



Optimization of Mutagenic Doses in Bitter Gourd (*Momordica charantia* L.) for EMS and Gamma (γ) Rays

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ABSTRACT

Background: Optimizing mutagen doses is a critical step in mutagenesis studies, as it allows researchers to balance the induction of genetic variations with the overall health and viability of the treated plants. The specific optimal doses may vary depending on the species and desired traits to be improved in bitter gourd.

Methods: Present research was conducted to create various attributes for selection like macro mutants and micro mutants. In the present study, bitter gourd cultivar Kahalgaon Local was treated with four concentrations of EMS viz., 0.1, 0.2, 0.3 and 0.4% and three doses of gamma rays irradiation viz., 0.25, 0.50 and 0.75 kGy. The germination percentage, survival rate and LD₅₀ value were estimated.

Result: Through induced mutagenesis and based on seed germination (%) and plant survival of treated material; it is concluded that, LD₅₀ dose of EMS and gamma rays were 0.2% and 0.25 kGy, respectively. The increase in the treatment dose of EMS and gamma rays, resulted in reduced germination and survival percentage. Among various EMS treatments tested, highest lethality was recorded at 0.4% EMS treatment and lowest lethality was recorded at 0.1% EMS treatment. Similarly, among various gamma irradiation treatments tested, highest lethality was recorded at 0.75 kGy of seed treatment and lowest lethality was recorded at 0.25 kGy of seed treatment.

Key words: Bitter gourd, EMS, Gamma (γ) rays, LD₅₀, Lethality, Mutagenesis.

INTRODUCTION

Bitter gourd, scientifically known as *Momordica charantia* L., is a tropical and subtropical vine belonging to the family cucurbitaceae. It is considered to be an old world species (Jadhav *et al.*, 2009). People widely cultivate it for its bittersweeting edible and medicinal fruit. It is monoecious in nature and native to South Asia (Pathak *et al.* 2014). Bitter gourd is low in calories and rich in essential nutrients, including vitamins A, C and K (Chaudhary, 1967). It also contains minerals like iron, potassium and dietary fibre. Chang *et al.* (1996) reported that oil contents of bitter gourd seed vary from 41 to 45% and the oils contain 22-27% stearic acid and 63-68% eleostearic acid. Fruit juice of bitter gourd has anti-diabetic properties which aid in retaining sugar levels in human body (Sreejayan and Rao, 1991). Cucurbitacin glucoside, which is present in bitter gourd, adds bitterness to the fruits. Among the cultivated cucurbits, it has been known as a prospective vegetable for foreign export by APEDA (Thangamani *et al.*, 2011).

Proper resource use efficiency is a challenge for Indian agriculture, given the growing population and the need to mitigate food demand in a sustainable manner (Karthik *et al.*, 2023). The productivity of bitter gourd in Bihar is reported to be lower than the national average. There is a noticeable difference in productivity, with Bihar struggling to achieve higher yields compared to the rest of the country. So, there is a need to develop high-yielding cultivars in Bihar to improve bitter gourd productivity. Developing cultivars with higher yield potential could potentially address the productivity gap and contribute to the region's overall agricultural output.

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In bitter gourd, gamma irradiation and EMS treatment led to an increased genetic variability in quantitative characters (Mallaiah and Zafar, 1986). Mutation breeding, involves the induction of mutations in plant propagules through chemical or physical mutagens. The resulting mutants are then selected for desirable changes and the process is repeated until the desired trait is stably expressed in subsequent generations. This method allows plant breeders to expand the genetic base of germplasm, creating new varieties or sources of variation for breeding programs. Mutation breeding depends on selfing mutants until the instigated character has a steady expression in the advanced mutant generations.

Mutation induction includes the treatment of plant propagules (seed or cutting or plant tissue) with chemical or physical mutagens. This is followed by selection for attractive (desirable) changes in the subsequent mutants. The plant breeders use mutation induction to expand the hereditary base of germplasm and utilize the mutant lines straightforwardly as new varieties or as sources of new variation. Mutation breeding has become an amazing strategy for creating novel plant genotypes (Penna *et al.*, 2012). Mutation screening is the choice of individuals from a sizeable transformed population that meet explicit selection criteria and mutant affirmation is re-evaluating (Oladosu *et al.*, 2016).

A bitter gourd landrace MC 013 was treated with gamma (γ) rays and advanced another cultivar MDU 1 which has improvement for yield, long greenish white fruits, tolerant to pumpkin insects, fruit flies and leaf spot diseases (Rajasekharan and Shanmugavelu, 1984). Moreover, DBGy-201 and DBGy-202: two gynocious lines of bitter gourd (*Momordica charantia* L.) confined from native source as a spontaneous mutations (Behera *et al.*, 2006). Overall, mutation breeding has proven to be an effective strategy for creating novel plant genotypes (Penna *et al.*, 2012) and enhancing agricultural trait variability. Given these findings, the primary focus of the present study was to optimize mutagenic doses for gamma rays and EMS in bitter gourd.

MATERIALS AND METHODS

For the pre sent investigation, Kahalgaon Local genotype was used which was collected from the farmer's field of Kahalgaon region in Bhagalpur district of Bihar and through pure line selection, stable homozygous inbred line was developed by Department of Horticulture (Vegetable and Floriculture), BAC, BAU, Sabour in the year 2018. It produces green colour fruits with sharp tubercles. Present research was conducted to create various attributes for selection like macro and micro mutants with this genotype. In the present study, this genotype was treated with four concentrations of EMS, viz., 0.1, 0.2, 0.3 and 0.4% and three doses of gamma ray irradiation, viz., 0.25, 0.50 and 0.75 kGy. The details of treatments are given hereunder.

Chemical treatment by using EMS

Treatment was done in the laboratory at Department of Plant Breeding and Genetics, BAU, Sabour. Initially, sufficient seeds for chemical treatment were soaked in distilled water for 16 hours in a beaker. After 16 hours of pre-soaking treatment, the seeds were removed from beaker and added 60 seeds each in five conical flasks for seed treatment. To maintain 7.4 pH dilution of the combined stock solutions of 40.1 ml of dipotassium phosphate (K_2HPO_4) and 9.9 ml of monopotassium phosphate (KH_2PO_4) to 1000 ml with distilled H_2O was done. Then filled the conical flask 1 with 100 ml of distilled water because it acted as control and remaining conical flasks

were filled with 100 ml of 0.05 M potassium phosphate buffer according to the experimental plan and then added required quantity of EMS solution i.e 100 μ l, 200 μ l, 300 μ l and 400 μ l to flask 2, flask 3, flask 4 and flask 5, respectively and all conical flasks were kept in mechanical shaker (140 rpm) for 8 hours. EMS was added to the conical flasks under laminar air flow chamber wearing mask, lab coat and gloves because it was a carcinogenic agent. Seed sowing was done immediately in pro-trays after treatment. For detoxifying EMS, we used 0.5 M NaOH solution. It was made by adding 20 g of NaOH in one litre of water. Solutions were decontaminated by disposal on to 2.5 times the volume of 0.5 M NaOH. They were left overnight and discarded in the hood on following days by rinsing the flasks for 30 minutes.

Physical treatment using gamma (γ) rays

Treatment was done at Regional Nuclear Agriculture Research Centre (RNARC), Bidhan Chandra Krishi Viswavidyalaya (BCKV), Haringhata, West Bengal, India. 300 seeds per treatment were treated and replicated thrice in completely randomized design. The concentrations of various treatments were 0.25, 0.50 and 0.75 kGy. The capacity of gamma chamber at RNARC was 4983.38 Gy per hour and penetration was up to 8 mm thickness of seed. The time taken for seed treatment of various concentrations viz., 0.25, 0.50 and 0.75 kGy was 3.01 minutes, 6.02 minutes and 9.03 minutes, respectively. After seed treatment, seed sowing was done immediately on next day in the protrays.

Observations made in M_1 generation

Seed germination was calculated by per cent ratio of total number of seeds sown to number of seeds germinated. Similarly, survival rate was calculated by per cent ratio of seedlings survived to number of seeds germinated.

Estimation of LD_{50} value

LD_{50} is the lethal dose of particular mutagen that will cause the death of 50% of the plant population being tested. LD_{50} value was calculated based on germination and survival plant percentage compared to control.

Lethality (%) =

$$LD_{50} \text{ value of control} - LD_{50} \text{ value of treatment}$$

RESULTS AND DISCUSSION

Germination and survival per centage

In the pre sent investigation, results regarding seed germination percentage revealed that per cent seed germination was decreased with an increase in concentration/dose of mutagens in M_1 generation. Higher concentrations of EMS and higher dose of gamma rays have put forth maximum inhibitory effect on seed germination percentage (Table 1). Among EMS treatments, the mean germination percentage was recorded highest

in seeds treated with 0.1% EMS (65.00%) and lowest with 0.4% EMS (26.66%). In gamma irradiation treatments, the mean germination percentage was recorded highest in 0.25 kGy gamma irradiation (54.66%) and lowest in seeds treated with 0.75 kGy gamma irradiation (8.33%). Reduction in germination was due to inhibition of genetic and physiological processes by mutagens which leads to lethality. Belele *et al.* (2002) have reported reduction in seed germination percentage in french bean while increasing the mutagenic doses *i.e.* 200 Gy to 250 Gy. Reduction in seed germination due to increasing doses of mutagens was also recorded in other crops by Kumar *et al.* (2010), Girija and Dhanavel (2009) in cowpea; Mahla *et al.* (2010) in cluster bean; Chaudhari (2002) in lentil and Mejri *et al.* (2012) in faba bean.

A negative correlation was observed between dose of the mutagens and survival percentage. Increase in dose of mutagen reduced plant survival percentage. Plant survival is one of the most reliable parameters for evaluating the effects of any mutagen. Among various EMS treatments, the highest and lowest survival percentage was recorded in 0.1% EMS and 0.4% EMS respectively (Table 1). In case

of gamma irradiation treatments, the highest and lowest survival percentages were recorded in 0.25 kGy and 0.75 kGy doses, respectively (Table 1). According to the experiments done by Shinde and More (2018) on cluster bean, it might happen due to point mutations or the injuries caused by the genetic material used, which eventually led to decrease the rate of respiration and energy production and finally a decrease in survival percentage. Similarly, an inverse relationship between dose of the mutagen and survival of plants has also been reported in cowpea by Kumar *et al.* (2010) and Mejri *et al.* (2012) in faba bean.

Kangarasu *et al.* (2014) opined that, LD₅₀ was the lethal dose of particular mutagen that will cause the death of 50% of the plant population being tested. In this experiment, the LD50 value (the concentration at which 50% of the plant population survives) for EMS in bitter gourd was found between 0.2% and 0.3% EMS treatments. Specifically, the survival rate at 0.2% EMS suggested that the LD₅₀ is approximately 61.62%, meaning that a concentration of around 0.2% EMS was close to the lethal dose for 50% of the plant population as shown in Table 2. In case of 0.3% EMS and 0.4% EMS, LD50 based on survival percentage

Table 1: Mean values of various observations recorded in M₁ generation.

Type of mutagen	Treatment	Dose of mutagen	Germination percentage (%)	Survival percentage (%)
No mutagen	T ₀	Non treated	75.00	74.00
EMS	T ₁	0.1%	65.00	59.33
	T ₂	0.2%	53.33	45.66
	T ₃	0.3%	36.33	31.00
	T ₄	0.4%	26.66	22.50
	T ₅	0.25 kGy	54.66	47.16
Gamma (γ) rays	T ₆	0.50 kGy	21.00	18.66
	T ₇	0.75 kGy	8.33	7.00
C.D. (P≤0.05)			2.17	1.53
SE(m) ±			0.72	0.51
C.V. (%)			2.95	2.31

Where, SE (m) ± = Standard error (mean); CD (P≤0.05) = Critical difference at 5% level of significance in completely randomized design; CV (%) = Coefficient of variation.

Table 2: Mean values of germination and survival percentage of seeds treated with EMS.

Mutagen	Treatment	Dose of mutagen	Germination percentage (%)	LD ₅₀ based on germination %	Survival percentage (%)	LD ₅₀ based on survival%
No mutagen	T ₀	Non - treated	75.00	100.00	74.00	100.00
EMS	T ₁	0.1%	65.00	86.66	59.33	80.17
	T ₂	0.2%	53.33	70.66	45.66	61.62
	T ₃	0.3%	36.33	48.00	31.00	41.18
	T ₄	0.4%	26.66	34.66	22.50	30.40
*Standard deviation (±)			19.90	26.88	20.84	28.30
**Standard error (±)			8.90	12.02	9.32	12.66

(Note: Lesser the LD₅₀ value, higher the death rate of plant population).

*Standard deviation is calculated by taking means of all treatments.

**Standard error is calculated by dividing the standard deviation by square root of number of treatments.

Table 3: Mean values of germination and survival percentage of seeds treated with Gamma (γ) rays.

Mutagen	Treatment	Dose of mutagen	Germination percentage (%)	LD ₅₀ based on germination %	Survival percentage (%)	LD ₅₀ based on survival %
No mutagen	T ₀	Non - treated	75.00	100.00 (transformed)	74.00	100.00 (transformed)
Gamma (γ) rays	T ₅	0.25 kGy	54.66	72.00	47.16	63.51
	T ₆	0.50 kGy	21.00	28.00	18.66	25.21
	T ₇	0.75 kGy	8.33	11.06	7.00	9.45
*Standard deviation ($\pm$)			30.57	40.64	30.05	40.58
**Standard error ($\pm$)			15.29	20.32	15.02	20.29

(Note: Lesser the LD₅₀ value, higher the death rate of plant population).

*Standard deviation is calculated by taking means of all treatments.

**Standard error is calculated by dividing the standard deviation by square root of number of treatments.

was less than 50% i.e. 41.18% and 30.40% respectively. Concentrations of 0.3% and 0.4% EMS were more toxic, resulting in survival rates below 50% as shown in Table 2. Among various EMS treatments tested, highest lethality was recorded in T₄ (0.4% EMS) and lowest lethality was recorded in T₁ (0.1% EMS). It showed linear increase with increasing concentration of EMS. The stimulatory effect of EMS at a lower dose is observed because at lower concentrations mutagens stimulate enzymes and growth hormones responsible for growth, yield and fruit quality, while, higher concentrations of mutagens had inhibitory effect (Sebastian *et al.*, 2023). The similar trends were also obtained by Kavithamani *et al.* (2008), Tyagi and Khan (2010) in soya bean; Girija and Dhanavel (2009) in cowpea; Mahla *et al.* (2010) and Velu *et al.* (2012) in cluster bean.

The LD₅₀ value for gamma (γ) rays in bitter gourd was observed at the concentration of 0.25 kGy (T₅) gamma rays where the survival plant population was 47.16% and LD₅₀ based on survival percentage was 63.51 % which was more than 50% survival percentage compared to treatment T₆ (0.50 kGy) and T₇ (0.75 kGy) as indicated in Table 3. In case of 0.50 kGy and 0.75 kGy, LD₅₀ based on survival percentage was less than 50% i.e. 25.21% and 9.45% respectively. Higher doses of gamma rays lead to lower germination and survival rates, highlighting the detrimental effects of radiation on plant growth and viability as evident from Table 3. For practical applications, this information would be crucial in determining safe exposure levels for gamma rays in agricultural practices or genetic studies involving mutation breeding on bitter gourd.

Among various gamma irradiation treatments tested, highest lethality was recorded in T₇ (0.75 kGy) and lowest lethality was recorded in T₅ (0.25 kGy). It showed linear increase with increasing doses of gamma rays. Similar trend was also obtained by Tyagi and Khan (2010), Kavithamani *et al.* (2008) in soya bean; Girija and Dhanavel (2009) in cowpea; Mahla *et al.* (2010), Velu *et al.* (2012) and Patil and Rane (2015) in cluster bean.

The greater sensitivity at higher doses of mutagens has been attributed to various factors such as changes in

the metabolic activity of the cells (Natarajan and Shivasankar, 1965), inhibitory effects of the mutagen (Ramulu, 1972) and to disturbances of balance between promoters and inhibitors of growth regulators (Meherchandani, 1975; Wani *et al.*, 2012). The reduction in biological criteria (plant height and survival) might be attributed to a drop in the auxin level, inhibition of auxin synthesis, chromosomal aberrations or due to reduction of assimilation mechanism (Girija and Dhanavel, 2009).

CONCLUSION

Higher concentrations of EMS (0.4%) and Gamma (γ) rays (0.75 kGy) recorded the highest lethality. This suggests that a higher concentration of EMS (0.4%) and Gamma (γ) rays (0.75 kGy) resulted in a more significant impact, at inducing mutations but also cause more harm, leading to increased plant death in bitter gourd. Lower concentrations of EMS (0.1%) and Gamma (γ) rays (0.25 kGy) recorded the lowest lethality. This indicates that a lower concentration of EMS and Gamma (γ) rays had a milder effect, causing fewer mutations and less harm, leading to higher survival rates. There appears to be a linear relationship between the concentration of mutagens and lethality. As the concentration of EMS increased from 0.1% to 0.4%, lethality increased in a consistent and proportional manner. Similarly, as the concentration of Gamma (γ) rays increased from 0.25 kGy to 0.75 kGy, lethality increased in a consistent and proportional manner. The LD₅₀ value for EMS and gamma (γ) rays in bitter gourd were observed at the concentration of 0.2 % EMS and 0.25 kGy, respectively. Both EMS and gamma rays are effective mutagens, but their effectiveness is dose-dependent. Higher doses result in higher lethality, likely due to an increased number of mutations or a greater extent of damage.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

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