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EFFECT OF COLCHICINE TREATMENT ON PLANT GROWTH AND FLOWER DEVELOPMENT IN *Zinnia elegans*

Performa Pertumbuhan dan Reproduksi Tanaman Refugia Bunga Kertas (*Zinnia elegans*) Hasil Induksi Kolkisin

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ABSTRACT

Refugia is a flowering plant that is intended to trap and destroy insects. Colchicine is reported to induce polyploidy in plants. This study aims to determine germination, growth, and refugia performance of colchicine induced Zinnia elegans at different immersion concentrations and times. This research was conducted experimentally with a completely randomized design. The treatment was immersion in colchicine solution at different times duration. Colchicine concentration 0.01% with immersion time of 6,12,24,48, and 72 hours. Colchicine induction with different immersion times showed a decrease in sprouts height ($p<0.05$), resulting in abnormal sprouts with three leaves and wider leaf area (correlation test r=0.560). The longer the colchicine immersion showed plant height (correlation test r=0.618). Morphologically, control and colchicine induction results were relatively the same, but flowers resulting from colchicine induction had higher flower heights (correlation test r=0.772) and flower diameter (correlation test r=0.815). Induction of colchicine immersion had a different effect on the growth and reproduction performance of Z. elegans.

***Keywords**: Zinnia elegans, Refugia, Colchicine, Growth, Development*

ABSTRAK

Refugia merupakan tanaman berbunga yang diperuntukan untuk menjebak serangga penghancur tanaman. Kolkisin dilaporkan dapat menginduksi poliploidi pada tanaman. Penelitian ini bertujuan untuk menentukan daya kecambah, pertumbuhan dan untuk menentukan kinerja refugia *Zinnia elegans* yang diinduksi kolkisin pada konsentrasi dan waktu perendaman yang berbeda. Penelitian ini dilakukan secara eksperimental dengan rancangan acak lengkap. Perlakuan berupa perendaman dalam larutan kolkisin dan pada waktu yang berbeda. Konsentrasi kolkisin 0,01% dengan waktu perendaman 6, 12,24,48,72 jam. Induksi kolkisin dengan waktu perendaman yang berbeda menunjukkan penurunan tinggi kecambah ($p<0.05$), menghasilkan kecambah abnormal dengan tiga daun dan luas daun yang lebih lebar (r = 0.560). Semakin lama perendaman kolkisin menunjukkan tinggi tanaman (r = 0,618). Secara morfologis, bunga kontrol dan hasil induksi kolkisin relatif sama, tetapi bunga yang dihasilkan dari induksi kolkisin memiliki tinggi bunga yang lebih tinggi (r = 0,772) dan diameter bunga (r = 0,815). Induksi perendaman kolkisin memiliki efek yang berbeda pada pertumbuhan dan kinerja reproduksi *Z. elegans*.

Kata Kunci: *Zinnia elegans*, Refugia, Kolkisin, pertumbuhan, perkembangan.

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INTRODUCTION

Refugia is an ornamental plant that is used as micro-habitat protection, a source of food for natural enemies such as predators and parasitoids. Refugia is expected to preserve ecosystems on agricultural land (Purwantiningsih et al. 2012, Mahanani et al. 2020). The characteristics of refugia plants include: having striking flower colors, fast plant regeneration, easy to plant, easy to obtain seeds, and being superimposed with other plants (Sinar Tani, 2016). One example is the paper flower (*Zinnia elegans*) (Sakir and Desinta 2018).

Z. elegans is a species of annual plant from the Asteraceae family and the Zinnia genus (Stimart and Boyle 2007). *Z. elegans* has tolerance to environmental drought and salinity (Carter and Grieve 2010). The morphological characters of *Z. elegans* have round stems that are herbaceous, the surface of the stems is downy and green. The leaves are alternately opposite, the leaf blade is oval in shape, the leaf tip is pointed, the leaf edge is flat, the leaf bone branches do not reach the leaf edge and the leaf surface is rough. Wreath with ribbon flowers, yellow tube flowers, lanceolate-shaped protective leaves, blunt protective leaf bases and cup-shaped flower bases (Hasanuddin and Fitriana 2014).

One effort to get superior plants is by way of chromosome engineering, namely the formation of polyploid plants (Murni 2010). Polyploid plants are plants that have more than two sets of chromosomes (Comai 2005). Polyploid plants have taller plant height, larger stem diameter, longer leaf length, wider leaf width, longer corolla length and wider corolla width (Murni, 2010), heavier fruit weight (Pradana and Hartatik 2019), larger stomata size (Nofitahesti and Daryono 2016; Saraswati et al. 2017), and have more resistance to pathogens compared to diploid plants (Comai 2005).

Colchicine is an alkaloid compound derived from the species *Colchicum autumnale* L which is toxic in nature and has an effect on the nucleus thereby inhibiting cell division (Sattler et al. 2016). Colchicine prevents the formation of spindle threads at the anaphase stage, so that cells with double chromosome content do not form a cell plate (Sundov et al. 2005). Polyploid plants can be induced with colchicine by immersion (Murni 2010; Rosmiati and Dani 2017; Dewi and Pharmawati 2018), dripping (Saraswati et al. 2017; Saraswati et al. 2018). These treatments can be given to seeds (Nofitahesti and Daryono, 2016), sprout roots (Dewi and Pharmawati 2018; Pradana and Hartatik 2019), stem tips (Widya et al. 2018), tubers (Rahayu et al. 2014) and plantlets from tissue-culture cultures (Ermayanti et al. 2018).

Successful polyploid plant induction using colchicine has been observed in the following species*: Capsicum annum* L (Murni 2010), *Glycine max* (L.) Merr (Nofitahesti and Daryono 2016), roses (Kermani et al. 2003), orchids (Miguel and Leonhardt 2011), and *Impatiens balsamina* L. (Wiendra et al. 2011). The effective concentration of colchicine is 0.01%-1.00% (Suryo 2007). It has been observed as well that immersing curly chili (*Capsicum annuum*) sprouts in 0.01% colchicine for 12 hours effectively induces polyploidy (Murni 2010). In this study, polyploidy was induced by soaking the seeds with colchicine. This study aimed to determine the growth and reproduction performance of *Z. elegans* induced by colchicine with different immersion durations.

MATERIALS AND METHODS

Location

This research was conducted at the Agribusiness Experimental Garden Laboratory of University of Muhammadiyah Bandung

Materials

The equipment used includes polybags with dimensions of 15x15x15cm^3, trowel, germination tray, millimeter block with accuracy of 1 mm, caliper, microtubes, conic tubes of 15 and 50 mL, 50 mL beaker glass, ruler, stationery, scales with accuracy of 1 g and camera (Sony Z5). The materials used included Zinnia seeds (*Z. elegans*), 0.01% colchicine indo biotech agro, distilled water, planting media (soil, husks, 5:5:1 manure), and tap water

Method

Research Method

For the experimental research method, we utilized Completely Randomized Design (CRD). The concentration of colchicine used was 0.01%. The independent variable was the duration of colchicine immersion with the control treatment level, namely immersion in mineral water for 6 hours (P0), immersion for 6 hours (P1), immersion for 12 hours (P2), immersion for 24 hours (P3), immersion for 48 hours, (P4) and immersion for 72 hours (P5). For each treatment, 5 plant units were subjected to the immersion. The phases of this research include:

Planting Media

Planting media includes soil, husks and compost, mixed with a ratio of 5:5:1. The three components were stirred until homogeneous. The resulting planting media was then put in a tray containing 128 holes for germination purposes and polybags with dimensions of 15x15x15 cm^3.

Colchicine Immersion

Zinnia seeds were immersed in 1.5 mL of 0,01% colchicine for 6, 12, 24, 48, and 72 hours. For the control group, mineral water was used to immerse the seeds for 6 hours. For each treatment, 5 units of seeds were used.

Germination

Seeds were germinated in a tray that fulfills the requirements for good germination process, which is a place that is moist and is not subjected to a direct sunlight. Nurturing the growth of the plant was done by sufficient watering every morning. Every week Weeds that grow in polybags were picked to maximize plant growth.

Data Collection

Germination data were collected on the 7th and 14th day after planting. Germination data included observations of the number of leaves, plumule size, stem length, radicle length and the colour of each organ. Growth data were observed every two weeks, namely at weeks 4, 6, and 8. Growth data included number of leaves, plant height and leaf size. At week 17, the wet weight of the plant and the dry weight of the plant were measured. Observed reproductive parameters were rate of flowering percentage, flower development, flower character and seed character.

Data Analysis

Qualitative data, such as germination phase and flower morphology, were described and displayed in the form of paragraph. The quantitative data such as germination profile, growth data, character of flowers and seeds, wet and dry weight of plants. Quantitative data were then analyzed by ANOVA and continued with Duncan's test with significance levels (α) =0.05. For each quantitative data, a correlation test was conducted to determine the relationship between the immersion duration and the measured quantitative data parameters. Correlation test was performed using Data Analyze in Microsoft Excel 2010.

RESULTS AND DISCUSSION

1. Performance Growth *Z. elegans* Results Induction of Colchicine With Different Soaking Lengths

Growth performance was observed on the germination and growth profile of *Zclr. elegans*. The germination morphology at P0 showed a normal morphology, which was indicated by greenish plumules and numbered in pairs. The color of the radicle is white and looks wavy. The color character of the plumule, radicle and stem in each treatment was the same. Morphology of P1 and P2 found one individual with three strands of plumule, with wavy and curled strands (Figure 1).

Chilli sprouts (*Capsicum annuum* L) aged 3 weeks soaked in 0.01% and 0.025% colchicine for 24 hours showed swollen root tips, while soaking at 0.05% showed necrosis at the root tips of the sprouts (Murni 2010). High concentrations or too long treatment duration can cause death of the meristem tissue so that the root tips undergo necrosis (Zeng et al. 2006). Two weeks old germination profile was observed for plant height, root length, and leaf area. Based on the results of observations of green plumules, there were white radicles that have branched, greenish white stems to green

(Figure 1). Sprout height ranges from 16-48 mm. The dimensions of the length x width of the leaves range from 6x4 mm to 12x 18 mm root length. Root length ranged from 21-65 mm.

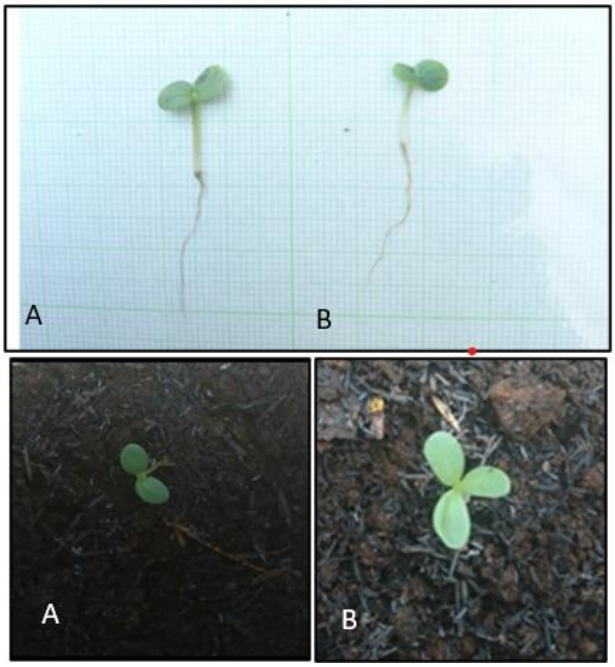

Figure 1. Normal (A) and abnormal (B) sprouts of *Z. elegans*.

Based on the ANOVA results, it was shown that the colchicine induction at two weeks of soaking duration differed significantly from the height of the two-week-old *Z. elegans* sprouts control group ($p<0.05$). P3, P4, P5 showed relatively the same results, but different from P0. P2 produced the highest sprouts. Based on the correlation test, it was shown that the longer the colchicine immersion duration, the shorter the height of the sprouts ($r=-0.566$). This is in accordance with Murni (2010), curly chili sprouts *C. annuum* L aged three weeks soaked in colchicine 0%, 0.01%, 0.025% and 0.05 for 24 hours showed the higher the concentration of colchicine resulted in sprouts with shorter height, but has larger stem diameter. The results of the research by Dewi and Pharmawati, 2018 showed that the Marigold (*Tagetes erecta L*.) sprouts treated with colchicine were shorter in size but had a larger stem diameter than the control sprouts.

Based on the results of ANOVA test, it was shown that the colchicine induction at different immersion duration with the dimensions of the leaf area (leaf length x width) of two weeks old *Z. elegans* sprouts have a significant difference ($p<0.05$). Based on further tests P0, P1, P2 and P3 showed relatively the same, but different from P4 and P5. P4 has the highest leaf dimension. Based on the correlation test, the longer the immersion duration, the wider the dimensions of the leaf area ($r=0.560$). Based on the research of Rahayu et al. (2014) the interaction between the concentration level treatment and the duration of colchicine immersion had a significant effect on covering the number and area of leaves. According to Rohmah et al. (2017), Administration of colchicine can affect leaf anatomy, namely the stomata size of olive leaves (*Olea europaea L.*).

Based on the ANOVA results, it was shown that the colchicine induction with different durations to *Z. elegans* of two-weeks old have no significant difference ($p>0.05$). Table 1 shows that colchicine induction at different immersion durations had relatively the same root length.

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Table 1. Growth performance of *Z. elegans* sprouts at different colchicine immersion durations

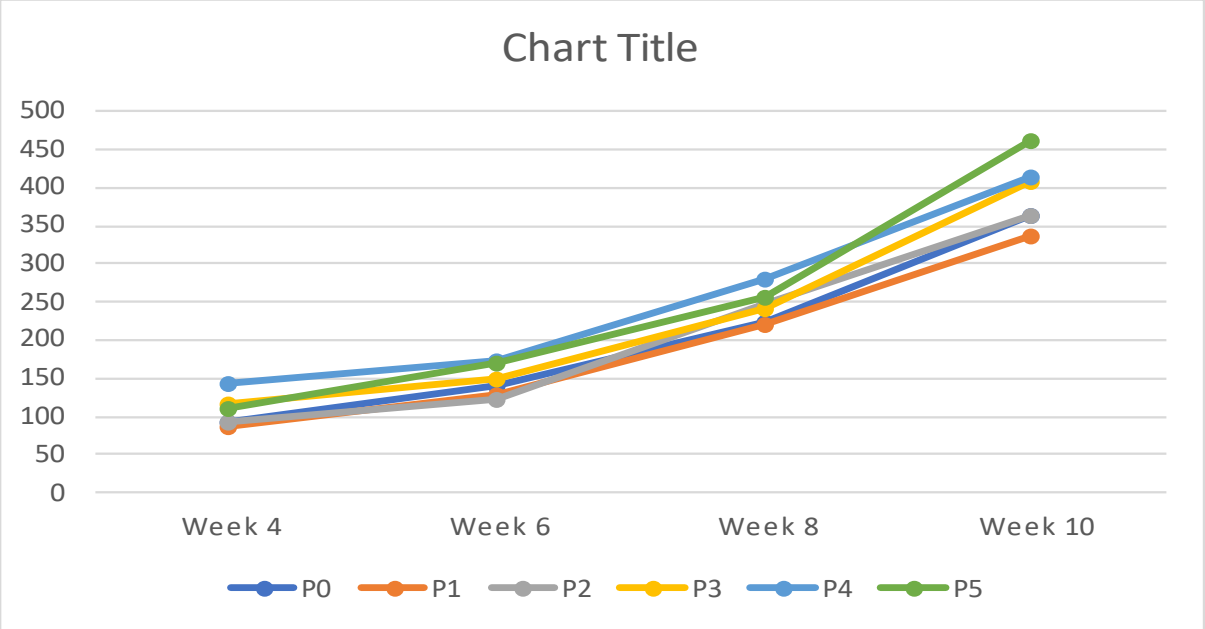
Treatment	Plant height (mm)	Leaf area: length x width (mm^2)	Root length (mm)
P0	37.00 ± 2.65^{AB}	74.67 ± 4.61^{AB}	49.67 ± 2.88^{A}
P1	38.67 ± 4.16^{AB}	76.67 ± 21.20^{AB}	41.33 ± 8.50^{A}
P2	43.33 ± 5.03^{B}	62.33 ± 8.02^{AB}	36.00 ± 8.72^{A}
P3	26.33 ± 3.79^{A}	45.00 ± 31.22^{A}	22.67 ± 2.51^{A}
P4	26.67 ± 9.71^{A}	160.67 ± 47.43^{C}	41.67 ± 17.10^{A}
P5	27.00 ± 10.15^{A}	112.00 ± 26.23^{B}	32.00 ± 4.00^{A}

Notes: different superscript letters indicate a significant Duncan"s test at the 95% confidence level.

Based on the results of the 4th, 6th, 8th, and 10th week of measurements, the plant height at each treatment level experienced an increase. The lowest growth was shown in P1, while the fastest growth was shown in P4. Growth data can be seen in Graphic 1.

Based on the results of ANOVA test, plant height at week 10 showed a significant difference between treatments ($p<0.05$). Growth in P0, P1, and P2 is relatively the same, but different from P4. P4 produced the highest plant height growth profile. Based on the correlation results, the longer duration of colchicine immersion on plant height growth at week 10 showed a positive correlation ($r=0.618$). This corresponds to the watermelon plant *Citrullus lanatus* (Thunb.) Matsum. et Nankai with 0.2% colchicine immersion treatment showed that the plants had a faster plant height compared to the control at 45 days after planting (Rosmaiti and Dani 2015).

Graphic 1. Height growth performance of *Z. elegans* plants at different colchicine immersion durations.



Notes: different superscript letters indicate a significant Duncan"s test at the 95% confidence level.

The number of leaves in the 1st week ranged from 2-3 leaves. At week 10 it ranged from 19-120 leaves. The results of ANOVA analysis showed that the number of leaves at weeks 1 and 12 was relatively the same ($p>0.05$). This shows that colchicine with different immersion durations did not give a significant difference (Table 3).

Table 3. Number of leaves of *Z. elegans* at different colchicine immersion durations

Treatment	Number of leaves on week 1	Number of leaves on week 12
P0	2.00±.,00^{A}	47.00±18.07^{A}
P1	2.33±0.58^{A}	56.25±6.85^{A}
P2	2.33±0.58^{A}	68.50±30.90^{A}
P3	2.00±0.00^{A}	66.00±37.48^{A}
P4	2.00±0.00^{A}	54.50±11.85^{A}
P5	2.00±0.00^{A}	79.00±11.37^{A}

Notes: different superscript letters indicate a significant Duncan' test at the 95% confidence level.

Based on the results of ANOVA, it was shown that the colchicine induction with different immersion durations against the wet weight of *Z. elegans* at 17 weeks of age was significantly different ($p<0.05$). P0 and P4 have relatively the same wet weight, but different from P1. The highest wet weight is found in P1 (Table 4). based on the correlation test of colchicine immersion duration on plant wet weight, there was no significant correlation ($r=-0.238$).

According to the ANOVA test results, it was shown that the colchicine induction with different immersion durations at two weeks old *Z. elegans* was not significant ($p<0.05$). This shows that all the dry weight treatments are relatively the same (Table 4). Based on the correlation test of colchicine immersion duration on plants' dry weight, there was no significant correlation observed ($r=-0.054$). The results of the research by Widya et al. (2018), showed that the induction treatment had an effect on the fresh weight and dry weight of the tuber of Javanese Ginseng (*Talin baiticulatum* Gaertn). The highest average yield on the parameters of fresh weight and dry weight of tubers was 6-hours treatment with a concentration of 0.01%.

Table 4. Wet and dry weights of *Z. elegans* on different colchicine immersion durations at 17 weeks of age.

Treatment	Wet weight (g)	Dry weight (g)
P0	48.25±17.86^{A}	9.25±3.40^{A}
P1	88.25±31.38^{B}	14.25±8.02^{A}
P2	70.67±19.14^{AB}	11.50±4.67^{A}
P3	55.00±6.68^{AB}	9.75±1.71^{A}
P4	40.25±6.95^{A}	7.00±2.00^{A}
P5	72.75±28.66^{AB}	13.88±5.78^{A}

Notes: different superscript letters indicate a significant Duncan"s test at the 95% confidence level.

Induction of colchicine with the right concentration and duration can induce polyploid plants. In addition to colchicine, orazine compounds have also been reported to induce polyploid plants (Wulansari et al. 2006). Colchicine has the effect of not forming spindle fibers, causing the separation of chromosomes at the anaphase stage to not occur and resulting in doubling of chromosomes without cell division. Chromosomes that are already in a state of doubling are not divided in opposite directions, thus forming a polyploid cell. Cell size in polyploid plants has a larger size (Escandon et al. 2006).

2. Reproductive Performance of *Z. elegans* Induced by Colchicine at Different Immersion Durations

The development of *Z. elegans* can be divided into seven stages. The first stage is covered with the criteria that there are prospective flowers with a light green round shape that is still covered by leaves. This structure has a sepal with a cycloid shape. The color of the sepals is light green. The size of this stage ranges from 1-2 (1.40±0.55 mm) (Figure number 2 a). Plain stage with the criteria for a round flower candidate that is still wrapped in flower sepals. The sepals are cycloid, bright green in color with black

tips. The size of this stage ranges from 2-4 (3.00±0.61 mm). At this stage, potential flowers have appeared with relatively long sepals (Figure number b). The emerging stage is that the flower candidate has a corolla on the top surface of the flower candidate. Sizes at this stage ranged from 6-8 (6.60±0.82 mm) (Figure number c).

The budding stage qualifies when the flower corolla is already visible on the surface. The corolla is perpendicular to the sepals. The corolla is still coiled and has not opened. The length of the corolla ranges from 10-20 mm. Meanwhile, the flower sepals ranged from 8-9 (8.80±0.27 mm) (Figure number d). The blooming stage is based on the criteria that the flower corolla has opened around the flower sepals. The size of the sepals ranged from 8-10 (9.30±0.83 mm) (Figure number. e).

The perfect bloom stage is marked by the flower corolla that is fully open. The diameter of the perfect blooming flower ranges from 54-71 mm. The size of the sepals ranges from 10-25 mm. Flower height 20-35 mm. The corolla length ranges from 18-24 mm and the corolla width ranges from 9-13 mm. The number of corollas is generally 13 strands (Figure number f). The wilting stage is characterized by a dull corolla color, with a wrinkled and dry shape. At this stage the corolla dries and falls. The remaining brown bractea contains seeds (Figure number g). And it is getting drier (Figure number h).

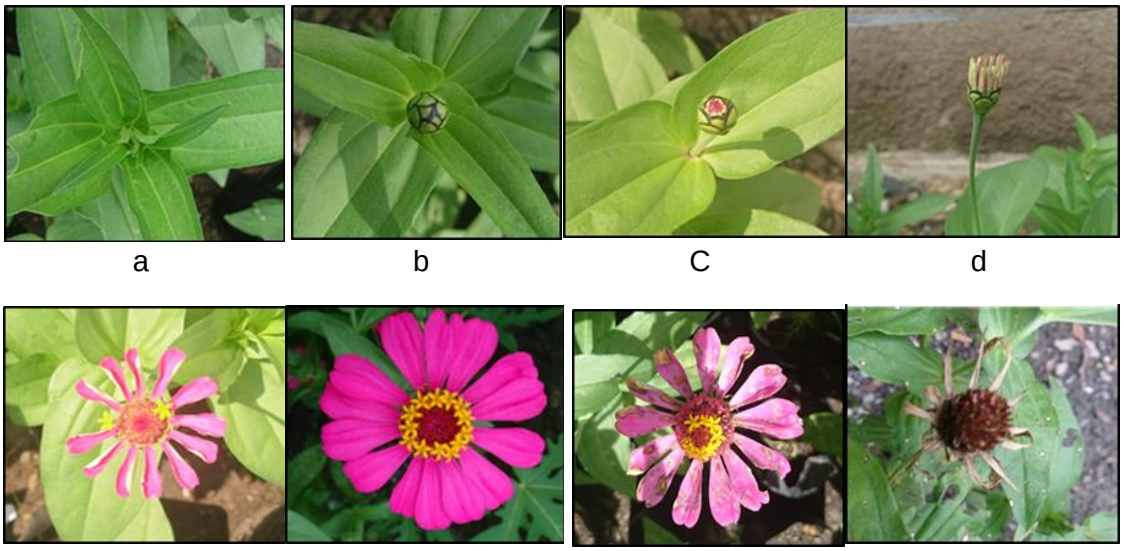


Figure 2. Developmental stages of *Z. Elegans*

At the end of the observation week 17, the number of flowers that appear was around 15-25 flowers. P0 and P2 have the least number of flowers, while P5 was the treatment that produces the most flowers. Based on the correlation test, the longer duration of colchicine immersion resulted in plants that produced more flowers (r= 0.553).

Table 5. Number of flowers of *Z. elegans* induced by colchicine at different immersion durations at week 17

Treatment	Covered	Plain	Appear	Bud	Bloom	Full	Aging	Total
P0	0	4	0	0	2	6	3	5
P1	3	6	1	0	1	10	0	21
P2	1	1	2	0	1	10	0	15
P3	4	4	1	0	0	11	0	23
P4	1	2	2	2	4	5	3	19
P5	6	2	1	1	3	8	4	25

Based on the observation of the flower characteristics, P4 produced the widest sepal diameter. The correlation test showed that longer duration of colchicine immersion did not make a difference to the sepal diameter (r=0.106). P4 produced the highest interest, the correlation test showed that the longer colchicine immersion resulted in the higher interest (r=0.772). P4 produced the widest flower diameter, correlation test showed that the immersion duration increased the flower diameter (r=0.815).

P1 resulted in the highest corolla length, correlation test showed that there was no significant difference in corolla length with colchicine immersion duration. P5 resulted in the highest corolla width, the correlation test showed a weak positive correlation between colchicine immersion time and corolla width (r=0.326). P0 produces the highest number of corollas. Based on the results, P4 produced flowers with the largest size (Table 6). According to Hayuatmaja et al. (2016), the flower diameter of *Z elegans* ranges from 32.45-61.61 mm, with the number of flowers per tree ranging from 2.59-11.39 flowers. The diameter of the flower to the number of flowers has a correlation coefficient of r = 0.49, while the diameter of the flower to the height of the plant has a correlation coefficient of r = 0.48. In this study, it was found that one plant produced pompom-type flowers (compound flower corolla) in P5 treatment.

Tabel 6. The character of *Z. elegans* flowers induced by colchicine at different immersion durations

Treatment	Ø Petals (mm)	flower height (mm)	Ø flower (mm)	Crown length (mm)	Crown width (mm)	Number of crowns (mm)
P0	17-21 (19.33±2.08)	24-28 (25.83±2.02)	56-61 (59.17±2.36)	19-22 (20.33±1.53)	9-11 (10.00±1.00)	13-17 (14.33±2.31)
P1	22 (22.00±0.00)	24-28 (26.67±2.31)	58-68 (62.33±5.13)	24-25 (24.33±0.58)	10-12 (11.00±1.00)	13 (13.00±0.00)
P2	18-23 (20.33±2.51)	23-30 (27.00±3.61)	58-65 (59.33±5.13)	19-21 (20.33±1.15)	9 (9.00±0.00)	13-14 (13.33±0.58)
P3	21-24 (22.33±1.53)	27-33 (30.00±3.00)	53-65 (60.33±6.29)	20-24 (22.00±2.00)	10 (10.00±0.00)	13 (13.00±0.00)
P4	21-25 (22.33±2.31)	27-35 (31.33±4.04)	58-68 (63.50±5.27)	21-23 (22.17±0.76)	9-11 (9.33±1.44)	13-15 (14.00±1.00)
P5	19-25 (21.33±1.53)	27-35 (30.67±4.04)	64-67 (65.33±3.21)	22-24 (23.17±0.76)	10-13 (11.33±1.53)	13 (13.00±0.00)

Another effort for breeding *Z. elegans* is by X-ray irradiation. Generation M5 of X-ray irradiated *Z. elegans* shows new phenotypic characters such as the appearance of new "tiered" flower bands on the flower buds, the presence of color gradations in the ribbon flowers, and abnormal leaf shapes (Gunawan et al. 2014). X-ray irradiation increased the activity of A+B function genes so that more ray florets were formed, besides that X-rays suppressed the activity of B+C function genes so that the flowers were of the pompom type without stamens (Gultom et al. 2012).

CONCLUSION

The growth profile of *Z. elegans* induced by colchicine showed a significant effect on germination length, leaf area dimensions, plant height and wet weight. Developmental profile of *Z. elegans* induced by colchicine resulted in a characteristically taller and larger flower diameters

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