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Morphological and Cytological Characteristics of Tomato Plants (*Solanum lycopersicum* L.) Induced by *Tapak Dara* (*Catharanthus roseus* (L.) G. Don) Extract

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Abstract

Polyploidy is commonly induced using antimitotic agents such as colchicine, which is imported and relatively expensive, therefore, alternative antimitotic agents derived from local resources are needed. There are three main classes of antimitotic agents: taxanes, vinca alkaloids, and colchicine. Taxanes are obtained only from the bark of the western yew plant, colchicine is derived from *Colchicum autumnale* L., and vinca alkaloids are obtained from the *tapak dara* plant (*Catharanthus roseus*). Therefore, the *tapak dara* plant has great potential as a source of antimitotic agents other than colchicine. This study aimed to determine the effect of *tapak dara* flower extract on the morphological and cytological characteristics of tomato plants (*Solanum lycopersicum* L.). The research used a completely randomized design with four treatment levels and five replications: P0 = control treatment, P1 = 0.05% concentration, P2 = 0.1% concentration, and P3 = 0.15% concentration, with a soaking duration of 10 hours. The observed parameters included germination percentage (%), plant height, number of leaves, number of flowers, and chromosome number. Data were analyzed using one-way ANOVA followed by Duncan's multiple range test at the 5% significance level. The results showed significant effects on the number of leaves, number of flowers, and chromosome number. Treatment P1, with a concentration of 0.05%, was the best treatment.

Keywords: Colchicine; Polyploidy; *Solanum lycopersicum* L.

INTRODUCTION

Plant breeding is a method for developing new plant varieties with the assistance of human intervention (Novak & Brunner, 1992). Plant breeding can be carried out through various approaches, such as plant hybridization, mutagen induction, and the use of molecular markers. Mutation breeding is one of the plant breeding methods used to enhance genetic variability in plants, in addition to hybridization, protoplast fusion, and genetic engineering (Husain et al., 2022). Mutagen induction in plants is generally aimed at producing polyploid plants. Polyploid plants obtained through mutagen induction are expected to exhibit superior characteristics compared to their diploid counterparts, such as improved disease resistance and higher productivity.

Colchicine is among the chemical mutagens that are widely used for inducing mutations in plants (Udofia et al., 2024). The use of colchicine for inducing polyploidy has been reported in several plant species, including *Rhododendron fortunei* Lindl., *Lilium regale*, golden berry, *Gladiolus grandiflorus*, and many others. However, the application of colchicine as a mutagen still faces constraints, particularly in Indonesia. The high cost of colchicine remains the main obstacle to its use for plant breeding purposes, both at the research scale and for direct application in the community. The price of colchicine can reach up to IDR 2.000.000 per gram. This high cost is due to the fact that the primary source plant of colchicine, *Colchicum autumnale*, only grows in certain regions and does not grow in Indonesia.

An alternative approach to overcome the problem of expensive colchicine mutagens is to search for plant species other than *Colchicum autumnale* that contain colchicine. The Madagascar periwinkle/*tapak dara* (*Catharanthus roseus* (L.) G. Don) is known to contain vinca alkaloids with antimitotic properties similar to those of *Colchicum autumnale*. The use of extracts from *Catharanthus roseus* in polyploid induction has been reported in several studies.

The selection of tomato plants as the subject for induction using tapak dara (*Catharanthus roseus*) extract is based on the fact that tomatoes are widely cultivated and highly favored by the community. Listiana (2016) also stated that tomatoes are known as plants with diverse

benefits and are therefore extensively used in daily life. According to data, tomato consumption in Indonesia in 2019 reached 1.020.333 tons, representing an increase of 4.46% compared to the previous year, which was 976.772 tons. The induction of tapak dara extract in tomatoes is expected to produce polyploid tomato plants that positively correlate with increased tomato productivity.

The analysis of the degree of polyploidy in plants resulting from mutagen induction can be conducted through various approaches, including morphological, cytological, and molecular observations. Polyploid plants generally exhibit better morphological characteristics than their diploid counterparts, such as broader and thicker leaves, larger stem diameters, larger flower diameters, and bigger and heavier fruits. Cytological observation is also one of the methods used to identify the ploidy level of plants produced through mutagen induction. Cytological analysis can be carried out by observing and counting chromosome numbers as well as examining stomatal characteristics on the leaf epidermis. This research has significance in the development of alternative mutagens to replace colchicine. Therefore, the researcher was interested in conducting a study entitled **"**Morphological and Cytological Characteristics of Tomato Plants (*Solanum lycopersicum* L.) Induced by tapak dara (*Catharanthus roseus* (L.) G. Don) Extract**."**

MATERIALS AND METHODS

Study Area

This research was conducted from September to November 2024 at the Greenhouse of Raden Intan State Islamic University of Lampung, and the preparation of *tapak dara* (*C. roseus*) flower extract was carried out in the laboratory of Lampung Polytechnic.

Procedures

Approach and Type of Research

The research approach and type used are descriptive quantitative. The experiment employed a completely randomized design (CRD) with four treatments. The samples consisted of 20 plants for all treatments. The combinations of tapak dara (*C. roseus*) flower extract concentration and tomato seed soaking duration tested were as follows:

1. A = Soaked in water for 10 hours (control).
2. B = Soaked in tapak dara flower extract at a concentration of 0.05% for 10 hours.
3. C = Soaked in tapak dara flower extract at a concentration of 0.1% for 10 hours.
4. D = Soaked in tapak dara flower extract at a concentration of 0.15% for 10 hours.

Research Procedure

The research procedure in this study was divided into three stages, namely preparation, implementation, and observation:

a. *Preparation*

At this initial stage, the researcher prepared the tools and materials to be used in the study. This was followed by the process of preparing the flower extract of *tapak dara*.

b. *Implementation*

1. Sample preparation
   The *tapak dara* flowers were separated from the stalks and other impurities, then dried in an oven at 40°C. After obtaining the dried tapak dara flower simplicia, it was stored in an airtight and non-humid container.
2. Preparation of *tapak dara* flower extract
   The *tapak dara* flower extract was prepared using the maceration method. A total of 400 g of *tapak dara* flower powder was dissolved in 2 liters of 96% ethanol. Maceration was carried out for 3 × 24 hours, after which the mixture was filtered using filter paper. The filtrate (macerate) was then evaporated using a rotary evaporator at a temperature of 50°C and a speed of 60 rpm until a viscous extract was obtained.
3. *Seed treatment and planting*
   Tomato seeds were soaked in the *tapak dara* flower extract solution according to the tested concentrations and soaking durations. After the treatment, the seeds were planted in polybags containing a planting medium composed of soil, manure, and rice husk in a ratio of 2:1:1.

c. *Observation*

There were two categories of observations in this study, as follows:

1. Phenotypic character measurements
   Plant height and leaf length were measured using a ruler with an accuracy of 1 mm.
2. Cytological observation of chromosomes
   Chromosome observations were carried out using the squash method, with the following procedures:
   a) The tips of germinated tomato roots were cut to a length of 0.5 cm, placed into flacon tubes, and immersed in 45% glacial acetic acid solution (45 ml glacial acetic acid mixed with 55 ml distilled water). The samples were left for 15 minutes at 4 °C and then rinsed three times with distilled water.
   b) The washed root tips were then soaked in 1 N HCl solution (1 ml HCl + 11 ml distilled water) for 11 minutes at 55°C.
   c) The root tips were subsequently stained by immersion in 1% aceto-orcein solution (1 g aceto-orcein dissolved in 100 ml of 45% acetic acid) for 20 minutes at room temperature.
   d) The stained root tips were placed on microscope slides and covered with cover slips. The cover slips were gently pressed until the root tissue was crushed, and the preparations were then observed under a microscope (Muhlisyah et al., 2014).

Parameters

The phenotypic data observation parameters include the following:

a. *Germination Percentage*

Germination capacity is a measure of seed viability that indicates the ability of seeds to germinate and grow into normal seedlings under optimal conditions. Observation of germination percentage was conducted for four days during the germination period using Petri dishes. The germination percentage was calculated using the following formula:

$$\text{Seed Viability} = \frac{\sum BK}{\sum TB} \times 100\%$$

Description:

∑BK= Number of germinated seeds

∑TB = Total number of seeds tested

b. *Plant Height (cm)*

Plant height was measured at 1, 2, 3, and 4 weeks after planting (WAP). Measurements were taken from the base of the stem to the highest growing point of the plant.

c. *Number of Leaves (leaves)*

The number of leaves was recorded at 1, 2, 3, and 4 WAP by counting all fully developed leaves on each plant.

d. *Number of Flowers (flowers)*

Flowering time was determined by the appearance of the first flower on the plant. The number of flowers was determined by counting all flowers that bloomed on each plant during the observation period.

Cytological Data

Cytological observations were conducted on root tip chromosomes collected when the roots had just begun to grow. Chromosome observation followed the squash technique. Root tips were excised and placed into a vial, followed by fixation using 45% glacial acetic acid. After fixation, the roots were rinsed with distilled water, then subjected to maceration and squashing until the preparations were ready for microscopic observation.

Tools and Materials

The tools and materials used in this study included 3-kg plastic bags, stationery, a camera, a ruler, a small hoe, a microscope, flasks, microscope slides, a graduated cylinder, petri dishes, *tapak dara* flower extract, distilled water, glacial acetic acid, 1% aceto-orcein, planting media (soil, rice husk, and manure), tomato seeds, and 1 N HCl solution.

Data Analysis

The data analysis test used in this study is the Analysis of Variance (ANOVA) test using the SPSS 26 program with One-Way ANOVA. If there is a significant difference between treatments, a follow-up test will be conducted using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

RESULTS AND DISCUSSION

Results

Observation of Morphological Characteristics

Germination Percentage

Observations on the growth of tomato seedlings over a 4-day period showed that almost all seedlings in each treatment emerged at 2 days after planting, with an average percentage of surviving plants across all treatments of 82%.

Table 1. Germination Percentage of Tomato Seeds (*Solanum lycopersicum* L.).

Treatment	Average Number of Germinated Seeds	Germination Percentage (%)
P0	0.84	84%
P1	0.88	88%
P2	0.80	80%
P3	0.76	76%

Plant Height

Plant height is the first morphological observation parameter. This observation was carried out by measuring the height of tomato plants from the base of the stem to the tip of the highest leaf from the first to the fourth week.

Table 2. Stem Height Data of Tomato Plants (*Solanum lycopersicum* L.) at 4 Weeks After Planting.

Treatment	Replicate					Total	Average
	1	2	3	4	5		
P0	20	18	19	22	25.2	104.2	20.84
P1	35	30	28	28	27.3	148.3	29.66
P2	21	20	24	21.7	23	109.7	21.94
P3	17	20	15	21	18.6	91.6	18.32

Number of Leaves

The third observation parameter was the number of leaves per tomato plant (*S. lycopersicum* L.). Observations were conducted once a week for four weeks.

Table 3. Leaf Number Data of Tomato Plants (*S. lycopersicum* L.) at 4 Weeks After Planting.

P	Replicate					Total	Average
	1	2	3	4	5		
P0	21	17	16	19	16	89	17.8
P1	39	35	31	38	43	186	37.2
P2	16	17	17	19	21	90	18
P3	12	11	11	14	16	64	12.8

Number of Flowers

The first parameter used to observe the productivity of tomato plants (*S. lycopersicum* L.) was recorded when the plants entered the generative phase, indicated by the onset of flowering.

Table 4. Average Number of Flowers of Tomato Plants (*S. lycopersicum* L.).

Treatment	N	Subset for alpha = 0.05		
		1	2	3
P3	5	1.6000		
P0	5		4.6000	
P2	5		5.6000	
P1	5			11.0000
Sig.		1.000	.330	1.000

Cytological Character Observation

Chromosome counting in mitotic root tip cells is an important method for confirming plant ploidy levels, particularly in newly formed cells following polyploidization treatments (Gamage & Schmidt, 2009) such as seed soaking with *tapak dara* flower extract in this study. Polyploidy may occur because the *tapak dara* flower extract inhibits chromosome migration during metaphase, resulting in the formation of polyploid cells. Tomato plants (*S. lycopersicum* L.) have a chromosome number of 2n = 24.

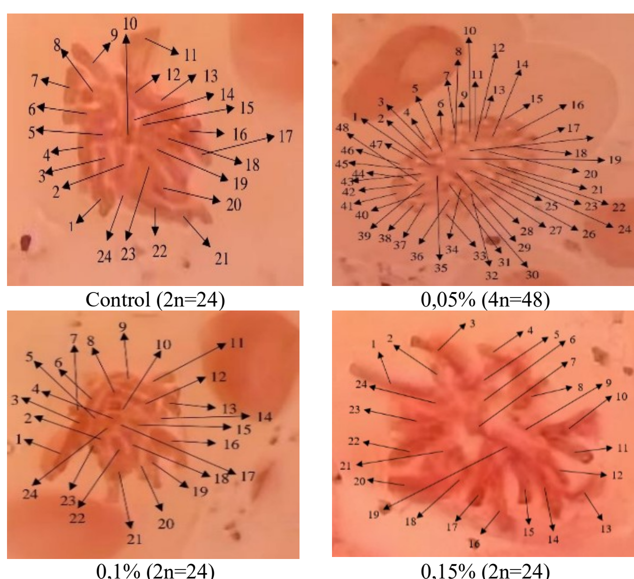

Figure 1. Chromosome Number of Tomato Plants (*S. lycopersicum* L.)

Discussion

Table 1 shows that treatment 1 (0.05%), the control treatment, and treatment 2 (0.1%) had significantly higher germination rates compared to treatment 3 (0.15%), which exhibited a germination percentage below 80%. Seeds are considered to have good germination capacity when the germination percentage exceeds 80%. Treatment 3, with a concentration of 0.15%, did not show a significant difference in germination rate. This is presumably because the application of the extract to tomato seeds did not have a significant effect on plant cells. Differences in the germination process are likely related to the mechanism of action of the *tapak dara* flower extract, which tends to be more effective on actively dividing cells (Soedjono, 2003). In line with this, Paul et al (2023) reported that the main compounds of *C. roseus*, such as vinca alkaloids (vincristine and vinblastine), act by inhibiting cell division (mitosis) through disruption of microtubule formation. The non-significant germination percentage indicates that plant cells were still able to withstand the negative effects of the extract through physiological tolerance mechanisms (Jamal & Ahmad, 2024). In addition, the sensitivity and response of each plant to the extract treatment may vary. *C. roseus* is known to produce various active secondary metabolites, such as alkaloids and flavonoids, which exhibit diverse biological activities (Jamal & Ahmad, 2024).

Treatment 1 (0.05%), the control treatment, and Treatment 2 (0.1%) showed significantly higher germination capacity compared to Treatment 3 (0.15%) (Table 2). This indicates that excessively high concentrations of vindoline and vindesine compounds in plants can inhibit growth. This study also demonstrates that increasing the concentration of *C. roseus* (periwinkle) flower extract has a direct effect on plant height. Treatments with higher concentrations (0.15%) resulted in lower plant height compared to lower concentrations. The reduction in plant height is presumed to be caused by the antimitotic properties of alkaloid compounds present in *C. roseus*, which can inhibit cell division in meristematic tissues by disrupting microtubule formation, thereby reducing cell elongation and stem growth (Banyal et al., 2023). High concentrations of colchicine can cause malformations and reduce the production of tetraploid plants, leading to inhibited growth (Manzoor et al., 2019). These high concentrations are also suspected to suppress overall cell

division activity, which consequently affects vegetative growth, such as plant height.

The treatment with the highest average number of leaves was observed at a concentration of 0.05%, whereas the lowest average leaf number occurred at a concentration of 0.15% (Table 3). This indicates that higher concentrations tend to cause tissue damage and inhibit plant growth. The highest leaf number in treatment 1 at a concentration of 0.05% was attributed to plants possessing a chromosome number of 4n = 48 (tetraploid), where an increased chromosome number may lead to enhanced plant height. Treatment 1 at a concentration of 0.05% produced a significantly higher number of leaves compared to treatment 2 (0.1%) and treatment 3 (0.15%). This result is attributed to the induction of antimitotic substances in the form of vincristine and vinblastine compounds, which play a role in enhancing leaf development and leaf number. In contrast, treatment 3, with a high flower extract concentration of 0.15%, inhibited cell division, resulting in reduced plant growth. Polyploid plants are able to produce more shoots than their diploid counterparts, and each shoot that emerges will grow into a stem. The taller the stem that develops, the greater the number of leaves produced by the plant. Plants treated with high concentrations of antimitotic agents show lower average growth compared to control plants, whereas growth increases when the treatment is applied at an optimal and balanced level (Kusnuriyanti dkk., 2017). Manipulation of plant ploidy levels can improve the genetic quality of plants. The application of an appropriate concentration can enhance plant quality; however, excessively high doses may inhibit growth or even cause damage to the plants (Wang et al., 2016).

Based on the results of the DMRT analysis on the flower parameter (Table 4), the highest number of flowers was observed in treatment P1, which was significantly different from treatment P3, while treatment P2 did not differ significantly from the control treatment (P0). The lowest number of flowers was found in treatment P3 with the highest concentration of 0.15%. This result is attributed to the use of high concentrations, which can disrupt the cell division process, thereby inhibiting plant growth (Purbosari et al., 2018). Zulfiqar et al. (2022) also reported high concentrations of colchicine combined with prolonged exposure can affect plant survival rates. In contrast, low concentrations applied in vitro are more effective in inducing polyploidy, leading to optimal morphological traits and increased plant productivity.

The optimum concentration and soaking duration depend on the treatment method applied (Figure 1.). Treatment 1 (0.05% concentration) showed an increase in ploidy level, indicating an increase in chromosome number to 4n = 48. In contrast, treatment 2 (0.1%) and treatment 3 (0.15%) did not show an increase in chromosome number. Figure 1. illustrates the cytological observations obtained using a soaking duration of 10 hours for the control treatment and treatment 1 (0.05%), 2 (0.1%), and 3 (0.15%). The soaking duration was the same for all treatments; therefore, extensive chromosome duplication did not occur. The control treatment, treatment 2 (0.1%), and treatment 3 (0.15%) exhibited 2n = 24 chromosomes (diploid). The success of polyploidization is indicated by chromosome duplication. Normal diploid tomato plants have a chromosome number of 2n = 24, whereas successful polyploid induction is characterized by the formation of tetraploid cells with 4n = 48. At concentrations of 0.1% and 0.15%, tetraploid cells were not formed, which is presumably due to toxic effects at higher concentrations that inhibit chromosome separation during mitosis through the suppression of microtubule polymerization by vinca alkaloids (Ainurrohmah et al., 2020). At a concentration of 0.05%, an increase in chromosome number was observed. In addition, this concentration is also presumed to have the potential to enhance plant phenotypic traits, including the size of vegetative organs and overall productivity. A study by Kwon et al (2016) showed that lower concentrations of colchicine (0.05% to 0.1%) are often more effective for chromosome doubling. For example, in *Codonopsis lanceolata*, the highest number of tetraploid plants was achieved at 0.05% colchicine. Similarly, in persimmon, a 0.05% colchicine treatment produced the best results for chromosome doubling (Bok Ma et al., 2018).

CONCLUSIONS

Five observed parameters indicated that three parameters had a highly significant effect. The leaf number parameter showed that treatment 1 with a *tapak dara* flower extract concentration of 0.05% had the greatest effect on tomato plant growth, with an average of 37.2 leaves. The flower number parameter also showed that Treatment 1 (0.05%) was significantly different from the other treatments, producing an average of 11 flowers. Microscopic observations demonstrated that treatment 1 (0.05%) was able to induce the formation of tetraploid cells with a chromosome number of 4n = 48. A *tapak dara* flower extract concentration of 0.05% was the most optimal concentration for inducing polyploidy in tomato plants based on both morphological and cytological parameters.

Further research is required to determine the effects of tapak dara flower extract using additional parameters, such as fruit number, fresh fruit weight, dry weight, and stomatal density, while also taking into account external factors including water availability, light intensity, temperature, and oxygen levels. In addition, the application of Plant Growth-Promoting Rhizobacteria (PGPR) utilizing *tapak dara* flower extract should be further investigated to evaluate its effectiveness in

enhancing plant resistance to various environmental stresses, such as drought, salinity, and extreme temperatures.

Competing Interests: The authors declare that there are no competing interests.

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