

Effect of gamma rays irradiation on seed germination, seedling growth and seedling survival in Sesame (*Sesamum indicum* L.)

Comment [k1]: Seedling survival % if not calculated then why the word used in title?

ABSTRACT

The present investigation was carried out with the aim to understand the effect of gamma irradiation on germination and survival of seedlings in *Sesamum indicum* L. during 2023-24. The pure, healthy and dry seeds of three sesame varieties viz., DS-5, Swetha and DSS-9 were irradiated with ten different doses of gamma rays i.e., 250, 300, 350, 400, 450, 500, 550, 600, 650 and 700 Gy for determination of their responses to gamma rays treatment and the effective radiation dose for induced mutation breeding. The M₁ generation was raised in the laboratory condition and the significant variation was observed for various growth parameters i.e., germination per cent, shoot length, root length, total seedling length and vigour index. The percentage of seed germination, shoot length and root length decreased progressively with increasing dose in all the three varieties. Among three genotypes, DSS-9 was observed more sensitive while, Swetha exhibited more resistance reaction for most of the traits. The results of the present study indicated that genotypes recorded varied performance for different doses of gamma rays and this kind of preliminary studies help in selection of best range of gamma rays which could be effectively utilized to create wide range of genetic variability for various quantitative traits in all the three sesame genotypes.

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Keywords: Sesame, germination, seedling morphology, gamma radiation

Introduction:

Sesame (*Sesamum indicum* L.) is one of the oldest oil seed crop domesticated by mankind and is being widely cultivated in tropical and subtropical parts of the world including India (Ashri, 1998). It is a member of the family Pedaliaceae having $2n=26$ chromosome number and considered to be originated in Africa as there exists diverse wild species. Sesame is commonly known as gingelly, til, simsim, benniseed etc. It is referred to as "Queen of Oil Seeds" because of its excellent oil quality offered by lignans - a natural antioxidants such as sesamol and sesamin present in its oil (Brar and Ahuja 1979, Kamal Eldin 1993) and the seeds are also rich in minerals (calcium, potassium, phosphorus, iron, magnesium, zinc), vitamins (E, C, B complex). Sesame has wider economical, industrial, nutritional and medicinal significances thus

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the demand for sesame seed and oil consumption is estimated to reach about 220.46 million tons by 2030. Hence, demands the crop improvement.

Sesame is grown widely in many countries, including China, India, Ethiopia, Uganda, Nigeria and Myanmar. As per the report of FOASTAT 2020, the productivity of sesame is low in India (431 kg/ha) as compared to world's average (790 kg/ha) and it is far below as compared to China (1620 kg/ha) which is being the highest (Telku., 2022).

Presence of genetic variation is the basic requirement in any crop for its genetic improvement. Among various breeding tools, one of the feasible and potential means of creation of these variations is through induced mutagenesis as spontaneous mutations occur in very low frequency in nature. In order to have mutations in crop plants, both physical and chemical mutagens are being used and subsequent improvement can be achieved through selection. Among the physical mutagens, gamma rays are commonly used mutagens that directly penetrate plant tissue. Usually radiation makes cuts in the plant's DNA and errors to DNA repairs lead to induced mutations. Doses of any physical mutagen that leads to 50% lethality or 50% growth reduction are considered as LD₅₀ or GR₅₀ respectively (Viana et al. 2019) which helps to understand the adequate gamma irradiation dose for mutations. Further, it is also considered that the optimum mutation frequency lies in between GR₃₀ and GR₅₀ values (Roy., 2019).

The degree of radio sensitivity of different genotypes and the extent of damage caused by the mutagens is observed by inhibition of seed germination in M₁ generation (Gaul., 1958). Previous studies indicate that the mutagen is very effective at creating genetic variation in sesame also. Hence, the present study was carried out to understand the influence of gamma rays on seed germination and seedling parameters in different sesame genotypes and there by to have scientific basis for sesame mutation breeding.

Materials and Methods

Genetically pure and healthy seeds of three promising sesame genotypes viz., DS-5 and DSS-9 and Swetha were irradiated with 10 different doses of gamma rays i.e., 250Gy, 300Gy, 350Gy, 400Gy, 450Gy, 500Gy, 550Gy, 600Gy, 650Gy and 700Gy from ⁶⁰Co at Bhabha Atomic Research Centre, Mumbai. About 100 seeds of each dose along with control in all the three genotypes were placed between moistened germination papers replicated twice for seed germination test and seedling parameters study in the laboratory at Seed Unit, UAS, Dharwad during 2023-24.

Seed germination:

The emergence of cotyledon leaf in each treatment was considered as the indication of germination. Germinated seeds count for each treatment was taken on seventh day after sowing according to ISTA (2004) rules and germination percentage was calculated.

Germination percent = [(Number of seeds germinated/ Total number of seeds taken) x 100]

Shoot length (cm):

The length of the shoot from the cotyledonary node to the tip of the shoot was measured in centimeter on ten randomly selected seedlings and averaged to get mean shoot length.

Root length (cm):

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Comment [k8]: From where these three genotype collected, it should be mention.

The root length from the cotyledonary node to the tip of the primary root was measured on ten randomly selected seedlings and average root length was expressed in cm.

Seedling Vigour index:

Seedling vigour indices were calculated by using the formula suggested by Abdul-Baki and Anderson (1973).

Seedling vigour index = Standard germination (%) x seedling length (cm)

The LD₅₀, GR₅₀ and is calculated using graphical analysis on Microsoft excel sheet.

Comment [k9]: Formula? Reference?

Comment [k10]: Seedling survival % if not calculated then why the word used in title?

Results and Discussions:

In any induced mutation breeding experiments assessment of the effects of mutagens in M₁ generation is a common procedure. In general physical mutagens cause physiological damages, gene mutations as point mutations and chromosomal mutation in terms of chromosomal aberrations in M₁ generation of the irradiated biological materials under study (Gaul, 1970). These biological damages caused by the mutagens is measured based on seed germination, survival reduction, plant growth reduction as well as fertility reduction or sterility. These damage caused could be considered as an indication of mutagenic effects (Gaul, 1970). Similar to aforesaid research results, different effects due to gamma rays have been studied in the present investigation among three sesame genotypes. The results of the present study are presented (Table 1) and discussed here.

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Germination percentage: In the present experiment, the results of germination test under laboratory condition revealed that the percentage of seed germination was increased progressively with the increasing dose of gamma rays at specific treatment and then decreased thereafter in all the three sesame varieties (Table 1). Many earlier workers viz., Bolbhatsadashiv et al. (2012) in horsegram, Dhakshanamoorthy et al. (2010) in *Jatropha curcas*, Khan and Wani (2005), Lavanya et al. (2011) and Rukesh et al. (2017) in green gram also reported similar results due to treatment of mutagens. However, Boranayak et al. (2010) and Raut et al. (2021) reported the decrease in percentage of seed germination in sesame varieties. Among the three sesame varieties, DS-5 recorded highest average germination percentage (87.7 %) followed by Swetha (86.1 %) and DSS-9 (85.5%).

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Shoot length: A considerable reduction in shoot length development was observed among all the sesame genotypes and rate of reduction was more as the dose of gamma rays increased in present study. Lower doses exhibited highest shoot length. The highest reduced shoot length was recorded at 700 Gy in all the three genotypes namely DS-5 (1.17 cm), DSS-9 (1.36 cm) and Swetha (2.27 cm). Similar results of reduced shoot length was reported by Raut et al. (2021) in their study.

Comment [k19]: Comparison with Control always mention.

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Root length: In this study, root length of gamma irradiated plants and control plants differed significantly in all the three sesame genotypes. Shoot length also decreased progressively with increase in dose of the gamma rays mutagen. It was observed that at 700 Gy highest reduced root length as compared to other doses and shortest root length was noticed in the variety DSS-9 (2.47 cm) followed by DS-5 (2.90 cm) and Swetha (4.85 cm). Decreased in shoot length witnessed in the present study was also in conformity with the observation of Raut et

al. (2021), Boranayaket al.(2010) and Kumari et al.(2016). The results of this study showed an inverse relationship between the mutagenic dosages and the reduction rate of both shoot and root development. Shoot and root growth is being related to many factors which may be due to chromosomal abnormalities, reduction in auxin levels and inhibition of its synthesis and chromosomal damage cum mitotic inhibition (Boranayaket al.,2010). Further, hampered protein synthesis in the embryonic cells may also prevent transfer of cells from G₁ onwards thereby retarding the emergence of root and shoot.

Comment [k21]: These discussion is related to physical basis or molecular level. If such molecular study is not done in this article, then why it is mentioned?

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Total seedling length: Wide range of variation for total seedling length was recorded. It ranged from 14.94 to 3.83cm in DSS-9 followed by DS-5 (13.79 – 4.07cm). The study also revealed that reduction in average total seedling length of M₁ plants with the increase in dose of gamma rays. Similar report was given by Raut et al.(2021). Among the different doses of gamma rays doses of mutagen, 700 Gy of gamma ray recorded the maximum total seedling length reduction (Table 1). In the present investigation, the seed germination got delayed and the seedlings were shorter at higher dosage of mutagens which might be due to the effect of mutagens.

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Seedling Vigour Index: Increase in dose of gamma radiation resulted in a gradual reduction in vigour index (Table 1). Minimum vigour index was witnessed at 700 Gy of gamma rays treatment irrespective of genotypes and showed wide range values. Among the three genotypes, Swetha genotype recorded maximum vigour index (1196.9) followed by DS-5 (1169.2) while, DSS-9 (321.0) showed least vigour index value. These results are supported by the research findings of Raut et al. (2021) and Boranayaket al.(2010).

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The radio-sensitivity test: For any successful mutation breeding programme, it is necessary to determine the lethal dose (LD₅₀) or growth reduction dose (GR₅₀) values which serve as a baseline for the subsequent doses that can be used to treat and study a larger population. The GR₅₀ value for all the three sesame genotypes were determined based on the seedling growth reduction percentage with different doses of gamma rays by adopting graphical excel analysis (Table 1 and Fig 1,2 and 3). Results of *in vitro* experiment of the present study revealed dose dependent gradual growth reduction in sesame seedlings. The calculated GR₅₀ was 608.1 Gy for DS-5 (Fig 1), 909.9 Gy for Swetha (Fig 2) and 585.7 Gy for DSS-9 (Fig 3) indicating that Swetha has less sensitivity to gamma rays irradiation followed by DS-5 and DSS-9 variety. These difference indicated that different genotypes of the same family can also vary considerably in their sensitivity response to gamma rays irradiation (Muhammad et al.,2021). The expected LD₅₀ value for DS-5, Swetha and DSS-9 was 493.2 Gy, 670.6Gy and 584.8 Gy respectively.

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Conclusion :

In the present study it was observed that, reduction in the seedling growth rates of treated seeds probably might be due to high genetic aberrations caused by higher doses of the gamma rays. The seed germination, seedling growth, root length and shoot lengths were inhibited by increasing doses of gamma rays. Thus the gamma rays found highly effective for modifying majority traits of the sesame crop plant.

Among three sesame genotypes Swetha was found more resistant whereas, DSS-9 was observed more sensitive for different doses of gamma radiation exposure. Overall results of the present study confirmed that gamma radiation induced can be used effectively to induce genetic

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variability for the genetic improvement of certain traits in the crop species. However, it should be kept in mind that induced mutagenesis is random events, not specific events and thus, established gamma rays irradiation conditions might probably not give the same result for different genotypes belonging to the same family.

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Table 1. Effect of gamma rays irradiation on growth parameters (Laboratory condition)

Genotypes	Dose of Gamma rays	Germination %	Shoot length (cm)	Root length (cm)	Total Seedling length (cm)	Vigour Index
DS-5	Control	91.5	4.05	9.74	13.79	1255.1
	250 Gy	89.5	3.98	9.23	13.21	1169.2
	300 Gy	92.5	3.60	9.57	13.17	1205.3
	350 Gy	93.0	3.25	8.34	11.59	1084.0
	400 Gy	95.0	3.29	8.06	11.35	1105.9
	450 Gy	96.0	2.79	7.15	9.94	951.6
	500Gy	89.0	2.01	6.00	8.01	745.5
	550 Gy	82.5	2.26	6.15	8.41	784.7
	600 Gy	80.0	1.36	3.96	5.32	463.4
	650 Gy	79.0	1.39	4.82	6.21	513.2
	700 Gy	76.5	1.17	2.90	4.07	342.4
	Mean	87.7	2.65	6.90	9.55	874.57
	S.Em. ±	2.15	0.13	0.29	0.41	35.38
	CD@5 %	6.77	0.42	0.91	1.28	111.48
CV %	3.33	7.13	5.94	6.04	5.72	
Swetha	Control	93.0	4.80	7.83	12.63	1173.8
	250 Gy	86.5	4.85	7.54	12.39	1196.9
	300 Gy	82.0	4.34	7.13	11.47	943.7
	350 Gy	84.5	4.00	8.16	12.16	1028.2
	400 Gy	88.5	4.08	8.30	12.38	1096.7
	450 Gy	86.5	4.18	7.70	11.88	974.2
	500Gy	92.0	3.81	7.29	11.1	960.6
	550 Gy	91.0	3.13	6.69	9.82	891.8
	600 Gy	87.5	3.29	5.72	9.01	788.2
	650 Gy	82.0	3.38	6.27	9.65	890.6
	700 Gy	73.0	2.27	4.85	7.12	647.9
	Mean	86.1	3.83	7.04	10.87	962.97
	S.Em. ±	2.57	0.27	0.48	0.56	72.56
	CD@5 %	8.11	0.84	1.52	1.78	228.65
CV %	4.11	9.81	9.68	7.33	10.66	
DSS-9	Control	91.0	4.38	10.56	14.94	1359.1
	250 Gy	84.5	4.16	8.58	12.74	1076.3
	300 Gy	83.0	4.01	8.84	12.85	1066.0
	350 Gy	87.5	3.77	7.89	11.66	1019.1
	400 Gy	89.5	3.79	8.32	12.11	1082.9
	450 Gy	90.5	3.32	7.46	10.78	932.0
	500Gy	88.5	2.19	6.75	8.94	790.8
	550 Gy	86.5	2.11	7.04	9.15	818.2
	600 Gy	84.0	1.64	4.14	5.78	486.0
	650 Gy	82.0	1.44	4.99	6.43	539.5
	700 Gy	74.0	1.36	2.47	3.83	321.0
	Mean	85.5	2.92	7.00	9.92	862.80
	S.Em. ±	2.29	0.09	0.23	0.18	29.11
	CD@5 %	7.21	0.29	0.72	0.58	91.73
CV %	3.74	4.53	4.59	2.61	4.77	

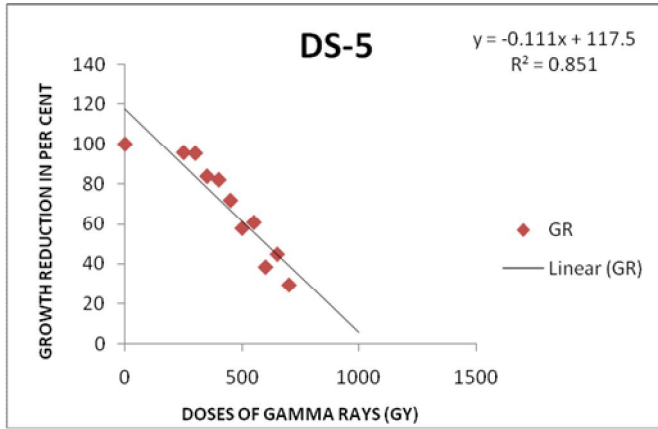


Fig.1 Effect of different doses of gamma irradiation on growth reduction in DS-5 genotype.

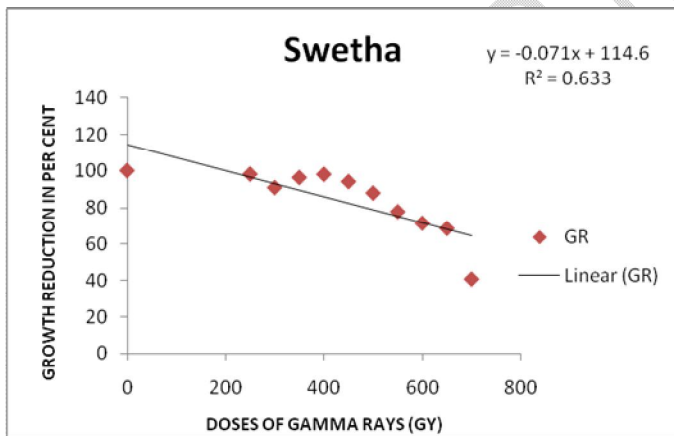


Fig.2 Effect of different doses of gamma irradiation on growth reduction in Swetha-5 genotype

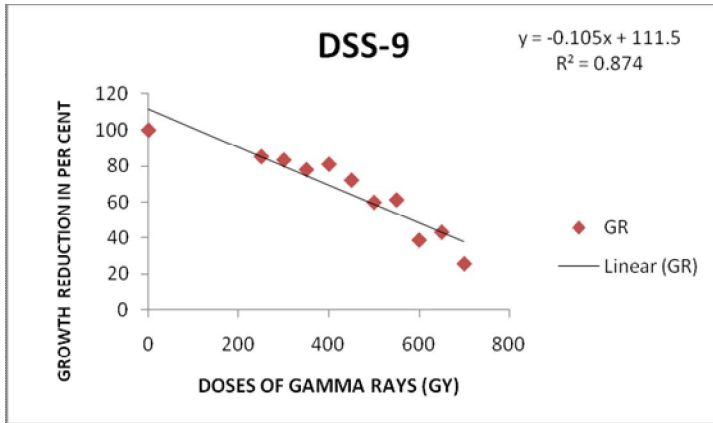


Fig.3 Effect of different doses of gamma irradiation on growth reduction in Swetha-5 genotype

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Reference:

- Abdallah, J., Said, S., Emad, A., Servet, A., Halil, H., & Adrian, C. B. (2024). Optimizing gamma irradiation seed treatment of sesame (*Sesamum indicum* L.) varieties for potential future application in mutation breeding. *Crop Breeding Genetics and Genome*, 6(3), e240004. <https://doi.org/10.20900/cbagg20240004> Comment [k29]: Not Found in Whole Article.
- Abdul-Baki, A. A., & Anderson, J. D. (1973). In: Physiological and biochemical deterioration of seeds. *Seed biology*. Academic Press, New York, 2:283-315.
- Ashri, A. (2007). Sesame (*Sesamum indicum* L.). In: SINGH R. J. (ed.), Genetic resources, chromosome engineering and crop improvement. Oilseed crop, CRC Press, Boca Raton, FL, USA, 4: 231-289. Comment [k30]: Year is mismatch
- Bolbhat, S. N., Bhoge, V. D., & Kondiam, D. N. (2012). Effect of mutagens on seed germination, plant survival and quantitative characters of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). *International Journal of Life Science and Pharma Research*, 2-4.
- Boranayaka, M. B., Kambegowda, R., Nandini, B., Satish, R. G., & Santoskumar, B. P. (2010). Influence of gamma rays and ethyle methane sulphonate on germination and seedling survival in sesame (*Sesamum indicum* L.). *International Journal of Plant Science*, 5, 655-59.
- Brar, G., & Abuja, R. (1979). Sesame: its culture, genetics, breeding and Biochemistry. In *Annual Review of Plant Sciences*, 285-313.
- Dhakshanamoorthy. (2010). Physical and chemical mutagenesis in *Jatropha curcas* L. to induce variability in seed germination, growth and yield traits. *Roman Journal of Biobiology-Plant Biobiology*, 55, 113-125.
- Gaul, H. (1958). Present aspects of induced mutation in Plant Breeding. *Euphytica*, 7, 275-289.
- ISTA (International Seed Testing Association). (2004). International rules for seed testing annexes. International Seed Testing Association (ISTA), Zurich, Switzerland.
- Kamal-Eldin, A. (1993). Seed oils of *Sesamum indicum* L. and some wild relatives. A compositional study of the fatty acids, acyl lipids, sterols, tocopherols and lignans. Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala.
- Khan, S., & Wani, M. R. (2005). Genetic variability and correlations studies in chickpea mutants. *Journal of Cytology and Genetics*, 2, 155-160.
- Kumari, V., Chaudhary, H. K., Prasad, R., Kumar, A., Singh, A., & Jambhulkar, S. (2016). Effect of mutagenesis on germination, growth and fertility in sesame (*Sesamum indicum* L.). *Annual Research & Review in Biology*, 10(6), 1-9.
- Lavanya. Sodium azide mutagenic effects on biological parameters and induced genetic variability in mungbean. *J. Food Leg.* 2011;24(1): 46-49 Comment [k31]: Publication year not mention
- Muhammad, I., Rafii, M. Y., Nazli, M. H., Ramlee, S. I., Harund, A. R., & Oladosu, Y. (2021). Determination of lethal (LD) and growth reduction (GR) doses on acute and chronic gamma- irradiated Bambara groundnut [*Vigna subterranea* (L.) Verdc.] varieties. *Journal of Radiation Research and Applied Sciences*, 4(1), 133-145.
- Priyanka, J. B., Balaji, S. T., & Vaibhav, J. G. (2020). Effect of gamma radiation on germination and seedling parameters of mung bean (*Vigna radiata*). *International Journal of Current Microbiology Applied Sciences*, 11, 1582-1587. Comment [k32]: Not Found in Whole Article.
- Roy, U., Basak, D., & Nath, S. (2019). Mutagenic sensitivity analysis of gamma irradiations in cowpea (*Vigna unguiculata* L. Walp). *Emergent Life Sciences Research*, 5: 12-16.

- Rukesh. (2017). Impact of gamma irradiation induced mutation on morphological and yield contributing traits of two genotypes of green gram *Vigna radiata* L.). *Journal of Pharmacology and Phytochemistry*, 6(6),1229-1234.
- Telku, D. D., Shimelis, H.,& Abady, S. (2022). Genetic improvement in sesame (*Sesamum indicum* L.): Progress and outlook: A Review. *Agronomy*, 12, 2144-50.
- Raut, Y., Vaidya, E. R., & Pallavi, S. (2021). Effect of gamma rays on germination and plant survival in sesame (*Sesamum indicum* L.). *The Pharma Innovation Journal*, 10(12), 392-394.

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