Effect of colchicine treatment on growth and yield traits of cauliflower (*Brassica oleraceae* var. *botrytis*) under Siquijor, Philippines condition

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ABSTRACT

Background and Objective: Cauliflower contains high levels of antioxidants and anticarcinogenic compounds. It is highly affected by extreme weather conditions in tropical countries resulting in hindrances to growth, immature fruit, and reduced yield. A study that has implications for polyploidy in cauliflower through colchicine induction is essential during such extreme weather to help farmers save their crops. The study investigated cauliflower seeds' germination and the morphological and yield responses of cauliflower affected by colchicine.

Methodology: The seeds were soaked in different concentrations for three hours: T_1 - control (untreated), T_2 - 0.05% colchicine, T_3 - 0.10% colchicine, T_4 - 0.20% colchicine, and T_5 - 0.50% colchicine, with three replications. They were laid out in a randomized complete block design (RCBD).

Main Results: Percentage germination ($P < 0.05$) was high in plants treated with 0.10% colchicine reaching 92.38%. Significant differences ($P < 0.05$) were observed in the morphological responses in plants treated with 0.10% colchicine with increased plant height (73.79 ± 0.36 cm) and number of leaves (27.34 ± 0.31 cm). Regarding leaf width, 0.20% colchicine application (23.64 ± 0.80 cm) was comparable to applying lower colchicine concentration (control, 0.05%, and 0.10%), but was significantly larger than the highest concentration (0.50%). The use of 0.10% colchicine showed promising results in above-ground fresh weight (1,839.58 ± 3.49 g), flower weight (777.08 ± 3.85 g), and inflorescence diameter (19.45 ± 0.65 cm). However, both 0.10% and 0.50% showed delays in the flower formation, with days to flower recorded at 61.46 ± 0.63 and 61.04 ± 0.46 days, respectively indicating a notable implication of polyploidy in plants.

Conclusions: This study showed that treatment with 0.10% colchicine had a significant positive effect, being the most effective treatment in improving cauliflower and increasing morphological and yield responses. These observed improvements encompass various implications associated with polyploidy, which are beneficial in places with extreme weather conditions like Siquijor, Philippines.

Keywords: Colchicine, cauliflower, plant growth, yield traits, flower weight

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INTRODUCTION

Cauliflower (*Brassica oleraceae* var. *botrytis*) is a functional vegetable that is known to contain high nutritional value because of its high levels of antioxidants and anticarcinogenic compounds, such as ascorbic acid (AA), carotenoids and glucosinolate, besides of vitamins and minerals (Gu *et al*., 2015). It has a high nutritional composition of water, sugar, protein, crude fiber, and fat. It contains compounds of high therapeutic value to fight cancers and illnesses and boost the immune system. The inflorescence is the edible part of the cauliflower, consisting of a shoot system with short internodes, branches apices, and bracts. The color of the inflorescence is usually white but can also be yellow, green, or purple because of the special combination of phytochemicals called carotenoids, tocopherols, and ascorbic acid (Belbase and Bc, 2020). These properties make it very attractive to the consumer, generating high demand and significant economic value.

The effects of climate change are already well visible by increasing air temperatures, increasing desertification, as well as by more frequent extreme weather events such as heat waves and droughts. Climate change is not globally uniform and affects some regions more than others. Climate change has already affected the province of Siquijor during the past 40 years. Plants are among the things that are highly affected by extreme weather conditions in tropical countries, resulting in hindrance in growth, unmatured fruit, and reduced yield. Often cauliflower gives severe negative responses to unfavorable environmental conditions like droughts that lead to economic reduction due to pre-mature inflorescence formation (Singh *et al*., 2013). Many researchers have used different methods to help plants cope with these problems. Mutation techniques have been used to derive many varieties of food crops, including cauliflower. These methods have proved useful in obtaining new traits, creating genetic variability, improving growth, increasing yield, and supplementing conventional breeding. This genetic variability is required for crop improvement (Aliero, 2006; Bolbhat *et al*., 2012) as variability existing in all organisms, including our crop plants, has been generated by mutation and subsequent recombination.

Colchicine inhibits mitosis in various plant and animal cells by interfering with the orientation and structure of the mitotic fibers and spindle (Finnie and Staden, 1991). Since microtubules drive chromosome segregation, colchicine is applied to interfere with mitosis and induce polyploidy and plant cell mutations (Essel *et al*., 2015). While polyploidy is fatal in animal cells, it is usually well tolerated in plant cells and mostly results in fruits and seeds that are larger, hardier, and faster growing, and more desirable, which can increase the yield and income of farmers (Finnie and Staden, 1991).

The ability of polyploid plants to establish themselves in a wide range of habitats and to survive in adverse environmental conditions makes them successful against their diploid ancestors due to the presence of additional alleles that increase their heterozygosity. A study that has implications on polyploidy in cauliflower is essential during this extreme weather to help farmers save their valuable crops. This is why this study induced cauliflower mutations using colchicine treatment under the Siquijor condition.

MATERIALS AND METHODS

Seed Preparation and Experimental Site

Cauliflower (*Brassica oleracea* var. *botrytis*) seeds were obtained from Ramgo International Corporation. Dry and quiescent cauliflower seeds were pre-soaked in distilled water for six hours at room temperature. The pre-soaked seeds were again soaked in the different concentrations of the colchicine for three hours. The colchicine-treated seeds were thoroughly washed under running tap water and immediately sowed in rows alongside controls (untreated). Transplanting of the seedling was done 30 days after sowing by hand pricking method on 22 cm high ridges to maintain row to a distance of 60 cm and plant to plant 60 cm with a deep of 2.25 cm to facilitate good root production. This was conducted in the greenhouse located in Barangay Caipilan, Siquijor, Siquijor (9°11’58” N, 123°29'9" E), and was carried out for three months from March 2021 to June 2021. The study has five treatments and three (replications). A total of fifteen plots were used with a gross plot size of 3.60 m × 1.20 m and plot-to-plot distance of 0.5 m.

Thinning, Weeding and Fertilizer Application

Thinning was carried out 15 days after transplanting to maintain adequate spacing and to promote optimal plant development. Any missing hills were replaced with thinned seedlings. Weeding was performed every three weeks using a bolo or by hand to ensure optimal cauliflower plant growth and minimize competition from other crops. The organic fertilizer in the form of vermicast was utilized with a full dose applied per hill at the rate of 120 grams per hill or 20 tons per hectare.

Morphological and Production Determinations

The experiment was terminated after three months. Each block was collected for stand count, and ten samples based on the colchicine dosage were used for the rest of the parameters to be gathered. The following parameters were collected in this study: %germination – was calculated as germinated samples over the total samples per treatment multiplied by 100; plant height (cm) – was recorded from the base just above the soil surface to the top of the plant; number of leaves – was counted every week in the whole plant; leaf length – was measured using a ruler from the tip of the longest leaf at one end of the leaf to the other end; leaf width – was measured using a ruler from the one side to the other side of the leaf; above ground flesh weight (g) – was measured using a digital balance right after the plants were removed from the soil, then washed off any loose soil and was wiped off gently with the use of a soft paper towel to remove any free surface moisture; inflorescence diameter (cm) – was measured and the diameter was calculated and recorded; flower weight (g) – was measured using a digital balance, and the average weight of the whole plant was calculated and recorded.

Statistical Analysis

This study was laid out using randomized complete block design (RCBD) with five treatments used: T_1 - control, T_2 - 0.05% colchicine, T_3 - 0.10% colchicine, T_4 - 0.20% colchicine, and T_5 - 0.50% colchicine. Each treatment was replicated three times. The data obtained with respect to all the characters were analyzed using analysis of variance, and further tests were done using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Seed Germination

The data shows that the germination percentage of treated seeds declined with an increase in the concentration of colchicine dosage used (Table 1). The 0.10% colchicine solution had the highest germination percentage at 92.38%. The lowest germination percentages were observed in the higher dosage at 0.20% and 0.50% colchicine solutions, with only 80.95% and 78.09% seeds germinating, respectively. This is because colchicine does not only have an effect on cell division but spreads through the cell, interfering with cellular mechanisms and causing toxicity at high concentrations.

Colchicine apparently impacts the viscosity of cytoplasm, so the cell cannot function normally (Kumar *et al*., 2020). It has been proved that when a high dose of colchicine is used as a mutation agent for plants, toxic contamination, phytotoxicity, and abnormality become the main cause of plant death. Similar observations were reported in cowpeas with the highest of 90.2% recorded in treatment at 0.05% comparable with the control garnered 89.3% and reduced germination percentages were observed with increasing concentrations of the mutagen (Essel *et al*., 2015). In the study of Kim *et al*. (2003), it was stated that the concentration of 0.01% and 0.05% colchicine treatments for 1 hour and 12 hours are suitable for obtaining the tetraploid in *Platycodon grandiflorum*.

Table 1 Effect of colchicine on the germination of cauliflower seeds

Treatments	%Germination
Control	89.52[a]
0.05%	88.57[ab]
0.10%	92.38[a]
0.20%	80.95[bc]
0.50%	78.09[c]

Note: Means not sharing common letters differ significantly according to Duncan's multiple range test at the 5% probability level.

Growth Parameters

In Table 2, it is evident that the plant treated with 0.10% colchicine exhibited significantly greater height ($P < 0.05$), measuring 73.79 ± 0.36 cm compared to the other treatments. Additionally, this treatment resulted in the development of more leaves with an average of 27.34 ± 0.31 leaves. Regarding leaf width, 0.20% colchicine application (23.64 ± 0.80 cm) was comparable to applying lower colchicine concentration (control, 0.05%, and 0.10%), but was significantly larger than the highest concentration (0.50%). The findings indicate that a 0.10% dosage of colchicine significantly promotes the growth of cauliflower.

Colchicine, an alkaloid, exerts its influence on microtubule organization, leading to the doubling of plant chromosomes and the creation of polyploid plants. A common trait observed in polyploid plants is a more robust stature, accompanied by an overall enlargement in various aspects of plant growth, including height, leaf size and number, flower, and yield (Ridwan and Witjaksono, 2020; Trojak-Goluch *et al*., 2021). In growth parameters, the result of this study corresponds with the research of Tammu *et al*. (2021) where a lower dosage of colchicine concentration has a more significant effect on plant height of Katokkon pepper than another high concentration dosage. It was also found on *Vicia cracca* that colchicine with several variations of concentration could cause an increase in plant height and performance. Abello *et al*. (2021) supported this result where plant height decreased as colchicine dosage was increased.

In terms of the number of leaves, the result of this study coincides with Kobayashi *et al*. (2008) where doubling through colchicine caused an increase in number of leaves, number of branches, plant height, and stem length in salvia (*Salvia coccinea* cv Coral Nymph). This was also in agreement with the study of Mastuti *et al*. (2022), where treated seeds produced more leaves than the untreated samples. This has the same result with *Chrysanthemum* seeds with colchicine in *in-vitro* cultivation which resulted in morphological characteristic variations and produced a better result than the untreated samples in terms of their number of leaves, number of nodes, root length, number of roots, and height of plantlet (Münzbergová, 2017). Wider leaves are one of the signs of a polyploid plant. The study results showed that the polyploid plants had gigantic characteristics, such as thicker, wider leaves with greater stomata size and larger flowers (Komariah *et al*., 2021). Colchicine-treated plants increased width at higher concentrations compared to the control in the study of Glazunova *et al*. (2020).

Table 2 Morphological responses (mean ± standard error) for plant height, number of leaves, leaf length, and leaf width of cauliflower influenced by colchicine

Treatments	Plant height (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
Control	62.60 ± 0.40^{b}	17.17 ± 0.56^{c}	50.07 ± 0.62	22.42 ± 0.33^{ab}
0.05%	64.00 ± 0.71^{b}	21.05 ± 0.03^{b}	49.86 ± 0.54	22.75 ± 0.24^{ab}
0.10%	73.79 ± 0.36^{a}	27.34 ± 0.31^{a}	51.18 ± 0.81	22.52 ± 0.52^{ab}
0.20%	62.13 ± 0.24^{b}	22.67 ± 0.37^{b}	52.81 ± 0.64	23.64 ± 0.80^{a}
0.50%	59.63 ± 0.96^{b}	22.13 ± 0.56^{b}	49.44 ± 0.43	21.40 ± 0.75^{b}

Note: Means not sharing common letters differ significantly according to Duncan's multiple range test at the 5% probability level.

Yield Parameters

The results shown in Table 3 demonstrate that the application of 0.10% colchicine solution resulted in a significant increase ($P < 0.05$) in above ground fresh weight, flower weight, and inflorescence diameter, with mean values of 1,839.58 ± 3.49 g, 777.08 ± 3.85 g, and 19.45 ± 0.65 cm, respectively. The result was supported by Kumar *et al*. (2020) where plants treated with colchicine produced bigger flower sizes at 2.93 cm^2 and 3.00 cm^2 than the untreated plants. Increased fresh weight is also an implication of polyploidy.

In a comparison of the number of days to flowering, the study revealed that cauliflower treated with 0.10% and 0.50% colchicine solution exhibited a longer ($P < 0.05$) period to flowering, with a mean of 61.46 and 61.04 days, respectively after transplanting, as compared to the control treatment. These results suggest that the initial treatment may have interfered with cauliflower plants' early maturity and flowering. Table 3 provides further details on the morphological responses observed in this study. This may mean a longer time for the flower to develop thus the plant will produce larger and heavier flowers, while early flowering would mean possible drought escape. Per the product label, the variety generally produces flowers 50 to 60 days after transplanting. Thus, all flowering time fell within the variety range to flower.

Table 3 Morphological responses (mean ± standard error) for above-ground fresh weight, flower weight, inflorescence diameter, and days to flower after transplanting of cauliflower influenced by colchicine

Treatments	Above ground fresh weight (g)	Flower weight (g)	Inflorescence diameter (cm)	Days to flower after transplanting
Control	1,395.83 ± 5.74^{b}	527.08 ± 1.54^{c}	16.62 ± 0.31^{c}	47.88 ± 0.84^{d}
0.05%	1,422.92 ± 6.33^{b}	641.67 ± 3.66^{b}	17.51 ± 0.24^{b}	56.21 ± 0.59^{b}
0.10%	1,839.58 ± 3.49^{a}	777.08 ± 3.85^{a}	19.45 ± 0.65^{a}	61.46 ± 0.63^{a}
0.20%	1,472.92 ± 4.14^{b}	733.33 ± 5.67^{a}	17.88 ± 0.89^{b}	53.96 ± 0.71^{c}
0.50%	1,389.58 ± 4.75^{b}	570.83 ± 3.49^{c}	17.87 ± 0.53^{b}	61.04 ± 0.46^{a}

Note: Means not sharing common letters differ significantly according to Duncan's multiple range test at the 5% probability level.

Sattler *et al*. (2016), stated that the process of induction polyploidy is called gigas effect, which is the most important result of polyploidy that increased cell size due to the addition of extra gene copies which would result in improved growth and yield. It was reported by Inthima and Sujipuli (2019) that 21 and 13 examined autotetraploid clones had significantly higher fresh and dry weights compared to diploid clones, respectively.

According to previous research by Dhakhanamoorthy *et al*. (2010), mutagenic effects may cause early flowering and fruit maturity. However, in the present study, cauliflower plants treated with colchicine exhibited delays in flowering, suggesting that the chemical may have interfered with maturity and early flowering. This result corresponded with the study of Zaffar *et al*. (2004), where studies on developmental, morphological, and leaf anatomical characters of C_0 and C_1 generation showed that the colchiploids exhibited delayed flower and leaf emergence. It was reported by Chen *et al*. (2016) that the phenological states were delayed largely including the flowering time in the second year in tetraploid plants, which may have allowed the tetraploid plants to grow for a longer period and also contributed to the increase of the total biomass yield.

CONCLUSIONS

Plants treated with 0.10% colchicine significantly increased the growth and yield weight responses of cauliflower which have implications of induced polyploidy. This method is beneficial in places with extreme weather conditions like Siquijor, Philippines.

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