RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (6) 2467-2477, 2025
http://doi.org/10.54910/sabrao2025.57.6.20
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

EVALUATING COLCHICINE-INDUCED MUTATION IN PINEAPPLE (*ANANAS COMOSUS* L.) BASED ON MORPHOLOGY

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SUMMARY

In plant breeding, mutation is one of the plant improvement strategies through the induction of genetic diversity. This study sought to evaluate the use of colchicine to induce mutations based on morphological characters in pineapple (*Ananas comosus* L.). The study employed a completely randomized factorial design, with the first factor comprising four pineapple genotypes and the second factor consisting of three concentrations of colchicine (300, 400, and 500 ppm) and a control. The results showed a colchicine concentration of 500 ppm changed the flesh color to golden yellow in the Q02 genotype. Interaction in 11 characters between genotypes and colchicine concentrations occurred. These characters included plant height, the number of leaves and crown leaves, leaf length, crown height and weight, stem diameter, fruit stalk and fruit diameter, fruit weight with and without crown, and edible part (%). This study concluded that colchicine 500 ppm produced golden-yellow flesh color and significant variations in vegetative characters. Therefore, it highly recommends colchicine concentrations of more than 500 ppm can be beneficial for improving pineapple plants.

Keywords: Pineapple (*A. comosus* L.), genotypes, colchicine concentrations, mutation, morphological traits, flesh color

Key findings: Colchicine of 500 ppm produced a golden yellow flesh color in the Q02 genotype and significant changes in vegetative characters of pineapple (*A. comosus* L.). It is superior to use colchicine concentrations of more than 500 ppm for future pineapple improvement.

Communicating Editor: Dr. Thiyagu Bin Devarajan

Manuscript received: May 29, 2025; Accepted: September 06, 2025.

Citation: Rosmaina, Aryanti E, and Zulfahmi (2025). Evaluating colchicine-induced mutation in pineapple (*Ananas comosus* L.) based on morphology. *SABRAO J. Breed. Genet.* 57(6): 2467-2477. http://doi.org/10.54910/sabrao2025.57.6.20.

INTRODUCTION

Pineapple (*Ananas comosus* L. Merr) is a tropical fruit with a broad market potential. Demand for pineapple increases in various countries through the consumption of fresh fruit, processed products, and derivative products such as juice and jam. Many tropical countries export pineapples to countries with climates that do not support pineapple growth, such as China, Japan, Singapore, the United States, Russia, and the United Arab Emirates (ITC, 2024). Pineapple is a favored fruit because it has a good taste and high nutritional value, being in great demand by the public. Indonesia is one of the world's largest pineapple-producing countries, with the highest pineapple production in Indonesia achieved on submarginal lands, such as peatlands (Rosmaina *et al.,* 2021a). This means a pineapple can grow and thrive even in unfavorable conditions. The more diverse and demanding consumers request continuous improvement in the quality of horticultural products, such as sweeter taste, more attractive color, non-itchy pineapple, and high nutritional content. It requires breeders to continue innovating to produce new varieties that are more desirable to consumers (Li *et al.*, 2022).

Genetic improvement of pineapple can succeed through conventional breeding, including crosses. However, pineapple has the problem of high self-incompatibility, making crosses within the same genotype unable to produce seeds. Crosses between genotypes can be applicable, but it requires a long time and necessitates planting arrangements to ensure flowers appear together, as they have different harvest ages (Hadiati *et al.,* 2011; Rosmaina *et al.,* 2021b). Mutation can improve plant characteristics in breeding, with 2,252 varieties derived from mutation breeding techniques currently released and circulating worldwide (Kharkwal, 2023), or 70% of all varieties (Wattoo *et al.,* 2024).

Mutation is one of the widely used techniques in plant breeding to increase diversity in the context of crop improvement. Several advantages of mutation breeding can improve specific characteristics without sacrificing agronomic traits (Ali and Suryakant, 2024). It is universal, allowing it to apply to all types of plants to increase diversity and resistance to global climate change. Additionally, it can take place at various stages of growth, making it not limited to the plant's age. Mutation technology is also usually a sustainable, flexible, and proven method to increase yields for both quality and quantity, as well as enhance resistance to biotic and abiotic stresses, including drought, salinity, and diseases. Mutation breeding is essential in pineapple plants because they have a highly self-incompatible nature and a vegetative propagation system that limits conventional breeding.

Colchicine is one of the mutagens widely used to induce diversity in many cultivated plants. Colchicine is an antimitotic agent that interferes with chromosome segregation during cell division by inhibiting spindle-thread formation during mitosis. This leads to failure of chromosome segregation and the generation of new cells with multiple sets of chromosomes, known as polyploidy (Kara and Yazar, 2022; Mangena and Mushadu, 2023), causing changes in the number of chromosomes impacting alterations in plant morphology (Rosmaina *et al.,* 2021; Cabahug *et al.,* 2022; Udofia *et al.,* 2024). Polyploid plants often show morphological changes compared with the original plant, such as increased leaf size, thicker, more succulent leaves (Zhu *et al.,* 2021), larger stomatal size (Baby *et al.,* 2023), and color changes in flowers and fruits (Peña-Morán *et al.,* 2022; Aisyah *et al.,* 2024). Polyploidy induction is valuable for breeding new plant varieties with desirable traits (Zou *et al.,* 2025).

The use of colchicine to induce mutation in pineapple plants has reports from Mujib (2005) through callus culture techniques, with the addition of 0.01% colchicine resulting in the production of several variants and albino plants. Tongpukerepri and Changjeraja (2012) reported that applying 0.10% colchicine increased the total sugar content in the Tatawei variety of pineapple. Plant improvement using colchicine is highly effective. It has been helpful in many crops, as reported on pear (Cabahug *et al.,* 2022), tomato (Hailu *et al.,* 2021), *Calendula officinalis* (Yassein *et al.,* 2021), and shallot (Zulfahmi *et al.,* 2022, 2024). This research sought to evaluate the use of colchicine as a mutagen to change the morphology of pineapple plants. The primary objective of this research is to develop pineapple genotypes that exhibit enhanced fruit quality, characterized by a golden-yellow flesh, increased sweetness, and reduced water content, resulting in a crispier texture. Mutation induction will facilitate the expression of these desirable traits.

MATERIALS AND METHODS

This research commenced in the Experimental Field and Genetics and Breeding Laboratory, Faculty of Agriculture and Animal Science, Sultan Syarif Kasim State Islamic University, Riau, from July 2022 to October 2023. It used a factorial completely randomized design, where the first factor was four pineapple genotypes—the Queen cultivar from Kampar (Q01), the Queen cultivar from Tembilahan (Q02), and the Smooth Cayenne from Tembilahan (SC01) and (SC02). The four genotypes used came from Pekanbaru since 2020. The second factor consisted of four concentrations of colchicine: 300, 400, and 500 ppm, and the control (0 ppm). Each plant received a total of 15 ml, administered in three 5-ml doses at weekly intervals, resulting in a total of 15 ml per plant throughout the study. There were 16 treatments, each repeated five times, with 80 experimental units. Pineapple seedlings measuring 40–50 cm entailed planting in 30 cm × 30 cm polybags. The soil used was a combination of peat soil and manure with a ratio of 4:1. Furthermore, fertilization took place three times with an interval of three months, i.e., at the ages of three, six, and nine months, each with an application of 20 g/plant. Colchicine application proceeded when the plants were three months old by dripping colchicine at the growing point according to the concentration. The treatment, as administered three times, had an interval of one week between each dose.

Observations continued on plants induced by colchicine mutagenesis (M0). The observation parameters included 12 qualitative characters (leaf, flower petal, and flower crown color; fruit shape; fruit color after ripening; presence of fruit spots; fruit eye profile, eye surface, flesh color, and stalk color; and fruit crown shape). The study also assessed 22 quantitative characters. These comprised plant height, leaf length and width, the number of leaves and stem buds, stem diameter, fruit weight with crown, fruit weight without crown, and fruit stalk diameter, length, and diameter. Other traits are eye depth, core diameter, crown height and weight, the number of crown leaves, and edible parts (%). Nutritional contents included vitamin C, total titratable acid (TTA), total dissolved solids (TDS), and TDS/TTA ratio. Observations proceeded following the Descriptors for Pineapple (IBPGR, 1991).

Data analysis ensued by ANOVA (analysis of variance) using the SAS program version 9.1. When the treatment showed a significant difference, further testing continued with the LSD (least significant difference) at the 5% level.

RESULTS AND DISCUSSION

Qualitative characters

The impact of the mutation induction of colchicine concentrations (300–500 ppm) on four pineapple genotypes is evident in the qualitative and quantitative characters. From the 12 qualitative characters observed, only the flesh color on the Queen Genotype (Q02) with 500 ppm colchicine gave different results, namely, bright yellow (Figure 1), in contrast with the control, which has a white fruit flesh color. The remaining 11 qualitative traits remained unchanged following colchicine treatments (therefore, no data presentation occurred). One of the qualities of the pineapple fruit is dependent on the attractive color of the fruit flesh, which is yellowish to yellow-orange (Nashima *et al.,* 2022). Consumers prefer yellow flesh because it links to sweetness and

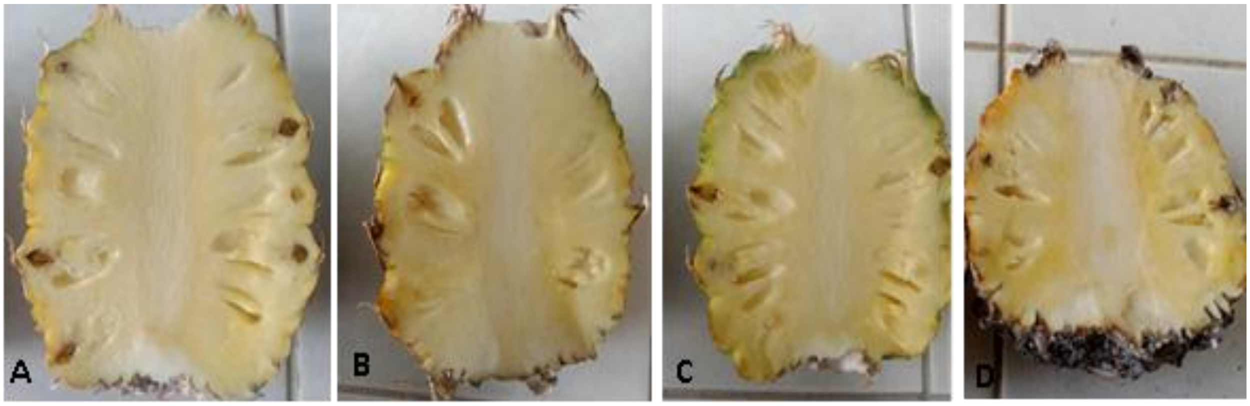

Figure 1. Fruit flesh color of the genotype cultivar Queen from Tembilahan (Q02): (a) control plant, (b) 300 ppm colchicine, (c) 400 ppm colchicine, and (d) 500 ppm colchicine.

high carotenoid content, improving taste and nutritional value, and positively correlates with total soluble solids (Zhao *et al.*, 2022; Kinsman *et al.,* 2024). In contrast, Japan has a popular cultivar, "Yugafu," which has a white flesh color and lower carotenoid content, which is still preferred because it has a milder taste (Nashima *et al.,* 2022). An increase in the yellow color of the fruit is indicative of the rise in carotenoids, which act as photosynthetic pigments and precursors of bioactive compounds such as abscisic acid (Moreno and Al-Babili, 2023). Colchicine is a mutagen that induces polyploidy, which can affect the accumulation of pigments, such as anthocyanins and carotenoids, thereby changing the color of golden berry, tomato, and strawberry fruits (Çömlekçioğlu and Özden, 2020; Xu *et al.,* 2023). The results of this study revealed that colchicine also influences the color of pineapple pulp, which has an association with the accumulation of yellow pigments (carotenoids).

Quantitative characters

Changes due to mutation are also noteworthy in quantitative characters; 11 qualities incurred significant effects from colchicine (Table 1). These characters are vegetative, namely, plant height, the number of leaves, leaf length, stem diameter, crown weight, the number of crown leaves, crown height, and fruit stalk diameter. Production characteristics, such as fruit weight, diameter, and length, had no significant effect. These results indicated that colchicine, given in general, only affects changes in growth characters of the four genotypes used. The effect of colchicine showed high influences from the concentration and genotype used. Some genotypes experienced an increase, but in other genotypes, the opposite was true (Figure 2). This result confirms previous studies where colchicine can alter morphology and plant growth, both inhibitory and stimulatory effects, depending on the concentration and genotype used (Singh *et al.,* 2020; Kushwah *et al.,* 2021; Rosmaina *et al.,* 2021b; Boonyawiwat *et al.,* 2023; Wang *et al.,* 2024).

Plant height, the number of leaves, and leaf length

An interaction between genotypes and colchicine concentration existed, affecting plant height, leaf length, and the number of leaves. Smooth Cayenne SC01 significantly decreased plant height at all colchicine concentrations; in contrast, SC02 plant height was only notably different at 300-ppm colchicine, while other treatments did not differ from the control (Table 1). The decrease in plant height in SC01 reached 14.76%, while SC02 was only 7.54% (Figure 2). In the Queen cultivar of the Q02 genotype, colchicine application increased plant

Table 1. Effect of colchicine, genotype, and interaction of both on plant height, the number of leaves, leaf length, stem diameter, crown height, the number of crown leaves, crown weight, fruit diameter, fruit weight with crown, fruit weight without crown, and edible part.

Character	Genotype	Colchicine Concentration (ppm)				Mean
		0	300	400	500	
Plant height (cm)	SC01	90.85	78.30	80.08	80.08	82.37
	SC02	69.00	63.80	72.00	69.00	68.45
	Q01	65.80	59.42	61.93	71.68	64.70
	Q02	64.60	64.20	68.25	70.62	66.92
	Mean	72.56	66.43	70.56	72.84	
	$LSD_{0.05}$ Genotype: 36.28, Colchicine: 13.62, Interaction: 13.14					
Number of leaves (blade)	SC01	30.52	28.94	33.10	34.00	31.64
	SC02	37.80	33.20	42.40	33.40	36.70
	Q01	49.40	45.60	53.25	51.00	49.81
	Q02	37.60	38.00	42.40	44.25	40.56
	Mean	38.83	36.43	42.78	40.66	
	$LSD_{0.05}$ Genotype: 34.65, Colchicine: 12.86, Interaction: 7.26					
Leaf length (cm)	SC01	88.26	75.70	78.72	76.44	79.78
	SC02	66.40	61.50	69.40	67.00	66.08
	Q01	50.64	48.42	49.68	52.05	50.20
	Q02	63.25	62.86	66.50	69.00	65.40
	Mean	67.14	62.12	66.08	66.13	
	$LSD_{0.05}$ Genotype: 54.40, Colchicine: 10.06, Interaction: 10.93					
Stem diameter (cm)	SC01	7.86	7.78	7.94	7.73	7.83
	SC02	7.30	7.80	8.17	8.05	7.83
	Q01	6.79	6.64	7.56	6.50	6.87
	Q02	8.02	7.90	8.23	7.93	8.02
	Mean	7.49	7.53	7.97	7.55	
	$LSD_{0.05}$ Genotype: 2.32, Colchicine: 1.03, Interaction: 0.83					
Crown height (cm)	SC01	27.50	29.67	31.05	28.20	29.10
	SC02	22.70	24.50	16.05	18.65	20.47
	Q01	24.47	29.58	26.43	32.35	28.20
	Q02	14.78	20.56	15.35	26.15	19.21
	Mean	22.36	26.07	22.22	26.33	
	$LSD_{0.05}$ Genotype: 23.08, Colchicine: 10.19, Interaction: 11.22					
Number of crowns leaves	SC01	108.80	118.00	86.55	93.87	101.68
	SC02	80.50	52.00	44.00	60.00	59.12
	Q01	90.60	93.40	94.25	104.55	95.68
	Q02	95.00	103.80	109.25	96.50	101.13
	Mean	93.06	91.80	83.51	88.71	
	$LSD_{0.05}$ Genotype: 91.68, Colchicine: 19.89, Interaction: 39.13					
Crown weight (g)	SC01	329.40	406.90	467.80	334.70	384.70
	SC02	155.43	145.39	82.53	146.68	132.50
	Q01	224.98	220.94	236.12	234.56	229.15
	Q02	171.84	185.70	195.55	212.75	191.46
	Mean	220.41	239.73	245.50	232.17	
	$LSD_{0.05}$ Genotype: 485.04, Colchicine: 48.77, Interaction: 134.84					
Fruit diameter (cm)	SC01	11.60	11.04	11.20	11.02	11.21
	SC02	6.90	7.43	8.37	7.51	7.54
	Q01	12.34	12.50	12.67	13.75	12.82
	Q02	8.88	10.21	8.84	7.77	8.92
	Mean	9.93	10.29	10.27	10.10	
	$LSD_{0.05}$ Genotype: 10.54, Colchicine: 0.83, Interaction: 2.41					

Table 1. (cont'd).

Character	Genotype	Colchicine Concentration (ppm)				Mean
		0	300	400	500	
Fruit weight with crown (g)	SC01	1175.70	1123.40	1089.50	993.40	1095.50
	SC02	778.43	693.01	711.59	973.18	789.05
	Q01	692.30	1008.70	908.30	735.40	836.17
	Q02	729.50	874.60	1033.00	729.40	841.62
	Mean	843.98	924.92	935.59	857.85	
	$LSD_{0.05}$ Genotype: 623.94, Colchicine: 208.67, Interaction: 439.45					
Fruit weight without crown (g)	SC01	846.10	714.40	622.30	655.30	709.52
	SC02	623.00	564.75	629.09	826.15	660.74
	Q01	757.60	787.80	671.80	500.70	679.47
	Q02	558.10	688.70	837.30	516.00	650.05
	Mean	696.20	688.91	690.12	624.56	
	$LSD_{0.05}$ Genotype: 117.29, Colchicine: 151.86, Interaction: 436.28					
Edible Part (%)	SC01	64.42	54.75	51.53	58.19	57.22
	SC02	62.48	65.11	65.44	68.69	65.43
	Q01	65.73	66.90	62.41	59.11	63.53
	Q02	64.10	66.04	63.34	61.62	63.77
	Mean	64.18	63.2	60.68	61.90	
	$LSD_{0.05}$ Genotype: 16.26, Colchicine: 6.86, Interaction: 12.15					

height up to 9.32%, but in genotype Q01, the increase in plant height only occurred at 500-ppm colchicine, from 65.80 cm in the control to 71.68 cm, or an increase of 8.94%. Colchicine did not alter the number of leaves in SC01. However, in SC02, a significant decrease resulted in the application of 300 and 500 ppm colchicine by 12.17% and 11.64%, respectively, and a considerable increase in the number of leaves occurred at 400 ppm colchicine to 42.40 strands (Table 1), or an increase of 12.17% versus the control plants (Figure 2b). In the type of Q01, colchicine only significantly increased the number of leaves at 400 ppm, while in Q02, a considerable rise in the number of leaves appeared at all concentrations used (1.06%–17.69%); the magnitude of the increase in number bore strong influences from the concentration used. Leaf length in the SC01 genotype significantly decreased, but not in the SC02 genotype. In the Queen Q01 genotype, the length of the leaves did not differ between treatments. However, in the Q02 genotype, a remarkable boost emerged only at the 500-ppm colchicine, which amounted to 2.78% (Figure 2c). This increase was relatively low.

The same cultivar but different genotypes produces different responses to colchicine application. The increase or decrease in each character receives strong influences from the concentration of colchicine and the genotype used. This study showed the 500-ppm colchicine concentration was the minimum concentration to increase plant height and the number and length of leaves in pineapple. The effect of colchicine has high effects from the genotype in tomatoes (Udofia *et al.,* 2024). In contrast, the results of research by Yassein *et al.* (2021) stated the higher the colchicine concentration, the fewer plants are produced. Colchicine is an inhibitor of the mitotic cell division process, which means it has phytotoxic properties. These properties can inhibit plant growth (Peña-Morán *et al.,* 2022). Colchicine has various effects, with great effects coming from the plant's genotype and concentration (Zulfiqar *et al.,* 2022).

Crown height, weight, and leaves

There existed an interaction between the genotype and colchicine concentration on crown height, the number of crown leaves, and crown weight. Colchicine increased crown height and weight in three genotypes (SC01, Q01, and Q02); only in SC02 was there a decrease in crown height and weight. The magnitude of the increase in crown height and weight varied depending on the genotype and

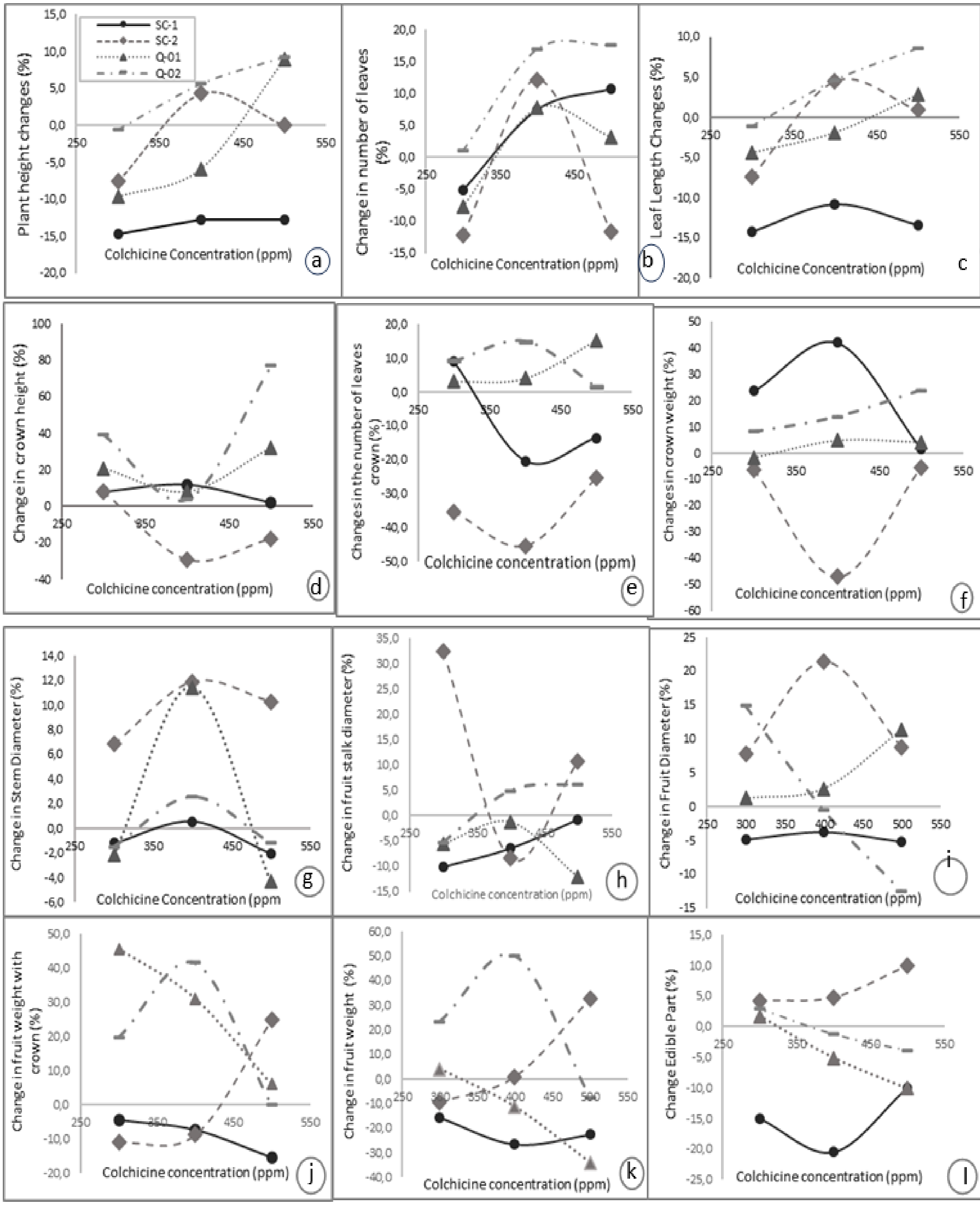

Figure 2. Percent variations in various characters are due to the colchicine mutation. a) plant height, b) leaf number, c) leaf length, d) crown height, e) changes in crown leaf number, f) crown weight, g) stem diameter, h) fruit stalk diameter, i) fruit diameter, j) fruit weight with crown, k) fruit weight, and l) edible part.

colchicine concentration used. The highest increase in crown height resulted in Q02, which reached 76.93% at 500 ppm (Figure 2d), while the maximum crown weight increase appeared in SC01, which amounted to 41.02% at 400 ppm colchicine (Figure 2f). The Queen cultivar significantly increased the number of crown leaves; the highest increase was 15.34% in Q02 with a dose of 500 ppm colchicine, while Q01 was at 400 ppm colchicine (Figure 2e). In contrast to the Queen genotype, Smooth Cayenne, especially SC02, experienced a decrease in the number of crown leaves, weight, and height due to colchicine application. These data indicate different genotypes give different responses to the application of colchicine. The crown is a collection of leaves found at the tip of the pineapple fruit. The crown's response to colchicine is the same as that of the leaves, where concentration and genotype are very influential. An increase in specific genotypes occurred, but in other genotypes, it causes a decrease in the trait. The effect of colchicine often changes the morphology of the leaves, both in shape and size (Seneviratne *et al.,* 2020; Zhu *et al.,* 2021).

Fruit stalk and stem diameters

There was an interaction between genotype and colchicine concentration on fruit stalk and stem diameters. The SQ01 genotype exhibited the highest increase in fruit stalk diameter (32.41% at 300 ppm and 10.67% at 500 ppm colchicine). In contrast, at 400 ppm colchicine, the stem diameter decreased by 8.31% compared with the control. An increase in stem diameter also occurred in Q02, while the other two genotypes, SQ02 and Q02, had a lower fruit stem diameter (Figure 2h). Colchicine significantly raised stem diameter in three genotypes (SC01, SC02, and Q02); the most significant rise occurred at a concentration of 400 ppm colchicine. The increase in stem diameter due to colchicine treatment could be due to colchicine's ability to induce the formation of larger cells (polyploid) in meristematic tissues. Just like other vegetative organs, fruit stalks and stem diameters' alterations also resulted from colchicine treatment. The magnitude of change gained strong influences from genotype and colchicine concentration (Boonyawiwat *et al.,* 2023; Zhu *et al.,* 2021). This causes an increase in cell volume and organ size, including stem diameter.

Some plants experienced growth inhibition when treated with colchicine. In gladiolus plants, a concentration of 0.6% significantly reduced bulb survival and induced chlorophyll mutants (Manzoor *et al.,* 2023). Similarly, concentrations above 30 mg/L in vetiver plants inhibited growth and caused explant death (Sinta and Widoretno, 2020). In this study, the increased response began to occur at a concentration of 400 ppm (0.04%), and qualitative character changes occurred at 500 ppm (0.05%), meaning the colchicine concentration for pineapple mutation needs to be increased. *Brassica juncea*'s effective concentration to increase growth and yield is 5% (Indriani *et al.,* 2024). In citrus, a report stated colchicine concentrations of less than 0.05% were ineffective in increasing cell division (Singh *et al.,* 2020). For tomato, colchicine ranging from 0.1 to 1.0 mM induced chromosomal anomalies and polyploidy, with a change in somatic chromosome number from 2n=24 to 2n=48 in treated cells, indicating successful mutation induction (Udofia *et al.,* 2024). In *Panicum miliaceum*, a 0.08% to 0.1% colchicine concentration was the most effective in inducing mutation modification (Zeinullina *et al.,* 2023). In gladiolus, concentrations of 0.17% to 0.34% were optimal for inducing mutations without significant toxicity (Manzoor *et al.,* 2023). However, in Japanese taro, concentrations higher than 0.3% caused a decrease in growth and increased mortality (Sudirman *et al.,* 2022). In citrus plants, a finding disclosed colchicine concentrations of less than 0.05% were ineffective in increasing cell division (Singh *et al.,* 2020). The results of this study indicate too low concentrations are also not effective in inducing mutations; conversely, too high concentrations cause cell death. Hence, it is essential to optimize the concentration of mutation induction in target plants because each genotype shows a different response (Boonyawiwat *et al.,* 2023).

Fruit weight and edible part

An interaction between genotype and colchicine concentration materialized on fruit weight with crown, fruit without crown, and the edible part. Colchicine caused an increase in fruit weight (with crown and without crown) in genotype Q02. Although there was an increase in fruit weight, the edible part decreased significantly. This means the increase in crown weight was higher than in fruit weight without the crown (Table 1). Colchicine in Q01 caused an upsurge in the weight of fruit with a crown, but the weight of fruit without a crown decreased by 33.91%. This indicates colchicine causes an increase in crown weight but hurts fruit weight (Figure 2k), resulting in a decrease in edible parts up to 10.07% (Figure 2l). The response was different in the SQ01 genotype, where colchicine caused a reduction in fruit weight both with and without crown and the edible part (Table 1). Meanwhile, in genotype SQ02, colchicine significantly boosted fruit weight and the edible part at 400 ppm concentration. These data showed the four genotypes used produced different responses. The results revealed the colchicine response varied among the various genotypes used. In Queen (Q01 and Q01), there was an increase in crown size but a decrease in fruit weight, whereas in Smooth Cayenne SQ02, colchicine raised fruit weight and the percent edible part. Research related to the mutation in pineapple using colchicine is limited. The results of previous studies report that colchicine does not directly increase fruit weight and edible parts (Tongpukerepri and Changjeraja, 2012). However, Istiqomah and Shofi (2018) found that there is potential for improving pineapple fruit quality through colchicine mutation, with increased sugar content.

CONCLUSIONS

The study concluded that colchicine at 500 ppm produced golden yellow flesh color in genotype Q02 and significant changes in vegetative characters. It is superior to use colchicine concentrations of more than 500 ppm for future pineapple improvement.

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