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Effect of Polyploidy Induction in Lettuce (*Lactuca sativa*) through Colchicine Treatment: Implications for inflorescence and flowering

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ABSTRACT
Lettuce holds significant economic importance as a staple food for a vast portion of the population worldwide. As a leafy vegetable, it is cultivated across various regions. This research aims to investigate the impact of polyploidy induction via colchicine treatment on the inflorescence and flowering traits of Lettuce (*Lactuca sativa*). Seed and seedling treatments were administered using five different concentrations of colchicine alongside a control group. Results revealed that lower concentrations of colchicine, particularly 0.1%, showed the most favorable response in lettuce, suggesting its potential utility in breeding programs. Regarding inflorescence and flowering traits, the study found that inflorescence in lettuce exhibited higher values at concentrations of 0.1% and 0.3% colchicine. This suggests that these concentrations may positively influence inflorescence development in lettuce plants. Additionally, inflorescence in Lettuce exhibited higher values at concentrations of 0.1% and 0.3%. The study recommends exercising caution in the use of colchicine for crop improvement endeavors.

Keywords: Polyploidy, Colchicine-Inducement, Lettuce, inflorescence, flowering.

INTRODUCTION
Genome duplication stands as a pivotal mechanism underpinning the acquisition of additional gene copies, thereby instigating genetic and evolutionary innovation within organisms. Polyploidy, a consequence of genome duplication, categorizes into euploids or aneuploidy based on chromosomal composition (Peterson and Queitsch, 2024). Euploids, characterized by multiples of the complete set of species-specific chromosomes, further divide into autopolyploids or allopolyploids depending on genome composition (Radionov, 2023). Among euploids, tetraploidy emerges as the predominant class (Harrison *et al*., 2014). Notably, polyploidy has left its mark on the evolutionary trajectory of numerous angiosperm species, with a strikingly high frequency of 1 in every 100,000 plants experiencing polyploidization, particularly prevalent in flowering plants (Doyle and Coate, 2019). Polyploidy proves detrimental in animal cells, plant cells typically tolerate it well, often resulting in larger, hardier, and faster-growing fruits and seeds, thereby enhancing desirability (Ferera and Dahanayake, 2014; Yetman *et al*., 2020). Consequently, ploidy manipulation has emerged as a valuable tool in the genetic enhancement of various plant species, including economically significant crops such as Cotton and Sugarcane (Trojak-Goluch *et al*., 2021; Li and Iqbal, 2024).
Colchicine ($C_{22}H_{25}O_6N$), an anti-mitotic agent extracted from *Colchicum autumnale* L. seeds and bulbs, serves as a potent inducer of polyploidy. Its mechanism of action involves binding to dimers, thereby impeding microtubule formation and subsequent spindle fiber development during cell division. Lettuce (*Lactuca sativa* L.), a dicotyledonous plant belonging to the Compositae family, boasts a rich history dating back to ancient Asia, where it was cultivated as early as 4500 BC.

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With over 100 species, lettuce emerges as a crop of significant economic importance and dietary staple for diverse populations worldwide (Eigenbrod and Gruda, 2015). Cultivated extensively for its leafy greens across various regions, the productive potential of lettuce hinges on genotype, environmental factors, and management practices such as spacing. The selection of appropriate cultivars plays a pivotal role in cultivation success.

Notably, lettuce is esteemed for its nutritional content, particularly abundant in Vitamins A and C, as well as essential minerals like calcium and iron. Darker green outer leaves tend to exhibit higher nutrient concentrations while being low in calories, making lettuce a valuable dietary component. This study is therefore, aimed at determining the effect of two colchicine application methods and five different concentrations on the production of inflorescence and overall flowering in lettuce.

MATERIALS AND METHODS

Study Site

The research encompassed three distinct locations. Seed treatment occurred in the Physiology Laboratory, while planting took place in the screen house, both situated at latitude 11.058°N and longitude 8.030°E within the Department of Plant Biology, Bayero University Kano. Cytological analysis was conducted at the Biological Laboratory, Umaru Musa Yaradua University, Katsina State.

Plant Materials

Seeds of lettuce (*Lactuca sativa* L.) variety "Dan Rani" were procured from a local seed vendor at Yankaba Market in Nassarawa Local Government Area of Kano State, Nigeria. The chemical mutagen, colchicine, utilized for the research, was acquired from Shugaba Scientific Supply located at 14A Murtala Muhammad Way, Katsina Road, near Federal Secretary, Kano. The colchicine was packaged by Kemi Laboratory, Lagos.

Seed Treatment (M1 generation)

Lettuce (*Lactuca sativa*) seeds were initially washed and presoaked in distilled water for one hour. Subsequently, the seeds were immersed in various concentrations of colchicine ($C_{22}H_{25}NO_6$) ranging from 0.0% to 0.5% and left at room temperature for 19 hours. Following immersion, the seeds underwent thorough rinsing with distilled water, and excess moisture was removed by blotting them on absorbent paper for 30 minutes. The remaining seeds were then placed in Petri dishes for cytological analysis.

M2 generations

The seeds harvested from M1 plants were planted. These seeds represented the M2 generation. The M2 plants were grown to maturity. Mutations segregated in the M2 generation, and homozygous mutations, which exhibited visible phenotypic changes, began to appear.

Seedling Treatment

Young lettuce seedlings (aged two weeks) were subjected to treatment with different concentrations of colchicine ($C_{22}H_{25}NO_6$), ranging from 0.0% to 0.5%. Sterilized cotton balls were dipped into each concentration of colchicine and placed on the growth apex of the seedlings. Treatments were administered consecutively for 12, 24, 36, 48, 52, and 72 hours.

Analysis of Flower Characteristics

On the first day, the day to 50% flowering, and at full inflorescence, observations were made, and measurements were taken for flower and inflorescence characteristics. These included counts for the number of flowers, determination of the time taken for 50% of the plants to reach flowering stage, and assessment of inflorescent weight.

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Experimental Design and Statistical Analysis

The experiment was laid out in Completely Randomized Design with each treatment replicated four times and data collected were subjected to One-Way ANOVA and means were separated using Pitcher's Least Significant Difference at P<0.05 using Genstat version 19.

RESULTS AND DISCUSSION

Days to First Flowering and 50% Flowering of Lettuce

The investigation into the impact of different concentrations of colchicine on the M2 generations of lettuce revealed significant differences in the days to first flowering and days to 50% flowering. In the M2 generation of treated seeds, the time taken for first flowering varied notably across different concentrations. Notably, the shortest duration to first flowering, 19.00 days, was observed at 0.1% concentration, closely followed by the control group at 22.00 days. Conversely, the longest time to first flowering, 47.00 days, was recorded at 0.5% concentration. Similarly, in the M2 generation, the control group exhibited the highest number of days to first flowering (57.00 days), while the lowest was observed at the 0.5% concentration.

Seedling treatments yielded similar trends, with the exception of the 0.1% concentration, which experienced a significant delay in days to first flowering compared to the control group at P < 0.05 in the M2 generation. It was also observed that, the 0.1% concentration displayed the highest number of days to first flowering, while the 0.4% concentration recorded the lowest (48.00 days). Days to 50% flowering, minor discrepancies were observed among concentrations. In the M2 generation, the 0.4% concentration exhibited the shortest duration (56.00 days), while the control and 0.3% concentration both recorded 60.00 days. The 0.1% concentration did not significantly differ from the 0.2% concentration in terms of days to 50% flowering in the M2 generation of seed treatment. Furthermore, comparing seedling treatments between the M2 generations, the former generally displayed shorter durations to 50% flowering. For instance, the M2 generation's shortest duration was 55.00 days at the 0.5% concentration, surpassing the M1 generation's highest value of 30.00 days at the 0.3% concentration.

Table 1: Effect of Colchicine on days to first and 50% flowering in both seeds and seedling treated with colchicine for M_2 generation in lettuce.

Colchicine concentrations	Days to 1st flower		Days to 50% flower	
	Seedling treatment	Seed treatment	Seedling treatment	Seed treatment
Control	57.00 [a]	54.00 [b]	60.00 [a]	63.00 [a]
0.1	53.00 [b]	61.00 [a]	59.00[b]	62.00 [a]
0.2	56.00 [a]	59.00 [a]	59.00[b]	63.00 [a]
0.3	51.00 [b]	49.00 [c]	60.00 [a]	60.00 [b]
0.4	53.00 [b]	48.00[c]	56.00 [c]	54.00 [c]
0.5	42.00 [c]	51.00[c]	61.00[a]	55.00 [c]

Means along column with the same letter are significantly similar using SNK at P<0.05

Lettuce Inflorescence

According to Table 2, the number of fruits was recorded, revealing higher inflorescence counts in treatments with concentrations of 0.3%, control, and 0.4% in the M2 generations. Interestingly, the number of inflorescence was greater in seedling treatments compared to seed treatments.

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Furthermore, the table demonstrates significant differences in the number of inflorescences across all treatments. Specifically, concentrations ranging from 0.1% to 0.3% exhibited the highest mean values at P < 0.05. However, it is worth noting that at higher concentrations, the values declined.

Table 2: Effect of Colchicine on number inflorescences in both seeds and seedling treated with colchicine for M2 generation in Lettuce

Colchicine concentrations	Seedling treatment	Seed treatment
Control	15.00[b]	10.0[d]
0.1	15.00[b]	12.00 [c]
0.2	14.00 [c]	14.00[b]
0.3	16.00 [a]	16.00 [a]
0.4	11.00[e]	12.00 [c]
0.5	12.00[d]	14.00[b]

Means along column with the same letter are significantly similar using SNK at P<0.05

DISCUSSION

In the comparative analysis of colchicine application methods, focusing on seed versus seedling treatment in lettuce, the study revealed a notable superiority of seed treatment, aligning with the findings of Dhamayanthi and Gotmare (2010). Their research similarly advocated for seed treatment as the preferred method over seedling or cutting treatments. However, contrasting results emerged from the study by Kumar *et al*. (2014), which favored seedling treatment for inducing polyploidy. This discrepancy underscores the complexity of colchicine application methods and their varied effects on lettuce morphology and physiology. Furthermore, the application of colchicine resulted in significant delays in both days to first flowering and 50% flowering in lettuce plants, particularly evident with higher concentrations. These findings are in line with observations made by Dhakhanamoorthy et al. (2015) and Essel *et al*. (2015), indicating a consistent interference of colchicine with flowering and maturity processes. Such delays underscore the intricate regulatory mechanisms governing plant development and the impact of external factors like colchicine on these processes.

Additionally, the number of inflorescences exhibited significant variation across different treatments. Concentrations ranging from 0.1% to 0.3% consistently yielded the highest mean values, indicating a favorable response of lettuce plants to these intermediate colchicine concentrations. However, higher concentrations led to a reduction in inflorescence numbers, echoing the findings of Simko and Zhao (2023). This highlights the nuanced relationship between colchicine concentration and inflorescence development, suggesting an optimal range for effective polyploidy induction while avoiding adverse effects on flowering traits.

The study has in the whole emphasizes the involved effects of colchicine treatment on inflorescence and flowering traits in lettuce, emphasizing the need for careful consideration of colchicine concentration levels in polyploidy induction strategies for crop improvement purposes. These findings provide valuable insights into optimizing colchicine application methods to enhance crop breeding and genetic improvement efforts.

CONCLUSION

The investigation into the impact of various concentrations of colchicine on the M2 generations of lettuce unveiled significant disparities in the days to first flowering and days to 50% flowering.

Notably, seed treatments revealed distinct trends in the time taken for first flowering across different concentrations. The shortest duration to first flowering was observed at 0.1% concentration (19.00 days), closely followed by the control group (22.00 days), while the longest time was recorded at 0.5% concentration (47.00 days). Similarly, the control group exhibited the highest number of days to first flowering (57.00 days), whereas the lowest was noted at the 0.5% concentration. Seedling treatments on another hand exhibited analogous patterns, except for the 0.1% concentration, which experienced a noteworthy delay in days to first flowering compared to the control group. Interestingly, the 0.1% concentration displayed the highest number of days to first flowering, while the 0.4% concentration recorded the lowest. In terms of days to 50% flowering, minor discrepancies were observed among concentrations in the M2 generation. The 0.4% concentration exhibited the shortest duration (56.00 days), while the control and 0.3% concentration both recorded 60.00 days. These findings highlight the elaborate relationship between colchicine concentration and flowering traits in lettuce, suggesting potential avenues for optimizing polyploidy induction strategies to enhance crop breeding and genetic improvement efforts. Further research is warranted to elucidate the underlying mechanisms driving these observed effects and to refine colchicine application methods for maximum efficacy in lettuce cultivation.

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