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Induction of Lili Hujan polyploid (*Zephyranthes rosea* Lindl.) with ethanolic extract of Tapak Dara leaf (*Catharanthus roseus* (L.) G. don.) to increase its economic value

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Abstract. The ornamental plant is a high economic-value commodity and highly prospective cultivated in Indonesia. One of them is Lili Hujan plant (*Zephyranthes rosea* Lindl.). However, this plant is less desirable because the size of the flowers is small, thin, and wilted quickly. Therefore, the genetic improvement to improve the quality of interest is necessary. One way is through polyploidy induction using chemicals such as vincristine, which is derived from an ethanolic extract of tapak dara leaf (*Catharanthus roseus* (L.) G. Don.). This research aims to know the potency of ethanolic extract of tapak Dara leaf in induction polyploidization of Lili hujan plant (duration of immersion) and its influence on the characters of the morphology and chromosomes. Tuber plants soaked (8, 16, and 24 hours) with 0.1% ethanolic extract of tapak dara leaf. The observed variables include morphological characters (root mass, length, and width of the leaves, the day of flower appearance, tuber diameter), stomata density and chromosomes. Extract of ethanolic from tapak data leaves 0,1% with 8, 16, and 24 hours immersion periods can induce polyploidization of Lili hujan plants with increasing number of chromosomes compared with control plants. Also, the root mass, number and broad leaves, and the stomata density also increased. Finally, the economic value of Lili hujan plant increased because of quality improvement phenotype.

1. Introduction

Ornamental plants are high economic value commodities and highly prospective cultivated. One of the cultivated ornamental plants is Lili Hujan (*Zephyranthes rosea* Lindl.). However, this plant is less attractive because the flowers are small, thin, and quickly withered, so the genetic improvement to enhance the quality of the flowers is very necessary. Plant breeding can be done with polyploidization using antimitotic agent compounds such as colchicine and vincristine from tapak dara leaves extract (*Catharanthus roseus* (L.) G. Don). Colchicine (C₂₂H₂₅O₆N) is the result of extraction from *Cochium autumnale* L. which retrieved simply from the subtropical regions. Colchicine used to create seedless fruit. It is generally imported from Japan at a high price.



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Base on the fact, we need to look for the alternative of the anti-mitotic agent to substitute colchicine which material obtained from a local source in Indonesia. The anti-mitotic agent can be obtained from tapak dara leaves extract in the form of the vincristine compound [1]. The extract ethanolic of tapak dara leaves can be used as an alternative compound for colchicine in chromosomes doubling process. Besides that, this plant lives throughout the year in the tropic region and thrives in Indonesia.

Tapak dara plant is only used as a traditional medicinal plant, and its existence is often considered less useful, so this is allowed to grow wild. The available scientific data about the success of giving of tapak dara leaves extract in polyploidization are still limited for onion [1] and melon [2]. To extract the tapak dara leaves can be utilized more as a substitute for colchicine, it is necessary to do further research on its influence on polyploidization on other plants such as Lili Hujan to accelerate its growth and produce a superior Lili Hujan cultivar.

The responses of mutagen treatment from each plant are different. Polyploidization succeeded by soaking the onion bulbs for 18 hours with a concentration of 0.1%. Meanwhile, the availability of scientific data about the efficiency of tapak dara leaves extract on polyploid induction of Lili Hujan plants is very limited, so the research about tapak dara leaves for polyploidization needs more to do.

The problems in this research are: 1) How is an ethanolic extract of tapak dara (*Catharanthus roseus* (L) G. Don) used as a polyploidization agent for Lili Hujan plant (*Zephyranthes rosea* Lindl.)?; 2) How long is the immersion of ethanolic extract of tapak dara (*Catharanthus roseus* (L) G. Don) to induce polyploidization of Lili Hujan plant (*Zephyranthes rosea* Lindl.) most effectively?; 3) How are chromosome and morphological characters of Lili Hujan plant (*Zephyranthes rosea* Lindl.) between the given ethanolic extract of tapak dara (*Catharanthus roseus* (L) G. Don) with control?. The purpose are: 1) Analyzing the potential of ethanolic extract of tapak dara (*Catharanthus roseus* (L) G. Don) as an alternative to replacement colchicine in inducing polyploidization of Lili Hujan plant (*Zephyranthes rosea* Lindl.); 2) Analyzing the long immersion of ethanolic extract of tapak dara (*Catharanthus roseus* (L) G. Don) which most effectively induces polyploidization of Lili Hujan plant (*Zephyranthes rosea* Lindl.); 3) Analyzing the chromosome and morphological characters of Lili Hujan plant (*Zephyranthes rosea* Lindl.) after giving ethanolic extract of tapak dara (*Catharanthus roseus* (L) G. Don); 4) Analyzing the adding economic value of Lili hujan after giving ethanolic extract of tapak dara (*Catharanthus roseus* (L) G. Don).

2. Materials and Method

2.1. Methods

The used plant materials in this research were the seed of Lili Hujan plant (*Zephyranthes rosea* Lindl.) and tapak dara (*Catharanthus roseus* (L) G. Don). The used chemical materials were aceto-orcein, acetic acid, aquadest, glycerol, HCl 1 N, safranin dyes, cotton, gauze, soil media, manure, polybag (size: 30 cm x 15 cm). The used tools were microscope (Yazumi), tweezers, rules, hot plate, preparation glass, cover glass, dropper drop, Petri dishes, blander, label papers, analytical scales, and so on. This research was conducted in six stages, they were : (1) The extraction of mutagen from tapak dara leaves; (2) preparation of mutagen solution; (3) preparation of planting media; (4) Soaking the seed; (5) planting, maintenance; and (6) measurement of observed variables.

2.2. Mutagen Extraction

Techniques extraction mutagen used is a technique modification of [3]. The old leaves were separated from the branches, dried in an oven for 72 hours with a temperature of 70°C, and then crushed using a blender. Once refined, the powder was soaked in a mixture of ethanol 90% 70 mL and water 30 mL for 24 hours, and then filtered with gauze. Then, the filter solution was heated by a hotplate with a temperature of 70°C to form solid green deposits. The procedure consists of the following: 1) The first step is making a mutagen solution. The concentration of ethanolic extract of tapak dara leaves used in this research was based on onion, i.e., 0.1% as the most effective concentration in inducing polyploids [1]. The second is the preparation of planting media. Planting media used in this research was a

mixture of soils and fertilizer enclosure with a ratio of 1:1. Mixed media inserted into polybags which have size 30 cm in diameter and 15 cm in height. The next step is soaking seed. The seeds of Lili Hujan plant used were selected with a uniform size and weight. And then soaked in a petri dish which containing a 0.1% mutagen solution with a different soaking periods, they were 8, 16, and 25 hours. As a control used a seed which soaked with aquadest. And the last step is planting and maintenance. The soaked seeds in accordance with treatment were planted in polybags. Every seed planted in a polybag with a depth of 5 to 7 cm. Watering was done once a day because the water needs of Lili Hujan plant are moderate. Hereafter they placed in someplace with sunlight throughout the day.

2.3. Variable Measurement

Variables to be observed include:

1. Morphological Characters

Observation of plant morphology is done by measuring the length of leaves each week until the plants are 10 weeks old. The measurement of length and diameter of the stem, and diameter flowers after flower stalks begin to appear. Counting the number of roots is done after 4 months old.

2. Stomatal density

If Lili Hujan leaves are 9 weeks after planting, they will cut and use as a basis for calculating the stomatal density. The stomatal density is observed by cleansing the surface of the leaves of Lili hujan plants, after that, slashing the undersurface of the leaf as thin as possible. The epidermal incision is immersed in a safranin dye for 3 – 5 minutes and then placed on the object glass. Further observed under a microscope. Equation (1) was used to determine the stomatal density:

$$\text{Stomatal density} = \frac{\text{st o s}}{\text{w f o v}} \quad (1)$$

3. Chromosome Characters

Chromosome observations use layered polybag techniques with the aim to avoid crop removal at taking root time. The tip of Lili hujan root is cut about 2 mm, then be fixed by 45% acetic acid. After that, allowed to stand at a temperature of 5°C for 15 minutes. Then, the root pieces are washed with aquadest. The next, hydrolyzed with HCl 1 N for 2 – 3 minutes at 60°C. And then, the tip of the root is placed on an object glass and dyed with chromosome dye, 1% aceto orcein and glycerol. After the dye permeated, the preparation glass is covered with a cover glass and squash by tapping and swiping with the flat part of the pencil, and then observed by using a microscope.

2.4. Data Analysis

The data are displayed in term of the average percentage of the somata index and the amount chromosome. For morphological data are analyzed using descriptive analysis. The contingent valuation method (CVM) is used to know the price of Lili hujan plant, both treated and untreated (control plant). After knowing the price, we compare by statical t-test to know the difference. If the Lili Hujan plant treated has a higher price then without treated, the calculation of adding economic value is started. The adding economic value is knowing from the difference in the price value between Lili hujan plant treated with an untreated.

3. Results and Discussion

3.1. Material Treatment

This research was experimental research using mutagen (0.1% for ethanolic extract of Tapak dara leaves) which treated on Lili hujan plants with long soaking (K0: control; K1: soaking 8 hours; K2: soaking 16 hours, and K3: soaking 24 hours). Growth parameters measured include the number and broad of the leaves, root mass, tuber diameter, stomatal density, and chromosome character. At the age

of 4 weeks after planting, plants began to show growth, both length and number of leaves. The number of leaves was more and more, more elongated and began to form flowers.

3.2. Morphological Characters

The results of the analysis showed that mutagen had a significant effect on the number and extent leaves, root mass, density stomata, as well as the number of chromosomes, but not significantly different from the diameter of Lili hujan plant (table 1).

Table 1. Morphological Parameter Data

Morphological Data	Length of immersion (hours)			
	0 (control)	8	16	24
Root mass (gram)	0.25	0.39	0.67*	0.89*
Number of leaves	4	8*	5	4
Leaf area (cm ²)	9.6	10.38	12	13.92*
Tuber diameter (cm)	1.68	2.07	2	1.81
Day of the appearance of the flower	-	-	-	4

* differs markedly at the level of 5%

On the character of root mass phenotypes that harvested after the plants reached a maximum state (already in bloom), the increased root mass of mutagen treatment showed better than control. The increased root mass of mutagen treatment had also been evidenced by the result of research on polyploidization on Bougainville (*Zinnia elegans*) [4]. At a concentration of 0.01% mutagen for 36 hours of soaking time, there was an increase in root mass from the control plants even in the treatment; the plants were not induced into polyploid plants yet. Other results as well are shown in polyploidization research on peanut plant (*Arachis hypogea*) [5]. The peanut plant was given a mutagen treatment of 0.2% for 12 hours of soaking time, and the mass of its root more increased compared to control. This research showed that mutagen from the ethanolic extract of tapak dara leaves was able to increase the number and extent of Lili hujan leaves. Based on the observation, the number of Lili hujan leaves which treated with mutagen showed a significant increase compared to control plants (Table 1). Plants which soaked in mutagen for 8 hours is known to form the largest number of leaves. The results of this research are in line with the research of [6] on patchouli plants. It is treated with mutagen 0.04% for 24 hours resulted in the largest number of leaves. The same case was also reported by [7], the culture of *Dendrobium spectabile* had more leaves after having mutagen treatment than control. Polyploidization on plants produce thicker leaves, older green color, and increase the length and number of leaves [8].

The increasing number of plant leaves will increase the rate of photosynthesis which resulted in the addition of leaves area of plants. Similarly, the leaf area parameter of Lili hujan plants which treated with mutagen were wider than control (Table 5.1). The results of the data analysis showed that the leaf area of Lili hujan plant was the widest at 24 hours soaking treatment (13.92 cm²). Increased leaf area due to treatment mutagen, such as ethanolic extract of tapak dara leaves similar to the research of [9] in the hikadi cultivar of a melon plant that had increased leaf area due to the treatment mutagen.

The size of the larger leaf in the mutagen treatment plant has a positive effect on the growth of Lili hujan plant. Larger leaves make absorption of sunlight much better, so the photosynthesis runs smoothly. If photosynthesis runs optimally, then the plant will produce more carbohydrates for its growth and development. [10] [11]. But instead, the provision of mutagen did not show any significant effect on the diameter of the tuber produced both between treatment and control. This matter indicated that ethanolic extract of tapak dara leaves could lead to interference to the growth of tuber diameter of Lili hujan plant. This is in line with [12] which states that mutagen (colchicine) had no significant effect on the diameter of large white ginger (*Zingiber officinale*). Furthermore on the character of the flowering age was known that mutagen could induce early flowering of Lili hujan plants (4 weeks after planting) (figure 1).



Figure 1. Lili hujan plant age 4 weeks after planting (K3: immersion of mutagen for 24 hours; K2: immersion for 16 hours; K1: immersion for 8 hours; K0: control/without immersion of mutagen)

Figure 1 showed the ethanolic extract of tapak dara leaves were made as the mutagen, the best-induced flowering on treatment with long immersion 24 hours. Flowering is the process of primordial change of stems into primordial flowers. The flowering process in this Lili hujan plant was suspected to be triggered by an external factor, namely mutagen which stimulates the flowering process of plants. It was suspected mutagen 0.1% with long soaking 24 hours very precisely to Lili hujan plants, so there was no delay in the mitosis process. If mitosis process did not delay, then it can speed up the creation of thread spindle, so flowering in plants can form more quickly [13].

3.3. Stomata Character

Changes like the plant are not only found in the number and extent of leaves and mass root but also found in the stomata character. The result showed that the treatment of mutagen concentration significantly affected the number of stomata (Figure 2).

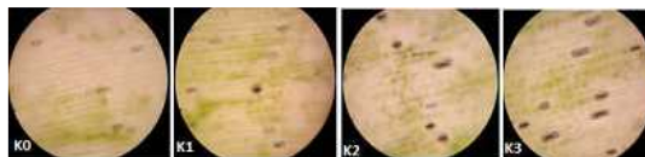


Figure 2. Comparison stomata of Lili Hujan which enlargement 400 times

Table 2. Comparison of the stomatal density of Lili hujan plants

Long Soaking	Stomatal density (mm ²)
K0 (control)	18.68
K1 (8 hours)	32.27
K2 (16 hours)	33.97
K3 (24 hours)	47.55*

* Real different at the level of 5%

As clearly shown in figure 2 and table 2, it is important to know the stomatal density in the treated plant is higher at 24 hours of immersion than in long treatment soaking 8 and 16 hours, as well as control. This is similar to [14], giving mutagen can affect the stomatal density of onion leaves (*Allium fistulosum*). Further [15] reported stomatal density was affected by size, where the length and width of the stomata can be used as an indicator of changes in polyploidy.

In general, the size of stomata has a positive correlation to success rate polyploidization in plants. As reported by [16] in his research that radish plants (*Raphanus sativus*) treated with mutagen had larger stomata size than the radish without treatment. This was due to the amount chromosomes in plant cells have multiplied to cause an increase in cell size. Mutagen can affect changes in the structure of cytoskeleton microtubules in the process of tissue morphogenesis found in leaves, especially tissue epidermis, thus causing a change size on cell companion of stomata [17].

3.4. Chromosome Character

The result of chromosome observation showed that mutagen affects the amount of chromosomes (figure 3).

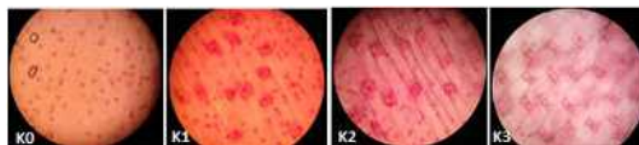


Figure 3. Comparison chromosome of Lili Hujan plant

Figure 3 showed that the part of a cell called a chromosome is solid, red and thicker than the rest of the cell. This is appropriate with the opinion of [18] which states that chromosomes can absorb substances the color of aceto orcein well, making it easier to observe. The shape of the chromosome on this observation still can not be identified yet according to some researches about chromosomes, that have been reported by [19], [20], and [21]. Lili hujan which considered polyploid is a plant that has 2 sets of chromosomes or $x = 10$. However, the number of chromosomes of polyploid plants observed through a microscope did not show all the sum of $x = 10$. This was probably because chromosomes still overlap each other and mutually accumulate. Chromosome observation in control plants showed the number of chromosomes from Lili hujan plant is as many as $2n = 10$. This is accordance with research of [22] which mention that Lili hujan plant has karyotype $2n = 10$. The karyotype is a set of chromosomes in an organized species which set to consider the number, size and shape of the completeness of the cell chromosome which each chromosome is identified according to the morphology in the related organism. Each type of plant and animal has a typical and specific karyotype [23].

The observation of chromosomes of Lili Hujan polyploid is shown in Figure 3. Chromosomes of treatment plants (K1, K2, and K3) showed different results with the chromosome of control plants. Ideally, Lili hujan plant with a double set of chromosomes will produce $2n = 4x = 20$. This possibility of inaccuracies caused by pile between chromosome at the time in observation. This indicates that the plant which observed as a polyploid plant. Research on polyploidization is supported by the observation of the number of chromosomes where the plant whose 2 sets of the chromosome will have more the number of chromosomes then before. Several research support it, [23] *Platycodon grandiflorus* plant ($2n = 2x = 18$) to ($2n = 4x = 36$) induced colchicine 0.05% for 24, 48, and 72 hours; and [19] on Vanda hybrid orchid plants ($2n = 2x = 38$) to ($2n = 4x = 76$) with colchicine 0.5% for 18 hours. Therefore, ethanolic extract of tapak dara leaves 0.1% with long soaking varies (8, 16, and 24 hours) can induce polyploidization of Lili hujan plants.

3.5. The adding economic value

The result of independent t-test about the price of Lili hujan plant treated and untreated showed there was a difference price between treated and untreated. The average price of Lili hujan untreated was Rp. 750.00 and the average price of Lili hujan plant treated was Rp. 1,750.00. So the adding economic value was Rp. 1,000.00 for a Lili hujan plant after getting treatment by mutagen (0.1% for ethanolic extract of Tapak dara leaves with long soaking 24 hours) although the Lili hujan plant has become better than before. The attractive plant is still lower than original ornamental plants, such as rose and orchid. So the people still give the lower price for it. But we found the mutagen (0.1% for ethanolic extract of Tapak dara leaves with long soaking 24 hours) can increase the economic value of Lili hujan plant.

4. Conclusion

The conclusions are: 1) Ethanolic extract of tapak dara leaves (*Catharanthus roseus* (L.) G. Don) can be used as polyploidization agent of Lili hujan plant (*Zephyranthes rosea* Lindl.); 2) Concentration 0.1% of ethanolic extract of tapak dara with long soaking 8, 16, and 24 hours can induce polyploidization of Lili hujan plants; 3) Lili hujan plant which treated by ethanolic extract of tapak dara leaves showed increasing in mass root, number, and area of leaves, stomatal density and number of chromosomes better than control plants; and 4) Economic value of Lili Hujan plant will increase by using mutagen (0.1% for ethanolic extract of Tapak dara leaves with long soaking 24 hours). Finally, we recommended the concentration and long soaking of ethanolic extract of tapak dara leaves which used as a mutagen in next research are not only in the concentration of 0.1%. So it can be known concentration and long soaking most effective for inducing polyploidization of Lili hujan plant. Also, it is necessary to optimize for root harvesting time so that the cell division can be known precisely.

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