*Original Research Article*

Induction of novel agronomic and biochemical variations in sunflower (*Helianthus annuus* L.) through EMS mutagenesis for enhanced yield and oil quality

ABSTRACT

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| Low genetic variability in the sunflower cultivar has been a bottleneck in the crop improvement programmes of this introduced crop in India; therefore, the generation of novel variations in this crop becomes highly desirable. To address this, a mutagenesis experiment in the sunflower was carried out at Punjab Agricultural University Ludhiana (30.9010º N and 75.85.73º E), in which the seeds of inbred line 67B were exposed to the chemical mutagen - EMS (ethyl methane sulfonate) @ 0.1% for four hours, and were grown over for two-generation (M1 and M2), during *Kharif* 2021 and *Rabi* 2021. Statistically significant improvements were observed in seed yield, oil content and oleic acid concentrations, with mutants such as M2-380-7 (42.09 g seed yield), M2-358-10 (37.52% oil content), and M2-63-1 (60.62% oleic acid) emerging as promising candidates. K-mean clustering identified two groups of mutants based on agronomic characters and 12 groups of mutants based on biochemical traits. This study highlights the potential of EMS mutagenesis for creating genetic diversity in sunflower and advancing breeding programs. The identified mutants offer practical value for sunflower varietal development and hybrid breeding aimed at improved productivity and oil quality. In conclusion, EMS-induced mutagenesis serves as a valuable tool to overcome genetic bottlenecks and accelerate targeted sunflower improvement. |

*Keywords: Sunflower, mutagenesis, EMS, fatty acid composition, K-means cluster*

1. INTRODUCTION

Sunflower oil is the fourth most-produced vegetable oil globally, following palm, soybean, and canola oil, contributing significantly to human nutrition and industry. In 2023, the global production of sunflower oil reached approximately 20 million metric tons, accounting for about 10% of the total vegetable oil market. However, due to continuous selection for targeted commercially important traits, numerous essential alleles present naturally in sunflowers are lost, especially in India where this crop was introduced in 1969 from the Russian Federation after recurrent selections and further selection were extensively practised, therefore cultivable species show a low amount of gene variability, therefore, the creation of novel genetic variabilities becomes essential in this crop.

Induced mutagenesis, particularly using chemical agents like EMS, provides an effective method for generating novel genetic variations (Fernández-Martínez et al. 1993, Ahloowalia et al. 2004 and Pessino et al. 2025), and this approach is well suited for developing sunflower genotypes with enhanced yield, oil quality and other agronomic traits (Jambhulkar and Shitre 2009, Cvejić et al. 2011 and Shamimuzzaman et al. 2023). Previous studies have also demonstrated the efficacy of mutagenesis in creating high-oleic acid lines, herbicide-resistant varieties, disease-tolerant lines and improved metal phytoextraction (Kolbas et al. 2011, Christov and Hristova-Cherbadzh 2020, and Fernández-Melero 2023). However, its application in the Indian sunflower breeding remains unexploited.

This study seeks to address these gaps by inducing mutations in sunflower using EMS and evaluating their impact on key agronomic and biochemical traits. These findings hold promise for improving sunflower genotypes and meeting future challenges such as climate resilience, quality improvement and disease resistance.

2. Materials and Methods

**2.1. Planting material and field layout**

A total of 4500 seeds of inbred 67B (obtained from the oilseed section, department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana) were treated with 0.1% dosage of EMS for four hours’ time durations as determined median lethal dose (LD50) in the experiment by Sharma et al. (2023) and were grown in the field alongside untreated seeds during Kharif 2021 (M1 generation) and Rabi 2022 (M2 generation), at the spacing of 60 × 30 cm at the sunflower breeding area, Punjab Agricultural University, Ludhiana (30.9010º N and 75.85.73º E).

**2.2 Observations recorded and statistical analysis**

Various quantitative and qualitative traits were observed for both generations, and statistical comparison between treated and control populations was performed using t-tests and confidence intervals (CI). Mutation frequency, efficiency and effectiveness were calculated to understand the extent of mutagenesis, and K-means clustering was employed to identify superior groups based on key traits using the NB clust package in R software.

where,

3. results and discussion

This EMS-induced mutagenesis experiment yielded significant variations in both quantitative as well as qualitative traits of sunflowers, demonstrating the potential of this approach for enhancing genetic diversity and improving agronomic and biochemical characteristics (Appendix Figure 1-13)), as also observed by Padmavathi et al. (2020). The results, supported by the comparative analysis with the control population, highlight the implication of induced mutations in sunflower breeding.

**3.1. Enhancement in Quantitative and Qualitative Traits**

The comparative analysis for quantitative traits for M1 and M2 generations is presented in Figures 1 to 12**,** which depicts that approximately 54.17% of the M2 population is below the CI for days to flowering. Notably, mutants such as M2-131-4 (58 days), M2-131-5 (58 days), and M2-131-7 (58 days) were identified as early-flowering and early maturing mutants. Moving on, 49.17% of plants had greater height, 41.40% had more head diameter, 44.74% had thicker stem girth, 49.57% had a greater number of leaves per plant, and 49.46% had more bracts per head in M2 generation in comparison to the control population. Consistent to this study, several other authors like Lazanyi et al. (1961), Savin and Stapanko (1968), Gundaev (1971), Surovikin (1973), Leclercq (1984), Encheva et al. (1993, 2002, 2003a, 2003b and 2003c), Srivastava and Srivastava (2004), Bharose (2014), Habib et al (2023) and Selvaraj and Jayakumar (2023), have also identified variation in structural characters in sunflower upon mutation.

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| Figure 1.: Box-plot diagram for time of flowering in both generations | Figure 2.: Box-plot diagram for plant height in both generations |
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| Figure 3.: Box-plot diagram for head diameter in both generations | Figure 4.: Box-plot diagram for stem girth in both generations |
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| Figure 5.: Box-plot diagram for number of leaves per plant in both generations | Figure 6.: Box-plot diagram for number of bracts per head in both generations |
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| Figure 7.: Box-plot diagram for biological yield per plant in M2 generations | Figure 8.: Box-plot diagram for economic yield per plant in M2 generations |
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| Figure 9.: Box-plot diagram for the number of seeds per head in both generations | Figure 10.: Box-plot diagram for harvest index in M2 generations |
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| Figure 11.: Box-plot diagram for oil content in M2 generations | Figure 12.: Box-plot diagram for fatty acid composition in M2 generations |
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Similarly, 30.86% of the M2 population was above CI for the seed yield per plant with mutants such as M2-380-7 (42.09 g), M2-204-1 (39.8 g), and M2-339-23 (39.72 g) being the high-yielding ones, while 33.11% of the population had greater biological weight per plant with high weighing mutants such as M2-179-1 (824 g), M2-245-1 (702 g), M2-380-9 (624 g) and M2-191-5 (614 g). Likewise, 37.19% population had more number of seeds per head with notable mutants like M2-204-1 (928), M2-525-13 (701) and M2-469-4 (655), giving rise to 19.89% of the population with a higher harvest index. These results align with the findings of Chandrappa (1980), Deshpande and Giriraj (1998), Jagadeesan et al. (2008) and Habib et al. (2023), who also reported similar yield gains in the sunflower following mutation breeding, however, it opposes the study of Chuiko (2022), who reported low yields in the sunflower lines upon mutation.

The oil content of 52.88% of the M2 plants surpassed the CI, with mutants like M2-358-10 (37.52 %), M2-144-6 (36.51 %), and M2-525-5 (35.77 %) showing maximum production of the same, thereby proving that this improvement reinforces the role of chemical mutagenesis in enhancing the economic value of the sunflower crop. Notably, oleic acid levels were increased in 50.44% of the M2 population, with M2-63-1 (60.62 %), M2-28-5 (60.06 %) and M2-78-20 (59.89 %) exhibiting superior fatty acid profiles. Other, biochemically superior mutants were also recognised for low palmitic acid (M2-395-3 (5.03 %), M2-114-6 (5.06 %) and M2-395-10 (5.12 %)), relatively high stearic acid content (M2-63-3 (5.83 %), M2-119-5 (4.82 %) and M2-28-11 (4.75 %)) and low linoleic acid content (M2-63-1 (25.25 %), M2-78-20 (27.87 %) and M2-380-7 (28.18 %)). These results mirror advancement in the oil quality achieved through mutagenesis as documented by Soldatov (1971), Voskoboinik and Soldatov (1974), Vranceanu and Stoenescu (1982), Kubler (1984), Osorio (1995), Vick and Miller (1996), Christov (1996), Miller and Vick (1999), Fernandez-Moya et al. (2002), Salas et al. (2004), Velasco et al. (2008) Natikar (2011), Leon et al. (2013 and 2022), Alberio et al. (2016 and 2018), Rozhon (2023) and Demurin et al. (2025).

The experiment also produced significant morphological variations (identified via DUS characteristics identified by Dhillion et al. (2013)) (Appendix Table 1**)** in leaf and stem traits, like anthocyanin pigmentation in leaves and bracts as well as leaf size and shape. Unique mutants like M2-63-1 exhibited heterochromatic leaves, indicating potential for ornamental or specialized breeding. Furthermore, variations in ray floret pigmentation and bract characteristics, including the presence of pale and heterochromatic florets were recorded. Such traits provide valuable genetic diversity for aesthetic sunflower breeding programs. Similar to this study, various other authors like Luczkiewicz (1975), Coppola (1986), Hermelin et al. (1987), Jan and Rutger (1988), Srivastava and Srivastava (2004), Soroka and Lyakh (2011), Encheva et al. (2011 and 2012), Yang et al. (2012) and Selvaraj and Jayakumar (2023), also reported variations in qualitative traits due to mutagen treatment in sunflower. Notably, Rozhon et al. (2023) reported changes in seed shape and size, while Mshelmbula et al. (2025) documented alterations in plant architecture and flowering patterns, similar to this study.

**3.2 Analysis for Mutation Frequency, Mutagenic Effectiveness and Efficiency**

Based on the study conducted by authors like Jayakumar and Selvaraj (2003), and Kumar and Venkat (2010) who also worked out the mutagenic frequency, effectiveness and efficiency in response to increasing dosage of mutagen in this oilseed crop, this study also calibrated these parameters based on number of mutants observed (Appendix Table 2). Mutation frequency serves as a key indicator of the number of observable genetic changes within a treated population. This study depicted that the mutation frequency for the quantitative characters was consistently high exceeding 85% for most quantitative traits in the M1 generation including characters like head diameter, number of bracts per head and the number of seeds per plant, however, a slight reduction in mutation frequency was observed in M2 generation, with values remaining above 70% for key traits. This trend indicates the stability of the induced mutations across generations, though some loss of mutant phenotypes has occurred due to natural selection pressure, as also indicated by Banakar (2011 and 2013). For qualitative traits, mutation frequency showed greater variability, with traits such as leaf anthocyanin pigmentation and petiole pigmentation exhibiting more than 90% of mutation frequency, while certain traits like seed-base colour displayed minimal or no mutations in M2 generation.

Furthermore, the mutation effectiveness was calculated to measure the ability of the mutagen to induce observable changes relative to the dosage and duration of exposure, which revealed that effectiveness is highest for the traits directly related to yields and reproductive fitness, like the number of leaves per plant (2.50 in M1 and 2.32 in M2) and time of flowering (2.49 in M1 and 2.38 in M2), demonstrating a consistent response to mutagenic treatment across generations. While, qualitative traits, like stem pigmentation (2.35 in M1 and 2.21 in M2) and leaf blistering (1.04 in M1 and 1.79 in M2) show variability in effectiveness, reflecting the differential sensitivity of traits to mutagenic stress.

To evaluate the proportion of induced mutations that are observable relative to the overall lethality, mutagenic efficiency was recorded which showed that it rapidly decreases from 30 days after sowing to 60 days to sowing to 90 days after sowing to harvest as the lethality increases (survival percentage decreases) due to various abiotic and biotic stresses faced by plant increases, as the crop progresses.

**3.3 Statistical Analysis for Pollen Viability (%) / Fertility**

Similar to many other scientists like Reddy et al. (1993), Kumar and Venkat (2010), and Demurin and Rubanova (2021) who reported reduced pollen fertility, and Natikar (2011) reported increased fertility due to mutagenesis, while Jan and Rutger (1988) who isolated male sterile plants formed due to mutagen treatment, this study also observed the ability of the plants to maintain fertility under mutagenic treatment, by constituently collecting the pollens of M2 generation at 8 am every day during the time of anthesis, and observing their viability using acetocarmine solution under light microscope.

The results depicted that 90.16% of plants in the mutated population had 100% pollen viability, suggesting that most mutants retained their reproductive capabilities, while 9.84% of plants showed reduced pollen fertility, with isolated cases of male sterility (M2-214-1, M2-131-6, M2-131-9, M2-131-10), non-viable white pollens (M2-228-4, M2-280-11, M2-446-2 and M2-592-1), partially sterile mixed white and yellow pollens (M2-389-17, M2-395-3, M2-395-10, M2-395-15, M2-395-17) and few headless plants (M2-21-17, M2-21-20, M2-21-22, M2-28-1, M2-246-17, M2-389-2, M2-389-16, M2-422-8, M2-477-2, M2-531-5, M2-538-3 and M2-538-5).

**3.4. K- mean clustering of mutants**

The K-mean clustering revealed distinct groups of mutants based on agronomic and biochemical traits. In M2 generation (Table 1, Figure 13 and Figure 14), Cluster I was identified as superior with traits like early flowering, higher seed yield and greater plant height, while in oil composition clustering (Table 2 and Figure 15), among 12 clusters, Cluster IV stood out with high oil content (29.57 %), elevated oleic acid content (58.46 %) and reduced linoleic acid content (30.47 %). Such fatty acid profiles are highly desirable in the edible oil industry, aligning with the trends noted by Fernández-Martínez et al. 1993.

Table 1: Cluster means for all the clusters in M1 and M2 generation

| **Traits under Study** | **M1 generation** | | **M2 generation** | |
| --- | --- | --- | --- | --- |
| **Cluster I** | **Cluster II** | **Cluster I** | **Cluster II** |
| Days to flowering | 54.797 | 59.045 | 77.326 | 82.738 |
| Plant Height | 34.828 | 25.617 | 78.621 | 66.323 |
| Head Diameter | 4.327 | 2.770 | 6.554 | 3.665 |
| Stem Girth | 2.626 | 1.935 | 2.518 | 2.026 |
| Number of Leaves per Plant | 12.729 | 10.139 | 15.442 | 12.492 |
| Number of Bracts per Head | 28.159 | 16.082 | 36.063 | 22.769 |
| Number of Seeds per Head | 15.715 | 0.107 | 73.853 | 26.262 |
| Harvest Index (%) | - | - | 8.095 | 5.986 |
| Pollen Viability | - | - | 97.453 | 93.369 |
| Biological Yield Per Plant | - | - | 53.270 | 25.615 |
| Economic Yield per Plant | - | - | 3.511 | 0.549 |
| HypocotylAnthocyanin Colouration | 1.705 | 1.980 | 1.116 | 1.077 |
| Leaf Anthocyanin Colouration on the margins of Young Leaves | - | - | 1.000 | 1.000 |
| Time of Flowering | 2.420 | 2.734 | 3.611 | 3.831 |
| Leaf size | 3.193 | 2.340 | 3.011 | 2.523 |
| Leaf Shape | 1.937 | 1.332 | 2.305 | 2.169 |
| Leaf Colour | 2.048 | 1.930 | 1.926 | 2.046 |
| Leaf Blistering | 2.261 | 1.885 | 2.747 | 2.231 |
| Leaf Fineness of Serrations | 2.295 | 2.660 | 1.926 | 2.277 |
| Leaf Angle of Lateral Veins | 1.507 | 1.168 | 1.684 | 1.431 |
| Leaf Height of the Tip of the Blade Compared to Insertion of Petiole (⅔ th height of plants) | 2.246 | 2.668 | 2.937 | 2.646 |
| Leaf Angle between Lower part of Petiole and Stem | 2.763 | 2.656 | 2.568 | 2.692 |
| Leaf Hairiness | 2.010 | 1.836 | 1.011 | 1.000 |
| Leaf Petiole Pigmentation | 1.430 | 1.451 | 1.979 | 2.000 |
| Stem Hairiness at the Top | 2.556 | 2.275 | 2.042 | 2.031 |
| Stem Pigmentation | 2.430 | 2.586 | 1.147 | 1.415 |
| Stem Number of Leaves on Main Stem | 1.019 | 1.000 | 1.137 | 1.046 |
| Ray Flower Number | 2.357 | 2.225 | 2.853 | 2.354 |
| Ray Flower Shape | 2.275 | 2.123 | 2.905 | 2.631 |
| Ray Flower Colour | 4.000 | 3.947 | 4.000 | 4.000 |
| Disc Flower Colour | 2.005 | 2.000 | 2.000 | 2.000 |
| Disc Flower Anthocyanin Colouration of Stigma | 3.068 | 2.713 | 2.000 | 2.000 |
| Disc Flower Pollen Colour | 3.995 | 3.980 | 3.989 | 4.000 |
| Head Number of Bracts on the Back | 3.208 | 2.336 | 3.695 | 2.938 |
| Bract Shape | 2.787 | 2.516 | 2.989 | 2.954 |
| Bract Anthocyanin Colouration | - | - | 2.000 | 2.000 |
| Head Attitude | 3.377 | 3.008 | 5.916 | 5.877 |
| Head Diameter | - | - | 1.042 | 1.000 |
| Head Shape of Grain Side | 3.715 | 3.770 | 3.905 | 3.831 |
| Plant Height | - | - | - | - |
| Plant Branching | 1.005 | 1.049 | 1.084 | 1.031 |
| Plant Natural Position of Closest Lateral Head to the Central Head (end of Flowering) - Branched | 1.010 | 1.148 | 1.168 | 1.077 |
| Plant Type of Branching | 1.010 | 1.078 | 1.168 | 1.062 |
| Seed Length | 2.961 | 1.041 | 3.516 | 1.000 |
| Seed Shape | 3.329 | 1.033 | 3.474 | 1.000 |
| Seed Base Colour | 4.903 | 1.115 | 3.000 | 1.000 |
| Seed Mottling | 2.406 | 1.041 | 2.011 | 1.000 |
| Seed Stripes | 2.580 | 1.049 | 2.947 | 1.000 |
| Seed Colour of Stripes | 3.952 | 1.070 | 3.158 | 1.000 |

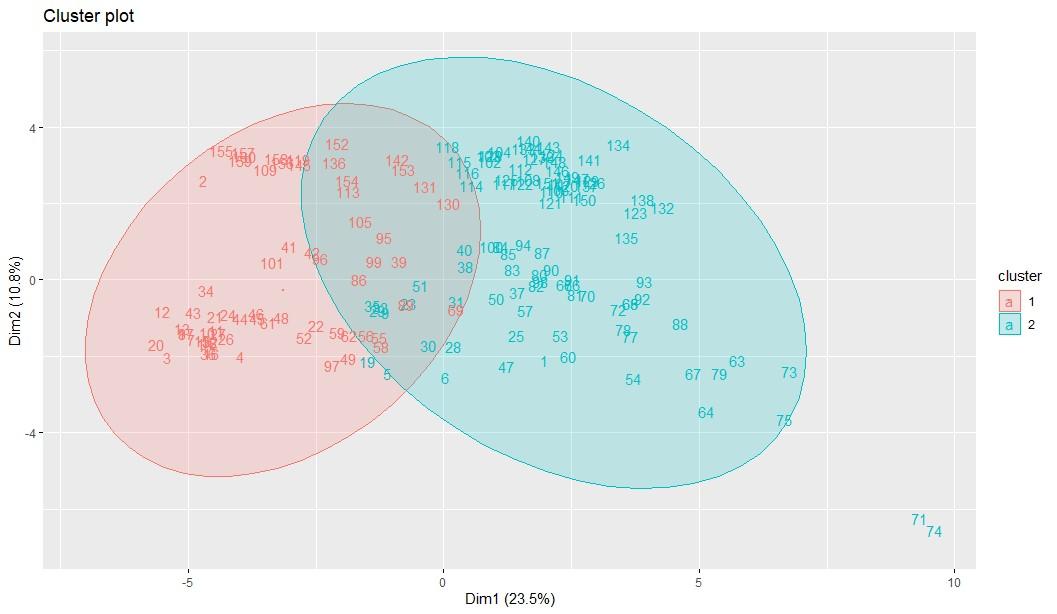


Figure 13.: Cluster - plot analysis for M1 generation

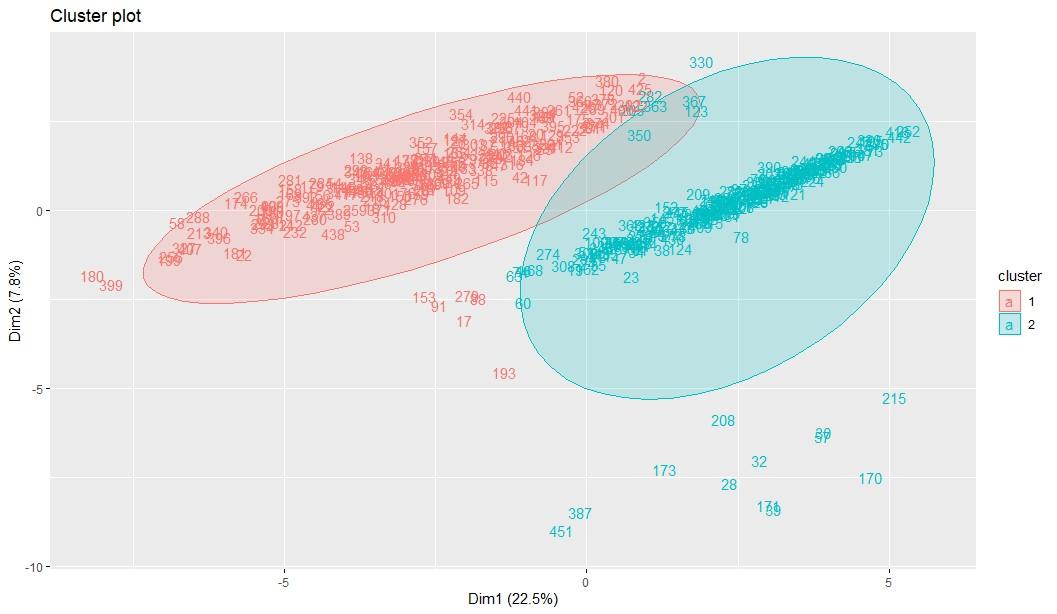


Figure 14.: Cluster - plot analysis for M2 generation

Table 2.: Cluster means for oil content and fatty acid composition in M2 generation

| **Cluster** | **Oil Content (%)** | **Palmitic Acid (%)** | **Stearic Acid (%)** | **Oleic Acid (%)** | **Linoleic Acid (%)** |
| --- | --- | --- | --- | --- | --- |
| I | 26.60 | 7.48 | 2.14 | 40.70 | 49.71 |
| II | 18.80 | 6.36 | 1.56 | 36.84 | 55.24 |
| III | 28.73 | 8.25 | 1.21 | 30.48 | 60.06 |
| IV | 29.57 | 6.73 | 3.75 | 58.46 | 30.47 |
| V | 24.25 | 7.05 | 3.91 | 49.70 | 39.33 |
| VI | 19.02 | 7.64 | 3.16 | 37.99 | 51.14 |
| VII | 28.59 | 5.98 | 0.94 | 34.94 | 58.14 |
| VIII | 25.39 | 9.88 | 2.35 | 39.10 | 48.66 |
| IX | 28.02 | 8.73 | 1.79 | 55.47 | 34.02 |
| X | 22.94 | 5.97 | 2.30 | 48.98 | 40.93 |
| XI | 18.92 | 7.64 | 2.44 | 26.87 | 63.08 |
| XII | 30.84 | 5.96 | 1.68 | 46.42 | 45.94 |

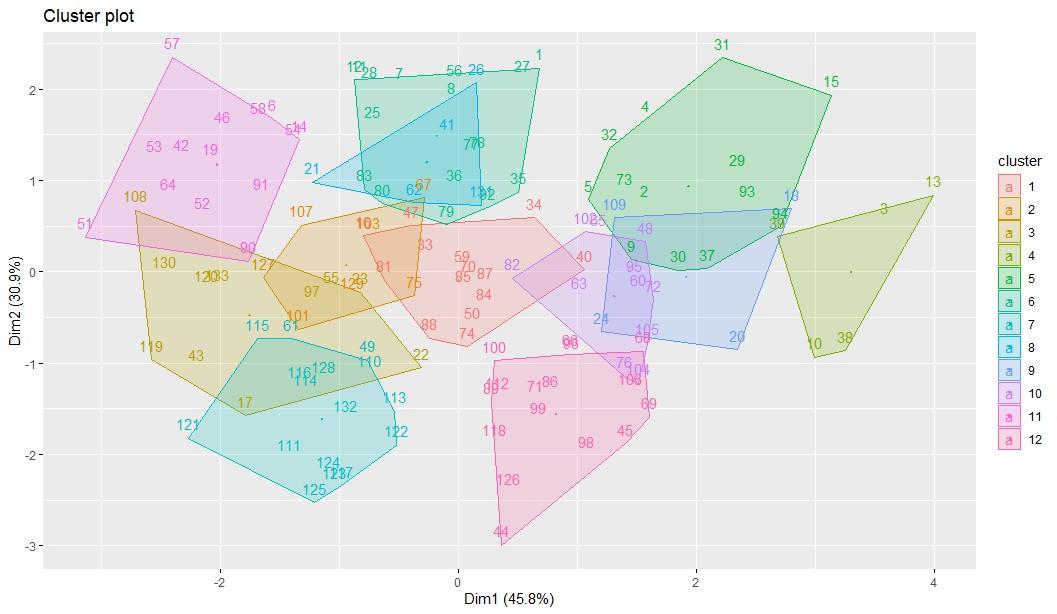


Figure 15.: Cluster-plot analysis for oil content and fatty acid composition M2 generation

4. Conclusion

This study demonstrated the effectiveness of EMS-induced mutagenesis in generating significant genetic diversity in sunflower, leading to improved agronomic and biochemical traits. High-yielding mutants like M2-380-7, oil-rich lines like M2-358-10 and the superior oleic acid profile of M2-63-1, make them highly valuable for breeding programs and industrial applications.

The results highlight the potential of EMS mutagenesis as a precise and efficient tool for addressing genetic bottlenecks for sunflower breeding, as by creating novel variations in economically important traits, this approach contributes to the development of high-performing cultivars suited to evolving agricultural and market demands. Future integration of these mutations into hybrid and molecular breeding could further amplify genetic gains, enhancing sunflower productivity and resilience globally.

Consent

Not applicable

Ethical approval

Not applicable

disclaimer (Artificial Intelligence)

Author(s) hereby declare that no generative AI technologies such as large language models (ChatGPT, Copilot, etc.) And text-to-image generators have been used during the writing or editing of this manuscript, except for Grammarly: a free AI writing assistance tool was downloaded and installed in the PC for grammar checks.

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APPENDIX

Table 1.: Characterization of M1 and M2 generation for qualitative traits in comparison to control

| [S. No.](http://s.no/) | **Trait** | **Characters observed in particular trait** | **M1 generation** | | **M2 generation** | |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Control Population** | **Mutants Population (%)** | **Control Population** | **Mutants Population (%)** |
| 1 | **Hypocotyl Anthocyanin Colouration** | Absent | ✔ | 27.273 | ✔ | 97.455 |
| Medium |  | 43.237 |  | 1.382 |
| Strong |  | 26.829 |  | 1.164 |
| 2 | **Leaf Anthocyanin Colouration on the margins of Young Leaves** | Absent | ✔ | 100 | ✔ | 99.927 |
| Present |  | 0 |  | 0.073 |
| 3 | **Time of Flowering** | Headless |  | 1.996 |  | 0.873 |
| Early | ✔ | 0.222 |  | 0.145 |
| Medium |  | 42.794 | ✔ | 35.636 |
| Late |  | 54.989 |  | 63.345 |
| 4 | **Leaf size** | Very Small |  | 5.543 |  | 0.073 |
| Small |  | 38.137 |  | 13.164 |
| Medium |  | 36.142 | ✔ | 21.382 |
| Large |  | 18.625 |  | 24.291 |
| Very Large | ✔ | 1.552 |  | 41.091 |
| 5 | **Leaf Shape** | Oblong |  | 60.31 | ✔ | 5.745 |
| Lanceolate |  | 21.951 |  | 46.473 |
| Triangular | ✔ | 16.186 |  | 39.127 |
| Cordate |  | 0 |  | 8 |
| Rounded |  | 1.552 |  | 0.582 |
| Ovate |  | 0 |  | 0.073 |
| 6 | **Leaf Colour** | Light Green |  | 28.603 |  | 12.073 |
| Medium Green |  | 44.568 | ✔ | 35.491 |
| Dark Green | ✔ | 26.829 |  | 52.364 |
| Heterochromatic |  | 0 |  | 0.073 |
| 7 | **Leaf Blistering** | Absent |  | 18.182 |  | 4.945 |
| Medium | ✔ | 58.093 | ✔ | 28.945 |
| Strong |  | 23.725 |  | 66.109 |
| 8 | **Leaf Fineness of Serrations** | Fine |  | 15.521 |  | 23.855 |
| Medium |  | 20.177 |  | 52.145 |
| Coarse | ✔ | 64.302 | ✔ | 24 |
| 9 | **Leaf Angle of Lateral Veins** | Acute |  | 72.506 |  | 29.091 |
| Right Angled or Nearly Right Angled | ✔ | 22.838 | ✔ | 54.327 |
| Obtuse |  | 4.656 |  | 16.582 |
| 10 | **Leaf Height of the Tip of the Blade Compared to Insertion of Petiole (⅔ rd height of plants)** | Very Low | ✔ | 24 |  | 0 |
| Low |  | 23.111 | ✔ | 46.182 |
| Medium |  | 36 |  | 18.618 |
| High |  | 14.889 |  | 35.127 |
| Very High |  | 2 |  | 0 |
| 11 | **Leaf Angle between Lower part of Petiole and Stem** | Small |  | 3.125 |  | 0.073 |
| Medium | ✔ | 22.098 |  | 55.709 |
| Large |  | 74.777 | ✔ | 44.218 |
| 12 | **Leaf Hairiness** | Absent | ✔ | 19.512 |  | 99.855 |
| Sparse |  | 69.18 | ✔ | 0.145 |
| Dense |  | 11.308 |  | 0 |
| 13 | **Leaf Petiole Pigmentation** | Absent |  | 56.098 | ✔ | 11.782 |
| Present | ✔ | 43.902 |  | 88.218 |
| 14 | **Stem Hairiness at the Top** | Absent |  | 2.882 |  | 0.437 |
| Medium | ✔ | 53.659 |  | 98.469 |
| Strong |  | 43.459 | ✔ | 1.093 |
| 15 | **Stem Pigmentation** | Absent |  | 23.556 | ✔ | 95.849 |
| Weak | ✔ | 27.333 |  | 1.675 |
| Medium |  | 22.889 |  | 1.602 |
| Dense |  | 26.222 |  | 0.874 |
| 16 | **Stem Number of Leaves on Main Stem** | Low | ✔ | 99.113 | ✔ | 85.08 |
| Medium |  | 0.887 |  | 11.499 |
| High |  | 0 |  | 3.421 |
| 17 | **Ray Flower Number** | Headless |  | 0.222 |  | 0.873 |
| Very Few |  | 72.284 |  | 21.033 |
| Few | ✔ | 27.051 |  | 53.13 |
| Medium |  | 0.222 |  | 15.284 |
| Many |  | 0 |  | 6.405 |
| Very Many |  | 0.222 | ✔ | 3.275 |
| 18 | **Ray Flower Shape** | Headless |  | 0.222 |  | 0.873 |
| Elongated | ✔ | 84.257 | ✔ | 21.891 |
| Ovoid |  | 11.752 |  | 63.2 |
| Rounded |  | 3.769 |  | 14.036 |
| 19 | **Ray Flower Colour** | Headless |  | 0.234 |  | 0.873 |
| Ivory |  | 0 |  | 0 |
| Pale Yellow |  | 4.215 |  | 0.073 |
| Yellow | ✔ | 94.678 | ✔ | 99.055 |
| Orange |  | 1.109 |  | 0 |
| Purple |  | 0 |  | 0 |
| Red Brown |  | 0 |  | 0 |
| Multicolour |  | 0 |  | 0 |
| 20 | **Disc Flower Colour** | Headless |  | 0.222 |  | 0.873 |
| Yellow | ✔ | 99.557 | ✔ | 99.127 |
| Red |  | 0.222 |  | 0 |
| Purple |  | 0 |  | 0 |
| 21 | **Disc Flower Anthocyanin Colouration of Stigma** | Headless |  | 0.222 |  | 0.873 |
| Absent | ✔ | 55.111 | ✔ | 97.818 |
| Weak |  | 16.667 |  | 0 |
| Medium |  | 12.889 |  | 0.727 |
| Strong |  | 15.111 |  | 0.582 |
| 22 | **Disc Flower Pollen Colour** | Headless |  | 0.226 |  | 0.873 |
| Male Sterile |  | 0.451 |  | 0.291 |
| White |  | 0.451 |  | 0.291 |
| Yellow | ✔ | 98.871 | ✔ | 98.182 |
| Half Yellow and Half White |  | 0 |  | 0.364 |
| 23 | **Head Number of Bracts on the Back** | Headless |  | 0.222 |  | 0.873 |
| Few |  | 54.767 |  | 21.164 |
| Medium |  | 16.851 |  | 11.345 |
| Many | ✔ | 28.16 | ✔ | 66.109 |
| 24 | **Bract Shape** | Headless |  | 0.222 |  | 0.873 |
| Elongated |  | 35.92 |  | 0.873 |
| Rounded | ✔ | 63.858 | ✔ | 98.255 |
| 25 | **Bract Anthocyanin Colouration** | Headless |  | 0.222 |  | 0.873 |
| Absent | ✔ | 99.778 | ✔ | 99.055 |
| Present |  | 0 |  | 0.073 |
| 26 | **Head Attitude** | Headless |  | 0.225 |  | 0.873 |
| Horizontal |  | 31.011 |  | 0.073 |
| Inclined |  | 34.382 |  | 0.8 |
| Vertical | ✔ | 24.944 |  | 2.036 |
| Half Turned Down |  | 5.843 |  | 4.873 |
| Turned Down |  | 3.596 | ✔ | 91.345 |
| 27 | **Head Diameter** | Headless |  | 0.222 |  | 0.873 |
| Small | ✔ | 99.778 | ✔ | 94.982 |
| Medium |  | 0 |  | 3.855 |
| Large |  | 0 |  | 0.291 |
| 28 | **Head Shape of Grain Side** | Headless |  | 0.223 |  | 0.873 |
| Concave |  | 9.152 |  | 4.436 |
| Flat |  | 12.723 |  | 1.818 |
| Convex | ✔ | 72.545 | ✔ | 92.509 |
| Mis-Shaped |  | 5.357 |  | 0.364 |
| 29 | **Plant Height** | Very Short | ✔ | 99.778 | ✔ | 40.8 |
| Short |  | 0 |  | 42.545 |
| Medium |  | 0 |  | 14.909 |
| Tall |  | 0 |  | 1.745 |
| Very Tall |  | 0.222 |  | 0 |
| 30 | **Plant Branching** | Absent | ✔ | 97.118 | ✔ | 95.636 |
| Present |  | 2.882 |  | 4.364 |
| 31 | **Plant Natural Position of Closest Lateral Head to the Central Head (end of Flowering) - Branched** | No Branching | ✔ | 97.118 | ✔ | 95.636 |
| Above |  | 1.109 |  | 0.291 |
| Below |  | 1.774 |  | 4.073 |
| 32 | **Plant Type of Branching** | No Branching | ✔ | 97.118 | ✔ | 95.636 |
| Basal Branching |  | 0.443 |  | 0.873 |
| Top Branching |  | 0.887 |  | 0.727 |
| Fully Branched with central head |  | 0 |  | 2.545 |
| Fully Branched without central head |  | 1.552 |  | 0.218 |
| 33 | **Seed Length** | Short |  | 29.952 |  | 8.188 |
| Medium |  | 42.029 | ✔ | 36.54 |
| Long | ✔ | 28.019 |  | 55.271 |
| 34 | **Seed Shape** | Elongated |  | 25.604 |  | 13.306 |
| Ovoid Elongated | ✔ | 34.3 |  | 29.99 |
| Ovoid Wide |  | 19.807 | ✔ | 53.122 |
| Rounded |  | 20.29 |  | 3.582 |
| 35 | **Seed Base Colour** | white |  | 0 |  | 0.102 |
| Grey |  | 0 |  | 0 |
| Brown |  | 0 |  | 0 |
| Black | ✔ | 100 | ✔ | 99.898 |
| 36 | **Seed Mottling** | Absent | ✔ | 55.556 |  | 99.693 |
| Present |  | 44.444 | ✔ | 0.307 |
| 37 | **Seed Stripes** | Absent |  | 38.164 |  | 1.842 |
| Present | ✔ | 61.836 | ✔ | 98.158 |
| 38 | **Seed Colour of Stripes** | Stripes Absent |  | 32.367 |  | 1.842 |
| White |  | 16.908 | ✔ | 0.205 |
| Grey | ✔ | 6.28 |  | 96.111 |
| Violet Grey |  | 7.246 |  | 1.842 |
| Brown |  | 36.715 |  | 0 |

Table 2.: Mutation frequency, mutagenic effectiveness and efficiency among M1 and M2 populations for various qualitative and quantitative traits

| [**S.No**](http://s.no/)**.** | **Trait** | **Mutagenic Frequency (%)** | | **Mutagenic Effectiveness** | | **Mutagenic Efficiency based on Germination (%) for M1 Population** | **Mutagenic Efficiency based on Survival (%) for M1 Population** | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **M1** | **M2** | **M1** | **M2** | **After 30 days of sowing** | **After 60 days of sowing** | **After 90 days of sowing** | **At Harvest** |
| 1 | **DF** | 96.026 | 95.007 | 2.401 | 2.375 | 1.111 | 5.752 | 4.132 | 2.978 | 1.452 |
| 2 | **PHT** | 94.667 | 92.291 | 2.367 | 2.307 | 1.095 | 5.671 | 4.073 | 2.936 | 1.432 |
| 3 | **HD** | 87.556 | 96.843 | 2.189 | 2.421 | 1.013 | 5.245 | 3.767 | 2.716 | 1.324 |
| 4 | **STG** | 90.687 | 96.073 | 2.267 | 2.402 | 1.049 | 5.432 | 3.902 | 2.813 | 1.371 |
| 5 | **NLPP** | 100.000 | 92.795 | 2.500 | 2.320 | 1.157 | 5.990 | 4.303 | 3.102 | 1.512 |
| 6 | **NB** | 94.000 | 95.448 | 2.350 | 2.386 | 1.088 | 5.631 | 4.045 | 2.915 | 1.422 |
| 7 | **PV** | - | 99.926 | - | 2.498 | - | - | - | - | - |
| 8 | **BW** | - | 95.310 | - | 2.383 | - | - | - | - | - |
| 9 | **SDY** | - | 91.198 | - | 2.280 | - | - | - | - | - |
| 10 | **NSP** | 90.291 | 95.496 | 2.257 | 2.387 | 1.045 | 5.409 | 3.885 | 2.800 | 1.365 |
| 11 | **HI** | - | 72.057 | - | 1.801 | – | - | - | - | – |
| 12 | **OC** | - | 79.894 | - | 1.997 | - | - | - | - | - |
| 13 | **PA** | - | 89.474 | - | 2.237 | - | - | - | - | - |
| 14 | **SA** | - | 91.729 | - | 2.293 | - | - | - | - | - |
| 15 | **OA** | - | 86.466 | - | 2.162 | - | - | - | - | - |
| 16 | **LA** | - | 84.211 | - | 2.105 | - | - | - | - | - |
| 17 | **HAC** | 72.727 | 2.545 | 1.818 | 0.064 | 0.842 | 4.356 | 3.129 | 2.256 | 1.100 |
| 18 | **LAC** | 0.000 | 0.073 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 19 | **PTF** | 99.778 | 35.782 | 2.494 | 0.895 | 1.155 | 5.977 | 4.293 | 3.095 | 1.509 |
| 20 | **LSi** | 98.448 | 78.618 | 2.461 | 1.965 | 1.139 | 5.897 | 4.236 | 3.053 | 1.489 |
| 21 | **LSh** | 83.814 | 94.255 | 2.095 | 2.356 | 0.970 | 5.021 | 3.606 | 2.600 | 1.268 |
| 22 | **LC** | 73.171 | 64.509 | 1.829 | 1.613 | 0.847 | 4.383 | 3.148 | 2.269 | 1.107 |
| 23 | **LB** | 41.907 | 71.709 | 1.048 | 1.793 | 0.485 | 2.510 | 1.803 | 1.300 | 0.634 |
| 24 | **LFS** | 35.698 | 76.000 | 0.892 | 1.900 | 0.413 | 2.138 | 1.536 | 1.107 | 0.540 |
| 25 | **LAV** | 77.162 | 45.673 | 1.929 | 1.142 | 0.893 | 4.622 | 3.320 | 2.393 | 1.167 |
| 26 | **LHT** | 76.000 | 53.818 | 1.900 | 1.345 | 0.879 | 4.553 | 3.270 | 2.357 | 1.149 |
| 27 | **LAPS** | 77.902 | 55.782 | 1.948 | 1.395 | 0.901 | 4.666 | 3.352 | 2.416 | 1.178 |
| 28 | **LH** | 80.488 | 99.855 | 2.012 | 2.496 | 0.931 | 4.821 | 3.463 | 2.496 | 1.217 |
| 29 | **LP** | 56.098 | 88.218 | 1.402 | 2.205 | 0.649 | 3.360 | 2.414 | 1.740 | 0.848 |
| 30 | **SH** | 46.341 | 98.907 | 1.159 | 2.473 | 0.536 | 2.776 | 1.994 | 1.437 | 0.701 |
| 31 | **SP** | 72.667 | 4.151 | 1.817 | 0.104 | 0.841 | 4.353 | 3.127 | 2.254 | 1.099 |
| 32 | **SN** | 0.887 | 14.920 | 0.022 | 0.373 | 0.010 | 0.053 | 0.038 | 0.028 | 0.013 |
| 33 | **RN** | 72.949 | 96.725 | 1.824 | 2.418 | 0.844 | 4.370 | 3.139 | 2.263 | 1.103 |
| 34 | **RS** | 15.743 | 78.109 | 0.394 | 1.953 | 0.182 | 0.943 | 0.677 | 0.488 | 0.238 |
| 35 | **RC** | 5.322 | 0.945 | 0.133 | 0.024 | 0.062 | 0.319 | 0.229 | 0.165 | 0.080 |
| 36 | **DC** | 0.443 | 0.873 | 0.011 | 0.022 | 0.005 | 0.027 | 0.019 | 0.014 | 0.007 |
| 37 | **DA** | 44.889 | 2.182 | 1.122 | 0.055 | 0.519 | 2.689 | 1.931 | 1.392 | 0.679 |
| 38 | **DP** | 1.129 | 1.818 | 0.028 | 0.045 | 0.013 | 0.068 | 0.049 | 0.035 | 0.017 |
| 39 | **BN** | 71.840 | 33.891 | 1.796 | 0.847 | 0.831 | 4.303 | 3.091 | 2.228 | 1.086 |
| 40 | **BS** | 36.142 | 1.745 | 0.904 | 0.044 | 0.418 | 2.165 | 1.555 | 1.121 | 0.547 |
| 41 | **BA** | 0.222 | 0.945 | 0.006 | 0.024 | 0.003 | 0.013 | 0.010 | 0.007 | 0.003 |
| 42 | **HA** | 75.056 | 8.655 | 1.876 | 0.216 | 0.868 | 4.496 | 3.229 | 2.328 | 1.135 |
| 43 | **HD** | 0.222 | 5.018 | 0.006 | 0.125 | 0.003 | 0.013 | 0.010 | 0.007 | 0.003 |
| 44 | **HS** | 27.455 | 7.491 | 0.686 | 0.187 | 0.318 | 1.645 | 1.181 | 0.852 | 0.415 |
| 45 | **PH** | 0.222 | 59.200 | 0.006 | 1.480 | 0.003 | 0.013 | 0.010 | 0.007 | 0.003 |
| 46 | **PB** | 2.882 | 4.364 | 0.072 | 0.109 | 0.033 | 0.173 | 0.124 | 0.089 | 0.044 |
| 47 | **PN** | 2.882 | 4.364 | 0.072 | 0.109 | 0.033 | 0.173 | 0.124 | 0.089 | 0.044 |
| 48 | **PTB** | 2.882 | 4.364 | 0.072 | 0.109 | 0.033 | 0.173 | 0.124 | 0.089 | 0.044 |
| 49 | **SL** | 33.038 | 45.091 | 0.826 | 1.127 | 0.382 | 1.979 | 1.422 | 1.025 | 0.500 |
| 50 | **SS** | 65.700 | 46.878 | 1.643 | 1.172 | 0.760 | 3.936 | 2.827 | 2.038 | 0.994 |
| 51 | **SC** | 0.000 | 0.102 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 52 | **SM** | 44.444 | 99.693 | 1.111 | 2.492 | 0.514 | 2.662 | 1.912 | 1.378 | 0.672 |
| 53 | **SSt** | 38.164 | 1.842 | 0.954 | 0.046 | 0.442 | 2.286 | 1.642 | 1.184 | 0.577 |
| 54 | **SCSt** | 93.720 | 99.795 | 2.343 | 2.495 | 1.084 | 5.614 | 4.033 | 2.907 | 1.417 |

Where, **DF** = Time of Flowering (in days), **PHT** = Plant Height (in cm), **HD** = Head Diameter (in cm), **STG** = Stem Girth (in cm), **NLPP** = Number of Leaves per Plant, **NBH** = Number of Bracts per Head, **PV** = Pollen Viability (%), **BW**= Biological weight (in g), **SDY** = Seed Yield per Plant or Economic Yield per Plant (in g), **NSP** = Number of Seeds per Plant, **HI** = Harvest Plant (%), **OC** = Oil Content (%), **PA** = Palmitic Acid (%), **SA** = Stearic Acid (%), **OA** = Oleic Acid (%), **LA** = Linoleic Acid (%), **HAC** = Hypocotyl: Anthocyanin Colouration, **LAC** = Leaf: Anthocyanin Colouration on the margins of Young Leaves, **PTF** = Plant: Time of Flowering, **LSi** = Leaf: Size, **LSh** = Leaf: Shape, **LC** = Leaf Colour, **LB** = Leaf Blistering, **LFS** = Leaf: Leaf Fineness of Serrations, **LAV** = Leaf: Angle of Lateral Veins, **LHT** = Leaf: Height of the Tip of the Blade Compared to Insertion of Petiole (⅔ th height of plants), **LAPS** = Leaf: Angle between Lower part of Petiole and Stem, **LH** = Leaf: Hairiness, **LP** = Leaf: Petiole Pigmentation, **SH** = Stem: Hairiness at the top, **SP** = Stem: Pigmentation, **SN** = Stem: Number of Leaves on the Main Stem, **RN** = Ray Flower: Number, **RS** = Ray Flower: Shape, **RC** = Ray Flower: Colour, **DC** = Disc Flower: Colour, **DA** =Disc Flower: Anthocyanin Colouration of Stigma, **DP** = Disc Flower: Pollen Colour, **BN** = Head: Number of Bracts on the Back, **BS** = Bract: Shape, **BA** = Bract: Anthocyanin Colouration, **HA** = Head: Attitude, **HD** = Head: Diameter, **HS** = Head: Shape of Grain Side, **PH** = Plant: Height, **PB** = Plant: Branching, **PN** = Plant: Natural Position of Closest Lateral Head to the Central Head (end of Flowering) - Branched, **PTB** = Plant: Type of Branching, **SL** = Seed: Length, **SS** = Seed: Shape, **SC** = Seed: Base Colour, **SM** = Seed: Mottling, **SSt** = Seed: Stripes, and **SCSt** = Seed: Colour of Stripes.

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| Some Mutagenic Variations | | | |
| Plate 1. Deformed seedling | | Plate 2. Seedling without chlorophyll pigmentation | |
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| Plate 3. Bushy appearance of the plant | | | |
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| Plate 4. Deformed head | | | |
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| Plate 5. Male sterility | | Plate 6. Lack of chlorophyll in head | |
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| Plate 7. Heterochromatic leaves | | Plate 8. White pollen | |
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| Plate 9. Half white and half yellow Pollen | | Plate 10. Pale ray florets | |
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| Plate 11. Headless plant | |
|  | |  | |
| Plate 12. Bushy Inflorescence | | Plate 13. Hairy Plant | |
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