



Differential Effects of Lead Nitrate and Cadmium Nitrate on Proline Accumulation and Associated Quantitative, and Morphological traits in M₂ generation of *Linum usitatissimum* L.

Nidhi Sharma*¹, Sana Choudhary¹, Nazarul Hasan¹, Neha Naaz¹, Parul Verma¹

¹Cytogenetics and Plant Breeding Lab, Department of Botany, Aligarh Muslim University, India

*corresponding author email: nidhisharma7517@gmail.com

Received: 12 July 2025, Revised: 18 October 2025, Accepted: 10 December 2025, Available Online: 15 December, 2025

<https://doi.org/10.5281/zenodo.19675307>

Abstract

This study evaluated the mutagenic effects of lead nitrate and cadmium nitrate on proline accumulation and various quantitative traits and morphological variations in M₁ and M₂ generations of *Linum usitatissimum* L. Both mutagens reduced plant height, branching, capsule number, seed weight, and yield, with cadmium nitrate resulting in stronger suppression. Proline content increased significantly in the M₁ generation of linseed under both heavy metal's treatment, indicating a strong biochemical response to heavy-metal stress. Although proline content declined in the M₂ generation, but the content remained higher than the control. This increased proline in second generation revealed a partial recovery towards the induced stress. Various distinct cotyledonary and floral mutations were recorded in treated populations, some of which were persisted into the M₂ generation. These findings shows heavy metal lead and cadmium nitrate although reduced the growth parameters but the mutants with high proline accumulation can serve as a better adaptor towards stress and may help in identifying mutant lines with improved stress tolerance for future breeding programmes.

Keywords:

Linum, Heavy metals, lead nitrate, cadmium nitrate, mutation, proline

1. Introduction

Linum usitatissimum, a valuable medicinal–oilseed crop that integrates high nutritional and industrial oilseed qualities with both therapeutic and pharmacological properties. The crop is termed as *flax* when cultivated mainly for fibre, *linseed* when grown for oil extraction, and *dual-purpose flax* when utilized for both i.e. fibre and oil. It is a self-pollinating, blue-

flowered rabi crop belonging to the family Linaceae. It has a chromosome number of $2n = 30$. Linseed seeds are highly rich in proteins, fats, dietary fibre, omega-3 fatty acids, phytoestrogens, and mucilage, and beyond its nutritional significance, flax is widely used for its pharmaceutical, medicinal, and industrial applications (Ansari *et al.*, 2019). The crop has relatively narrow genetic base which indicates the need for broader variability. For creating variability, mutation breeding delivers an efficient strategy to generate new heritable variation which gives far better and faster results than conventional germplasm method or interspecific hybridization.

Mutation breeding is a renowned tool for developing improved genotypes with superior yield potential. It is especially used where polygenic traits are involved. Over the last three decades, increasing attention has been directed toward environmental pollution, particularly heavy metals. This is due to their toxic influence on biological systems and their broader ecological impact on living world. Continuous deposition of these metals into soil, water, and air results in their gradual enhancement in plant tissues, resulting in range of morphological, physiological, biochemical, and ultrastructural disturbances (Arif *et al.*, 2016; Ghori *et al.*, 2019). Heavy metals such as lead and cadmium exhibit strong genotoxic and mutagenic activity, often interrupting normal mitotic behaviour and impairing root growth in plants (Shahwar *et al.*, 2019; Sharma *et al.*, 2024; Hasan *et al.*, 2025a). In some reports plants even respond a bit differently, reflecting species-specific sensitivity.

The present study evaluated the impact of lead nitrate and cadmium nitrate on biological injury, morpho-physiological attributes, and quantitative traits in the M_1 and M_2 generations of *Linum usitatissimum*. Particular emphasis was placed on proline accumulation, which is a biochemical indicator of stress tolerance and mutation-induced variability. The outcomes assist in identifying beneficial mutations that may contribute to the development of improved flax lines for medicinal and agricultural purposes, although sometimes the response seems slightly variable.

2. Materials and Methods

Certified and healthy M_0 seeds of *Linum usitatissimum* L. (var. Shekhar) were sourced from ICAR–NBPGR, New Delhi. These seeds were soaked in double-distilled water for 24 hours prior to their treatment with mutagens. Different concentrations of lead nitrate and cadmium nitrate (20, 40, 60, 80, and 100 ppm) were used for mutation induction. Untreated seeds were maintained as the control set. Both treated and control seeds were cultivated in four replications to raise the M_1 generation. Randomly selected seeds from each treatment of M_1 generation were used under similar field conditions to raise the M_2 generation. At this stage, plants were systematically evaluated for morphological and quantitative parameters, including plant height, number of branches, number of capsules, seeds per capsule, seed weight, and total seed yield. The mutants showing stable and noticeable variations were also documented. One thing were noted that some traits appeared more stable in M_2 , reflecting heritable changes.

For proline estimation, the ninhydrin-based colorimetric assay of Bates *et al.* (1973) was adopted, following the procedural refinements described by Verma *et al.* (2024). This method was employed to quantify stress-induced changes in proline accumulation across treatments.

3. Results

3.1. Quantitative traits

3.1.1. Plant Height (cm)

Plant height was found to be decreased with increasing concentrations of $\text{Pb}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2$ in both generations. In the M_1 generation, height decreased from 94.94 cm in the control to 81.06 cm at 100 ppm $\text{Pb}(\text{NO}_3)_2$, and to 80.78 cm at 100 ppm $\text{Cd}(\text{NO}_3)_2$. A similar trend continued in M_2 , where height ranged from 95.05 cm in the control to 81.79 cm under the highest $\text{Pb}(\text{NO}_3)_2$ dose and 81.53 cm with $\text{Cd}(\text{NO}_3)_2$. In M_2 generation, reduction in plant height persisted, but the values were slightly higher than those of M_1 , indicating minor recovery (Table No. 1 and 2).

3.1.2. Number of Branches per Plant

Both mutagens caused a dose-dependent reduction in branch number. In M_1 , branches decreased from 4.64 in the control to 3.19 at 100 ppm $\text{Pb}(\text{NO}_3)_2$ and to 2.73 under $\text{Cd}(\text{NO}_3)_2$. Similar reductions occurred in M_2 , where branch number declined from 4.70 in the control to 3.38 with $\text{Pb}(\text{NO}_3)_2$ and 2.97 with $\text{Cd}(\text{NO}_3)_2$. A slight improvement in M_2 compared to M_1 suggests partial stabilization of branching (Table No. 1 and 2).

3.1.3. Number of Capsules per Plant

A clear decline in capsule production was observed with higher doses of both mutagens. In M_1 , capsule number reduced from 48.98 in the control to 39.87 under $\text{Pb}(\text{NO}_3)_2$ and 35.17 under $\text{Cd}(\text{NO}_3)_2$. In M_2 , values ranged from 49.29 in the control to 40.97 with $\text{Pb}(\text{NO}_3)_2$ and 36.28 with $\text{Cd}(\text{NO}_3)_2$. Although M_2 plants performed slightly better, both mutagens still caused considerable reduction (Table No. 1 and 2).

3.1.4. Number of Seeds per Capsule

Seed number per capsule declined progressively with increasing mutagen doses. In M_1 , values dropped from 7.96 in the control to 6.44 under $\text{Pb}(\text{NO}_3)_2$ and 6.21 under $\text{Cd}(\text{NO}_3)_2$. In M_2 , the range decreased from 8.01 in the control to 6.56 with $\text{Pb}(\text{NO}_3)_2$ and 6.34 with $\text{Cd}(\text{NO}_3)_2$. A minor improvement in M_2 was visible but reductions remained significant (Table No. 1 and 2).

3.1.5. 1000-Seed Weight (g)

Seed weight also showed a downward trend across treatments. In M_1 , the weight decreased from 7.26 g in the control to 6.74 g under $\text{Pb}(\text{NO}_3)_2$ and 6.58 g with $\text{Cd}(\text{NO}_3)_2$. In M_2 , values ranged from 7.28 g in the control to 6.78 g under $\text{Pb}(\text{NO}_3)_2$ and 6.61 g under $\text{Cd}(\text{NO}_3)_2$. Though slightly higher in M_2 than M_1 , the overall reduction persisted (Table No. 1 and 2).

3.1.6. Seed Yield per Plant (g)

Seed yield was markedly reduced at all treatment levels. In M_1 , yield fell from 2.83 g in the control to 1.73 g at the highest $\text{Pb}(\text{NO}_3)_2$ concentration and 1.44 g under $\text{Cd}(\text{NO}_3)_2$. A similar pattern was noticed in M_2 , where yield ranged from 2.87 g in the control to 1.82 g in $\text{Pb}(\text{NO}_3)_2$ plants and 1.52 g in $\text{Cd}(\text{NO}_3)_2$ plants. $\text{Cd}(\text{NO}_3)_2$ were causing more reduction in both generations than $\text{Pb}(\text{NO}_3)_2$ (Table No. 1 and 2).

3.2. Biochemical parameter

3.2.1 Proline Content ($\mu\text{g g}^{-1}$)

Proline content increased sharply under both mutagens in the M_1 generation, rising from 10.57 in the control to 25.04 with $\text{Pb}(\text{NO}_3)_2$ and 27.68 with $\text{Cd}(\text{NO}_3)_2$ (Table No. 3). In contrast, M_2 plants showed a noticeable decrease compared to M_1 , although proline levels remained higher than the control. Values ranged from 10.55 in the control to 23.73 in $\text{Pb}(\text{NO}_3)_2$ -treated plants and 25.14 in $\text{Cd}(\text{NO}_3)_2$ -treated plants (Table No. 3). This

reduction from M_1 to M_2 indicates partial physiological recovery and adaptation in the succeeding generation.

3.3. Morphological mutations

3.3.1. Cotyledonary leaves mutations

In the M_1 generation, mutagen treatments produced noticeable alterations in cotyledonary and early vegetative leaf formation, some of which were transmitted to the M_2 generation. In untreated plants, cotyledonary leaves appeared as a pair of opposite, elliptical, green, entire, obtuse, and smooth leaves of uniform size (Figure 1A). Various cotyledonary mutants were observed in treated population viz., three cotyledonary leaves, unequal cotyledonary leaves with one bigger elliptical while other small, one cotyledonary leaf, leaves get curved toward lower side, cotyledonary leaves with notched and irregular margin, both leaves oriented towards one side, cotyledonary leaves with burned tip, chlorina mutated cotyledonary leaves with burned margins (Figure 1B-I).

3.3.2. Flower mutations

In control plants, the flowers exhibited pale bluish-purple corollas composed of five petals with darker streaks and smooth margins, arranged in twisted aestivation (Figure 2A). Various flower mutants were recorded in treated population viz., light colored flower with six petals, dark blue flower with seven petals, dark purple flower, cup shaped flower, flask shaped flower, white flower with curved petals, white flower with six petals, and slightly closed white flower (Figure 1B-I).



Figure 1: A- Control Cotyledonary leaves, B-I - Mutated cotyledonary leaves



Figure 2: A- Control flower, B-I - Mutated flower

Table 1: Growth and yield studies in Pb(NO₃)₂ and Cd(NO₃)₂ treated *Linum usitatissimum* L. (M₁ generation).

Treatment	Conc.	Plant height	Number of branches /plant	Number of capsules/ plant	No. of seeds/ capsule	1000 seed weight	Seed yield/plant
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control		94.94±1.43	4.64±0.16	48.98±1.29	7.96±0.29	7.26±0.14	2.83±0.19
Pb(NO₃)₂	20	90.93±2.75	4.18**±0.26	47.23±1.63	7.69±0.52	7.12±0.27	2.57±0.27
	40	89.85*±3.24	3.81**±0.28	45.86*±2.14	7.22±0.66	7.06±0.31	2.34*±0.29
	60	85.86**±3.83	3.64**±0.31	42.07**±2.29	6.91*±0.74	6.91±0.37	2.01**±0.31
	80	83.68**±4.28	3.42**±0.33	41.29**±2.47	6.65**±0.82	6.83±0.41	1.88**±0.32
	100	81.06**±4.76	3.19**±0.36	39.87**±2.58	6.44**±0.91	6.74*±0.45	1.73**±0.34
Cd(NO₃)₂	20	87.48**±2.89	4.11*±0.29	45.84*±1.71	7.47±0.56	7.08±0.29	2.43*±0.29
	40	85.57**±3.37	3.67**±0.31	43.77**±2.23	7.06±0.71	6.87±0.34	2.12**±0.31
	60	84.15**±3.91	3.34**±0.33	40.68**±2.38	6.73*±0.81	6.76*±0.39	1.85**±0.33
	80	82.39**±4.42	3.11**±0.35	38.43**±2.59	6.46**±0.89	6.64*±0.45	1.65**±0.35
	100	80.78**±4.94	2.73**±0.39	35.17**±2.67	6.21**±0.99	6.58**±0.48	1.44**±0.37

Table 2: Growth and yield studies in Pb(NO₃)₂ and Cd(NO₃)₂ treated *Linum usitatissimum* L. (M₂ generation).

Treatment	Conc.	Plant height	Number of branches/plant	Number of capsules/plant	No. of seeds/capsule	1000 seed weight	Seed yield/plant
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control		95.05±1.41	4.70±0.15	49.29±1.25	8.01±0.26	7.28±0.13	2.87±0.18
Pb(NO₃)₂	20	91.66±2.69	4.35*±0.24	48.22±1.61	7.67±0.45	7.15±0.25	2.65±0.25
	40	90.71±3.18	3.96**±0.26	46.19*±2.12	7.35±0.59	7.09±0.29	2.41*±0.27
	60	86.68**±3.77	3.88**±0.29	43.56**±2.27	7.01*±0.68	6.96±0.35	2.13**±0.29
	80	84.34**±4.21	3.59**±0.31	42.56**±2.45	6.78**±0.75	6.87*±0.38	1.98**±0.31
	100	81.79**±4.67	3.38**±0.34	40.97**±2.55	6.56**±0.82	6.78*±0.42	1.82**±0.33
Cd(NO₃)₂	20	88.22**±2.75	4.28*±0.27	46.31*±1.68	7.58±0.49	7.11±0.27	2.50±0.27
	40	86.41**±3.29	3.86**±0.29	44.27**±2.21	7.18±0.63	6.92±0.31	2.20**±0.29
	60	85.04**±3.84	3.51**±0.3	41.54**±2.36	6.85**±0.72	6.81*±0.37	1.94**±0.31
	80	83.11**±4.35	3.33**±0.32	39.75**±2.57	6.59**±0.79	6.69*±0.41	1.75**±0.34
	100	81.53**±4.83	2.97**±0.36	36.28**±2.65	6.34**±0.87	6.61**±0.45	1.52**±0.35

Table 3: Effect of Pb(NO₃)₂ and Cd(NO₃)₂ on Proline content of *Linum usitatissimum* L. (M₁ and M₂ generation).

Treatment	Conc.	Proline content $\mu\text{g g}^{-1}$ (M ₁ generation)	Proline content $\mu\text{g g}^{-1}$ (M ₂ generation)
		Mean \pm SD	Mean \pm SD
Control		10.57 \pm 1.04	10.55 \pm 1.06
Pb(NO ₃) ₂	20	13.66* \pm 1.64	11.34* \pm 1.58
	40	17.14** \pm 2.03	15.89** \pm 1.95
	60	20.45** \pm 2.37	18.99** \pm 2.29
	80	22.29** \pm 2.65	20.82** \pm 2.56
	100	25.04** \pm 2.88	23.73** \pm 2.81
Cd(NO ₃) ₂	20	15.34** \pm 1.72	13.02** \pm 1.64
	40	19.11** \pm 2.11	17.76** \pm 2.01
	60	23.47** \pm 2.48	21.91** \pm 2.38
	80	25.92** \pm 2.79	23.68** \pm 2.67
	100	27.68** \pm 2.96	25.14** \pm 2.89

4. Discussion

Heavy metals such as cadmium and lead are potent mutagenic agents capable of interacting with cellular components, disrupting nucleic acid metabolism, and inducing genetic alterations (Hasan *et al.*, 2022). In mutation breeding, controlled exposure to such agents helps generate novel variability (Arisha *et al.*, 2014). While subsequent generations (e.g., M₆–M₇) undergo natural selection that removes deleterious effects of the mutagens. This ensures the emergence of genetically stable and safe lines for further crop improvement.

In the current study, quantitative traits showed a consistent decline with increasing levels of Pb(NO₃)₂ and Cd(NO₃)₂ in both generations. Almost all parameters decreased progressively with higher treatment doses, with Cd(NO₃)₂ causing greater reductions than Pb(NO₃)₂. Previous studies have shown that reduced plant height after exposure to heavy-metal stress is primarily associated with mitotic disturbances and chromosomal abnormalities. These interfere with normal cell division and subsequently suppress overall vegetative growth (El Rasafi *et al.*, 2022). Abdo *et al.* (2012) reported that reductions in number of branches under cadmium stress arise due to the inhibitory effects of the metal on key regulatory enzymes, which disrupt normal physiological processes and overall plant growth. Similar reductions in vegetative growth of plants due to mutagenic exposures were also reported by Sharma *et al.* (2022), Naaz *et al.* (2024), and Hasan *et al.* (2025b). In mutation breeding, yield is considered as a critical selection criterion. For a breeder, improving seed productivity and its associated components remains their primary objective. In the

present investigation, yield-related attributes including, capsules per plant, seeds per capsule, 1000-seed weight, and seed yield per plant showed pronounced reductions relative to the control even at the lower concentrations of both the mutagens. Similar suppressive effects of heavy metals on vegetative and reproductive performance have been associated with impaired physiological activity and growth that reduced seed yield in multiple crop species. Zeeshan *et al.* (2020) reported that reductions in plant growth and yield parameters under exposure to heavy metals such as Ni, Cd and Pb may result from their ability to damage chloroplast structure and diminish chlorophyll a and b content, which ultimately lowers photosynthetic activity and transpiration efficiency.

Heavy metal exposure can substantially impair plant biomass and yield by disturbing several core physiological processes. These metals negatively affect photosynthetic performance through structural damage to chloroplasts (Rodriguez *et al.*, 2012) and restrict the uptake of essential nutrients that support normal vegetative growth (Mukhopadhyay and Mondal, 2015). They also disrupt internal water balance which is necessary for maintaining metabolic activities that are fundamental for plant development and growth. Together such disturbances weaken vegetative vigor and that results in reduced reproductive output and overall yield. Additionally heavy metals lead to the excessive production of reactive oxygen species (ROS), which disrupts the plant's antioxidant defense system and exaggerates oxidative stress that ultimately results in impaired cellular homeostasis (Nanda & Agrawal, 2016; Rui *et al.*, 2016). High concentrations of mutagens also induce chromosomal abnormalities, hinder chromosome pairing, delay DNA replication, and interfere with spindle formation during cell division. These genotoxicities induce by heavy metal mutagens triggers cytological disturbances, increase pollen sterility and contribute to a significant decline of yield (Hasan *et al.*, 2021).

Proline is an amino acid with distinctive conformational rigidity which majorly plays a key role in plant primary metabolism. Its accumulation intensifies under any environmental stresses like heavy metal exposure (Reddy *et al.*, 2024). In the present study, proline content in leaves increased proportionally with the concentration of both mutagens, with the highest accumulation recorded in Cd(NO₃)₂ followed by Pb(NO₃)₂. This elevation was most pronounced in the M₁ generation and showed a gradual decrease in the M₂ generation. This indicates that the plants might start to recover, likely because the stress pressure reduces in the advance generation. The increased proline accumulation indicates that plants are activating metabolic defense pathways to counteract the toxic influence of heavy metals. These findings align with earlier reports in other crops, including *Capsicum* (Hasan *et al.*, 2022) and *Trigonella* (Naaz *et al.*, 2023).

The mutagenic treatments with heavy metals also resulted in visible morphological alterations in cotyledonary leaves and floral structures. The observed abnormalities likely arise due to disruptions in major metabolic pathways, which alter normal developmental processes. More and Jagtap (2016) explained that changes in leaf characteristics emerged from spontaneous genetic mutations and recombination events during the course of evolution. Various floral variations including, petal colour, form, and structural arrangement were also identified in the current study, consistent with previous mutation-induced studies across various plant species (Asif & Ansari, 2019; Yousuf *et al.*, 2023; Sharma *et al.*, 2025). Devi & Mullainathan (2012) reported that floral variations originate due to the modifications in regulatory genes responsible for floral induction and organ development, leading to deviations in corolla pigmentation, irregular petal shapes, or altered floral architecture. These kind of alterations sometimes occurs even within the same treatment group, highlighting the variable nature of induced mutations.

5. Conclusion

The present study demonstrated the mutagenic effect of Pb(NO₃)₂ and Cd(NO₃)₂ in M₁ and M₂ generations of *Linum usitatissimum*. Significant reductions in key quantitative traits along with a range of morphological mutations were observed in treated populations. Despite the decline in overall yield, a marked increase in proline content was recorded in M₁ generation, which indicates the activation of a strong biochemical defense response toward heavy metals. The partial decline of proline content in the M₂ generation further reflects the physiological recovery over the successive generations. These findings shows heavy metal lead and cadmium nitrate although reduced the growth parameters but the mutants with high proline accumulation can serve as a better adaptor towards stress and may help in identifying mutant lines with improved stress tolerance for future breeding programmes

Acknowledgment

- Authors are thankful to Chairman of Department of Botany, AMU, for providing library, laboratory and research field facilities.

Conflict of Interest

- The authors declare no conflict of interest.

References

1. Abdo, F. A., Nassar, D. M., Gomaa, E. F., & Nassar, R. M. (2012). Minimizing the harmful effects of cadmium on vegetative growth, leaf anatomy, yield and physiological characteristics of soybean plant [*Glycine max* (L.) Merrill] by foliar spray with active yeast extract or with garlic cloves extract.
2. Ansari, R., Zarshenas, M. M., & Dadbakhsh, A. H. (2019). A Review on Pharmacological and Clinical Aspects of *Linum usitatissimum* L. *Current drug discovery technologies*, 16(2), 148-158.
3. Arif, N., Yadav, V., Singh, S., Singh, S., Ahmad, P., Mishra, R. K., ... & Chauhan, D. K. (2016). Influence of high and low levels of plant-beneficial heavy metal ions on plant growth and development. *Frontiers in environmental science*, 4, 69.
4. Arisha, M. H., Liang, B. K., Shah, S. M., Gong, Z. H., & Li, D. W. (2014). Kill curve analysis and response of first generation *Capsicum annuum* L. B12 cultivar to ethyl methane sulfonate. *Genet. Mol. Res*, 13(4), 10049-10061.
5. Asif, A., & Ansari, M. Y. K. (2019). Generation of mutant lines of *Nigella sativa* L. by induced mutagenesis for improved seed yield. *Industrial Crops and Products*, 139, 111552.
6. Devi, A. S., & Mullainathan, L. (2012). Effect of gamma rays and ethyl methane sulphonate (EMS) in M3 generation of blackgram (*Vigna mungo* L. Hepper). *African Journal of Biotechnology*, 11(15), 3548-3552.
7. El Rasafi, T., Oukarroum, A., Haddioui, A., Song, H., Kwon, E. E., Bolan, N., ... & Rinklebe, J. (2022). Cadmium stress in plants: A critical review of the effects, mechanisms, and tolerance strategies. *Critical Reviews in Environmental Science and Technology*, 52(5), 675-726.

8. Ghori, N. H., Ghori, T., Hayat, M. Q., Imadi, S. R., Gul, A., Altay, V., & Ozturk, M. (2019). Heavy metal stress and responses in plants. *International journal of environmental science and technology*, 16(3), 1807-1828.
9. Hasan, N., Choudhary, S., Joshi, D. C., Naaz, N., Sharma, N., Bhinda, M. S., & Laskar, R. A. (2025b). A comparative study of mutagenic efficacy of EMS and MMS in inducing quantitative and cytological variations in *Capsicum annuum* L. var. NS1101. *Discover Plants*, 2(1), 184.
10. Hasan, N., Choudhary, S., Laskar, R. A., 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.
11. Hasan, N., Choudhary, S., Naaz, N., & Sharma, N. (2021). The mechanism of heavy metal elements in various biological process and its deteriorate effects on the productivity of different crop plants. *Int. J. Waste Resour*, 11, 403.
12. Hasan, N., Choudhary, S., Naaz, N., Sharma, N., Budakoti, M., Joshi, D. C., ... & Laskar, R. A. (2025a). Qualitative and Quantitative Characterization of Mutations and Genetic Diversity Analysis in M2 Populations of Chilli (*Capsicum annuum* L.). *Crop Design*, 100108.
13. More, A. D., & Jagtap, S. S. (2016). Induction of morphological leaf mutations in *Lablab purpureus* (L.) sweet through chemical and physical mutagens. *International Journal of Current Microbiology and Applied Sciences*, 5(10), 592-597.
14. Mukhopadhyay, M., & Mondal, T. K. (2015). Effect of zinc and boron on growth and water relations of *Camellia sinensis* (L.) O. Kuntze cv. T-78. *National Academy Science Letters*, 38(3), 283-286.
15. Naaz, N., Choudhary, S., Hasan, N., Sharma, N., Alharbi, K., & Abd El Moneim, D. (2024). Enhancing genetic variability in *Trigonella* species through sodium azide induction: morpho-physiological and chromosomal amelioration. *Frontiers in Genetics*, 15, 1378368.
16. Naaz, N., Choudhary, S., Sharma, N., Hasan, N., Al Shaye, N. A., & 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.
17. Nanda, R., & Agrawal, V. (2016). Elucidation of zinc and copper induced oxidative stress, DNA damage and activation of defence system during seed germination in *Cassia angustifolia* Vahl. *Environmental and Experimental Botany*, 125, 31-41.
18. Reddy, S. H., Al-kalbani, H., Al-Qalhati, S., Al-Kahtani, A. A., Al Hoqani, U., Azmi, S. N. H., ... & Settaluri, V. S. (2024). Proline and other physiological changes as an indicator of abiotic stress caused by heavy metal contamination. *Journal of King Saud University-Science*, 36(8), 103313.
19. Rodriguez, E., Santos, C., Azevedo, R., Moutinho-Pereira, J., Correia, C., & Dias, M. C. (2012). Chromium (VI) induces toxicity at different photosynthetic levels in pea. *Plant Physiology and Biochemistry*, 53, 94-100.
20. Rui, W., Guan, L., Zhang, F., Zhang, W., & Ding, W. (2016). PM2. 5-induced oxidative stress increases adhesion molecules expression in human endothelial cells through the ERK/AKT/NF-κB-dependent pathway. *Journal of Applied Toxicology*, 36(1), 48-59.
21. Shahwar, D., Ansari, M. Y. K., & Choudhary, S. (2019). Induction of phenotypic diversity in mutagenized population of lentil (*Lens culinaris* Medik) by using heavy metal. *Heliyon*, 5(5).

22. Sharma, N., Choudhary, S., Naaz, N., Hasan, N., & Verma, P. (2024). Impact of Cadmium Nitrate on Physio-Morphological Characteristics of *Linum usitatissimum* in the M₂ Generation. *Journal Of Advanced Sciences Vol*, 3(2).
23. Sharma, N., Choudhary, S., Hasan, N., Verma, P., Marraiki, N., & Abd El-Moneim, D. (2025). Induced mutagenesis for the development of high-yielding mutant lines of linseed (*Linum usitatissimum* L.) using caffeine and sodium azide. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 53(1), 14262-14262.
24. 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.
25. Verma, P., Choudhary, S., Naaz, N., Sharma, N., Nora, M., Safhi, F., & Abd, D. (2024). Investigating the Mutagenic Impact of Cadmium Nitrate on Cytomorphological and Physiological Attributes in. *Phyton*, 93(12), 3347.
26. Yousuf, J., Raina, A., Rasik, S., Reshi, Z. A., & 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).
27. Zeeshan, M., Ahmad, W., Hussain, F., Ahamd, W., Numan, M., Shah, M., & Ahmad, I. (2020). Phytostabalization of the heavy metals in the soil with biochar applications, the impact on chlorophyll, carotene, soil fertility and tomato crop yield. *Journal of Cleaner Production*, 255, 120318.