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Effect of colchicine on chromosome number, morphological character and ß-carotene production of *Amaranthus tricolor*'s red giti cultivar

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Abstract. *Arindyaswari A, Etikawati N, Suratman. 2021. Effect of colchicine on chromosome number, morphological character and ß-carotene production of* Amaranthus tricolor*'s red giti cultivar. Cell Biol Dev 5: 18-24. Amaranthus tricolor* L. (bayam cabut or pulled spinach) is a vegetable consisting of ß-carotene, which acts as an antioxidant. Therefore, amaranths have potential as a functional food. ß-carotene production in a plant can be enhanced with mutation, one of which is mutation induction with colchicine. The aim of this research was to understand the effect of colchicine on chromosome number, morphological character and production of ß-carotene in *A. tricolor* of red giti cultivar and to find out which variation of treatment was optimum for producing polyploidy. This research was expected to give information about colchicine induced in amaranths, which had higher nutritional aspects and quality to be consumed. Amaranths seeds with colchicine treatment were done with various concentrations (0, 50, 100, 200, 500) ppm with soaking time variation of 6 and 12 hours, and then planted up to 40 days later. The number of chromosomes was analyzed by making preparation using the squash method, then observed under a microscope. Morphological characters were observed by measuring leaf length and width, stem diameter, and plant height. ß-carotene content was analyzed using a UV-Vis spectrophotometer with a wavelength of 450 nm. Data on leaf size, stem diameter, plant height, chromosome number, and ß-carotene content were analyzed using a one-way Analysis of Variance (ANOVA). The results obtained were amaranths with the treatment of concentration 50 ppm with 6 hours soaked in colchicine and concentration 100 ppm with 12 hours soaked in colchicine showed significant changes to the induction of colchicine, which had a polyploidy character with several 3n = 51. Amaranths with the treatment of concentration 100 ppm with 12 hours soaked in colchicine solution were more effective in increasing the size of length, width of amaranths leaf, stem diameter, and stem height, while treatment of concentration 50 ppm with 6 hours soaked in colchicine solution was effective in increasing ß-carotene production up to 908,40±116,800 mg/kg. Statistical analysis of each morphological character and ß-carotene production of colchicine-induced amaranths shows significant results.

Keywords: *Amaranthus tricolor*, chromosome, colchicine, morphology, red giti, ß-carotene

INTRODUCTION

Secondary metabolites are bioactive compounds that are very useful in treating and preventing human diseases (Dogra et al. 2015; Putra et al. 2020). Secondary metabolites compounds in Plants play a role in defending themselves from unfavorable environmental conditions, such as adaptation against drought and overcoming pests and diseases. These compounds also can treat some diseases, including humans. These compounds are Terpenoids, specialized nitrogen metabolites (including protein amino acids, amines, cyanogenic glycosides, glucosinolates, and alkaloids), phenolics, tannins, and flavonoids (Singh 2015). Amaranth is a plant that produces secondary metabolites; there are two species of amaranths in Indonesia, i.e., bayam cabut (*Amaranthus tricolor* L.) and bayam petik (*Amaranthus hybridus* L.). The *A. tricolor* is common for consumption, it has three cultivars, i.e.,: red giti, green giti, and raja. The cultivar used in this research was the red giti cultivar.

The *A. tricolor* of red giti cultivar contains more nutrients than other cultivars. It contains more calories, 51 kcal, while green giti only contains 36 kcal per 100 g. The protein content in the red giti cultivar is 46 g, while in the green giti cultivar in only 35 g per 100 g (DEPKES 1980). Red giti is also well-known as a vegetable that has antioxidant efficacy due to the presence of vitamin A, which is formed from provitamin A, one of which is ß-carotene (ß-carotene) (Madhavi et al. 1996). The presence of ß-carotene in the red giti cultivar makes this vegetable high nutritional. ß-carotene can act as an antioxidant and helps repel free radical molecules (Phillip et al. 2002). Free radicals can be defined as molecular or molecular fragments containing one or more unpaired electrons in their atomic orbitals or outermost orbitals. These molecules can be highly reactive and initiate chain reactions (Sen et al. 2010). Free radicals molecules can be generated from external factors such as smoke from a cigarette, certain pollutants, organic solvents, anesthetics, and pesticides. Free radicals can react with important macromolecules in the human body and cause cell damage and disruption of homeostasis (Mohammed et al. 2015). Free radicals can bring out negative effects if they continue to exist inside the human body, so humans need to consume fruits and vegetables that contain antioxidant properties such as ß-carotene. Red giti cultivar contains a carotenoid that acts as an antioxidant (Amin et al. 2006).

As cited by Wong et al. (2006), *A. tricolor* of red giti cultivar has a higher potential for antioxidant activity than celery (*Apium graveolens* L.) and rosella leaf (*Hibiscus sabdariffa* L.). This vegetable is commonly cultivated for direct consumption. However, it has the potential to become a functional food ingredient that has more value because of its properties. To maximize the potential of red giti can be done by increasing the quality of the plant, such as increasing its biomass or plant size and secondary metabolic production. Activities that can be carried out to support these theories are plant breeding.

According to Song et al. (2012), plant breeding activities can be done through mutations, one of which is polyploidy induction. Also known as chromosome doubling, it is a phenomenon of increasing the number of chromosomes set in an individual cell. Polyploidy can produce plants with a bigger size and more biomass than the normal ones, are more resistant to biotic and abiotic stress conditions, and produce more secondary metabolites compounds such as ß-carotene. Polyploidy can be induced by injecting mutagen compounds into an individual. The mutagens that can be used for this method are chemical mutagens from the anti-microtubule dinitroaniline group, such as colchicine, oryzalin, and trifluralin. This mutagen has the ability to inhibit the formation of spindle threads during cell division, developing into chromosome doubling (Ascough and Staden 2008). The compound used in this research is Colchicine. Colchicine ($C_{22}H_{25}O_6N$) is a white alkaloid obtained from the tuber of *Colchicum autumnale* L. (Liliaceae) (Eigsti and Dustin 1957). Colchicine solution can prevent the formation of microtubules so that the transfer of chromosomes at the anaphase stage of mitotic division does not proceed and cause chromosome doubling (Nagahatenna and Peiris 2008). Chromosome doubling that occurs due to colchicine induction has the potential to produce polyploid individuals and will affect the number of chromosomes, morphological characters, and the production of secondary metabolites.

This research is expected to provide insight and information about the effect of colchicine on chromosome number, morphological characteristics, and betaß-carotene production and determine the effect of treatment with optimum concentration and duration of immersion in colchicine to produce polyploidy cells in the red giti cultivar.

MATERIALS AND METHODS

Procedures

The study was conducted using a completely randomized design (CRD) factorial pattern with 2 factors and 5 repetitions. The first factor is the concentration of colchicine (K) with 5 variations (K_1= 0, K_2= 50, K_3= 100, K_4= 200, K_5= 500) ppm and the second factor is the difference in the length of time (W) of soaking the seeds with variations, namely W_1= 6 hours and W_2=12 hours (Table 1).

Preparation of colchicine solution

Colchicine solution was made of 10 mL each in 4 different concentrations, 50 ppm; 10 ppm; 200 ppm, and 500 ppm. The 500 ppm colchicine solution was prepared by dissolving 10 mg of colchicine in 20 mL distilled water. The next concentration was made by diluting 500 ppm concentration solution with distilled water; a concentration of 200 ppm requires 4 mL of 500 ppm solution, 2 mL of 500 ppm solution for a concentration of 100 ppm, and 1 mL of 500 ppm solution to create 50 ppm concentration. Then each of them was added with distilled water up to 10 mL.

Colchicine treatments and planting

The activity starts with soaking 100 seeds of *A. tricolor*'s red giti cultivar in distilled water to clean the dirt. Seeds were soaked in colchicine solution with various concentrations (0, 50, 100, 200, 500) ppm for 6 and 12 hours at room temperature (25-27°C). Next, seeds are then sown on wet cotton for 10 days, then planted in polybags with a media mixture of soil, manure, and husk charcoal in a ratio of 2:1:1. Watering while still in the seedling stage is done twice a day. Watering of adult plants was conducted once a day, in the morning. Plants were maintained by watering 150 mL of water per polybag containing one individual sprout. Amaranth plants can be harvested 40 days after planting and then observed for the number of chromosomes, morphological character, and levels of ß-carotene.

Analysis of chromosomes number

The number of chromosomes was analyzed according to the modified method of Sinha et al. (2016). The first step is to pick young leaves and cut the ends of the leaf at the time of cell division. With some trials conducted before, the time for mitotic cell division for red giti is 5.12 am Jakarta time. Samples were stored in flacon bottles containing 0.2% colchicine solution for 4 hours at room temperature to stop cell division activity. Samples that had been soaked in 0.2% colchicine were then washed with distilled water three times and fixed with 45% glacial acetic acid (AAG) for 15 minutes. The next step is hydrolysis; before then, the sample was washed three times with distilled water, then hydrolysis using 1N HCl solution for 30 minutes at 60°C. The sample was then immersed in a 2% acetoorcein solution for 24 hours, crushed with the Squash method, and placed in a sample glass. The sample was then dripped with glycerin and covered with a cover glass. Observations were carried out with a light microscope. The chromosome image was then observed, and the number of chromosomes contained was calculated.

Observation of morphological character

Observations of morphological character were conducted on leaf size, stem diameter, and plant height on amaranths after 40 days of planting. Leaf size was observed by taking the first grown leaf and tracing it on millimeter blocks. Measurements were made on the length and the width. Stem diameter is measured at the widest part of the stem and is measured with a caliper. Plant height measurements were carried out from the base of the stem to the tip of the farthest part of the stem.

Table 1. Experimental design of colchicine concentration and treatment time

Colchicine / Time	K_0	K_1	K_2	K_3	K_4
W_1	K_0 W_1	K_1 W_1	K_2 W_1	K_3 W_1	K_4 W_1
W_2	K_0 W_2	K_1 W_2	K_2 W_2	K_3 W_2	K_4 W_2

ß-Carotene determination

Amaranth leaf extraction

An extraction requires approximately 1 g of wet amaranths leaves, then added with 0.04 g of Magnesium Carbonate ($MgCO_3$) and then crushed using a mortar and pestle. Extraction was conducted by maceration method using 8 mL of 90% acetone solvent, which had been mixed with 0.01% Butyl Hydroxy Toluene (BHT). The sample was then immersed in a mixture of acetone solution for 15 minutes and filtered using Whatman filter paper no. 41. The resulting filtrate is restored first, while the remaining dregs are added with the same solvent, and this step is carried out three times. The filtrate resulting from the three repetitions of these steps was combined and put into a 250 mL separating funnel. The filtrate was then added with 16 mL of Petroleum Ether (PE) Solution and 100 mL of distilled water, then let it sit for a while until the PE phase and the water phase were formed. The aqueous phase was discarded, and in the PE phase, 50 mL of distilled water was added. This step was proceeded twice to produce a clean PE phase. It was then filtered using Whatman filter paper no. 41 with the addition of 15 g anhydrous sodium sulfate (Na_2SO_4), which acts as a filtering agent. The filtered PE phase was then added with PE solution to reach a volume of 20 mL. Samples with a clean PE phase were ready for absorbance measurement with Uv-Vis Spectrophotometer.

ß-carotene levels

The levels of ß-carotene were calculated based on the linear regression equation y=a+bx, obtained from the ß-carotene standard curve, which was analyzed using a UV-Vis Spectrophotometer. The equation calculates the content of ß-carotene:

$$\text{ß-carotene concentration } \left(\frac{mg}{kg}\right) = \frac{\text{C x V x Fp}}{\text{W}}$$

Where:

C : Concentration of ß- carotene in the sample (mg/L) which is read from standard curve

W : Weight of sample used (kg)

V : Volume of flask used (L)

Fp : Dilution factor

RESULTS AND DISCUSSION

Chromosomes number

The number of chromosomes in *A. tricolor*'s red giti cultivar was observed on plants with colchicine treatment and plants without colchicine treatment. Chromosome number observation with a microscope showed a change in the number of chromosomes in some plants that were given the colchicine treatment. Plants without colchicine treatment had a chromosome number of 2n= 34. They were in accordance with research conducted by Andini et al. (2002) and Grubben (2004) that stated the number of chromosomes of *A. tricolor* was indeed a 2n= 34 or diploid. Meanwhile, results of some plants that were treated with colchicine had different numbers, 3n= 51 or triploid (Table 2 and 3).

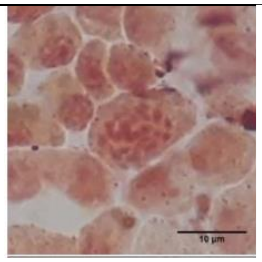
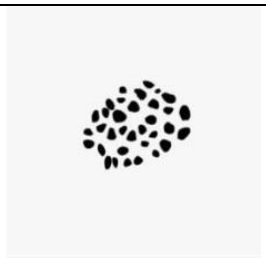
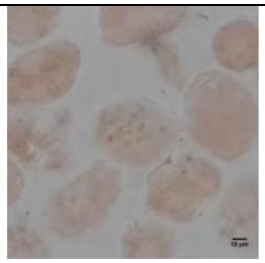
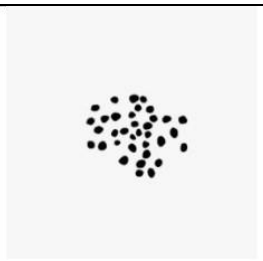
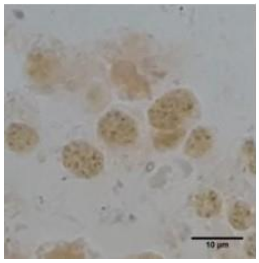
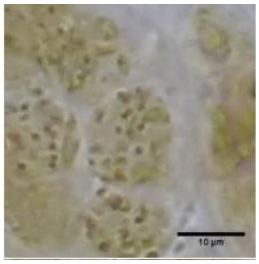
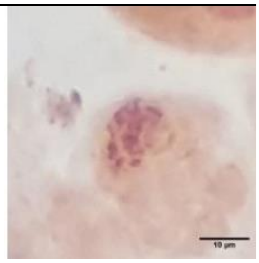
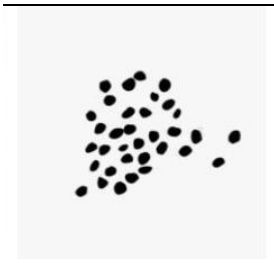
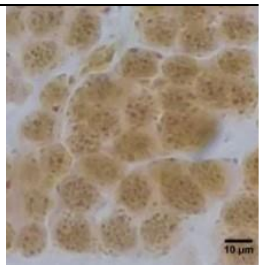
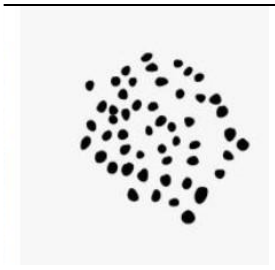
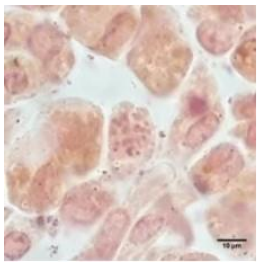
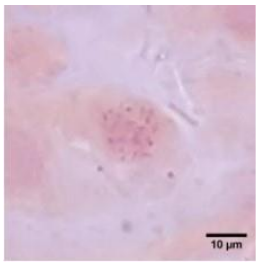
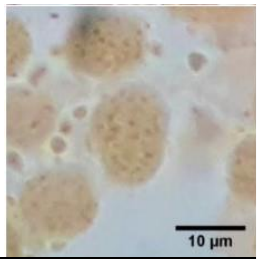
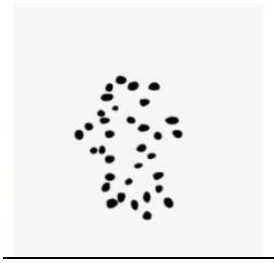
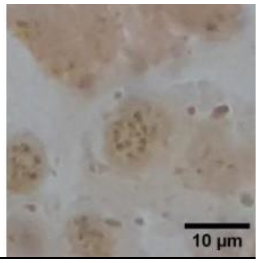
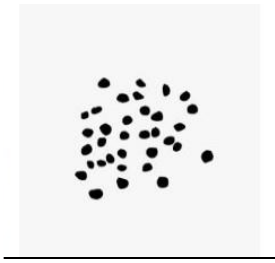
Plants treated with 50 ppm colchicine concentration and soaked for 6 hours and treatment of 100 ppm and soaked for 12 hours showed a change in the number of chromosomes, becoming triploid with an amount of 3n= 51 (Table 2). The doubling of the number of chromosomes is caused by the activity of colchicine in inhibiting the formation of spindle fibers during the anaphase stage. This causes the failure of cell separation that should occur at the anaphase stage, affecting the increasing number of chromosomes without forming a cell wall (Sundov et al. 2005).

According to Friska and Daryono (2017), plant cells have higher cell resistance to colchicine than animal cells. However, each individual plant has its resistance to colchicine compounds, and the resulting response can be different even in the same species. Inappropriate colchicine treatment can cause plant cell toxicity, affecting cell damage and death (Burun and Emiroglu 2008). Finkelstein et al. (2010) also stated that colchicine could cause apoptosis in cells due to failure of protein synthesis, thereby reducing the number of cells in plants.

Table 2. Variations in the chromosomes number of *Amaranthus tricolor* of red giti cultivar as a result of the variation in concentration and duration of colchicine immersion

Colchicine conc. (ppm)	Immersion time (hour)			
	6 (W_1)		12 (W_2)	
	Chromosome number	Notes	Chromosome number	Notes
0 (K_0)	2n=34	Diploid	2n=34	Diploid
50 (K_1)	3n=51	Triploid	2n=34	Diploid
100 (K_2)	2n=34	Diploid	3n=51	Triploid
200 (K_3)	2n=34	Diploid	2n=34	Diploid
500 (K_4)	2n=34	Diploid	2n=34	Diploid

Table 3. Variations in the chromosomes number of *Amaranthus tricolor* of red giti cultivar as a result of the variation in concentration and duration of colchicine immersion

Colchicine concentration (ppm)	Immersion time (hour)			
	6 (W_1)		12 (W_2)	
0 (K_0)				
50 (K_1)				
100 (K_2)				
200 (K_3)				
500 (K_4)				

The performance of colchicine on plant cells to inhibit the process of cell division is also influenced by several factors. Allum et al. (2007) stated that the effectiveness of inhibitory compounds is highly dependent on the applied concentration, exposure time, type of plant, and absorption of the compound. If the applied concentration is too high, chromosomal replication can occur in only a few cells before the inhibitory compound is completely dispersed throughout the meristem cells. The exposure time should be long enough to maximize the meristematic cell population exposed to the inhibitory compound but short enough to maintain plant viability.

Colchicine induction in actively dividing cells requires the right duration of time to induce polyploidy (Limera et al. 2016). Kehr (1996) stated that excessive exposure or not in accordance with the ability of plants could have bad consequences and even plant death. This is what happened to other colchicine treatment plants; variations in the

concentration of colchicine treatment given if it is incorrect, then polyploidy will not occur. Seeds with colchicine soaking for 6 hours and 12 hours were not all able to produce polyploidy. A concentration of 50 ppm at 6 hours of immersion and a concentration of 100 ppm at 12 hours of immersion showed a change in the number of chromosomes from diploid 2n= 34 to triploid 3n= 51.

Morphological characteristics

Leaf length

The leaves with the longest size were produced by colchicine treatment with a concentration of 100 ppm and soaked for 12 hours, where the leaf length reached 82.2 ± 11.692 mm (Table 4). The graph shows that the 12 hour immersion treatment with a concentration of 100 ppm colchicine was more effective in increasing the leaf length of the red giti. Colchicine treatment of 100 ppm for 6 hours had shorter leaf lengths than plants without colchicine treatment. Overall, changes in the length of the red giti with variations in the colchicine treatment showed a significant value.

Leaf width

The 12 hour soaking treatment with a concentration of 100 ppm resulted in an average leaf width much larger than the other treatments. The treatment, which resulted in the widest leaf size of 71.8 ± 8.319 mm, was not significant compared with the treatment of 50 ppm colchicine concentration by soaking for 12 hours with a size of 65.4 ± 6.841 mm, but the results obtained showed a fairly large difference (Table 5). The data showed that a 100 ppm treatment with 12 hours of immersion was more effective in increasing leaf width than other treatments. The colchicine immersion treatment of 100 ppm for 6 hours had a smaller leaf width than plants without colchicine treatment of 33.8 ± 2,490 mm. Based on the statistical analysis of the leaf width of the *A. tricolor* of red giti cultivar, the results of colchicine treatment and without colchicine showed significant results.

Stem diameter

The stems with the largest diameter were plants seed soaked for 12 hours with concentrations of 50 ppm and 100 ppm, namely 3.4 ± 0.548 mm. A follow-up test showed that the two treatments did not significantly differ. The results of this treatment were also not significant with the immersion treatment of 50 ppm colchicine concentration for 6 hours (Table 6). The results obtained were indeed higher in the two previous treatments, so it could be concluded that the effective treatment in increasing the size of the stem diameter was the 12 hour soaking treatment with concentrations of 50 ppm and 100 ppm. Like the previous characters, the smallest diameter size for this character was also produced by plants treated with 100 ppm colchicine immersion for 6 hours, 1.6 ± 0.5477 mm. Overall, the effect of colchicine and no colchicine treatment on the stem diameter of the *A. tricolor* of red giti cultivar showed a significant value.

Stem height

Plants with colchicine concentration of 0 ppm and soaked for 6 hours only had stem height of 64.2 ± 7.694 mm, while the tallest plants resulted from colchicine immersion treatment with 100 ppm concentration for 12 hours, namely 113.80 ± 15.975 mm (Table 7). Further tests showed significant differences between the two treatments. Plants with the lowest stem height, as before, were also produced by soaking 100 ppm colchicine for 6 hours with a height of 58.2 ± 7.259 mm.

Table 4. The leaves length of *Amaranthus tricolor* of red giti cultivar as the result of a variation in concentration and duration of colchicine immersion ($\bar{x}$±SD) (mm)

Colchicine conc. (ppm)	Immersion time (hour)	
	6 (W_1)	12 (W_2)
0 (K_0)	51.2^{a} ± 2.387	68^{b} ± 5.701
50 (K_1)	69.2^{b} ± 3.114	78^{c} ± 5.958
100 (K_2)	44.8^{a} ± 2.280	82.2^{c} ± 11.692
200 (K_3)	64.8^{b} ± 8.585	62.2^{b} ± 8.643
500 (K_4)	67.4^{b} ± 7.369	60.0^{b} ± 4.827

Note: Numbers in the same column followed by the same letter indicate no significant difference in the 5% DMRT test. $\bar{x}$= Mean, SD= Standard Deviation

Table 5. The leaves width of *Amaranthus tricolor* of red giti cultivar as a results of the variation in concentration and duration of colchicine immersion ($\bar{x}$±SD) (mm)

Colchicine conc. (ppm)	Immersion time (hour)	
	6 (W_1)	12 (W_2)
0 (K_0)	39.4^{a} ± 3.345	59.6^{bc} ± 3.507
50 (K_1)	61^{d} ± 3.464	65.4^{de} ± 6.841
100 (K_2)	33.8^{a} ± 2.490	71.8^{e} ± 8.319
200 (K_3)	51.2^{b} ± 10.134	51.6^{b} ± 6.348
500 (K_4)	58.6^{bc} ± 9.990	50.6^{b} ± 4.450

Note: Numbers in the same column followed by the same letter indicate no significant difference in the 5% DMRT test. $\bar{x}$= Mean, SD= Standard Deviation

Table 6. The stem diameter of *Amaranthus tricolor* of red giti cultivar as a result of variation in concentration and duration of colchicine immersion ($\bar{x}$±SD) (mm)

Colchicine conc. (ppm)	Immersion time (hour)	
	6 (W_1)	12 (W_2)
0 (K_0)	2^{ab} ± 0	2.6^{bcd} ± 0.548
50 (K_1)	3.2^{de} ± 0.447	3.4^{e} ± 0.548
100 (K_2)	1.6^{a} ± 0.548	3.4^{e} ± 0.548
200 (K_3)	2.8^{cde} ± 0.447	2.4^{bc} ± 0.548
500 (K_4)	2.8^{cde} ± 0.447	2.6^{bcd} ± 0.548

Note: Numbers in the same column followed by the same letter indicate no significant difference in the 5% DMRT test. $\bar{x}$= Mean, SD= Standard Deviation.

Appropriate colchicine induction treatment can trigger mutations in an individual, such as polyploidy. The formed cells have a more robust character and have larger roots, stems, and leaves than diploid plants (Suryo 2007). This can be seen in the study results, where plants without colchicine treatment had a smaller morphological size than plants with optimum treatment, such as 12 hours of soaking treatment in a concentration of 100 ppm. Giving colchicine also does not always produce plants with the expected quality. Concentrations of colchicine that are too high or not in accordance with the ability of plants to receive colchicine substances can be bad for plants. One of them is worsening the condition of the seeds so that the resulting growth is not good or worse than plants without colchicine treatment. Based on Table 2-7, colchicine immersion treatment with a concentration of 100 ppm for 6 hours had worse results than plants without colchicine treatment. This is in accordance with the opinion expressed by Finkelstein et al. (2010) that colchicine can cause apoptosis in cells which causes a decrease in the number of cells.

Levels of ß-carotene

ß-carotene is the most prominent member of the group of carotenoids, natural colorants present in the human diet. ß-carotene, responsible for the orange to red color, shows the main absorption peak in the maximum wavelength spectrum at 450 nm (Britton et al. 2008). The compound ß-carotene is formed from the condensation of two geranylgeranyl diphosphates by phytoene synthase (PSY) to produce the first colorless 15-cis-phytoene carotenoid. Horticultural crops such as spinach usually contain 2-3 PSY genes that show tissue-specific expression (Goodwin 1980). The biosynthetic pathway of ß-carotene starts from the carotenoid Phytoene C40, which is derived from the condensation of two molecules of C20 geranylgeranyl diphosphate (GGPP), produced from the isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Phytoene is converted to lycopene through a series of desaturation and isomerization. Lycopene β-cyclase LCY processes lycopene) and lycopene ε–cyclase (ε-LCY) to produce αß-carotene or ββ-carotene (Cazzonelli et al. 2010).

Carusoa et al. (2011) argue that the doubling of chromosomes that occurs causes an increase in the number of genes which results in an increase in the concentration and activity of several enzymes so that the biosynthesis process of a compound will change according to the expression of genes and enzymes that are affected. The effect of chromosomal doubling is an increase in the production of ββ-carotene compounds as in plants treated with 50 ppm colchicine immersion for 6 hours (Table 8).

The levels of ββ-carotene were calculated by measuring the absorbance using a UV-Vis spectrophotometer at a wavelength of 450 nm, referring to Amaya and Kimura (2004). They stated that the wavelength that gave the maximum absorption of ß-carotene was 450 nm.

The highest levels of ββ-carotene were produced by spinach with 50 ppm concentration treatment with 6 hours soaking time, which was 908.40 ± 116.800 mg/kg. The lowest levels of ββ-carotene produced by plants with colchicine concentration of 100 ppm with 6 hours immersion were 284.80 ± 98.993 mg/kg. These results indicate that diploid plants contain less ββ-carotene than polyploid plants. The results of this study are in accordance with Song et al. (2012). They state that plants indicated by polyploidy due to the mutagen induction of colchicine compounds can produce more secondary metabolite products. Based on statistical analysis (Table 8), the 6 hour soaking treatment with a concentration of 50 ppm or the treatment with the highest betaß-carotene content had significant results against other treatments but not for the 6 hour soaking treatment in 200 ppm colchicine and 12 hour soaking treatment in 50 ppm colchicine.

Based on the results of the research on the effect of colchicine on the number of chromosomes, morphological characters, and production of ß-carotene in *A. tricolor* from the red giti cultivar, several conclusions can be drawn, namely: Induction of colchicine with the right treatment affects the number of chromosomes in *A. tricolor* with red giti cultivar to become triploid 3n= 51. Changes in the morphological character of the red giti cultivar have leaves with longer and wider sizes, larger stem diameters, and taller plants. The level of ββ-carotene in *A. tricolor* from the colchicine-induced red giti cultivar was higher. The colchicine treatment with a concentration of 50 ppm with an immersion time of 6 hours was the optimum treatment because there was an increase in the number of chromosomes, the size of the morphology, and an increase in the production of ββ-carotene.

Table 7. The stem height of *Amaranthus tricolor* of red giti cultivar as a result of the variation in concentration and duration of colchicine immersion ($\bar{x}$±SD) (mm)

Colchicine conc. (ppm)	Immersion time (hour)	
	6 (W_1)	12 (W_2)
0 (K_0)	64.2^{ab} ± 7.694	90.6^{cd} ± 10.621
50 (K_1)	108.40^{de} ± 12.857	97^{de} ± 11.662
100 (K_2)	58.2^{a} ± 7.259	113.80^{e} ± 15.975
200 (K_3)	96^{de} ± 10.440	95.4^{d} ± 20.095
500 (K_4)	98.4^{de} ± 14.519	78^{bc} ± 10.320

Note: Numbers in the same column followed by the same letter indicate no significant difference in the 5% DMRT test. $\bar{x}$= Mean, SD= Standard Deviation

Table 8. Levels of ß-carotene in *Amaranthus tricolor* of red giti cultivar as a result of the variations in concentration and duration of colchicine immersion ($\bar{x}$±SD) (mg/kg)

Colchicine conc. (ppm)	Immersion time (hour)	
	6 (W_1)	12 (W_2)
0 (K_0)	515.20^{b} ± 119.194	708.80^{cd} ± 150.812
50 (K_1)	908.40^{c} ± 116.800	861.40^{de} ± 64.229
100 (K_2)	284.80^{a} ± 98.993	756.40^{cd} ± 78.717
200 (K_3)	794.80^{cde} ± 129.972	736.40^{cd} ± 64.010
500 (K_4)	693.00^{c} ± 138.852	746.20^{cd} ± 56.888

Note: Numbers in the same column followed by the same letter indicate no significant difference in the 5% DMRT test. $\bar{x}$= Mean, SD= Standard Deviation

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