

Chemical mutagens and their impact in modifying the growth of chrysanthemum (*Dendranthema grandiflorum*) var. Flirt

Original Research Article

Abstract

The present research was conducted at Experimental Farm, Kharora, Department of Agriculture, Mata Gujri College, Fatehgarh Sahib, Punjab, during autumn of the year 2023-2024. The study was carried out in a randomized block design with seven treatments and three replications. Different doses of the chemical mutagens Ethyl methanesulphonate (E.M.S.) and colchicine were utilized in the experiment, with the specific doses being T₁ i.e., E.M.S. 0.75%, T₂ i.e., E.M.S. 1.0%, T₃ i.e., E.M.S. 1.25%, T₄ colchicine 0.02%, T₅ i.e., colchicine 0.03%, T₆ i.e., colchicine 0.04%, and T₇ i.e., control. Applying E.M.S. and colchicine treatments to rooted chrysanthemum cuttings led to significant changes in vegetative and flowering characteristics, creating variability. Both E.M.S. and colchicine treatments resulted in a significant reduction among vegetative parameters (plant height, number of branches, plant spread, diameter of main stem and number of leaves per plant) on the contrary the leaf area (65.34 cm²) increased in response to colchicine treatment and decreased in response to E.M.S. treatment compared to the control group treatment. Notably, an increase in the concentration of E.M.S. (E.M.S 1.25%) and colchicine (colchicine 0.04%) resulted in a decrease in vegetative and flowering parameters. Maximum plant height (52.00 cm), number of branches per plant (13.29), plant spread (29.39 cm²), main stem diameter (8.85 mm), and number of leaves per plant (111.22). These findings suggest that E.M.S. and colchicine can be effectively utilized to induce beneficial mutations in chrysanthemums potentially contributing to develop new dwarf plant varieties with desired traits, breeding program and scientific research to study plant development. This research highlights the importance of optimizing mutagen doses to achieve desired horticultural outcomes. Treated plants exhibit valuable ornamental traits suitable for different purposes for instance, the dwarf plants.

Keywords: Chrysanthemum, E.M.S., colchicine, Chemical mutagen, growth and flowering.

INTRODUCTION

Chrysanthemum, the 'Queen of the East', is not just one of the oldest and most beautiful flowering plants but also a symbol of royalty in Japan. Chrysanthemums, with their unique trait of blooming early in the fall, are a favourite flower for November. In today's world, where diverse tastes in flowers, including their colour, shape, size, and unique features, are celebrated, the ongoing need for new and improved flower varieties is a testament to the evolution in horticulture. One significant benefit of using mutation in chrysanthemums is its ability to alter specific traits of a superior cultivar without affecting the rest of its genetic composition. Genetic mutation breeding provides a valuable method for increasing the diversity of plants reproduced vegetatively. It also expedites the production of sports, which can be rare or slow to occur in specific cultivars. According to Wasscher (1956), nearly 30 % of chrysanthemum varieties originated as sports. In India, exceptional chrysanthemum cultivars have been developed by employing various chemical and physical mutagens to induce mutation. Mutagens can cause changes to the DNA and causes genotoxic. They can affect the transcription and replication of the DNA, which in severe cases can lead to cell death.

Multiple researchers highlight that the deliberate induction of mutations using ethyl methane sulphonate (E.M.S.) colchicine (Col.) offers a way to address the limitations of variability in plants. E.M.S. is an alkylating mutagenic chemical generally in liquids, highly soluble in

organic solvent. E.M.S. has been widely used in plants because it causes a high frequency of gene mutations and a low frequency of chromosome aberrations (Ingelbrecht *et al.* 2018). Colchicine is utilized in the breeding process to deliberately cause morphological alterations, in the investigation conducted on the impact of Colchicine on inducing polyploidy in various plant species (Eng & Ho, 2019).

The highest mutagenic efficiency was recorded in Col. the effectiveness of the three chemicals on Dianthus is ranked as E.M.S.>Col>SA (Roychowdhury and Tah 2011). Colchicine has been effectively employed to induce flower colour mutation in Chrysanthemum Cv. "Sharad Bahar". Originally, the flowers were purple, but the introduction of colchicine resulted in a mutation that produced flowers in a Terracotta Red hue. This mutation was subsequently released under the name "Colchi Bahar" (Datta, 1987).

Material and Method

The present field experiment was conducted at Mata Gujri College's Experimental Research Farm in Fatehgarh Sahib, Punjab, during the 2023-2024 autumn season. The coordinates of the site are 30.6435° North latitude and 76.3970° East longitude, with an altitude of 246 meters above mean sea level. Cuttings of the Chrysanthemum variety Flirt were obtained from the Department of Horticulture, Punjab Agriculture University, Ludhiana (Punjab).

All intercultural operations and plant protection measures were meticulously carried out as required to ensuring the experiment's thoroughness. Different doses of the chemical mutagens Ethyl methanesulphonate (E.M.S.) and colchicine were utilized in the experiment, with the specific doses being T₁ i.e., (E.M.S. 0.75%), T₂ i.e., (E.M.S. 1.0%), T₃ i.e., (E.M.S. 1.25%), T₄ i.e., (colchicine 0.02%), T₅ i.e., (colchicine 0.03%), T₆ i.e., (colchicine 0.04%), and T₇ i.e., (control). Experimental land was ploughed one to two times and harrowed to bring the soil to an acceptable state. The soil was turned over and smoothed out after the beds were made using bed maker. The soil was loosened, and a raised bed (1.5 × 1 meter) was prepared. The recommended dosage of farmyard manure and chemical fertilizers for chrysanthemums is 15 tons per hectare of farmyard manure (FYM) and 300 kg 200 kg 200 kg per hectare of NPK (Nitrogen, Phosphorus, and Potassium) respectively. After transplanting, a one-third dose of nitrogen and a total dose of phosphorus and potassium will be applied. The remaining nitrogen dose will be applied one month after transplanting.

Uniform and healthy rooted cuttings were selected for transplanting. The rooted cuttings were treated with different concentrations of Ethyl methane sulphonate (E.M.S.) and colchicine. Solutions were prepared in laboratory using E.M.S and colchicine mutagens. Required amount of mutagen was dissolved in distilled water according to treatments. Cuttings were immersed in Ethyl methane sulphonate (E.M.S.) and colchicine solution for 2 and 4 hours, respectively. After the treatments, cuttings were then given a brief rinse under running water from the faucet before transplanting into the field. The treated cuttings were transplanted on the main field. These cuttings were planted at a 45 × 30 cm distance on raised beds at experimental field in Randomized Block Design (RBD) with three replications and seven blocks in a row. All the standard cultural practices were followed, except disbudding operations. Disbudding can be defined as removing of buds or shoots to control growth and improve quality of plant.

Result and Discussion

A significant decrease is observed in the plant height, number of branches per plant, plant spread, diameter of the main stem, and number of leaves per plant in chrysanthemum plants treated with both E.M.S. and colchicine. Conversely, the leaf area increased in the colchicine treated population and decreased in the E.M.S. treated population compared to the control

group. The plant height was measured by meter scale from the base of the plant to the growing tip of each plant. Among the treatments maximum plant height of chrysanthemum plant was observed in T₇ i.e., control (52.00 cm) which is statistically superior to other treatments. Minimum plant height was found T₃ i.e., 1.25% E.M.S. (41.53 cm) which is statistically at par with T₂ i.e., 1.0% E.M.S. (41.62 cm) and T₆ i.e., 0.04% colchicine (42.88 cm). Due to chemical mutagens, treated plants exhibited a decrease in cell multiplication rate resulting in dwarfness, while the control plants showed an increase in height due to continuous cell multiplication. Similar patterns of decreased plant height have been witnessed in treated plants in finding of Ghormade *et al.* (2020), Vaidya *et al.* (2016), Kapadiya *et al.* (2014) and Kang *et al.* (2007).

There was significant decreased in number of branches per plant over control. Maximum branch count per chrysanthemum plant was observed in T₇ i.e., control (13.29) which is statistically superior to other treatments. Minimum number of branches was found in T₃ i.e., 1.25% E.M.S (10.44). Increased E.M.S. concentrations are harmful because it resulted in reduced shoot growth and cell division. The exact mechanisms E.M.S induced branching reduction may be disruption of auxins transport, increased oxidative stress also effect pathway of auxins leading to change in gene expression it could be attributed to the reduction in number of branches. These results are in conformity with the findings Ghormade *et al.* (2020), Rafiq *et al.* (2017) and Kazi (2012).

The plant spread was measured by a meter scale along both the direction i.e. East-West and North-South at full bloom stage. Plant spread was decreased due to the E.M.S. and colchicines treated population of chrysanthemum over control in T₇ (29.39 cm²). The reduction in the number of branches caused by E.M.S. and colchicine ultimately limited the spread of the chrysanthemum plant, length and width of the leaves decreased with higher dosage. E.M.S can alter auxins biosyntheses plant cell elongation and division ultimately affects plant spread. The results obtained in present investigation are in conformity with the findings of Palekar *et al.* (2022), Verma *et al.* (2018) and Padmadevi and Janaharlal (2011).

Diameter of main stem decreased due to the E.M.S. and colchicines treated chrysanthemum. Maximum main stem diameter was observed in T₇ i.e., control (8.85 mm) which is statistically superior to other treatments. Minimum diameter of main stem was found in T₃ 1.25% E.M.S (6.62 mm) which was statistically at par with T₆ 0.04% colchicine (6.80 mm). This issue may arise from chromosomal aberrations and disruptions in the synthesis of auxins, E.M.S induces point mutations in the DNA of plants leading to changes in stem diameter with increase in E.M.S concentration. The results obtained are in conformity with the findings of Verma *et al.* (2018) and Prabhukumar *et al.* (2015).

Number of leaves per plant was decreased due to the E.M.S. and colchicines treated population. Maximum number of leaves was recorded in T₇ i.e., control (111.22) which is statistically at par with T₁ i.e., 0.75% E.M.S. (111.03), T₂ i.e., 1.0% E.M.S. (105.85), T₃ i.e., 1.25% E.M.S. (100.33) and T₄ i.e., 0.02% colchicine (105.11). Minimum number of leaves was found in T₅ i.e., 0.03% colchicine (95.59) which is statistically at par with T₆ i.e., 0.04% colchicine (97.64). Decreased plant height and physiological dwarfing prompted the use of chemical mutagens to stimulate leaf area. Colchicine induced polyploidy that may have influenced leaf development, cell expansion and growth patterns among chrysanthemum leaves resulting in larger leaves size. The results obtained are in conformity with the findings of Rathod (2018), Rafiq *et al.* (2017), Bhajantari and Patil (2013) and Amiri *et al.* (2010). Increased in leaf area with higher dose of colchicine this was probably due to the fact

that decreased plant height and physiological dwarfing prompted the use of chemical mutagens to stimulate leaf area. Colchicine induced polyploidy that may have influenced leaf development, cell expansion and growth patterns among chrysanthemum leaves resulting in larger leaves size. This increase in size may translate to an increase in plant and its organs Kushwah *et al.* (2018) and He *et al.* (2016). The decline in leaf area resulting from higher E.M.S. levels may stem from inactivation, diminished auxins levels, or disruptions in auxins synthesis. The results obtained are in conformity with the findings of Ghormade *et al.* (2020), Vaidya *et al.* (2016) and Kapadiya *et al.* (2014).

Table 1: Effect of chemical mutagens on growth of chrysanthemum (*Dendranthema grandiflorum*) variety Flirt

Treatments	Plant height (cm)	No. of branches	Plant spread (cm ²)	Diameter of main stem (mm)	Leaf area (cm ²)	No. of leaves per plant
T ₁ E.M.S. 0.75%	46.10	12.14	26.68	7.77	60.70	111.03
T ₂ E.M.S. 1.0 %	41.62	11.33	24.61	7.29	54.61	105.85
T ₃ E.M.S. 1.25%	41.53	10.44	25.90	6.62	50.53	100.33
T ₄ Colchicine 0.02%	46.82	12.70	26.47	7.88	63.21	105.11
T ₅ Colchicine 0.03%	44.26	11.70	25.16	7.17	64.48	98.48
T ₆ Colchicine 0.04%	42.88	11.50	24.77	6.80	65.34	97.64
T ₇ Control	52.00	13.29	29.39	8.85	59.46	111.22
SE(m)±	0.71	0.27	0.77	0.16	0.54	4.00
CD _{0.05}	2.19	0.83	2.36	0.49	1.66	12.33

CONCLUSION

The present investigation revealed that T₇ i.e., control responded maximum among all the treatments of vegetative parameters including plant height (52.00 cm), number of branches per plant (13.29), plant spread (29.39 cm²), diameter of main stem (8.85 mm), number of leaves per plant (111.22). In contrast leaf area increased with increase in colchicine concentration. It was recorded maximum in T₆ i.e., colchicine 0.04% (54.90 cm²).

These findings suggest that E.M.S. and colchicine can be effectively utilized to induce beneficial mutations in chrysanthemums potentially contributing to develop new dwarf plant varieties with desired traits, breeding program and scientific research to study plant development. This research highlights the importance of optimizing mutagen doses to achieve desired horticultural outcomes. Treated plants exhibit valuable ornamental traits suitable for different purposes.

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