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RESEARCH ARTICLE

Gamma ray-induced mutations in hemp (*Cannabis sativa* L.) for enhanced industrial and medicinal traits

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Abstract

Mutation breeding is a powerful tool for inducing genetic diversity and facilitating the development of crops with novel and desirable traits. These genetic variations will consequently expand available germplasm of a crop. This study aimed to create hemp (*Cannabis sativa* L.) mutants with enhanced industrial and medicinal characteristics through gamma irradiation. Two seed groups i.e., pre-irradiation hydropriming (G_1) and post-irradiation hydropriming (G_2) were subjected to four gamma radiation doses (150 Gy, 300 Gy, 450 Gy and 600 Gy) using a Co^{60} source. Seedswere hydro primed before radiation exposure in G_1 and after irradiation in G_2 for 12 hrs. Higher doses of radiation led to greater phenotypic variation among the surviving plants. Out of forty isolated mutants, six exhibited significant improvements. Mutant 'M_1' (G_1D_1) showed superior industrial traits including increased plant height, stem thickness and shoot weight, while, 'M_{31}' (G_2D_3) demonstrated medicinal traits such as enhanced axillary shoots, trichome number and trichome size. These mutants will be advanced for potential use as new industrial and medicinal varieties of hemp. Variants with superior traits may be included in future breeding programs as well.

Keywords: gamma irradiation; genetic diversity; hemp mutants; hydropriming; industrial traits; medicinal traits; mutation breeding

Introduction

Mutation breeding is a highly efficient approach to generate improved and novel plant varieties within a short time frame (1-3) particularly for crops with limited genetic variability (4). It involves permanent changes in a plant's DNA sequence induced by exposure to physical, chemical or biological mutagens. These mutagens can introduce multiple mutations in a single plant, resulting in random genetic variations across the entire genome (5). Mutation breeding has become a vital tool in plant breeding programs, as it effectively generates genetic diversity for desirable traits (3), thereby expanding the available germplasm (6, 7). Moreover, unlike genetically modified organisms (GMOs), mutation breeding is not subjected to regulatory restrictions (8).

Physical mutagens, such as gamma (γ) rays, X-rays, ion beams and UV-rays, account for approximately 89 % of all registered mutant varieties (6, 9). Of the 3433 registered mutant varieties, agronomic food crops constitute the largest share (66 %), followed by flowers and ornamental plants (21 %) and fruits and vegetables (4.6 %). However, no mutant varieties of hemp (*Cannabis sativa* L.) have been registered to date (10). Gamma rays, a form of ionizing radiation, are considered ideal and robust for mutation induction due to their high penetration, high mutation frequency and minimal damaging effects (11, 12).

The key factors in gamma irradiation are the total dose level and dose rate, which influence plant growth, lethality, fecundity and other traits (13, 14). While mild doses of γ-rays have a stimulatory effect, high doses can be inhibitory due to the production of reactive radicals that negatively affect a plant's morphology, physiology, anatomy and biochemistry (15). Plant breeders have increasingly utilized low doses of gamma rays to improve crop varieties with poor qualitative and polygenic traits, as well as stress tolerance (16).

Gamma irradiation has been extensively employed to develop superior plant varieties with traits such as early maturity, compact growth habit, improved plant height, variegated leaves, variations in flower color and shape and resistance to biotic and abiotic stresses. Some studies have explored gamma-induced mutations in hemp (*Cannabis sativa* L.), including sex alteration, stem bending (17) and reduced pollen viability at higher dose rates (18). Enhancing phenotypic traits in hemp, such as plant height, flower size and plant biomass, could lead to increased fibre production, improved staple length, higher CBD potency and greater seed oil content.

The research was designed to investigate induced genetic variation in local hemp mutants with superior industrial and medicinal traits using gamma irradiation. The goal is to identify mutants with desirable variations that could serve as the foundations for developing new hemp varieties tailored to local needs.

Materials and Methods

Local hemp seeds (*Cannabis sativa* L.) were subjected to gamma irradiation using a Co-60 source at the Pakistan Radiation Services (PARAS), Lahore, at a dose rate of 230 Gy/hr. Two groups of seeds were hydro-primed: one group before irradiation (G_1) and the other group after irradiation (G_2). Seeds were hydro-primed in distilled water for 12 hrs. Four levels of gamma radiation were applied (150 Gy, 300 Gy, 450 Gy and 600 Gy) (17, 19) and non-irradiated seeds served as controls (Table 1). After treatment, seeds were sown in 10-inch earthen pots filled with a 1:1:1 mixture of soil, sand and cocopeat. The pots were maintained under semi controlled conditions in a lath house at the Plant Propagation Unit, Department of Horticulture, PMAS Arid Agriculture University, Rawalpindi. The experimental design was a Completely Randomized Design (CRD) arranged factorially with three replications for each treatment.

Evaluation of parameters

Irradiated plants were assessed for morphological, physiological and chemical traits. The evaluated parameters included:

Morphological traits

Survival percentages, plant height, stem thickness, leaf area index, number of leaves and axillary shoots.

Physiological traits

Dry shoot weight, dry root weight, flowering time, trichome density and trichome size.

Chemical traits

Total chlorophyll content, total anthocyanin content [measured with slight modification according to previous studies (20)] and total flavonoid content (21).

Statistical analysis

Data were subjected to statistical analysis using SPSS version 16. An independent sample *t*-test ($p \leq 0.05$) was initially employed to compare the differences between isolated gamma mutants and control plants. As the intentions were to compare mutants of a particular dosage to control group with unequal means and different number of individuals in each group. *t*-test is preferred in such conditions as supported by literature (22). For multiple group comparisons, a CRD Factorial design ANOVA was conducted to assess the significance of dose levels on phenotypic traits. Post-hoc comparisons using Tukey's HSD test were applied where appropriate. Dose-response relationships were evaluated using regression analysis and principal component analysis (PCA) was performed to visualize trait variation across treatments.

Results and Discussion

Irradiated plants were under observation throughout the growing cycle to identify variation in their phenotypic traits from wild type. Plant showing any single visible variation was tagged as mutant in each treatment group. Throughout the research, 40 gamma-irradiated *Cannabis sativa* plants were identified as mutants, but many did not survive. Ultimately, 16 stable mutants exhibiting diverse growth patterns were isolated. This indicated a significant effect of hemp seed priming and dose levels of γ-rays on their growth and development.

Phenotypic variation in gamma irradiated plants

Variation percentage is number of mutants obtained from survived irradiated plants. Among G_1 plant population, dose level D_3 (450 Gy) exhibited highest variation percentage of 100 % while D_4 (600 Gy) gave more distinct phenotypic changes with 40 % variation in G_2 plants (Table 2). However, maximum number of mutants was obtained @ 150 Gy among all treatment groups as low dose of gamma rays may have positive impact on the plant tissues and result in phenotypic variations rather than biological injuries. It was also observed that γ-rays had detrimental impact at high doses and enhances the growth of mutants at low doses. In this study, highest dose level of 600 Gy gave 0 % survival rate in G_1 and only 10 % in G_2. These results are at par with previous studies where low survival % was observed at high doses of γ-

Table 1. Treatment groups for γ-irradiation of hemp (*Cannabis sativa* L.) at different dose levels

S. No.	Treatment groups	Dose level (Gy)	Total exposure time (hrs)
1	G_0D_0	0 Gy	0
2	G_1D_1	150 Gy	0.69
3	G_1D_2	300 Gy	1.38
4	G_1D_3	450 Gy	2.07
5	G_1D_4	600 Gy	2.76
6	G_2D_1	150 Gy	0.69
7	G_2D_2	300 Gy	1.38
8	G_2D_3	450 Gy	2.07
9	G_2D_4	600 Gy	2.76

*****Seed batch size **=** 50 seeds per treatment; Gy = gray; hrs = hours.

Table 2. Degree of variation percentage among survived plants of hemp (*Cannabis sativa* L.)

Treatments	Total plants	Survived plants (n)	Survival percentage (%)	Phenotypic variation	Phenotypic variation among survived plants (%)
G_0D_0	50	48	96 %	0	0 %
G_1D_1	50	31	62 %	12	39 %
G_1D_2	50	9	18 %	5	55 %
G_1D_3	50	3	5 %	3	100 %
G_1D_4	50	0	0 %	0	0 %
G_2D_1	50	44	88 %	5	11 %
G_2D_2	50	43	86 %	6	14 %
G_2D_3	50	29	58 %	7	24 %
G_2D_4	50	5	10 %	2	40 %

*Where, G_0D_0 = 0 Gy; G_1D_1, G_2D_1 = 150 Gy; G_1D_2, G_2D_2 = 300 Gy; G_1D_3, G_2D_3 = 450 Gy; G_1D_4, G_2D_4 = 600 Gy; n = number; % = percentage.

rays in soyabean (15) and long beans (23).

After statistical analysis, significant variations were evident among isolated mutants and wild type in evaluated parameters. G_1 mutants showed highly significant differences at lowest dose level (D_1) from wild type (Table 3). Comparatively, G_2 mutants exhibited better variation in recorded parameters from wild type population at all dose levels, however, D_4 was at par to other treatments (Table 4).

Comparison of mutants with parent plant

From weekly data growth patterns, more robust growth was observed in mutants as compared to wild type particularly from low dose groups. This resulted in mutants with superior traits like plant height, stem thickness, leaf area, number of leaves and axillary shoots. During the study, different variations were also observed like variegated leaves (Semi xantha), malformed leaves (Fig. 1) and growth pattern (three and four leaves at single node, opposite leaves, double stem) (Fig. 2). Variations in leaf color and shape were unstable and vanished later during maturity. These morphological changes could be further explored for their potential industrial implications, such as enhanced fibre yield.

Different γ-rays' induced leaf variants were identified in *Salvia hispanica*, like shape variants (leaf bifurcation, rolling, leathery leaves), colour variants (Xantha and Albino) and pattern variants (three leaves at a single node or bunches of leaves clustered at a node) (24). Leaf variegations after gamma irradiation were also reported in Wandering Jew (25); chrysanthemum (26) and tuberose (27). Rose has several mutant varieties with leaf (i.e., Caiyemingxin) and flower color variations (i.e., Beijingzhichun, Beiyumudan, Bridal Sonia, Paula and September wedding) (28). Out of the 16 isolated mutants, seven mutants showed noticeable diversity from the wild type in specific traits, their phenotypic variations are evident from Table 5.

Industrially important traits of isolated hemp mutants

Hemp is a vital industrial crop with a high global demand due to its long and strong bast fibres and high biomass yields (29). Hemp fibre has wide range of industrial applications such as textile, fibreboard, automotive, composite, heat-insulating material (30), fibre-reinforced concrete (30, 31) and sound insulation (32). Out of the assessed morphological and physiological parameters, substantial results were obtained regarding important industrial traits (plant height, stem thickness, leaf area index and shoot weight) of hemp comparing with its wild type. Bast fibre is obtained from stalk of a hemp plant, therefore, all these traits may positively contribute to overall fibre content, hence, play an important role in enhancing its industrial value.

Plant height (cm)

Hemp fibre content depends on length of its erected and hollow stems (33). Tall plants are preferred in industrial hemp as it has more cellulose and fibre. In the current study, a remarkable increase was found in the height of hemp mutants in response to gamma irradiation. Mutants M_1 (187 cm) from G_1D_1 and M_{31} (213 cm) and M_{34} (236 cm) from G_2D_3 and G_2D_4 respectively, showed significantly increased heights when compared to wild type (M_0) (102 cm) (Table 5). It is usually found that high doses suppress the growth of a plant and reduce its stem length by affecting growth regulators e.g., auxins that results in slow cell division and elongation (34, 35). However, positive impact of high doses was also observed on mutant growth in this study. According to slope of regression model Y = 13.231x + 101.69, a positive trend was recorded in plant height of hemp mutants though not impeccably linear. Coefficient of determination (R^2) is 0.4387 which shows a moderate variation of 43 % among treatment group in plant height (Fig. 4A).

Stem thickness (mm)

The stem thickness is influenced by the activity of essential hormones like auxins, gibberellins and cytokinin in plants. Exposure to ionizing rays may have positive affect on hormone levels by activating metabolic pathways or affecting gene expression relevant to hormone production and signalling. Out of all isolated mutants, M_1 and M_{19} were most prominent mutants from D_1 groups with 6.67 mm and 5.56 mm stem thickness respectively, in comparison to its wild type M_0 (3.79 mm) (Table 5). It is evident from the obtained results that dose level of γ-rays and seed priming had substantial influence on *Cannabis* mutation. Low doses and unprimed seed as planting material offered considerable enhancement in stem grid.

The reduction in stem thickness at high dose levels might be attributed to damaged cell components, reduced levels of growth hormones, altered enzyme activity and disturbance of different physiological processes in plants (36, 37). In case of regression model (Y = -0.0563x + 4.8946), negative slope is indicating a declining trend across mutants for stem grid. The coefficient of determination value (R^2) is showing a weak relation between stem thickness and treatment group because mutants are behaving as independent variables here and do not follow any linear trend of variation (Fig. 4B).

Leaf area index (cm^2)

Leaf area is linked with rapid plant growth by capturing more sunlight and CO_2, therefore, enhancing photosynthetic activity and nutrient assimilation which eventually gave vigorous plant growth and yield (38). Majority of isolated mutants showed significant variation in their leaf area from the wild type (M_0). Highly valuable results were recorded in M_{19} (107.3 cm^2) and M_{34} (101.0 cm^2) as compared to M_0 (68.5 cm^2) (Fig. 3). Large leaves in G_2 mutants might be due to more resilience of unprimed hemp seeds towards gamma irradiations than imbibed seed's tissues. R^2 value indicated a very low variation of 2.09 % in the leaf area of mutants from all treatment groups. Overall, a positive, nearly flat and weak trend line was recorded in the selected hemp mutants (Fig. 4C).

Previous studies reported small plant leaves at high dose levels due to cellular damages, reduced nuclear DNA content (12, 39) and breakdown of indole acetic acid (IAA) by indole acetaldehyde dehydrogenase enzyme inhibition. Similar decline in leaf area and thickness with increasing dose levels was reported in soyabean (15), mangosteen plants (40), fig (34), orchid (41) and rose (42).

Shoot weight (gm)

Mutants M_1 (49.0 gm) from G_1D_1 and M_{31} (55.0 gm) from G_2D_3 stood out by displaying significantly high shoot weight compared to wild type M_0 (30.2 gm) (Table 5). Maximum shoot weight in these mutants is attributed to the formation of large leaves, a greater number of branches and leaves, long and thick stem. Overall, a diverse response was observed among mutants in terms of shoot weight. This growth promoting potential of γ-

Table 3. Phenotypic variations in isolated gamma mutants of hemp from G_1 (independent sample *t*-test)

Independent sample *t*-test									
	G_1D_1 (150 Gy)			G_1D_2 (300 Gy)			G_1D_3 (450 Gy)		
Parameter	***p*-Value**	**t-Value**	**Sig. (2-tailed)**	***p*-Value**	***t*-Value**	**Sig. (2-tailed)**	***p*-Value**	***t*-Value**	**Sig. (2-tailed)**
Plant height	.164	-3.00	.040	.215	-.546	.614	.176	.765	.487
Stem thickness	.038*	-5.26	.006	.101	.117	.913	.105	3.69	.021
No. of leaves	.029*	-3.20	.033	.138	-1.36	.244	.022*	-1.11	.328
Leaf area index	.035*	-2.63	.058	.028*	-.355	.740	.037*	5.73	.005
Axillary shoots	.038*	-4.20	.014	.186	-1.57	.190	.022*	-.670	.537
Dry shoot weight	.111	-.826	.455	.228	-.648	.552	.079	2.88	.045
Dry root weight	.524	-2.23	.089	.338	-1.64	.175	.058	-.270	.800
Flowering time	.186	-1.33	.253	.470	-9.29	.001	.448	-.870	.433
Trichome density	.195	-2.92	.043	.405	-4.07	.015	.101	-5.88	.004
Trichome length	.034*	-2.73	.052	.619	1.86	.136	.743	5.52	.005
Trichome width	.412	-2.07	.106	.156	3.68	.021	.244	13.2	.000
Chlorophyll a	.022*	-1.75	.155	.030*	-8.97	.001	.045*	-.332	.757
Chlorophyll b	.021*	-1.49	.210	.095	-6.11	.004	.649	-5.09	.007
TCC	.021*	-1.58	.188	.065	-7.01	.002	.142	-1.94	.124
TAC	.229	2.55	.063	.057	3.07	.037	.103	5.54	.005
TFC	.134	-.979	.383	.145	-1.62	.179	.154	-.660	.540

*Significance at 0.05 % level, TCC = total chlorophyll content; TAC = total anthocyanin content; TFC = total flavonoid content.

Table 4. Phenotypic variations in isolated gamma mutants of hemp from G_2 (independent sample *t*-test)

Independent sample *t*-test												
	G_2D_1 (150 Gy)			G_2D_2 (300 Gy)			G_2D_3 (450 Gy)			G_2D_4 (600 Gy)		
Parameter	***p*-Value**	***t*-Value**	**Sig. (2-tailed)**	***p*-Value**	***t*-Value**	**Sig. (2-tailed)**	***p*-Value**	***t*-Value**	**Sig. (2-tailed)**	***p*-Value**	***t*-Value**	**Sig. (2-tailed)**
Plant height	.024*	-18.34	.000	.034*	-13.34	.000	.132	-2.60	.060	.000*	-.93	.421
Stem thickness	.109	-20.59	.000	.358	-2.56	.062	.070	-.388	.718	.000*	-.19	.859
No. of leaves	.491	-37.85	.000	.223	-1.27	.272	.018*	-1.10	.332	.000*	-1.26	.294
Leaf area index	.593	-24.05	.000	.060	11.02	.000	.125	1.99	.117	.000*	-.38	.723
Axillary shoots	.642	-21.72	.000	.279	-2.48	.068	.023*	-.86	.437	.000*	-.97	.404
Dry shoot weight	.369	-5.46	.005	.078	-4.98	.008	.586	-3.90	.017	.242	-3.59	.037
Dry root weight	.357	-1.71	.161	.297	-3.67	.021	.504	-4.69	.009	.515	-1.97	.143
Flowering time	.166	3.35	.028	.166	-12.68	.000	.111	-6.09	.004	.041*	-1.34	.271
Trichome density	.027*	-9.22	.001	.026*	-5.15	.007	.228	-6.51	.003	.584	-2.08	.128
Trichome length	.282	9.25	.001	.020*	-1.64	.175	.157	2.18	.094	.001*	-.02	.981
Trichome width	.896	12.17	.000	.038*	3.74	.020	.083	3.39	.011	.001*	.15	.890
Chlorophyll a	.024*	-335.77	.000	.024*	-102.88	.000	.019*	-4.45	.011	.001*	-16.35	.000
Chlorophyll b	.072	-744.17	.000	.072	-256.07	.000	.021*	-4.10	.015	.000*	-10.52	.002
TCC	.211	-131.40	.000	.156	-22.08	.000	.020*	-4.24	.013	.000*	-12.11	.001
TAC	.032*	14.80	.000	.024*	12.21	.000	.043*	3.36	.028	.526	8.44	.003
TFC	.021*	-12.75	.000	.021*	-1.55	.196	.411	-4.26	.013	.049*	-2.70	.073

*Significance at 0.05 % level, TCC = total chlorophyll content; TAC = total anthocyanin content; TFC = total flavonoid content.

Table 5. A comparison between isolated hemp mutants and wild type

Treatment Group	Mutant	Plant Height (cm)	Stem Thickness (mm)	Leaf Area Index (cm^2)	Shoot Weight (gm)	Axillary Shoots (n)	Trichome Density/ mm^2	Trichome Size (μm^2)
G_0D_0	M_0	102	3.79	68.5	30.2	5	1755.5	78.3
G_1D_1	M_1	187	6.67	98.9	49.0	24	3066.6	105.8
	M_3	154	5.50	79.6	28.0	15	4066.6	101.2
G_1D_2	M_{10}	110	3.59	60.1	31.2	5	3206.0	77.8
G_1D_3	M_{13}	120	2.88	36.7	22.5	33	2833.3	57.1
G_2D_1	M_{19}	167	5.56	107.3	46.5	24	3266.6	48.9
G_2D_3	M_{31}	213	4.15	65.4	55.0	29	3333.3	81.2
G_2D_4	M_{34}	236	4.99	101.0	43.3	37	2433.3	64.1

*Where, G_0D_0 = 0 Gy; G_1D_1, G_2D_1 = 150 Gy; G_1D_2 = 300 Gy; G_1D_3, G_2D_3 = 450 Gy; G_2D_4 = 600 Gy.

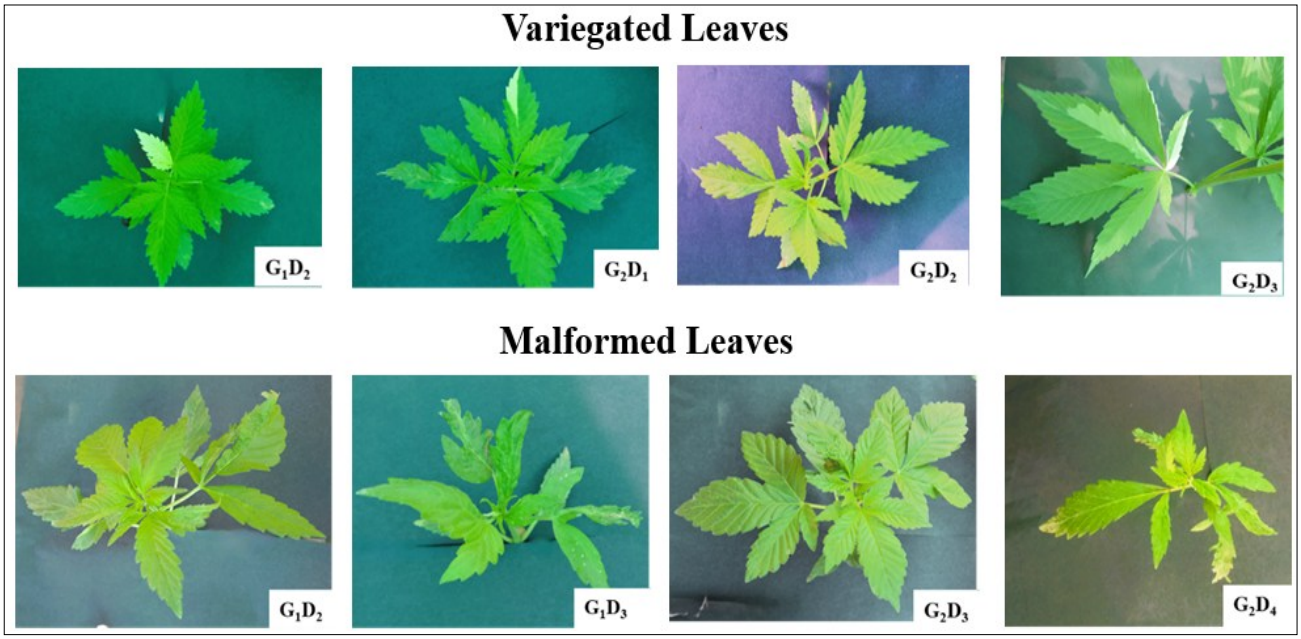

Fig. 1. Color variations and malformation in leaves among treatment groups.

*Where, G_1D_2 = 300 Gy; G_1D_3, G_2D_3 = 450 Gy; G_2D_4 = 600 Gy.

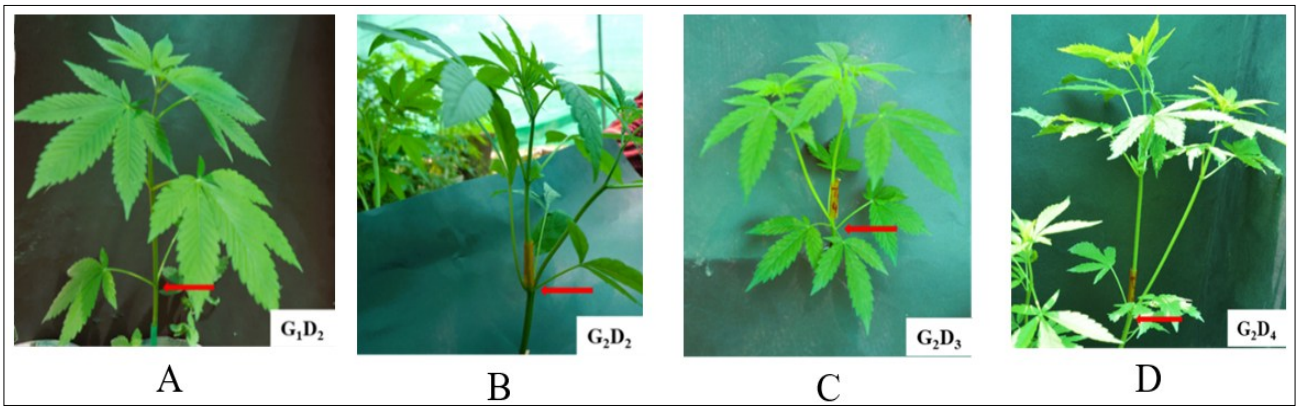

Fig. 2. Growth pattern variations induced by gamma irradiation: (A) Two opposite leaves; (B) Three leaves per node; (C) Four leaves per node; (D) Double stem formation.

*Where, G_1D_2, G_2D_2 = 300 Gy; G_2D_3 = 450 Gy; G_2D_4 = 600 Gy.

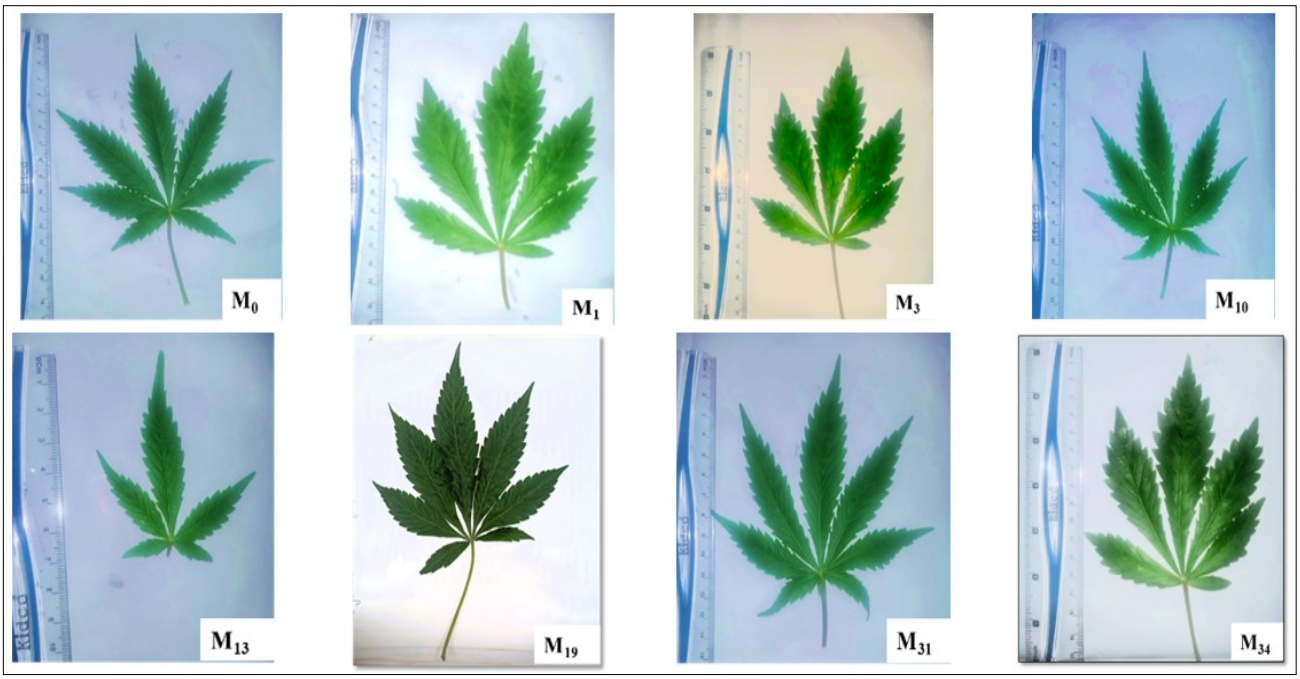

Fig. 3. Phenotypic variation in leaf area among mutants of hemp.

*Where, M_0 = G_0D_0 (0 Gy); M_1, M_3 = G_1D_1 (150 Gy); M_{10} = G_1D_2 (300 Gy), M_{13} = G_1D_3 (450 Gy); M_{19} = G_2D_1 (150 Gy); M31= G_2D_3 (450 Gy); M_{34} = G_2D_4 (600 Gy).

rays, through physiological and morphogenetic changes in cells and tissues, is dose dependent (43). Positive effect of γ-rays on plant weight could also be due to diverse genetic makeup and environmental factors (44).

A positive relationship existed between shoot weight and mutants obtained from different treatment groups and R^2 value indicated approximately 17.65 % variation in the shoot weight of mutants due to the impact of dose levels (Fig. 4D). High dose levels alter a plant's vulnerability by lowering hormones' level, particularly cytokinin either through breakdown or inhibiting their synthesis. Previous studies reported plant growth stimulation at low dose rates and growth retardation at high dose rates of γ-rays like in green gram (14), *Arabidopsis* plants (45, 46), pigeon pea (47) and orchid (41).

Medicinally important traits of isolated hemp mutants

In medicinal hemp, female plants are of great economic and medicinal importance due to the presence of special metabolites i.e., cannabinoids and terpenes (33, 48, 49). These compounds are profusely produced in the glandular trichomes on their flowers (50, 51). Compared with wild type, significant outcomes were recorded in some medicinally vital traits of mutants i.e., number of axillary branches, size and density of trichomes. These traits have direct relation with the CBD content of a hemp plant, hence, contribute to enhancing its medicinal value.

Axillary shoots (n)

Wild type (M_0) was served as a baseline against which all hemp mutants were evaluated. Results showed that exposure to γ-rays had potentially positive response on the branching patterns of hemp. Significantly higher variations were recorded in M_{13} (33 n) from G_1D_3, M_{31} (29 n) and M_{34} (37 n) from G_2D_3 and G_2D_4 respectively, in comparison to M_0 with only 5 average number of axillary shoots (Table 5). Therefore, mutants from high dose treatment groups produced distinct outcomes in enhancing the lateral shoot growth which led to more floral biomass. According to weekly data growth pattern, early and more robust branch formation was observed in mutants just after 10 weeks of plantation. However, branch formation started late in M_0. Similar increase in primary branches' ratio was recorded in linseed at high doses (52).

In *Arabidopsis* plants, chronic irradiations (5, 10 and 15 days with 10 hr/day) enhanced their branching while acute irradiations (1 and 24 hrs) inhibited them (45). This reduction in shoot number is due to inhibitory impact of high doses of γ-rays on proteins and endogenous growth hormones concentration especially cytokinin (53). Regression model depicted a strong positive relationship between X and Y; each unit increase in dose will bring 3.6107 units increase in shoot number of mutants significantly. However, coefficient of determination value (R^2) gave moderate to strong variability of 53.44 % among both variables (Fig. 5A).

Trichome density and trichome size (µm²)

In Cannabis, glandular trichomes are the key sites to produce cannabinoids and terpenes (48) and referred as 'cell factories' (54, 55). Comparison of isolated hemp mutants with wild type revealed significant variation in terms of trichome characteristics (Fig. 6 and 7). Among all mutants, M_3 from G_1D_1 and M_{31} from G_2D_3 exhibited maximum trichome density of i.e., 4066.6 and 3333.3 trichomes/mm² and trichome size of 101.2

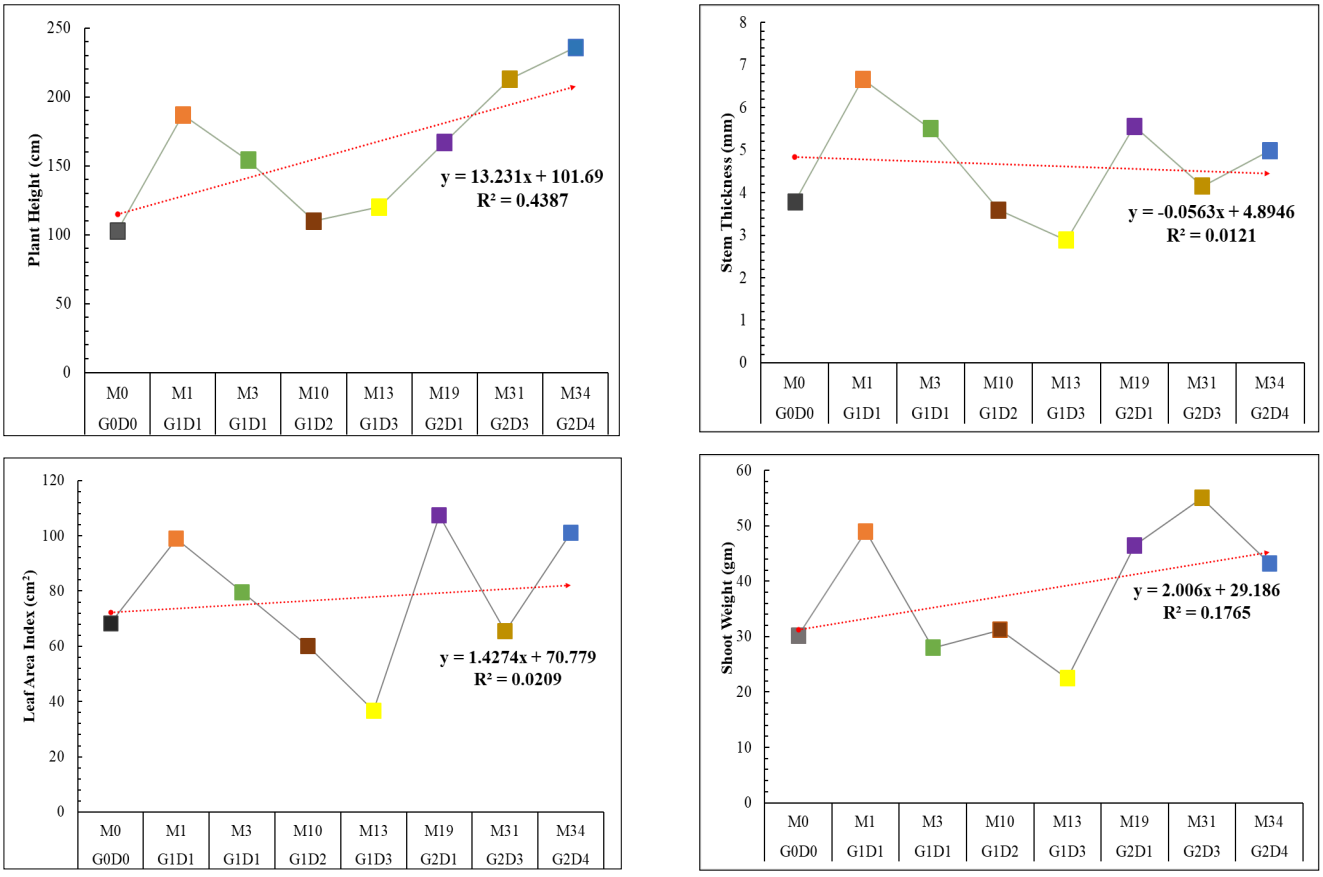

Fig. 4. Linear regression analysis chart for industrial traits of isolated hemp mutants: (A) Plant height; (B) Stem thickness; (C) Leaf area index; (D) Shoot weight. Mutants are represented by different colors here.

*Where, G_0D_0 = 0 Gy; G_1D_1, G_2D_1 = 150 Gy; G_1D_2 = 300 Gy; G_1D_3, G_2D_3 = 450 Gy; G_2D_4 = 600 Gy.

µm² and 81.2 µm²when compared with M_0 with only 1755.5 trichomes/mm² and 78.3 µm² size. High trichome density in hemp flower makes it a metabolic powerhouse (33, 56). An increase in trichome characters is reported after exposure to γ-rays on soybean (57) and *Arabidopsis* plant (45). These rays also affect terpene content in hemp trichomes that impact bioavailability of cannabinoids (58).

Fig. 5 (B, C) is showing regression trend for trichome density and size of selected hemp mutants. In case of trichome density, extremely low variability was explained by R^2 value (1.99 %). The reason behind poor trend is the individual behavior of all obtained mutants regardless of the impact of different treatment groups. However, a negative trend was observed in trichome size that showed a reduction in mutants' trichome size with increase in dose level and only 34 % of this change was attributed to given treatments.

Principle component analysis (PCA)

PCA generated seven principal components (PCs) of interest for detecting patterns among different traits across hemp mutants isolated from different treatment groups. PC1 accounted for highest data variation (46.9 %) followed by PC2 (24 %), PC3 (13.7 %) till PC7 (0 %) with no variation (Fig. 8A). PCA for individuals showed closed relation between G_1D_1 mutants in PC1 and among all G_2 mutants in PC2. However, M_{10} has maximum similarity with wild type (M_0) comparatively to other mutants. No similarity is detected between M_{13} and other mutants.

All variables are co-related; there is no negative correlation existed among recorded variables. Small angles between medicinal traits (trichome size TS and trichome density TD) and industrial traits (ST and LAI, SW and PH) shows strong correlation between them. From biplots, PC1 shows strong contribution of stem thickness (ST) and leaf area index (LAI) in mutant M_1 proving it an outlier for industrial traits. Similarly, PC2 depicts high values of medicinal trait i.e., axillary shoots (AS) in G_2 mutants M_{31} and M_{34} (Fig. 8B).

Conclusion

This study demonstrated the positive effects of seed priming, gamma irradiation dose levels and their interactions on medicinally and industrially significant traits of hemp. Gamma irradiation of hemp seed induced notable phenotypic variations,

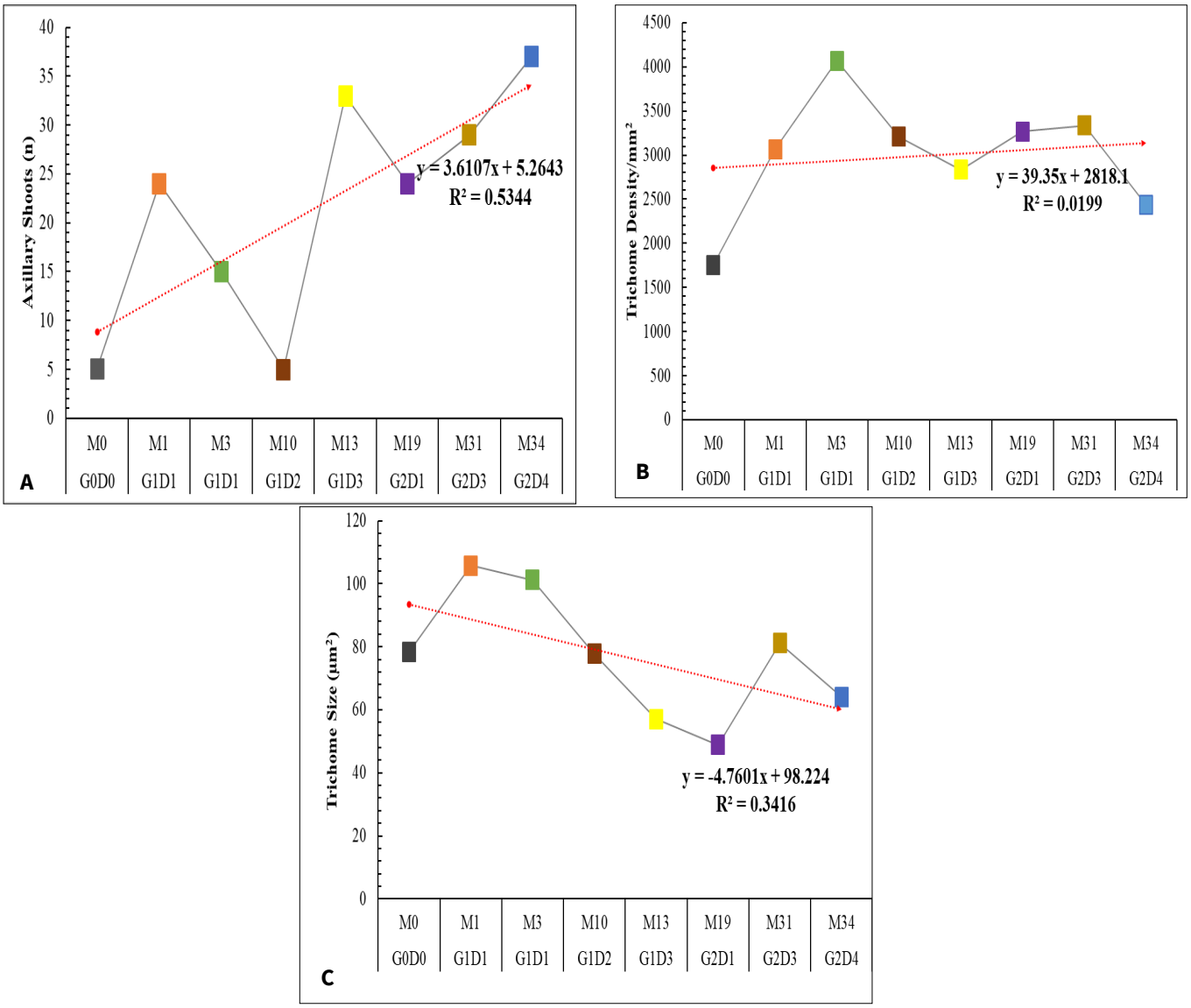

Fig. 5. Linear regression analysis chart for medicinal traits of isolated hemp mutants: (A) axillary shoots; (B) trichome density; (C) trichome size. Mutants are represented by different colors here.

*Where, G_0D_0 = 0 Gy; G_1D_1, G_2D_1 = 150 Gy; G_1D_2 = 300 Gy; G_1D_3, G_2D_3 = 450 Gy; G_2D_4 = 600 Gy.

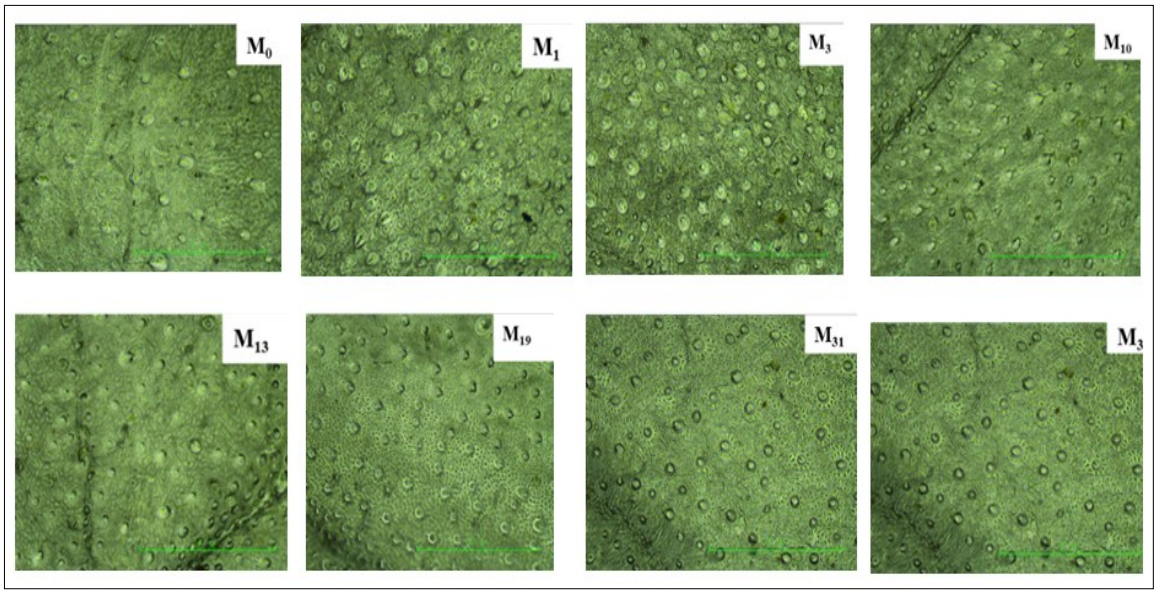

Fig. 6. Phenotypic variation in trichome density among isolated mutants of hemp (*Cannabis sativa* L.).

*Where, *G0D0* = 0 Gy; *G1D1*, *G2D1* = 150 Gy; *G1D2* = 300 Gy; *G1D3*, *G2D3* = 450 Gy; *G2D4* = 600 Gy.

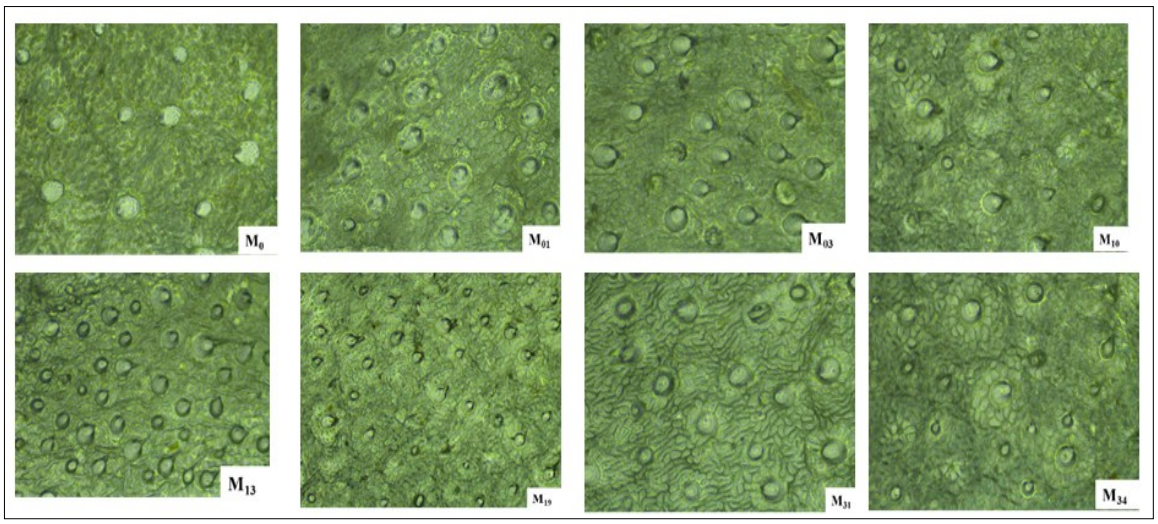

Fig. 7. Phenotypic variation in trichome size among isolated mutants of hemp (*Cannabis sativa* L.).

*Where, G_0D_0 = 0 Gy; G_1D_1, G_2D_1 = 150 Gy; G_1D_2 = 300 Gy; G_1D_3, G_2D_3 = 450 Gy; G_2D_4 = 600 Gy.

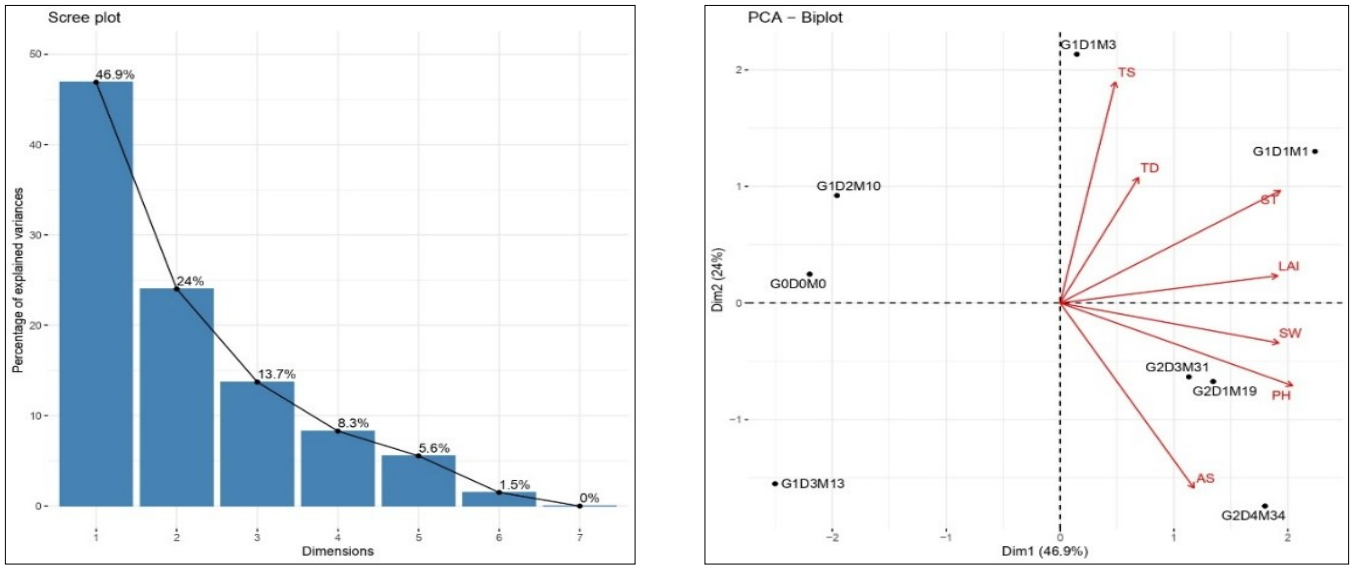

Fig. 8. Percentage of explained variances (%) and combined biplots of a principal component analysis (PCA) to detect differences in industrial and medicinal traits between hemp mutants.

*Where, G_0D_0 = 0 Gy; G_1D_1, G_2D_1 = 150 Gy; G_1D_2 = 300 Gy; G_1D_3, G_2D_3 = 450 Gy; G_2D_4 = 600 Gy; PH = plant height (cm); ST = stem thickness (mm); SW = shoot weight (gm); LAI = leaf area index (cm^2); AS = axillary shoots (n); TD = trichome density; TS = trichome size (μm^2).

with the highest variation observed in the G_1D_3 treatment (pre-irradiation hydropriming at 450 Gy). Mutant M_1 exhibited key industrial traits, including increased plant height and stem girth, while mutant M_{31} showed promising medicinal traits, such as enhanced branching and greater floral biomass. The use of gamma irradiation proved to be an effective method for generating hemp mutants with improved fibre yield and CBD oil content, offering valuable applications for both the textile and pharmaceutical industries. The selected mutants from this study will undergo further evaluation and screening, with the goal of developing new hemp varieties tailored for specific industrial and medicinal purposes.

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Authors' contributions

RT carried out the gamma irradiation studies, participated in the experimental design, research activities and drafted the manuscript. UH participated in the design of the study, resource provision and helped in statistical interpretation and manuscript editing. MAK and RMR conceived the study and participated in its design and coordination. MY helped for data collection and manuscript development. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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