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Abdullah Rahman Zain
Doctoral Program Students, Tadulako University, Palu, Indonesia

Mahfudz Zainuddin Basri
Lecturer at the Faculty of Agriculture, Tadulako University, Palu, Indonesia

Corresponding Author:
Abdullah Rahman Zain
Doctoral Program Students, Tadulako University, Palu, Indonesia

Induction of polyploids in cayenne pepper (*Capsicum frutescens* L.) through various concentrations of colchicine

Abdullah Rahman Zain and Mahfudz Zainuddin Basri

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Abstract
Polyploidy was declared as an attempt to obtain a new variety with the hope of increasing the quality of diploid (2n) plants by changing the chromosome arrangement to become tetraploid (4n) using the 2n chili seed soaking method for 30 days at a concentration of colchicine, namely K0 = 0.0% (control); K1 = 0.1%; K2 = 0.2%; and K3 = 0.3%. Data from the analysis of variance (ANOVA) stated that there was a very significant effect (**) on all observed variables. The results of the analysis on the variable percentage of seed germination showed that the treatment with a concentration of 0.0% was 92.20% with the number of chromosomes 24 (2n) and 0.1% as much as 26.20% with the number of chromosomes 48 (4n), while the increase in colchicine concentration (0.2% to 0.3%) has a lethal effect so that no chili seeds germinate (0.00). The average concentration of 0.1% treatment was higher with a large stem diameter, a large number of branches, a small number of leaves, more chlorophyll content with a dark green dun color, a larger number of stomata, a smaller leaf area, shorter flowering, more number of stomata, smaller leaf area, age shorter flowering, more glossy fruit color, shorter physiological maturity, longer fruit stalk size, longer fruit size, larger fruit diameter, heavier fruit, a higher number of harvested fruits, a heavier harvested fruit, number of seeds per fruit, and the weight per 100 seeds is heavier when compared to the control treatment (K0 = 0.0), which is very different.

Keywords: Cayenne pepper, polyploid, tetraploid (4n), colchicine

Introduction
Cayenne pepper (*Capsicum frutescens* L.) is one of the horticultural commodities needed by the community as a flavor enhancer for various types of food menus (Murni, 2010) [43]. The distinctively spicy taste of cayenne pepper is caused by the presence of capsaicin (C18H27NO3) contained in the fruit (Silvia *et al*., 2016) [35]. Differences in capsaicin content are often used as a differentiator between types of characters in cayenne pepper plants (Colney *et al*., 2018) [5].

Cayenne pepper productivity has been increased in various ways, including through fertilization (Hapsoh *at al*., 2017) [11] pest and disease control (Herison *et al*., 2014) [13], mechanization (Setyanto *et al*., 2020) [33], irrigation (Zaitun *et al*., 2021) [42], and the use of superior cayenne pepper varieties (seeds) (Andriani *et al*., 2020) [2].

The intensification program using superior cayenne pepper seeds is the first step to increasing production (Murni, 2010) [43]. According to Effendi *et al*. (2018) [6], efforts to improve productivity through the use of superior cayenne pepper seeds require several stages of breeding, including expanding genetic diversity, analyzing character inheritance, and selecting and releasing varieties. Superior cayenne pepper seeds to date are generally produced through breeding activities by crossing cayenne pepper elders who have superior characters so that hybrids are obtained that carry a combination of superior characters from the two crossed elders (Herison *et al*., 2014) [13]. In addition, superior cayenne pepper seeds can also be obtained through genetic material engineering, such as by carrying out polyploid operations, either triploid (Bakhtiar *et al*., 2002) [4] or tetraploid (Lelanga and Seran, 2020) [17].

Polyploidy in plants can occur naturally or artificially. Natural polyploids occur through exposure to lightning in plants or through *allogamy* (crossing) between similar plants, whereas artificial polyploids can be carried out through electrical shock or exposure to mutagens, particularly antimitotic compounds such as colchicine and oryzalins (Sukamto *et al*., 2010) [38].

Colchicine is a mutagen that is often used for polyploid purposes in plants. The effectiveness of colchicine in manipulating the number of chromosomes is largely determined by the concentration and method of exposure to colchicine and the part or type of plant used. The concentration of colchicine that has a polyploid effect on plants varies between 0.01% to 1% (Suminah *et al*., 2002) [39], while the methods of exposure to colchicine can be through soaking, dipping, dripping, greasing, injecting, and spraying on seeds, sprouts, stems, plantlets, or flowers (Mahyuni *et al*., 2015) [19].

The application of colchicine to diploid plants, that have two sets of chromosomes (2n), can lead to polyploidy, such as the production of tetrapolyd plants, that have four sets of chromosomes (4n). Murni (2010) [43], stated that the polyploid effect can be observed in morphological, anatomical, physiological, and cytological aspects; as shown by changes in the roots, stems, leaves, flowers, fruit, and composition of proteins, carbohydrates, and vitamins in plants (Sofia, 2008) [37].

This study aims to examine the effect of colchicine concentration on polyploid induction in cayenne pepper plants.

Materials and Methods

This research will be conducted in Lasoani Sub-District, Mantikolore District, Palu City, Central Sulawesi Province, Indonesia (Fig 1) at a geographical position of 0054'15" South Latitude and 119054'1" East Longitude with an altitude of ±135 m above sea level (Habbachi, 2022) [9]. This research will take place from August to December 2022.

The tools used were digital scales, analytical balances, a light microscope (1000x magnification), a UV VIS spectrophotometer (ME-UV1100), centrifuges, Eppendorf tubes, glass cuvettes, refrigerators, Petri dishes, spatulas, porcelain cups, vials, tweezers, pipettes, object glass, deglass, a razor, a hotplate, a measuring cup (100 ml, 500 ml, and 1000 ml), a thermometer, a tray (5.5 cm x 3.5 cm x 2.0 cm), scissors, hands prayer, and stationery.

The materials used are colchicine, cayenne pepper seeds (diploid or 2n) (sakagen trademark), cayenne pepper leaves, carnoy solution, HCl solution, Acetocarmine, 96% ethanol, 45% acetic acid, nail polish, masking tape, soil (top soil), rice husk, chicken manure, NPK Mutiara fertilizer, Demolish 18 EC pesticide, polybag (30 cm x 30 cm), label, tissue, and distilled water.

This study used a single-factor Completely Randomized Design (CRD), with the experimental treatment being colchicine concentration. There are four concentration levels of colchicine (K), (Fig 2) tested, namely:

K0 = Control (without colchicine)
K1 = Concentration of 0.1% colchicine (b/v)
K2 = Concentration of 0.2% colchicine (b/v)
K3 = Concentration of 0.3% colchicine (b/v)

Each treatment was repeated five times, so that there were 20 experimental units. Each experimental unit used 25 cayenne pepper seeds; thus, the total number of seeds used was 500 seeds.

The data obtained from this study will be analyzed using analysis of variance (ANOVA). The results of the analysis of variance that show a significant or very significant effect will be tested further using the Honest Significant Difference Test (5% HSD) to compare the average values of all treatments tried (Nuryadi *et al*. 2017) [24].

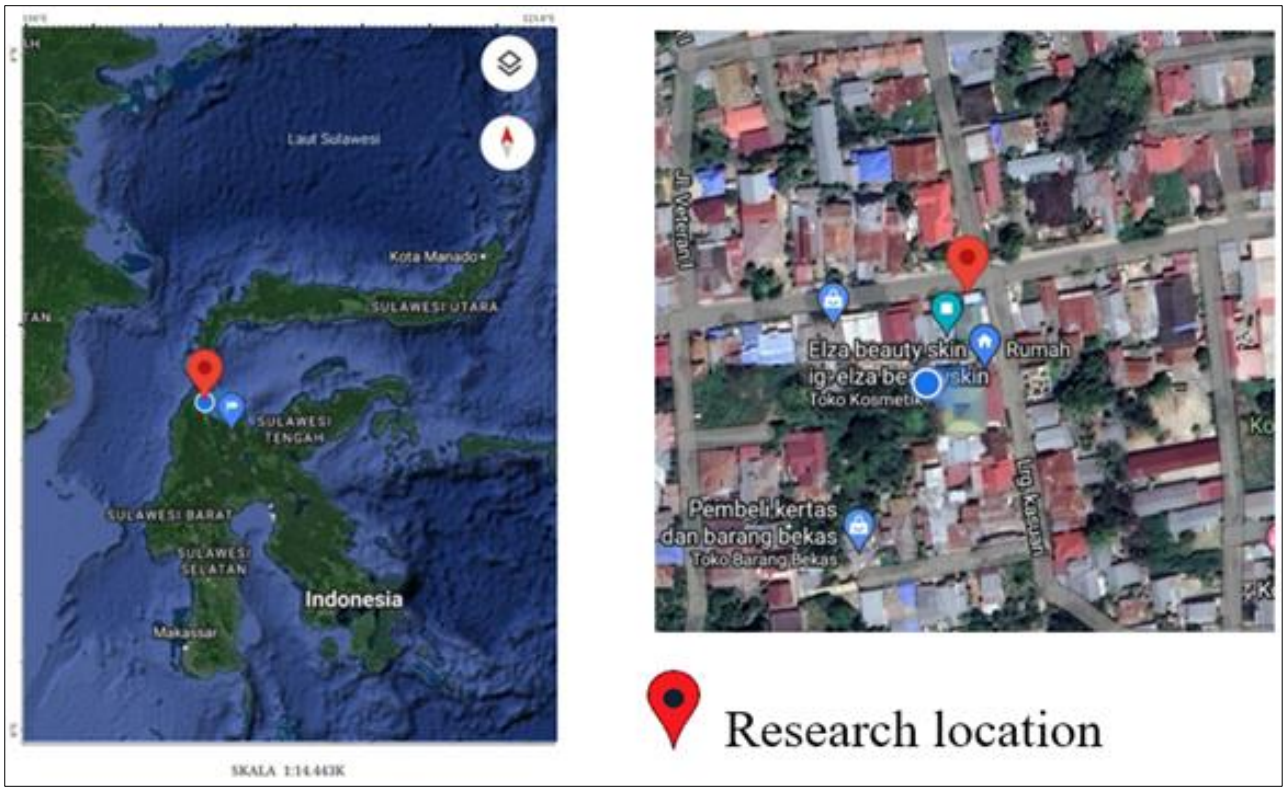


Fig 1: Research Location, Palu City, Central Sulawesi, Indonesia

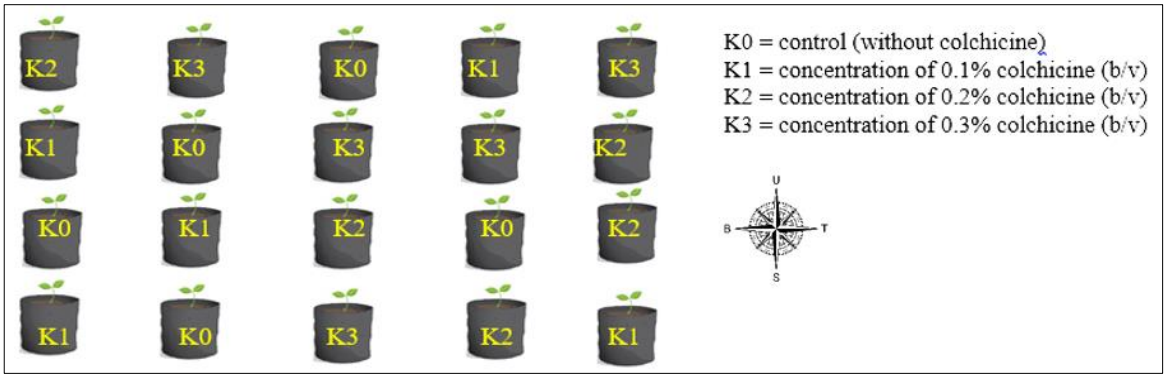


Fig 2: Layout Plan of the Experimental Unit.

Results

The results of the analysis of varian (ANOVA) in the treatment of colchicine concentrations from various observational variables are shown in Table 1.

Table 1: Results of the Analysis of Variance (ANOVA) in the treatment of colchicine concentrations from various observational variables

Variable	Observation	Colchicine Concentration (%)			
		K0 = 0,0	K1 = 0,1	K2 = 0,2	K3 = 0,3
Germination percentage	30 Day	**			
Plant heigh	4 WAP				
	8 WAP	**			
	12 WAP				
Stem diameter	4 WAP				
	8 WAP	**			
	12 WAP				
Number of branches	4 WAP				
	8 WAP	**			
	12 WAP				
Number of leaves	Strands	**			
Leaf chlorophyll content	Plant 1				
	Plant 2	**			
	Plant 3				
Number of stomata	Plant 1				
	Plant 2	**			
	Plant 3				
Leaf area	2nd leaf	**			
	3nd leaf				
Flowering age	Day	**			
Age of physiologically ripe fruit	Day	**			
Length of fruit stalk	Ripe fruit	**			
Fruit length	Ripe fruit	**			
Fruit diameter	Ripe fruit	**			
Fruit weight	Ripe fruit	**			
Number of harvested fruit	Ripe fruit	**			
Weight of harvested fruit	Ripe fruit	**			
Number of seed per fruit	Ripe fruit	**			
Weight per 100 seeds	Ripe fruit	**			

Note: ** = Very real; WAP = weeks after planting.

Discussion

Effect of Colchicine Concentration on Polyploid Induction in Cayenne Pepper Plants

Colchicine is an antimitotic substance that is used in manipulating genetic material, especially in engineering at the chromosomal level. According to Rochmat *et al.* (2017) [30], administration of antimitotic compounds can cause mutations in all parts of the plant, from the growing point to the generative organs. The administration of colchicine concentrations showed differences in morphology that could be directly observed during the vegetative phase when compared to diploid plants (Aili *et al*., 2016) [1]. Based on the results of the analysis of diversity, it was stated that the treatment of cayenne pepper seeds, which were soaked at concentrations of 0.0% colchicine, 0.1%, 0.2%, and 0.3% for 30 days, had a very significant effect on all observed variables. This is in accordance with the opinion of Gultom (2016) [8], namely that giving the right concentration will result in a change in the vegetative character. The higher the concentration given in polyploid induction, the more cells are exposed and damaged, which can cause morphological changes in the endoplasmic reticulum and Golgi bodies so that cell development becomes abnormal and takes time to grow back to normal (Langhans *et al*., 2009) [16].

Plants obtained from 0.1% coccine immersion showed a tetraploid effect with a doubling of the number of

chromosomes and an increase in coccine concentration to 0.2% and 0.3%, causing inhibition (a lethal effect) on cayenne pepper seed germination. According to Sartika *et al.* (2020) [32], the higher the concentration of colchicine, the higher the percentage of tetraploid cells, but the higher the percentage of sprout death. Data analysis of the percentage of seeds germinating stated that the 0.0% concentration treatment averaged 92.20% more than the 0.1% treatment, which only averaged 26.20%, while the increase in colchicine concentration (0.2% to 0.3%) gives a lethal effect so that no cayenne pepper seeds germinate "0.00" (Fig 3). According to Murni (2010) [43], high concentrations of colchicine and long soaking times can reduce the ability of seeds to germinate, this is due to the toxicity contained in them, causing cell death. According to Miguel and Leonhardt (2011) [21], effective polyploid induction is a treatment that produces a lot of polyploidy but does not kill plant cells.

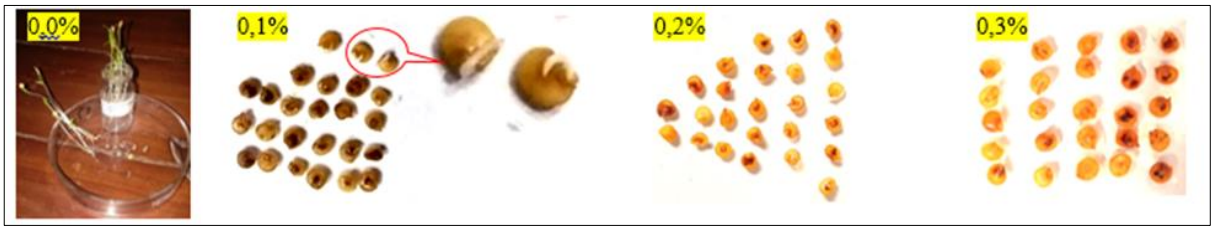


Fig 3: Germinated seeds of chili plants from various concentrations of colchicine

Nofitahesti and Daryono (2016) [23], reported that soybean seeds (g*lycine max* (L.) Merr.) at colchicine concentrations of 0.025% - 0.05% - 0.075% - 0.1% - 0.15% - 0.2%, and 0.25% with soaking times of 6-8-12-16-18 and 24 hours failed to germinate, whereas in a study conducted by Murni (2010) [43] on curly chilli (*Capsicum annuum* L.) sprouts at a concentration of 0.05% for 24 hours stated that as many as 13% of the sprouts had dead root tips. From the results of this analysis, it can be seen that an increase in colchicine concentration and soaking time results in a decrease in the ability of seeds to germinate. This is in accordance with the statement of Omidbaigi *et al.* (2010) [25], concerning the toxicity of colchicine, which can cause lethal effects, namely reactions that occur when substances or chemicals interfere with cell or sub-cell processes in living things, which can result in death, so that further observations, including analysis of the number of chromosomes, were only carried out on treatments with 0.0% and 0.1% colchicine concentrations.

Saadah (2021) [31], stated that soaking seeds in concentrations of colchicine for a long time can cause the chromosome structure to agglomerate and shrink, which is caused by the absorption of too much colchicine fluid (*absorption*). The results of chromosome analysis (Fig 4), in the treatment with a concentration of 0.0%, it was found that there were 24 (2n) with short spindle thread forms, while in the 0.1% colchicine concentration treatment, there were 48 (4n) with long and wrinkled spindle shapes.

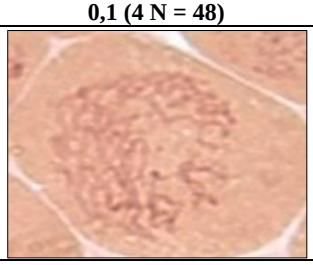
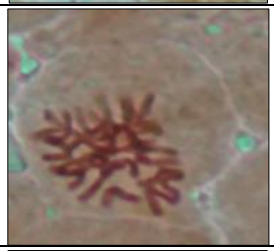
Test	Colchicine concentration (%)	
	0,0 (2 N = 24)	0,1 (4 N = 48)
1		
2		
3		

Fig 4: Shape and Number of Chromosomes Treatment 0.0% and Colchicine Concentration 0.1%. Cayenne pepper sprouts without colchicine treatment had a natural number of chromosomes (2n = 24) colchicine treatment at a concentration of 0.1% (K1) was able to cause a doubling of the number of chromosomes in chili peppers to 48 (4n = 48). Giving colchicine at higher concentrations causes lethal effects so that chromosome analysis in sprouts cannot be carried out

According to Omidbaigi *et al*. (2010) [25], polyploid engineering aims to produce plants with larger morphological sizes and that are resistant to the environment, so from plant height data from various treatments, the concentration of colchicine is 0.1% at 4 WAP, an average of 35.00 cm, 8 WAP, an average of 58.90 cm and 12 WAP, an average of 62.82 cm, higher than the 0% treatment at 4 WAP, an average of 22.20 cm; at 8 WAP, the average was 38.52 and at 12 WAP, the average was 57.90 cm. Zuyasna *et al*. (2023) [42], stated that the application of the right concentration of colchicine can have a positive impact on plant tissues and does not cause a decline in growth, resulting in tetraploid plants that are able to grow and develop faster than diploid plants.

Polyploid plants have large xylem and phloem transport bundles, so the transport of assimilation products becomes more numerous, which has an impact on the very fast growth and development of stem diameter, so tetraploid plants have a larger volume of wood compared to diploid plants (Zaitun *et al*., 2021) [41]. Based on stem diameter data, it was stated that in the 0.1% colchicine treatment, the average age of 4 WAP was 3.72 mm, 8 WAP averaged 4.66 mm and 12 WAP averaged 5.64 mm, has a larger stem size than in the 0.0% treatment, where the age of 4 WAP averaged 2.30 mm, 8 WAP averaged 3.28 mm and 12 WAP averaged 4.76 mm. While the data on the number of branches stated that the 0.1% colchicine treatment at 4 WAP averaged 0.60, 8 WAP averaged 1.40; and 12 WAP had an average of 2.00 while the 0.0% treatment at 4 WAP averaged 0.00, 8 WAP averaged 0.00 and 12 WAP averaged 0.80.

Leaves are the organ that determines the assimilation produced for the purposes of plant growth and development. Based on data on the number of leaves in the 0.0% concentration treatment, the average was 34.40, which was 34.40 more compared to the 0.1% colchicine concentration treatment, where the average was 28.00. This is in accordance with the opinion of Mahyuni *et al*. (2015) [19], which states that the application of colchicine can affect leaf formation, the higher the dose given, the fewer leaves are formed.

Polyploid plants contain more chlorophyll than diploid plants, this is indicated by the greener leaf color (Liu *et al*., 2011) [18]. Based on data on leaf chlorophyll content from the treatment of 0.1% colchicine concentration in plant 1, the average was 3.72 g/l, plant 2 averaged 4.66 g/l and plant 3 averaged 5.64 g/l, more than the 0.0% treatment, which in plant 1 averaged 2.30 g/l, plant 2 averaged 3.28 g/l and plant 3 averaged 4.76 g/l.

The success of colchicine in producing polyploid plants can be determined by observing the shape, size, and number of stomata, which have increased in number compared to diploid plants (Rocmat *et al*., 2017) [30]. Based on data on the number of stomata, the average 0.1% concentration of colchicine treatment was 98.00 more than the 0.0% treatment average of 69.33 (Fig 5). The existence of stomata is related to the plant's ability to adapt to the environment in terms of transpiration. The more number of stomata, the more parts of the leaf that produce Co2 for the photosynthesis process so that the more chlorophyll (green substance in leaves) is produced (Ere, 2018) [7].

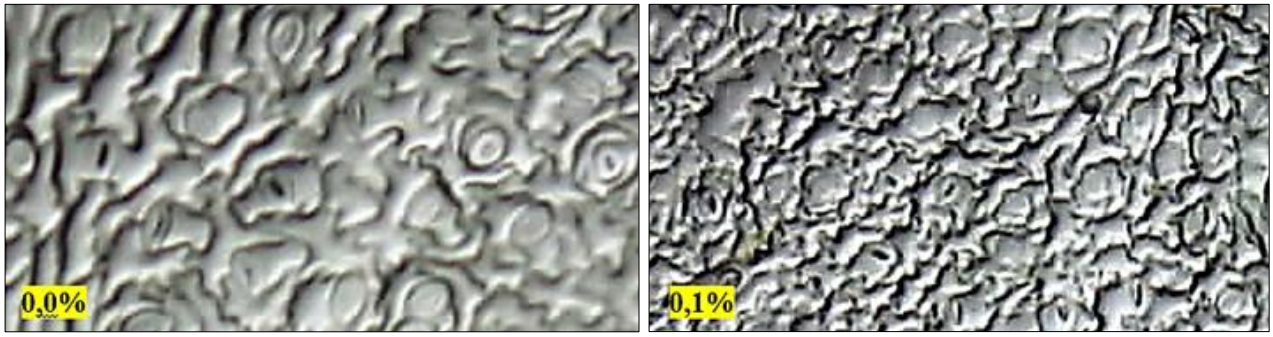


Fig 5: Observation of leaf structure with a magnification of 1000x at a concentration of 0.0%, the number of stomata is small, the shape looks large, the distance is far apart and the concentration of colchicine is 0.1%, the number of stomata is large, the shape looks small, the distance is close together

Maulana *et al*. (2021) [20], stated that polyploids from several types of plants have larger leaf sizes compared to diploid plants, but in this study, the concentration of colchicine on leaf area stated that the 0.0% treatment on the second leaf averaged 8.92 mm^2 and the 3rd leaf averaged 12.47 mm^2 wider than the 0.1% treatment, which had the 2nd leaf area an average of 5.97 mm^2 and the 3rd leaf had an average of 8.45 mm^2. This is in accordance with the statement of Sartika *et al*. (2020) [32], Mutations due to colchicine cause changes in shape, number, and leaf area compared to controls, with a greener color due to increased chlorophyll content (Fig 6).

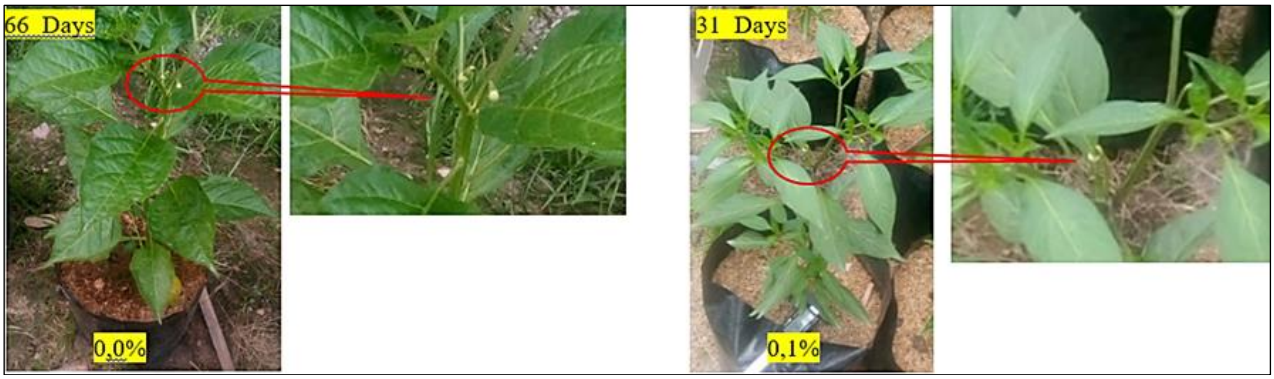


Fig 6: Plant Morphology at a concentration of 0.0%, has a large leaf area with a wavy surface, shiny green color, begins to flower at 66 days old and 0.1% colchicine concentration, has a smaller leaf area with a smooth surface, dark green color fades, starts flower at 31 days

Assess the effect of polyploidy (diploid [2n] and tetraploid [4n]) on the yield of cayenne pepper

According to Sa'adah (2021) [31], plants that experience changes in the arrangement of chromosomes will experience changes in chemical content, one of which is growth hormones such as auxins, cytokinins, and gibberellins, which can affect flowering rates. Syaifudin *et al.* (2013) [40], stated that polyploid plants have cells with large transport bundle sizes, causing the process of transporting assimilation products and water to run better at 0.1% on average, 36.60 days faster flowering than the 0.0% concentration treatment on average, 66.00 days longer (Fig 6).

Hasimi *et al*. (2016) [12], stated that the natural color change of fruit is influenced by ethylene gas, temperature, fruit cultivars, and chlorophyll content, which undergoes degradation so that carotenoid pigments emerge, which enzymatically appear little by little. Pigment groups contained in fruit, such as chlorophyll, carotenoids, and flavonoids (*Antocyanins and anthoxantins*), can affect fruit color, which is bright and fades (Pardede, 2013) [26]. Based on the results of visual observations of changes in fruit color (Fig 7) in the 0.0% treatment, when the ovary comes out of the calyx, it is light green; when it starts to develop,it is yellowish green; when it starts to ripen, it is faded yellowish red; and when it is ripe or ready to be picked, it is faded dark red. While for the treatment the concentration of colchicine is 0.1% when the ovary comes out of the dark green calyx, when it starts to develop it is shiny green, when it starts to ripen it is shiny dark orange, and when it is ripe or ready to be picked it is shiny dark red.

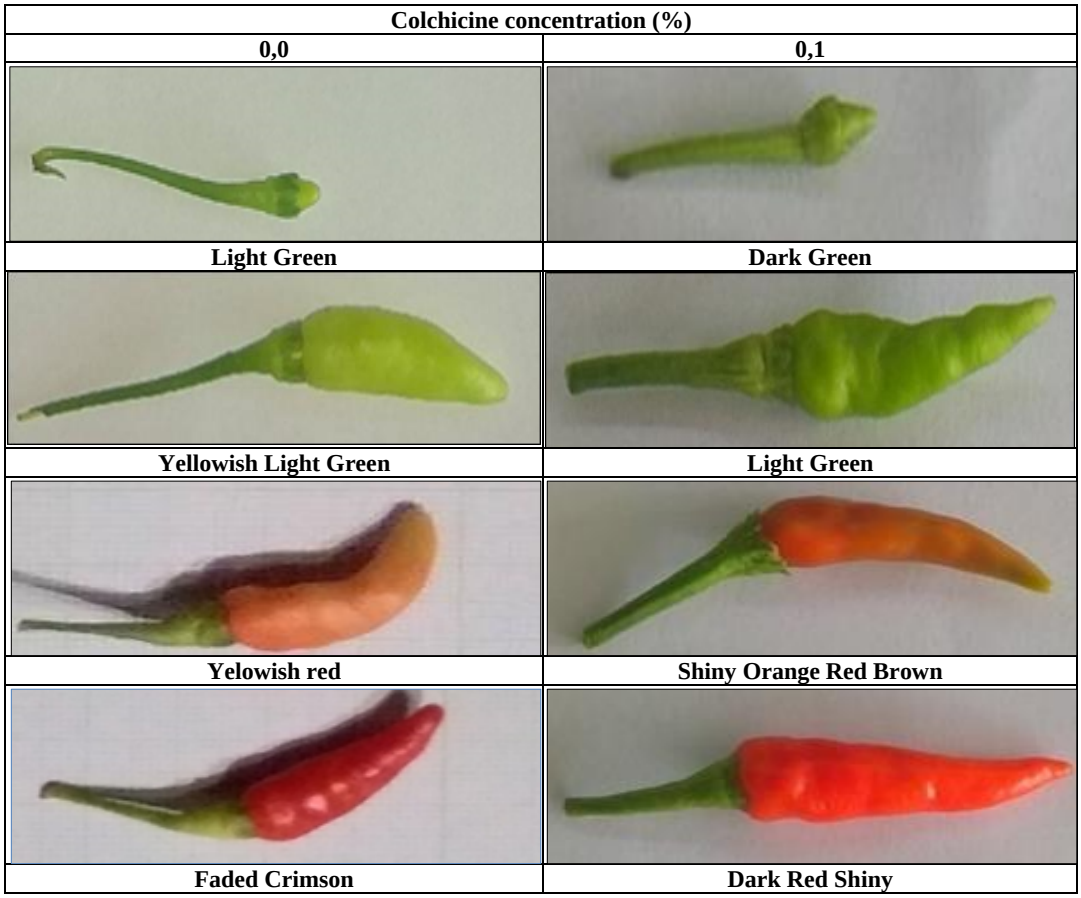


Fig 7: Observation results changes in fruit color from formation to physiological maturity at concentrations of 0.0% and 0.1% Colchicine

According to Handayani *et al*. (2017) [10], polyploid plants contain more secondary metabolites, a faster metabolism, and greater biomass, resulting in brighter fruit colors and faster physiological maturity. This is in accordance with physiologically ripe fruit age data in the 0.0% treatment, it takes an average of 45 days longer than the 0.1% colchicine concentration treatment, which takes an average of 35 days.
According to Ramadhani *et al*. (2013) [28], the morphological shape of the fruit in each variety varies, this is due to genetic factors, causing differences in traits or genotypes. The fruit morphology of polyploid plants can be used as an indicator of the success of colchicine application (Anggraito, 2004) [3]. Based on data on fruit stalk length in the 0.1% colchicine concentration treatment, the average was 26.43mm longer than the 0.0% treatment, which was an average of 21.91mm. Simonsvsks *et al*. (2016) [36], stated that the fruit stalk serves as a place for attachment and release of the fruit from the flower petals, besides that, the fruit stalk is closely related to fruit quality, and the short length of the fruit stalk will affect 36 velotyl compounds, which function as fruit aroma enhancers, and storage time.
According to Kosmiatin and Husni (2018) [15], fruit shape and size are influenced by the genotype of each variety as well as environmental factors that support fertilization. Data on fruit length in the 0.1% colchicine concentration treatment averaged 34.56mm longer compared to the 0.0% treatment with an average of 28.20mm (Fig 8). Data on fruit diameter in the 0.1% colchicine concentration treatment averaged 8.99 mm larger than the 0.0% treatment with an average of 8.75 mm (Fig 9). Fruit weight data in the 0.1% colchicine concentration treatment averaged 1.26 g heavier than the 0.0% treatment with an average of 0.66 g (Fig 10). Data on the number of fruits harvested in the treatment with 0.1% colchicine concentration averaged 37.00 more than the yield in the 0.0% treatment, with an average of 28.00. Harvest fruit weight data in the 0.1% colchicine concentration treatment averaged 38.35g heavier than the 0.0% treatment with an average of 20.94 g.

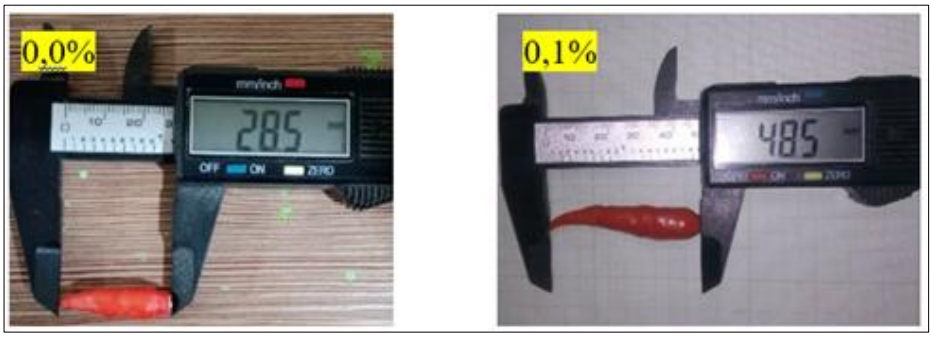


Fig 8: Length of cayenne pepper plant fruit from treatment with concentrations of 0.0% and 0.1% Colchicine

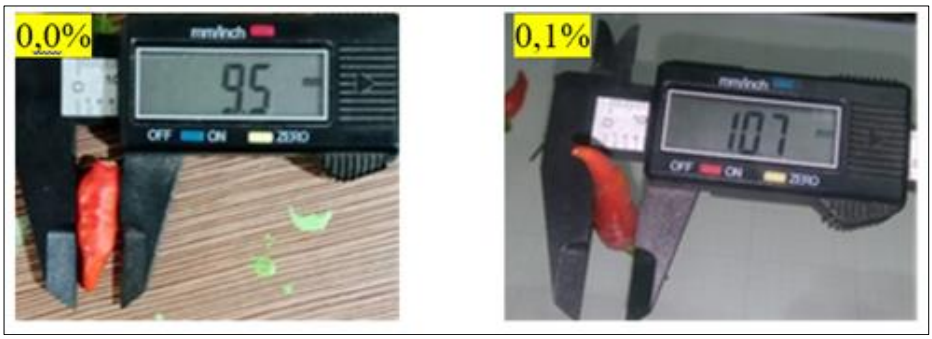


Fig 9: Fruit Diameter of cayenne pepper plants from treatment with 0.0% and 0.1% Colchicine Concentrations

Fig 10: Cayenne pepper plant fruit weight from treatment concentrations of 0.0% and 0.1% Colchicine

According to Rahajeng and Rahayuningsih (2013) [27], seed formation in tetraploid plants is influenced by two factors, namely genetic incompatibility and ineffective fertilization, so that it will produce fruit with a large or small number of seeds. Setyawan *et al*. (2018) [34], stated that pollination carried out at cultivation sites with an altitude of ±200 masl with an average temperature of 19-32 °C produced tetraploid fruit with a large number of seeds, while according to Riandoni *et al*. (2020) [29], if it was carried out at an altitude of ± 610 masl with a temperature average of 16-29 °C, it would produce tetraploid fruit with a small number of seeds. From the data on the number of seeds per pod, with the height of the study site ± 135 masl, the highest number of seeds was obtained on average at 29.73 in the 0.1% colchicine concentration treatment, while for the 0.0% treatment the average was 23.67. According to Iqbal *et al*. (2015) [14], the shape and size of seeds can affect seed viability in general. Large seed sizes have food reserves (*endosperm*) such as carbohydrates, proteins, fats, and minerals that function as energy for the embryo during germination, while small seeds and shrivels will experience difficulties during germination. Based on data on weight per 100 seeds, in the 0.1% colchicine concentration treatment, the average was 0.38g heavier than in the 0.0% treatment, where the average was 0.26g (Fig 11). According to Setyanto (2020) [33], after fertilization, the seeds will experience an increase in size and weight until they reach physiological maturity and then become lighter as the water content decreases due to the increased level of fruit maturity.

Fig 11: Weight of 100 cayenne pepper seeds from 0.0% and 0.1% colchicine concentration treatments

Conclusion

- Results of analysis of cayenne pepper seeds soaked in 0.0% colchicine concentration; 0.1%; 0.2% and 0.3% for 30 days indicated that all observation variables had a very significant effect.
- Cayenne pepper seeds that managed to germinate in the colchicine concentration treatment for 30 days of immersion, stated that the 0.0% treatment germinated as much as 92.20% and the 0.1% treatment as much as 26.20%, while the increase in colchicine concentration (0.2% to 0.3%) gives a lethal effect so that no chili seeds germinate "0.00".
- The results of chromosome analysis in the 0.0% treatment amounted to 24 = diploid (2n) with short spindle thread shapes, and the 0.1% colchicine concentration treatment totaled 48 = tetraploid (4n) with spindle thread shapes experiencing clumping and shrinkage caused by absorption of too much colchicine fluid (*absorption*).
- A greener leaf color has more chlorophyll content (0.1%) compared to a leaf that has control (0.0%). The number of stomata is more (0.1%) than in the control treatment (0.0%).
- The 0.1% colchicine concentration treatment had morphological forms, the plants were taller with a larger stem diameter than the control (0.0%), the number of branches formed was higher (0.1%) when compared to the control treatment (0, 0%), the number of leaves was higher (0.1%) than the control (0.0%), and the leaf area was smaller (0.1%) when compared to the control (0.0%), which is larger. The age of flowering treatment was 0.1% shorter with an average of 36,60 days compared to the control treatment (0.0%) with an average of 66.00.
- Based on fruit morphology, the 0.1% colchicine treatment had longer fruit stalks, longer fruit sizes, larger diameters, and had more fruit weight than the control (0.0%). The 0.0% treatment required a long time to achieve the criteria for physiological ripeness, while the fruit treated with a concentration of 0.1% colchicine required a short average time of 34.87 to meet the criteria for physiological maturity with a shiny red color.
- Based on data on the number of fruits harvested, the 0.1% treatment was more effective than the 0% treatment.
- Based on the results of the analysis, the average weight of the harvested fruit stated that the 0.1% treatment was heavier than the 0% treatment.
- The results of the calculation of the number of seeds in the 0.1% colchicine treated fruit had a higher number than the control treated fruit (0.0%).

- The weight per 100 seeds in the 0.1% colchicine treatment stated that the average was heavier than the control treatment (0.0%).

Acknowledgments
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