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Determination of LD_{50}, germination percentage and seedling survivability for induced mutagenesis through gamma rays in French marigold (*Tagetes patula* L.)

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Abstract
Marigold known as loose flower crop belongs to Asteraceae family. The impact of gamma radiations in developing new marigold mutants was carried out at Department of Horticulture, University of Agricultural Sciences, GKVK, Bengaluru. Seeds of French marigold var Arka madhu were collected and irradiated with various dosages *viz*. 50, 100, 150, 200, 250 and 300 Grays of gamma rays and control (untreated) to induce mutation. Seeds were sown in the nursery bed just after irradiation. LD_{50}, seed germination percent and seed survivability percent was determined. LD_{50} was determined on survival basis. LD_{50} it is a dose that causes 50 percent mortality to the seeds. LD_{50} gives an indication of the response on different types of explants to mutagen so that the right dose to obtain beneficial mutants with minimal population loss. All the untreated control has shown 100 per survivability, but in case of gamma irradiated seeds, survivability was decreasing with increasing dosage of gamma ray. Thus, 50 percent survivability was observed in 150 Gy (49.46). The maximum germination, 93 percent was seen in untreated seeds i.e control followed by 85 percent in treatment comprising of 50 Gy gamma irradiation with 8 percent reduction in germination over the control. The maximum survival percentage was recorded in untreated seeds control group (97.47%) followed by 87.58 percent in treatment comprising of 50 Gy gamma irradiation. It was concluded that as the dosage increases, seed germination percent and survivability percent decreased.

Keywords: Marigold, lethal dose (LD_{50}), gamma rays, mutagens

Introduction
Marigold is a promising ornamental plant of family Asteraeae, grown commercially in distant parts of the world retrieved from different species of *Tagetes viz.*, *T. tenuifolia*, *T. erecta*, *T. patula*, *T. lucida.* French marigold (*Tagetes patula* L.) native of Central and South America, especially, Mexico. This is an annual, upright, dense, moderately sized herb, height ranging from 1 to 3 ft having odd- pinnately compound, dentate, oblong green leaves. Flowers are brightly coloured, showy, capitulate inflorescence having ray and disc florets. Marigold plants thrive best in hot and dry as well as humid weather conditions. They grow best throughout the year under both tropical and subtropical conditions, but require mild climatic conditions for optimum growth and flowering. The colours of marigold range from yellow to gold to orange, red and mahogany.

Mutation breeding has paved a way to create genetic diversity and induce desirable characters in existing varieties. Conventional breeding is a time-consuming process for genetic improvement of the floriculture crops. Mutation breeding has emerged as an alternative, efficient and an innovative methodology to produce heritable changes particularly for flower color and quality. Genetic variation is essential in any plant breeding programme for crop improvement. Induced mutations are highly effective to enhance natural genetic resources (Jain, 2006) [8]. 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 (Smitha *et al*., 2022) [15].

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. (Alka *et al*., 2013 and Suna *et al*., 2016) [2, 16]. 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 ensuring plants. The dose needed for prime agent potency depends on properties of the mutagenic agents and material treated (Jayashree *et al*., 2022) [9]. 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 (Badere *et al*., 2007) [6]. 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 Aney (2013) [3]. Therefore, this study was carried out to assess the LD_{50} of French marigold for gamma rays and its effect on seed germination and survival of seedlings.

Materials and Methods

The present investigation was carried out at the Floriculture and Ornamental Section, Department of Horticulture, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru. The experiment material comprised of healthy and good quality seeds of marigold, which were taken for the mutagenic treatment: Gamma rays (^{60}Co).

The seeds of marigold were treated with different dosage of gamma rays. Seeds were irradiated at Gamma chamber 5000 installed at the ICAR-IIHR, Bangalore at various gamma dosages *viz*. 50, 100, 200, 300, 400, 500 and 600 Gy. The treated and untreated (control) seeds were sown in nursery bed and media containing sand, soil and Farm yard manure (FYM) in the ratio 1:2:1 with coir pith on top. Observation on to find out LD_{50} dose, germination percentage and survival percentage were recorded.

Results and Discussion

To find out LD_{50} for gamma irradiation

Determination of LD_{50} is crucial parameter to study and to develop M_1 population in marigold. 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. These results are in accordance with findings of (Dhange *et al*., 2023) [7] in stevia.

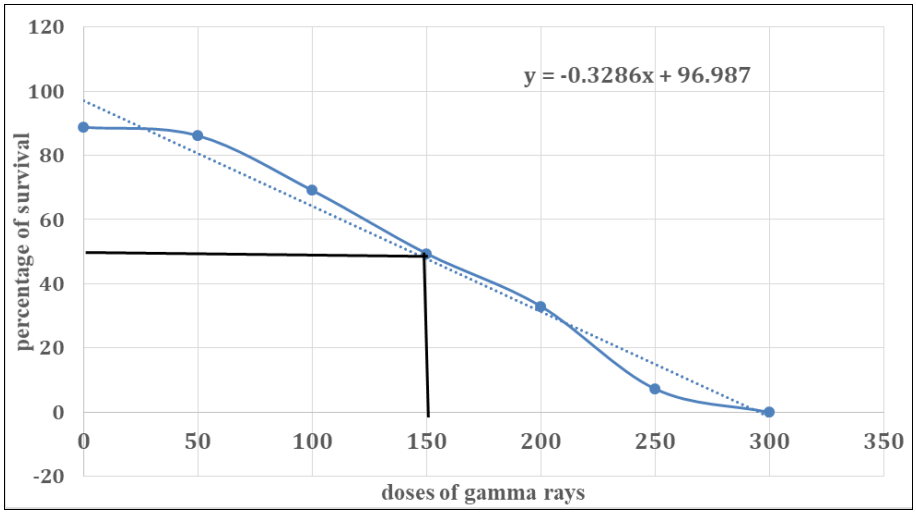

Fig 1: Plot of log doses of gamma v/s survival percentage of plants to determine LD_{50}

Table 1: Effect of gamma irradiation on LD_{50} of marigold seeds

Treatment Doses of gamma rays (Gy)	Lethality (%)	Seed germination (%)	Survival (%)
T_1 (control)	0.0	93	97.47
T_2 (50 Gy)	7.32	85	87.58
T_3 (100 Gy)	33.05	62	72.22
T_4 (150 Gy)	49.46	51	63.07
T_5 (200 Gy)	69.12	27	50.16
T_6 (250 Gy)	86.14	10	43.86
T_7 (300 Gy)	88.78	8	41.82

Seed Germination (%)

The effect of gamma irradiation on germination of marigold seeds was presented in Table 1. The maximum germination (93%) was seen in the untreated seeds i.e control followed by 85 percent in treatment comprising of 50 gy gamma irradiation with 8 percent reduction in germination as compared to the control. As the radiation levels increased, seed germination (%) decreased. At 100 Gy, there was a 62 percent reduction, while at, 150 Gy, 200 Gy, 250 Gy and 300 Gy seed germination percentages decreased by 51 percent, 27 percent, 10 percent and 8 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 300 Gy (8%). Lower percent of germination was recorded in most of the mutagen treated seeds and with

increased dose of gamma radiations. Apart from the control plant, the highest germination rate was observed in the lowest concentration whereas the lowest germination rate was observed in the highest concentration which was significantly reported by previous workers in little millet Ramkumar & Dhanavel (2020) [14], *Andrographis paniculata* Kasthuri and Dhanavel (2020) [10] and Roselle Priyanka and Dhanavel (2020) [13].

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. and Abdullaha *et al*., 2021) [11, 1].

Survival percentage

The Maximum survival percentage was recorded in untreated seeds i.e control (97.47%) followed by treatment comprising of 50 gy gamma irradiation (87.58%). As the radiation levels increased, survival (%) decreased. At 100 Gy, there was a 72.22 percent reduction, while at, 150 Gy, 200 Gy, 250 Gy and 300 Gy survival percentage decreased by 63.07 percent, 50.16 percent, 43.86 percent and 41.82 percent respectively, as compared to the control. 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 Latha and Dharmatti, 2018 [12], Aravind and Dhanavel 2021 [4].

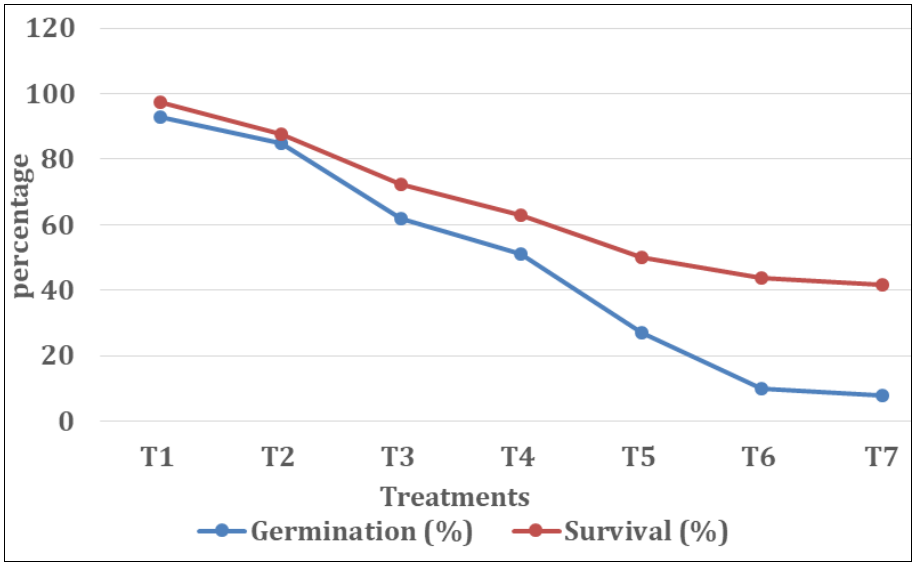

Fig 2: Effect of gamma irradiation on germination (%) and survival (%) in French marigold

Conclusion

To develop M_1 populations of marigold, determination of LD_{50}, seed germination and survival percentages are important parameters. It is evident from findings of present study that, the increased dose of gamma irradiation has 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 marigold using seeds, so the findings of this study on LD_{50} could be used as reference for initiating mutation breeding in other cultivars of marigold and also for improvement of specific traits by mutation breeding.

Conflict of Interest

Authors have declared that no competing interests exist.

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