta* var Antiquorum in the treatment c3t1 (2.00 leaves) was significantly different from c2t1 (4.00 leaves), c1t1 (5.67 leaves), c0t1 (18.67 leaves).

Number of roots

The results of the DMRT test ($\alpha = 0.05$) in Fig. 4 showed the number of roots of the best *Colocasia esculenta* var Antiquorum in the treatment c0t2 with 13.00 roots were significantly different from c2t2, c3t2, and c1t2 with 4.00, 2.67, and 2.33 leaves, respectively. The lowest mean number of roots of *Colocasia esculenta* var Antiquorum in the treatment c3t1 with 1.00 roots was significantly different from the 5.00 roots of c0t1 but not from c1t1 and c2t1 at 2.33 and 1.67 roots, respectively.

Number of compound shoots

The results of the DMRT test ($\alpha = 0.05$) in Fig. 5 showed the number of the best *Colocasia esculenta* var. Antiquorum compound shoots in the treatment c0t2 with 32.00 shoots were significantly different from c3t2 (17.67 shoots), c2t2 (16.33 shoots), c1t2 (9.00 shoots), but c3t2 (17.67 shoots) was not different from c2t2 (16.33 shoots). The lowest average number of shoots of *Colocasia esculenta* var. Antiquorum in the treatment c3t1 with 5.67 shoots was significantly different from c1t1, c2t1, and c0t1 with 14.00, 19.33, and 29.67 shoots, respectively.

Polyploid analysis of plants *Colocasia esculenta* var. Antiquorum

Polyploid analysis using flow cytometry on *Colocasia esculenta* var. Antiquorum in Table 3 showed that the difference in concentration and duration of colchicine immersion affected plant chromosomes. The treatments, namely c0t1, c1t1, c2t1, c3t1, c0t2, c1t2, and c3t1b showed an increase in the chromosomes from 2n-4n = 24 (diploid) to 48 (tetraploid). In the c2t2 treatment (0.075% colchicine, immersion 16 hours) the detected chromosomes ranged from 2n-3n-4n = 24 (diploid), 36 (triploid) to 48 (tetraploid).

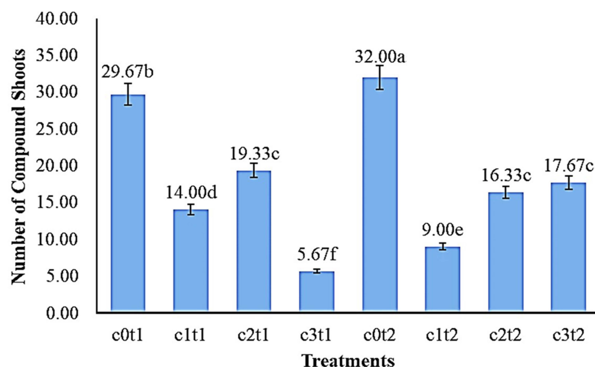

Fig. 5. The effect of colchicine concentration and immersion time on the number of compound shoots of *Colocasia esculenta* var. antiquorum plants *In vitro*. Numbers followed by the same letter (a-f) mean that they are not significantly different in the DMRT test with a level of = 0.05.

Based on the percentage of gated chromosomes in the treatment c0t1 and c0t2, the dominant chromosome was 2n (diploid) with a percentage of 67.70%, which was significantly different from the 4n chromosome (tetraploid) at 4.17%. Therefore, it can be concluded that the chromosomes in the treatments were diploid. In the c1t1 treatment (0.05% colchicine, immersion 8 hours), the dominant chromosome was 4n (tetraploid) with a percentage of 36.97% but not significantly different from 2n (diploid) at 28.35%. This showed that the chromosome was putative tetraploid because it allowed ploidy selection to occur. In c2t1 and c3t1, the dominant chromosomes were 2n (diploid) with a percentage of 52.94% and 55.26%, different from 4n (tetraploid) at 24.99% and 16.25%, which was mixoploid. In c1t2 and c3t2, the dominant chromosome was 4n (tetraploid) with a percentage of 54.10% and 44.36%, significantly different from 2n (diploid) at 20.27% and 28.24%, which did not allow ploidy selection to occur, namely tetraploid. In c2t2 treatment (0.075% colchicine, immersion 16 hours), the dominant chromosome was 4n (tetraploid) at 35.50% but not significantly different from chromosome 2n (diploid) at 29.94% and 3n (triploid) at 15.39%, making it putative-tetraploid because it allowed ploidy selection.

The results of the flow cytometry analysis in Fig. 6 showed the chromosome peaks in *Colocasia esculenta* var.

Table 3. Results of polyploidy analysis with flow cytometry in *Colocasia esculenta* var. Antiquorum plants *In vitro* induction of colchicine.

Treatment	Chromosomes detected	% gated	Dominant chromosomes	Explanation (*)
Control (c0t1, c0t2)	2n-4n	67.70 + 4.17	2n	Diploid
c1t1	2n-4n	28.35 + 36.97	4n	Putatif-Tetraploid
c2t1	2n-4n	52.94 + 24.99	2n	Mixoploid
c3t1	2n-4n	55.26 + 16.25	2n	Mixoploid
c1t2	2n-4n	20.27 + 54.10	4n	Tetraploid
c2t2	2n-3n-4n	29.94 + 15.39 + 35.50	4n	Putatif-Tetraploid
c3t2	2n-4n	28.24 + 44.36	4n	Tetraploid

(*) refers to Shahriari-Ahmadi *et al.* 2008 and based on the difference between diploid and tetraploid ≤ 1.50).
2n (diploid); 3n (triploid); 4n (tetraploid); c0t1 (0% colchicine, immersion 8 hours); c1t1 (colchicine 0.05%, immersion 8 hours); c2t1 (colchicine 0.075%, immersion 8 hours); c3t1 (colchicine 0.1%, immersion 8 hours); c0t2 (0% colchicine, immersion 16 hours); c1t2 (colchicine 0.05%, immersion 16 hours); c2t2 (colchicine 0.075%, immersion 16 hours); c3t2 (colchicine 0.1%, immersion 16 hours).

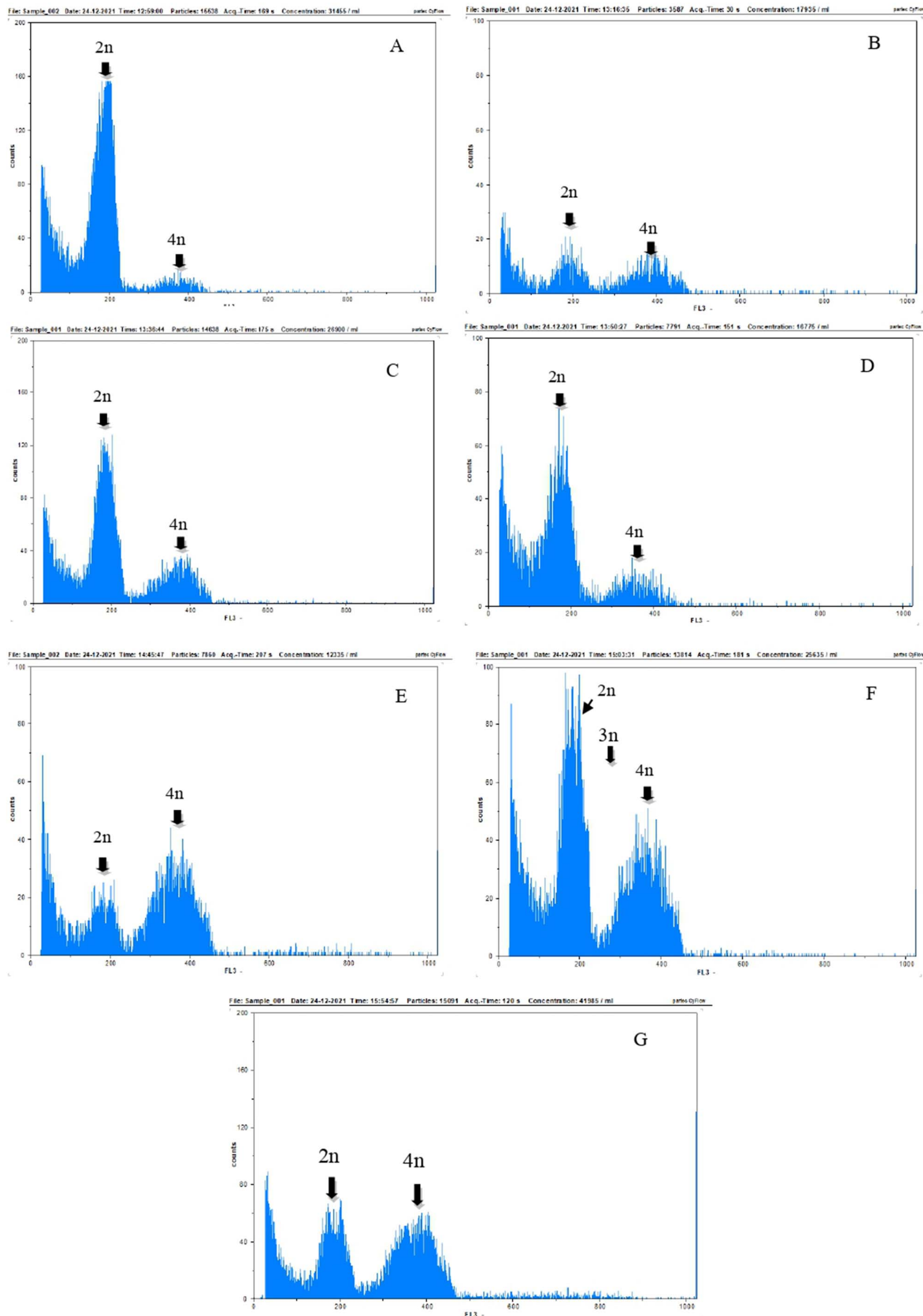

Fig. 6. Histogram of ploidy degree results with flow cytometry of *Colocasia esculenta* var. antiquorum plant resulting in *In vitro* colchicine induction. (A) c0t1 (0% colchicine, immersion 8 hours) and c0t2 (0% colchicine, immersion 16 hours); (B) c1t1 (colchicine 0.05%, immersion 8 hours); (C) c2t1 (colchicine 0.075%, immersion 8 hours); (D) c3t1 (colchicine 0.1%, immersion 8 hours); (E) c1t2 (colchicine 0.05%, immersion 16 hours); (F) c2t2 (colchicine 0.075%, immersion 16 hours); (G) c3t2 (colchicine 0.1%, immersion 16 hours).

Antiquorum plants in all treatments, which were interpreted as standards for diploid (2n), tetraploid (4n), and triploid cells (3n) were also seen. Although all seven treatments exhibited diploid (2n), triploid (3n), and tetraploid (4n) cells, the peak height of each cell was different among treatments.

Correlation analysis

The results of the correlation analysis in Table 4 showed the relationship between the number of leaf parameters, which can be evaluated *In vitro*, in polyploid of *Colocasia esculenta* var. Antiquorum. The number of leaves was positively correlated with the number of roots and compound shoots at 0.80 and 0.75, respectively.

DISCUSSION

Polyploid is a plant breeding technique carried out using mutagens such as colchicine to obtain plants with a chromosome number of more than 2n (diploid). Colchicine is one of the chemicals known to induce somaclonal variation in polyploidization in cultured plant cells. In plants, colchicine binds to tubulin, one of the main constituents of microtubules, which inhibits the development of spindle fibers and further mitotic division, leading to induction of polyploid (Kharde *et al*. 2017). It has also been used for several years to induce polyploid, which increases the potential for metabolite production and facilitates the generation of valuable compounds in plants (Bernard *et al*. 2012).

Flow cytometry analysis is a fast and efficient modern method for determining the ploidy level in almost all plant tissue regeneration and is particularly suitable for studies involving large sample sizes (De *et al*. 2010; Eeckhaut *et al*. 2005). For example, Jones *et al*. (2008) and De *et al*. (2004) used flow cytometry to determine the ploidy level and genome size of 200 diverse species and Rhododendron cultivars, creating a valuable database for breeders. This method has also been used to determine the ploidy level of induced polyploid adventitious shoots from three Rhododendron hybrids.

The results of flow cytometry analysis in Table 3 and Fig. 6 showed that the number of chromosomes in the plant cell of *Colocasia esculenta* var. Antiquorum doubled to tetraploid (4n). This indicated that the plant can be induced using polyploid by treating with colchicine concentration and immersion time, with the multiplication of chromosomes varied in each treatment. Visual observations of plantlets in Fig. 1 confirmed the doubling of chromosomes in *Colocasia esculenta* var. Antiquorum plant. The increase in leaf size and petiole was observed in the c1t2 treatment (0.05% colchicine, immersion 16 hours), with an improvement in the size of shoots formed. This result was supported by Mahyuni *et al*. (2015), who stated that diploid plants had a smaller morphology size than polyploid plants.

This study showed that the doubling of the chromosomes occurred randomly, leading to a non-uniform effect on an individual cell. The best chromosomal doubling occurred at low concentrations, namely 0.05% colchicine with 16 hours immersion time. This led to a significantly higher percentage of 54.10% compared to 44.36% and 35.50% at 0.1% and 0.075% colchicine concentration, respectively. Similarly, at 8 hours of immersion, more tetraploid chromosomes were formed at a low concentration of 0.05% by 36.97%, indicating different mutations in the same plant. Heo *et al*. (2016) also concluded that the most prolonged immersion with low colchicine concentrations induced the highest percentage of polyploid production efficiency among other treatments on L. leichtlinii plants. In orchid

Table 4. Results of correlation analysis on all observation parameters of *Colocasia esculenta* var. Antiquorum plants at various concentrations and duration of *In vitro* immersion of colchicine.

	Number of compound shoots	Number of roots	Number of living leaves
Number of compound shoots	1.00	0.79**	0.75*
Number of roots		1.00	0.80**
Number of leaves			1.00

Numbers followed by a sign mean that they are significantly different from the table *r 0.05 = 0.62; **r 0.01 = 0.79.

plants, higher concentrations of colchicine produced shorter pods, with some dry and dead pods due to colchicine toxicity at high concentrations (1000 and 2000 mg/L) (Sudirman *et al*. 2021).

Table 3 and Fig. 6 showed that the doubling of the chromosomes formed were diploid, mixoploid, and tetraploid. Mixoploid plants had diploid and tetraploid nuclei, assessed based on the relative number of nuclei (Cimen 2020; Koutoulis *et al*. 2005). In polyploid induction, a high percentage of mixoploid yield was a procedure drawback because the unstable polyploid state often returned wholly or partially to diploid state after successive cell division cycles (Esfahani *et al*. 2020). Furthermore, developing two different cells in a mixoploid bud caused competition during its growth. According to Kainth and Grosser (2010), diploid cells had a faster division rate than autotetraploid cells, allowing the resulting mixoploid shoots to become diploid again.

Tetraploid are polyploid containing four sets of chromosomes that can produce more secondary metabolites and biomass compared to diploid and are also drought-tolerant and disease-resistant (Sattler *et al*. 2016; Xu *et al*. 2014). Tetraploid can be artificially induced using mitotic spindle inhibitors such as colchicine (Wang *et al*. 2016), with their plants having giant leaf and shoot sizes than diploid (Kurtz *et al*. 2020). Wulandari *et al*. (2015) conclude that 0.1% colchicine treatment for 1-hour is the most efficient in producing tetraploid Pumelo Nambangan citrus plants.

Asif (2001) and Tesfaye (2005) reported that chromosomes were doubled in Musa balbisiana, various Musa acuminata subspecies, and Ensete ventricosum species using seeds, respectively. In polyploid induction, colchicine was found to be very efficient (Ganga 2002) depending on the variable amount of media, antimitotic agent, type of explant, exposure time, and concentration. Flow cytometry was the pre-eminent method used to induce evaluation polyploidization, while other methods such as chromosome number and morphology observations had also been applied.

Urwin (2014) reported that a concentration of 0.1% and 6 hours of immersion can duplicate chromosomes in Lavandins plants (*Lavandula* × *intermedia* cvs. Grosso and Seal). According to Sadhukhan *et al*. (2014), colchicine at a concentration of 0.0005% with 6 hours of immersion duplicated chromosomes in African marigold (*Tagetes erecta*) plants. The method was carried out by placing the entire plant with roots in colchicine solution and applying it to the apical shoots on cotton soaked in colchicine.

In this study, different concentrations of colchicine treatment and immersion time were found to affect morphology characteristics of regenerated plants under *In vitro* conditions. According to Jala (2014), the effect of colchicine concentration and duration of time for immersion in D. muscipula can be observed in changes in morphology and growth rate, survival rate, and variations that occurred at high concentrations of colchicine and immersion time.

The results of the DMRT analysis in Figs. 3-5 showed differences in the number of living leaves, roots, and compound shoots. The graph indicated that the highest data were obtained in the c0t2 treatment (0.0% colchicine, immersion 16 hours) and the lowest was in the c3t1 treatment (0.1% colchicine, immersion 8 hours). This indicated that adding a small concentration of colchicine and increasing immersion time significantly affected morphology of *Colocasia esculenta* var. Antiquorum, caused more exposed cells to be damaged or fail during growth. This was supported by Allum *et al*. (2007), who stated that at the concentration, the chromosomes will be doubled in the cell.

The best morphology in this study was obtained at a concentration of 0.0% due to the stress on the plant in colchicine treatment. However, in subsequent subcultures, other differences were observed between the concentration of 0.0% and colchicine at 0.05%, 0.075%, and 0.1% because the plants were no longer under stress due to colchicine administration. The nature of colchicine mutagen influenced morphology differences in each treatment due to its high toxicity to plants. Therefore, low doses with a long exposure period were considered reliable to reduce its toxic effect and increase the rate of polyploid production (Sajjad *et al*. 2013). The optimal amount of colchicine used for polyploid output varied widely, with concentrations ranging from 0.01% (Thao *et al*. 2003) to 1.0% (Demtsu *et al*. 2013).

In the correlation analysis in Table 4, the closeness between the variables was measured without considering

the influence or the magnitude of one variable on others. In this study, the main character was the number of leaves because it can be used as a character to identify tetraploid plants. The results of the correlation analysis showed that four characters were positively correlated, including a very significant positive correlation on the number of roots (0.80) and shoots (0.75), speed of shoots (0.69), and speed of rooting (0.66). This correlation proved the extent to which a relationship exists between one or more variables (Astuti 2017). According to (Rohaeni 2012), correlation with strong character conditions can be used as an indirect selection tool for the main character.

Based on the results, colchicine with the best effect on the formation of polyploid of Safira taro was the interaction between 0.05% colchicine concentration and 16 hours immersion time (c1t2), which resulted in tetraploid (4n) of 54.10%. This was followed by the combination of 0.1% colchicine concentration with 16 hours of immersion (c3t2), yielding tetraploid (4n) of 44.36%. Furthermore, colchicine with the best effect on morphology parameters was obtained from the interaction between colchicine concentration of 0.075% with 16 hours of immersion (c2t2), which produced 20.33 leaves and 4.00 roots, while a concentration of 0.075% with 8 hours of immersion (c2t1) yielded 19.33 shoots.

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