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Original Research Paper

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THE EFFECT OF SINGLE AND COMBINED USE OF GAMMA RADIATION AND ETHYL METHANE SULFONATE ON EARLY GROWTH PARAMETERS IN TRIGONELLA FOENUM-GRAECUM L.

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ABSTRACT Mutation breeding is a suitable strategy for developing new horticultural crop varieties with improved phenotypic and genotypic characteristics. Mutations are the primary cause of genetic differences found in plants. Mutagens, both physical and chemical are mostly utilized to induce desirable traits. Mutation induction has proven to be a trustworthy method of producing variation within a crop variety. It allows for the induction of desired characteristics that are either not found in nature or have been lost during evolution. Success in inducing the genetic variation through mutagenic agents is dependent on the source and effective dose of application. The objective of this study was to determine the optimum doses of a single and combined use of gamma radiation and Ethyl methane sulfonate (EMS) for effective mutation breeding in Trigonella. The experimental genotype selected for the present investigation was Trigonella foenum-graecum L. variety - Phule Kasturi, commonly known as Trigonella. In Marathi it is known as Methi. The experimental seed material of Trigonella was collected from Maharashtra Agro Seed Private Limited, Market yard, Pune released by Mahatma Phule Agricultural University, Rahuri, Dist: Ahmednagar (M. S.) The different concentrations of EMS were used for the chemical mutagenic treatments as 0.25%, 0.50%, 0.75% and 1%. For radiation treatment of Gamma rays the seed samples were exposed to doses of 240 Gy, 300 Gy, 360 Gy and 420 Gy. In the combination treatment of Gamma rays and EMS used as 240 Gy + 1%, 300 Gy + 0.75%, 360 Gy + 0.50% and 420 Gy + 0.25%. The above dose rates are useful to induce genetic variation in the tested genotypes for greater mutation events in Trigonella breeding programs.

KEYWORDS : Mutation breeding; Ethyl methane sulfonate; Gamma radiation; Trigonella.

INTRODUCTION

When desired variation is absent, plant breeding requires genetic variation of useful traits for crop improvement. Mutagenic agents, such as radiation and certain chemicals, can thus be used to induce mutations and produce genetic variations from which favorite mutants can be chosen. When no gene or genes for disease resistance or stress tolerance can be found in the available gene pool, plant breeders have no choice to go for mutation induction. Mutation occurs in plants when the appearance of the phenotype changes in response to a change in genotype. It can also be explained as a heritable change in a cell's genetic material.

The main objective of Trigonella mutation breeding program is to develop genotypes with high yield and good agronomic traits. The objective of this research was to evaluate the selected traits used in Trigonella breeding program and to determine their correlation with seed yield. It was thought desirable to undertake a detailed study involving induction of mutations in the system of Trigonella by physical mutagen like Gamma rays, chemical mutagen like EMS and combination of both Gamma rays and EMS.

Trigonella is one of the most important pulse crops in India. The seeds are major source of dietary protein in developing countries. Research and development in this area of investigation is necessary for quality crop improvement with increased nutritive value in Trigonella. It is an annual legume, diploid plant (2n = 16) [1].

Morphologically, it is an erect, aromatic annual closely resembling large clover. Trigonella crop improvement through hybridization is extremely difficult due to the delicate flower structure [3].

The above review of the relevant literature clearly indicates that, despite of many positive attributes, the Trigonella foenum-graecum L. has received a less attention of the scientists of our country [15]. Very few Indian scientists have shown interest in cytological, genetical study and mutational breeding aspects of this plant. Hence it was considered very much relevant and appropriate to study the induced mutation breeding in Trigonella.

The mutant variety database (MVD) holds records of 3320 varieties officially released across 73 countries and 228 plant species by different nations of the world [9]. The combined use of physical and chemical mutagens has been used to develop 37 of the mutant plant varieties in the MVD. However, there is a limited record of Trigonella mutant varieties developed by combined treatment of gamma radiation and EMS in the MVD. Reports reveal that combined use of gamma radiation and EMS might yield higher mutation frequency than using a single mutagenic agent [13, 17].

MATERIALS AND METHODS

The fresh, aqueous solutions of the mutagen were prepared prior to treatments. The different concentrations used for the chemical mutagenic treatments such as 0.25%, 0.50%, 0.75% and 1%. After presoaking, the seeds were immersed in the mutagenic solutions for 4 hours with continuous shaking. The volume of the chemical solutions used were five times more than that of the seeds to facilitate uniform absorption. Seeds soaked in distilled water for 6 hours served as control. Immediately after the completion of treatment, the seeds were washed thoroughly under running tap water for 3 to 4 times. Later on, they were subjected to post soaking in distilled water for 4 hours.

Healthy, uniform size and dry seeds of the Trigonella variety – Phule Kasturi were packed in polythene bags and sealed them for the Gamma radiation. Electromagnetic, ionizing radiations were applied from Como source of irradiation. Gamma radiation was carried out at Nuclear Chemistry Division, Department of Chemistry, University of Pune, Ganeshkhind, and Pune 411007. The seed samples were exposed to doses of 240Gy, 300Gy, 360Gy and 420Gy of Gamma rays.

For the combination treatments Gamma rays irradiated seed samples were used. After the physical mutagenic treatment, the chemical mutagenic treatment of EMS was conducted on the same seed samples. In the combination Gamma rays and EMS used like 240 Gy+1%, 300 Gy+0.75%, 360 Gy+0.50%

and 420 Gy+0.25%. For each treatment, a batch of 500 seeds were used. The objective of this study was to determine the effect of a single and combined use of physical and chemical mutagens for effective mutagenesis and breeding in Trigonella for following parameters.

Germination Percentage: After 7 days, the number of seeds showing emergence of radical were counted from the hundred seeds kept in petriplates lined with moist filter paper and expressed as germination percentage.

Survival of Plants: The survival of plants was recorded at the time of harvesting of the crop.

Shoot Length: The shoot length was recorded after 15 days of germination of seeds with moist filter paper.

RESULTS AND DISCUSSION

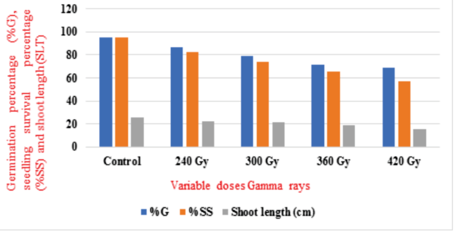
Experiment I:

Effect Of Doses Of Gamma Rays

Highly significant interaction effects ($p < 0.01$) were recorded between genotype and gamma rays dosage on germination percentage, survival percentage and shoot length (Table 1). This suggests that the optimum dose of Gamma rays in Trigonella mutation breeding is significantly influenced by the genotype. The summary of the mean of seedling germination percentage, survival rate and shoot length. Results showed a significant reduction in assessed traits with increased doses of gamma rays when compared with their respective controls (Table 1).

Table 1. Mean values and significance tests for germination percentage (%G), seedling survival percentage (%SS) and shoot length (SLT) of Trigonella genotypes evaluated with variable doses of gamma rays.

Source of Mutagen	Mutagenic Treatments	% G	% SS	SLT (cm)
Control		95.29	94.75	25.54
Gamma rays	240 Gy	86.83	82.10	22.60
	300 Gy	79.08	74.40	21.47
	360 Gy	71.35	65.35	18.59
	420 Gy	69.30	57.25	15.62



Graph: 1. Mean values and significance tests for germination percentage (%G), seedling survival percentage (%SS) and shoot length (SLT) of Trigonella genotypes evaluated with variable doses of gamma rays.

Trigonella seeds germinated on the seventh day after sowing in the control group. In the control group, the average germination percentage was 95.29. Mutagenic treatments have a negative effect on seed germination. In the case of all mutagenic treatments, the germination percentage decreased gradually from lower to higher dose. It was 86.83, 79.08, 71.35 and 59.30 at 240Gy, 300Gy, 360Gy, and 420Gy doses of gamma rays respectively. The highest germination percentage was 86.83 at 240Gy, while the lowest was 69.30 at 420Gy of gamma rays. Mutagenic treatments of gamma rays revealed negative effect on seed germination (Table 1).

Plant survival at maturity was 94.75% in the control. Survival rates in gamma rays ranged from 82.10 % to 57.25%. The highest survival percentage was 86.83 at 240Gy, while the lowest was 57.25 at 420Gy of gamma rays. Mutagenic treatments of gamma rays revealed negative effect on survival percentage. Almost all treatments produce quite satisfactory results in terms of plant survival at maturity (Table 1).

The values for seedling height showed a wide range of variation. The seedling height in the control group was 25.54 cm. Seedling heights were 22.60 cm at 240Gy and 15.62 cm at 420Gy doses of gamma radiation. Higher doses generally result in a decrease in seedling height. According to the data, seedling height decreased in a dose-dependent manner after all mutagenic treatments of gamma rays (Table 1).

Effect of Single Doses of EMS

Significant interactions ($p < 0.01$) were observed between genotype and EMS concentrations on germination percentage, survival rate and shoot length. This suggests that the optimum EMS dose in Trigonella mutation breeding is significantly influenced by the genotype. The means of germination percentage, survival percentage and shoot length showed a significant reduction with increased EMS concentration (Table 2)

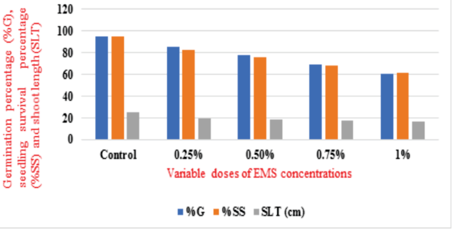
In case of all mutagenic treatments, the germination percentage decreased gradually from lower to higher dose. It was 85.21, 78.14, 69.52 and 60.29 of EMS treatments at concentrations of 0.25%, 0.50%, 0.75%, and 0.1%, respectively (Table 2).

Survival rates in EMS ranged from 82.17% to 61.40% at various concentrations. The values for shoot length showed a wide range of variation (Table 2).

The shoot length in the control was 25.54cm. The higher concentration of EMS, such as 0.1 %, showed shoot length of 19.62cm, while the lower concentration of EMS, such as 0.25 %, results about of 16.57cm (Table 2).

Table 2. Mean values and significance tests for germination percentage (%G), seedling survival percentage (%SS) and shoot length (SLT) of Trigonella genotypes evaluated with variable doses of EMS concentrations.

Source of Mutagen	Mutagenic Treatments	% G	% SS	SLT (cm)
Control		95.29	94.75	25.54
EMS	0.25%	85.21	82.17	19.62
	0.50%	78.14	76.18	18.61
	0.75%	69.52	67.80	17.48
	1%	60.29	61.40	16.57



Graph: 2. Mean values and significance tests for germination percentage (%G), seedling survival percentage (%SS) and shoot length (SLT) of Trigonella genotypes evaluated with variable doses of EMS concentrations.

Effect Of Variable Doses Of Combined Treatments

Highly significant interactions ($p < 0.01$) were observed between genotype and combined use of gamma radiation doses and EMS concentrations on germination percentage, survival percentage and shoot length. The mean of germination percentage, survival percentage and shoot length showed a significant reduction with increased combined dosage (Table 3).

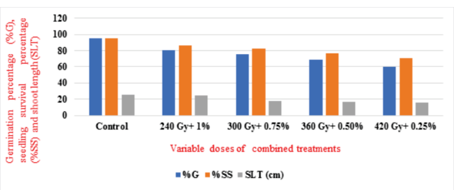
In case of combined mutagenic treatments, the results are as follows: 80.41%, 75.73%, 68.91%, and 59.99% for 240Gy+0.1% EMS, 300Gy+0.75% EMS, 360Gy+0.50% EMS and 420Gy+0.25 % EMS respectively. The germination percentage decreased after the EMS treatments as the concentration of the mutagen increased. The highest germination percentage was 80.41 at 240Gy+1%, while the lowest was 59.99 at 420Gy+0.25% for combined treatment (Table 3).

Almost all treatments produce quite satisfactory results in terms of plant survival at maturity. The maximum survival rate was 86.50% at 240Gy+1% and the minimum survival rate was 70.90% at 420Gy+0.25% of combined treatment. Survival values were lower in all the treatments when compared to the control. In contrast, the survival values are slightly decreasing when the dose of gamma rays increased and concentration of EMS decreased in combination (Table 3).

In combination treatment of gamma radiation and EMS concentration showed shoot length about 24.50 cm. at 240Gy+0.1% and 15.59 cm. at 420Gy+0.25%. Higher doses generally result in a decrease in shoot length (Table 3).

Table 3. Mean values and significance tests for germination percentage (%G), seedling survival percentage (%SS) and shoot length (SLT) of Trigonella genotypes evaluated with variable doses of combined treatments.

Source of Mutagen	Mutagenic Treatments	% G	% SS	SLT (cm)
Control		95.29	94.75	25.54
Gy + EMS	240 Gy + 1%	80.41	86.50	24.50
	300 Gy + 0.75%	75.73	82.65	17.62
	360 Gy + 0.50%	68.91	76.25	16.65
	420 Gy + 0.25%	59.99	70.90	15.59



Graph: 3. Mean values and significance tests for germination percentage (%G), seedling survival percentage (%SS) and shoot length (SLT) of Trigonella genotypes evaluated with variable doses of combined treatments.

DISCUSSION

In Trigonella, induction of inheritable variability hrough hybridization is delicate because of the veritably delicate and small-sized flower structure. The induced mutation through physical and chemical mutagen is effective tool to bring in changes for significant characters. Induced mutagenesis may bring about changes in the morphology and physiology of the plant. Accordingly, the present investigation was accepted for Mutational studies in Trigonella foenum-graecum L. induced through physical and chemical mutagens.

1. Seed Germination Percentage (%) Of Trigonella Genotypes:

The seed germination percentage was decreased with the increases in the dose/concentration of the mutagens like EMS, Gamma rays and combination (EMS and Gamma rays). The similar trend of the decreased in germination percentage was observed in all the mutagenic treatments. The same results were studied in seed germination percentage of French bean by [16, 2]. Mutagenic treatments inhibited growth related to seed germination percentage. The reduction of the seed germination may be due to the effect of the mutagens on the radicle and plumule meristematic region was reported by [4]. The chemical mutagens might be disturbing the formation of enzymes involved in the germination of the seeds reported by Kulkarni, [6].

2. Seedling Survival Percentage (%ss) Of Trigonella Genotypes:

The survival of plants at maturity shows decreased due to the mutagenic treatment. All the mutagenic treatments like EMS, Gamma rays and combination treatment shows the decreased in the survival of plants at the maturity as compared to the control. The increases in the percentage of the lethality at the higher dose or concentration of the mutagenic treatment were caused by the injury due to the physiological imbalance or the chromosomal aberrations were reported by [8, 12] also reported that decreased in the survival of plants in Lablab purpureus (L.)

3. Shoot Length Of Trigonella Genotypes

In the present investigation the shoot length was decreased with increases in the dose or concentration of the chemical and physical mutagens. In combination treatment, the shoot length was initially increased at the 200Gy+1% treatment and it was decreased again in the same concentration of the mutagen. The seedling injury was increased with the increases in the dose or concentration of the mutagens, except for combination treatment. Similar results were observed by [7, 18]. In Lablab purpureus (L) by using the EMS and Gamma rays treatment shows the reduction in the root and shoot length at the increasing dose or concentration of the mutagens [10, 11].

Data Collection

The following traits were assessed following the methods described by FAO/IAEA [5]. Germination percentage was recorded by counting sprouted seeds per treatment after 7 days using the moist paper towel germination method under room temperature as described by Sako et al. [14]. Seedling survival percentage was recorded by counting the number of surviving seedlings at 2 the time of harvesting of the crop. The shoot length was recorded from measuring five randomly selected plants per plot from the base to the tip of the top leaf, and the average was expressed in cm 15 days after 50% emergence.

Data Analysis

Data on germination percentage, seedling survival percentage and shoot length were subjected to analysis of variance (ANOVA) using GenStat (18^{th} edition) statistical software [19]. Mean comparisons were conducted using Fisher's least significant difference procedure when significant differences were detected in the ANOVA. The mean lethal dose (LD_{50}) was estimated through the simple linear regression model.

CONCLUSION

The current mutation breeding programme was studied over three generations. M1 generation studies included data collection on M_1 biological parameters such as seed germination percentage, seedling survival and shoot length. The results showed that EMS and Gamma rays had an inhibitory effect on seed germination. The height of seedlings decreased as the concentrations/doses of EMS and Gamma rays increased. Plant survival at maturity decreases in EMS treatment compared to Gamma rays treatment.

This study determined doses of gamma radiation and EMS singly or in combination in Trigonella based on early growth parameters. Combined doses of gamma radiation and EMS produced poor seedling germination, seedling survival and shoot length below the target of LD_{50}. Therefore, combined doses were not recommended for large scale mutation induction in Trigonella.

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