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BIO-CATHARANTIN EFFECTS ON PHENOTYPIC TRAITS AND CHROMOSOME NUMBER OF SHALLOTS (*ALLIUM CEPA* L. VAR. *ASCALONICUM* 'TAJUK')

A.T. BILLA[1], S.S. LESTARI[1], B.S. DARYONO[1], and A.S. SUBIASTUTI[2*]

[1]Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Indonesia
[2]Laboratory of Microbiology, Faculty of Biology, Universitas Gadjah Mada, Indonesia
*Corresponding author's email: apriliasufi@ugm.ac.id
Email addresses of co-authors: adhinda.t.b@mail.ugm.ac.id, sslestari31@mail.ugm.ac.id, bs_daryono@mail.ugm.ac.id

SUMMARY

The seasonal production of *Allium cepa* var. *ascalonicum* causes a rise in its demand during the off-season. Consumers mostly prefer onion cultivars like the 'Super Philip', because of their high productivity, large and round bulbs, shiny appearance, and less spicy taste. In plant breeding, polyploidy induction through mutagens is a technique often used to produce shallot cultivars of better quality. Bio-Catharantin from the leaf extract of *Catharanthus roseus* L. is used as a polyploid induction agent instead of colchicine. The latest study aimed to determine the effect of Bio-Catharantin concentration (0.2% and 0.4%) on phenotypic traits (plant height, bulb mass, and the number of bulbs), and the chromosome number to determine the minimum concentration that could cause polyploidization in shallots. The research was conducted from December 2020 to February 2021 in a greenhouse in Madurejo, Prambanan, and the Laboratory of Genetics and Breeding, Faculty of Biology, Gadjah Mada University, Indonesia. Bio-Catharantin concentration did not affect plant height which was comparable with the control. Both treatments caused an increase in bulb mass up to 37.7 and 41.76 g at the concentrations of 0.2% and 0.4%, respectively, compared with the control (31.47 g). The number of bulbs increased up to 10.6 and 9.8 g for 0.2% and 0.4% concentrations, respectively, compared with 8.8 in the control. The ploidy level of cells was increased from 2n (16) to 3n (24) at 2% and 4n (32) at 4% Bio-Catharantin.

Keywords: Shallots (*Allium cepa* L.), *Catharanthus roseus* L., bio-catharantin, polyploid, chromosome number, phenotypic traits

Key findings: As a polyploid agent derived from the leaves extract of the *Catharanthus roseus* L., Bio-Catharantin had a significant effect on the phenotypic traits of shallots. The concentration of 0.2% significantly improved the quality of the shallot phenotype. It was able to increase the ploidy level of cells to 3n with 0.2% and to 4n with 0.4% concentration.

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INTRODUCTION

The seasonal production of shallots causes a rise in demand during the off-season. Moreover, many shallot cultivars are poor yielders with high susceptibility to different diseases (Permatasari *et al*., 2017). Consumers also prefer certain shallot cultivars like 'Super Philip' due to their high productivity, large and round bulbs, shiny skin, and less spicy taste. By using plant breeding techniques, the yield of shallots can be

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enhanced significantly (Ren *et al*., 2018; Fairuzia *et al*., 2022). Phenotypic characters can be improved with mutagenic induction of polyploidy in the superior cultivar of *Allium cepa* L. var. *ascalonicum*.

In Indonesia, shallots can be found in various parts ranging from Eastern to Western Indonesia. Shallots are one of the most in-demand commodities, as almost every typical Indonesian dish, and are used either as a condiment or as a flavoring. Another use of shallots is in traditional medicines (Nemtinov *et al*., 2021). It can be used to relieve fever by rubbing the oil all over the body. The shallot and garlic are seasonal crops, widely cultivated in lowland areas near the coast with relatively high temperatures (Sandhu *et al*., 2015).

In plant breeding, chromosomal manipulation is one way of achieving breeding goals. Polyploidization causes changes in the genome that lead to changes in the anatomical structure. Polyploidization results are often different for each taxa level in the same species or between species. Polyploidization can produce mixoploid, triploid, or tetraploid organisms (Trojak-Goluch *et al*., 2021). However, the polyploid process can be carried out on a variety of plants, to enhance the ploidy level to triploid or tetraploid as a strategy for increasing the quality of phenotypic characters and producing the desired characters. Polyploidization is commonly used as a strategy to treat the non-viability and infertility of interspecific hybrids, improve phenotypic quality without crossing, obtain seedless polyploid cultivars, and increase resistance/tolerance to both biotic and abiotic factors. The product of a successful polyploidization process is an organism with a higher ploidy level than a diploid. Changes in the ploidy level to a higher level will cause organisms to tend to have better morphological characteristics, and produce larger tubers, rhizomes, and roots than diploids (Jaillon *et al*., 2007; Hegarty *et al.,* 2013).

Polyploidy induction in plants has been widely carried out using the anti-mitotic agents, such as, colchicine, extracted from the corm of Autumn crocus (*Colchicum autumnale* L.), by applying it to the meristem tissues. Past research has revealed that anti-mitotic abilities are caused mostly by alkaloids (Becvarova *et al.,* 2006). Colchicine, which is commonly used as an anti-mitotic agent, affects the arrangement of microtubules in the mitotic metaphase, and the chromosomes that were duplicated in the S phase cannot be separated into daughter cells during mitosis, resulting in quasi-tetraploid plants (Jaillon *et al*., 2007). The effective concentration of colchicine and treatment duration depends on the plant material because it differs from one material to another (Listiawan *et al*., 2009). Colchicine can be applied on seeds, protocorms (tissue that will grow as the first shoots), and sprouts with the cotton swab method (wrapping with cotton). The use of colchicine as a chromosomal multiplier agent still dominates. However, these compounds are dangerous, and this recent research is aimed at finding safer alternatives to be used as anti-mitotic agents. Therefore, there is a need for a study to find a substitute for colchicine which can multiply chromosomes. This compound will greatly help plant breeding efforts to be more effective. Similar studies have been done to find out natural material that have the potential as polyploidization agents, such as, Oryzalin (Trifluralin) (Zlesak *et al*., 2005; Allum *et al*., 2007), dinitroanilines, and phosphorothioamidates (Yemets and Blume, 2008).

Another alkaloid that has similar properties can be found in the leaf of *Catharanthus roseus* L. The Faculty of Biology, Gadjah Mada University, Indonesia discovered Bio-Catharantin, a polyploid agent derived from the leaf extract of 'tapak dara' (also called Madagascar periwinkle), as a substitute for colchicine at a more affordable production price (Listiawan *et al*., 2009). This product has the patent number IDPP000038766 obtained in 2015 from the Ministry of Agriculture, Indonesia. The active compound in catharanthine is vindoline alkaloid, which is an anti-cancer agent. Vinca-alkaloids are classified as vinblastine, vincristine, vinorelbine, and vinflunine, which can be used as effective inhibitors of mitotic division (Sertel *et al*., 2011). This study aimed to determine the effect of Bio-Catharantin on the phenotypic and ploidy characters of shallot cells and to determine the minimum concentration that causes polyploidization in shallots.

MATERIALS AND METHODS

The recent research was conducted from December 2020 to February 2021 in a greenhouse in Madurejo, Prambanan, and the Laboratory of Genetics and Breeding, Faculty of Biology, Gadjah Mada University, Indonesia.

Germination and planting of shallots

In this research, *Allium cepa* L. var. *ascalonicum* 'Tajuk' was used as samples. As many as 150 shallot bulbs were allowed to root before planting by sowing the bulbs in a container filled with water. The bulbs with roots were soaked in distilled water as the control, and in 0.2% and 0.4% solutions of Bio-Catharantin for 12 hours. Each treatment used 75 shallots. The shallots were then ready to be planted in the greenhouse randomly. The research design used is completely randomized. Before planting, the soil was loosened, and fertilizer was applied to provide nutrition for the shallots. Furthermore, the land was mulched to reduce weed growth. Then, planting was carried out in the soil medium by sticking the germinated shallot bulbs with a distance of 25 cm between them. Plants in the greenhouse were watered every two days. Fertilization and pesticide spraying were performed every two weeks. Soil cleaning and removal of weeds were conducted every three weeks.

Harvesting and recording of phenotypic characters

After 55 days of planting, the shallots were harvested by removing the bulbs and their leaves. All crop yields were analyzed for phenotypic characters. Phenotypic characteristics observed were: plant height, bulb mass (fresh weight of the bulbs), and the number of bulbs per plant. Observation of quantitative characters aimed to determine the phenotypic changes in shallots treated with 0.2% and 0.4% Bio-Catharantin, compared with the control. The plant height was measured with a ruler as the distance from the base of the bulb to the tip of the longest leaf in cm. The fresh weight of bulbs was measured with an analytical scale, and the number of bulbs per plant was counted. The data obtained were then analyzed using SPSS-25 with the one-way ANOVA – Duncan analysis method at a confidence level of 5%, to determine the significant differences among the treatments.

Observation of chromosomes

Chromosome observations were conducted by the squash method as described by Aristya and Alyza (2019). Roots, initiated in distilled water and then treated with 0.2% and 0.4% Bio-Catharantin for 12 hours, were used. For each treatment, 10 root tips of shallot were used. Root tips were harvested between 08.00–10.00 am and fixed in 45% glacial acetic acid for 15 min at 4 °C. Subsequently, the root tips were rinsed three times with distilled water, macerated using 1N HCL for 5 min at 60 °C, then washed three additional times with distilled water, and finally stained with 1% aceto orcein at room temperature for 30 min. The stained shallot roots were squashed on a glass coverslip, which was dipped in glycerin, then sealed, and observed under a microscope. Chromosomes were observed using a light microscope (Olympus BX-41TF) with 100 × 10 magnification.

Karyotyping and idiogram

Karyotype formulas were established based on measurements of the metaphase chromosomes in photomicrographs. There are five to seven cells analyzed for each sample. Then, the chromosomes were sorted from the longest to the shortest mean of the absolute chromosome arm. Karyotyping was performed using the software Image Raster 3 and Microsoft Excel 2016 (Aristya and Alyza, 2019). For studying the nomenclature of the chromosome type, and the classification of the karyotype symmetry, the methodology of Saensouk and Saensouk (2021) was followed.

RESULTS AND DISCUSSION

In the present study, shallot plants grown from bulbs treated with Bio-Catharantin at the concentrations of 0.2% and 0.4% for 12 hours were compared with the control (without Bio-Catharantin treatment) for three phenotypic traits: plant height, bulb mass, and the number of the bulbs. Based on the data, the average plant height did not show a significant difference among the treatments (Table 1). The average bulb mass and the number of bulbs of treated plants was significantly higher compared with the control, although there was no significant difference between the two concentrations of Bio-Catharantin. These results indicated that Bio-Catharantin was able to increase the mass and number of shallot bulbs with a minimum concentration of 0.2%.

A previous study from Sholikhah (2020) has treated *Allium cepa* L. var. *ascalonicum* 'Tiron' plants with Bio-Catharantin concentrations of 0.2%, 0.4%, 0.6%, and 0.8% for 12, 20, and 24 h. The result revealed that optimal multiplication of chromosomes was induced at the concentrations of 0.2% and 0.4% through the polyploidization process.

Table 1. Phenotypic characters until the harvest period after treatment with Bio-Catharantin in shallots (*Allium cepa* L. var. *ascalonicum* 'Tajuk').

Characters	Concentrations of Bio-Catharantin		
	Control	0.2%	0.4%
Plant height (cm)	36.8667 ± 0.7760	38.6667 ± 0.7193	38.9 ± 0.6330
Bulb mass (g)*	31.4667 ± 1.9062[a]	37.7 ± 2.0189[b]	41.7667 ± 1.795[b]
Number of bulbs*	8.7667 ± 0.6620[a]	10.5667 ± 0.5747[b]	9.8333 ± 0.5695[b]

*real different characters; Lowercase letters indicate the subset difference at the 5% significance level and the ± sign indicates the standard deviation value. The samples that were used in the measurements were 30 for each treatment

These two concentrations of Bio-Catharantin can be used to induce chromosomal changes and to determine their effects on phenotypic traits, such as, plant height, bulb weight, bulb color, and texture in shallots. According to Karim *et al*. (2019), polyploids tend to have phenotypic traits of larger size and better adaptability to the diverse environment than diploid and haploid plants.

The shallot bulb size of different treatments was compared (Figure 1). Shallots with Bio-Catharantin treatments showed a greater enhancement of bulb mass compared with the control. The weight increases in bulbs that had been treated with Bio-Catharantin were due to polyploidy induction. Polyploidy induction in plants can cause the cells to grow larger so that plant growth can be more dramatic with better quality. Polyploidy is usually achieved with mutagenic, and anti-mitotic substances, such as, colchicine because the substances contained in colchicine can inhibit the formation of spindle threads which help in cell division (Khoiroh *et al*., 2015). Therefore, if the division does not occur, the cells that will form become larger, and the chromosomes undergo imperfect multiplication and can express larger and higher-quality phenotypic characters (Hidaka *et al*., 1979).

The application of Bio-Catharatin with concentrations of 0.2%–0.4% was known to have not been able to increase the height of shallot plants. Based on Firmansyah and Bermana (2019), plant height and number of bulbs are determined by genetic factors. However, plant growth is closely related to environmental factors. Adding IAA and GA hormones, as well as rhizobacteria in shallot plants are known to significantly increase plant height. Possibly, it is necessary to increase the concentration of Bio-Catharantin to have a significant effect on shallot plant height. The success of polyploidization induction is not only influenced by the concentration of the solution but also the duration of immersion (A'yun *et al.,* 2021). The effect of induction on each tissue can also be different if the combination of solution concentration and soaking time is not appropriate.

Figure 1. The comparison of bulb size of shallots (*Allium cepa* L. var. *ascalonicum* 'Tajuk') between control and after Bio-Catharantin treatment.

Determination of the dose of chemical compounds as polyploidizing agents needs to take into account the possibility of higher penetration under *in vitro* conditions compared with *in vivo* conditions, media composition, and duration of treatment. Media components, such as, sucrose or growth regulators, may interact with polyploidization agents possibly causing an increase or decrease in its effectiveness (Lattier *et al*., 2013; Niu *et al*., 2016). Some literature shows that the treatment time of the polyploidization agent affects the degree of ploidy produced. Niu *et al*. (2016) reported that soaking the seeds of *Jatropha curcas* in 0.1% colchicine solution for 24 h did not induce polyploidy. However, the increase in exposure time, of four to seven days, increases the polyploid induction rate from 10% to 15%, respectively.

Bio-Catharantin contains vincristine and vinblastine, which can act as anti-mitotic compounds on the chromosomes of plants. According to Mohammed (2016), vincristine and vinblastine function to suppress microtubule activity through the β-tubulin side binding mechanism. This suppressive mechanism begins with the forming of spiral filaments of β-tubulin, which can interact laterally and form a paracrystalline structure. This process is similar to the anti-mitotic mechanism that occurs in plants treated with colchicine. The difference is in the paclitaxel structure, where this process stimulates the polymerization of β-tubulin polymers to form abnormal microtubule structures that are less stable (Sertel *et al*., 2011). Vincristine and vinblastine contained in Bio-Catharantin function as a stimulant for the formation of paracrystalline groups from β-tubulin, which causes microtubule depolymerization. With depolymerization, the spindle thread formation is inhibited. Consequently, the chromosomes fail to divide during mitosis but continue to multiply (Purbosari and Puspitasari, 2018). Therefore, it is a polyploidization process. Vincristine has higher tubulin binding activity than vinblastine. In contrast to vinblastine, vincristine can induce denaturation of tubulin at neutral pH (Sevenet, 1999).

To determine the existence of polyploidy, two methods can be used, i.e., microscopic observation of chromosomes and flow cytometry. Chromosome observation using the squash technique directly revealed the increased number of chromosomes in treated plants (Figure 2). Bio-Catharantin treatment of 0.2% contained cells with 3n or triploid chromosomes, while the concentration of 0.4% of Bio-Catharantin produced tetraploid (4n) cells. Artificial polyploidization is often used in plant breeding to produce individuals with superior phenotypic characters and improved quality (Susianti *et al*., 2015). A study of colchicine treatment with 0.05% colchicine immersion for 12 h resulted in mixoploid red ginger (2n = 2x + 4x) (Friska and Daryono, 2017). In another study, colchicine treatment of strawberry plants resulted in an increased size of the strawberry fruit by 0.05% treatment and vegetative organs of the plant by 0.01% treatment (Friska and Daryono, 2017).

In the shallot control, all of the observed cells had chromosomes of 2n (16) (Figure 2a), five of seven observed cells from Bio-Catharantin 0.2% treatment has chromosomes 3n (24) (Figure 2b), and all of the observed cells of Bio-Catharantin 0.4% treatment has 4n (32) chromosomes (Figure 2c). The cleavage phase was observed during prometaphase (Figure 2). The prometaphase phase is the best division phase for

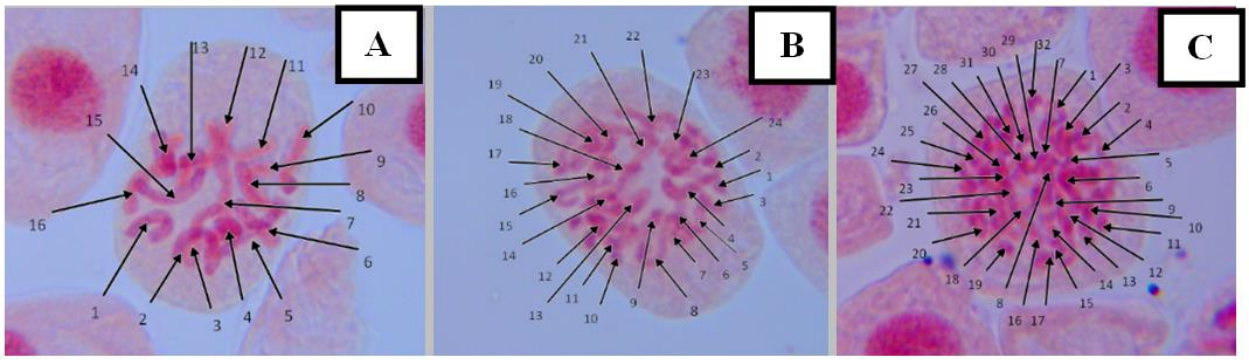

Figure 2. The number of chromosomes found in shallots (*Allium cepa* L. var. *ascalonicum* 'Tajuk'). (A) control = 2n (16), (B) 0.2% Bio-Catharantin = 3n (24), and (C) 0.4% Bio-Catharantin = 4n (32).

chromosome observation and analysis. This is because in the prometaphase, more precisely the initial chromatid metaphase, the chromosomes are moving toward the equator plane so that the chromosomes do not overlap and can be seen clearly (Gultom, 2016). Polyploidy can occur because of the presence of anti-mitotic substances that can prevent the formation of spindle threads in the prophase so that the chromosomes fail to separate (non-disjunction) (Rasineni *et al*., 2010). Separation of chromatids, which indicates the shift from metaphase to anaphase, does not take place causing chromosome duplication without cell wall formation (Tsukazaki *et al*., 2010; Marlin *et al*., 2018). Polyploid induction can produce plants with larger morphological sizes (Sevenet, 1999; Royani *et al*., 2015).

The formula obtained for this karyotype was 2n = 2x = 14M + 2SM, which means that on average, cells in the control treatment were diploid (2n), and contained seven metacentric pairs of chromosomes and one submetacentric pair (Figure 3a). The karyotype for Bio-Catharantin treatment of 0.2% with a chromosome formula of 3n = 3x = 24M, meaning that in treatment 0.2%, on average, cells were triploid with 24 chromosomes (Figure 3b). The chromosome formula and karyotype for Bio-Catharantin treatment of 0.4% is indicated as 4n = 4x = 32M, or tetraploid (Figure 3c). These three karyotypes revealed that shallot plants have symmetrical karyotypes. The symmetrical karyotype is a karyotype with a uniform chromosome type and is considered more primitive than an asymmetric karyotype (Ermayanti *et al*., 2019; Nemtinov *et al*., 2021).

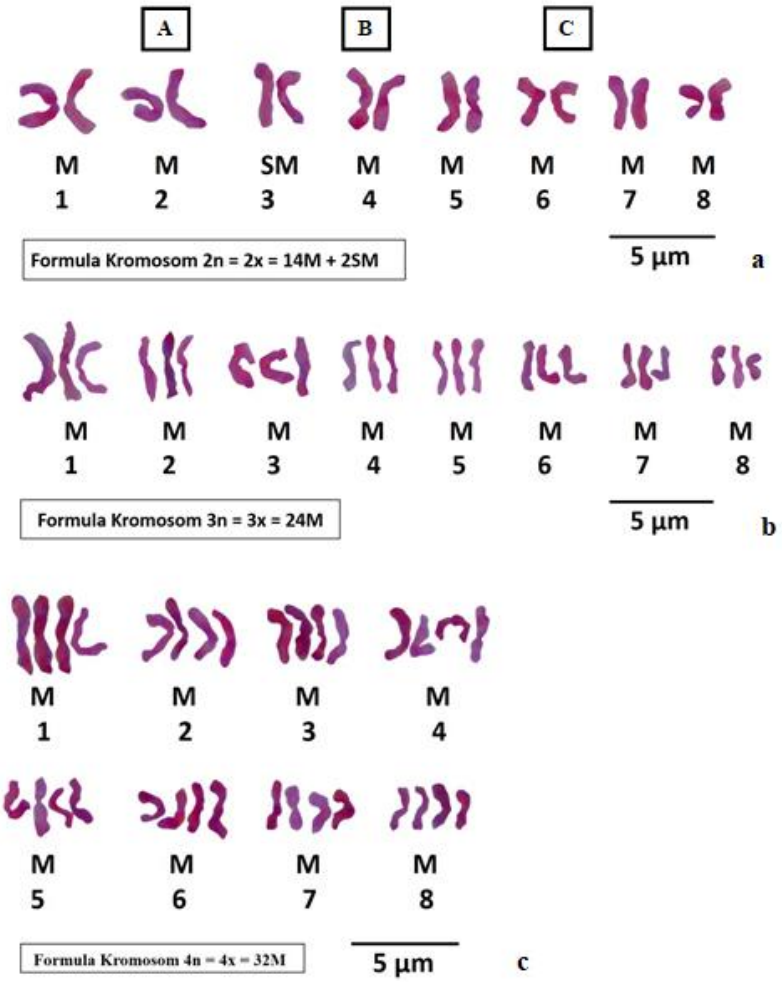

Figure 3. Shallots (*Allium cepa* L var. *ascalonicum* 'Tajuk'). karyotype with control treatment (a), 0.2% Bio- Catharantin treatment (b), and 0.4% Bio- Catharantin treatment (c).

The ideogram results revealed that the chromosome lengths between control and Bio-Catharantin treatments were different. However, it tended to be the same between the treatments using 0.2% and 0.4% of Bio-Catharantin (Figures 4a-c). The ideogram with the longer average graph was at 0.2% treatment (p = 4.9 µm, q = 5.52 µm) compared with control (p = 3.48 µm, q = 5.60 µm) and 0.4% treatment (p = 3.48 µm, q = 5.60 µm). The shortest ideogram graph was also at the concentration 0.2% (p = 2.77 µm, q = 3.44 µm) compared with control (p = 2.95 µm, q = 3.97 µm), and the concentration 0.4% (p = 2.94 µm, q = 3.63µm).

The differences in the length of the various ideogram graphs proved that each treated individual has a different genetic background. The main variations that can be observed in comparisons of interrelated species can be seen by comparing the absolute arms, chromosome staining properties, chromosome number, and phenotypic shapes. On the ideogram, the chromosomes observed with a longer graphic form might also contain more genetic information than chromosomes with shorter absolute lengths (Sulistyaningsih *et al*., 2006).

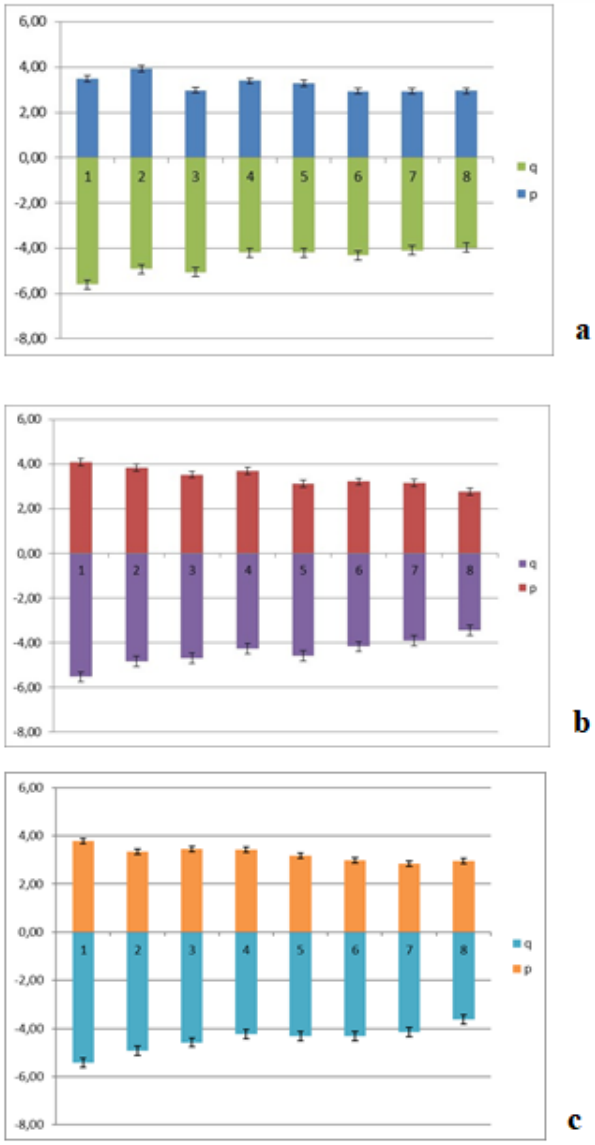

Figure 4. Ideogram of shallots (*Allium cepa* L var. *ascalonicum* 'Tajuk'). with control treatment (a), 0.2% Bio- Catharantin treatment (b), and 0.4% Bio- Catharantin treatment (c).

CONCLUSIONS

Based on the results, Bio-Catharantin can produce a polyploid in the shallot at both 0.2% and 0.4% concentrations. These concentrations may have different results if induced in other plants. The administration of anti-mitotic substances during polyploidization treatment can cause genomic shock, resulting in changes in genome components. A large number of genomic changes due to polyploidization are proven to play a vital role in plant evolution. Using Bio-Catharantin as the polyploid agent, it is hoped that it will be another local alternative commodity for the nation's future generations that can produce basic crops, not only with higher yield, but also best quality.

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REFERENCES

Allum JF, Bringloe DH, Roberts AV (2007) Chromosome doubling in a *Rosa rugosa* Thunb. hybrid by exposure of *in vitro* nodes to oryzalin: The effects of node length, oryzalin concentration, and exposure time. *Plant Cell Rep*. 26(4): 1977-1984.

Aristya GR, Alyza R (2019). Chromosome characterization of festival strawberry (*Fragaria* × *ananassa* D. var. *Festival*) result of polyploidization. *Biogenesis J. Ilm. Biol*. 7(1): 1-8.

Becvarova P, Skorpikova J, Janish R, Novy J (2006) A vinca alkaloid effect on microtubules of hela cells. *Script Medica* (BRNO). 79(1):19-34.

Ermayanti TM, Rantau DE, Wulansari A, Martin AF, Haflizh EA (2019). Variasi jumlah kromosom talas bentul (*Colocasia esculenta* (L.) Schott) *in vitro* hasil perlakuan orizalin. *J. Biologi. Indones*. 15(1): 53-64.

Friska M, Daryono BS (2017). Derajat ploidi jahe merah (*Zingiber officinale* Roxb. var. rubrum Rosc.) hasil induksi dengan kolkisin. *Biogenesis J. Ilm. Biol*. 5(1): 49-54.

Gultom T (2016). Pengaruh pemberian kolkisin terhadap jumlah kromosom bawang putih (*Allium sativum*) lokal kultivar Doulu. *J. Biosains.* 2(3): 165-172.

Handayani T, Hidayat IM (2012). Keragaman genetik dan heritabilitas beberapa karakter utama pada kedelai sayur dan implikasinya untuk seleksi perbaikan produksi. *J. Hortic*. 22(4): 327-333.

Hegarty MJ, Coate S, Sherman-Broyles R, Abbott RJ, Hiscock S, Doyle J (2013). Lessons from natural and artificial polyploids in higher plants. *Cytogenet. Genome Res*. 140(2-4): 204-225.

Hidaka T, Yamada Y, Shichijo T (1979). *In vitro* differentiation of haploid plants by anther culture in *Poncirus trifoliata* (L.). *Japan J. Breed*. 29(3): 248-254.

Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C, Vezzi A, Legeai F, Hugueney P, Dasilva C, Horner D, Mica E, Jublot D, Poulain J, Bruyère C, Billault A, Segurens B, Gouyvenoux M, Ugarte E, Cattonaro F, Anthouard V, Vico V, Del Fabbro C, Alaux M, Di Gaspero G, Dumas V, Felice N, Paillard S, Juman I, Moroldo M, Scalabrin S, Canaguier A, Le Clainche I, Malacrida G, Durand E, Pesole G, Laucou V, Chatelet P, Merdinoglu D, Delledonne M, Pezzotti M, Lecharny A, Scarpelli C, Artiguenave F, Pè ME, Valle G, Morgante M, Caboche M, Adam-Blondon AF, Weissenbach J, Quétier F, Wincker P (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449(7161): 463-467.

Karim HA, Jamal A, Sutrisno T (2019). Respon pemberian pupuk mikrobat dengan berat umbi berbeda terhadap pertumbuhan dan produksi tanaman bawang merah (*Allium ascalonicum* L). *Agrovital J. Ilmu. Pertanian* 4(1): 24-29.

Khoiroh R, Aristya GR, Sutikno S, Handayani NSN (2015). Karakterisasi kromosom stroberi (*Fragaria vesca* L. subsp. *californica* Cham. & Schltdl. cv. *Californica*) hasil poliploidisasi. *Biogenesis J. Ilm. Biol*. 3(2): 87-95.

Lattier JD, Touchell DH, Ranney TG, Smith JC (2013). Micropropagation and polyploid induction in *Acer platanoides* "Crimson Sentry". *J. Environ. Hortic*. 31(4): 246-252.

Listiawan DA, Indraningsih E, Septantri AN, Wibowo AT, Darojat UW, Daryono BS (2009). Potensi ekstrak etanolik daun tapak dara (*Catharanthus roseus* (L.) D. Don) sebagai alternatif pengganti kolkhisin poliploidisasi tanaman. *J. Biologi Indonesiq* 5(4): 423-430.

Marlin, Maharijaya A, Purwito A, Sobir (2018). Molecular diversity of the flowering-related gene (LEAFY) on shallot (*Allium cepa* var. Aggregatum) and *Allium* relatives. *SABRAO J. Breed. Genet*. 50(3): 313-328.

Mohammed IH (2016). A vinca alkaloid effect on microtubules of CHO (China Hamster Ovary). *Am. J. Biomed. Sci*. 4(6): 227-235.

Nemtinov VI, Kostanchuk YN, Pashtetskiy VS, Motyleva SM, Bokhan AI, Caruso G, Katskaya AG, Timasheva LA, Pekhova OA (2021).Biochemical and cytological features of onion bulbs and leaves collected from various ecogeographical origins. *SABRAO J.*

Breed. Genet. 53(4): 543-560. https://doi.org/10.54910/sabrao2021.53.4.1.

Niu L, Tao Y, Chen M, Fu Q, Dong Y, He H, Xu Z (2016). Identification and characterization of tetraploid and octoploid *Jatropha curcas* induced by colchicine. *Caryologia*. 69(1): 58-66.

Permatasari NA, Yuliasih I, Suryani A (2017). Proses pembuatan pasta bawang merah (*Allium cepa* var. *aggregatum*) dan penentuan umur simpannya dalam kemasan gelas. *J. Teknol. Ind. Pertan. Indonesia* 27(2): 200-208.

Purbosari PP, Puspitasari ED (2018). Pengaruh ekstrak etanol daun tapak dara (*Catharanthus roseus* L.) dan kolkisin terhadap perkecambahan biji cabai rawit hibrida (*Capsicum annuum*). *Bioedukasi: J. Pendidik. Biol*. 9(2): 181-187

Rasineni K, Bellamkonda R, Singareddy RS, Desireddy S (2010). Antihyperglycemic activity of *Catharanthus roseus* leaf powder in streptozotocin-induced diabetic rats. *Pharmacogn. Res*. 2(3): 195-201.

Ren J, Wu X, Song C, Liang Y, Gao W, Wang Y (2018). Induction of polyploid tillered onion using colchicine and pendimethalin. *Sains Malays*. 47(11): 2617-2624.

Royani I, Zulkifli L, Sedijani P (2015). Induksi kalus kacang tanah (*Arachis hypogaea* L.) varietas kelinci dengan perlakuan 2,4-D DAN BAP. *J. Penel. Pend. IPA*. 1(2): 70-76.

Saensouk S, Saensouk P (2021). Karyotype analysis of three species of Allium (*Amaryllidaceae*) from Thailand. *Biodiversitas* 22(8): 3458-3466.

Sandhu SS, Brar PS, Dhall RK (2015). Variability of agronomic and quality characteristics of garlic (*Allium sativum* L.) ecotypes. *SABRAO J. Breed. Genet*. 47(2): 133-142.

Sertel S, Fu Y, Zu Y, Rebacz B, Konkimalla B, Plinkert PK, Krämer A, Gertsch J, Efferth T (2011). Molecular docking and pharmacogenomics of vinca alkaloids and their monomeric precursors, vindoline, and catharanthine. *Biochem. Pharmacol.* 81(6): 723-735.

Sevenet T (1999). Antimitotic and cytotoxic compounds from tropical plants. *Nig. J. Nat. Prod. And Med*. 3(1): 9-14.

Sholikhah A (2020). Analisis induksi poiliploid bawang merah (*Allium cepa* L. 'Tiron') mengunakan bio-catharanthine dan in-silico senyawa anti-viral. Thesis, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Sulistyaningsih E, Aoyagi Y, Tashiro Y (2006). Flower bud culture of shallot (*Allium cepa* L. *Aggregatum* group) with cytogenetic analysis of resulting gynogenic plants and somaclones. *Plant Cell Tissue Organ Cult.* 86(2): 249-255.

Susianti A, Aristya GR, Sutikno S, Kasiamdari RS (2015). Karakterisasi morfologi dan anatomi stroberi (*Fragaria x ananassa* D. cv. Festival) hasil induksi kolkisin. *Biogenesis J. Ilm. Biol.* 3(2): 66-75.

Trojak-Goluch A, Kawka-Lipińska M, Wielgusz K, Praczyk M (2021). Polyploidy in industrial crops: Applications and perspectives in plant breeding. *Agronomy* 11(12): 2574-2600.

Tsukazaki H, Yamashita K, Yaguchi S, Yamashita K, Hagihara T, Shigyo M, Kojima A, Wako T (2010). Direct determination of the chromosomal location of bunching onion and bulb onion markers using bunching onion–shallot monosomic additions and allotriploid-bunching onion single alien deletions. *Theor. Appl. Genet.* 122(3): 501-510.

Yemets, A, Blume, Y (2008). Progress in plant polyploidization based on antimicrotubular drugs. *The Open Hortic. J*. 1(3): 15-20.

Zlesak DC, Thill CA, Anderson NO (2005) Trifluralin-mediated polyploidization of *Rosa chinensis* minima (Sims) Voss seedlings. *Euphytica* 141(7): 281-290.