Mutagenesis study to unleash the genetic potential of Guava cv Allahabad Safeda through Irradiation of seeds by EMS and Gamma rays

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ABSTRACT

In the current investigation, guava *cv* Allahabad Safeda seeds underwent irradiation using varying concentrations of EMS (1% to 2%) and different doses of gamma rays (0.15 to 0.25 k Gy). The objective was to assess the impact on germination, as well as morphological and physiological parameters of the mutants, in comparison to the control group. Results indicated a negative correlation between seed germination and growth parameters (such as plant height, internodal length, leaf number, and leaf dimensions) with both gamma irradiation and EMS. The number of branches exhibited a significant increase up to a certain dosage, after which it decreased with higher concentrations of mutagens. Stomatal frequency, chlorophyll content, and trichome count on both the abaxial and adaxial surfaces of mutants decreased with an elevated dosage or concentration of mutagens. These findings offer valuable insights for initiating guava mutagenesis efforts. Moreover, they provide potential morphological and physiological markers for the early identification of favorable mutants

*Keywords: Guava, EMS, Gamma rays, germination, morphological, physiological*

1. INTRODUCTION

Guava (*Psidium guajava* L.), a perennial fruit tree belonging to the Myrtaceae family, is a cross-pollinated species with a chromosome number of 2n = 22 (Kumar and Ranade, 1952). Originating from tropical America, it was introduced to India by the Portuguese in the 17th century, becoming a significant fruit crop both in terms of nutrition and commerce (Menzel, 1985). Renowned for its adaptability to various soil types, early fruiting, and easy propagation, guava is often referred to as the "tropical apple" and the "apple of the poor" (Nakasone and Paull, 1998). Its nutritional content, delightful flavour, and affordability have made it a popular fruit in India, earning it a reputation as one of the most nutritious and robust fruits. Guava is rich in niacin, riboflavin, thiamine, and dietary fiber. It is particularly high in vitamin C (250 mg/100 g fresh fruits), carbohydrates (13%), and essential minerals such as calcium (29 mg), phosphorus (10 mg), and iron (0.5 mg/100 mg). The vitamin C content in guava is notably higher (4-6 times) than that found in citrus fruits, making it an accessible and economical source of this essential vitamin.

As a hardy and nutritious fruit tree, guava thrives in both humid and dry regions, making it one of the most nutrient-dense crops. However, commercial guava varieties face challenges such as small and misshapen fruits in triploid seedless varieties, susceptibility to diseases like wilt and anthracnose, excessive seed content, and low yield and quality. Traditional guava breeding methods are time-consuming and labour-intensive, relying on the random gene rearrangement between parent plants. To address these challenges, mutation breeding has emerged as a crucial strategy to eliminate undesirable traits and promote economically advantageous characteristics at an early stage. Over the past eight decades, mutation breeding has demonstrated success in supplementing and enhancing conventional plant breeding endeavors (Amin et al., 2015). Induced mutation serves as a valuable tool for modifying the genetics of crop plants, particularly those challenging to alter through cross-breeding and other conventional techniques (Khan and Wani, 2004). This approach effectively produces horticultural varieties with improved phenotypic and genotypic traits. The majority of genetic variations in plants stem from mutations, with physical and chemical mutagens commonly employed to induce favorable characteristics in crops.

Induced mutagenesis and related breeding techniques offer a quicker means of enhancing both the quantitative and qualitative attributes of crops compared to traditional breeding methods. The global influence of plant varieties developed through mutant breeding underscores the versatility and utility of mutation breeding for various crops (Yali and Mitiku, 2022). Consequently, the current study was initiated to produce guava mutants with commercially significant traits. Given that diversification is essential for the gradual enhancement of guava, mutation breeding was employed to instigate variations. This strategy aims to explore the potential for obtaining valuable guava variants, allowing for thorough analysis at both morphological and physiological levels.

2. material and methods

This experiment was carried out in College of Horticulture, Bengaluru, Karnataka during the year 2022-2023. Seeds of guava cv Allahabad Safeda were subjected to both physical mutagen and chemical mutagen. Seeds were treated with gamma rays at 3 different concentration viz.. 0.15k Gy, 0.20k Gy and 0.25k Gy. Physical irradiation was taken place at BARC, Mumbai. With respect to chemical mutagen, EMS (ethyl methane sulphonate) was treated to seeds at three different concentration 1%, 1.5% and 2% EMS respectively.

**2.1 Procedure followed**:

Pre-soaking of guava seeds in water for 18 hours

Treat the seeds with 0.1M Phosphate buffer containing 1%, 1.5% and 2% EMS solution

Leave the seeds in EMS solution for 12 hours

Incubate the seeds at 20-25°C for 1 hour

Wash the seeds for 3-5 times with normal water

Sow the seeds in portrays (Veena et al., 2014)

Protrays, each with a capacity of 98 wells, were filled with growing media. A single seed was placed in each cell, covered with media and watered. After stacking the sowing trays for 3 to 4 days, a polythene sheet was used to cover the entire stack, ensuring moisture and humidity were maintained until germination. Daily light irrigation of the trays was carried out, adjusting based on weather conditions. To foster optimal seedling growth, water-soluble fertilizer 19:19:19 was applied at a concentration of 1g/L every 3 weeks. After 3 months, when the seedlings reached a height of 5-7 cm with 5 to 6 leaves, they were transplanted into 6×6-inch polybags. The seedlings were nurtured using appropriate cultural techniques for 5–6 months within the polybags. Following transplantation, water-soluble fertilizers were provided at 20-day intervals, and daily watering was implemented. Subsequently, after 6 months, these seedlings were transplanted into the field, with proper nutrient application adhering to recommended practices.

**2.2 Germination parameters recorded are:**

1. Days for initiation of germination: The time taken for the initiation of germination was observed and recorded
2. Days to 50 percent germination: The time taken for 50% germination of seeds was recorded.
3. Number of under developed seedlings: The number of seedlings which are not fully developed/ under developed were counted and recorded.
4. Germination percentage of mutants: The number of seeds germinated out of total number of seeds sown was calculated to know the percentage germination using the below formula.

Germination per cent = Number of seeds germinated/Total number of seeds sown × 100

**2.3 Morphological parameters recorded are:**

1. Seedling height (cm): Height of the individual seedling was measured with the help of measuring scale from the base, near to soil surface to the tallest point of the plant and was expressed in centimetre (cm).
2. Internodal length (cm): The distance between the nodes were recorded with the help of measuring scale (cm).
3. Number of branches: Number of branches in each seedling was counted and recorded.
4. Number of leaves: Number of leaves in each seedling was counted and recorded.
5. Leaf length (cm): Average length of five fully developed leaves excluding petiole were taken using the measuring scale and expressed in centimetre (cm).
6. Leaf breadth (cm): The breadth of leaf was measured from the middle of the leaf and expressed in centimetre (cm).

**2.4 Physiological parameters recorded are:**

1. **Stomatal frequency (Number of stomata on abaxial surface per unit area):** The term stomatal frequency describes how many stomata present per square millimetre of leaf either on the adaxial or abaxial surface measured by the thermocol-xylene impression method (Wolf *et al*., 1979).
2. **Chlorophyll content (SPAD value)**: A portable KONICA MINOLTA chlorophyll meter SPAD-502Plus metre was clamped into the leaf at various positions on various matured leaves of the plant and the SPAD readings were measured. SCMR (SPAD Chlorophyll Metre Reading), which is an indication of chlorophyll status in the leaf. The average of SPAD values were calculated from the SPAD units (Ling *et al*.,2011)
3. **Number of trichomes per unit area:** The density of trichomes present on adaxial and abaxial surface within the interveinal area near the center of each leaflet was determined using microscope at 40x and Scanning Electron Microscope using 300x magnification

**2.5 Statistical analysis:**

Descriptive statistics was performed using the excel spreadsheet to find the mean of quantitative parameters at 0.05 level of significance (Nick, 2007)

3. results and discussion

**3.1 Effect of EMS and gamma rays on germination parameters**

In the present investigation, days for initiation of germination and days to 50 percent germination tends to generally increase with increase in EMS and gamma irradiation as elaborated in Table 1. Treatment 4 (2% EMS) took almost 30 days for germination initiation. In contrast, the control treatment exhibited the shortest initiation period, germinating in a minimum of 20 days among all the treatments. Whereas the treatment 7 (0.25 k Gy) has recorded maximum of 49 days for its 50 percent germination and the lowest days for 50 percent germination was found to be in the treatment control with 26 days respectively. The treatment with the highest count of underdeveloped seedlings was Treatment 7 (0.25 k Gy) with 9 seedlings, in contrast, Treatment 1 (control) exhibited the lowest number of underdeveloped seedlings, with only 2 seedlings recorded.

With increasing dose/concentration of mutagens, seed germination decreased linearly. When compared to non-irradiated plants, mutant seedlings showed a considerable difference in the rate of seed germination which varied from 78.10 to 22.20 percentage. The germination percentage was recorded highest in treatment EMS which had least dosage among different EMS treatments and the least germination was recorded in treatment 0.25 k Gy. The irradiated seedlings with highest dosage showed least germination percentage, and the treatments with highest dosage of EMS as well as gamma source showed least germination of seeds compared to other treatments.

**Table 1**. **Effect of different dozes of EMS and gamma rays on germination parameters of guava cv. Allahabad Safeda**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatments** | **Initiation of germination (days)** | **Days to 50 percent  germination** | **Number of under  developed seedlings** | **Germination  percentage** |
| **T1 - Control** | 20 | 26 | 2 | 56.12 |
| **T2 – 1% EMS** | 22 | 34 | 4 | 78.23 |
| **T3 - 1.5%EMS** | 27 | 37 | 5 | 76.19 |
| **T4 - 2% EMS** | 30 | 42 | 7 | 74.31 |
| **T5 - 0.15 k Gy** | 21 | 28 | 3 | 78.10 |
| **T6 - 0.20 k Gy** | 25 | 40 | 6 | 72.04 |
| **T7 – 0.25 k Gy** | 29 | 49 | 9 | 22.20 |
| **Mean** | 24.85 | 36.57 | 5.14 | 65.31 |
| **Standard error** | 1.50 | 3.03 | 0.91 | 7.74 |
| **Range** | 10 | 23 | 7 | 56.03 |

Increased dosage/ concentration of mutagens significantly decreased germination percentage, lead to development of more underdeveloped seedlings and mutated seeds took more time for initiation of germination and this is due the reason that Control treatment which were not exposed to any mutagens showed early germination (Kushwah *et al*., 2010), indicating that there were no negative effects of mutagens on embryo development. According to Kushwah *et al.* (2010), increasing EMS concentration and dose of gamma resulted in longer time for germination. They also reported a higher number of days for germination when high concentration of EMS and gamma rays was used.

The germination percentage recorded in control was about 56.12 due to its hard seed coat as it was sowed without GA3 treatment. According to Kumar and Mishra (2004), the percentage of germination in okra (*Abelmoschus esculentus*) reduced with increasing doses / concentrations of EMS and gamma rays. The higher dosages used in the current investigation have the potential to interfere with hormone balance, gas and water exchange, enzyme function, and the production of free radicals through cell water hydrolysis (Kiong *et al*., 2008 and Asare *et al*., 2017). The decrease in germination with increase in dose might be due to higher combination of mutant genes which do not allow germination of the seeds. In some cases, mutagens effect become a manifest at the germination stage itself, while in the other cases germination do take place but the plants die after making poor growth. Inverse relationship between mutagen dose and seed germination had been reported in general by Gustafsson (1947).

Various explanations have been offered to account for the inhibition of germination following mutagenic treatments, which might be due to auxin destruction (Caldecott, 1955 and Reddy and Smith 1983) or due to inhibition of auxin synthesis by the mutagens (Gardon, 1954). But Gunkel (1957) considered it to be inhibition of mitosis on growing points. The decrease in germination at higher doses of the mutagens may also be attributed to disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by mutagenic treatments particularly chemical mutagens like EMS leading to decrease in germination.

**3.2 Effect of EMS and gamma rays on morphological parameters**

The data pertaining the effect of mutagens on seedling growth and internodal length was presented in Table 2. The results revealed that the 2% EMS treatment had the lowest mean seedling height (69.79 cm), which was considerably lower than the treatment control (86.25 cm). The highest internodal length was recorded in the treatment control (4.52 cm) and the least length was noted in the treatment 2% EMS (4.34 cm).

**Table 2. Effect of different dozes of EMS and gamma rays on seedling height and internodal length of guava cv. Allahabad Safeda at various stages of growth.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Seedling height (cm)** | | | | | **Internodal length (cm)** | | | | |
| **60 days** | **120 days** | **180 days** | **240 days** | **Mean** | **60 days** | **120 days** | **180 days** | **240 days** | **Mean** |
| **T1 - Control** | 11.09 | 34.83 | 65.29 | 86.25 | **43.92** | 0.83 | 2.34 | 3.46 | 4.52 | **2.53** |
| **T2 - 1% EMS** | 10.94 | 31.66 | 62.42 | 76.87 | **40.36** | 0.79 | 2.28 | 3.41 | 4.43 | **2.48** |
| **T3 - 1.5%EMS** | 10.49 | 29.96 | 57.58 | 72.81 | **38.13** | 0.77 | 2.25 | 3.37 | 4.39 | **2.45** |
| **T4 - 2% EMS** | 9.56 | 27.64 | 55.72 | 69.79 | **36.49** | 0.75 | 2.22 | 3.32 | 4.34 | **2.41** |
| **T5 - 0.15 k Gy** | 11.00 | 32.73 | 63.05 | 80.88 | **41.57** | 0.81 | 2.32 | 3.43 | 4.45 | **2.50** |
| **T6 - 0.20 k Gy** | 10.68 | 30.01 | 58.33 | 75.34 | **39.07** | 0.78 | 2.28 | 3.39 | 4.40 | **2.47** |
| **T7 - 0.25 k Gy** | 9.63 | 28.80 | 56.57 | 70.82 | **37.44** | 0.76 | 2.24 | 3.34 | 4.35 | **2.43** |
| **Mean** | 10.48 | 30.80 | 59.85 | 76.11 |  | 0.78 | 2.28 | 3.39 | 4.41 |  |
| **Standard error** | 0.24 | 0.93 | 1.39 | 2.21 |  | 0.01 | 0.02 | 0.02 | 0.02 |  |
| **Range** | 1.53 | 7.19 | 9.57 | 16.46 |  | 0.08 | 0.12 | 0.14 | 0.18 |  |
| **Minimum** | 9.56 | 27.64 | 55.72 | 69.79 |  | 0.75 | 2.22 | 3.32 | 4.34 |  |
| **Maximum** | 11.09 | 34.83 | 65.29 | 86.25 |  | 0.83 | 2.34 | 3.46 | 4.52 |  |

The data pertaining the effect of mutagens on number of branches and leaves was presented in Table 3. There were no significant differences in the number of branches among mutants during the initial stages of growth. However, after a certain period of observation, it was noted that the treatment with 1.5% EMS (2.00) resulted in the highest branch count compared to all other treatments. Conversely, the lowest branch count was recorded in the treatment with 0.25 k Gy (1.36).The treatment control (26.56 cm) recorded the highest number of leaves and the minimum number was observed in the treatment 2% EMS (20.88 cm).

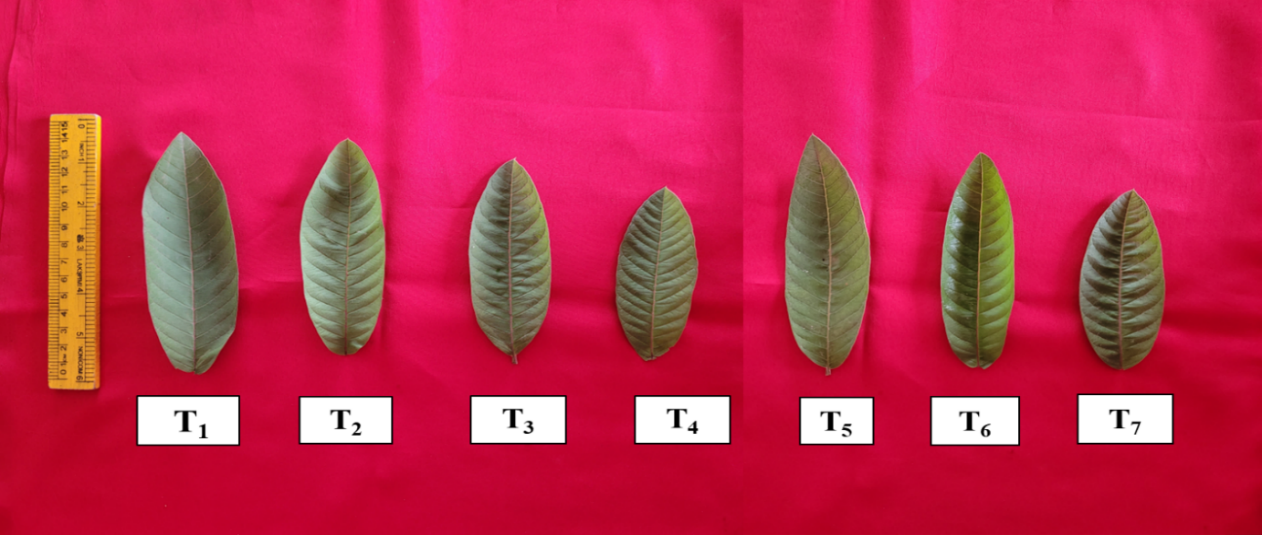
**Table 3. Effect of different dozes of EMS and gamma rays on number of branches and leaves of guava cv. Allahabad Safeda at various stages of growth**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Number of branches** | | | | | **Number of leaves** | | | | |
| **60 days** | **120 days** | **180 days** | **240 days** | **Mean** | **60 days** | **120 days** | **180 days** | **240 days** | **Mean** |
| **T1 - Control** | 1.00 | 1.20 | 1.28 | 1.72 | **1.27** | 12.24 | 18.16 | 20.88 | 26.56 | **18.33** |
| **T2 - 1% EMS** | 1.04 | 1.28 | 1.52 | 1.92 | **1.40** | 11.20 | 16.24 | 18.08 | 23.92 | **16.33** |
| **T3 - 1.5%EMS** | 1.12 | 1.36 | 1.68 | 2.00 | **1.45** | 10.56 | 15.40 | 17.68 | 22.44 | **15.38** |
| **T4 - 2% EMS** | 1.04 | 1.28 | 1.36 | 1.84 | **1.34** | 10.12 | 13.04 | 16.88 | 20.88 | **14.23** |
| **T5 - 0.15 k Gy** | 1.08 | 1.20 | 1.24 | 1.48 | **1.21** | 12.00 | 17.40 | 19.96 | 25.40 | **17.35** |
| **T6 - 0.20 k Gy** | 1.12 | 1.28 | 1.36 | 1.60 | **1.29** | 11.00 | 15.80 | 18.48 | 23.48 | **16.02** |
| **T7 - 0.25 k Gy** | 1.00 | 1.16 | 1.20 | 1.36 | **1.16** | 10.40 | 13.66 | 17.88 | 21.48 | **14.97** |
| **Mean** | 1.07 | 1.25 | 1.35 | 1.70 |  | 11.07 | 15.66 | 18.55 | 23.45 |  |
| **Standard error** | 0.02 | 0.03 | 0.05 | 0.09 |  | 0.30 | 0.70 | 0.53 | 0.77 |  |
| **Range** | 0.12 | 0.20 | 0.32 | 0.64 |  | 2.12 | 5.12 | 4.00 | 5.68 |  |
| **Minimum** | 1.00 | 1.12 | 1.20 | 1.36 |  | 10.12 | 13.04 | 18.24 | 20.88 |  |
| **Maximum** | 1.12 | 1.36 | 1.68 | 2.00 |  | 12.24 | 18.16 | 20.88 | 26.56 |  |

The data pertaining the effect of mutagens on leaf length and breadth was presented in Table 4. The highest length of leaf was recorded in the treatment control (9.68 cm) and the lowest length of leaf was noted in the treatment 2% EMS (9.43 cm). And the treatment control (4.94 cm) had the highest leaf breadth, whereas the treatment 2% EMS (4.67 cm) had the shortest leaf breadth (Fig 1).

**Table 4. Effect of different dozes of EMS and gamma rays on leaf length and breadth of guava cv. Allahabad Safeda at various stages of growth**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Leaf length** | | | | | **Leaf breadth** | | | | |
| **60 days** | **120 days** | **180 days** | **240 days** | **Mean** | **60 days** | **120 days** | **180 days** | **240 days** | **Mean** |
| **T1 - Control** | 2.63 | 6.00 | 7.45 | 9.68 | **5.86** | 1.75 | 3.06 | 4.24 | 4.94 | **3.25** |
| **T2 - 1% EMS** | 2.52 | 5.94 | 7.39 | 9.51 | **5.76** | 1.70 | 2.85 | 4.21 | 4.81 | **3.15** |
| **T3 - 1.5%EMS** | 2.40 | 5.89 | 7.37 | 9.48 | **5.67** | 1.61 | 2.74 | 4.18 | 4.72 | **3.08** |
| **T4 - 2% EMS** | 2.34 | 5.86 | 7.34 | 9.43 | **5.60** | 1.53 | 2.56 | 4.17 | 4.67 | **3.03** |
| **T5 - 0.15 k Gy** | 2.61 | 5.97 | 7.43 | 9.61 | **5.82** | 1.72 | 2.93 | 4.22 | 4.87 | **3.19** |
| **T6 - 0.20 k Gy** | 2.46 | 5.93 | 7.42 | 9.54 | **5.72** | 1.64 | 2.76 | 4.20 | 4.79 | **3.11** |
| **T7 - 0.25 k Gy** | 2.36 | 5.89 | 7.41 | 9.46 | **5.63** | 1.55 | 2.59 | 4.18 | 4.75 | **3.04** |
| **Mean** | 2.49 | 5.93 | 7.40 | 9.53 |  | 1.64 | 2.78 | 4.20 | 4.79 |  |
| **Standard error** | 0.05 | 0.02 | 0.01 | 0.03 |  | 0.03 | 0.07 | 0.01 | 0.03 |  |
| **Range** | 0.29 | 0.14 | 0.11 | 0.25 |  | 0.22 | 0.50 | 0.07 | 0.27 |  |
| **Minimum** | 2.34 | 5.86 | 7.34 | 9.43 |  | 1.53 | 2.56 | 4.17 | 4.67 |  |
| **Maximum** | 2.63 | 6.00 | 7.45 | 9.68 |  | 1.75 | 3.06 | 4.24 | 4.94 |  |



**Fig 1. Effect of EMS and gamma rays on leaf length and breadth of guava cv. Allahabad Safeda**

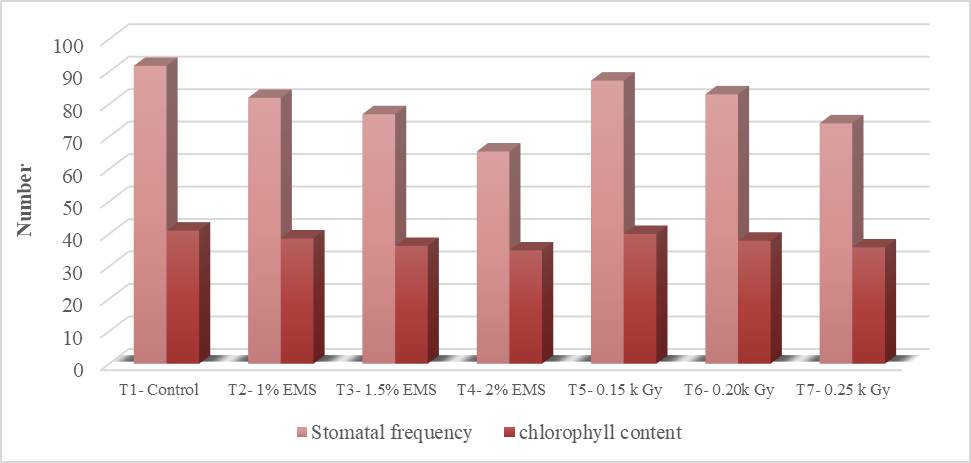
The results revealed that there is an decreasing trend with respect to seedling height with the increase in concentration of mutagens. With increasing dosage or concentration of gamma rays and EMS, mutagens had an inhibitory effect on seedling length that may have been caused due to the reduction in the length of the root and shoot. Several researchers studying fruit plants have reported making comparable observations (Arthur *et al*., 2011, Coban *et al*., 2002, Selvi *et al*., 2007 and Singh *et al*., 2008). This is due to an increased cell division rates and an activation of growth hormone such as auxin, were the presumed causes of these irradiation-induced stimulations. The same decreasing trend was observed with respect to internodal length. In both higher concentration of EMS and gamma rays, the length of internodes of mutants were found to be least. The variability in internode length in guava plants from different treatments could be related to environmental fluctuation. Additionally, Tah (2006) found that mungbean plants treated with gamma source resulted in compact growth due to reduced internodal length. Similar results were also obtained by Kumar and Kumar Rai (2007), Kaur and Rattanpal (2010) and L.K. Sharma et al., (2012) in rough lemon where due to the high dosage of gamma rays the seedling height and internodal length decreased considerably. Same kind of results were obtained, with the increasing level of EMS there was a decrease in the internodal length and ultimately plant height in tomato (Akhtar, 2014), mulberry (Kumar et al., 2013). (Arisha et al., 2015) isolated dwarf mutant with short internodes in peppers (Capsicum annuum L.) by inducing mutation with EMS.

It was found that no comparable changes were observed in number of branches at the initial period. Later, to a certain extent of dosage, the mutants showed increased number of branches. At the highest concentration it showed a declining trend. The same observation was recorded with respect to number leaves. This observation aligns with previous research findings by Predieri and Virgilio, (2007) who reported morphological effects in branches and also on leaves due to radiation treatments. It was observed that irradiating physiologically active meristems led to higher mutation frequencies compared to treating less active tissue. Gamma irradiation caused different types of changes in plant structure and function. It depends upon the dose rate and time period of irradiation (Piri et al., 2011). Higher doses might have stopped the enzymes that are necessary for leaves initiation. Another reason found that higher doses blocked cell division by decreasing the rate of physiological processes and genetic material was also damaged (Neary et al., 1957)

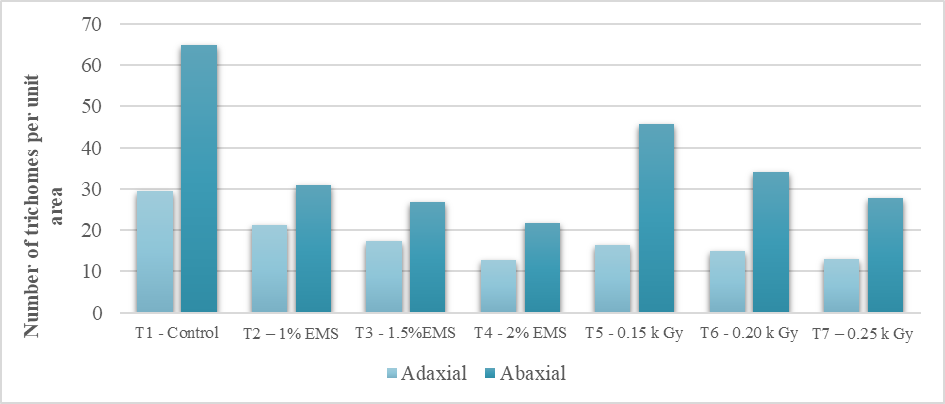
Higher dosage/concentration of mutagens have their morphological effect even on leaf dimensions. Average leaf length and leaf breadth were found to be negatively correlated with concentration of mutagens. The results were similar with Maan *et al*. (2021) and Singh *et al*. (2008). The morphological characteristics of the altered population were affected by chemicals and radiation, which led to the mutant population having shorter leaf length and breadth than the control population. Similar report was given by Prabhukumar *et al*., 2015 where the leaf length of EMS treated C. Inodora leaf increased to 22.5 cm in comparison to 14.5 cm in control plants and Rime *et al*., 2019 reported the same outcomes in mango.

**3.3 Effect of EMS and gamma rays on physiological parameters**

Number of stomata per mm2 was observed for control and mutated population, the highest stomatal count per unit area was noted in the treatment control (91.72) and the least stomatal count per unit area was noted in the treatment 2% EMS (65.36). Chlorophyll content of the irradiated seedlings was considerably lower than non-irradiated seedlings, and the minimum was found at a higher dose of EMS as well as gamma rays. The highest chlorophyll content (SPAD value) was recorded in control (41.01) and minimum chlorophyll content was noted in 2% EMS (34.90) (Fig. 2).



**Fig. 2. Effect of EMS and gamma rays on physiological parameters of guava cv. Allahabad Safeda**

Number of trichomes per unit area was observed for guava population of both irradiated and non - irradiated seedlings. The trichome count was found highest in the treatment control in both adaxial (29.40) and abaxial leaf surface (65) respectively where as the least trichome count was observed in treatment 2% EMS on adaxial (12.64) and abaxial (21.64) surface of guava leaf (Fig. 3).

**Fig. 3.** **Effect of EMS and gamma rays on number of trichomes per unit area of guava cv. Allahabad Safeda.**

The observations recorded revealed that mutagens had their negative effect on physiological traits of mutants like stomatal frequency, chlorophyll content and trichome count on both surface of leaves. The potency of modifications in stomatal characteristics of chemical mutagens was nearly equivalent. Indicators of dwarfism included a decreased stomatal density and frequency per unit leaf area (Kok, 2011). It has been recorded that photosynthesis and stomatal movements are mutually dependent, and higher stomatal frequency may increase photosynthetic activity, resulting in the accumulation of more carbohydrates, which may contribute to an increase in plant vigour (Chen *et al*., 2015; Kok, 2011). The reduction in leaf length and leaf breadth may also resulted in reduced stomata per unit area. However, a reduced stomatal density and frequency per unit area is an indicator of dwarfism. Reduction and enhancement in stomata length and width at higher doses of mutagen might be due to induced genetic damage and mutation frequencies (Fig. A close-up of cells under a microscope

Description automatically generatedA close-up of a cell

Description automatically generated4)

**Fig 4.Effect of EMS and gamma rays on stomatal frequency of guava cv. Allahabad Safeda a. T1- control b. T2- 2% EMS**

Chlorophyll content of the irradiated seedlings was considerably lower than non-irradiated seedlings, and the minimum was found at a higher dose of EMS as well as gamma rays. Chlorophyll-related characteristics were used as a phenotypic marker for the dwarfness trait in field crops, and the effect of gamma source irradiation on leaf chlorophyll content was profound in the current experiment. Ling *et al*. (2008) similarly noted a diminishing tendency in the total chlorophyll content of sweet orange plantlets that had undergone gamma radiation. Same findings were also achieved in papaya by Mahadevamma *et al*. (2012). With respect to trichome count because of the effect of mutagens, the trichome count decreased considerably from control to high doses of treated plants (Fig. 5) where these results were contradictory to the findings of Nagata *et al*. (1999) where irradiation of gamma rays lead to the increased formation of lead trichome in Arabidopsis.

Close-up of a magnified view of a group of worms

Description automatically generatedClose-up of a microscope view of a microscopic view of a human body

Description automatically generated with medium confidence

**Abaxial**

**Adaxial**

A close-up of a microscope

Description automatically generatedA close-up of a microscope

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**Abaxial**

**Adaxial**

**Fig 5. Effect of EMS and gamma rays on trichome content of guava cv. Allahabad Safeda a. T1- control b. T2- 2% EMS**

4. Conclusion

Different treatments affected seed germination rates and plant development. Control and specific treatments demonstrated variations in plant height, internodal length, number of branches, number of leaves, leaf dimensions, stomatal frequency, trichome density, and chlorophyll content. The treatment control was superior in all growth and physiological parameters, where as 2% EMS showed least results in many of the parameters. The study demonstrated the effectiveness of mutation breeding in guava, emphasizing the importance of diverse genetic pools for breeding purposes. The detailed characterization of mutated populations provided valuable insights into guava's genetic variations. The variants so likely observed may hold promise in near future particularly with respect to development of improved guava varieties with traits of commerce.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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