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Research Article

Induced mutagenesis for the development of high-yielding mutant lines of linseed (*Linum usitatissimum* L.) using caffeine and sodium azide

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Abstract

Generating variability is key for enhancing crops in any plant breeding initiative. This study aimed to create mutant lines with improved yield through induced mutation. *Linum usitatissimum* L. (variety 'Shekhar') was subjected to various concentrations of caffeine and sodium azide (0.10, 0.25, 0.50, 0.75, and 1.00%) before being sown to produce the M_1 generation. After harvesting and screening the M_1 variants, they were planted to cultivate the M_2 generation. The M_2 mutants undertook a thorough morphological examination for different mutation types and were then planted to develop the M_3 generation. Across the three generations, various parameters were analyzed, including quantitative traits (plant height, number of branches), morphological traits (cotyledonary, vegetative leaf, and flower mutants), physiological traits (chlorophyll and carotenoid content), cytological aspects, and yield characteristics. The results indicated that caffeine was more effective than sodium azide in generating beneficial mutants. In the M_3 generation, nine high-yielding mutant lines, labelled A, B1, B2, C1, C2, D, E, F1, and F2, were selected based on their morphological and yield-related traits. Also, SEM analysis was conducted on selected high-yielding mutants to examine stomatal variations in their leaves and to assess their seed morphology. These mutant lines have considerable breeding significance and should be advanced to future generations to promote their trait stability, ultimately creating new linseed cultivars with enhanced yield and better adaptability.

Keywords: caffeine; high-yielding mutants; *Linum*; mutagenesis; mutation breeding; M_1 generation; M_2 generation; M_3 generation; sodium azide

Introduction

Since the latter half of the 19th century, food systems have been influenced by globalization, which has increased the number of intermediaries between producers and consumers (Soria-Lopez *et al*., 2023).

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According to the United Nations Department of Economic and Social Affairs, Population Division (2024), the global population is projected to keep increasing over the next 50 to 60 years, reaching a peak of approximately 10.3 billion by the mid-2080s, rising from 8.2 billion in 2024. In addressing this contest, the Food and Agriculture Organization (FAO) recognizes the need to ensure adequate and reasonable food by promoting sustainable agricultural practices, as detailed in their report, "The Future of Food and Agriculture: Trends and Challenges." A substantial portion of the population in developing countries relies on herbal medicines sourced from nature at minimal cost. The expanding global herbal healthcare and wellness sector, including in India, has significantly increased the demand for medicinal plants, whether wild-harvested or cultivated (Goraya and Ved, 2017). The growing demand for wild resources raises concerns, as it may result in irreversible declines in species reproductive capabilities (Hasan *et al*., 2024a). Conserving these plants is crucial for maintaining a steady supply of raw materials for pharmaceutical companies and fostering economic growth and development (Shukla, 2023).

Linum usitatissimum L. (linseed or flaxseed), is a rich source of antioxidant and antidiabetic phytochemical compounds such as lignans, phenolic acids, and flavonoids and hence has a significant role in medicinal field (Sarfraz and Ahmad, 2024). Primarily cultivated as an oilseed crop, linseed has appeared as an alluring dietary component due to its extremely high concentration of bioactive chemicals. Secoisolariciresinol diglycoside (SDG) is one of the main bioactive components with promising pharmacological properties (Akter *et al*., 2021). It is widely used in treating various diseases like cardiovascular diseases, rheumatoid arthritis, inflammation, high cholesterol, diabetes, obesity, hypertension, kidney disorders, tumors, and cancer (Goyal *et al*., 2018).

Linseed, a self-pollinated, blue-flowering rabi crop belonging to the Linaceae family, is cultivated worldwide for its edible seeds, seed oil, and fibers extracted from its stems (Sarfraz and Ahmad, 2023). Flaxseeds contain approximately 35-45% lipids, 30% dietary fiber, 20-30% protein, 10% mucilage, and glycoside linamarin along with 5-6% gum. The protein content in flaxseeds primarily consists of about 80% globulins (linin and conlinin) and 20% glutelin (Arslanoğlu and Aytaç, 2020). According to FAOSTAT data 2022, global production of flaxseed increased by 38% from 2017 to 2022 with Russian Federation, Kazakhstan, Canada, China, and India among the leading producers (FAOSTAT data assessed on 27 August 2024). The flax market is estimated to be expanding rapidly, reflecting renewed interest likely driven by recent research advancements and the growing recognition of flax's diverse applications (Stavropoulos *et al*., 2023). Additionally, the increasing global demand and exploitation of linseed for several use researchers are focused on boosting linseed production. To achieve this, efforts are being directed toward developing improved crop varieties through hybridization techniques and induced mutagenesis (Jahan *et al*., 2020).

Evaluating the genetic diversity of germplasm is essential for identifying genotypes that exhibit greater diversity and excel in particular conditions. Over the years, the continuous selection for superior traits of linseed by conventional breeding methods has led to a loss of genetic diversity due to fixation. This has made breeders increasingly reliant on a smaller pool of superior genotypes, resulting in successive bottlenecks (Thakur *et al*., 2020). To overcome this mutation breeding programmes are adopted to widen the narrow genetic base of *Linum usitatissimum* L. The physical and chemical mutagens have been employed successfully in genetic improvement of many crops i.e. linseed (Kumar *et al*., 2021), *Trigonella* (Naaz *et al*., 2024a), chilli (Karim *et al*., 2022), *Lens culinaris* (Sharma *et al*., 2022). According to the FAO/IAEA Mutant Variety Database, mutation breeding has led to the development of several mutant varieties of *Linum usitatissimum* L. globally, enhancing traits such as disease resistance, early maturity, and high yield. The Indian mutant variety DLV 20, developed in 2019, was derived through EMS treatment of the NL-115 variety. This variety exhibits early maturity, compact growth, and significantly higher seed yield compared to the parent variety. Other significant mutant varieties include 'Baltyuchai' and 'M-5' from Russia, known for their disease resistance, 'Linola 989' from Canada, which improves oil quality, and 'Heiya 6' from China, noted for its resistance to salinity and lodging. Chemical mutagens deliver the advantage of creating mutant populations with high mutation densities

(Szarejko *et al.*, 2017) causing physiological damage, macro- and micromutations in genes, and chromosomal anomalies in the population, often inducing point mutations. Caffeine (1,3,7-trimethylxanthine), a purine alkaloid, occurs naturally in plants like coffee (*Coffea*), tea (*Camellia sinensis*), etc. (Alkhatib *et al.*, 2018). Its interaction with DNA alters its certain physical properties, such as DNA denaturation, which increases the frequency of spontaneous mutations, disrupts DNA repair mechanisms, and promotes DNA–DNA or DNA–protein cross-linking (Chung, 2021). Shahwar *et al.* (2017a) observed the mutagenic effect of caffeine on lentil. Studies on the genotoxicity of sodium azide (SA) (NaN_3) in different organisms confirmed the induction of gene mutation, AT→GC base pair transition and transversion chromosome aberrations (Gruszka *et al.*, 2012). The mutagenicity of SA is due to the development of azide compound that enters into the nucleus, interacts with DNA, and cause point mutation in the genome (Ingle *et al.*, 2018; Chaudhary *et al.*, 2021).

In the current study, chemical mutagens caffeine and sodium azide were employed to induce genetic variability in *Linum usitatissimum* L. and isolate promising high-yielding M_3 mutants and their characterization on basis of cyto-morphological, physiological and yield parameters.

Materials and Methods

Plant material

Certified healthy seeds (M_0) of *Linum usitatissimum* L. (var. Shekhar) were procured from the Indian Council of Agricultural Research (ICAR)- National Bureau of Plant Genetic Resources, New Delhi.

M_1 generation

Linum M_0 seeds pre-soaked in double distilled water (DDW) for 24 hours, were subjected to five different concentrations of caffeine and SA (0.10, 0.25, 0.50, 0.75 and 1.00%) at room temperature for 9 hrs. The chemicals used, caffeine and sodium azide, were purchased from Sigma-Aldrich, Mumbai, India. Control (untreated) and treated seeds were sown in 4 replications (25 seeds each) in earthen pots during the rabi season (mid-October 2021) to raise the M_1 generation. The plants were regularly irrigated and weeded throughout the cultivation process.

M_2 generation

The seeds of M_1 generation were harvested separately according to the concentration of both the mutagens and the control plants. Randomly selected healthy seeds were sown in mid-October 2022 to induce the M_2 generation. During this stage, treated plants were screened, leading to the identification of 25 high-yielding mutant lines. Plants with distinctive phenotypes were chosen based on stable traits, including plant height (cm), branches per plant, capsules per plant, seeds per capsule, seed weight (g), and total seed yield per plant (g). The selected mutant lines were then advanced for further study in the M_3 generation.

M_3 generation

All seeds of M_2 generation were harvested separately and 25 seeds from each selected high-yielding mutant lines were sown in mid-October 2023 to grow M_3 generation. Nine high-yielding mutants (denoted as Mutant A, Mutant B1, Mutant B2, Mutant C1, Mutant C2, Mutant D, Mutant E, Mutant F1, Mutant F2) were selected based on their plant yield. The control and these mutants underwent evaluations, including morphological, physiological, cytological and yield parameters, and Scanning Electron Microscopy of their stomata and seeds.

Biological damage

Seed germination, plant survival, and pollen fertility were recorded for each generation and were calculated using formula:

$$\text{Seed germination \%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100 \quad (1)$$

$$\text{Plant survival \%} = \frac{\text{Number of plants at maturity}}{\text{Number of seeds germinated}} \times 100 \quad (2)$$

$$\text{Pollen fertility \%} = \frac{\text{Number of fertile pollens}}{\text{Total number of pollens}} \times 100 \quad (3)$$

Chlorophyll and carotenoid content

The pigment content of leaves in M_1-M_3 generation was determined as suggested by Mackinney (1941) using formula:

$$\text{Total chlorophyll content (g}^{-1}\text{ fresh leaves)} = \frac{[20.2\ (OD645) + 8.02\ (OD663)] \times V}{(1000 \times w)} \quad (4)$$

$$\text{Carotenoid content (g}^{-1}\text{ fresh leaves)} = \frac{[7.6\ (OD480) - 1.49\ (OD510)] \times V}{(1000 \times w \times d)} \quad (5)$$

Wherever, V, the volume of an extract; W, the mass of leaf tissues; d, the length of light path (1.4cm).

Mutagenic effectiveness and efficiency

Mutation frequency:

$$\text{\% mutated plant progeny (Mp)} = \frac{\text{Noumber of mutant plant progrenies segregating in M2}}{\text{Total number of M1 plant progenies}} \quad (6)$$

Mutagenic effectiveness and efficiency were determined in the M_2 generation as proposed by Konzak *et al.* (1965).

$$\text{Mutagenic effectiveness} = \frac{\text{Percentage of mutated plant progenies(Mp)}}{\text{Mutagen concentration} \times \text{treatment duration(h)}} \quad (7)$$

$$\text{Mutagenic efficiency} = \frac{\text{Percentage of mutated plant progenies(Mp)}}{\text{Biological damage}} \quad (8)$$

Biological damage was based on chromosomal abnormalities.

High-yielding M_3 mutants

High-yielding mutants were selected based on morphological (such as growth habit, plant height, shape and colour of leaves and flowers, and shape, size and colour of capsules and seeds), physiological (chlorophyll mutants), and yield parameters (number of capsules per plant, number of seeds per capsule, seed weight in grams, and total seed yield per plant in grams).

Scanning electron microscopy (SEM)

Scanning electron microscopy was employed to analyze both stomatal apertures and seed micromorphology of isolated high-yielding mutants. The procedure followed the protocol described by Naaz *et al.* (2023). The analysis was conducted using a JEOL JSM-6510LV SEM (JAPAN) microscope at 15kV, with various magnifications at the USIF, Aligarh Muslim University, Aligarh.

Statistical analysis

Experiments were performed in four replicates for each treatment, with data presented as the mean ± standard error. Statistical analysis was carried out using R software (R Studio), applying one-way ANOVA to

determine the least significant difference (LSD) between treatments at significance levels of $p < 0.05$ and $p < 0.01$.

Results

Seed germination, plant survival and pollen fertility in M_1, M_2 and M_3 generation

The seed germination percentages across three generations (M_1, M_2, and M_3) were analyzed for both caffeine and SA treatments. The untreated plants show the highest seed germination rates across all generations, with a mean of 94.00% (±1.88) for both caffeine and SA in the M_1 generation, 95% (±1.87) in the M_2 generation, and 96% (±1.86) in the M_3 generation. Following the control, the subsequent lines show the effects of increasing concentrations of caffeine and SA on seed germination. As the concentration of these chemicals increases, there is a significant decline in germination rates. In the M_1 generation, the germination rate for caffeine-treated seeds decreases from 86.00% (±2.59) to 52.00% (±5.03), and for SA-treated seeds, from 81.00% (±2.71) to 49.00% (±5.16). This trend continues similarly in the M_2 and M_3 generations. In the M_2 generation, the germination rate for caffeine-treated seeds drops from 89% (±2.52) to 62% (±4.91) as concentration increases, while for SA-treated seeds, it decreases from 85% (±2.62) to 58% (±5.08). Similarly, in the M_3 generation, the germination rate for caffeine-treated seeds declines from 92% (±2.42) to 74% (±4.80), and for SA-treated seeds, from 89% (±2.53) to 68% (±4.90) with rising concentrations. This consistent pattern across M_1, M_2, and M_3 generations indicates that higher concentrations of caffeine and SA lead to reduced seed germination rates.

A similar decreasing pattern was observed for plant survival and pollen fertility across the three generations (Figure 1).

Plant height and number of fertile branches per plant

In M_1 generation plant height in untreated plants recorded as 94.94 cm (±0.64). As the concentration of mutagens increases, the plant height rises significantly in lower concentrations and then declines. For caffeine-treated plants, the average plant height decreases from 101.03 (±0.98) to 87.88 cm (±1.78), and for SA-treated plants, it decreases from 100.46 (±1.06) to 86.38 cm (±2.04). In M_2 the average plant height of control was 95.05 cm (±0.63) which decreases from 102.23 (±0.94) to 88.78 cm (±1.74) and 101.07 (±1.04) to 87.16 cm (±2.00) in caffeine and SA treated plants respectively. Similar trend observed in M_3 generation where the average plant height of control was 95.59 cm (±0.61) which decreases from 102.57 (±0.91) to 89.15 cm (±1.70) and 101.49 (±1.02) to 87.93 cm (±1.96) in caffeine and SA treated plants respectively.

The number of branches per plant across three generations (M_1, M_2, and M_3) under caffeine and SA treatments were recorded. In the M_1 generation, the control group shows a mean number of branches per plant of 4.64 (±0.07). As the concentration of mutagens increases, the number of branches significantly rises initially and then significantly declines at higher concentrations. For caffeine-treated plants, it decreases from 5.68 (±0.10) to 3.88 (±0.13), and for SA-treated plants, it decreases from 5.34 (±0.11) to 3.69 (±0.15). In the M_2 generation, the control group maintains a mean of 4.7 (±0.06) branches per plant. With increasing concentrations, caffeine-treated plants show a mean number of branches ranging from 5.71 (±0.09) to 3.96 (±0.12), while SA-treated plants show a mean of 5.41 (±0.10) to 3.76 (±014). For the M_3 generation, the control group's mean remains at 4.68 (±0.06) branches per plant. The caffeine-treated plants exhibit a mean number of branches from 5.79 (±0.08) to 4.05 (±0.11), and the SA-treated plants range from 5.51 (±0.09) to 3.81 (±0.13).

Overall, while the plant height and number of branches per plant initially increases with low concentrations of caffeine and SA, it decreases at higher concentrations, consistent across the M_1, M_2, and M_3 generations (Figure 2).

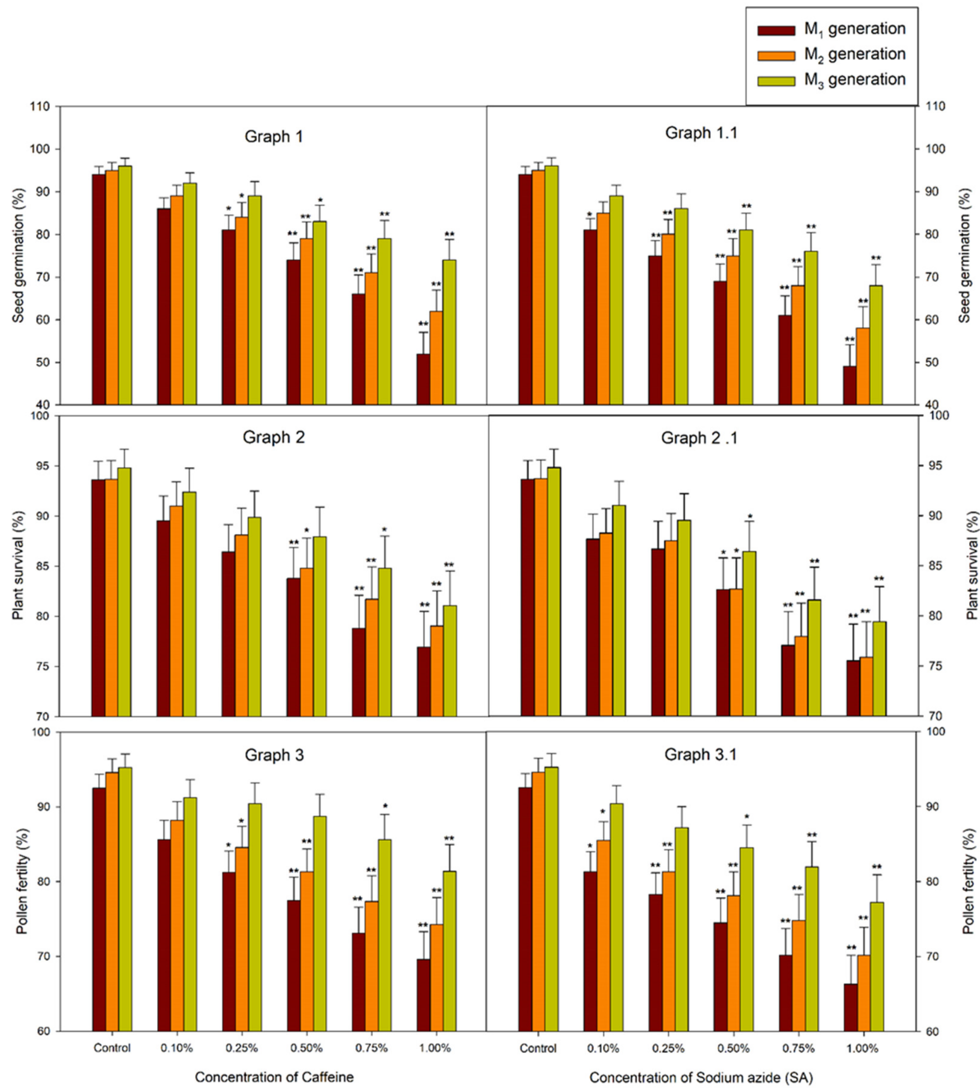

Figure 1. Seed germination (%), plant survival (%) and pollen fertility of control and mutagen (caffeine and sodium azide) treated plants in M_1, M_2 and M_3 generation

The results are expressed as the mean ± SE, and the * and ** denote the significant difference among treatments at ($p < 0.05$) and ($p < 0.01$) respectively, according to LSD tests.

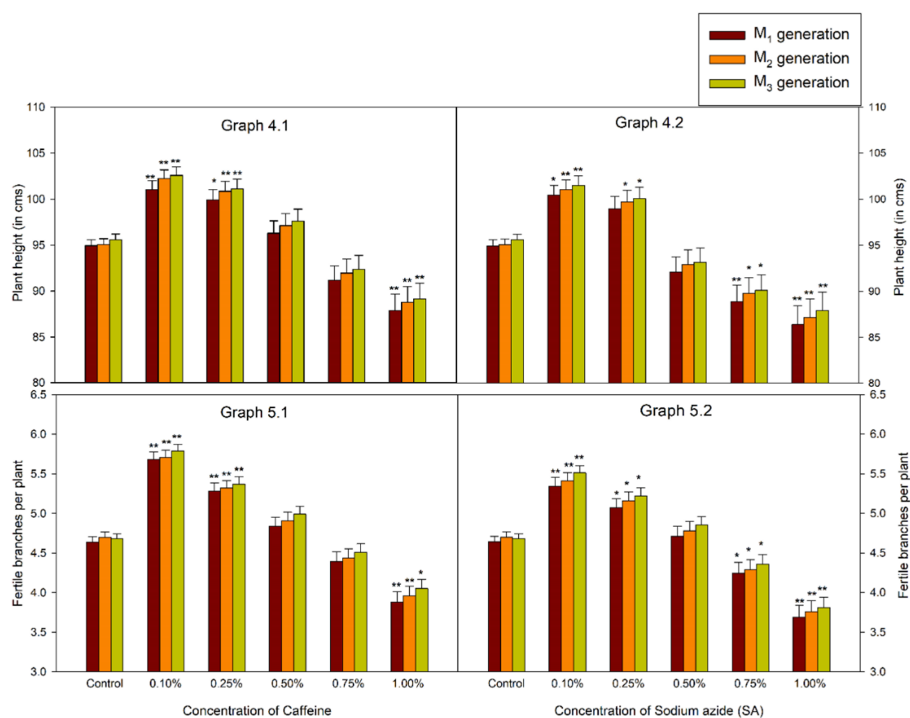

Figure 2. Plant height and number of fertile branches per plant of control and mutagen (caffeine and sodium azide) treated plants in M_1, M_2 and M_3 generation

The results are expressed as the mean ± SE, and the * and ** denote the significant difference among treatments at (p < 0.05) and (p <0.01) respectively, according to LSD tests.

Yield parameters

Different yield parameters like number of capsules per plant, number of seeds per capsules, weight of 1000 seeds (g) and total yield per plant (g) were recorded for three generations. Initially these parameters increased with lower dose of caffeine and SA, and get decreased as the dose increases. In the M_1 generation, the untreated plants show a mean number of capsules per plant of 48.98 (±0.57). As the concentration of mutagens increases, the number of capsules significantly rises in lower concentration and then significantly declines at increasing higher concentrations. For caffeine-treated plants, it decreases from 52.11 (±0.66) to 46.33 (±1.08), and for SA-treated plants, it decreases from 51.24 (±0.70) to 41.22 (±1.09). In the M_2 generation, the control group maintains a mean of 49.29 (±0.56) capsules per plant. With increasing concentrations, caffeine-treated plants show a mean number of capsules ranging from 53.18 (±0.65) to 47.73 (±1.06), while SA-treated plants show a mean of 52.26 (±0.69) to 42.66 (±1.07). For the M_3 generation, the control group's mean remains at 49.53 (±0.55) capsules per plant. The caffeine-treated plants exhibit a mean number of capsules from 54.07 (±0.64) to 48.88 (±1.04), and the SA-treated plants range from 53.14 (±0.67) to 43.76 (±1.05).

The number of seeds per capsule across three generations were analyzed for both caffeine and SA treatments. In the M_1 generation, the untreated plants show a mean number of seeds per capsule of 7.96 (±0.13). As the concentration of mutagens increases, the number of seeds rises in lower concentrations and then declines. For caffeine-treated plants, the average number of seeds per capsules decreases from 8.45 (±0.19) to 6.67 (±0.36), and for SA-treated plants, it decreases from 8.27 (±0.20) to 6.56 (±0.37). In M_2 the average seeds per capsule of control was 8.01 (±0.1) which decreases from 8.57 (±0.16) to 6.74 (±0.33) and 8.34 (±0.18) to 6.67 (±0.35) in caffeine and SA treated plants respectively. Similar trend observed in M_3 generation where the average plant height of control was 7.98 (±0.11) which decreases from 8.69 (±0.15) to 6.81 (±0.32) and 8.41 (±0.17) to 6.72 (±0.33) in caffeine and SA treated plants respectively.

Weight of 1000 seeds (g) and total yield per plant (g) were recorded for three generations and it was observed that the weight get increased in lower doses and then declined gradually in higher doses of mutagens. In the M_1 generation, the control plants show average weight of 1000 seeds and total yield per plant of 6.36 (±0.08) and 2.47 (±0.09) respectively. For caffeine-treated plants, the average weight of 1000 seeds (g) and total yield per plant (g) decreases from 6.48 (±0.10) to 5.98 (±0.16) and 2.86 (±0.11) to 1.82 (±0.14) respectively. For SA-treated plants, 1000 seeds weight and total yield decreases from 6.45 (±0.11) to 5.81 (±0.18) and 2.75 (±0.11) to 1.59 (±0.15) respectively. In M_2 the average weight of 1000 seeds (g) and total yield per plant (g) of control was 6.39 (±0.07) and 2.51 (±0.08) respectively. For caffeine-treated plants, the average weight of 1000 seeds (g) and total yield per plant (g) decreases from 6.53 (±0.09) to 6.04 (±0.15) and 2.99 (±0.10) to 1.93 (±0.13) respectively. For SA-treated plants, 1000 seeds weight and total yield decreases from 6.49 (±0.10) to 5.85 (±0.16) and 2.85 (±0.11) to 1.67 (±0.14) respectively. Similarly, in M_3 generation the average weight of 1000 seeds (g) and total yield per plant (g) of control was 6.41 (±0.06) and 2.53 (±0.07) respectively. For caffeine-treated plants, the average weight of 1000 seeds (g) and total yield per plant (g) decreases from 6.59 (±0.08) to 6.09 (±0.13) and 3.11 (±0.09) to 2.01 (±0.13) respectively. For SA-treated plants, 1000 seeds weight and total yield decreases from 6.53 (±0.09) to 5.90 (±0.15) and 2.94 (±0.10) to 1.72 (±0.14) respectively.

Overall, while the yield parameters usually increase significantly with low concentrations of caffeine and SA, it decreases at higher concentrations, consistent across the all three generations (Figure 3).

Chlorophyll and carotenoid contents

Physiological parameters viz., total chlorophyll and carotenoid content were examined across three generations. Result revealed that the treatments with caffeine and SA had a significant impact on chlorophyll and carotenoid levels. In the M_1 generation, the control plants show chlorophyll and carotenoid 1.33 (±0.03) and 0.82 (±0.02) mg/g respectively. Chlorophyll content decreases from 1.53 (±0.05) to 1.19 (±0.10) mg/g and 1.48 (±0.06) to 1.11 (±0.11) mg/g, while carotenoid levels decrease from 0.94 (±0.03) to 0.72 (±0.06) mg/g and 0.91 (±0.04) to (±0.07) 0.69 mg/g in caffeine and SA treated population respectively. In the M_2 generation, the control had chlorophyll and carotenoid level of 1.31 (±0.03) and 0.83 (±0.02) mg/g respectively. Chlorophyll content decreases from 1.58 (±0.04) to 1.20 (±0.09) mg/g and 1.54 (±0.05) to 1.16 (±0.10) mg/g, while carotenoid levels decrease from 0.98 (±0.02) to 0.76 (±0.05) mg/g and 0.96 (±0.03) to 0.72 (±0.06) mg/g in caffeine and SA treated population respectively. In the M_3 generation, the control had chlorophyll and carotenoid level of 1.35 (±0.02) and 0.85 (±0.03) mg/g respectively. Chlorophyll content decreases from 1.62 (±0.04) to 1.24 (±0.08) mg/g and 1.58 (±0.05) to 1.23 (±0.09) mg/g, while carotenoid levels decrease from 1.01 (±0.02) to 0.79 (±0.05) mg/g and 0.97 (±0.03) to 0.75 (±0.06) mg/g in caffeine and SA treated population respectively. Overall, both the physiological parameters increase significantly with lower concentrations of caffeine and SA with decreased value at higher concentrations, consistent across the all three generations (Figure 4).

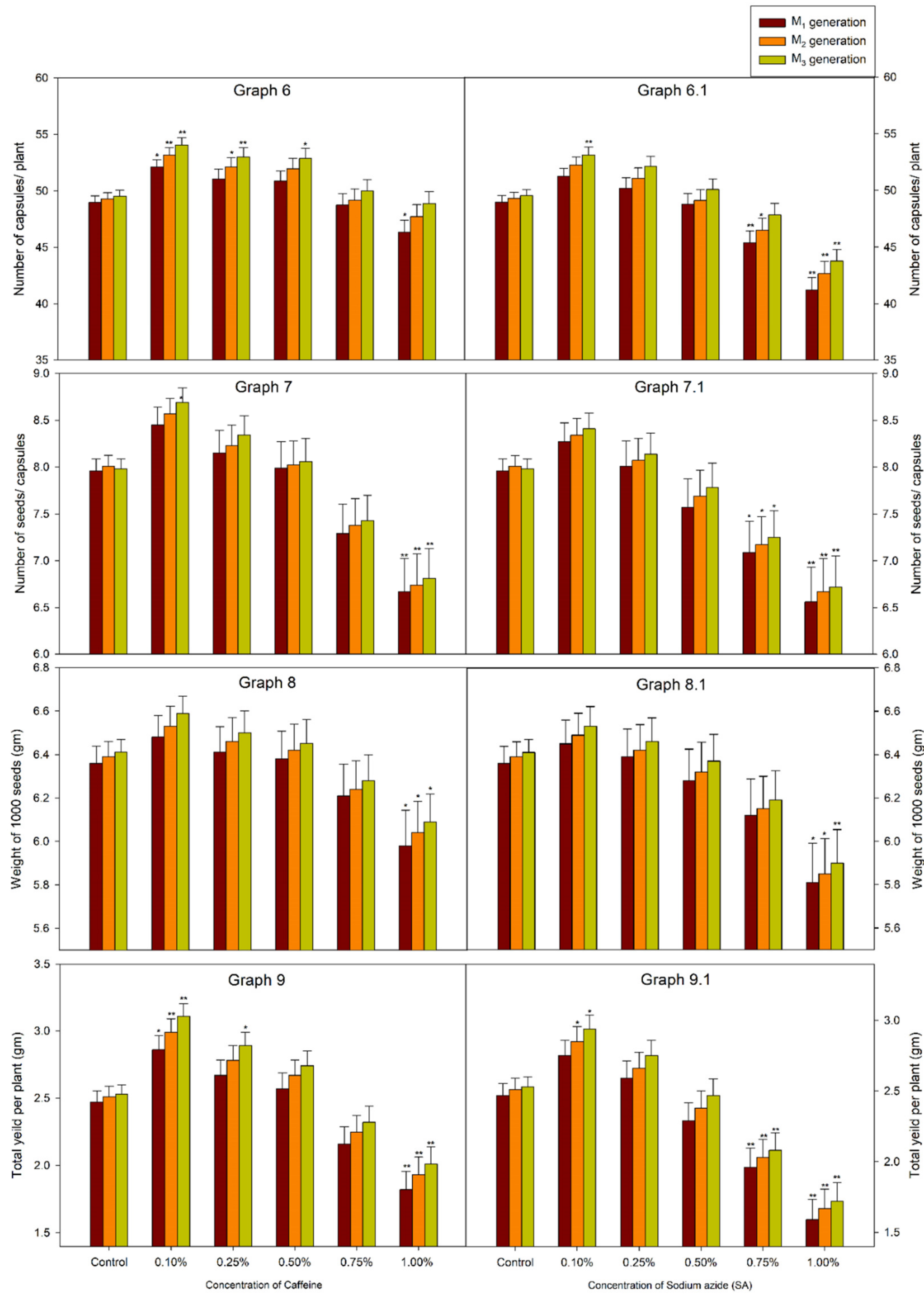

Figure 3. Yield parameters of control and mutagen (caffeine and sodium azide) treated plants in M_1, M_2 and M_3 generation

The results are expressed as the mean ± SE, and the * and ** denote the significant difference among treatments at (p < 0.05) and (p <0.01) respectively, according to LSD tests

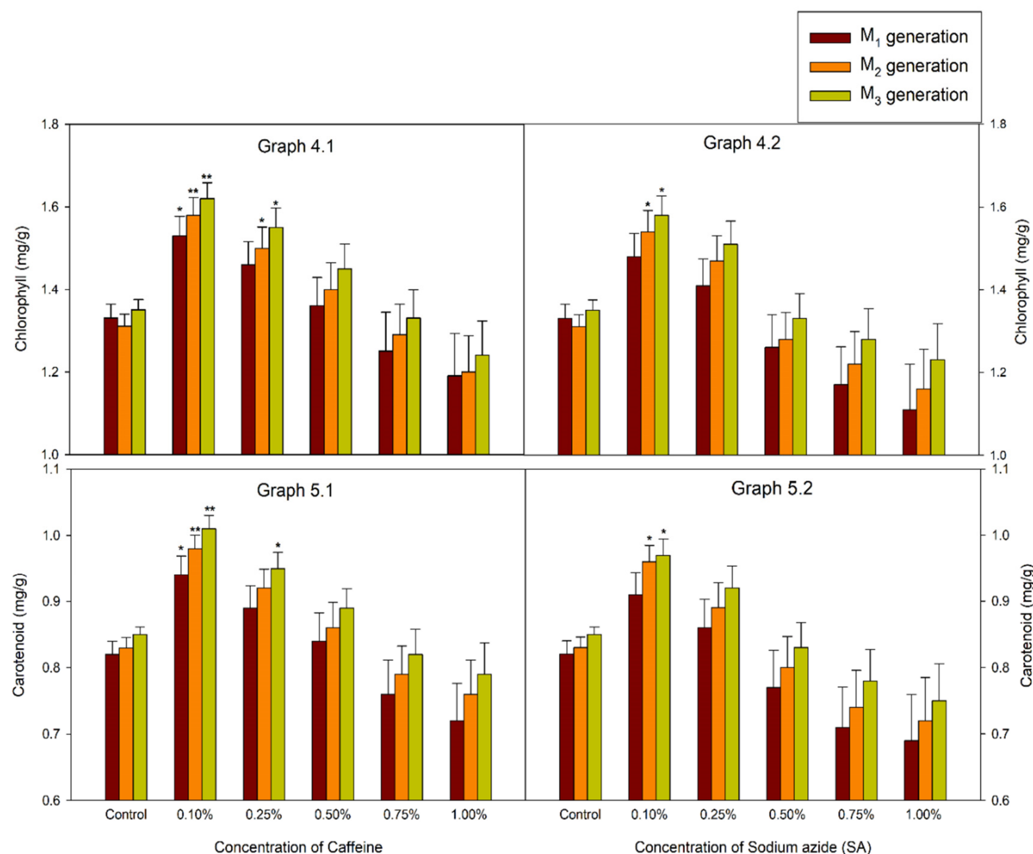

Figure 4. Chlorophyll and carotenoid content of control and mutagen (caffeine and sodium azide) treated plants in M_1, M_2 and M_3 generation

The results are expressed as the mean ± SE, and the * and ** denote the significant difference among treatments at (p < 0.05) and (p <0.01) respectively, according to LSD test.

Morphological variants/mutants

The immediate effects of mutagens were observed in the formation of cotyledonary and vegetative leaves in the M_1 generation, with some of these traits being inherited in the M_2 and M_3 generations. In the control group, cotyledonary leaves were characterized as two opposite, elliptical, green, entire, obtuse, and smooth leaves of equal size (Figure 5A). Various cotyledonary variants observed in M_1 generation viz., number get increased to three and four leaves, one leaf get bifurcated, leaves get curved at apex, chlorophyll variant with curved at middle, deformed leaves, both leaves oriented towards one side with long hypocotyl, etc. (Figure 5B-H). Cotyledonary mutants observed in M_2 and M_3 generation are three leaves, notching at apex, leaves get rolled toward lower side, deformed leaves with both oriented to one side, elongated leaves, small leaves with chlorophyll mutant, kidney shaped, heart shaped, small cotyledonary leaves with elongated hypocotyl, etc. (Figure 5I-T).

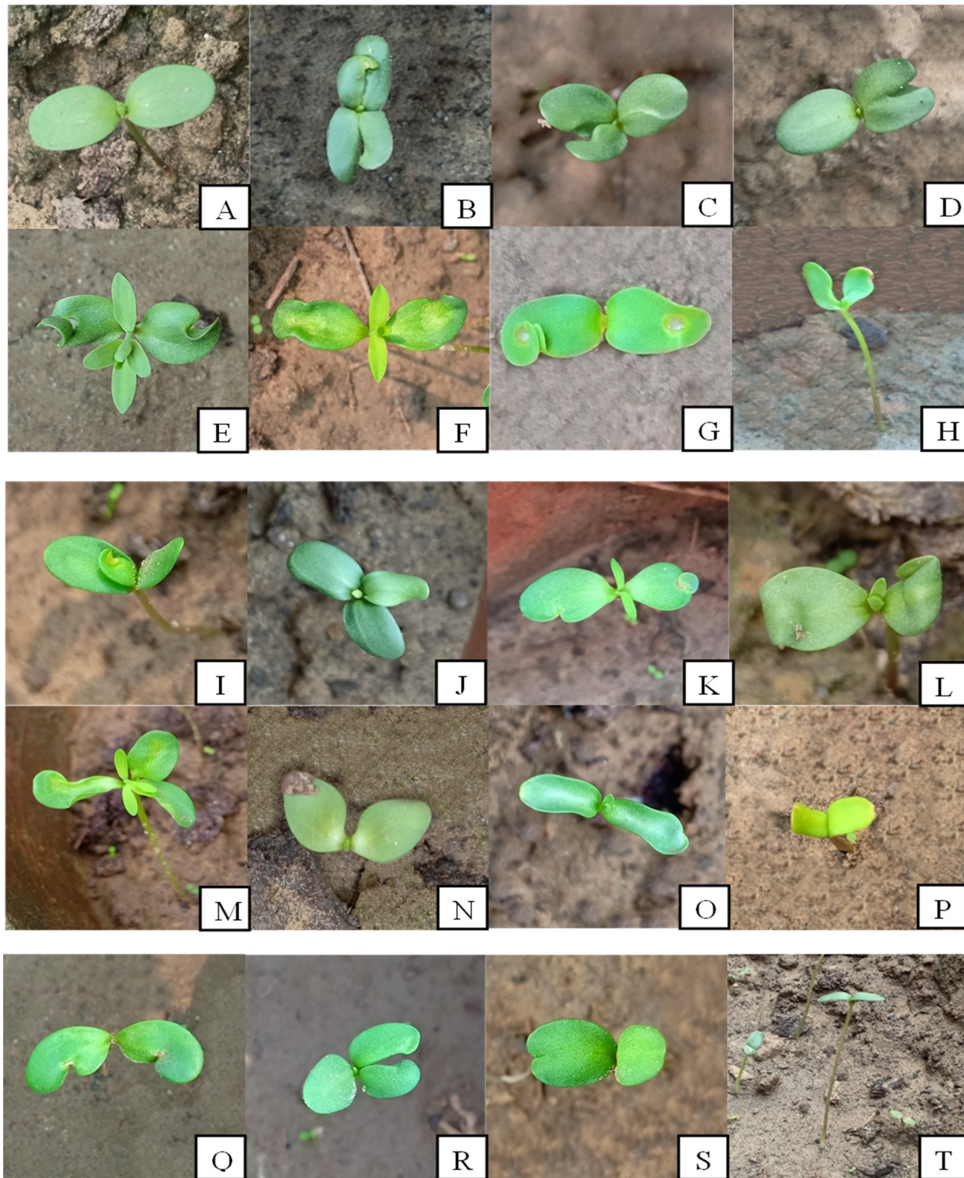

Figure 5. (A) Control cotyledonary leaves, (B-H) Cotyledonary leaf variants in M_1 generation, (I-P) Cotyledonary leaf mutants in M_2 generation, (Q-T) Cotyledonary leaf mutants in M_3 generation

Vegetative leaves of control plants were lanceolate, green, entire, acute, smooth and arranged in an alternate pattern along the stem (Figure 6A). Vegetative leaf variations were observed in M_1 generation are four leaves (2+2) born at a node with two leaves fused completely and other two fused partially, fused heart shaped leaves, two leaves fused along their middle surface, three fused leaves of which two fused completely and one fused at base, four fused leaves, shorting of internode at apex forming bushy appearance, etc. (Figure 6B-H). Abnormalities in mutants leaves in M_2 and M_3 generations were leaf with broad base, small leaves with distorted shape and margins, three leaves fused out of which two leaves fused completely and one fused partially, four leaves (3+1) fused with three fused and one born oppositely, two fused leaves one with broad base, two narrow leaves fused at base, deformed leaves with broad apex, vegetative leaves with xantha chlorophyll mutants, sickle shaped small leaves, vegetative leaves with shortened internode born on flattened stem, vegetative leaves with chlorina chlorophyll mutants, leaves forming bushy appearance at apex, etc. (Figure 6I-T).

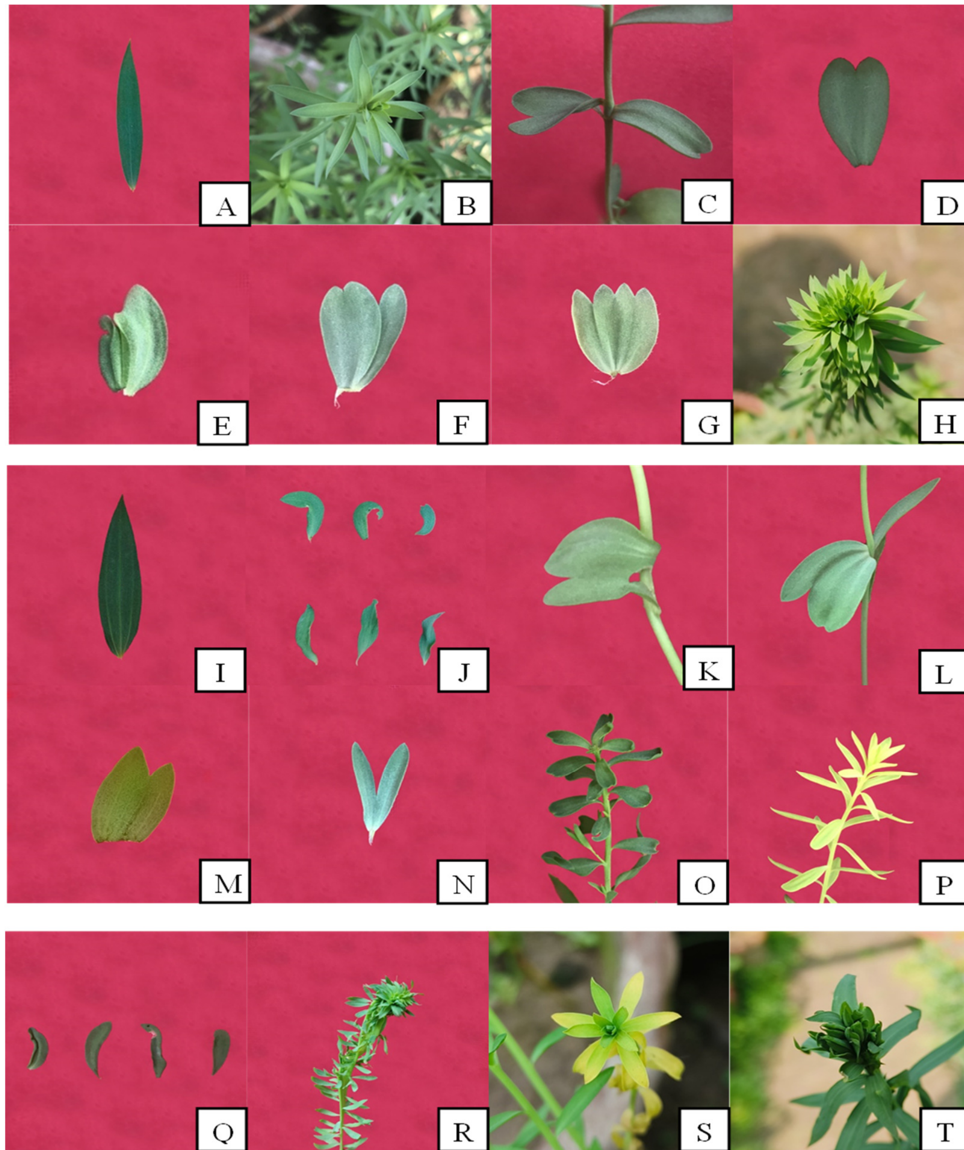

Figure 6. (A) Control vegetative leaf, (B) Control vegetative leaves at apex, (C-H) Vegetative leaf variants in M_1 generation, (I-P) Vegetative leaf mutants in M_2 generation, (Q-T) Vegetative leaf mutants in M_3 generation

In control plants flowers were pale bluish-purple in colour with 5 petals (corolla) with darker streaks, smooth margin with twisted astivation and five blue-tipped stamens and a white centre with a green ovary (Figure 7A). The variations observed in M_1 generations were flower with distorted shape and astivation, flower with six, seven, and nine petals, flower with dark blue and dark purple petals with distorted astivation, flower with white petals, etc. (Figure 7B-H). In M_2 and M_3 generations mutant flower have various abnormalities viz, petals of different size and shape with distorted astivation, circular holes at tip of petals, smaller size flower, flower with six petals, flask shaped flower, flower with six and dark blue petals, flower with dark blue and white petals, flower with bold and thick white-coloured stamens, flask shaped flower, light blue flower with six petals, white coloured flower with distorted astivation, etc. (Figure 7I-T).

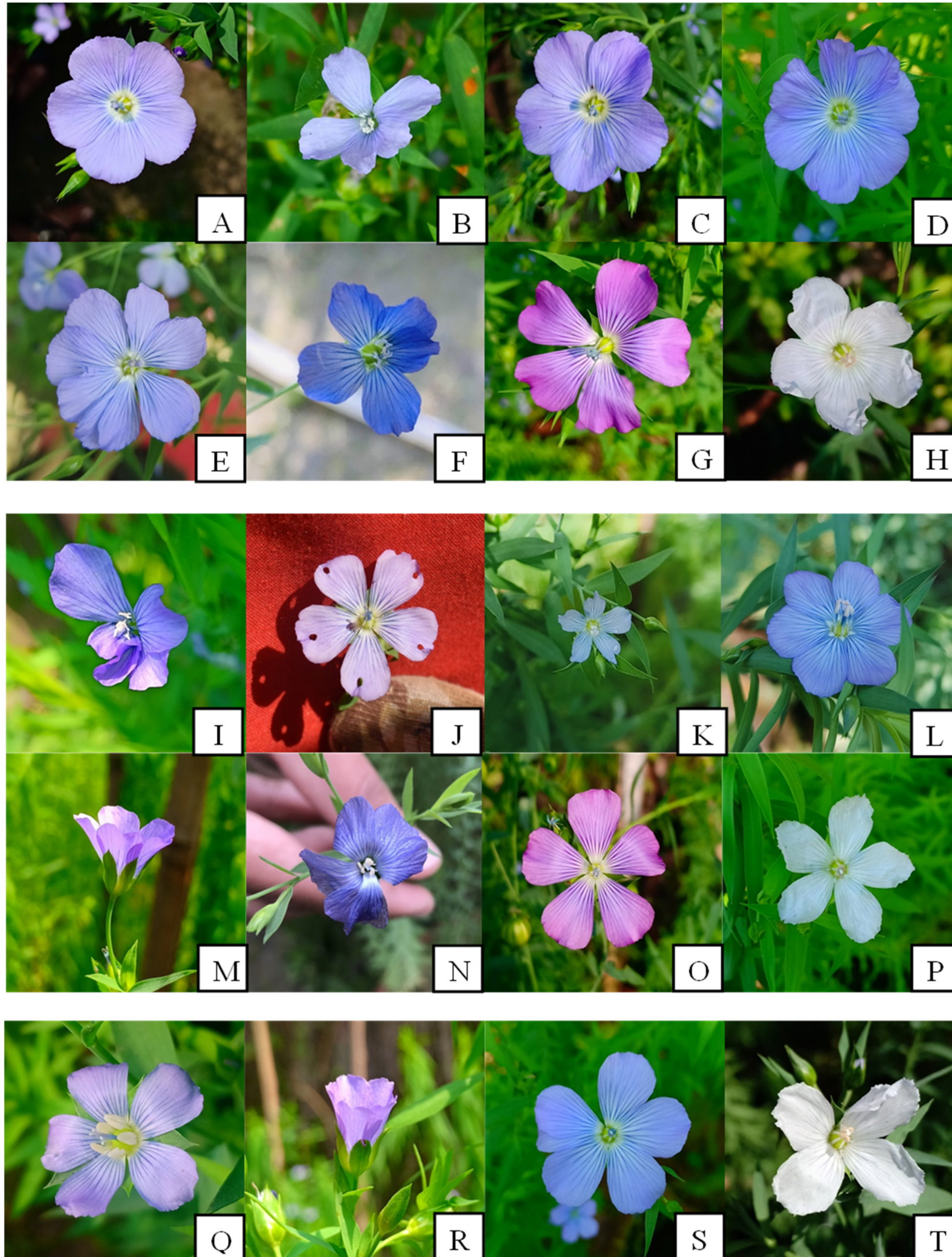

Figure 7. (A) Flower (Control), (B-H) Flower variants in M_1 generation, (I-P) Flower mutants in M_2 generation, (Q-T) Flower mutants in M_3 generation

Cytological abnormalities

Control plant showed normal meiotic division with 15 pairs of ring bivalents (2n=30) at diakinesis. At metaphase-I, all 15 bivalents were normally arranged at equator, followed by the separation of 15-15 chromosomes (univalents) to their respective poles at anaphase-I. Telophase-I showed two groups of 15 chromosomes at each pole. Metaphase-II displayed two groups of normal chromosomes at two equatorial planes, followed by four groups of chromosomes moving towards opposite poles at anaphase-II. At telophase-II, four groups of chromosomes were present on their respective poles. Various chromosomal abnormalities were recorded in different stages of meiosis across three generations viz., prophase (univalents, multivalents),

metaphase I/II (univalents, multivalents, precocious movements of chromosomes, stray chromosomes, stickiness), anaphase I/II (laggards, bridges, stickiness, unequal separation of chromosomes), telophase I/II (laggards, bridges, micro- and multi nucleate conditions, disturbed polarity and cytomixis). In M_1 generation, the percentage of total abnormal cells increased from 3.40 to 24.02% and 5.24 to 30.59% in in caffeine and SA treated population respectively. In M_2 generation, the percentage of total abnormal cells increased from 2.62 to 20.16% and 3.50 to 24.81% in in caffeine and SA treated population respectively. In M_3 generation, the percentage of total abnormal cells increased from 1.49 to 17.93% and 2.64 to 21.12% in caffeine and SA treated population respectively (Table 1-3).

Table 1. Frequency of chromosomal abnormalities induced by caffeine and SA treatments in M_1 generation

Generation M_1		**Caffeine**						**SA**					
Treatments		Cont.	0.10%	0.25%	0.50%	0.75%	1.00%	Cont.	0.10%	0.25%	0.50%	0.75%	1.00%
Total no. of PMCs observed		284	265	261	257	255	254	284	267	256	255	253	255
Prophase (diakinesis)	Univalents				1	2	2		1	1	2	3	3
	Multivalents		1	2	2	2	3		1	2	3	3	4
	Abnormal PMCs (%)		**0.38**	**0.77**	**1.17**	**1.57**	**1.97**		**0.75**	**1.17**	**1.96**	**2.37**	**2.75**
Metaphase I/II	Univalents				2	2	4				3	3	4
	Multivalents		1	1	2	3	4		1	1	3	4	5
	Precocious Movements				1	2	3				2	3	4
	Stray chromosomes		1	1	2	2	3		1	2	3	3	4
	Stickiness		1	2	3	4	4		1	2	3	4	4
	Abnormal PMCs (%)		**1.13**	**1.53**	**3.89**	**5.10**	**7.09**		**1.12**	**1.95**	**5.49**	**6.72**	**8.24**
Anaphase I/II	Laggards		1	2	2	3	5		2	2	3	4	5
	Bridges		1	1	2	3	4		1	1	2	3	4
	Stickiness				2	2	3			1	2	3	5
	Unequal separation				1	2	3		1	1	2	3	4
	Abnormal PMCs (%)		**0.75**	**1.15**	**2.72**	**3.92**	**5.51**		**1.50**	**1.95**	**3.53**	**5.14**	**7.06**
Telophase I/II	Laggards		1	1	2	3	5		2	2	3	4	5
	Bridges				1	2	3				2	3	5
	Unequal separation		1	1	2	2	3		2	1	2	3	4
	Micro-nucleate		1	1	2	2	2			2	3	3	5
	Multi-nucleate			1	2	3	4			1	3	4	5
	Disturbed Polarity			1	1	2	3		1	1	3	4	4
	Cytomixis				1	2	3			1	1	3	4
	Abnormal PMCs (%)		**1.13**	**1.92**	**4.28**	**6.27**	**9.06**		**1.87**	**3.13**	**6.67**	**9.49**	**12.55**
Total no. of abnormal PMCs			9	14	31	43	61		14	21	45	60	78
Total % of abnormal PMCs			**3.40**	**5.36**	**12.06**	**16.86**	**24.02**		**5.24**	**8.20**	**17.65**	**23.72**	**30.59**

Table 2. Frequency of chromosomal abnormalities induced by caffeine and SA treatments in M_2 generation

Generation M_2		**Caffeine**						**SA**					
Treatments		**Cont.**	**0.10%**	**0.25%**	**0.50%**	**0.75%**	**1.00%**	**Cont.**	**0.10%**	**0.25%**	**0.50%**	**0.75%**	**1.00%**
Total no. of PMCs observed		285	267	263	255	257	258	285	257	261	254	257	258
Prophase (diakinesis)	Univalents				1	1	2			1	1	2	2
	Multivalents			1	1	2	3		1	1	2	2	3
	Abnormal PMCs (%)		**0**	**0.38**	**0.78**	**1.17**	**1.94**		**0.39**	**0.77**	**1.18**	**1.56**	**1.94**
Metaphase I/II	Univalents				2	2	3				2	3	3
	Multivalents			1	1	2	3		1	1	2	3	4
	Precocious Movements				1	2	2			1	1	2	3
	Stray chromosomes		1	1	2	2	3		1	1	2	2	2
	Stickiness		1	1	2	3	4		1	2	3	3	4
	Abnormal PMCs (%)		**0.75**	**1.14**	**3.14**	**4.28**	**5.81**		**1.17**	**1.92**	**3.94**	**5.06**	**6.20**
Anaphase I/II	Laggards		1	1	2	2	3		1	1	3	3	4
	Bridges			1	1	2	4		1	1	2	2	4
	Stickiness		1	1	2	3	3			2	1	2	4
	Unequal separation				1	2	3			1	2	3	3
	Abnormal PMCs (%)		**0.75**	**1.14**	**2.35**	**3.50**	**5.04**		**0.78**	**1.92**	**3.15**	**3.89**	**5.81**
Telophase I/II	Laggards			1	2	3	4		1	2	3	4	5
	Bridges				1	2	2				2	3	4
	Unequal separation		1	1	2	3	3		1	2	2	4	4
	Micro-nucleate		1	1	1	2	3			1	2	3	3
	Multi-nucleate				1	2	2				2	3	4
	Disturbed Polarity		1	1	2	2	3		1	2	3	4	5
	Cytomixis			1	1	2	2			1	2	2	3
	Abnormal PMCs (%)		**1.12**	**1.90**	**3.92**	**6.23**	**7.36**		**1.17**	**3.07**	**6.30**	**8.95**	**10.85**
Total no. of abnormal PMCs			7	12	26	39	52		9	20	37	50	64
Total % of abnormal PMCs			**2.62**	**4.56**	**10.20**	**15.18**	**20.16**		**3.50**	**7.66**	**14.57**	**19.46**	**24.81**

Table 3. Frequency of chromosomal abnormalities induced by caffeine and SA treatments in M_3 generation

Generation M_3		Caffeine						SA					
Treatments		Cont.	0.10%	0.25%	0.50%	0.75%	1.00%	Cont.	0.10%	0.25%	0.50%	0.75%	1.00%
Total no. of PMCs observed		287	269	267	259	258	251	287	262	259	257	257	258
Prophase (diakinesis)	Univalents				1	1	2				1	1	2
	Multivalents			1	1	2	2			1	1	2	2
	Abnormal PMCs (%)		**0.00**	**0.37**	**0.77**	**1.16**	**1.59**		**0.00**	**0.39**	**0.78**	**1.18**	**1.59**
Metaphase I/II	Univalents				1	2	3				1	1	3
	Multivalents			1	1	2	2			1	1	2	2
	Precocious Movements			1	1	2	2			1	1	2	2
	Stray chromosomes				1	1	2		1	1	2	2	2
	Stickiness		1	1	2	2	3		1	1	2	2	3
	Abnormal PMCs (%)		**0.37**	**1.12**	**2.32**	**3.49**	**4.78**		**0.75**	**1.57**	**2.75**	**3.54**	**4.78**
Anaphase I/II	Laggards				1	2	3			1	2	2	3
	Bridges			1	1	2	3			1	1	2	3
	Stickiness		1	1	2	3	3		1	1	1	2	4
	Unequal separation				1	1	2		1	1	2	3	3
	Abnormal PMCs (%)		**0.37**	**0.75**	**1.93**	**3.10**	**4.38**		**0.75**	**1.57**	**2.35**	**3.54**	**5.18**
Telophase I/II	Laggards			1	1	2	3			1	2	3	4
	Bridges				1	2	2				2	3	3
	Unequal separation				2	3	3		1	1	2	3	4
	Micro-nucleate		1	1	2	2	3		1	1	2	3	3
	Multi-nucleate			1	1	2	2				1	3	3
	Disturbed Polarity		1	1	2	2	3		1	2	3	3	4
	Cytomixis			1	1	2	2			1	2	2	3
	Abnormal PMCs (%)		**0.74**	**1.87**	**3.86**	**5.81**	**7.17**		**1.13**	**2.36**	**5.49**	**7.87**	**9.56**
Total no. of abnormal PMCs			4	11	23	35	45		7	15	29	41	53
Total % of abnormal PMCs			**1.49**	**4.12**	**8.88**	**13.57**	**17.93**		**2.64**	**5.91**	**11.37**	**16.14**	**21.12**

Control plants showed normal meiotic stages (Figure 8A-H). Cytological anomalies recorded in treated plants of M_1 generation were 6^{II} rings + 9^{II} rods present at prophase I (diakinesis), chromatin bridges with 2 chromosomes separating precociously at anaphase I, and stickiness and 5 laggards at metaphase II (Figure 8I-L). In M_2 generation 6^{II} rings + 8^{II} rods + 2^{I} present at prophase I (diakinesis), stickiness and 1 laggard at anaphase I, unequal separation (14+16) at anaphase I, and cytomixix at telophase I were observed (Figure 8M-P). In M_3 generations unequal separation (18+12) at anaphase I, 3 laggards at telophase, trinucleate condition, and cytomixix at telophase II were recorded (Figure 8Q-T).

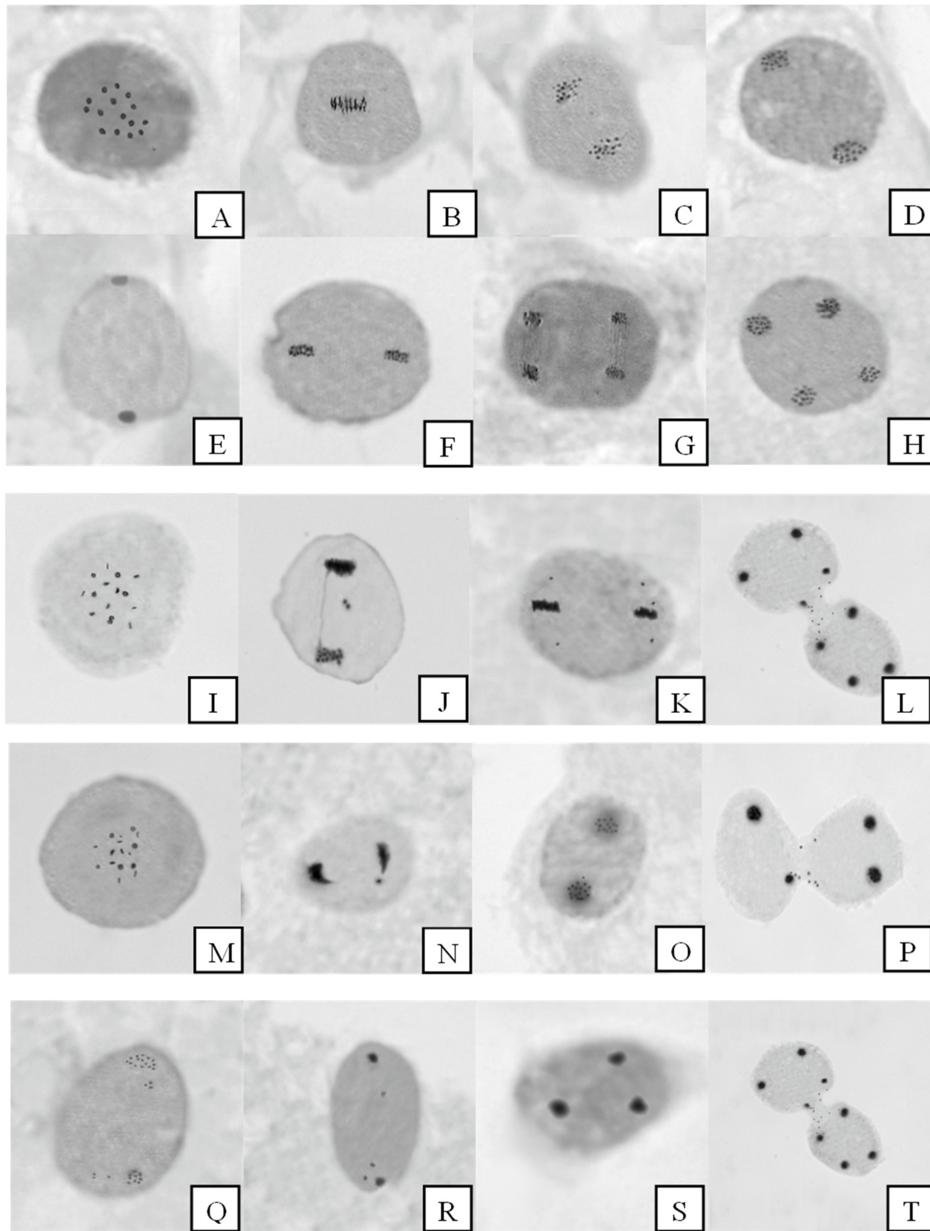

Figure 8. (A-H) Cytology(meiosis) of control, (I-L) Cytological aberrations in M_1 generation, (M-P) Cytological aberrations in M_2 generation, (Q-T) Cytological aberrations in M_3 generation

Mutagenic effectiveness and efficiency

Mutagenic effectiveness and efficiency were determined in the M_2 generation which varied according to mutagens. The results indicated that both effectiveness and efficiency of the mutagen treatments were highest at lower concentrations of mutagens. Among the mutagens, SA exhibited the greatest mutagenic effectiveness, followed by caffeine. Effectiveness declined from 6.61 to 2.44 in caffeine concentrations ranging from 0.10% to 1.00%, and from 9.97 to 2.78 in SA over the same concentration range. Mutagenic efficiency, calculated based on the extent of biological damage (i.e., chromosomal abnormalities), also decreased. For caffeine, efficiency dropped from 1.75 to 0.92, while for SA it decreased from 1.71 to 0.82 at concentrations from 0.10% to 1.00% (Table 4).

Table 4. Mutagenic effectiveness, and efficiency induced by caffeine and sodium azide (SA) in M_2 generation

Mutagen treatments	Number of seeds sown in M_1	Number of seeds germinated in M_1	Number of fertile M_1 plants progeny	Number of seeds sown in M_2	Number of seeds germinated in M_2	Number of fertile M_2 plants	Number of M_2 mutants	Mutated plant (%) Mp	Biological damage in M_1 generation (% meiotic abnormalities) Me	Mutagenic effectiveness	Mutagenic efficiency (Mp/Me)
Control	100	94	93	100	95	93	-	-	-	-	-
Caffeine											
0.10	100	86	84	100	89	87	5	5.95	3.40	6.61	1.75
0.25	100	81	79	100	84	83	7	8.86	5.36	3.94	1.65
0.50	100	74	71	100	79	76	9	12.68	12.06	2.82	1.05
0.75	100	66	63	100	71	69	10	15.87	16.86	2.35	0.94
1.00	100	52	50	100	62	58	11	22.00	24.02	2.44	0.92
SA											
0.10	100	81	78	100	85	82	7	8.97	5.24	9.97	1.71
0.25	100	75	73	100	80	78	8	10.96	8.20	4.87	1.34
0.50	100	69	67	100	75	72	10	14.93	17.65	3.32	0.85
0.75	100	61	56	100	68	66	11	19.64	23.72	2.91	0.83
1.00	100	49	48	100	58	55	12	25.00	30.59	2.78	0.82

Isolation of high-yielding mutant lines

The M_3 high-yielding mutant lines exhibited significant variability in quantitative traits, including plant height and the number of fertile branches, as well as various yield parameters such as the number of capsules per plant, seeds per capsule, total seeds per plant, and total yield (in grams). Nine high-yielding mutants, designated as A, B1, B2, C1, C2, D, E, F1, and F2, were selected based on their morphology and yield parameters. Five mutants (A, B1, B2, C1, and C2) were selected from the caffeine-treated populations, while four (D, E, F1, and F2) were chosen from the SA-treated populations. The quantitative and yield data for the selected mutant lines are presented in Table 5.

Table 5. Selected high-yielding M3 mutant lines and their mean yield attributes traits

S. No.	Mutant Code	Concentration of Mutagen used	Plant height (cm)	Number of fertile branches	Number of capsules	Number of seeds per capsule	Total number of seeds	Total yield (g)	Main characters
1	Control		95.78	4	51	7.69	392	2.51	Normal height with average yield
2	**A**	Caffeine 0.10%	115.64	7	98	8.12	796	4.87	Tall and bushy mutant with bold seeds and increased yield
3	**B1**	Caffeine 0.25%	114.32	11	92	8.09	744	4.61	Tall and bushy mutant with bold seeds and increased yield
4	**B2**	Caffeine 0.25%	112.93	5	71	8.06	572	3.52	Tall mutant with black seeds and moderately increased yield
5	**C1**	Caffeine 0.50%	73.41	6	112	7.87	881	3.79	Semi dwarf bushy mutant with small seeds and high yield
6	**C2**	Caffeine 0.50%	64.32	12	108	7.74	836	3.43	Semi dwarf bushy mutant with small seeds and moderately increased yield
7	**D**	SA 0.10%	110.12	7	74	8.08	598	3.71	Tall mutant with bold seeds and high yield
8	**E**	SA 0.25%	107.88	4	63	7.98	503	3.26	Tall mutants with moderately increased yield
9	**F1**	SA 0.50%	78.35	8	99	7.76	768	3.02	Semi dwarf bushy mutant with small seeds and moderately increased yield
10	**F2**	SA 0.50%	72.87	7	95	7.69	731	2.89	Semi dwarf bushy mutant with small seeds and slightly increased yield

The control plant exhibited an average height, with blue flowers, normal sized capsules, and brown seeds. Mutant A, selected from population treated with 0.10% caffeine, was characterized by its tall and bushy growth habit, darker bluish-purple flowers, larger capsules, and bold, shiny brown seeds and increased yield. Mutant

B1, selected from 0.25% caffeine treated population was tall, bushy, white flowers, large capsules with dark stripes, bold brown seeds, and increased yield. Mutant B2, selected from 0.25% caffeine treated population was tall, bushy, light purple flowers, darker capsules, bold brown seeds, and moderately increased yield. Mutant C1, selected from 0.50% caffeine treated population was semi dwarf, bushy, sky blue flask shaped flowers, light brown capsules, smaller seeds, and high yield. Mutant C2, selected from 0.50% caffeine treated population was semi dwarf, bushy, bluish-purple flowers, light brown small capsules, smaller seeds, and moderately increased yield. Mutants selected from SA treated population shows relatively lower yield than mutants of caffeine. Mutant D, selected from population treated with 0.10% SA was tall, bushy, dark purple flowers, larger capsules, bold seeds, and increased yield. Mutant E, selected from 0.25% SA treated population was tall, bluish-purple flowers, capsules with darker stripe, bold seeds, and moderately increased yield. Mutant F1, selected from 0.50% SA treated population was semi dwarf, bushy, dark purple flowers, light brown small capsules, smaller seeds, and moderately increased yield. Mutant F2, selected from 0.50% SA treated population was semi dwarf, bushy, white flowers, light brown small capsules, smaller seeds, and slightly increased yield (Figures 9-11).

Figure 9. Control plant, high-yielding mutants: Mutant A (Tall and bushy), Mutant B1 (Tall and bushy), Mutant B2 (Tall)

Figure 10. High-yielding mutants: Mutant C1 (semi dwarf and bushy), Mutant C2 (semi dwarf and bushy), Mutant D (tall), Mutant E (tall)

Figure 11. High-yielding mutants: Mutant F1 (semi dwarf and bushy), Mutant F2 (semi dwarf and bushy)

Scanning Electron Microscopy (SEM) analysis of stomatal behaviour and seed morphology in selected mutants

Scanning electron microscopy (SEM) of selected high-yielding mutants was carried out to study their stomatal behaviour and seed micromorphology. In the analysis, stomata size was found to be increased in all high-yielding mutants. The control plant has a stomatal aperture of size 9.975 µm in length and 1.480 µm in width. In caffeine treated population, mutant A exhibited remarkable increase in size of stomata with 24.207 µm in length and 1.804 µm in width, while mutant D displayed largest stomatal aperture among SA treated population with 21.271 µm in length and 1.480 µm in width. Mutant B1 exhibit maximum number of stomata per field and has stomatal aperture of 16.160 µm in length and 2.019 µm in width. Mutant B2 has 15.401 µm in length and 2.001 µm in width. Mutant C1 and C2 has stomatal length of 14.673 µm and 14.137 µm respectively and width of 2.071 µm and 2.737 µm respectively. Mutant E displayed stomatal aperture of 12.634 µm in length and 1.360 µm in width. Mutant F1 and F2 exhibited slight increase in stomatal aperture with length of 11.361 µm and 11.239 µm respectively and width of 1.760 µm and 1.924 µm respectively. The variations in the shape and size of stomatal pores are displayed in Figures 12-13.

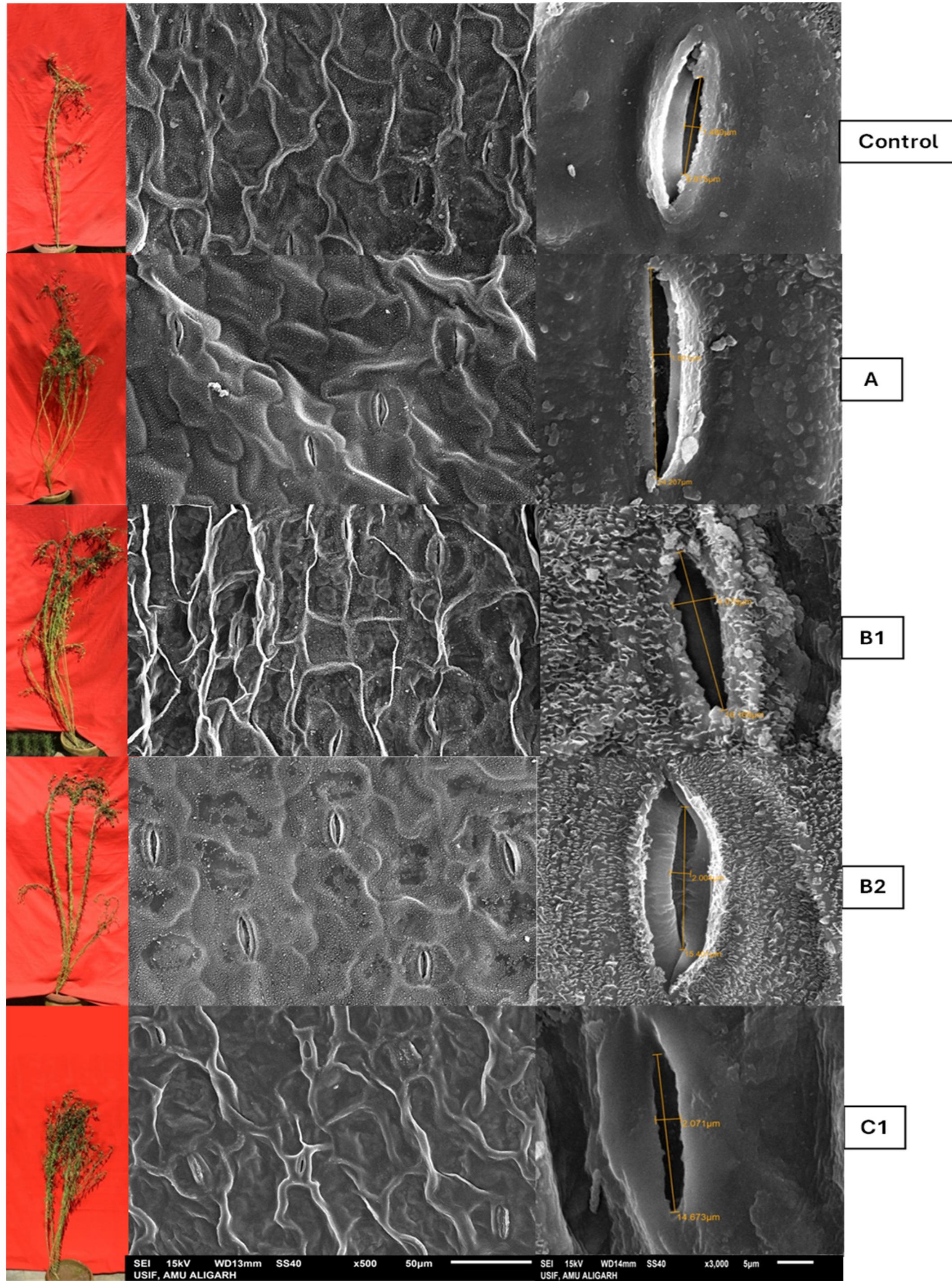

Figure 12. SEM images of leaves showing number of stomata per field and dimensions of stomatal aperture of control and selected mutant lines (A, B1, B2, and C1)

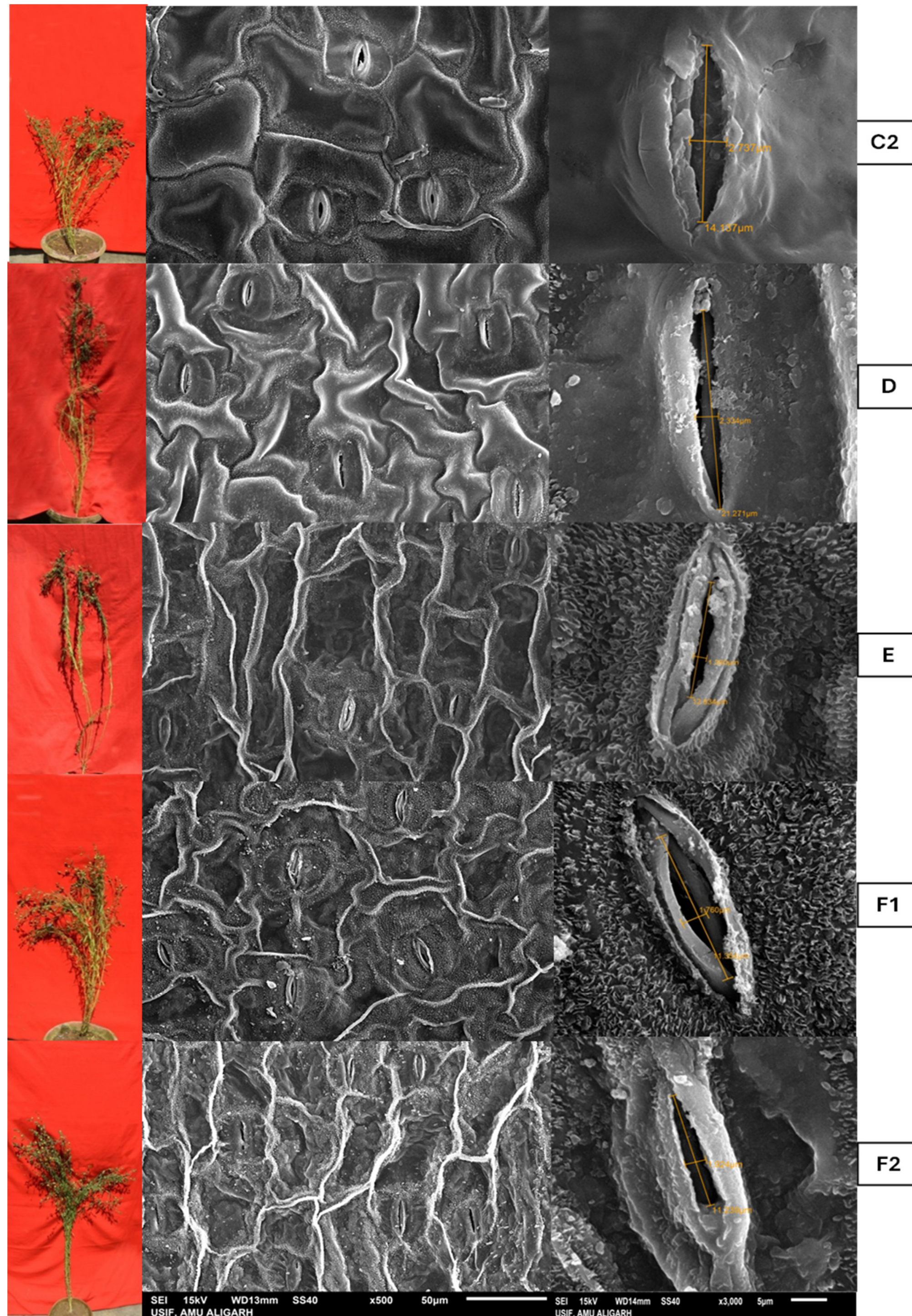

Figure 13. SEM images of leaves showing number of stomata per field and dimensions of stomatal aperture of selected mutant lines (C2, D, E, F1 and F2)

SEM analysis of seed micromorphology revealed variations in morphological characteristics such as seed shape, size, surface texture, and arrangement of surface cells. Mutants of both species exhibit notable variations in length and width of the seed. Control seeds had 4.713 mm length and 2.487 mm width. Among all mutants,

mutant A exhibit highest seed length and width of 6.007 mm and 2.786 mm respectively. Mutant B1, B2, D and E exhibit larger size than control with length 5.690, 5.292, 5.336, and 5.101 mm respectively. Semi dwarf mutants C1, C2, F1, and F2 had relatively smaller size than control with length of 4.460, 4.271, 3.951, and 3.337. In flaxseed, the gum is found in the mucous epidermis beneath the seed's cuticle layer. The control seeds exhibit smooth surface with pentagonal cells. Mutant A, B1, and B2 displayed smooth surface while other has relatively rough surface. Mutant D and E exhibit larger size pentagonal cells, while mutant F1 exhibit prominent small pentagonal cells all over the seed surface (Figures 14-15).

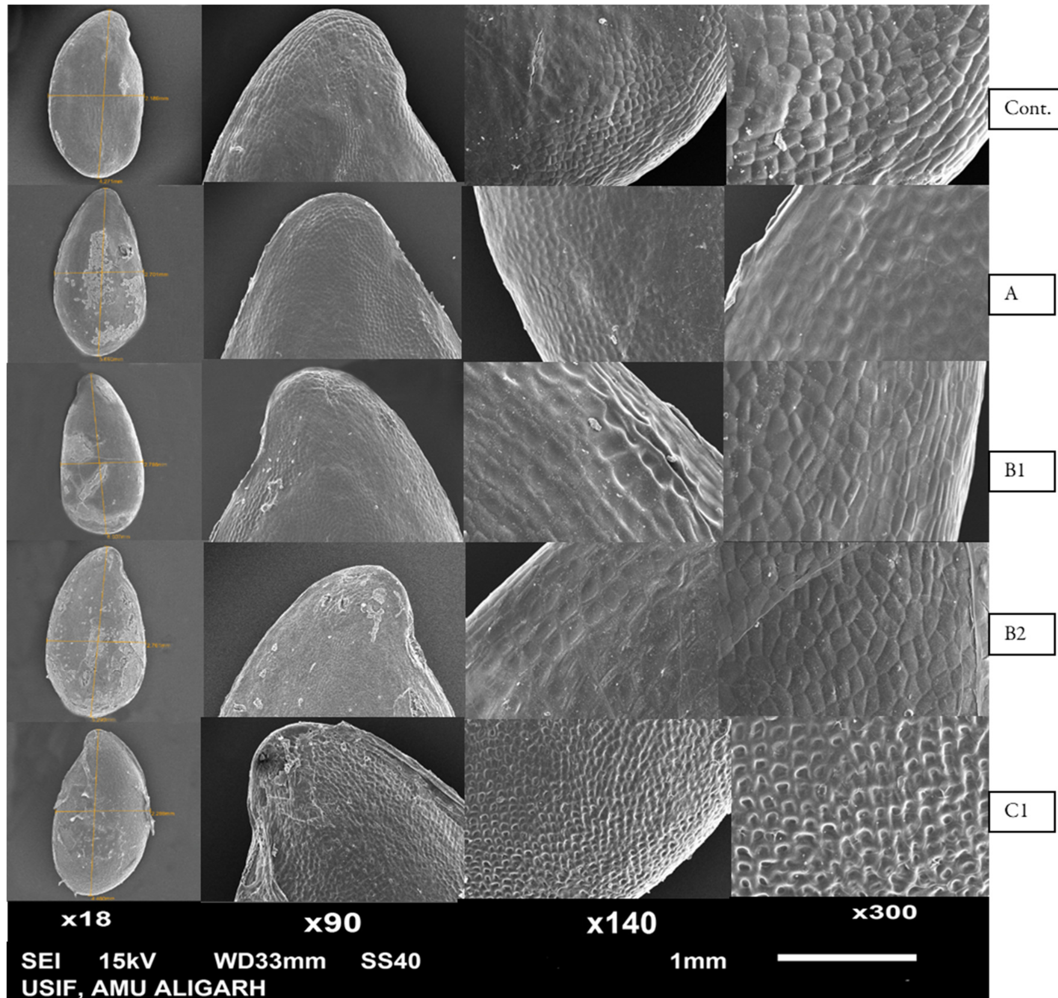

Figure 14. SEM images of seeds showing seed morphology and seed coat pattern of control and selected mutant lines (A, B1, B2, and C1)

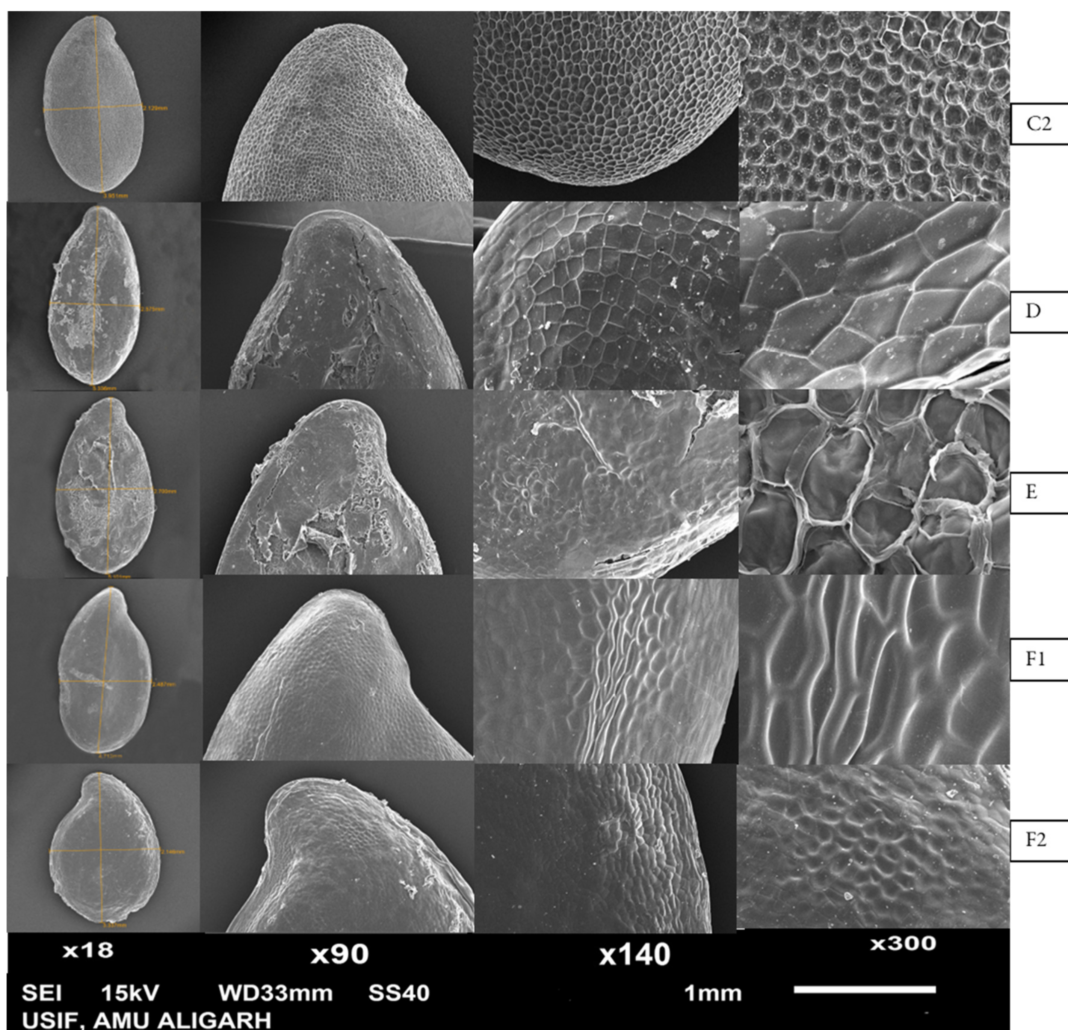

Figure 14. SEM images of seeds showing seed morphology and seed coat pattern of selected mutant lines (C2, D, E, F1, and F2)

Discussion

The current findings demonstrated the application of chemical mutagens (caffeine and sodium azide) in enhancing the morpho-physiological traits, quality and yield parameters of *Linum usitatissimum*. In M_2 and M_3 generations, the likelihood of detecting visible changes or phenotypic mutations is higher which may be attributed primarily due to genetic factors. As a result, mutations observed in these generation are generally considered more stable (Parry *et al*., 2009). The overall goal is to isolate high-yielding mutant lines of *Linum* in M_3 generation and discover their potential for creating novel genotypes with worthwhile agricultural traits.

In the present study, treatment of caffeine and SA exhibited a severe reduction in various biological parameters viz., seed germination, plant survival, and pollen fertility across all three generations. Germination is marked when seed begins to grow after a period of dormancy, the resumption of its active metabolism, resulting in visible growth. This is an important criterion for evaluating the plant's response to mutagenic treatments (Bhat *et al*., 2017). In present study, suppression in germination rate may be ascribed to chromosomal aberrations/delay in DNA synthesis/delayed metabolic process (Aslam *et al*., 2017). Similar

findings have also been reported by Gaswanto *et al*. (2016) in chilli, Asare *et al*. (2017) in okra, and Rasik *et al*. (2022) in cowpea. Reduced plant survival in treated populations may be due to factors such as cytogenetic damage, physiological disturbances (Sato and Gaul, 1967), or imbalances between growth inhibitors and promoters (Maherchandani, 1975). Similar results have also been reported by Naaz *et al*. (2023) in fenugreek. Results showed drop in pollen fertility across the generations after mutagen treatments, as reported earlier by Veni *et al*. (2017) in *Vigna*, Ulukapi and Ozmen (2018) in *Phaseolus*, and Karim *et al*. (2022) in *Capsicum*. Shahwar *et al*. (2020) found that increasing chromosomal abnormalities significantly affected microsporogenesis, leading to the production of non-viable gametes and subsequent reduction in pollen fertility.

Quantitative traits are controlled by small additive effects of multiple genes and their expressions are also influenced by environmental factors. Therefore, statistical analysis of these traits is necessary to assess the significance of the data. In the present study, the improvement in quantitative traits observed in plants treated with lower and intermediate levels of mutagens can be attributed to the induction of beneficial mutations (Raina and Khan, 2023) whereas higher doses showed the inhibitory effects. Similar results have been reported by Yousuf *et al*. (2023) in lentil. The decrease in plant height could be attributed to alterations in ascorbic acid levels and physiological disruptions (Dhamayanthi and Reddy, 2000). Additionally, certain pleiotropic genes may play a role in regulating plant height (Yano *et al*., 2016). The mean fertile branches per plant raised significantly at lower doses of mutagens in all three generations. These results align with previous findings of Choudhary *et al*. (2012) in *Trigonella*, Shahwar *et al*. (2016) in *Vicia faba*, and Hasan *et al*. (2020) in chilli. The rise in the number of fertile branches per plant in populations treated with lower doses of mutagens may be linked to decreased strigolactone synthesis.

In a breeding program, yield is regarded as a key parameter, with the primary goal of the plant breeder being to improve yield and its associated traits. The study found that yield parameters increased at lower and intermediate concentrations of both mutagens, with caffeine proving to be more effective than SA. Previous studies have also reported significant raise in the number of capsules per plant in crops like sesame (Pradhan and Paul, 2019) and black cumin (Amin *et al*., 2019), positively correlating with the total yield per plant. Increased number of seeds per capsule has been reported by Arisha *et al*. (2015) in *Capsicum*, Shahwar *et al*. (2017b) in *Vicia faba*, Amin *et al*. (2019) and Hassan *et al*. (2018) in *Trigonella*. The increase in seed yield and yield-related traits can be attributed to mutagen-induced enhancement of mitotic division, alterations in physiological, biochemical, and metabolic pathways, as well as the interaction of mutagens with genes that regulate yield (Goyal *et al*., 2022). In this study, at higher concentrations of mutagens showed an inhibitory effect on total yield. This reduction may be due to disruptions in meiosis, changes in microspore and megaspore frequency, physiological imbalances, chromosomal damage, spindle formation abnormalities, impaired pairing, extended DNA synthesis, and high pollen sterility. A reduction in yield parameters following mutagenic treatments has also been observed in several plant species, such as *Lens culinaris* (Laskar and Khan, 2017), *Trigonella* (Naaz *et al*., 2020), and *Capsicum* (Arumingtyas and Ahyar, 2022).

The study showed a substantial increase in the mean values of chlorophyll and carotenoid contents across the generations with their respective controls. Similar increase has been reported in plants like *Brassica* (Chen *et al*., 2018), *Triticum* (Abaza *et al*., 2020), and *Trigonella* (Naaz *et al*., 2024b). The rise in total chlorophyll and carotenoid levels in the mutants may be due to an increase in chlorophyll-a and β-carotene content (Tomlekova *et al*., 2009). Furthermore, chlorophyll development is controlled by multiple genes located on various chromosomes, particularly near the centromere and the proximal regions of the chromosomes (Swaminathan, 1965; Shahwar *et al*., 2023).

Morphological variants or mutants play a crucial role in the selection of high-yielding lines. The visible mutants directly indicate there may be positive genetic changes, suggesting enhanced performance or adaptation. These mutants provide a diverse pool of traits that can be evaluated for desirable characteristics, such as improved yield, better adaptability and resistance to environmental stresses, making them key targets

in breeding programs aimed at selecting high-yielding mutant lines. In this study various cotyledonary and vegetative leaves mutants have been identified. Similar variations have been observed by workers like Shahwar *et al*. (2019) in *Lens culinaris*, Azizan *et al*. (2023) in *Stevia* and Opoku Gyamfi *et al*. (2022) in *Vigna unguiculata*. These indicated that the mutation may have affected the genes controlling the shape of *Linum* leaves. Also, leaf abnormalities are attributed to chromosomal damage, disrupted enzymatic activities, and imbalances in protein and mineral metabolism (Grover and Virk, 1984). Variations in floral morphology like flower colour, shape, aestivation, increased number of petals have been recorded. Several researchers also reported the flower mutants in crops such as *Vigna* (Diouf *et al*., 2020), *Medicago* (Jade *et al*., 2023), and cowpea (Dabiré *et al*., 2024). These mutations may be attributed to mutagen-induced physiological or biochemical changes affecting the differentiation of floral whorl (Thombre and Mehetre, 1981).

In the present investigation, various chromosomal abnormalities such as univalents, multivalents, precocious movements of chromosomes, stray chromosomes, stickiness, laggards, bridges, stickiness, unequal separation of chromosomes, micro- and multi nucleate conditions, disturbed polarity and cytomixis have been reported. The formation of univalents caused by mutagens may result from the failure of chiasma formation during chromosome desynapsis in meiosis-I, as well as from pairing disturbances (Goyal and Khan, 2010). The occurrence of univalents and multivalents has also been reported by Khursheed *et al*. (2015) in *Vicia*, and Alam *et al*. (2022) in *Triticum*. Unoriented or stray chromosomes may result from disruptions in spindle activity, leading to abnormal chromosome alignment during metaphase (Khah and Verma, 2017). Stray chromosomes have also been observed in crops like *Cichorium* (Khan *et al*., 2009), and *Vigna* (Gandhi *et al*., 2013). The occurrence of chromatin bridges during anaphase I/II may be linked to chromosome end stickiness, breakage, or reunion, as observed in *Capsicum* (Kumar and Gupta, 2009; Hasan *et al*., 2022), *Linum* (Alka *et al*., 2012), and *Nigella* (Mukherjee and Datta, 2011). Micronucleus formation occurs when non-oriented or lagging chromosomes fail to reach the poles (Utsunomiya *et al*., 2002). Cytomixis involves the transfer of chromatin from one cell to another (Gulfishan *et al*., 2012), leading to the production of aneuploid and polyploid gametes. Cytomixix have previously been observed by Raina *et al*. (2023) in cowpea.

In this study, nine high-yielding mutants were selected based on their morphology and yield parameters after treatments with caffeine and sodium azide. Several researchers have also selected various mutants based on their morphology, growth habit and yield parameters in plants such as *Cicer arietinum* (Wani and Anis, 2008), *Lens culinaris* (Raina *et al*., 2022), *Vigna mungo* (Goyal *et al*., 2022), *Trigonella* (Naaz *et al*., 2024b), *Capsicum* (Hasan *et al*., 2024b). It was also noted that morphological variants proved useful in mapping studies and in understanding the evolutionary processes of crops (Gaur and Gour, 2003).

Present investigation recorded variations in stomatal aperture size of selected mutants in SEM analysis. An increase in stomatal size enhances the transpiration rate, which in turn boosts photosynthesis, thereby promoting plant growth and ultimately improving yield. The opening of stomatal pores is stimulated by sufficient photosynthetic light, high humidity, and low CO_2 concentrations, while stomatal closure is induced by darkness, high temperatures, low humidity, and elevated CO_2 levels (Shimazaki *et al*., 2007). The enlarged stomatal length and width were previously reported by Mahadevamma *et al*. (2012) and Mallick *et al*. (2016). The changes in stomatal characteristics observed in the high-yielding mutants may be linked to genetic damage and mutations induced by the mutagenic agents employed in the study. Seed morphology and seed coat patterns were also investigated through SEM analysis in selected mutants of *Linum*. In this research, distinct differences in the micromorphology of seeds were observed, consistent with findings reported by Naaz *et al*. (2023).

Conclusions

The current findings emphasized the effectiveness of chemical mutagens (caffeine and sodium azide) in improving the morpho-physiological traits, quality, and yield parameters of *Linum usitatissimum* L. in M_1, M_2, and M_3 generations. Caffeine has been found to be more efficient in producing positive mutants than SA. Furthermore, the selected high-yielding mutant lines in the M_3 generation, isolated from caffeine and sodium azide treatments, demonstrated higher yields and enhanced physiological activity compared to the untreated parent genotype. These were demonstrated to be highly effective in increasing genetic variability for yield and yield-related traits. It has been well-known that selecting for yield traits joint with the genetic enhancement of the nutritional content of linseed, can be potentially leading to the development of mutant varieties. Therefore, these mutant lines embrace significant breeding importance and should be advanced to subsequent generations to ensure further trait stability, ultimately leading to the development of new linseed cultivars with improved yield and nutrient quality.

Authors' Contributions

Conceptualization: NS and NN; Data curation: NS; Formal analysis: NS and SC; Funding acquisition: NM and DA; Investigation: SC and NH; Methodology: NS and PV; Project administration: SC; Resources: SC and NN; Software: NS; Supervision: SC; Validation: NN, NH, PV, NM, and DA; Writing—original draft: NS; writing—review and editing: NS, NN, NH, SC, NM, and DA. All authors have read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abaza GMSM, Awaad HA, Attia ZM, Abdel-Lateif KS, Gomaa MA, Abaza SMSM, Mansour E (2020). Inducing potential mutants in bread wheat using different doses of certain physical and chemical mutagens. Plant Breeding and Biotechnology 8(3):252-264. *https://doi.org/10.9787/PBB.2020.8.3.252*

Akter Y, Junaid M, Afrose SS, Nahrin A, Alam MS, Sharmin T, Akter R, Hosen SM (2021). A comprehensive review on *Linum usitatissimum* medicinal plant: its phytochemistry, pharmacology, and ethnomedicinal uses. Mini Reviews in Medicinal Chemistry 21(18):2801-2834. *https://doi.org/10.2174/1389557521666210203153436*

Alam Q, Khah MA, ZR AAA (2022). Comparative analysis of different chemical mutagens in inducing chromosomal aberrations in meiotic cells of *Triticum aestivum* L. Cytologia 87(2):99-105. *https://doi.org/10.1508/cytologia.87.99*

Alka A, MYK, Bhat TM, Choudhary S, Aslam R (2012). Genotoxic effect of ethylmethane sulphonate and sodium azide in *Linum usitatissimum* L. International Journal of Plant, Animal and Environmental Sciences 2(3):1-6.

Alkhatib R, Alkhatib B, Al-Eitan L, Abdo N, Tadros M, Bsoul E (2018). Physio-anatomical responses of tobacco under caffeine stress. Photosynthetica 56:1140-1146. *https://doi.org/10.1007/s11099-018-0798-4*

Amin R, Wani MR, Raina A, Khursheed S, Khan S (2019). Induced morphological and chromosomal diversity in the mutagenized population of black cumin (*Nigella sativa* L.) Using single and combination treatments of gamma rays and ethyl methane sulfonate. Jordan Journal of Biological Sciences 12(1).

Arisha MH, Shah SN, Gong ZH, Jing H, Li C, Zhang HX (2015). Ethyl methane sulfonate induced mutations in M2 generation and physiological variations in M1 generation of peppers (*Capsicum annuum* L.). Frontiers in Plant Science 6:399. *https://doi.org/10.3389/fpls.2015.00399*

Arslanoğlu F, Aytaç S (2020). The important in terms of health of flax (*Linum usitatissimum* L.). International Journal of Life Sciences and Biotechnology 3(1):95-107.

Arumingtyas EL, Ahyar AN (2022). Genetic diversity of chili pepper mutant (*Capsicum frutescens* L.) resulted from gamma-ray radiation. In: IOP Conference Series: Earth and Environmental Science 1097:012059. IOP Publishing. *https://doi.org/10.1088/1755-1315/1097/1/012059*

Asare AT, Mensah F, Acheampong S, Asare-Bediako E, Armah J (2017). Effects of gamma irradiation on agromorphological characteristics of okra (*Abelmoschus esculentus* L. Moench.). Advances in Agriculture 2017(1):2385106. *https://doi.org/10.1155/2017/2385106*

Aslam R, Bhat TM, Choudhary S, Ansari MYK, Shahwar D (2017). Estimation of genetic variability, mutagenic effectiveness and efficiency in M2 flower mutant lines of *Capsicum annuum* L. treated with caffeine and their analysis through RAPD markers. Journal of King Saud University-Science 29(3):274-283. *https://doi.org/10.1016/j.jksus.2016.04.008*

Azizan NI, Shamsiah A, Hasan NA, Hussein S (2021). Morphological characterization of colchicine-induced mutants in *Stevia rebaudiana*. In: IOP Conference Series: Earth and Environmental Science 757:012006. IOP Publishing. *https://doi.org/10.1088/1755-1315/757/1/012006*

Bhat IA, Pandit UJ, Sheikh IA, Hassan ZU (2017). Physical and chemical mutagenesis in *Linum usitatissimum* L. to induce variability in seed germination, survival and growth rate traits. Current Botany 7:28-32. *https://doi.org/10.19071/cb.2016.v7.3054*

Chaudhary L, Sharma R, Kumar M (2021). Estimation of LD50 and effect of sodium azide on germination and seedling parameters of different cultivars of *Cajanus cajan* (L.) Millspaugh. Toxicology and Environmental Health Sciences 13(3):279-285. *https://doi.org/10.1007/s13530-021-00105-6*

Chen W, Wan S, Shen L, Zhou Y, Huang C, Chu P, Guan R (2018). Histological, physiological, and comparative proteomic analyses provide insights into leaf rolling in *Brassica napus*. Journal of Proteome Research 17(5):1761-1772. *https://doi.org/10.1021/acs.jproteome.7b00744*

Choudhary S, Ansari MYK, Khan Z, Gupta H (2012). Cytotoxic action of lead nitrate on cytomorphology of *Trigonella foenum-graecum* L. Turkish Journal of Biology 36(3):267-273. *https://doi.org/10.3906/biy-1010-167*

Chung WH (2021). Pleiotropic effects of caffeine leading to chromosome instability and cytotoxicity in eukaryotic microorganisms. Journal of Microbiology and Biotechnology 31(2):171. *https://doi.org/10.4014/jmb.2011.11042*

Crops and Livestock Products (2024). Retrieved 2024 August 27 form: *https://www.fao.org/faostat/en/#data/QCL*

Dabiré MT, Nikié MP, Yonli D, Sanna S, Josué W, Traoré H, Batieno JT, … Hingane AJ (2024). Genetic enhancement of indigenous cowpea with gamma-ray induced trait variation. American Journal of Plant Sciences 15(8):651-676. *https://doi.org/10.4236/ajps.2024.158044*

Dhamayanthi KPM, Reddy VRK (2000). Cytogenetic effects of gamma rays and ethyl methane sulphonate in chilli pepper (*Capsicum annuum* L.). Cytologia (Tokyo) 65:129-133. *https://doi.org/10.1508/cytologia.65.129*

Diouf M, Diallo S, Badiane FA, Diack O, Diouf D (2020). Development of new cowpea (*Vigna unguiculata*) mutant genotypes, analysis of their agromorphological variation, genetic diversity and population structure. bioRxiv 2020-05. *https://doi.org/10.1101/2020.05.22.109041*

FAO (2017). The Future of Food and Agriculture: Trends and Challenges; FAO: Rome, Italy.

Gandhi S, Umavathi S, Mullainathan L (2013). Gamma rays and ethyl methanesulphonate induced cytotoxicity in green gram *Vigna radiata* (L.) Wilczek. Journal of Current Research in Science 1(6):518.

Gaswanto R, Syukur M, Purwoko BS, Hidayat SH (2016). Induced mutation by gamma rays irradiation to increase chilli resistance to Begomovirus. AGRIVITA Journal of Agricultural Science 38(1):24-32. *http://doi.org/10.17503/agrivita.v38i1.581*

Gaur PM, Gour VK (2003). Broad-few-leaflets and outwardly curved wings: two new mutants of chickpea. Plant Breeding 122(2):192-194.

Goraya GS, Ved DK (2017). Medicinal plants in India: an assessment of their demand and supply. National Medicinal Plants Board, Ministry of AYUSH, Government of India, New Delhi and Indian Council of Forestry Research and Education, Dehradun, 1-430.

Goyal A, Patel A, Sihag MK, Shah N, Tanwar B (2018). Therapeutic potential of flaxseed. In: Therapeutic, probiotic, and unconventional foods. Academic Press, pp 255-274. *https://doi.org/10.1016/B978-0-12-814625-5.00013-3*

Goyal S, Khan S (2010). Cytology of induced morphological mutants in *Vigna mungo* (L.) Hepper. Egyptian Journal of Biology 12(2):81-85.

Goyal S, Wani MR, Raina A, Laskar RA, Khan S (2022). Quantitative assessments on induced high yielding mutant lines in urdbean [*Vigna mungo* (L.) hepper]. Legume Science 4(2):e125. *https://doi.org/10.1002/leg3.125*

Grover IS, Virk GS (1984). Induced chlorophyll mutants in mungbean *Vigna-radiata*. Acta Botanica Indica.

Gruszka D, Szarejko I, Maluszynski M (2012). Sodium azide as a mutagen. In: Plant mutation breeding and biotechnology. Wallingford UK: CABI, pp 159-166.

Gulfishan M, Khan AH, Jafri IF, Bhat TA (2012). Assessment of mutagenicity induced by MMS and DES in *Capsicum annuum* L. Saudi Journal of Biological Sciences 19(2):251-255. *https://doi.org/10.1016/j.sjbs.2012.01.008*

Hasan N, Choudhary S, Laskar RA, Naaz N, Sharma N (2022). Comparative study of cadmium nitrate and lead nitrate [Cd (NO3) 2 and Pb (NO3) 2] stress in cyto-physiological parameters of *Capsicum annuum* L. Horticulture, Environment, and Biotechnology 63(5):627-641. *https://doi.org/10.1007/s13580-021-00417-z*

Hasan N, Choudhary S, Naaz N, Sharma N, Farooqui SA, Budakoti M, Joshi DC (2024b). Identification and characterization of *Capsicum* mutants using, biochemical, physiological, and single sequence repeat (SSR) markers. Journal of Genetic Engineering and Biotechnology 22(4):100447. *https://doi.org/10.1016/j.jgeb.2024.100447*

Hasan N, Choudhry S, Laskar RA (2020). Studies on qualitative and quantitative characters of mutagenised chili populations induced through MMS and EMS. Vegetos 33(4):793-799. *https://doi.org/10.1007/s42535-020-00164-z*

Hasan N, Laskar RA, Farooqui SA, Naaz N, Sharma N, Budakoti M, Joshi DC, Choudhary S, Bhinda MS (2024a). Genetic improvement of medicinal and aromatic plant species: breeding techniques, conservative practices and future prospects. Crop Design 100080. *https://doi.org/10.1016/j.cropd.2024.100080*

Hassan N, Laskar RA, Raina A, Khan S (2018). Maleic hydrazide induced variability in fenugreek (*Trigonella foenum-graecum* L.) cultivars CO1 and Rmt-1. Research & Reviews: Journal of Botanical Sciences 7(1):19-28.

Ingle AT, Sable AD, Zote RK (2018). Studies on morphological and phytochemical variation between two varieties of fenugreek (*Trigonella foenum-graecum* L.) at different concentration of sodium azide. International Journal of Current Microbiology and Applied Sciences 7:1655-1661. *https://doi.org/10.20546/ijcmas.2018.710.189*

Jade SS, Gaikwad VN, Jadhav SP, Takawale PS, Bahulikar RA (2023). Phenotypic variations in M2 generation by ethyl methanesulfonate mutagenesis in lucerne (*Medicago sativa* L.). Crop and Pasture Science 75(1). *https://doi.org/10.1071/CP23047*

Jahan R, Ansari SB, Malik S, khan S (2020). Cytological aberrations in M2 morphological mutants of *Linum usitatissimum* (L.) induced by physical and chemical mutagens. Plant Archives 20:1343-1348.

Karim KR, Rafii MY, Misran A, Ismail MF, Harun AR, Ridzuan R, Chowdhury MFN, Hosen M, Yusuff O, Haque MA (2022) Genetic diversity analysis among *Capsicum annuum* mutants based on morpho-physiological and yield traits. Agronomy 12(10):2436. *https://doi.org/10.3390/agronomy12102436*

Khah MA,Verma RC (2017). Effect of gamma irradiation on seed germination and chromosomal behaviour at meiotic division in bread wheat (*Triticum aestivum* L.). The Journal of Indian Botanical Society 96(3-4):198-204.

Khan Z, Gupta H, Ansari MYK, Chaudhary S (2009). Methyl methanesulphonate induced chromosomal variations in a medicinal plant *Cichorium intybus* L. during microsporogenesis. Biology and Medicine 1(2):66-69.

Khursheed S, Laskar RA, Raina A, Amin R, Khan S (2015). Comparative analysis of cytological abnormalities induced in *Vicia faba* L. genotypes using physical and chemical mutagenesis. Chromosome Science 18(3-4):47-51. *https://doi.org/10.11352/scr.18.47*

Konzak CF, Nilan RA, Wagner J, Foster RJ (1965). Efficient chemical mutagenesis. In: The Use of Induced Mutations in Plant Breeding Radiation Botany. Pergamon, Oxford 5:49-70.

Kumar A, Paul S, Sood VK, Thakur G, Thakur R (2021). Effectiveness and efficiency of gamma rays and EMS (Ethyl methane sulphonate) in linseed (*Linum usitatissimum* L.). Himachal Journal of Agricultural Research 47(2):163-168.

Kumar G, Gupta P (2009). Induced karyomorphological variations in three phenodeviants of *Capsicum annuum* L. Turkish Journal of Biology 33(2):123-128. *https://doi.org/10.3906/biy-0807-29*

Laskar RA, Khan S (2017). Assessment on induced genetic variability and divergence in the mutagenized lentil populations of microsperma and macrosperma cultivars developed using physical and chemical mutagenesis. PloS One 12(9):e0184598. *https://doi.org/10.1371/journal.pone.0184598*

Mackinney G (1941). Absorption of light by chlorophyll solutions. Journal of Biological Chemistry 140(2):315-322. *https://doi.org/10.1016/S0021-9258(18)51320-X*

Mahadevamma M, Dinesh MR, Kumari VR, Shankarappa TH (2012). Evaluation of induced variability in papaya (*Carica papaya* L.) by physical mutagenesis. CIBTech Journal of Biotechnology 1(1):66-71.

Maherchandani N (1975). Effects of gamma radiation on the dormant seed of *Avena fatua* L. Radiation Botany 15(4):439-443. *https://doi.org/10.1016/0033-7560(75)90018-6*

Mallick M, Awasthi OP, Paul V, Verma MK, Jha G (2016). Effect of physical and chemical mutagens on leaf sclerophylly and stomatal characteristics of Kinnow mandarin mutants. Indian Journal of Horticulture 73(2):291-293. *https://doi.org/10.5958/0974-0112.2016.00063.3*

Mukherjee S, Datta AK (2011). Mitotic and meiotic consequences of gamma irradiations on dry seeds of *Nigella sativa* L. (black cumin). Journal of Plant Development Sciences 3:233-238.

Naaz N, Ansari SB, Khan S, Choudhary S, Jahan R (2020). Physio-morphological Variations induced by methyl methane Sulphonate in *Trigonella foenum-graecum* L. International Journal of Botany Studies 5(6):37-42.

Naaz N, Choudhary S, Hasan N, Sharma N, Al Aboud NM, Shehata WF (2024b). Biochemical and molecular profiling of induced high yielding M3 mutant lines of two *Trigonella* species: Insights into improved yield potential. PloS One 19(7):e0305691. *https://doi.org/10.1371/journal.pone.0305691*

Naaz N, Choudhary S, Hasan N, Sharma N, Alharbi K, Abd El Moneim D (2024a). Enhancing genetic variability in *Trigonella* species through sodium azide induction: morpho-physiological and chromosomal amelioration. Frontiers in Genetics 15:1378368. *https://doi.org/10.3389/fgene.2024.1378368*

Naaz N, Choudhary S, Sharma N, Hasan N, Al Shaye NA, Abd El-Moneim D (2023). Frequency and spectrum of M2 mutants and genetic variability in cyto-agronomic characteristics of fenugreek induced by caffeine and sodium azide. Frontiers in Plant Science,13:1030772. *https://doi.org/10.3389/fpls.2022.1030772*

Opoku Gyamfi M, Eleblu JSY, Sarfoa LG, Asante IK, Opoku-Agyemang F, Danquah EY (2022). Induced variations of ethyl methane sulfonate mutagenized cowpea (*Vigna unguiculata* L. walp) plants. Frontiers in Plant Science 13:952247. *https://doi.org/10.3389/fpls.2022.952247*

Parry MA, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, … Phillips AL (2009). Mutation discovery for crop improvement. Journal of Experimental Botany 60(10):2817-2825. *https://doi.org/10.1093/jxb/erp189*

Pradhan M, Paul A (2019). Induced genetic variability for different quantitative characters in sesame (*Sesamum indicum*). Journal of Pharmacognosy and Phytochemistry 8(3):1026-1031.

Raina A, Khan S (2023). Field assessment of yield and its contributing traits in cowpea treated with lower, intermediate, and higher doses of gamma rays and sodium azide. Frontiers in Plant Science 14:1188077. *https://doi.org/10.3389/fpls.2023.1188077*

Raina A, Tantray YR, Khan S (2023). Assessment of Bio-physiological damages and cytological aberrations in cowpea varieties treated with gamma rays and sodium azide. PloS One 18(7):e0288590. *https://doi.org/10.1371/journal.pone.0288590*

Raina A, Wani MR, Laskar RA, Khan S (2022). Chemical mutagenesis: Role in breeding and biofortification of lentil (*Lens culinaris* Medik) mutant lines. Molecular Biology Reports 49(12):11313-11325.

Rasik S, Raina A, Laskar RA, Wani MR, Reshi ZA, Khan S, Ndhlala AR (2022). Lower doses of sodium azide and methyl methanesulphonate improved yield and pigment contents in vegetable cowpea [*Vigna unguiculata* (L.) Walp.]. South African Journal of Botany 148:727-736. *https://doi.org/10.1016/j.sajb.2022.04.034*

Sarfraz H, Ahmad IZ (2023). A systematic review on the pharmacological potential of *Linum usitatissimum* L.: a significant nutraceutical plant. Journal of Herbal Medicine 100755. *https://doi.org/10.1016/j.hermed.2023.100755*

Sarfraz H, Ahmad IZ (2024). Antidiabetic properties of *Linum usitatissimum* L. seed: A promising medicinal plant. In: Antidiabetic Medicinal Plants. Academic Press, pp 551-563. *https://doi.org/10.1016/b978-0-323-95719-9.00019-7*

Sato M, Gaul H (1967). Effect of ethyl methanesulfonate on the fertility of barley. Radiation Botany 7(1):7-15. *https://doi.org/10.1016/0033-7560(67)90028-2*

Shahwar D, Ansari MYK, Bhat TM (2016). Assessment of genetic variability, morphology and productivity response of *Vicia faba* under the stress of lead nitrate. International Journal of Advanced Life Sciences 9:58-65.

Shahwar D, Ansari MYK, Bhat TM, Choudhary S, Aslam R (2017a). Evaluation of high yielding mutant of lentil developed through caffeine of an exotic germplasm. Journal of Plant Breeding and Genetics 11(2):55-62. *https://doi.org/10.1016/j.heliyon.2019.e01722*

Shahwar D, Ansari MYK, Choudhary S (2019). Induction of phenotypic diversity in mutagenized population of lentil (*Lens culinaris* Medik) by using heavy metal. Heliyon 5(5). *https://doi.org/10.3923/ijpbg.2017.55.62*

Shahwar D, Ansari MYK, Choudhary S, Aslam R (2017b). Evaluation of yield attributing variants developed through ethyl methane sulphonate in an important proteinaceous crop-*Vicia faba*. Asian Journal of Crop Science 9:20-27. *https://doi.org/10.3923/ajcs.2017.20.27*

Shahwar D, Khan Z, Ansari MY, Park Y. (2023) Relative mutagenic effectiveness and efficiency of chemical mutagens (Caffeine and EMS) and heavy metals [(Pb (NO3) 2 and Cd (NO3) 2)] in developing chlorophyll and morphological mutants in lentil. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 1:503668. *https://doi.org/10.1016/j.mrgentox.2023.503668*

Shahwar D, Khan Z, Ansari MYK (2020). Evaluation of mutagenized lentil populations by caffeine and EMS for exploration of agronomic traits and mutant phenotyping. Ecological Genetics and Genomics 14:100049. *https://doi.org/10.1016/j.egg.2019.100049*

Sharma N, Shahwar D, Choudhary S (2022). Induction of chromosomal and morphological amelioration in lentil (*Lens culinaris* Medik.) mutagenized population developed through chemical mutagenesis. Vegetos 35(2):474-483. *https://doi.org/10.1007/s42535-021-00319-6*

Shimazaki KI, Doi M, Assmann SM, Kinoshita T (2007). Light regulation of stomatal movement. Annual Review of Plant Biology 58(1):219-247.

Shukla SK (2023). Conservation of medicinal plants: challenges and opportunities. Journal of Medicinal Botany 7:5-10.

Soria-Lopez A, Garcia-Perez P, Carpena M, Garcia-Oliveira P, Otero P, Fraga-Corral M, Cao H, Prieto MA, Simal-Gandara J (2023). Challenges for future food systems: From the Green Revolution to food supply chains with a special focus on sustainability. Food Frontiers 4(1):9-20. *https://doi.org/10.1002/fft2.173*

Stavropoulos P, Mavroeidis A, Papadopoulos G, Roussis I, Bilalis D, Kakabouki I (2023). On the path towards a "greener" EU: A mini review on flax (*Linum usitatissimum* L.) as a case study. Plants 12(5):1102. *https://doi.org/10.38001/ijlsb.690295*

Swaminathan MS (1965). The experimental manipulation of genes. Current Science 34(4):108-111.

Szarejko I, Szurman-Zubrzycka M, Nawrot M, Marzec M, Gruszka D, Kurowska M, ... Maluszynski M (2017). Creation of a TILLING population in barley after chemical mutagenesis with sodium azide and MNU. Biotechnologies for Plant Mutation Breeding: Protocols 91-111.

Thakur G, Paul S, Kumar A (2020). Mutagenic effectiveness and efficiency of ethyl methane sulphonate (EMS) mutagen in linseed (*Linum usitatissimum* L.). Indian Society of Oilseeds Research 37:260-266.

Thombre MV, Mehetre SS (1981). A pistillate mutant in cotton. Indian Journal of Genetics and Plant Breeding 40(2):388-390.

Tomlekova N, Todorova V, Petkova V, Yancheva S, Nikolova V, Panchev I, Penchev E (2009). Creation and evaluation of induced mutants and valuable tools for pepper breeding programmes. Induced Plant Mutations in the Genomics Era, Rome, Italy: Food and Agriculture Organization of the United Nations, pp 187-90.

Ulukapi K, Ozmen SF (2018). Study of the effect of irradiation (60Co) on M1 plants of common bean (*Phaseolus vulgaris* L.) cultivars and determined of proper doses for mutation breeding. Journal of Radiation Research and Applied Sciences 11(2):157-161. *https://doi.org/10.1016/j.jrras.2017.12.004*

United Nations Department of Economic and Social Affairs, Population Division (2024). World Population Prospects 2024: Summary of Results (UN DESA/POP/2024/TR/NO. 9).

Utsunomiya KS, Bione NCP, Pagliarini MS (2002). How many different kinds of meiotic abnormalities could be found in a unique endogamous maize plant. Cytologia 67(2):169-176. *https://doi.org/10.1508/cytologia.67.169*

Veni K, Vanniarajan C, Souframanien J (2017). Probit analysis and effect of electron beam and gamma rays in blackgram (*Vigna mungo* (L.) Hepper). Electronic Journal of Plant Breeding 8(3):950-955. *https://doi.org/10.5958/0975-928X.2017.00112.0*

Wani AA, Anis M (2008). Gamma ray-and EMS-induced bold-seeded high-yielding mutants in chickpea (*Cicer arietinum* L.). Turkish Journal of Biology 32(3):161-166.

Yano K, Yamamoto E, Aya K, Takeuchi H, Lo PC, Hu L, Yamasaki M, Yoshida S, Kitano H, Hirano K, Matsuoka M (2016). Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice. Nature Genetics 48(8):927-934.

Yousuf J, Raina A, Rasik S, Reshi ZA, Shahwar D (2023). Comparative effects of caffeine and lead nitrate on the bio-physiological and yield associated traits of lentil (*Lens culinaris* Medik.). Heliyon 9(6). *https://doi.org/10.1016/j.heliyon.2023.e16351*

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