

Effectiveness of Bio-Catharantin Induction to Increase Red Spinach (*Alternanthera amoena* Voss.) Production

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ABSTRACT

Red spinach (*Alternanthera amoena* Voss.) is a plant with high nutritional value and contains anthocyanin compounds that act as antioxidant compounds to prevent the formation of free radicals in the body, besides that anthocyanin can also treat anemia. The increase in demand for red spinach in the market has not been matched by the amount of production. The productivity of spinach can be increased by polyploid induction. The advantages of polyploid plants are increased quality (taste, aroma, and other metabolites), size of vegetative and generative structures, pest resistance, and more adaptability. Based on the latest research, it was found that Bio-Catharantin as a periwinkle leaf extract product can induce polyploidization in shallot (*Allium cepa* L.) and watermelon (*Citrullus lanatus*) plants. This study was carried out from July to August 2021. The treatments include control, seed induction by Bio-Catharantin 0.2% solution, and seed induction by Bio-Catharantin 0.4% solution for 12 hours, 24 hours, and 48 hours. The results showed that induction with Bio-Catharantin in plants was followed by changes in morphological characters. The results of ANOVA-Duncan analysis showed that there was a significant difference in seed induction by Bio-Catharantin 0.2% solution for 12 hours treatment. Chromosomal observations could not be identified because the chromosome size was not visible and hardly seen under the microscope. Flow cytometry test showed that plants treated with Bio-Catharantin were unable to produce polyploidization of red spinach. The result of the chlorophyll test is in line with the morphological characters.

Keywords: *Bio-Catharantin, Periwinkle, Polyploidization, Red spinach.*

1. INTRODUCTION

Indonesia has a variety of vegetables for daily consumption, and one of those vegetables is red spinach. Red spinach (*Alternanthera amoena* Voss.) is a plant with high nutritional value and contains anthocyanin compounds which are characterized by the appearance of the reddish color on the leaves and stems. Anthocyanins can increase the immune system by helping to prevent the formation of free radicals. Based on data from the Indonesian Central Statistics Agency in 2012, red spinach production reached 155,118 tons and the demand is increasing every year [1].

The high demand for red spinach is unable to be satisfied with the amount of production. This is because red spinach has not been widely developed by the community and is not as popular as green spinach which

is widely circulated in traditional markets. This is what affects the availability of red spinach seeds. Considering the high anthocyanin and other nutritional content of red spinach, it is beneficial to increase the production of red spinach.

Red spinach leaves are single leaf, generally ovoid, with slightly convex tips, and pronounced leaf veins. The leaves can also be round, pointed, stiff or soft, and wide or narrow. Red spinach leaves are different from wild spinach leaves, which are rough and thorny. The colors of the leaves can be light green, whitish green to red. The red color of this spinach is the darkest red which combines with green compared to other varieties. Red spinach is also known as "the king of vegetables" because its high nutritional value [2].

The demand for red spinach in Indonesia continues to increase from year to year for consumption and seed source. One of the efforts that can be done to increase the productivity of red spinach is through the induction of plant polyploidy with mutagen compounds. This method can produce plants with an increased size of vegetative and generative structures, improved quality (taste, aroma, and other metabolite substances), increased resistance to pests and diseases, and can make plants more adaptive to environmental changes compared to diploid plants [3]. Polyploidization generally uses chemical compounds as mutagens such as colchicine. Treatment with colchicine with 0.025%–0.1% concentration significantly affected the ploidy level of Katokkon pepper (*Capsicum annuum* L.) plants to become mixoploid [4]. However, the use of chemical compounds could have a toxic impact on plants and the environment. The periwinkle plant contains many active compounds such as terpenoids, bioflavonoids, alkaloids, and tannins [5].

Periwinkle is an important medicinal plant of the Apocynaceae family that contains more than 70 types of alkaloids and chemotherapy agents that are effective in treating various types of breast cancer, lung cancer, uterine cancer, melanoma, Hodgkin lymphoma, and non-Hodgkin's. It also contains a large number of volatile and phenolic compounds including caffeoylquinic acid and flavonol glycosides that are known to be antioxidant against reactive oxygen species (ROS), which is harmful by forming such products through normal cell aerobic respiration [6]. Based on the latest research, it was found that the periwinkle plant (*Catharanthus roseus* [L.] G. Don.) has vincristine compounds that act as antimitotic agents so that it can cause polyploidization in plants [7].

Plant cells that already duplicated their chromosomes are unable to divide in the presence of antimitotic compounds during mitosis [8]. As a result, colli-tetraploid plants are formed. Based on the previous research, Bio-Catharantin as periwinkle leaves extract, can increase the ploidy of melon (*Cucumis melo* L.) [9] and peanut (*Arachis hypogaea* L.) [10]. Using 0.05% Bio-Catharantin with 8 hours incubation can doubled the chromosome number and increase the morphological size of habitus and fruit of melon cultivar Melodi Gama-1 [9]. The chromosome number of peanut was also doubled using 0.15% Bio-Catharantin with 24 hours of treatment [10]. Another Bio-Catharantin application on *Citrus aurantiifolia* and *Citrus maxima* cv. Gula-Gula (Rutaceae) also resulted in a larger phenotype character through incubation for 6 hours with 0.05% Bio-Catharantin [11]. This study was conducted to provide information regarding the effectiveness of Bio-Catharantin as a polyploidization agent in the red spinach and to encourage the development of red spinach production.

2. MATERIALS AND METHOD

2.1. Sample Preparation and Bio-Catharantin Treatments

We performed polyploidy induction on red spinach seed cultivar Century Merah (CV. Sastra Bina, Indonesia). The seeds were treated with 0.2% and 0.4% Bio-Catharantin solution (w/v). Distilled water was used as control.

The red spinach seeds were incubated in a 100 mL dark bottle filled with 0.2% and 0.4% Bio-Catharantin solutions for 12 hours, 24 hours, and 48 hours. Before planting, the seeds were washed with distilled water then planted in polybags containing mixed planting medium. In this study, 10 plants from each treatment sample were used for further data analysis.

2.2. Phenotypic Observation and Data Collection

Plant phenotype was observed and measured at the age of 35 days. The morphological data include plant height, number of leaves, leaf length, leaf width and productivity observations by measuring the wet weight per plant. Phenotypic data were analyzed using One Way ANOVA-Duncan with SPSS version 24 (IBM Corp., US) at a significance value of 5%.

2.3. Ploidy Level Analysis with Flow Cytometry

The ploidy level analysis was carried out using fresh leaf samples at Indonesian Institute of Sciences (LIPI) Laboratory (Bogor, Indonesia). Leaves were cut to a size of 0.5 x 0.5 cm, then 100 mg of leaves tissue added with 1.5 mL of Galbraith's buffer supplemented with 0.1% w/v Triton® X-100 [12]. The homogenate was filtered through the 30- μ m nylon filter. The homogenate (0.5 mL) was added to a labeled tube containing 2.5 μ L of 10 mg/mL of DNase-free RNase A and incubated on ice for 15 minutes. Propidium iodide was then added to a final concentration of 50 μ g/mL. The stained samples were incubated on ice in darkness for 30 minutes prior to analysis. Samples were analyzed with BD Accuri™ C6 Cytometer (BD Biosciences, UK). The ploidy level was determined based on the peak pattern obtained [13].

2.4. Chlorophyll Content Analysis with spectrophotometry

The chlorophyll content analysis is also carried out using fresh leaf samples. The 0.5 g of fresh leaves for every treatment was homogenized in a homogenizer with 10 mL of 80% acetone. The samples were centrifuged at 10,000 rpm for 15 minutes at 40°C. The 0.5 mL of supernatant is collected and mixed with 4.5 mL of solvent. The mixture was analyzed for chlorophyll-a

(Cha-a) content in a spectrophotometer (Parkin) at a wavelength of 663.2 nm and calculated using Equation (1) below [14].

$$\text{Ch-a} = 12.25A_{663.2} - 279A_{646.8} \quad (1) \quad [14]$$

3. RESULTS AND DISCUSSION

In most plant species, doubling the number of chromosomes can generate larger cell and organs. The number of chromosomes can also have a positive influence on the levels and composition of constituent elements in plants, such as the content of secondary metabolites [15]. Phenotype occurs due to unique gene interactions in every organism thus can be fundamental in predicting gene changes. This character is influenced by the interaction of genotype factors and environmental factors.

The red spinach chromosome induced with Bio-Catharantin (0.2% and 0.4%) (control) are compared. Polyploidization using Bio-Catharantin in plants is expected to be accompanied by changes in morphological character. Polyploidization occurs by disrupting the process of microtubule formation. Further, the disruption thwarts the formation of a separation wall. Chromosomes that have been doubled during interphase fail to separate so that the number of chromosomes in each cell increases. Higher ploidy nucleus is often associated with an increase in cell size. Plant achieved this increase in cell size through increasing their ploidy level by successive rounds of DNA replication with endoreduplication mechanism. Then cell size and cell number also help to coordinate a total organ size [16].

In this study, the ploidy level of red spinach was analyzed using flow cytometry. Flow cytometry is a widely used technique to estimate core DNA content that is particularly useful in plant taxonomy for filtering ploidy levels and for determining genome size. Sample preparation usually takes only a few minutes and rarely requires expensive reagents [17]. The differences

between flow cytometer results of diploid and mixoploid plants can be observed from the peaks of a histogram (Figure 1).

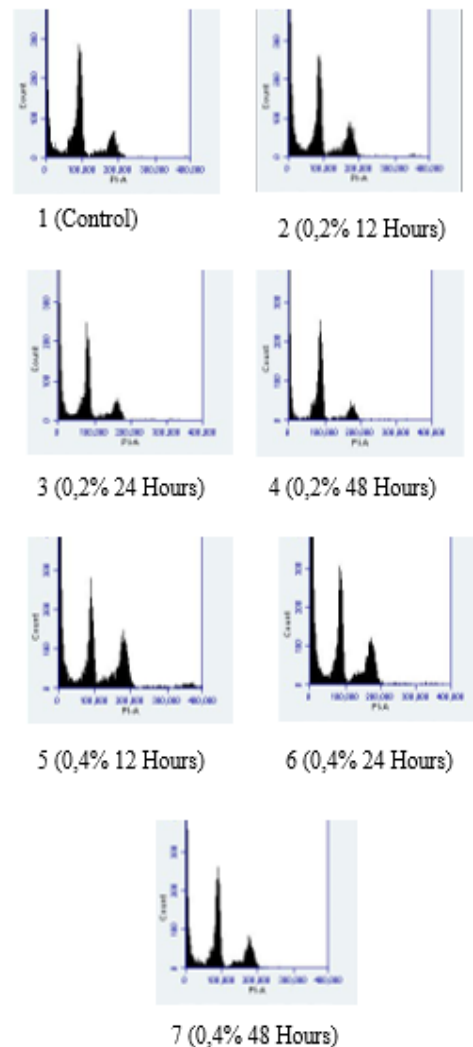


Figure 1. The ploidy levels of red spinach (control and Bio-Catharantin treatment) by flow cytometry.

Table 1. Red spinach morphology analysis on Bio-Catharantin treatment

Treatment	Stem Length (cm)	Number of Leaves	Leaf length (cm)	Leaf Width (cm)	Wet Weight (g)
Control	6.55 ± 0.29 ^a	7.00 ± 0.26 ^{bc}	3.17 ± 0.13 ^b	2.46 ± 0.12 ^b	0.45 ± 0.05 ^b
0,2% 12 Hours	8.40 ± 0.48 ^b	7.40 ± 0.31 ^c	3.74 ± 0.19 ^c	2.86 ± 0.16 ^c	0.62 ± 0.09 ^c
0,2% 24 Hours	6.77 ± 0.25 ^a	6.40 ± 0.16 ^{ab}	2.94 ± 0.14 ^{ab}	2.18 ± 0.14 ^{ab}	0.34 ± 0.04 ^{ab}
0,2% 48 Hours	6.32 ± 0.25 ^a	6.50 ± 0.17 ^{ab}	2.77 ± 0.09 ^{ab}	2.16 ± 0.04 ^{ab}	0.30 ± 0.02 ^a
0,4% 12 Hours	5.81 ± 0.53 ^a	6.30 ± 0.30 ^{ab}	2.95 ± 0.23 ^{ab}	2.40 ± 0.21 ^{ab}	0.38 ± 0.05 ^{ab}
0,4% 24 Hours	6.98 ± 0.26 ^a	6.50 ± 0.17 ^{ab}	3.03 ± 0.07 ^{ab}	2.40 ± 0.08 ^{ab}	0.40 ± 0.03 ^{ab}
0,4% 48 Hours	6.15 ± 0.43 ^a	6.10 ± 0.28 ^a	2.61 ± 0.14 ^a	2.00 ± 0.11 ^a	0.30 ± 0.03 ^a

Description = superscript alphabet letters show a subset difference at a significance level of 5%; and the ± mark indicates the standard error value.

Based on the Figure 1, all treatments (2, 3, 4, 5, 6, 7) having the same ploidy (2n) with controls (1) indicated with approximately similar peak value. All plants had one peak detected at channel ± 85 and one peak detected at ± 175 . Based on that results, Bio-Catharantin concentration of 0.2% and 0.4% were unable to induce polyploidization of red spinach plants.

Morphological analysis showed that some treatments have statistical difference to the control. Our data (Table 1) shows that treatment 2 (0,2% Bio-Catharantin for 12 hours) has superior character compared to other treatments. The morphological characters of stem length, leaf length, leaf width, and wet weight are higher and shows statistical difference to control. The phenotypic data obtained from this study are in line with three previous studies [9, 10, 18]. Meanwhile based on studies in peanut plants, any level of periwinkle extract application can boost the performance of peanut plants [10]. The presence of vincristine and vinblastine compounds in periwinkle plants is assumed to have the ability to double the set of chromosomes in plants. Those comparison show how sensitivity to mitotic agents could differ between plant species [18].

The results of statistical analysis showed that the chlorophyll-a content of treated red spinach are 0.4%, 12 hours > 0.2%, 12 hours > 0.4%, 24 hours > control > 0.4%, 48 hours > 0.2%, 24 hours > 0.25%, 48 hours. There are 3 treatments which content is greater than control (Figure 2), one of them is a plant with a treatment of 0.2% 12 hours induction. This is in line with the morphological data obtained on Table 1, showing that the treatment is statistically different from other treatments. However, the treatment of 0.4% Bio-Catharantin, 12 hours has the highest average chlorophyll-a content value of 177,966 mg/L. Bio-Catharantin induction treatment is thought to have a positive influence on the chlorophyll-a content because some treatments showed improved phenotypes compared to the control. Other factors that may affect the results of chlorophyll content are nutrients from the planting medium [18]. This research showed the widely available periwinkle is promising as a bio-booster

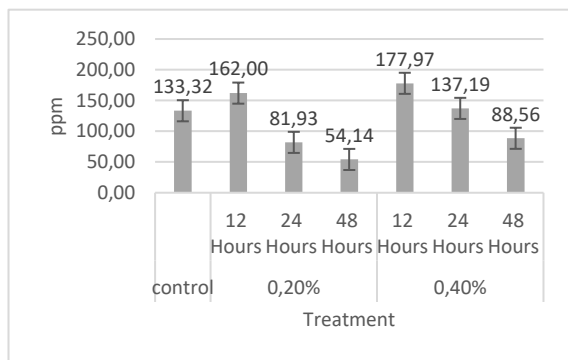


Figure 2. Chlorophyll-a content of red spinach with Bio-Catharantin induction.

agent. Further research is needed to confirm those effects on other plants.

Based on the results of this research, application of 0.2% Bio-Catharantin for 12 hours can boost the growth of red spinach plants but unable to induce polyploidization.

AUTHORS' CONTRIBUTIONS

NS and BSD designed the research. NS, LNJ, and MSH performed the research. NS and LNJ analyzed the data. All authors wrote the manuscript.

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