Exploring of Lethal Dose (LD_{50}) using gamma rays and its impact on seed germination and seedlingsurvival in *Stevia rebaudiana*Bertoni cv. CIM-Madhu

ABSTRACT

*Stevia rebaudiana*Bertoni an natural alternative to sugar and is known for production of sweet glycosides. The impact of gamma radiations in developing new stevia mutants was carried out at Department of Horticulture,University of Agricultural Sciences, GKVK, Bengaluru. Seeds of stevia cv. CIM-Madhu were collected and irradiated with various dosages of gamma rays *viz*. 50, 100, 200, 300, 400, 500 and 600 Gyto assess the LD_{50} value. The maximum germination (65%) was seen in the non-mutagenized control group followed by 61 percent in treatment comprising of 50 gy gamma irradiation with 6.15 per cent reduction in germination over the control. The maximum survival percentage was recorded in non-mutagenized control group (89%).Non-mutagenized control group took significantly lesser number of days (12 days) for germination and the treatment comprising of 200 Gy and 500 Gy took maximum number of days for germination (20 days). LD_{50} a dose that causes 50% mortality to the seeds was found at 150 Gy. This LD_{50} dosecan be used as reference for initiating mutation breeding in other cultivars of stevia.

Key word: Gamma rays,Lethal dose (LD_{50}), Stevia, Mutagens

1. INTRODUCTION

Stevia (*Stevia rebaudiana*Bertoni)a member of the Asteraceae family, originates from Paraguay and South-West Brazil, commonly known as sweet leaf, sugar leaf, sweet honey leaf, rebiana, sweet herb, and methitulsi, this plant gained global recognition for its remarkably sweet-tasting leaves and aqueous extracts. The extraordinary sweetness can be attributed to diterpene glycosides, including Stevioside and Rebaudioside A, B, C, D, M, along with six other compounds. Notably, these components exhibit insulin-balancing properties, contributing to stevia's appeal as a natural sweetener with potential health benefits[1].

In India, a growing desire for natural sweeteners and the rising diabetic population has spurred farmers to embrace stevia cultivation. Key stevia-producing states include Madhya Pradesh, Punjab, Andhra Pradesh, Karnataka, Chhattisgarh, and Maharashtra [2]. However, stevia cultivation faces challenges in India due to early flowering under local photoperiod conditions, resulting in suboptimal leaf yields. Additionally, meager breeding work has been done in developing suitable cultivars.

The initial phase in any crop enhancement initiative involves evaluating genetic variability, achievable through hybridization or induced mutation. Induced mutagenesis emerges as a potent mechanism for instigating intrinsic genetic diversity, crucial for cultivating high-yielding varieties. Mutation breeding employs both chemical and physical mutagens to induce novel recombinations, fostering variability [3]. Mutations may arise spontaneously or due to exposure to radiation or chemicals. Extensive studies across various crops underscore the efficacy of mutation in provoking variability and crafting cultivars with enhanced traits. This approach plays a pivotal role in crop improvement programs, contributing to the development of resilient and high-performing plant varieties [4-5].

Mutagenic agent like gamma has been widely used for the development of assorted traits of crops but the success of mutation depends on its dose applied. Usually, mutagen treatments scale back seed germination, rate of growth, vigour and fertility. There's substantial killing of plants throughout completely different stages of development, so significantly reduces the survival of ensuing plants. The dose needed for prime agent potency depends on properties of the mutagenic agents and material treated [6] Hence, an overdose may kill too many treated individuals and lesser dose can turn out fewer mutations. The optimum dose can turn out the high frequency of mutations and cause minimum killing that varies with crop species and agent used [7]. Therefore, assessment of the LD_{50} (Lethal Dose), a dose that causes 50% mortality to the seeds is critical. The LD_{50} is completely different between species and varieties in a species [8]. Therefore, this study was carried out to assess the LD_{50} of stevia cultivar CIM-Madhu for gamma rays and its effect on seed germination and survival of seedlings.

2. MATERIALS AND METHODS

2.1 Seed source: Seeds of stevia cv. CIM-Madhu were procured from Central Institute of Medicinal and Aromatic Plants-CIMAP, Reginal Centre, Bangalore. This variety was developed by applying half sib family selection followed by clonal breeding approach. CIM – Madhu is having closed growth habit, highly vigorous, dark green medium size leaves and dark green stem. Fresh (13.37t/ha) and dry leaf yield (4.30 t/ha) with high stevioside (12.57%) and rebaudioside (5.8%) with low dulcoside –A content (0.20 %) [9].

2.2 Treatment of stevia seeds with various dosages ofγ irradiation:

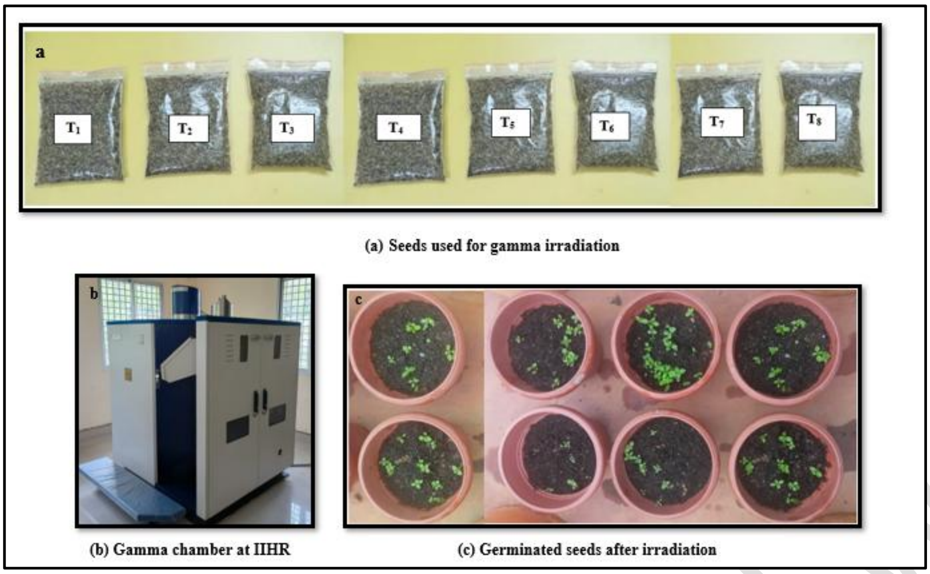
The seeds of stevia cv. CIM- Madhu were treated with different dosage of gamma rays. Based on the color of the seeds,viable (dark colored**)** and non-viable (pale or clear colored) seeds were separated manually. 85 viable seeds were used for each treatment with 3 replications. Seeds were irradiated at Gamma chamber 5000 installedat the ICAR-IIHR, Bangaloreat various gamma dosages *viz.* 50, 100, 200, 300, 400, 500 and 600 Gy (Table 1). The treated and untreated (control) seedswere sown in media containing sand, soil and Farm yard manure (FYM) in the ratio 1:2:1 with coir pith on top (Plate 1). Observation on germination percentage, days taken for germination, survival percentage and LD_{50} value were recorded.

2.3 Statistical analysis:

The mean values of germination percentage,days taken for germination and survival percentage, of seeds in each replication were used for Fisher's method of analysis of variance (ANOVA). The analysis of variance for individual character was carried out using the percentage values of replications following the method given by Panse and Sukhatme[10]. The significance of the differences among all the treated lines was tested by F-test using the error variance. The complete data was analysed using completely randomized design (CRD) and OP-Stat software.

Table 1. Treatment details of gamma radiation induction of stevia cv. CIM-Madhu

Sl. No.	**Radiations**	**Dosages**
T_1	Non-mutagenized - CIM-Madhu	(0.0Gy, control)
T_2	Gamma-ray (Co^{60})	50 Gy
T_3	Gamma-ray (Co^{60})	100 Gy
T_4	Gamma-ray (Co^{60})	200 Gy
T_5	Gamma-ray (Co^{60})	300 Gy
T_6	Gamma-ray (Co^{60})	400 Gy
T_7	Gamma-ray (Co^{60})	500 Gy
T_8	Gamma-ray (Co^{60})	600 Gy



(a) Seeds used for gamma irradiation

(b) Gamma chamber at IIHR

(c) Germinated seeds after irradiation

Plate 1: Treatment details of gamma radiation induction of stevia cv. CIM-Madhu

3. RESULTS AND DISCUSSION

3.1 Germination percentage:

The effect of gamma irradiation on germination of stevia seeds was presented in Table 2. The maximum germination (65%) was seen in the non-mutagenized control group followed by 61 percent in treatment comprising of 50 gy gamma irradiation with 6.15 per cent reduction in germination as compared to the control. As the radiation levels increased, a dose-dependent reduction in germination became evident. At 100 Gy, there was a 23.08 percent reduction, while at, 200 Gy, 300 Gy, 400 Gy and 500 Gy germination percentages decreased by 35.38 percent, 41.54 percent, 58.46 percent and 55.38 percent respectively, as compared to the control. These findings underscore the sensitivity of seed germination to ionizing radiation and suggest a pronounced dose-response relationship. The lowest germination was recorded in gamma irradiations at 600 Gy (26%). Lower per cent of germination was recorded in most of the mutagen treated seeds and with increased dose of gamma radiations.

The possible reasons for decrease in germination, may be because gamma rays have high energy and can penetrate deeply into biological tissues, including seeds. When gamma rays interact with the DNA within the seed cells, they can cause various types of damage, such as breaks in the DNA strands, cross-linking of DNA, and mutations. This DNA damage can disrupt the genetic information necessary for proper seed germination.These results are in accordance with Khalida*et al*. (2022), Abdullaha*et al*. (2021) Singh *et al*. (2020) and Khalil*et al*. (2014)[11-14].

Table 2: Effect of gamma irradiation on germination percentage of stevia seeds

Treatment	No of seeds germinated out of 85 seeds sown	Germination %	Percent reduction over control
T_1 -Control	55	65	0.00
T_2 -50 Gy	52	61	6.15
T_3-100 Gy	43	50	23.08
T_4-200 Gy	36	42	35.38
T_5-300 Gy	32	38	41.54
T_6-400 Gy	23	27	58.46
T_7-500 Gy	25	29	55.38
T_8-600 Gy	22	26	60.00
C.D.	2.853	3.359	-
SE(m)	0.931	1.097	-
SE(d)	1.317	1.551	-
C.V.	4.481	4.497	-

3.2 Days taken for germination:

It was evident from present study that there was significant difference in the days taken for germination among different mutagenic treatments (Table 3). The days taken for germination ranged from 12 to 20 days. Non-mutagenized control group took significantly lesser number of days (12 days) for germination and the treatment comprisingof 200 Gy and 500 Gy took maximum number of days for germination (20 days).Gamma radiation can disrupt the cell division processes in seeds, particularly in the root tip meristems where cell division is most active. This can result in slower or abnormal cell division, leading to delayed germination or poor seedling development. These results are in accordance with findings of other researchersKhalil*et al*. (2014), Snehaland Madhukar, (2011) in stevia[14-15].

Table 3: Influence of gamma irradiation days taken for germination of stevia seeds

Treatment	Days taken for germination
T_1 -Control	12
T_2 -50 Gy	14
T_3-100 Gy	15
T_4-200 Gy	20
T_5-300 Gy	15
T_6-400 Gy	16

T_7-500 Gy	20
T_8-600 Gy	18
C.D.	0.633
SE(m)	0.207
SE(d)	0.292
C.V.	2.202

3.3 Survival percentage:

Similar results were observed for survival percentage as that of germination percentage (Table 4). The Maximum survival percentage was recorded in non-mutagenized control group (89%) followed by treatment comprising of 50 gy gamma irradiation (67%). None of the seedlings survived at 400 gy and above dose of gamma irradiation though there was considerable germination at these dosages.The possible reasons for death of seedlings may be because gamma irradiation generates free radicals that may bring metabolic disorders in the seeds leading to growth retardation. Similar results were found by findings of with Khalida*et al*. (2022), Abdullaha*et al*. (2021) Singh *et al*. (2020), Khalil*et al*. (2014), Snehal and Madhukar, (2011) in stevia[11-15].

Table 4: Effect of gamma irradiation on survival percentage of stevia

Treatment	Survival %	Percent reduction over control
T_1 -Control	89	0.00
T_2 -50 Gy	67	24.72
T_3-100 Gy	53	40.45
T_4-200 Gy	40	55.06
T_5-300 Gy	32	64.04
T_6-400 Gy	0	100.00
T_7-500 Gy	0	100.00
T_8-600 Gy	0	100.00
C.D.	**7.453**	-
SE(m)	**2.434**	-
SE(d)	**3.442**	-
C.V.	**12.001**	-

3.4 Lethal dose 50 percent (LD_{50}):

Determination of LD_{50} is crucial parameter to study to develop M_1 population of stevia cv. CIM-Madhu. The LD_{50} (Lethal Dose) was determined by plotting a simple regression graph of seedling survival percentage against gamma dosage (Fig.1). The LD_{50} was noted to be at 150 Gy for gamma irradiations, above which there was a maximum lethality in cv. CIM-Madhu after mutation induction. These results are in accordance with findings of Khalida*et al*., 2022 in stevia [11].

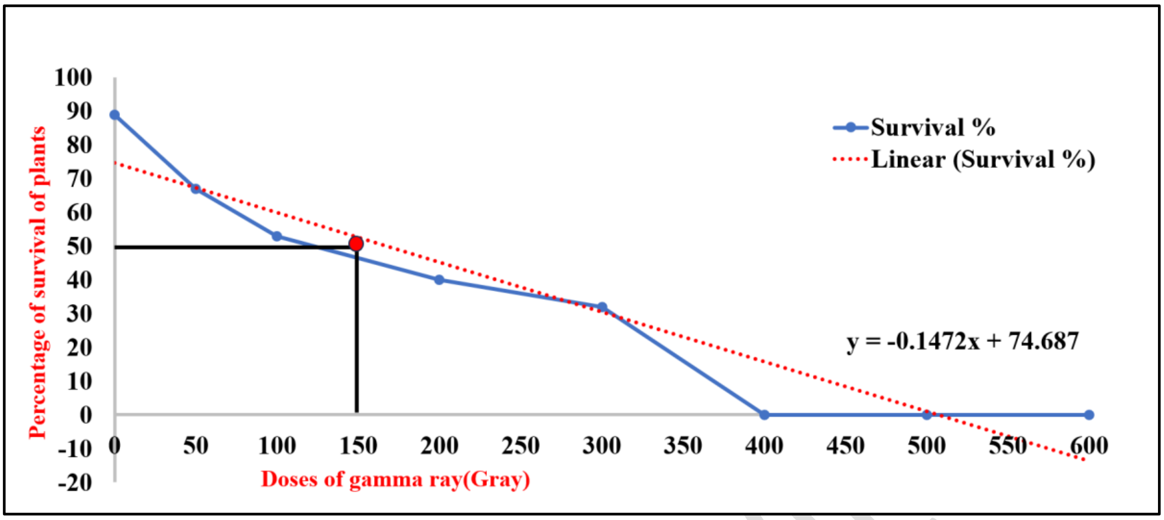


Figure 1: Plot of log doses of gamma v/s survival percentage of plants to determine LD_{50}

4. CONCLUSION

To develop M_1populations of steviacv. CIM-Madhu, determination of LD_{50}, germination and survival percentagesare crucial parameters.It is evident from findings of present study that, the increased dose of gamma irradiationhas resulted in lower germination and survival percentage as compared to the control plants. There are limited studies on determination of $LD_{50}$for gamma irradiation in stevia using seeds, sothe findings of this study on LD_{50}could be used as reference for initiating mutation breeding in other cultivars of stevia and also for improvement of specific traits by mutation breeding.

5. REFERENCES

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