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Enhancing Floral and Post-harvest Traits in Gladiolus through EMS-Induced Mutagenesis

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Abstract

Gladiolus (Gladiolus grandiflora L.) cv. 'White Prosperity' is a commercially important cut flower, valued for its glossy white colored spikes and excellent vase life. Among cut flowers, it stands second in ranking after roses, but a narrow genetic base limits its breeding, which creates potential for improving its floral and postharvest characters. This study aimed to induce genetic variation by using different concentrations of chemical mutagen, Ethyl methanesulfonate (EMS) (control, @ 0.25%, @ 0.50%, @ 0.75% and @ 1.00%) for enhancing floral and postharvest attributes. Using a Randomized Complete Block Design (RCBD), significant variations were observed and recorded. The data showed that treatment T1 (0.25 %) showed the overall best results with the longest vase life (12.4 days), good stalk length (43.33 cm), number of florets (7.5), intermediate flower size with length of 5.37 cm and width of 53.56 mm. Also, maintained higher flower quality based on the rubric scoring method. EMS treatment T3 (0.75 %) showed moderate results by delaying senescence (3.79 days) while T4 (1.00 %) enhanced stalk length (43.40 cm) and also promoted earliest spike emergence (118.67 days). However, T3 and T4 negatively affected floret number, flower size and vase life. Overall, low concentration (0.25 %) emerged as most effective for improving both ornamental and postharvest longevity, making it a promising treatment for mutant selection and future gladiolus breeding. This study successfully demonstrates the potential of EMS mutagenesis for enhancing commercially important traits in gladiolus cultivation.

Keywords: Cut flower; Ethyl methanesulfonate (EMS); Mutagenesis; Postharvest Longevity.

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Introduction

Floriculture, a vital branch of horticulture, has emerged as a globally significant and rapidly expanding industry (Haider & Javed, 2023). It comprehends the cultivation, management, and marketing of flowering and ornamental plants, particularly for landscape beautification, floral arrangements, and indoor décor (Ahmad et al., 2025; Yousaf & Riaz, 2025). Among these, *Gladiolus grandiflora L.*, commonly known as sword lily, holds prominent commercial and aesthetic value due to its vibrant floral spikes, long vase life, and adaptability to diverse agro-climatic conditions (Akram et al., 2021; Zhang et al., 2024).

Originating from South Africa and Eurasia, gladiolus has evolved into a centrepiece of the global bulbous ornamental market, earning the title "Queen of bulbous ornamentals" (Kumar et al., 2019; Kashyap & Saha, 2023). The cultivar "White Prosperity" is one of the most favoured varieties for commercial floriculture, appreciated for its elegant white blossoms and consistent spike quality. In Pakistan, this crop thrives under a range of environmental conditions and is cultivated throughout the year, especially in Punjab and northern hilly regions (Usman et al., 2015; Ali et al., 2016).

Despite its commercial potential, *Gladiolus grandiflora* suffers from limited genetic variability, posing a significant hurdle to breeding programs aimed at improving floral traits and postharvest quality (Kazemzadeh et al., 2018; Silva & Costa, 2023). Postharvest senescence in floriculture is a critical limitation. Cut flowers are highly perishable, and their degradation is rapid during storage and transportation. In gladiolus, postharvest life is 6–7 days, which restricts its marketability in distant regions (Akram et al., 2021; Gul & Shahid, 2024). Global demand for high-quality cut flowers continues to rise; international floral markets were valued at $57.5 billion in 2024 and projected to reach $109.1 billion by 2032 (Javaid et al., 2025).

Therefore, there is a need to develop resilient cultivars with extended vase life and improved morphology for both marketability and consumer satisfaction (Kim et al., 2022). Traditional breeding methods offer limited scope for enhancing genetic diversity and are time-consuming (Kole & Meher, 2005; Khan et al., 2023). In contrast, induced mutagenesis has proven to be an efficient and cost-effective tool for generating novel phenotypes in ornamental crops (Datta, 2014; Kumari et al., 2025). Ethyl methanesulfonate (EMS), a chemical mutagen and alkylating agent, introduces point mutations by alkylating guanine bases in DNA, leading to genetic variation without the introduction of foreign genes (Tirkey & Singh, 2019; Dhiman et al., 2022). EMS has been successfully used to alter flower colour, size, spike length, and postharvest performance in multiple floricultural species (Ali et al., 2015; Kumari & Kumar, 2015; Ahmed et al., 2024).

Goals

To investigate the role of EMS-induced mutagenesis in Gladiolus grandiflora for floral and postharvest variability. To identify the optimal dose of EMS concentration with desired morphological and floral characteristics. To successfully generate useful genetic variation for breeding and commercial use.

Research questions

Different EMS concentrations showed dose-dependent effects on floral traits. Low concentration of EMS enhanced spike length and longevity, while higher doses reduced floret size, diameter and overall flower quality. Post-harvest longevity was improved at the dose of 0.25 % EMS, where it extends the vase life up to 12.40 days. At higher concentrations, deterioration was observed. Floral traits were affected at higher EMS concentrations, as a decline was observed in floret number and flower diameter, whereas lower doses significantly improved the senescence and vase life. EMS mutagenesis proved to be a vital tool for breeding programs to create novel varieties with marketable desired phenotypes.

Innovation

The gladiolus variety is genetically narrow, which makes it useful for breeding programs. Use of the EMS application can improve both pre- and post-harvest traits in this commercially important variety. Visual traits were assessed through rubric scoring and morphological combined data. The concentration of 0.25 % to 0.50 % found to be the best suited without disturbing aesthetic appeal.

Materials and Methods

The present study was conducted in the experimental field area under the Department of Horticulture, PMAS Arid Agriculture University, Rawalpindi, during the 2024 - 25 year. The university is located at 33.64 °N latitude and 73.07 °E longitude, and the altitude above sea level is approximately 1640-1800 ft. The average weather conditions of Rawalpindi between March to June during this research are mild to hot summers with an average temperature of 15 - 40 °C, and rainfall is moderate throughout this research season, which is 1.26 mm per month, whereas pre-monsoon showers start from late June.

Plant specimens and chemical agents

The corms of Gladiolus grandiflora L. "White Prosperity" were purchased from NNA Rapid Solution (Agriculture Products & Pest Control Services), Nouman Plaza, Lehtrar Road, Islamabad. The chemical ethyl methanesulfonate (EMS) used in this study was procured from Qaiser Scientific Store, Saddar, Rawalpindi. Manufactured by Macklin Chemicals (China), with a purity of 99 %, the molecular formula is C3H8O3S and with a molecular weight of 124.16 g/mol.

EMS preparation and application. The corms were soaked in the 3 ml/L (Propiconazole) fungicide solution for 5 minutes to remove the fungal infection and then air-dried. After that EMS solution of different concentrations (control, 0.25 %, 0.5 %, 0.75 % and 1 %) was made using a pipette by adding 2.5 mL, 5 mL, 7.5 mL, and 10 mL of EMS, respectively, in 5 different beakers of 1000 mL, having distilled water required for each treatment. The corms were treated by soaking them for 5 minutes, then rinsing well under tap water for half an hour, followed by air drying. Then the corms were transplanted to the experimental field. The depth of the corms planted in the soil was double the size, for better growth and development, necessary practices were followed accordingly.

Treatment	EMS Concentration
T_0	Control
T_1	0.25 %
T_2	0.50 %
T_3	0.75 %
T_4	1.00 %

Table 1: Different concentrations of ethyl methanesulfonate (EMS) used in the experiment

Experimental layout

The experiment was laid on raised beds of sandy loamy soil with a total area of 20 ft2 (L= 10 ft. × W 2 ft.). The distance between plant to plants and row to rows was 1 ft respectively. The total number of 150 corms was used, which includes 30 corms per treatment with three replications, each having 10 corms. The Experiment was conducted according to the Randomized Complete Block Design (RCBD).

Post-harvest variables and floral traits

The post-harvest variables and floral traits were measured in this research. The floral traits were (days to spike emergence, flower stock length, no. of florets per spike, flower length and flower diameter) and postharvest (vase life, flower quality, days to senescence). Data was collected randomly and analyzed according to the standard procedures.

Days to spike emergence (count)

The number of days from planting to the emergence of the first floret spike from the leaf sheath was recorded from five selected plants of each treatment, and the average was taken, using the given formula below:

$$DSEAvg=(D1+D2+D3+...n)5$$

Flower stalk length (cm)

Flower stalk length was measured from the base of the plant to the tip of the last floret in cm using a wooden meter rod (model: UNIQUE, made in China). Data was recorded from five selected plants from each treatment and recorded. The average was calculated using the formula below:

$$FSLAvg=(SL1+SL2+SL3+...n)5$$

Number of florets per spike (count)

The total number of florets on each fully developed spike was counted from five selected plants of each treatment, then the average floret number was calculated and recorded.

$$FPSAvg=(NF1+NF2+NF3+...n)5$$

Flower Length (cm)

The length of the flower is measured in centimetres (cm) of the fully opened flower using a Digital calliper; the jaws were positioned across the edges of the flower perpendicularly. The average of the representative five flowers was recorded and calculated by the given formula:

$$FLAvg=(FL1+FL2+FL3+...n)5$$

Flower diameter (mm)

The diameter is measured in millimetres (mm) from fully opened five representative flowers using a Digital calliper, the jaws were positioned horizontally to the axis of the flower. The average was calculated and recorded using the given formula:

$$FDAvg=(FD1+FD2+FD3+...n)5$$

Days to start senescence (count)

The number of days was recorded until the first visible onset of aging, characterized by a decline in appearance and vitality (e.g., wilting, browning of petals). The data was recorded in days taken to senescence of the representative five plants of each treatment, and the average no. of days was calculated, using the given formula:

$$DSAvg=(DS1+DS2+DS3+...n)5$$

Vase life (count)

The vase life was assessed by observing the start of senescence in the last floret of the fully bloomed stalk. The number of days till the last floret remained in good condition was recorded, and the average of 5 selected plants from each treatment was calculated.

$$VLAvg=(VL1+VL2+VL3+...n)5$$

Flower quality scoring

Judges analytically rate qualitative traits like colour, colour hues, fragrance, petal shape and petal appearance, on a defined scale of 1 to 9. Scoring shows (7-9) excellent quality, (4-6) average quality and (1-3) poor quality.

STATISTICAL ANALYSIS

Statistical analysis was done by using Statistics 8.1 and SPSS software. One-way Analysis of Variance (ANOVA) was conducted to determine the significance of treatments at $P \leq 0.05$, and Least Significant Differences (LSD) was applied at 5 % probability level. Descriptive statistics for standard deviation (SD) and standard error (SE) were calculated to assess precision and variability.

Results

Results of the following post-harvest and floral parameters were recorded and discussed below:

Days to spike emergence

Significant differences were observed in the number of days to spike emergence among different ethyl methanesulfonate (EMS) treatments in *Gladiolus grandiflora*, as shown in Figure 2 (a). The earliest spike emergence was observed in T_4 (1.00 % EMS) and T_0 (control), with 118.67 and 120.13 days. The most delayed spike emergence was recorded in T_2 (0.50 % EMS), with a mean of 127.43 days, significantly higher than all other treatments. This was followed by T_3 (0.75 %) with 125.20 days and T_1 (0.25 %) at 121.77 days, respectively.

Flower stock length

The flower stock length (cm) showed a significant response to ethyl methanesulfonate (EMS) treatments, as shown in Figure 2 (b). The longest flower stocks were recorded in T_4 (1.00 % EMS), T_1 (0.25 %), and T_2 (0.50 %), with mean values of 43.40 cm, 43.33 cm, and 41.60 cm, respectively. These treatments indicated no significant difference among them. In contrast, T_0 (control) and T_3 (0.75 %) showed shorter flower stocks at 39.33 cm and 38.43 cm, respectively and were significantly different from T_4 and T_1.

Florets per spike

The number of florets per spike (count) in *Gladiolus grandiflora* was significantly influenced by ethyl methanesulfonate (EMS) treatments, as shown in Figure 2 (c). The highest number of florets was recorded in the control T_2 (0. 50 %) with 7.6 florets, significantly greater than all other treatments. This was followed by T_1 (0.25 %), having a mean of 7.5 florets. The lowest number of florets per spike was observed in T_4 (1.00 %) and T_3 (0.75 %), with 4.10 and 4.27 florets, which is statistically different from all other treatments. Whereas, T_1 (control) show intermediate results having 7.16 florets per spike.

Flower Length

The flower length (cm) of *Gladiolus grandiflora* showed a highly significant response to ethyl methanesulfonate (EMS) treatment levels, as shown in Figure 2 (d). T_0 (control) produced the longest flowers, with a mean of 5.80 cm, significantly greater than all other treatments. Shortest flowers were produced in T_3 (0.75 %) and T_4 (1.00 %), with 4.73 cm and 4.43 cm. As the EMS concentration increased, flower length decreased progressively. Whereas, T_1 (0.25 %) and T_2 (0.50 %) recorded 5.37 cm and 5.03 cm flower lengths, respectively.

Flower Diameter

A highly significant variation in flower diameter (mm) was observed among ethyl methanesulfonate (EMS) treatments and control plants of *Gladiolus grandiflora,* as shown in Figure 2 (e). T_0 (control) produced the widest flowers, with a mean diameter of 58.00 mm and significantly superior to all other treatments. The smallest diameters were recorded in T_3 (0.75 %) and T_4 (1.00 %) with 48.07 mm and 46.87 mm, respectively. It is clear that by increasing EMS concentration, a consistent decline in flower diameter was recorded. While T_1 (0.25 %) with 53.57 mm and T_2 (0.50 %) with 50.17 mm show an intermediate effect.

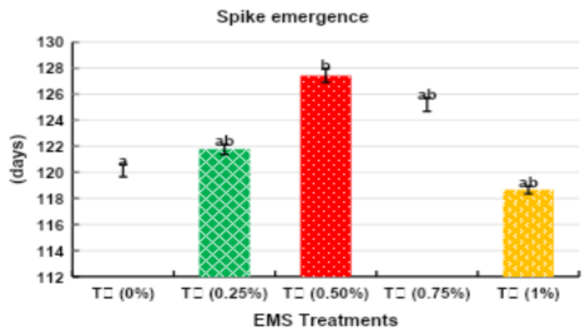

Figure 2(a): Days to spike emergence across EMS treatments

Days to start senescence

The analysis of variance revealed that days to senescence in *Gladiolus grandiflora* were significantly affected by ethyl methanesulfonate (EMS) treatments, as shown in Figure 3 (a). The maximum duration before senescence was recorded in T_3 (0.75 % EMS), with a mean of 3.79 days, which was significantly higher than in T_1, T_2, and T_4. The minimum duration (earliest senescence) was observed in T_0 (control) with a mean of 3.37 days. The T_1 (0.25 %) showed results following T_3, with a mean of 3.62 days.

Vase life

Vase life, a critical trait determining the commercial and ornamental value of *Gladiolus grandiflora* as a cut flower, was significantly affected by ethyl methanesulfonate (EMS) treatments as shown in Figure 3(b). The T_1 (0.25 %) showed the maximum vase life of 12.40 days, significantly higher than all EMS treatments. Increase in EMS concentration showed a negative effect as T_4 (1.00 %) and T_2 (0.50 %) recorded the shortest vase lives of 6.13 and 7.5 days. Whereas T_3 (0.75 %) and T_0 (control) showed intermediate effects in vase life, which are 8.90 and 8.53 days, respectively.

Flower Quality

The flower quality traits of *Gladiolus grandiflora L. "White Prosperity"* were evaluated based on five parameters: colour, colour hues, fragrance, petal shape, and petal appearance using a rubric-based scoring method (1 = poor, 5 = average, 9 = excellent). As shown in Figure 4 (a), the control treatment (T_0) showed superior performance across all parameters, with scores of 7 for colour, 6 for colour hues, 3 for fragrance, 7 for petal shape, and 7 for petal appearance. T1, treated with a low EMS dose, also maintained high aesthetic quality, with scores close to the control. As the EMS concentration increased from T_2 to T_4, a gradual decline was observed in all traits. The lowest scores were recorded in T_4 (colour = 3, colour hues = 3, fragrance = 1, petal shape = 4, petal appearance = 4), indicating a deterioration in flower quality with higher EMS exposure.

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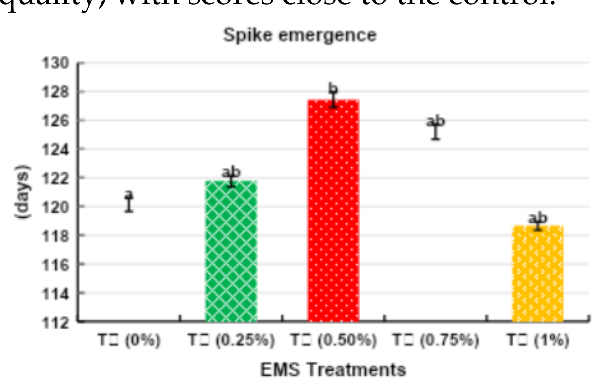

Figure 2(a): Days to spike emergence across EMS treatments

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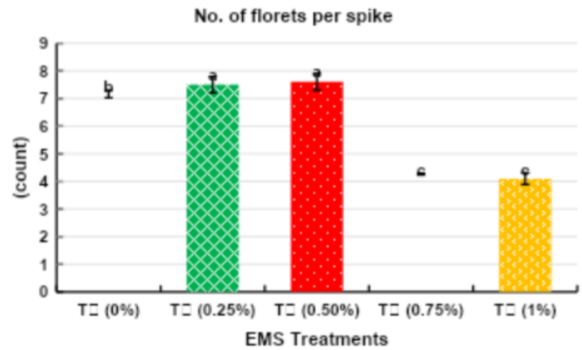
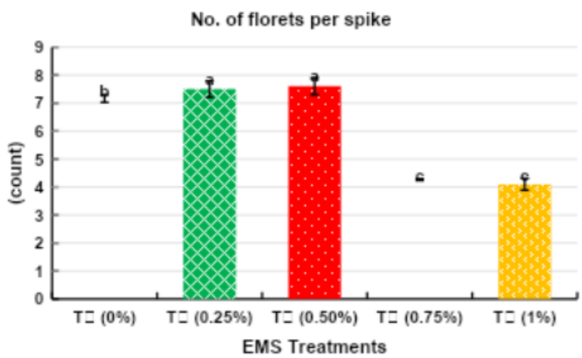


Figure 2(c): Showing number of florets per spike across EMS treatments



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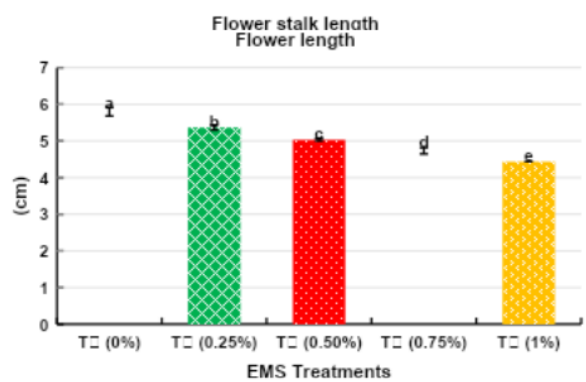

Figure 2(b): Showing flower stalk length across EMS treatments

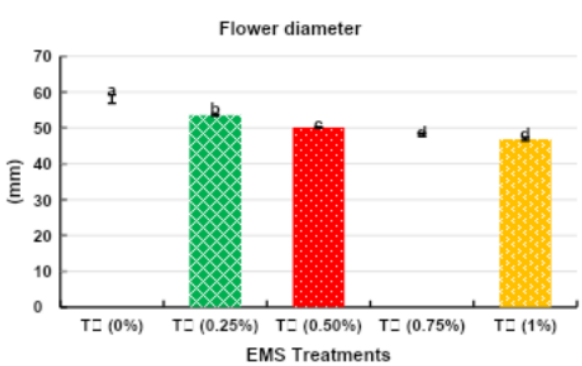

Figure 2(e): Showing flower diameter across EMS treatments

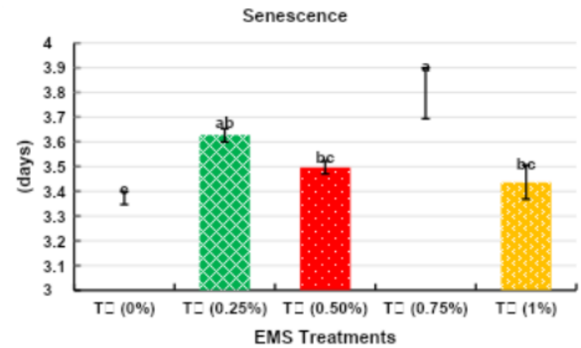

Figure 2(d): Showing flower lengths across EMS treatments

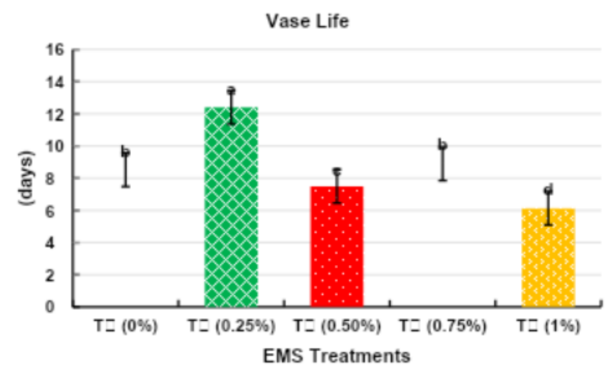

Figure 3(b): Showing vase life across EMS treatments

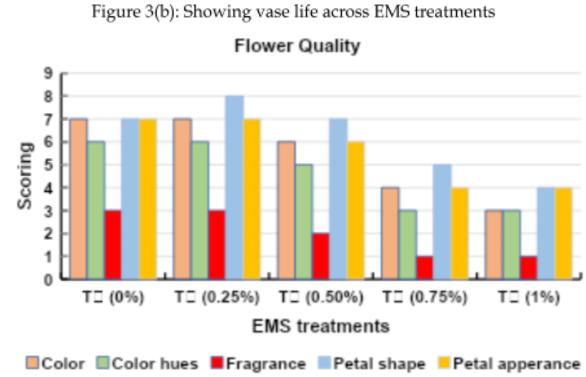

Figure 3(a): Showing senescence across EMS treatments

Discussions

Increase in doses of ethyl methanesulfonate (EMS) induces early spike initiation, which is probably due to absorption of more nutrients, due in which higher amount of photosynthesis occurs which ultimately results in early sprouting, good growth, and hence, early spike emergence and flowering. These findings align with Cantor and Korosfoy (2002). Lower doses of EMS enhance early flowering in plants because of physiological changes (fast metabolic activity and altered gene expression). Whereas delayed flowering occurs at higher doses of EMS due to inhibitory effect, it might also be due to a reduction in the rate of physiological processes (EL-Nashar & Asrar, 2016; Pooja, 2016; Moustafa et al., 2018).

Moderate ethyl methanesulfonate (EMS) doses often enhance cell elongation and meristem activity, resulting in promoting elongation in plant structures like flower stalks (Siddique et al., 2020; Gulzar et al., 2024). The response pattern observed in this study is due to auxin sensitivity or altered gibberellin synthesis due to EMS, as it directly influences these pathways (Lenatway et al., 2022). Low to moderate stress influences stem elongation while higher doses reduce it due to a hormetic response (Siddique et al., 2020).

In conclusion, EMS exhibits a concentration-dependent effect on flower stalk elongation. Hence, an optimal dose is essential for maximizing beneficial morphological mutations.

The results confirm earlier reports that ethyl methanesulfonate (EMS) can cause cytological and developmental disruptions at higher doses and negatively affect reproductive traits such as flower or floret number, as it interferes with genes regulating floral meristem development and organogenesis (Jabeen & Mirza, 2004; Subramaniam & Kumar, 2023). In *Gladiolus*, floret development is strongly regulated by a complex genetic and hormonal balance. Moderate EMS concentrations introduce beneficial mutations or slight stress, which enhance floret differences by triggering developmental pathways. Similar patterns have been observed in EMS mutagenized *Chrysanthemum* and *Tuberose* (Puripunyavanich et al., 2023; Patil et al., 2025). Whereas higher EMS concentrations inhibit mitotic division and disrupt hormonal signalling that reduces floral formation (Kumar, 2023). Furthermore, Jyothsna et al. (2024) highlighted that higher concentrations often induce malformed or fewer floral structures due to disrupted meristematic activity. In summary, EMS employs a concentration-dependent effect on the number of florets per spike in *Gladiolus*, with lower doses maintaining reproductive performance and higher doses causing noticeable reductions.

The findings of the study supported by the article, indicate that ethyl methanesulfonate (EMS) can negatively affect the floral organ size due to its mutagenic impact on genes regulating cell division, elongation, and organogenesis (Jabeen & Mirza, 2004; Kumar, 2023). EMS-induced mutations can hinder the expression of key regulatory genes or disrupt hormonal pathways that influence flower morphogenesis, including auxin and gibberellin signalling. It is observed that flower length in EMS-treated *okra* plants reduced significantly with increasing EMS concentration, paralleling the present findings in *Gladiolus* (Gulzar et al., 2024). In *Chrysanthemum*, Nasri et al. (2022) described that higher doses of EMS compromised petal development, thus reducing overall flower size. Subramaniam et al. (2023) observed a reduction in flower length at T_3 and T_4 likely results from EMS-induced genotoxic stress, impairing cell expansion and meristematic tissue growth in floral buds. In comparison, the moderate reductions in T_1 and T_2 treatments suggest that low EMS exposure may induce slight mutations without severely disrupting developmental processes. However, even at 0.25 %, a measurable decrease compared to the control was evident, indicating that floral size is a sensitive morphological trait to EMS exposure.

Ethyl methanesulfonate (EMS) is a potent alkylating agent; it can disrupt gene functions which involved in cell elongation, expansion and overall floral organ development (Jain et al., 2006; Nasri et al., 2022). Reduction observed in flower diameter possibly results from EMS mutations affecting cell proliferation and turgor-regulated growth processes within petal tissues (Jabeen & Mirza, 2004). Studies in ornamental species like *Chrysanthemum* and *Petunia* also reported a significant decrease in size and shape of flowers under EMS treatments, mostly at concentrations above 0.5% (Popatanasov et al., 2023). These effects are attributed to EMS-induced point mutations that may alter hormone-responsive genes. In *Gladiolus*, ethyl methanesulfonate (EMS) appears to have a threshold effect. Lower concentrations (T_1 and T_2) cause slight reductions in diameter but preserve some floral characteristics, suggesting partial mutagenic pressure. However, higher concentrations at 0.75 % exceed the tolerance level for optimal floral growth, leading to stunted floral organs. This is consistent with the findings of Gulzar et al. (2024), who documented a decline in petal spread and floral symmetry in treated *okra* flowers. Such changes in flower diameter are critical in the context of floriculture breeding, as visual appeal and flower size are primary market traits.

Disrupting or enhancing genes related to hormonal regulation and stress responses is done through inducing mutagenesis by EMS (alkylating agent). Flower senescence is mainly ethylene-mediated in many ornamental plants, including *Gladiolus* (Singh, 2008). EMS at specific concentrations might accidentally affect ethylene biosynthesis, resulting in delaying the aging of petals. According to a study on EMS mutagenized *Antirrhinum majus*, certain concentrations exhibited altered ethylene response and prolonged vase life, attributed to mutations in ethylene receptor genes (Heffron & Korban, 2023). It is reported that EMS combined with growth regulators prolonged vase life in chrysanthemum, supporting the idea that EMS can decrease senescence through indirect physiological changes. However, excessive EMS T_4 (1.00 %) appears to reverse these gains, due to collective genetic damage beyond the repair threshold, resulting in earlier tissue degradation. This aligns with comprehensive observations in mutation breeding where higher concentrations reduce cell viability and functional longevity (Din et al., 2023).

These findings are in line with the present literature that associates ethyl methanesulfonate (EMS) treatment with the decline of postharvest floral quality, particularly vase life. Being an effective chemical mutagen, it can disrupt genes responsible for longevity traits such as water balance regulation, antioxidative defence and ethylene biosynthesis. Flower senescence is strongly controlled by hormonal signals, especially ethylene and ABA. Induced mutations in these pathways likely accelerate wilting and petal degradation in chrysanthemum (Heffron & Korban, 2023). In snapdragon (*Antirrhinum majus*), it is reported that EMS treatments reduced vase life significantly at concentrations above 0.5 %, a pattern reflected here in *Gladiolus*. These effects are due to reduced uptake of the vase solution due to vascular blockage, increased cellular membrane leakage and premature petal senescence. Whereas, extensive mutagenic damage affects membrane stability and stress tolerance mechanisms, which are crucial for vase life (Mehrabi et al., 2022; Baraiya et al., 2022).

Assessment of flower quality revealed that low ethyl methanesulfonate (EMS) concentration (T_1) positively influenced traits such as petal shape, colour and appearance, resulting in enhanced floral aesthetics but without improving fragrance. In contrast, higher EMS doses (T_3 and T_4) significantly declined flower quality, particularly fragrance and petal structure, likely due to mutagen-induced developmental stress. Scoring is done by using the rubric method as evaluated by Qureshi et al. (2025).

Conclusion

A study showed that EMS mutagenesis had a dose-dependent effect on Gladiolus grandiflora cv. 'White Prosperity'. Lower dose (0.25 %) enhanced post-harvest life (12.4 days), stalk length and floret number while maintaining good flower quality, making it most effective for breeding. Moderate doses (0.5 % and 0.75 %) caused delayed flowering and reduced flower size but slightly prolonged senescence, whereas higher concentrations (1%) promoted early spike emergence and stalk elongation but negatively affected floret number, size, vase life and quality. Overall, EMS at low concentration proved most suitable for creating useful variation without compromising ornamental value.

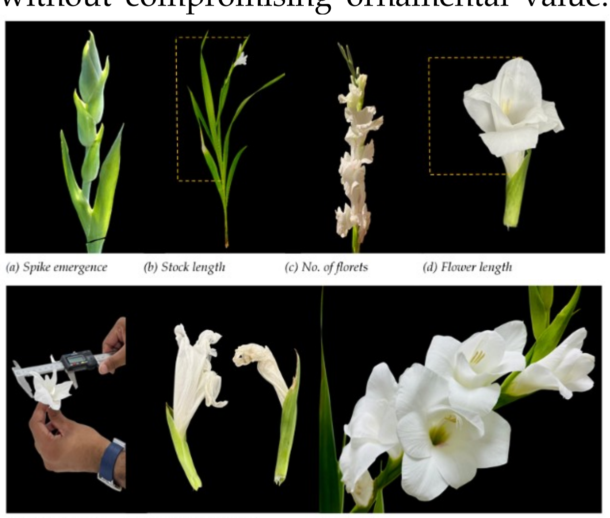

Figure 6: Visual representation taken from representative plants of different treatments.

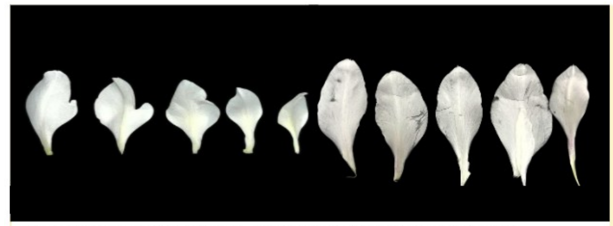

Figure 5: Comparison of petal symmetry across all treatments for visual characterization

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