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Effect of colchicine mutagen on phenotype and genotype of *Vigna unguiculata* var. *sesquipedalis* the 7th generation

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Abstract. *Fathurrahman F, Mardaleni, Krisianto A. 2023. Title Effect of colchicine mutagen on phenotype and genotype of* Vigna unguiculata *var.* Sesquipedalis the 7th generation. *Biodiversitas 24: 1408-1416.* The problem with long bean species is the low diversity of varieties, so obtaining them using chromosomal mutations was necessary. One of the ingredients used was colchicine. The aim of this study was to determine the effectiveness of the mutagen colchicine against mutations that occur in *Vigna unguiculata* var. *sesquipedalis* the 7th generation (renek long bean). The design used was a factorial Completely Randomized Design consisting of two factors. The first factor was four colchicine concentration levels: including without colchicine (the control) and colchicine concentration (0.01%, 0.05%, and 0.09%). The second factor was the soaking of the *V. unguiculata* var. *sesquipedalis* seeds, namely without soaking of seeds and soaking seeds (6, 12, 18, and 24 hours). Further phenotype analysis was tested statistically using the DMRT test at $p < 0.05$, while genotypes were analyzed using karyotypes and statistical T-test at $p < 0.05$. The results showed that treatment with 0.09% of colchicine and soaking for 24 hours was the best combination. Phenotype analysis showed increased growth with colchicine treatment: plant height increased by 20.59%, pod weight increased by 0.88%, the number of pods increased by 47.93%, pod length increased by 22.50%, and 100 seed weight increased by 15.91%. While Genotype analysis obtained a total of 11 karyotype chromosomes and 2n = 22 chromosomes. Chromosome karyotypes obtained two types, namely metacentric and telocentric. The arm length (p+q) in the 0.09% colchicine treatment sample and 24-hour soaking time was 1.77 μm longer than the control sample, which was 1.53 μm. It turns out that colchicine can increase the growth and changes in the structure of chromosomes so that a mutated *V. unguiculata* var. *sesquipedalis* species is obtained.

Keywords: Colchicine, genotyping, mutations, phenotyping, *Vigna unguiculata* var. *sesquipedalis*

INTRODUCTION

Vegetable plants rich in vitamins include long beans (*Vigna unguiculata*), which are widely cultivated as shrubs with creeping or vines and require rods for propagation. Protein content in nuts length is quite high, namely 22.3% in dry seeds, 4.1% in leaves, and 2.7% on young pods. This leads to the development of an upright variety by breeding the long bean plants at the Faculty of Agriculture, Universitas Islam Riau, Indonesia. The breed obtained does not require vines, has low planting costs, and can produce up to 50 pods per plant. Furthermore, the upright type of long beans developed is entering the 7th generation with the original name of the renek long bean variety (*Vigna unguiculata* var. *sesquipedalis*).

Therefore long beans are a source of inexpensive protein and are easy to develop in various regions. Besides that, other nutritional compositions are thiamin, vitamin A, riboflavin, iron, phosphorus, potassium, ascorbic acid, folic acid, magnesium, and manganese. Every 100 grams of long beans contain 62 mg or 15% of the total daily folate requirement (Kaswinarni et al. 2014). This substance, which works with vitamin B12, is an important component of DNA synthesis and cell division. Even long beans have become an industrial ingredient and have been canned to be used in the vegetable industry in other countries.

Because the *V. unguiculata* var. *sesquipedalis* is relatively new in Indonesia, there are problems; it has not been identified genetically, and its production is still low. Furthermore, this bean production needs to be increased through chromosomal mutations that can become polyploid plants. Thus it can add an introduction to genetic resources. Information on V. *unguiculata* var. *sesquipedalis* must be completed based on its potential and benefits. However, research on genetic aspects of *V. unguiculata* var. *sesquipedalis*, especially growth and chromosome field, is poorly reported. The number of *V. unguiculata* var. *sesquipedalis* chromosomes was 2n=22. Culturing this type of plant requires additional costs, namely the provision of trellises.

Since *V. unguiculata* var. *sesquipedalis* seeds are relatively new, it is necessary to conduct research to improve quality (Suwandi et al. 2019) through plant breeding. The type of plant breeding that can be applied is chromosomal engineering through mutagen colchicine. According to Gultom et al. (2016), colchicine with the chemical formula $C_{22}H_{25}O_6N$ is an alkaloid with microtubules. One of its effects is to cause a doubling of plant chromosomes, which can form polyploid plants. The mutation techniques have been used to get various varieties of plants, including *V. unguiculata* var. *sesquipedalis*. This method can prove useful in obtaining genotype traits or characters and new phenotypes to form genetic variation and add references in conventional breeding (Anbarasan et al. 2013). This genetic diversity is necessary for plant breeding; if other mutations can occur in organisms, plants can also experience mutations (Shilpa et al. 2021). Colchicine inhibits mitosis in various plant and animal cells by disrupting the orientation and structure of mitotic division and spindles (Kumar and Rani 2013). Colchicine is given in parts of plants that divide at the point of vegetative growth, such as seeds, sprouts, and plant stem tips (Corneillie et al. 2019).

According to Shilpa et al. (2021), genetic diversity can be increased from various plant genetic sources, including old and new cultivars. Cultivars, such as local races and close and distant relatives, are cultivated and germplasm collections and mutations. Colchicine inhibits the metaphase, prevents the polymerization of tubulin into microtubulin, and prevents the tubulin from becoming a functional yarn fiber. Thus, the anaphase stage for separating chromosomes does not occur. As a result, the separation wall failed to form without bobbin thread so that the chromosomes and their duplicates remain in the same cell. Furthermore, according to Sajjad et al. (2013), cell division does not occur immediately; division begins with diploid cells and ends with the formation of tetraploid cells. Therefore, the concentration and Soaking time of colchicine affect polyploidy induction.

Polyploidy can be induced artificially in plants using the most widely effective chemical, colchicine. This is because colchicine dissolves easily in water and can change the number of chromosomes in cells (Manzoor et al. 2019). It also affects plant physiology, making plants appear bigger and stronger. However, the long-term application can cause stunted growth. This makes obtaining the effective concentration and appropriate soaking time necessary for maximum and profitable plant growth and production. Plant growth and development can be observed in characteristics such as height, number of pods, pod length and weight. The chromosomal mutations can also occur with the influence of colchicine mutagens which cause changes in phenotype and genotype.

Colchicine can induce plants to become polyploid plants at the right concentration and time. Generally, polyploidy is a condition in an organism having more than one pair of chromosomes. At a certain concentration, colchicine will weaken the arrangement of spindle thread microtubules, inhibiting mitosis (Curuk et al. 2020). Therefore, this research was conducted to determine the effect of the colchicine mutagen on phenotypes and genotypes of *V. unguiculata* var. *sesquipedalis*.

MATERIALS AND METHODS

This research was carried out in September-December 2022 at the Air Dingin location, the Experimental garden of the Faculty of Agriculture, Universitas Islam Riau. The materials used were *V. unguiculata* var. *sesquipedalis* seeds the 7th generation, chemicals for chromosome analysis, and a camera from the Laboratory of Biotechnology Faculty of Agriculture Universitas Islam Riau. Colchicine from Sigma Aldrich Catalog C9754 25 mg from Jakarta. The compost fertilizer from the Faculty of Agriculture, Universitas Islam Riau. NPK Mutiara 16:16:16 and TSP fertilizer from the farm shop of Pekanbaru Riau.

The design was a factorial Completely Randomized Design (CRD) consisting of two factors: the concentration of colchicine (Factor C), namely, without colchicine, 0.01%, 0.05%, and 0.09% colchicine, and the soaking time (Factor T) namely without soaking times, 6, 12, 18 and 24 hours. The administration of colchicine consisted of 4 treatment levels, with each soaking time making a total of 16 treatments. Each treatment consisted of 3 replications to obtain 48 experimental units. Subsequently, each experimental unit comprised four plants, and two were used as sample plants.

The implementation consisted of land preparation, soil processing I and II, and making plots of 80 cm x140 cm. The seeds were planted by digging in the planting hole to a depth of 2 cm with a spacing of 40 cm x 70 cm. That was followed by plant maintenance consisting of watering, replanting, weeding, hilling, and controlling pests and diseases. The compost was given 2 kg per plot and NPK Mutiara 16:16:16 30 g, while labeling was carried out two days before treatment. The prepared labels were attached according to the treatment in each plot based on the research plan. Furthermore, 50 cm in length stakes were installed two weeks after planting to avoid damage such as falling and support fruit because *V. unguiculata* var. *sesquipedalis* plants easily fall over. Pest and disease control during the experiment was carried out in a preventive and curative manner. The phenotype parameters were plant height (cm), pod weight, and number of pods per plot, as well as pod length and weight of 100 seeds. The research used was a randomized block design, and data on growth morphology were statistically analyzed using Analysis of Variance (ANOVA) with SAS 9.1.3 software. When the treatment had a significant effect, it was continued with the Duncan Multiple Range Test (DMRT) at $p < 0.05$. The linear model is as follows:

$$Yct = \mu + Cc + Tt + \Sigma(ctn)$$

Where:
Yct : The observed variable from colchicine the t level and the immersion time of the c level
μ : The effect of the mean value
Cc : effect of the T factor on the t level
Tt : Effect of factor C on level c
CcTt : effect of interaction between the C factor at the level to - c and the T factor to the t level
Σ(ctn) : Error effect of factor C at the c level and the T factor at the t level and repetition up to n
C : 0, 1, 2, 3 (Colchicine)
T : 1, 2, 3, 4 (Soaking time)
n : 1, 2, 3 (Repeat)

Three seeds were obtained from the control sample and 0.24% colchicine treatment group from the first crop generation. That was to observe changes in the karyotype structure of chromosomes in biological samples. The basic staining procedure followed the method that Sharma and Sharma developed (1980). The sample was tested in the laboratory to observe the karyotype and number of chromosomes. The samples of meristematic root tips are taken from plants at 8:00 am. The root tip was then processed in distilled water and stored at a low temperature of 4-5°C for 24 hours. The fixation with 45% glacial acetic acid solution for one hour at room temperature was done. After that, the roots were washed using distilled water with three repetitions and then hydrolyzed using 1N HCL for 10 minutes at room temperature. Before the final treatment, root ends must be washed with distilled water three times. Root tip staining using 2% aceto-orcein was then stored for 24 hours at room temperature. Coloring works chromosomes observed and compared to the cytoplasm (Ahmad et al. 1983). The preparation method for observation is the squash method, a simple and fast way to visualize chromosomes and cell nuclei (Chirino et al. 2014). The squash method aims to get a runny preparation and can be observed for a long time. Samples used for observation were six samples, obtained for each consisting of 3 samples of the control and colchicine. The root tip that has been colored is then dripped with 45% acetic acid and followed by maceration (squash) using a brush tip or rubber eraser previously covered with a cover glass. Observation of root tip preparations seen using a microscope and Optilab. The microscope magnification used is 1000x. The data needed at that observation time was recorded. Chromosomes that appear on observations with a microscope are captured, and the number of chromosomes counted from the images can be analyzed. The type of chromosome was determined based on the method by Levan et al. (1964). Furthermore, observational data were analyzed statistically and presented in tabular form. Then, the measurement data of the short-arm (p), long-arm (q), and long karyotype were measured and continued with the T-test using the formula:

$$t = \frac{Md}{\sqrt{\frac{\sum x^2 d}{N(N-1)}}}$$

Where:

Md = Mean of the difference between the control sample and the colchicine treatment

Xd = deviation of each subject (d-Md)

$\sum x^2d$ = sum of squared deviations

N = Subjects in the sample

RESULTS AND DISCUSSION

Plant height

The phenotypic observation of the interaction of colchicine treatment and soaking time in Table 1 showed significant differences in the parameters of plant height. Figure 1 shows the height growth of the *V. unguiculata* var. *sesquipedalis* plant in the generative phase. The best treatment was the interaction of colchicine 0.09% and 12 hours of soaking time, then by 0.09% and 24 hours, and 0.05 % with 18 and 24 hours. Meanwhile, the main effect of colchicine was to produce significantly different heights. Based on the results, the highest treatment produced 0.09% and 0.05% colchicine, that significantly different from the 0.01% colchicine treatment and the control. Meanwhile, the main effect of soaking time which produced the best plant height was 12 hours, followed by 18 hours and 24 hours, significantly different from 6 hours.

The height of the control plant without colchicine was 35.20 cm, while the combination of 0.09% colchicine treatment and 24 hours of soaking time yielded a 24.46% increase and a height of 43.81 cm, as presented in Table 1. This showed that these combination treatments have the main effect of increased plant height. Colchicine is an alkaloid that affects the arrangement of microtubules, doubling the number of plant chromosomes or the formation of polyploid plants. The general characteristic of polyploid plants was that they became more stocky, while the roots, stems, leaves, flowers, and fruits were also enlarged (Ridwan and Witjaksono 2020; Trojak et al. 2021). Generally, this chemical is often used to induce polyploidy in plants. According to Girsang et al. (2021), colchicine solution at a certain critical concentration will hinder microtubule arrangement from the spindle fibers. That would cause irregularities in mitosis.

Pod weight

The next phenotypic observation in Table 2 showed that the interaction of colchicine treatment and soaking time had a significant difference in the parameters of pod weight. The highest treatment was 0.09% colchicine interaction and 24 hours of soaking. However, the interaction with others, such as 0.05% and 0.09%, with the notation equal to 0.09%. The main effect of colchicine was to produce significantly different heights. In this research, the highest treatment was produced at 0.09% colchicine. The main effect of soaking time, which led to pod weight, was not significantly different.

The main effect of the interaction and treatment was a 0.88% increase in pod weight in *V. unguiculata* var. *sesquipedalis* yielding 513.17 g per plant with 0.09% colchicine and 24 hours of soaking time compared to the control, which only produced 508.67 g per plant. Generally, colchicine is given to the part of the plant that is dividing, namely at the point of vegetative growth, for example, in seeds, sprouts, and plant stem tips (Grumet et al. 2023).

Figure 1. The height growth of the *Vigna unguiculata* var. *sesquipedalis* the 7th generation plant in the generative phase

Colchicine could inhibit the metaphase stage, the tubulin polymerization into microtubulin, and then become functional thread fibers (spindle threads). Thereby it's preventing the occurrence of the anaphase stage for chromosome separation. Without the spindle fibers, dividing walls fail to be formed. Therefore, the chromosomes and their duplicates remain in the same cell. This will prevent cell division, which begins with diploid cells ending with the formation of tetraploid cells (Shenk and Ganem 2016).

Number of pods

Based on the phenotypic observations in Table 3, colchicine treatment and soaking time interaction showed significant differences in the observed parameters of the number of pods. The highest treatment was 0.09% colchicine interaction and 24 hours of soaking. However, the interaction with other colchicine percentages, such as 0.05%, with 18 and 24 hours of soaking time was not significantly different. For the main effect of colchicine, the number of pods significantly differed from the control, where the highest colchicine treatment was 0.09%. However, the main effect of soaking time, which produced the number of pods, was not significantly different from without colchicine soaking.

The results showed that the combined treatments and the main effect increased the number of pods on short *V. unguiculata* var. *sesquipedalis*. It was also discovered that the combination of 0.09% colchicine treatment and 24 hours of soaking time produced 41.17 pods, while the control yielded 27.83 pods. The appropriate colchicine can increase the production of chickpea pods to 47.93%. This is because colchicine affects the morphology of plants that look stocky and increases organic matter in cells, such as protein and vitamins, the total weight, and the number of cells. However, using colchicine at high concentrations and for a long time will cause stunted plant growth (Ayu et al. 2019). This makes it necessary to search for the appropriate concentration of colchicine and the length of time for effective and efficient application/soaking.

Pods length

Based on the interaction of colchicine treatment and soaking time in Table 4, there were significant differences in the observed parameters of pod length. The best treatment was the interaction of colchicine 0.05% and 24 hours soaking time, followed by 0.09% and 24 hours, 0.01% and 12 hours, as well as 18 hours soaking time. Meanwhile, the main effect of colchicine that produced significantly different heights was obtained at 0.09%, followed by 0.05% and 0.01%. These concentrations differed from 0.01% colchicine treatment and 18 hours of soaking. The main effect of soaking time producing the best pod length was immersion of 24 hours followed by 12 and 18 hours.

The main effect of the combined treatment was increased pod length of *V. unguiculata* var. *sesquipedalis*. In control, pod length was 35.37 cm, which increased to 43.33 cm (22.50%) due to the administration of 0.05% colchicine treatment and 24 hours of soaking time. Meanwhile, the main effect of colchicine in the best treatment was the concentration of 0.09%. However, it was not significantly different from the concentrations of 0.05% and 0.01%, but significantly different from that without colchicine (control). Likewise, the main effect of soaking time of 24 hours produces the highest pod length.

This makes it necessary to search for appropriate concentration and an effective and efficient application/soaking time. Changes that occur in plants due to the appropriateness of colchicine can vary. This is because some plants experience mutations in almost all parts, from the growing point to the generative organs, while others only mutate in a few organs. Therefore, possible that colchicine given to each plant does not affect all plant cells but only some of the cells. Colchicine is a mitotic inhibitor widely used to induce plant polyploidy during cell division by inhibiting chromosome segregation (Manzoor et al. 2019).

Weight 100 seeds

Observations on the interaction of colchicine treatment and soaking time showed significant differences in the parameters of pod length, as shown in Table 5. The best treatment was 0.09% colchicine interaction and 24 hours of soaking time, yielding a seed weight of 68.11 g, followed by 0.05% and 0.01%. The main effect of colchicine was to produce significantly different heights. The highest value was obtained in 0.09% colchicine, followed by 0.05% and 0.01%. However, the length of time for soaking the seeds was not significantly different between treatments.

The weight of 100 seeds in control yielded 58.76 g, while the highest combination treatment of 0.09% colchicine and 24 hours soaking time produced 68.11 g. Furthermore, there was a 15.91% increase in the weight of 100 seeds in the treatment sample. Based on the statistical analysis of all parameters observed, there was also an increase in the growth and production of the *V. unguiculata* var. *sesquipedalis*. The combined treatment and the main effect of the percentage of colchicine increased the weight of 100 *V. unguiculata* var. *sesquipedalis* seeds. Previous investigations showed that plant growth response to the weight of 100 seeds in control yielded 58.76 g, while the highest combination treatment of 0.09% colchicine and 20 hours soaking time produced 68.11 g. Furthermore, there was a 15.91% increase in the weight of 100 seeds in the treatment sample. Based on the statistical analysis of all parameters observed, there was also an increase in the growth and production of the *V. unguiculata* var. *sesquipedalis*.

The combined treatment and the main effect of the percentage of colchicine increased the weight of 100 *V. unguiculata* var. *sesquipedalis* seeds. Previous investigations showed that the response of plant growth to the appropriation of colchicine varies. According to Fathurrahman (2016), the highest yield was given at 0.1% for all parameters, namely flowering age, plant height, seed weight, and weight of 100 black soybean seeds. However, treated peanut plants showed a better phenotype in the number of pods than the negative control (Ahmad et al. 2021). Also, Ikhsanudin and Budi (2020) discovered that the concentration of 0.1 % colchicine in peanuts caused high monomorphic levels. Furthermore, the appropriation of 0.01% and 0.02% colchicine concentrations with a treatment time of 10 hours affected the increase in stomata size, plant height, and weight of 100 Anjasmoro soybean seeds (Ni and Made 2019).

Table 1. Plant height (cm) of *V. unguiculata* var. *sesquipedalis* given colchicine mutagen at 28 days of age

Percentage colchicine (%)	Soaking time (hour)				Average
	6 (T1)	**12 (T2)**	**18 (T3)**	**24 (T4)**	
Without colchicine (C0)	35.20 g	35.48 fg	33.70 g	33.35 g	34.43 c
0.01 (C1)	38.71 de	41.12 bcd	42.12 bc	40.25 cde	40.55 b
0.05 (C2)	37.93 ef	41.30 bcd	43.30 ab	43.30 ab	41.46 ab
0.09 (C3)	38.66 de	44.99 a	42.35 abc	43.81 ab	42.45 a
Average	37.62 b	40.72 a	40.36 a	40.17 a	

Note: Numbers followed by different lowercase letters at the column and row are significant according to the DMRT follow-up test at 0.05

Table 2. Pod weight (g) of *V. unguiculata* var. *sesquipedalis* plant given colchicine mutagen

Percentage colchicine (%)	Soaking time (hour)				Average
	6 (T1)	**12 (T2)**	**18 (T3)**	**24 (T4)**	
Without colchicine (C0)	423.32 bcd	396.32 d	388.64 d	508.67 ab	429.24 b
0.01 (C1)	436.50 a-d	459.17 a-d	403.17 d	420.17 cd	429.75 b
0.05 (C2)	405.00 d	470.00 a-d	434.83 a-d	416.83 cd	431.67 b
0.09 (C3)	465.67 a-d	491.17 abc	493.67 abc	513.17 a	490.92 a
Average	432.62	454.16	430.08	464.71	

Note: Numbers followed by different lowercase letters at the column and row are significant according to DMRT at $p < 0.05$

Table 3. Number of *V. unguiculata* var. *sesquipedalis* pods given colchicine mutagen

Percentage colchicine (%)	Soaking time (hour)				Average
	6 (T1)	**12 (T2)**	**18 (T3)**	**24 (T4)**	
Without colchicine (C0)	29.50 cd	29.00 cd	27.33 d	27.83 d	28.42 b
0.01 (C1)	29.83 cd	37.00 ab	34.17 bc	32.00 bcd	33.25 a
0.05 (C2)	29.33 cd	32.00 bcd	37.00 ab	37.17 ab	33.88 a
0.09 (C3)	30.67 cd	34.33 bc	34.67 bc	41.17 a	35.21 a
Average	29.83 b	33.08 ab	33.29 a	34.54 a	

Note: Numbers followed by different lowercase letters at the column and row are significant according to DMRT at $p < 0.05$

Table 4. Pod length (cm) of *V. unguiculata* var. *sesquipedalis* given colchicine mutagen

Percentage colchicine (%)	Soaking time (hour)				Average
	6 (T1)	**12 (T2)**	**18 (T3)**	**24 (T4)**	
Without colchicine (C0)	35.37 g	37.22 fg	38.28 def	37.96 efg	37.20 b
0.01 (C1)	41.33 abc	41.70 abc	39.51 c-f	40.54 b-e	40.77 a
0.05 (C2)	38.33 def	40.57 b-e	41.50 abc	43.33 a	40.93 a
0.09 (C3)	39.13 c-f	41.64 abc	40.74 a-d	43.25 ab	41.19 a
Average	38.54 b	40.28 ab	40.00 ab	41.27 a	

Note: Numbers followed by different lowercase letters at the column and row are significant according to DMRT at $p < 0.05$

Table 5. The weight of 100 seeds (g) of *V. unguiculata* var. *sesquipedalis* given colchicine mutagen

Percentage colchicine (%)	Soaking time (hour)				Average
	6 (T1)	**12 (T2)**	**18 (T3)**	**24 (T4)**	
Without colchicine (C0)	58.76 g	59.74 f	61.91 cd	59.85 f	60.06 c
0.01 (C1)	61.00 de	62.85 c	59.86 f	62.08 c	61.09 b
0.05 (C2)	60.68 ef	60.68 ef	61.84 cd	61.83 cd	61.61 b
0.09 (C3)	66.93 b	66.83 b	67.07 b	68.11 a	67.23 a
Average	62.19	62.52	62.67	62.62	

Note: Numbers followed by different lowercase letters at the column and row are significant according to DMRT at $p < 0.05$

Chromosomal karyotype

The *V. unguiculata* var. *sesquipedalis* seed samples used in the karyotype analysis of chromosomes were derived from 0.09% colchicine treatment and 24-hour soaking time. Based on the character morphological observations, a greater difference in the growth and yield of baby beans was discovered by comparing the control with the 0.01% and 0.05% treatments. Therefore, to obtain more accurate data, three seeds were taken from each of the biological samples, which were germinated, and the karyotype was analyzed. The observations on the field of view plate under the microscope showed that all test samples had a ploidy degree of 2n = 22 (diploid), as presented in Table 6. Furthermore, based on the test results, the number of chromosomes in the control *V. unguiculata* var. *sesquipedalis* samples (C1, C2, C3) and those treated with 0.09% colchicine and 24 hours soaking time (T1, T2, and T3) showed diploid status as illustrated in Figure 2.

Comparing karyotype analysis on biological samples between 3 seeds in the control sample (C1-C3) and 0.09% colchicine treatment and 24-hour seed soaking (T1-T3) yielded 11 karyotypes of chromosomes with a ploidy of 2n = 22 chromosomes. There was no polyploid in samples treated with colchicine. Previous studies using colchicine mutants can increase ploidy (Gantait et al. 2011). Whereas that did not occur in ajowan (*Trachysper mumammi* L.), which showed no increase in the number of chromosomes after increased concentration and the soaking time (Noori et al. 2017). The *Stevia rebaudiana* plant also shows an increase in the level of chromosome ploidy (Zhang et al. 2018).

Although the number of chromosomes did not experience polyploidy, structurally, the karyotype had changed. Table 6 shows two types of *V. unguiculata* var. *sesquipedalis* chromosomes based on the location of the centromere, namely metacentric and telocentric. The results of measuring the length of the chromosome arms and karyotype show that there are chromosomal formulas in all the different test samples. In all test samples, it is known that there are variations in the shape of the chromosomes. The chromosomal formula in all test samples is as follows:

Treatment of concentration and soaking time of colchicine induction of all test samples can change the length of the chromosome karyotype arms. However, there were differences in size and shape, namely the length of the short arm (p) and the long arm (q), as shown in Figure 3. There were also differences in the chromosomes' karyotypes in the C1-C3 control and T1-T3 colchicine treatment samples, but the number of chromosome pairs was 11 sets. A chromosomal karyotype can be seen and counted at the prometaphase stage of mitotic division. At this stage, jerky movements of the chromosomes occur, causing the condensed chromosomes to disperse into the cytoplasm (Ganies et al. 2019).

The analysis of control samples results and 0.09% colchicine treatment on the karyotype of the *V. unguiculata* var. *sesquipedalis* chromosome were presented in Figures 4 and 5. It was discovered that there were differences in karyotype with the arm length (p+q). Furthermore, the average value of each karyotype of the same size and the length of the arm was longer in the sample treated with 0.09% colchicine. This difference in length affected the phenotype, as shown in Tables 1-5.

The results of the T-test statistic in Figure 6 showed that the average length of the chromosomal arms of the 0.09% colchicine treatment sample was 1.77 μm longer than 1.53 μm the control sample. Therefore, a -7.246 was obtained in the control sample based on the T-test analysis data. This was lower than the colchicine treatment sample, with a value of p (T<=t) two tails, namely 3.868×10^{-7}, which was $p < 0.05$. Therefore, the appropriation of 0.09% colchicine treatment to the *V. unguiculata* var. *sesquipedalis* seeds increased the length of the chromosomes and affected the antitypic properties.

When there is a difference in the chromosomes' size and arrangement, the genes' location will be affected at the molecular level. Differences at the level of chromosome structure can have a major effect on the phenotypes expressed (Ganies et al. 2019). Based on this research, the chromosomal structure was different between the control sample and the 0.09% colchicine treatment. This was indicated in the difference in growth and production as found in the criteria: height, the number of pods, pod length, and weight, and the weight of 100 seeds in Tables 1-5. Variations influenced the differences in phenotypic characters in the arrangement of genes on chromosomes, which can express different phenotypic characters between samples, as Orgogozo et al. (2015) reported. The exposure of explants to colchicine at the doses used rose to aneuploid plants (monosomic and trisomic). These showed different morphological characteristics from the wild genotypes in anthurium plants (Lopez et al. 2022).

Table 6. The number and shape of the *V. unguiculata* var. *sesquipedalis* chromosomes in the control (C1-C3) and treatment samples of 0.09% colchicine treatment and the soaking time of *V. unguiculata* var. *sesquipedalis* seeds for 24 hours (T1-T3)

Samples	Formula of chromosome	Shape of chromosome
C1	2n = 2x = 22	20M + 2 T
C2	2n = 2x = 22	22 M
C3	2n = 2x = 22	22 M
T1	2n = 2x = 22	22 M
T2	2n = 2x = 22	20 M + 2 T
T3	2n = 2x = 22	22 M

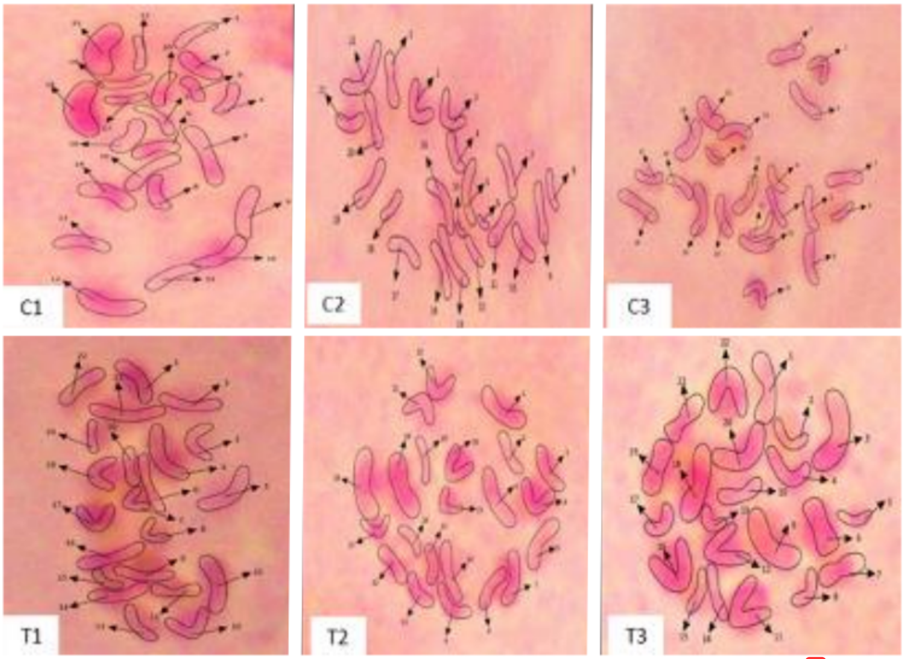

Figure 2. The number of chromosomes in the control sample (C1-C3), the 0.09% colchicine treatment, and the soaking time of *V. unguiculata* var. *sesquipedalis* seeds for 24 hours (T1-T3)

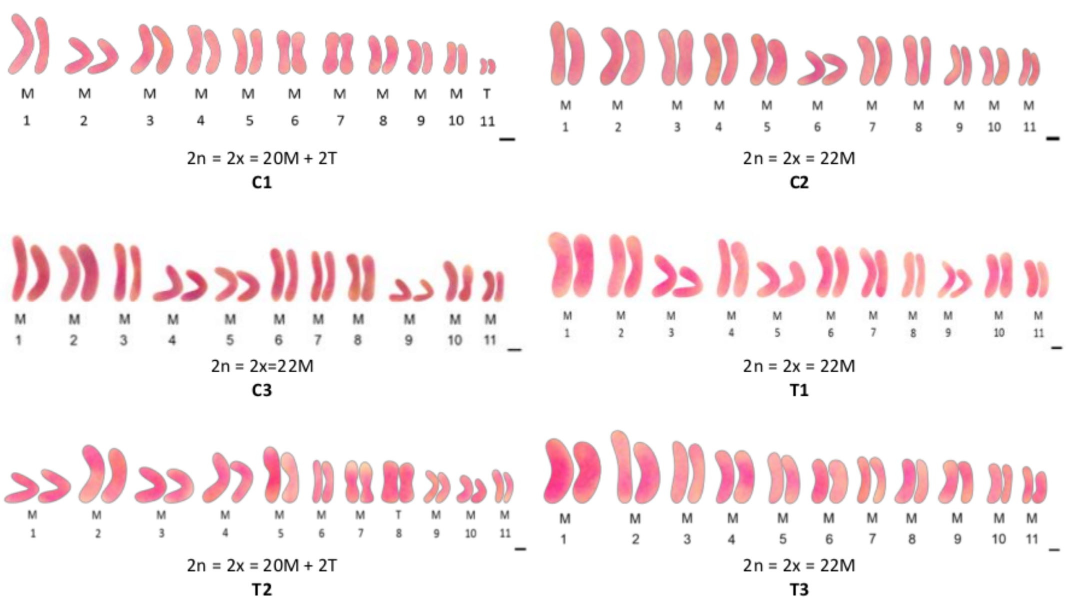

Figure 3. Karyotype of *V. unguiculata* var. *sesquipedalis* chromosomes in control samples (C1-C3) and samples treated with 0.09% colchicine and 24-hour seed soaking (T1-T3). Bar = 1 μm

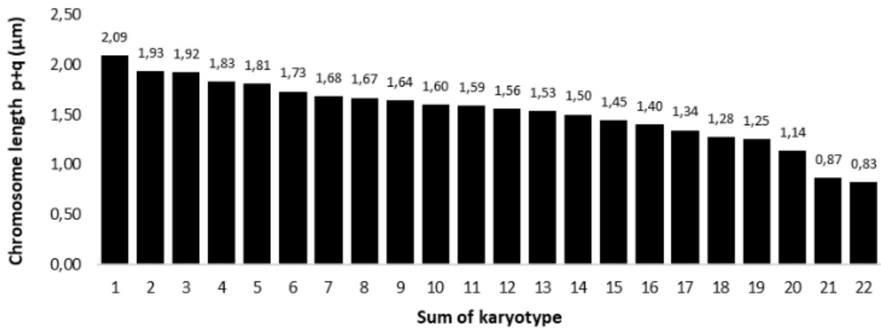

Figure 4. Short-arm (p) and long-arm (q) karyotype lengths in the control sample. Notes: T is the error bar at $p < 0.05$

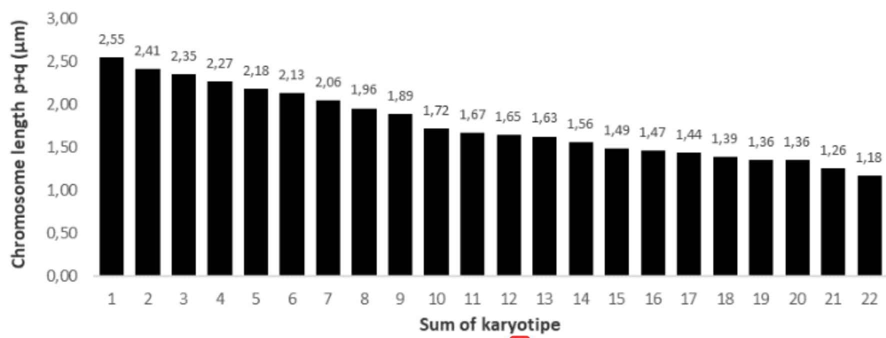

Figure 5. The length of the short arm (p) and long arm (q) karyotype in the 0.09% colchicine treatment sample and 24 hours of soaking time. Note: T is the error bar at $p < 0.05$

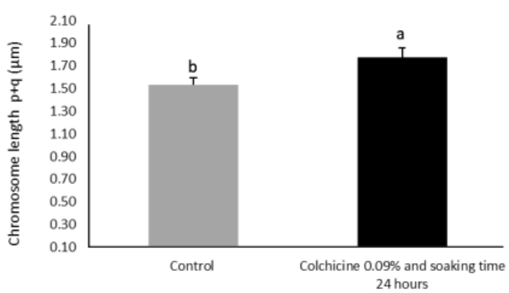

Figure 6. The average length of the karyotype arm of the control sample and the 0.09% colchicine treatment. Note: T is the error bar at $p < 0.05$

In further research, it is necessary to optimize the concentration of colchicine, which can be increased by 1.5-2 and 2-2.5 times the initial soaking time. During the plant growth stage, colchicine can be administered in the vegetative phase by spraying or wiping the solution on the shoots at the apical ends of leaves and stems after soaking the root tips.

In conclusion, the results of colchicine administration and soaking time showed that the treatment could increase the phenotyping in *V. unguiculata* var. *sesquipedalis* with a significant effect on the parameters of plant height, pod weight, and length, as well as the number of pods, and weight of 100 seeds. Furthermore, colchicine did not cause polyploidy; it made the chromosomes' karyotype longer than the control. This indicated gene DNA duplication and changes in the structure of the chromosomes in the sample.

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