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Mutation induction in the pineapple (*Ananas comosus* L. Merr) using colchicine

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Abstract. Pineapple is a tropical fruit that has high economic value. Mutation is a method to increase plant diversity which plays an essential role in plant improvement. This study aimed to induce mutations in pineapple using colchicine. This study was arranged in a factorial completely randomized design. The first factor was pineapple genotypes (i.e., Gemilang, Bangka, Queen, and Suska Kualu) and the second factor was colchicine concentration (i.e., 0.03%, 0.04%, 0.05%, and control). There were 16 treatments with five repetitions so the total was 80 experimental units. Observations were made for three months after treatment in the vegetative phase. Observation parameters included leaf shape, leaf color, plant height, number of leaves, leaf width, and length of stomata. This study indicated that the interaction between genotype and colchicine significantly differed in plant height and the number of leaves. Colchicine significantly increased the length of stomata, and genotype significantly affected all observed parameters. This study concluded that 0.05% colchicine significantly increased plant height (26.67%) and the number of leaves (48.98%) in the Gemilang genotype but decreased plant height and leaf number in other genotypes. This study suggests the need for observation of the flowering phase and fruit quality due to colchicine treatment.

1. Background

Pineapple (*Ananas comosus* L. Merr.) is one of the tropical fruits with high economic value after bananas. One of the pineapple-producing areas in Indonesia is Riau Province, where pineapples are cultivated in the peatlands [1]. Some of the problems in pineapple cultivation are the varying fruit size and spines on leaves. Fruit size is the primary standard determining the price of a variety in the market, while the spines make the cultivation process difficult. The ideal Idiotype of pineapple desired by farmers, the canned fruit industry, and consumers is a uniform size, good taste, and non-spines leaves.

The improvement of pineapple can be made through conventional breeding programs, crossing, or mutation induction. The limitation of conventional breeding in pineapples was because it was highly self-incompatible. The selfing of pineapple never produces seeds [2–4]. While crosses between different cultivars can produce seeds, the obstacle that arises is the difference in flowering age between cultivars. So that breeding through mutational biotechnology is the primary alternative for pineapple plants.

Currently, there are 2,252 varieties released and circulating in the world market derived from mutation breeding techniques [5,6] or 70% of all varieties [7]. Mutation induction would be done physically and chemically. The physical mutations were done using gamma rays, x-rays, beta, and



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neutrons. While chemical mutations can hold using EMS (*Ethyl methane sulphonate*) and *colchicine* [7,8]. The use of colchicine as a mutagen is prevalent today, such as the report on pear [9], garlic [10], tomato [11], and *Calendula officinalis* [12]. The advantage of mutation induction can produce varieties faster than conventional breeding [8], so that mutation induction played an essential role in pineapple diversity.

Colchicine is an alkaloid extracted from the seeds and tubers of the *Colchicum autumnal*. It is one of the mutagenic substances used in the polyploidization process. Colchicine works by inhibiting the formation of chromatin spindles during cell division, which causes chromosome doubling in these cells [13]. The increase of chromosome number causes the size of the cell and nucleus to increase. Giant cells produce more plant organs such as leaves, flowers, and fruit and can experience changes in plant morphological characters. Chromosomal doubling is also referred to as polyploidy. Polyploidy induction will impact larger fruit size because the number of chromosomes in the cell is double. Polyploid induction has been implemented on watermelon, soybeans, onions, tomatoes, and strawberries [10,14,15]. Another effect of polyploidy is to produce seedless fruits because there are unpaired chromosomes that cause seed production failure [15–17].

Istiqomah and Shofi [18] have reported the use of colchicine in pineapple plant mutations. Induction using 0.1% colchicine is the optimal concentration that can induce polyploidy explants in pineapple callus. Hannweg et al. [19] demonstrated some of the methods of induction of colchicine. The author concluded that the most effective way is splitting the tip of the pineapple plant and then applying colchicine. 500 ppm of colchicine concentration produced fruit larger than control plants [20]. Mujib [21] reported that colchicine treatment on callus resulted in 5% albino plantlets. The response of plants to colchicine mutation induction varies between plants and between genotypes induced. So far, there has been no report on how effective doses for mutation induction in a pineapple in some genotypes in peatland. In this study, mutation induction in pineapple plants will be done using various concentrations of colchicine.

2. Methodology

The research was carried out in February-Juni 2021 at the experimental field of the Faculty of Agriculture and Animal Husbandry, Sultan Syarif Kasim Riau. The plant materials used in this study were four pineapple genotypes, namely the Suska Kualu, Gemilang, Bubur, and Bangka, which were two months old. This study used a factorial completely randomized design, where the first factor was four pineapple genotypes and the second factor was four concentrations of colchicine (0.03%, 0.04%, 0.05%, and control (0%)). With a total of 16 treatments, each treatment was repeated five times so that there were 80 experimental units. The application of colchicine was carried out on the pineapple shoots according to the concentration of the treatment. Observation parameters include plant height, leaves number, leaf width, number of stomata, stomata length, and stomata width. Stomata analysis used Microscope Nikon Elepsi 50i Observations were made every month for three months. The data obtained were then analyzed by ANOVA using SAS version 9.1.

3. Results and discussion

3.1. Plant height and number of leaves

Genotype and the interaction between genotype and colchicine concentration differed significantly in plant height and number of leaves. The Bangka genotype had the highest plant height in the control treatment (80.77 cm), and the Gemilang genotype had the lowest plant height (55.64 cm) (Table 1). The colchicine treatment significantly reduced plant height in the three tested genotypes (Bangka, Bubur, and Suska Kualu). In contrast, the Gemilang genotype experienced an increase in the number of leaves due to the colchicine treatment. The percentage reduction in plant height was different for each genotype tested. The highly significant decrease in plant height due to colchicine treatment was found in the Bangka genotype at 0.04%, 14.40% lower than the control plant. Only 1.26% of the control plants showed a minor reduction in plant height in the Bubur genotype. Genotype Gemilang experienced an

increase in plant height due to colchicine treatment. The highest increase in plant height was 0.05% of colchicine (26.67%) compared to control plants (Figure 1A).

Table 1. Effect of colchicine concentration and pineapple genotype on the average plant height and number of pineapple leaves at five months of age (3 months after colchicine application).

Colchicine Concentration (%)	Genotype			
	Bangka	Bubur	Suska	Gemilang
-----Plant Hight (cm)-----				
0	80.77a	78.02b	61.10l	55.64p
0.03	71.20g	76.02d	58.66n	64.94k
0.04	69.14i	74.22e	57.10o	67.90j
0.05	73.10f	77.04c	59.12m	70.48h
----- Number of Leaves -----				
0	34.80ab	25.80de	34.80ab	27.00de
	28.40bcd			
0.03	e	26.80de	31.20bcd	34.00bc
0.04	27.80cde	28.00cde	31.60bcde	32.60bcd
0.05	27.80cde	27.00de	31.00bcde	40.20a

A decrease in plant height due to colchicine treatment was also reported by Cabahug et al. [9] in *Echeveria peerless* and Abello et al. [11] in tomato plants. The optimum colchicine concentration to increase plant height in tomatoes was 0.05%, and more than 0.05% will inhibit the plant growth (Abello et al. [11]). This date indicates that each genotype has a different response to mutation induction at various concentrations of colchicine.

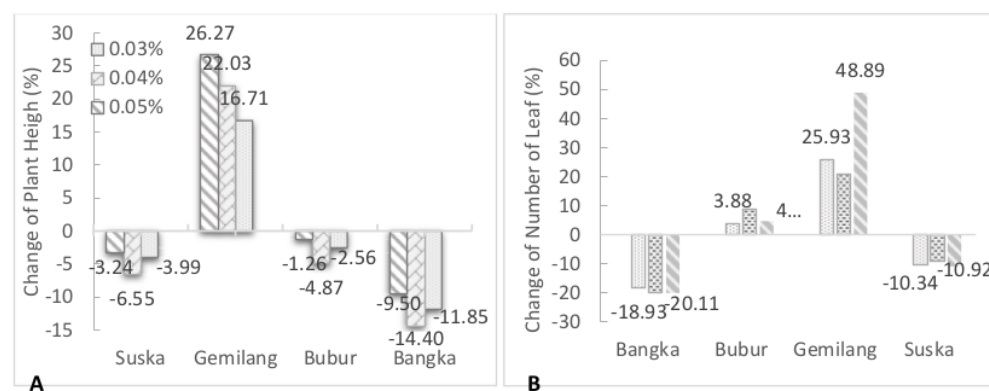


Figure 1. Graph of changes in plant height in four pineapple genotypes, due to colchicine treatment [A], and leaf number [B] in four pineapple genotypes in Riau peatlands due to colchicine treatment.

The number of leaves was influenced by the concentration of colchicine and the genotype used. The number of leaves significantly decreased in the Bangka and Suska Kualu genotypes but increased in the Bubur and Gemilang genotypes. Gemilang genotype has the highest number of leaves at 0.05% colchicine treatment, 48.89% or an increase of 13 leaves compared to control plants. In contrast, the most significant decrease in leaf number occurred in the Bangka genotype, namely 20.11% at 0.04% and 0.05% colchicine. From the plant height and number of leaves, it can be seen that pineapples with 0.05% colchicine treatment increased plant height by 26.67% and the number of leaves by 48.89% (Figure 1).

Colchicine concentration of 0.05% changes morphology in grapes which produced chimera leaves and tetraploid plants [22], increased leaf width and plant height in chrysanthemums [23], changes in shape and size of chrysanthemums, the size of *Stevia* [24]. Furthermore, Azizan et al. [24] reported that mutation induction occurred at a concentration of 0.2%, while lower concentrations could not induce mutations in *Stevia rebaudiana*. The right concentration will significantly determine the success of colchicine mutation induction, and some reports state that an inappropriate concentration can inhibit plant growth [25].

3.2. Width of leaves, number of stomata, and stomata width

The genotype used had a significant effect on leaf width, the number of stomata, and stomata width. Bubur genotype had the largest leaf size, namely 8.01 cm. In contrast, Gemilang was the genotype with the lowest leaf width (5.95 cm) which was significantly different from the Bangka and Suska Kualu genotypes

Table 2. Effect of genotype on leaf width, number of stomata, and stomata width of pineapple plants at five months of age (three months after colchicine application).

Parameter	Genotype			
	Bangka	Bubur	Suska Kualu	Gemilang
Leaf width (cm)	6.96b	8.01a	6.53b	5.95c
Number of stomata	114.00bc	104.75c	118.20ab	126.85a
Stomata width (μm)	209.71a	205.31a	209.73a	192.47b

The highest number of stomata was found in the Gemilang genotype, 126.85 stomata/field of view, while Bubur was the genotype with the least number of stomata, namely 104.75 stomata/field of view (Table 2). The smallest stomata width was obtained in the Gemilang genotype, which was 192.47 μm, which was significantly different from the other three genotypes. Gemilang has a relatively high number of stomata but has a smaller size. This study was in line with those reported by Cabahuq et al. [9] that found colchicine mutation induction decreases the number of stomata but increases the size of the stomata. In contrast, Abello et al. [11] obtained colchicine treatment that increased the number and density of stomata. Azizan et al. [24] found an increased number of stomata in stevia at a colchicine concentration of 1.5%-2.0%. This study used a concentration of 0.05% colchicine; it may be deficient, so colchicine applied was not able to increase the number of stomata as a response to colchicine treatment. Istiqomah and Shofi [18] support this result; the colchicine concentration of 0.1% can induce mutations in pineapple plants.

3.3. Stomata length

The colchicine concentration of 0.05% influenced stomata length, as well as increased stomatal size up to 5% greater than control plants (Figure 2). Identification of diversity in plants can be seen from changes in stomata [26,27]. The number and size of stomata have been widely reported to indicate polyploid plants due to colchicine treatment [9,28]. Abello et al. [11] reported that 0.05% colchicine increased stomatal length in tomato plants. In addition, 0.05% colchicine treatment resulted in tetraploid plants in grapes. The increase in stomata size is closely related to polyploid induction; cell size can occur due to the diploidization process, which causes organ growth to become more prominent [29,30].

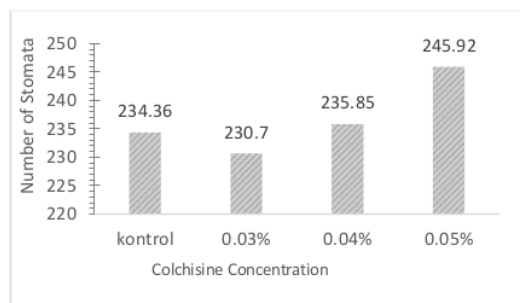


Figure 2. Graph of stomata length in pineapple plants at various concentrations of colchicine.

4. Conclusion

Based on the result of this study, it is concluded that an interaction between genotypes and colchicine concentration changes in pineapple morphology. Different genotypes responded differently to the given concentration of colchicine. The concentration of 0.05% colchicine increased plant height, the number of leaves, and increased stomata length in the Gemilang genotype. Since observations were carried out in the vegetative phase, this study suggested observing the level of diversity and changes in the morphology of the fruit and fruit quality.

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