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Harnessing gamma radiation-induced mutagenesis for enhanced essential oil yield and morphological improvement in lemongrass (*Cymbopogon flexuosus* Nees ex Steud)

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ABSTRACT

Lemongrass (*Cymbopogon* spp.) is a medicinally important aromatic crop, but conventional breeding approaches face limitations due to low genetic variability and lengthy breeding cycles. Induced mutagenesis through gamma radiation has potential to accelerate improvement of quantitative and qualitative traits in medicinal and aromatic plants. This study evaluated the effects of gamma radiation-induced mutation on morphological and yield characteristics of lemongrass. Uniform lemongrass slips (15-20 cm long) were subjected to nine gamma radiation doses (1-9 kR) using ⁶⁰Co isotope in a Complete Randomized Design with three replications. An untreated control was maintained for comparison. Observations were recorded at 30-day intervals until 120 days after planting. Results demonstrated that low-dose gamma radiation significantly enhanced yield attributes. Treatment T_2 (2 kR) produced the maximum herbage yield (11,100 kg ha⁻¹), essential oil yield (44.10 kg ha⁻¹), and oil percentage (0.397%), compared to control herbage yield of lower magnitude. Plant survival, morphological parameters (plant height, stem girth, number of tillers), and leaf characteristics (number, length, breadth, area, and dry weight) increased progressively from control through 2 kR treatment, then declined at higher doses. Pigmentation changes were noted at doses $\geq$ 6 kR. The lethal dose (LD₅₀) was established above 6 kR for all parameters studied. The findings indicate that 2 kR gamma radiation is the optimal dose for lemongrass improvement, enhancing essential oil yield and related agronomic traits without compromising plant survival, thereby establishing gamma irradiation as an effective tool for mutation breeding in vegetatively propagated aromatic crops.

Keywords: Induced mutagenesis, *Cymbopogon* spp., Essential oil, Gamma radiation, Optimization

Introduction

India possesses a remarkable diversity of plant species, with a significant portion identified as medicinal plants. This has made India a global leader in traditional medicine, particularly Ayurveda, which utilizes herbal remedies for various ailments and is known as the "botanical garden of the world" and is the world's largest producer of medicinal herbs (Grover *et al.*, 2002) [1]. Lemongrass, a valuable aromatic plant, is one of the nine mandated crops for development of appropriate production, protection and processing technologies (ICAR-DMAPR, 2024) [2]. Lemongrass, scientifically known as *Cymbopogon* spp., is a perennial grass with distinctive aromatic properties and it belongs to the family Poaceae, its three species are identified (Gupta, 1969) [3] viz; *Cymbopogon flexuosus*, *Cymbopogon citratus* and *Cymbopogon pendulus*. It is widely cultivated in India for its essential oil, which is rich in citral, a compound responsible for its lemon-like fragrance (Dutta *et al.*, 2016) [4]. Its oil has been traditionally used for its medicinal properties, including anti-bacterial, antifungal, and anti-inflammatory effects (Shah *et al.*, 2011) [5].

Conventional breeding methods, while effective for improving certain crops viz. cereals, pulses, oilseed etc. but face limitations when applied to medicinal and aromatic plants like lemongrass. These limitations include low genetic variability, self-incompatibility and the lengthy time required to achieve desired traits. This has prompted researchers to explore alternative approaches. Induced mutagenesis has the ability to improve both quantitative and qualitative qualities in crops in a shorter amount of time than traditional breeding (Lal *et al.*, 2016) [6]. Plant genetic variation, particularly alkaloid content and essential oil quality, as well as the economic aspects of medicinal and aromatic plants, have been demonstrated to be improved via mutation breeding (Kolakar *et al.*, 2018) [7]. It can lead to the development of lemongrass varieties with higher levels of essential oil, including its composition and aroma and certain morphological characteristics. Gamma irradiation utilizing ⁶⁰Co, a type of ionizing radiation, has been used effectively in mutation breeding and it is often classified as low light emission transfer (LET) radiation. It can induce genetic changes in plants, leading to a wide range of variations. Studies have shown that gamma radiation treatment can im

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prove both the oil quality and morphological traits of lemongrass. By keep all the points in view induced mutagenesis had accepted as a tool for improvement of the economic traits of medicinal and aromatic plant especially in vegetative propagated crops (Velmurugan *et al.*, 2020) [8].

Materials and methods

The present investigation was carried out at College of Horticulture, RVSKVV, Mandsaur in the Malwa plateau region of Madhya Pradesh during 2023-24. The slips of lemongrass were procured from “Herbal Garden” College of Horticulture Mandsaur, Madhya Pradesh in uniform size (15-20 cm long, 2-2.50 girth) and were obtained from well-established and healthy clumps. Lemongrass slips were treated with gamma irradiation in the gamma chamber on 18 July 2023 at Nuclear Research Laboratory, IARI, New Delhi. The source of gamma radiation was ^{60}Co isotope and the capacity gamma chamber was 5000 cc. Nine gamma radiation doses ranging from 1kR to 9kR were used in the study, along with three replications of each dose in CRD (Complete Randomized Design) and a radiation-free control. For comparison, untreated slips were used as a control and planted in the pots. All the desired traits were observed from date of planting to 120 days after planting with a fixed interval of 30 days. Following the data collected during the experiment, the ANOVA approach was used to statistically analyze the significance of various treatments at the 5% level of probability.

Results and discussion

It was confirmed from Figure 1 that, there was no major difference in T_0 -control (2.00 & 5.33 days) and T_2 – 2kR (2.00 & 6.33 days) for days to initiation and 50% sprouting, respectively, then the remaining treatments took significantly more days for the same traits. Improved sprouting at lower radiation doses was associated with increased auxin and gibberellin activity, as well as the suppression of the inhibitors. Misra *et al.* (1983) [9], Awad *et al.* (1986) [10], and Pranom *et al.* (1986) [11] found similarly minor earliness in sprouting with gamma radiation. Misra *et al.* (1983) [9], Awad *et al.* (1986) [10], and Pranom *et al.* (1986) [11] found the same comparatively minor earliness in sprouting with gamma radiation. Moreover, the survival was higher (73.33, 70.00 and 69.67 %) in T_2 - 2kR with the advancement of growth stages; the survival percentage decreased over T_0 - control (70.00, 65.67 and 64.00 %) at 60, 90 and 120 days after planting, respectively. This could be because gamma radiation positively impacts lemongrass. However, Roongtanakial *et al.* (2012) [12] likewise showed a decrease in the survival percentage in vetiver grass as gamma radiation doses were raised. The antagonistic impact of gamma radiation on grass may be the cause of variations in survival percentage as radiation doses rise. According to Mutlu *et al.* (2015) [13], higher gamma radiation doses have a well-known effect on lowering survival rates in Bermuda grass. Similar results have been found in other Poaceae species (Krishna *et al.*, 1984; Zaka *et al.*, 2002) [14, 15].

Different doses of gamma radiation had a notable effect on morphological parameters across life-span stages. Figure 2 indicates that, the incorporation of the gamma radiation causes no major impact on plant height, and number of tillers as the maximum plant height (50.92, 60.59 and 73.40 cm), stem length (9.73, 51.09 and 64.20 cm), girth of stem (4.37, 4.51 and 5.41 mm), number of tillers (6.44, 7.22 and 7.97) number of internodes (2.33, 4.39 and 5.39) and length of internodes (3.46, 11.49 and 11.79 cm) were recorded in T_0 - control while, minimum plant height (23.45, 25.16, 25.33 and 34.28 cm) and stem length (4.11, 15.83 and 25.08 cm) and girth of stem (3.28, 3.50 and 4.32 mm) number of tillers (1.00, 2.17, 2.72 and 3.06) number of internodes (1.00, 1.89 and 2.89) and length of internodes (2.70, 8.38 and 8.68 cm) in T_9 - 9kR and both were drastically decreased with the increment in gamma radiation doses at 60, 90, and 120 days after

planting, respectively. The results showed similarities with the findings of Lal *et al.* (2000) [16]. According to Misra *et al.* (1983) [9], mutagens disrupt the action of enzymes, which are essential for several plant metabolic processes that lead to improved plant development. According to Kainthura *et al.* (2015) [17], a reduction in the amount of auxin in leaves as a result of the enzyme system being destroyed or the enzyme system being inhibited by various radiation dosages may lead to a reduction in the vegetative growth of crops.

Different doses of gamma radiation significantly influenced all the leaf parameters and data are shown in Table 2. The more numbers of leaf (32.22, 42.33 and 46.67), maximum leaf length (42.48, 46.51 and 48.21 cm), leaf breadth (1.54, 1.67 and 1.77 cm), leaf area (65.56, 96.65 and 138.65 cm²), fresh weight of leaf (16.55, 35.92 and 46.05 g) and dry weight of leaf (4.96, 10.78 and 13.81 g) were confirmed in T_2 - 2 kR while, less numbers of leaf (18.22, 25.78 and 29.28), minimum leaf length (18.45, 20.15, 23.93 and 25.32 cm), leaf breadth (0.66, 1.02, 1.17 and 1.27 cm), leaf area (19.44, 35.59, 60.91 and 93.31 cm²), fresh weight of leaf (0.19, 10.40, 22.40 and 32.52 g) and dry weight of leaf (0.06, 3.12, 6.72 and 9.76 g) were recorded in T_9 - 9kR at 60, 90 and 120 days after planting, respectively. This variation may be related to the inherent genetic traits of the various cultivars and varying rates of photosynthesis (Pandey *et al.*, 2012) [18], as well as environmental influences (Safeena *et al.*, 2019) [19]. Both cell elongation and uneven cell wall growth are responsible for the difference in leaf length with an increase in gamma dose. DNA damage, variances in cell division, and alterations in cell structure and metabolism are just a few of the many effects that gamma radiation can have on plant tissues. According to Reiter *et al.* (1993) [20] and Takahashi *et al.* (1995) [21], the aforementioned effects can cause disturbances in typical growth and development processes.

The various gamma radiation dosages had a considerable impact on all yield parameters. The notable variations in the lemongrass were observed from Table 1 due to the induction of gamma radiation on lemongrass slips. There was no change in original leaf pigment in T_1 - 1kR to T_5 - 5kR and similar to T_0 - control (green), but changed from green to yellowish green from treatment T_6 - 6kR onwards, while, stem pigment was changed from yellowish green to purplish green and purple in different doses of gamma radiation. The change in pigmentation may be due the enhancement of the gamma radiation doses as they alter the morphology of pigmenting cells (Mutlu *et al.*, 2015) [13]. Among all the consequences, we found that highest herbage (11100 kg ha⁻¹), oil yield (44.10 kg ha⁻¹) and oil percent (0.397 %) were in T_2 - 2 kR though, the minimum herbage yield (6110 kg ha⁻¹) and oil yield (11.60 kg ha⁻¹) were taken by treatment T_9 - 9kR while, oil percent (0.223 %) was taken by T_8 - 8kR. Although radiation reduces growth characteristics, it can change oil quality and increase oil content (Lal *et al.*, 1998) [22]. Chemical and physical mutagens can generate economic trait variability in a variety of crops (Lal *et al.*, 2018) [23]. Low levels of gamma radiation have a major impact on the yield of different crops, according to research by Fowler *et al.* (1972) [24]. Vinutha *et al.* (2014) [25] also reported the fluctuation in essential oil yield with SUV-B treatment. *Mentha piperita* (Maffei *et al.*, 2000) [26], *Ocimum basilicum* (Jhonson *et al.*, 1999, Nitz and Schnitzler, 2004) [27], and *Mentha spicata* (Karousou *et al.*, 1998) [28] all demonstrated an increase in oil yield with short daily SUV-B treatment.

Conclusion

The properties of aromatic crops and their yield of essential oils were significantly impacted by the influence of gamma radiation-induced mutation. However, the current study concluded that, of all the gamma radiation doses applied, the yield attributes were found to be higher than control with T_2 -2 kR dose of gamma radiation. Accordingly, T_2 -2 kR is the optimum dose of gamma radiation for lemongrass, and minor changes were also noted in the pigmentation of the leaves and stem. However, we found that the LD₅₀ dose for every characteristic of lemongrass under study was greater than 6 kR.

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Figure 1. Effect of different doses of gamma radiation on days taken

to sprout initiation, days taken to 50% sprouting and survival percent.

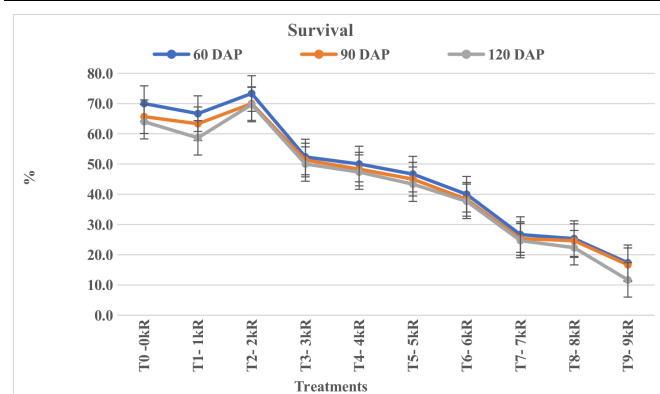


Table 1. Effect of different doses of gamma radiation on herbage yield, oil yield, oil percent and pigmentations

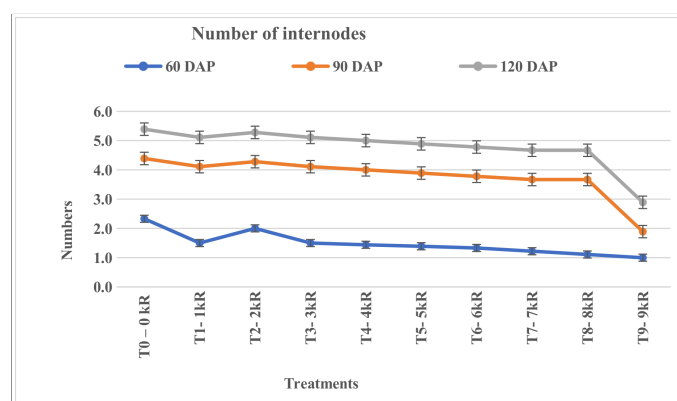
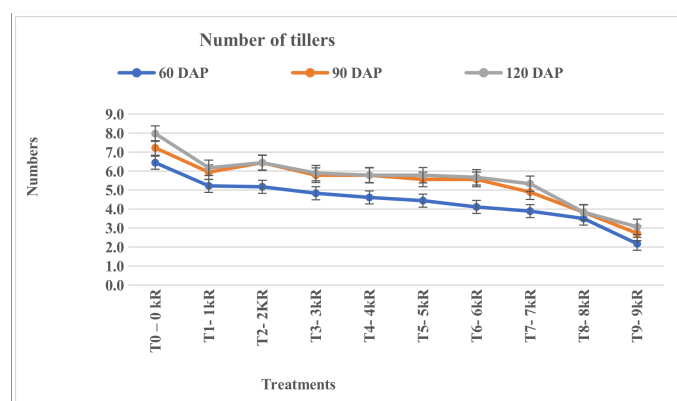
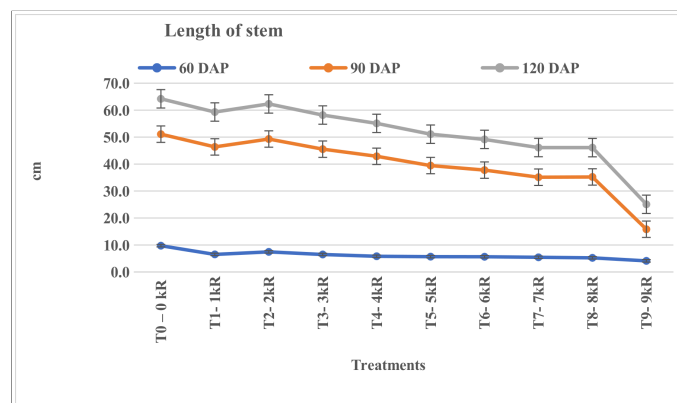
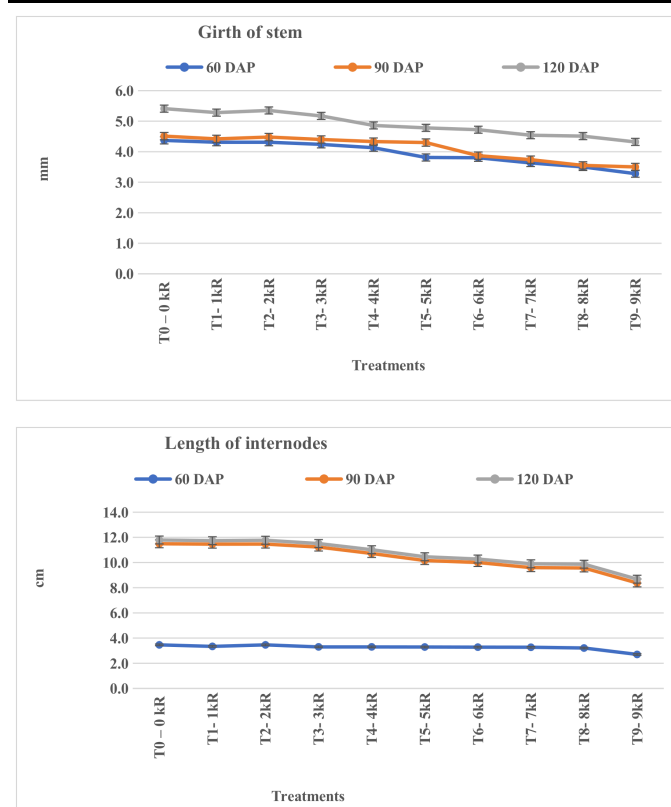
Treatments		Herbage yield (kg ha ⁻¹)	Oil yield (kg ha ⁻¹)	Essential Oil (%)	Pigmentation variation	
					Leaf	Stem
T ₀	0kR	10,567.00	34.85	0.330	Green	Yellowish Green
T ₁	1kR	9,000.00	29.06	0.337	Green	Purplish Green
T ₂	2kR	11,100.00	44.10	0.397	Green	Purplish Green
T ₃	3kR	8,300.00	27.99	0.323	Green	Purple
T ₄	4kR	7,900.00	26.86	0.340	Green	Green
T ₅	5kR	7,400.00	26.70	0.361	Green	Purplish Green
T ₆	6kR	7,100.00	24.92	0.351	Yellowish Green	Purplish Green
T ₇	7kR	6,893.33	23.19	0.336	Yellowish Green	Purplish Green
T ₈	8kR	6,600.00	12.48	0.223	Yellowish Green	Purplish Green
T ₉	9kR	6,110.00	11.60	0.227	Yellowish Green	Purple
S.Em. (±)		42.766	0.161	0.003	-	-
CD (5%)		126.158	0.475	0.007	-	-

Table 2. Effect of different doses of gamma radiation on numbers, length, breadth, area and dry weight of leaves

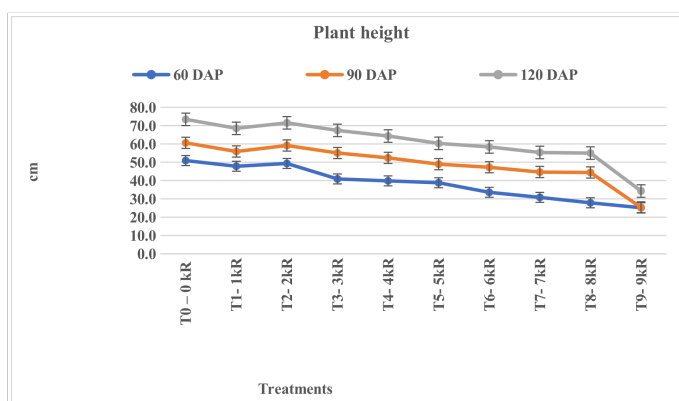
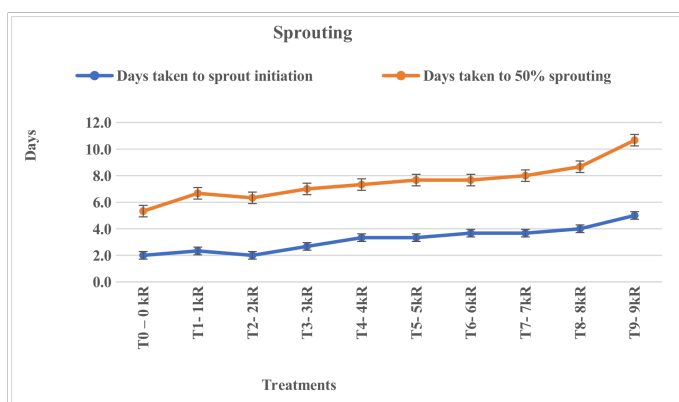
Treatments		Numbers (leaf plant ⁻¹)			Length (cm leaf ⁻¹)			Breadth (cm leaf ⁻¹)			Leaf area (cm ²)			Dry weight of leaves (g plant ⁻¹)		
		60 DAP	90 DAP	120 DAP	60 DAP	90 DAP	120 DAP	60 DAP	90 DAP	120 DAP	60 DAP	90 DAP	120 DAP	60 DAP	90 DAP	120 DAP
T ₀	0kR	31.22	40.28	43.00	40.80	46.46	48.41	1.45	1.63	1.73	64.43	96.65	134.07	4.93	10.68	13.72
T ₁	1kR	30.67	39.00	42.33	39.68	45.87	47.57	1.43	1.58	1.68	61.93	87.81	129.81	4.72	9.37	12.40
T ₂	2kR	32.22	42.33	46.67	42.48	46.51	48.21	1.54	1.67	1.77	65.56	96.65	138.65	4.96	10.78	13.81
T ₃	3kR	29.50	38.22	42.33	34.54	40.52	42.22	1.41	1.57	1.67	60.19	87.54	128.02	4.54	9.22	12.27
T ₄	4kR	27.44	36.39	39.94	34.35	38.42	40.12	1.38	1.57	1.67	59.15	87.25	126.48	4.44	9.21	12.26
T ₅	5kR	22.39	34.44	38.67	29.71	37.71	39.41	1.31	1.51	1.61	58.87	84.74	126.41	4.40	9.06	12.09
T ₆	6kR	22.22	29.83	33.44	27.77	32.17	33.87	1.18	1.38	1.48	58.45	82.84	124.50	4.25	8.23	11.92
T ₇	7kR	21.67	28.78	32.89	24.56	30.38	32.08	1.12	1.35	1.45	43.09	68.35	110.35	4.13	7.26	11.26
T ₈	8kR	19.67	25.83	29.33	20.28	25.60	27.50	1.03	1.20	1.30	38.94	64.25	95.03	3.38	7.07	10.30
T ₉	9kR	18.22	25.78	29.28	20.15	23.93	25.32	1.02	1.17	1.27	35.59	60.91	93.31	3.12	6.72	9.76
S.Em. (±)		1.85	2.32	2.60	2.30	2.49	3.23	0.07	0.08	0.06	1.43	1.53	2.74	0.07	0.47	0.51
CD (5%)		5.46	6.84	7.67	6.79	7.34	9.52	0.21	0.24	0.18	4.21	4.51	8.08	0.21	1.38	1.50

(continued on next page)

Figure 2. Effect of different doses of gamma radiation on plant height, length of stem, girth of stem, number of tillers, number of internodes and length of internodes



Figures



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