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Morpho-Floral and Postharvest Responses of *Iris kashmiriana* to Ethyl Methanesulfonate (EMS) Mutagenesis

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Abstract

Iris kashmiriana, a hardy perennial flowering cut flower native to the northern region of Pakistan and a member of the Iridaceae family, which exhibits high floral, commercial and medicinal value. The present study aimed to induce phenotypic variation using ethyl methanesulfonate (EMS), a potent chemical mutagen known to cause point mutations in DNA. Five concentrations (control, 0.25 %, 0.5 %, 0.75 %, and 1.0 %) were applied with a dipping duration of 5 minutes. A range of morphological, floral and postharvest characteristics was evaluated to assess the mutagenic impact of EMS. Results show that EMS application of 0.5 % (T₃), maximum improvement in multiple parameters including rhizome diameter (3.16 cm), leaf length (29.1 cm), number of leaves (8.4), plant height (32.8 cm), stalk length (28.26 cm), and flower count (2.26). T₃ also showed delayed floral senescence (2.46 days), extended vase life (4.66 days), and quality score (7.5), indicating improved post-harvest longevity. EMS application of 0.25 % (T₂) shows moderate results in parameters, rhizome diameter (2.66 cm), leaf length (26.4 cm), number of leaves (6.1), plant height (30.1 cm), stalk length (27.63 cm), and flower count (1.5). On the other hand, the highest concentration (T₄) constantly resulted in decreased growth and floral traits, like reduced flower diameter (0.7 cm), petal area (3 cm²) and vase life (0.7 days). These findings recommend that the EMS application at 0.5 % is effective in enhancing desirable morphological, floral and post-harvest traits in *Iris kashmiriana*.

Keywords: Chemical concentration; Vegetative variation; Vase life; Ethyl methanesulfonate (EMS); Cut flower quality

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Introduction

The genus *Iris*, a member of the family Iridaceae, contains more than 300 species (Khatib et al., 2022), of which 16 are indigenous to Pakistan, and is valued for its medicinal properties and striking floral structures. The name "*Iris*" comes from the Greek word for rainbow, reflecting the wide range of colours seen across species (Karpenko, 2015). *Iris kashmiriana* is endemic to the Western Himalayas and is distributed in Pakistan, Afghanistan, Nepal and India (Farooq et al., 2019). In Pakistan, *Iris kashmiriana* is found in the northern areas such as Swat, Chitral, Gilgit-Baltistan, Hazara Division, and Azad Jammu and Kashmir and grows at elevations ranging from 1600 to 2200 meters above sea level (Rahman & Akhtar, 2023). It is cultivated for cut flowers, fragrant, whitish flowers and rhizome containing medicinal compounds used in folk medicine (Mykhailenko et al., 2017). In folk medicine, particularly in regions like Kashmir, the rhizomes have been used as anti-inflammatory, expectorant, diuretic, and antimicrobial agents (Chalotra et al., 2022; Chandni et al., 2024). Ethnoveterinary applications include remedies for general body weakness, liver disorders, and improving milk production in cattle (Anderson et al., 2008; Mir, 2014; Agrawal & Kumar, 2021). Its aroma and therapeutic properties also make it valuable in perfumery and cosmetic products. However, despite its high potential, the plant remains underused commercially due to limited genetic diversity, mainly caused by clonal propagation through rhizomes.

Floriculture, a rapidly growing sector of ornamental horticulture, is driven by consumer demand for new flower forms, colours and improved vase life (Devrani et al., 2023). To address this demand and expand the genetic diversity of ornamental

plants, induced mutagenesis has become a powerful technique. Mutagenesis application of chemical agents like ethyl methanesulfonate (EMS) (Mehrabi et al., 2022), superior cultivars with improved commercial and aesthetic qualities can be achieved (Kumar, 2023; Larraburu et al., 2025). Role of mutagenesis producing phenotypic variation in *Iris kashmiriana*, an underexploited yet promising ornamental species native to the Himalayan region.

Ethyl methylsulfonate (EMS) is a chemical mutagen on plants because of its high ability to induce mutations, and since it is a simple compound (Gulzar et al., 2024). EMS alkylates induce point mutations. This mutagen attaches its alkyl groups to the oxygen bonded to guanine through hydrogen bonds and produces O-6 alkylguanine that pairs with thymine instead of cytosine and replaces A/T by G/C (Abd EL-Moneim et al., 2021). EMS influences a very short segment of the chromosome that carries one or several genes, and can affect the cytological, genetic, physiological, and morphological traits of plant tissues and cells (Kumar et al., 2024).

To overcome this limitation, ethyl methanesulfonate (EMS) induced mutagenesis provides an effective method for introducing genetic diversity (Din et al., 2023). These mutations can modify genes involved in flower morphology, pigment production, fragrance intensity, and postharvest behaviour, all of which are important traits for the floriculture industry (More, 2024). In Pakistan, where the export of cut flowers and trade of ornamental plants are still growing, native ornamentals like *Iris kashmiriana*, through scientific breeding, can create significant economic opportunities (Ahmad et al., 2020). The most desirable ornamental improvements were observed at moderate

EMS concentrations, which enhanced floral display without causing excessive phytotoxicity and provided the best balance, inducing beneficial morphological and floral variations without causing severe physiological damage. Additionally, postharvest evaluation revealed that certain mutants exhibited significantly extended vase life and delayed senescence, indicating potential for improved marketability. Different EMS concentrations produced distinct morphological and floral responses. Lower concentrations generally promoted favourable traits such as increased flower diameter, enhanced pigmentation, and stronger stems, while higher concentrations resulted in growth suppression, chlorosis, and reduced flowering due to mutagenic stress and the 0.5 % EMS concentration. This research represents the first documented attempt in Pakistan to optimize EMS mutagenesis in *Iris kashmiriana* and supports the development of new cultivars better suited for both local landscaping and international markets. This study aims to assess the effects of EMS-induced mutagenesis on phenotypic and postharvest traits in *Iris kashmiriana*, establishing the basis for cultivar development and broader use of mutation breeding in ornamental horticulture.

Materials and Methods

The present study was conducted during the 2024-2025 growing season at the *Iris* germplasm Unit, Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi, Pakistan. During the research period, the weather conditions of Rawalpindi: average temperature of day and night is 15–40 °C, moderate rainfall averaging 1.26 mm per month, and the pH range of 6 to 7.5. Certified rhizomes of *Iris kashmiriana* were procured from Muslim Ullah Nursery, Swat (Khyber

Pakhtunkhwa), while the chemical mutagen, Ethyl methanesulfonate (EMS), manufactured by Macklin (China), was procured from Qaiser Scientific Store, Saddar, Rawalpindi. The experiment was performed on raised beds, prepared with sandy loam soil, covering a total area of 20 ft² (10 ft in length × 2 ft in width). A uniform spacing of 1 ft² was maintained both plant-to-plant and row-to-row to ensure proper aeration and root development. The study involved a total of 75 rhizomes were used, divided into five treatments with 15 rhizomes each, having three replications each with five rhizomes, under a Randomized Complete Block Design (RCBD). Before mutagenic treatment, rhizomes were disinfected by soaking them in a 3 mL solution of propiconazole (a broad-spectrum fungicide) for 5 minutes to eliminate potential fungal contamination. The treated rhizomes were then air-dried at room temperature. Afterward, EMS solutions of varying concentrations (as shown in Table 1) were prepared by dissolving them into 1,000 mL of distilled water (required per treatment). Rhizomes were soaked in the respective EMS solutions for 5 minutes. After treatment, the rhizomes were rinsed thoroughly under running distilled water to remove residual mutagen and then air-dried. The treated rhizomes were immediately transplanted onto the prepared beds in the open field. Standard agronomic practices, including irrigation, weeding and pest control, were applied uniformly throughout the growth period to ensure optimal plant development.

Treatments	Concentrations
T ₀	0 %
T ₁	0.25 %
T ₂	0.5 %
T ₃	0.75 %
T ₄	1.0 %

Table 1. Different concentrations of ethyl methanesulfonate (EMS) solution Data for Morphological, Floral and Postharvest Parameters

The morphological, floral and postharvest parameters were recorded and evaluated for the following parameters.

Rhizome Diameter (cm)

The diameter of the rhizome was measured in centimetres (cm) using a vernier calliper (model: AOS: 500-196-30, Mitutoyo-Japan) from the widest point of each rhizome, and the average of five readings per treatment was calculated.

Rhizome Weight (g)

To determine the weight of a rhizome in grams (g) using an electronic weighing balance (model: FA2204), the average of five rhizomes was calculated.

Leaf Length (cm)

Leaf length was measured in centimetres (cm) using a steel scale (model: "NEXT") from the leaf base to the tip of the leaf, and the average of five leaf lengths of a representative plant were calculated.

Leaf Width (cm)

Leaf width was measured in centimetres (cm) using a steel scale (model: "NEXT") from the left side of the leaf to the right side, and the average of five leaf widths from the representative plants of treatments were calculated.

Number of Leaves (count)

The number of leaves was counted for five plants per treatment, and their average was calculated using the given formula. The data were recorded.

$$NLav = \frac{(NL1 + NL2 + NL3 + \dots .n)}{NLt}$$

NLav indicates the average number of leaves, NL1, NL2, NL3, ..., NLn represent the number of leaves on individual plants, and NLt stands for the total number of plants.

Plant Height (cm)

The plant height was measured in centimetres (cm) using a steel scale (model: "NEXT") from the plant base (near soil surface) to the tip of the highest part of the plant (spike or leaf), and the average of five plants were calculated (Sim et al., 2020).

$$PHav = \frac{(PH1 + PH2 + PH3 + \dots .n)}{PHt}$$

PHav indicates the average of plant height, PH1, PH2, PH3, ..., PHn represent the plant height of individual plants, and PHt stands for the total number of plants.

Flower Stalk Length (cm)

The stalk length was recorded in centimetres (cm) from the base of the plant to the highest point of the floret using a steel scale (model: "NEXT"). Five randomly selected flower stalk lengths were measured, and then the average was recorded for each treatment.

Flower Diameter (cm)

The diameter of the *Iris kashmiriana* flowers was measured in centimetres (cm) using a Vernier calliper (model: AOS: 500-196-30, Mitutoyo-Japan) at full bloom. Measurement was taken across the widest part of the flower tips. The average is calculated from the data of five selected flowers, and the result is recorded.

Flower Length (cm)

The flower length was measured from the base of the ovary to the tip of the uppermost petal using a vernier calliper (model: AOS: 500-196-30, Mitutoyo-Japan). Measurements were taken from fully bloomed flowers during the peak flowering stage (Brock & Weinig, 2007). Five randomly selected flowers per plant were measured, and the average was recorded for each treatment.

Petal area (cm²)

The area of each petal was measured using a wooden scale. The length and width of the petal were recorded in centimetres (cm²) (model: Li-3100), and

using the formula to find the petal area, the average was calculated (Zhao et al., 2024).

$$\text{Petal area} = \pi \times \frac{L}{2} \times \frac{W}{2}$$

where L is the petal length, W is the petal width, and π (pi) = 3.1416.

Number of Flower Buds (count)

The number of flower buds was counted for five plants per treatment, and their average was calculated using the average formula. The data were recorded.

$$\text{FBav} = \frac{(\text{BN1} + \text{BN2} + \text{BN3} + \dots .n)}{\text{BNt}}$$

FBav indicates the average number of flower buds, BN1, BN2, BN3, ..., BNn represent the number of flower buds on individual plants, and BNt stands for the total number of plants.

Number of Flowers (count)

The number of flowers was counted for five plants per treatment, and their average was calculated using the average formula. The data were recorded.

$$\text{NFav} = \frac{(\text{FN1} + \text{FN2} + \text{FN3} + \dots .n)}{\text{FNt}}$$

NFav indicates the average number of flowers, FN1, FN2, FN3, ..., FNn represent the number of flowers on individual plants, and FNt stands for the total number of plants.

Days to Start Senescence (count)

To examine the number of days till flower senescence, the method described by Wu et al. (2017) was followed. Five flower stalks were placed in a glass vase filled with distilled water and monitored daily. The days until aging were recorded when the flowers showed signs such as colour changes, dehydration, or the appearance of dark spots.

Vase Life (count)

The vase life of *Iris kashmiriana* flowers was assessed after harvest; the flowers show wilting petals or lose their original appearance and which leads to senescence of flowers. The number of days was

counted to estimate the vase life of *Iris* cut flowers (Gul et al., 2013).

Quality (scoring)

The quality of ethyl methanesulfonate (EMS) treated flowers was assessed by several floral attributes, including bearded, fragrance, colour and appearance. Evaluation was done by 50 judges following the method explained by Copper and Spokas (1991). Each attribute was scored, and the average was calculated to rate the quality of each flower. Based on the overall average, flowers were classified on a scale from 1 to 9 (Qureshi et al., 2025).

1= Imperfect Quality, 5= Average Quality, and 9= Excellent Quality

Statistical analysis

The data obtained from the experiment were analyzed by using a Randomized Complete Block Design (RCBD) through Analysis of Variance (ANOVA) with the help of Statistix 8.1 software (Steel, 1997).

Results

Rhizome diameter (cm)

The maximum rhizome diameter was observed in treatment T_3 (0.5%), 3.16 cm, followed by T_2 (0.25 %), 2.66 cm, T_4 (0.75 %), 2.53 cm, and T_5 (1 %), 2.4 cm. The minimum diameter was observed in the control group, T_1 , 2.26 cm. These results indicate that moderate ethyl methanesulfonate (EMS) concentrations, particularly 0.5 %, positively influenced rhizome thickness, with statistically significant differences among treatments in Figure (a).

Rhizome weight (g)

The maximum rhizome weight was recorded in T_2 (0.25 %), 29.5 g, followed by T_3 (0.5 %), 27.6 g, T_5 (1 %), 26.3 g, and T_4 (0.75 %), 26.1 g. The minimum rhizome weight was observed in the control treatment, T_1 , with an average of 20.4 g. These results indicate that all ethyl methanesulfonate (EMS) treatments

enhanced rhizome development compared to the control, with T₂ showing the most significant effect, as described in Figure (b).

Leaf Length (cm)

The maximum leaf length was observed in treatment T₃ (0.5 %) 29.1 cm, followed by T₂ (0.25 %) 26.4 cm, T₅ (1 %) 26.2 cm and T₄ (0.75 %) 21.9 cm. While the minimum leaf length was recorded in the control group, T₁, 18.3 cm. The data indicated a significant difference among treatments, with T₃ showing the effect, as shown in Figure (c).

Leaf Width (cm)

The maximum leaf widths were observed in treatments T₂ (0.25 %) and T₃ (0.5 %), which were statistically similar, 2.6 cm, followed by T₄ (0.75 %) and T₅ (1 %), 2.0 cm. The lowest leaf width was recorded in the control group T₁ at 1.6 cm. Statistical analysis revealed a highly significant difference, as shown in Figure (d).

Number of Leaves (count)

The maximum number of leaves was observed in T₃ (0.5 %), with 8.4 leaves, which was greater than in all other treatments. T₂ (0.25 %) 6.1, control T₁ 5.9, and T₅ (1%) 5.7. The minimum number of leaves was observed in T₄ (0.75 %), 4.3. From the recorded data, it can be concluded that there was a statistically significant effect of the different ethyl methanesulfonate (EMS) concentrations applied. At the same time, the results of T₃ were highly significant, as shown in Figure (e).

Plant Height (cm)

The *Iris kashmiriana* exhibited a significant variation in plant height in response to different ethyl methanesulfonate (EMS) concentrations (Figure f). The maximum plant height was observed in treatment T₃ (0.5 %), 32.8 cm, followed by T₂ (0.25 %), 30.1 cm, and T₅ (1 %), 28.5 cm. Treatment T₄ (0.75 %) attained a moderate height of 24.8 cm. However, the minimum plant

height was recorded in the control group T₁, 21.9 cm. The results shown by T₃ were highly significant compared to the other treatments.

(Annexure A)

Flower stalk length (cm)

The maximum stalk length was recorded in treatment T₃ (0.5 %), measuring 28.26 cm were followed by T₂ (0.25 %), 27.63 cm, T₄ (0.75%), 19.2 cm, and the control group, T₁, 15.85 cm. The minimum flower stalk length was observed in T₅ (1 %), 5.66 cm. The results were statistically significant, indicating a clear decline in Stalk elongation at higher ethyl methanesulfonate (EMS) concentrations, as presented in Figure (a).

Number of flowers (count)

The highest flower count was recorded in T₃ (0.5 %), 2.26 flowers per plant. Was followed by the control group T₁ 1.96, T₂ (0.25 %) 1.5 flowers, and T₄ (0.75 %) 1.2 flowers. The minimum flower production was observed in T₅ (1 %) with only 0.3 flowers per plant. The results showed a statistically significant difference. T₃ demonstrates the most effective response for enhancing flower production, as shown in Figure (b).

Flower diameter (cm)

The maximum flower diameter was recorded in control treatment T₁ (0 %) 6.43 cm, T₃ (0.5 %) 6.26 cm, T₂ (0.25 %) 5.2 cm and T₄ (0.75 %) 5.03 cm. The minimum flower diameter was observed in T₅ (1 %), 0.7 cm. These results suggest that while moderate ethyl methanesulfonate (EMS) exposure (0.5 %) maintained flower size, with statistically significant differences among treatments as shown in Figure (c).

Flower length (cm)

The maximum flower length was recorded in the control treatment, T₁ 6.03 cm, T₃ (0.5 %) 5.86 cm, while T₄ (0.75 %) 4.6 cm and T₂ (0.25 %) 3.33 cm, respectively. The minimum flower length

was recorded in T₅ (1 %), 1.23 cm. The results were statistically significant, as illustrated in Figure (d).

Petal area (cm²)

The highest petal area was recorded in the control treatment T₁, measuring 17.93 cm², followed closely by T₃ (0.5 %) 16.86 cm², T₂ (0.25 %) 13.76 cm², and T₄ (0.75 %) 12 cm², respectively. The lowest petal area was found in T₅ (1 %), which showed a significantly reduced value of 3 cm², while T₃ maintained petal size near the control, as shown in Figure (e).

Number of flower buds (count)

The highest number of buds was recorded in T₃ (0.5 %), 2.26 buds per plant. This was followed by control T₁ 1.96 buds, T₂ (0.25 %) 1.5 buds, and T₄ (0.75 %) 1.2 buds per plant. The minimum number of buds was recorded in T₅ (1 %) with only 0.3 buds per plant. Particularly 0.5 %, enhances bud formation, while higher concentrations suppress it significantly, as shown in Figure (f).

(Annexure B)

Vase life (count)

The maximum vase life was recorded in treatment T₃ (0.5 %), 4.66 days, control T₁, 3.8 days, T₂ (0.25 %), 3.23 days, and T₄ (0.75 %), 3.33 days. The shortest vase life was observed in T₅ (1 %), 0.7 days. These results indicate that 0.5 % ethyl methanesulfonate (EMS) positively influenced post-harvest longevity. The results were statistically significant, as shown in Figure (a).

Days to start senescence (count)

The maximum delay in senescence initiation was observed in treatment T₃ (0.5 %), 2.46 days, control T₁, 2.03 days, T₂ (0.25 %), 1.76 days, and T₄ (0.75 %), 1.6 days, respectively. The earliest onset of senescence was noted in T₅ (1 % EMS), with a significantly reduced time of 0.4 days. Whereas a 0.5 % ethyl methanesulfonate (EMS) treatment effectively delayed its onset, as shown in Figure (b).

Quality (scoring)

The highest quality score was recorded in treatment T₃ (0.5 %) with a score of 7.5, followed by T₂ (0.25 %) with 6.2 score, control T₁ 5.5 score, T₄ (0.75 %) 4.8 score, while the lowest flower quality was noted in T₅ (1 %), recording a score of 1.66 as shown in Figure (a).

(Annexure C)

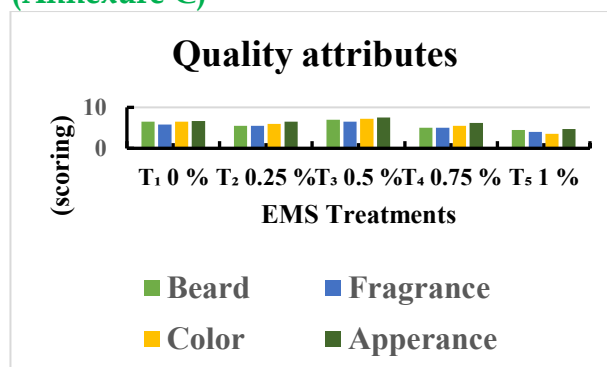


Figure 4. Quality attributes of *Iris kashmiriana* using the rubric method.

Discussion

The significantly rhizome diameter, with the maximum value observed at T₃ in *Iris kashmiriana*, followed by T₂. This moderate concentration is improved, attributed to enhanced cell division and expansion induced by EMS at sub-toxic levels. Bhajantri and Patil (2023) reported similar observations in *Gladiolus*, where 0.4–0.6 % EMS treatments increased corm diameter compared with the control. Whereas higher concentrations reduced these traits in *Chrysanthemum indicum* (Ghormade et al., 2020). The reduction in rhizome diameter at 1 % EMS may cause cytotoxic damage and reduced meristematic activity.

The maximum rhizome weight observed in T₂ indicates that slightly lower EMS concentrations effectively stimulated rhizome development by enhancing storage tissue formation. The same findings were observed in *Gladiolus*, where lower doses produced high-weight corms, possibly due to the low mutational

concentration allowing continued assimilate storage (Yoosumran et al., 2025). In *Lilium* (Kumar, 2023), at higher doses, cellular damage likely reduces translocation efficiency, resulting in the observed weight reduction (Huang et al., 2025).

The longest leaves were recorded at T₃; the moderate EMS doses can increase vegetative growth, because of enhanced cell elongation and division. Similar observations were reported in gladiolus, where moderate concentrations improved leaf length (Yang et al., 2025) and in chrysanthemum. In roses, the moderate concentration of EMS increased vegetative traits owing to balanced mutagenic stimulation and low toxicity (Zayed et al., 2014). In contrast, the reduced leaf length in the control group aligns with reports that the absence of mutagenic stimulation may limit hormonal activity, such as auxin production (Habib et al., 2021).

The leaf width of *Iris kashmiriana* is significantly enhanced in T₂ EMS concentrations, indicating that mutagenesis enhances cell growth and vegetative development. Similar research has been observed in ornamentals, where low EMS induced valuable morphological variability in gladiolus (*Gladiolus grandiflorus*) (Tiwari & Pandey, 2024) and chrysanthemum (*Dendranthema grandiflora*) (Shalini et al., 2023). These studies confirm that, when EMS is applied at optimal concentrations, it can be an effective tool for improving floral and vegetative traits. At the same time, the growth is suppressed at higher doses.

The highest number of leaves was in T₃, greater than all other treatments. This suggests that moderate EMS may induce genetic changes that promote vegetative branching or leaf initiation rates through cell division and meristem activity. In

Chrysanthemum, EMS has been reported to increase shoot number at moderate concentrations, contributing to more leaf counts (Shinoyama et al., 2012). In gladiolus, the number of leaves declines due to inhibited mitotic cell division, causing ill effects on auxin synthesis at higher EMS levels, as previously reported by Monika et al. (2016).

The significant increase in plant height was noted in T₃ EMS, indicating that this EMS level supports cell division and elongation. Similar enhancement of plant height at moderate EMS concentrations was observed in *Gladiolus* Turkey & Singh (2019). Dwarfing at high EMS levels may result from decreased auxin synthesis, chromosomal damage, or impaired assimilation. In tuberose, similar findings have been reported in (Kaur & Kumar, 2018).

The maximum flower stalk length was recorded in T₃, while the minimum was recorded in T₅. These results suggest that 0.5 % ethyl methanesulfonate (EMS) concentrations effectively promoted stalk elongation through enhanced cell division (Lenawaty et al., 2022). In contrast, the higher concentration likely caused cytotoxic effects, resulting in growth inhibition. The results are similar in chrysanthemum; moderate EMS doses respond positively, whereas they negatively impact growth and development at excessive concentrations (Purente et al., 2020).

The maximum flower diameter was observed in the control treatment (T₁), while the minimum was observed in T₅ at the highest concentration. This shows that increasing ethyl methanesulfonate (EMS) levels can negatively affect flower size, due to mutagen-induced cellular damage or disruption of floral development. Although moderate ethyl

methanesulfonate (EMS) concentrations exhibited some reduction in flower diameter, the effect was less severe compared to higher doses (Suryawati et al., 2023). In *Chrysanthemum*, EMS treatment induced significant morphological variations. At moderate EMS concentrations, enhanced flower size was observed, while higher concentrations reduced flower size (Puripunyanich et al., 2023).

The maximum flower length was recorded in the control (T_1), while the minimum was in T_5 . This shows that higher ethyl methanesulfonate (EMS) concentrations harmfully affected flower length, because of cellular damage or growth inhibition caused by excessive mutagenic stress. The flower length in *Iris kashmiriana* is sensitive to ethyl methanesulfonate (EMS) at higher concentrations (Singh et al., 2015). A similar result was found in *Ceratostigma willmottianum*, where at low concentrations of EMS, the result of flower length is enhanced, whereas with higher concentrations, it causes stunted growth and reduced floral dimensions, confirming the sensitivity of reproductive traits to excessive EMS exposure (Guo et al., 2025).

The maximum petal area was recorded in the control treatment, while the minimum was recorded in T_5 , at higher ethyl methanesulfonate (EMS) concentrations, severely decreasing petal size. This reduction is due to mutagenic stress affecting cell division and expansion in petal tissues. Moderate ethyl methanesulfonate (EMS) levels resulted in smaller reductions. The petal area in *Iris kashmiriana* is sensitive to ethyl methanesulfonate (EMS) concentration, with excessive exposure harming floral morphology. In African violet, EMS has

been reported similarly (Ghimire & Fang, 2023).

The maximum number of buds in T_3 concentration stimulates bud initiation, possibly by increasing meristematic activity or promoting favourable genetic mutations. Ethyl methanesulfonate (EMS) at lower levels may activate growth without causing excessive cellular damage. The decrease in bud count in T_5 at higher ethyl methanesulfonate (EMS) concentrations restricts reproductive development due to toxicity or disrupted hormonal balance (Rodge et al., 2024). In the gladiolus cv. PINK BEAUTY, moderate treatment of EMS produced the most favourable results, including a higher number of florets per plant, which is directly related to bud number (Monika et al., 2016).

The maximum flower count in T_3 indicates that moderate ethyl methanesulfonate (EMS) concentration effectively increased flower production, by cell division and floral meristem activity, and at this level, beneficial mutations that promote reproductive. The lowest flower count in T_5 suggests that excessive ethyl methanesulfonate (EMS) exposure restricts flowering, due to toxicity or disruption of genetic regulation (Gulzar et al., 2024). These findings are similar to China aster, where observed that moderate EMS treatments enhanced flower number, but higher concentrations suppressed floral output (Shivaswamy et al., 2025).

The maximum days of delay in senescence observed in T_3 suggest that moderate ethyl methanesulfonate (EMS) concentration positively influences physiological longevity by increasing stress tolerance or extending cellular aging processes. Such treatments involve beneficial mutations that slow down senescence. The earliest senescence in T_5

indicates that high ethyl methanesulfonate (EMS) levels accelerate flower aging due to cellular damage or disturbance of hormonal balance (Liu et al., 2016). In *Antirrhinum majus* (snapdragon), moderate concentrations of EMS produced viable plants with extended floral longevity, while higher EMS concentrations enhanced senescence and reduced plant vigour (Heffron & Korban, 2022).

The maximum vase life recorded in T₃ at moderate ethyl methanesulfonate (EMS) concentration increased post-harvest longevity, by extending senescence and maintaining cellular integrity. Such treatments may promote positive genetic or physiological changes that slow down the aging process in harvested flowers. The shortest vase life shows that high ethyl methanesulfonate (EMS) levels accelerate senescence, possibly due to metabolic imbalance or to oxidative stress (Macnish et al., 2010). Similarly, in tuberose, where mutation breeding enhances vase life and floral quality, the moderate EMS prolonged vase life, while higher concentrations decreased it (Eisa et al., 2025).

Rubric-based evaluation indicates that moderate ethyl methanesulfonate (EMS) treatment T₃ significantly improved the quality attributes of *Iris kashmiriana*. Similar observations were reported by Madhuri et al. (2017), where intermediate EMS doses enhanced floral traits in gladiolus and marigold, respectively.

Conversely, higher EMS treatments, T₄ and T₅, led to a notable deterioration in quality. The reduced scores for beard formation, colour vibrancy, and fragrance at these levels suggest cellular or genetic damage caused by excessive mutagenic stress, a similar finding noted in chrysanthemum (Din et al., 2023).

Conclusion

In conclusion, the present study proves that ethyl methanesulfonate (EMS) is an effective chemical mutagen for inducing phenotypic variability in *Iris kashmiriana*. Various concentrations of ethyl methanesulfonate (EMS) (0.25 %, 0.5 %, 0.75 %, and 1 %) were applied to evaluate their effect on morphological and floral traits. Among all treatments, the 0.5% concentration (T₃) constantly produced the most favourable outcomes, enhancing key vegetative and reproductive parameters, including rhizome diameter and weight, leaf length, plant height, number of leaves, flower stalk length, number of buds and flowers, vase life, and overall quality score. The T₃ also showed the longest delay in floral senescence, reflecting improved post-harvest longevity.

In contrast, the highest ethyl methanesulfonate (EMS) concentration (1 %) showed phytotoxic effects, resulting in a decline in growth, flower traits, and quality. The control (0 % EMS) showed inferior performance compared to moderate ethyl methanesulfonate (EMS) treatments, the mutagen's role in generating positive variation.

Overall, the results confirm that moderate ethyl methanesulfonate (EMS) treatment, especially at 0.5 %, can be a valuable tool in floriculture breeding programs that developing better *Iris kashmiriana* varieties.

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Annexure A

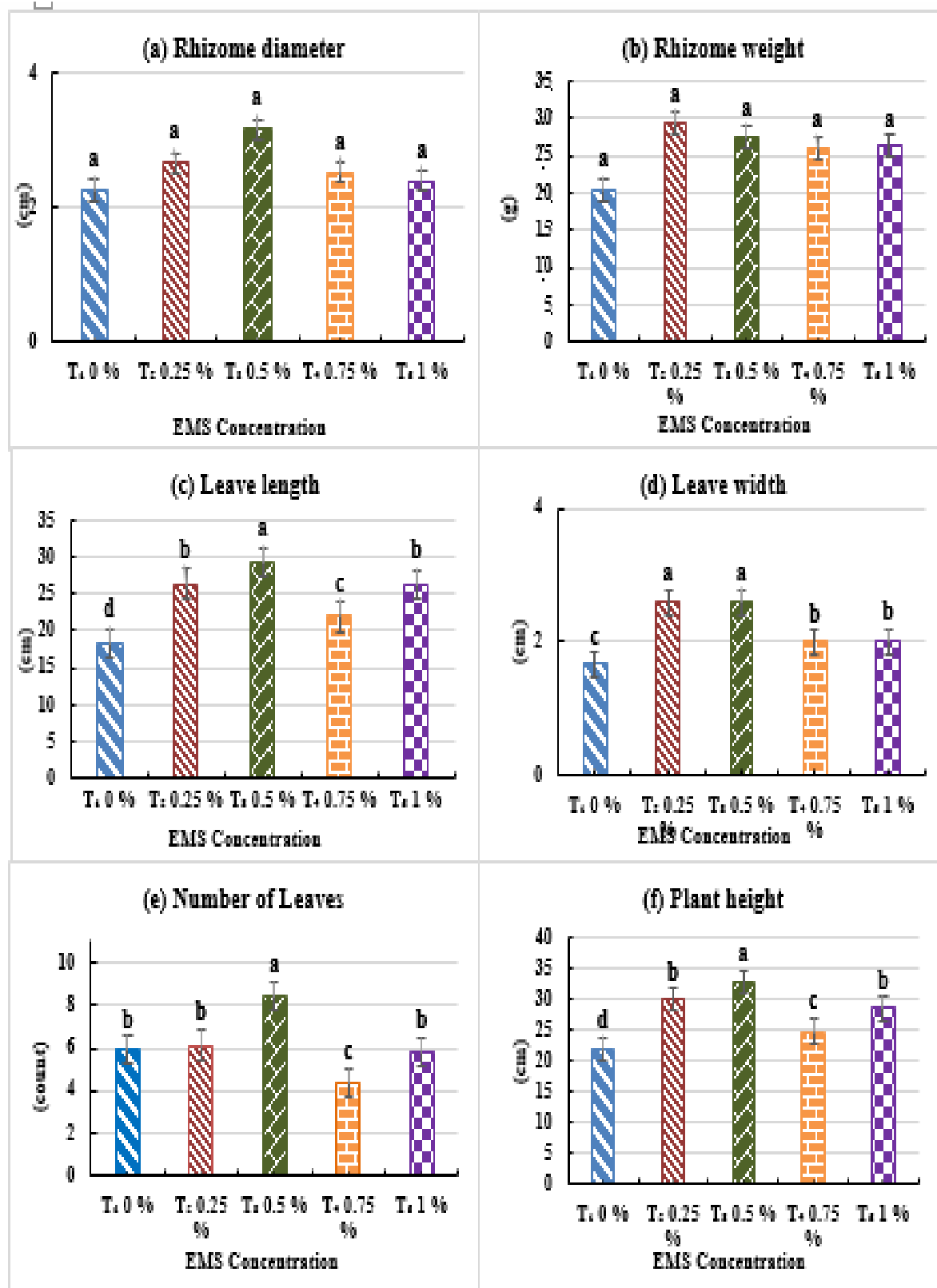


Figure 1: Stimulation of morphological attributes (a) rhizome diameter (cm) (b) rhizome weight (g) (c) leaf length (cm) (d) leaf width (cm) (e) number of leaves (count) (f) plant height (cm). Variability induced by EMS in *Iris kashmiriana* showed statistically significant results at the LSD value ($p < 0.05$)

Anneure B

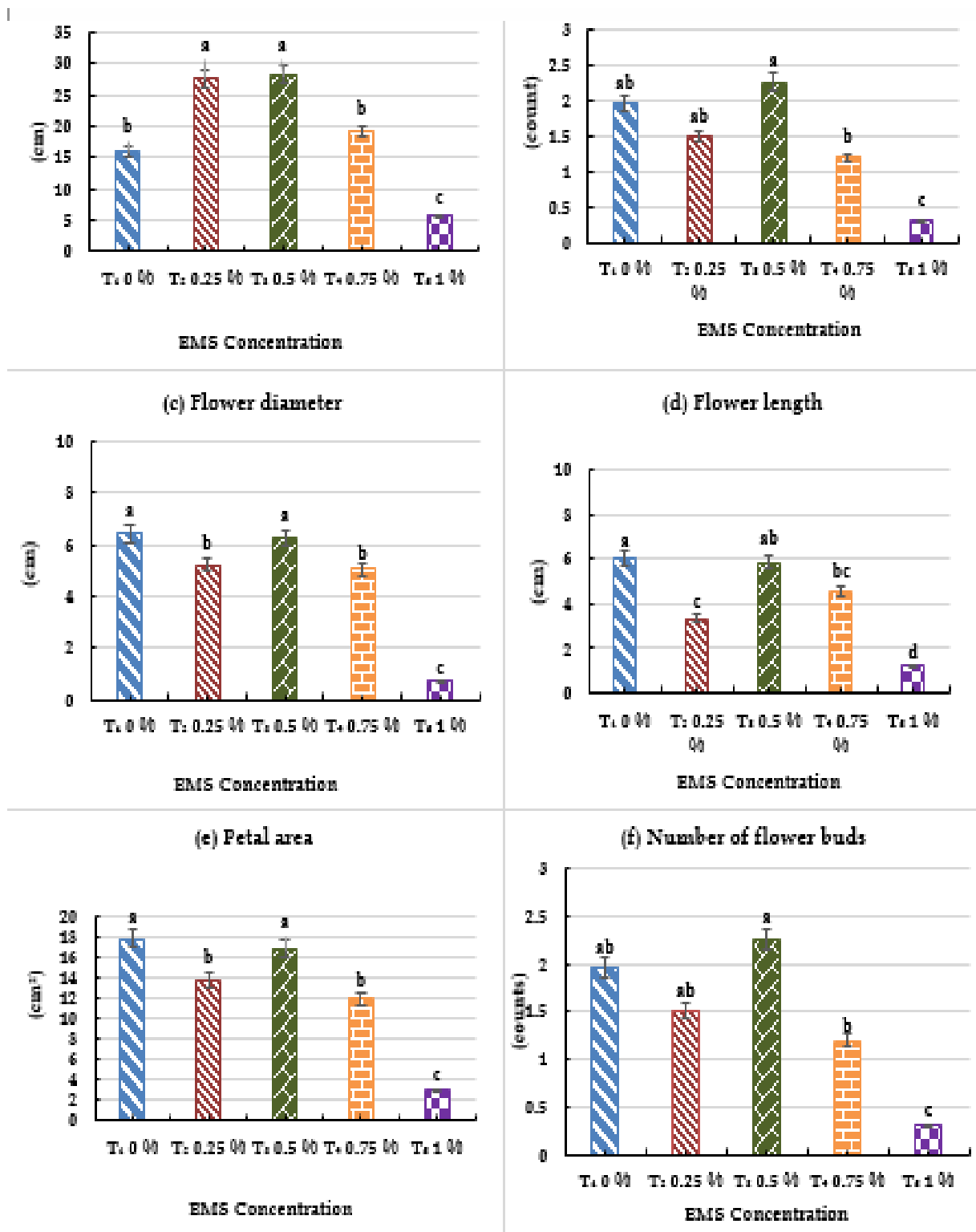


Figure 2 Stimulation of floral attributes (a) flower stalk length (cm) (b) number of flower (count) (c) flower diameter (cm) (d) flower length (cm) (e) petal area (cm²) (f) number of flower buds (counts). Variability induced by EMS in *Iris germanica* showed statistically significant result at the LSD value ($p < 0.05$)

Anneure B

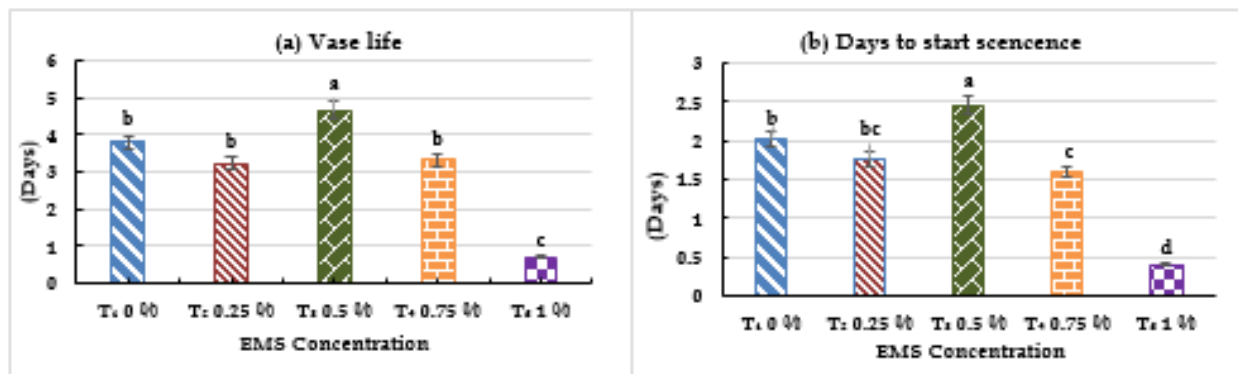


Figure 3 Stimulation of post-harvest (a) vase life (days) (b) days to start senescence (days). Variability induced by EMS in (*Iris kashmiriana*) showed statistically significant results at the LSD value ($p < 0.05$)