

Determination of mutagenic effectiveness and efficiency of gamma rays and EMS in Coriander

C. Sarada *

Department of Horticulture, College of Horticulture, Chinalataripi - Dr. YSRHU, India.

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Abstract

Coriander is a dual purpose vegetable used as leaf as well as a spice, along with medicinal properties. Owing to its compound umbel and difficulty in crossing programme, mutation breeding is the available source to create variability. Mutagenesis is a well-recognized tool to expand genetic variability. The present investigation involves mutagenesis in coriander cv. Sindhu with physical (gamma rays) and chemical mutagens (EMS) and their combinations. The mutation frequency, mutagenic effectiveness and mutagenic efficiency were calculated for the gamma rays, EMS and their combination doses. In gamma rays, a drop in mutagenic effectiveness was observed at 10Kr followed by increase with higher concentration. Mutagenic effectiveness due to gamma rays, ranged from 6.5 to 8.51 due to EMS ranged from 42.09 to 49.55 and due to combination treatments ranged from 12.17 to 18.80 (5kr+0.2% EMS) in coriander variety, Sindhu. The mutagenic efficiency based on survival ranged from 1.78 to 4.0, injury ranged from 3.83 to 8.2, sterility ranged from 2.37 to 5.18 indicating differential response to different mutagens. The combination of gamma rays and EMS at lower and moderate doses expressed its worth to create higher mutation frequency by manifesting higher efficiency than the individual mutagens single-handed.

Keywords: Coriander; EMS; Gamma rays; Variability; Mutagenic efficiency; Mutagenic effectiveness

1. Introduction

Coriander (*Coriandrum sativum* L.) is an important spice crop belonging to family Apiaceae. It is believed that coriander originated from around the Mediterranean region and cultivated globally, mainly in the tropics. India has the prime position in the cultivation and production of coriander. Its matured fruits and fresh leaves are used as spices and traditional medicine. (Shahwar et al., 2012; Izgi, 2020), Coriander is used in several culinary purposes and also for treatment of several diseases. India is the largest producer of coriander. It is prominently cultivated in Rajasthan, Andhra Pradesh, Gujarat and Madhya Pradesh. Approximately 80% of the world total coriander seed is produced in India. Genetic variation is the most essential prerequisite for successful crop improvement programme as it provide spectrum of variants for the effective selection. This can be achieved through the process of mutation [Yugandhar, 2018]. In general, lower concentration of chemical mutagens was more effective and efficient in inducing mutation with low biological damages and high mutation frequency. Mutation breeding is powerful tool to enrich variation particularly for attributes of economical importance in crop like coriander where hybridization is difficult. Mutation is a physical or chemical agent that changes the genetic material usually DNA of an organism. Physical and chemical mutagen induces physiological changes (injury), gene mutation (point mutations) in the biological material in M_1 generation [Gual, 1970]. Effectiveness of mutation breeding programme depends on the effectiveness and efficacy of mutagenic treatments. Hence, the present investigation in coriander (*Coriandrum sativum* L.) was undertaken with physical (gamma rays) and chemical mutagen (Ethyl Methane Sulphonate i.e. EMS) and their combination at different concentrations to assess their effectiveness and efficacy in inducing mutation.

* Corresponding author C. Sarada

2. Material and methods:

Genetically pure and uniform size seeds of coriander variety Sindhu were treated in different concentrations of gamma rays (5Kr, 10Kr, 15Kr), EMS (for four hours) and combination of EMS with 5Kr gamma rays under normal room temperature. Prior to treatment of chemical mutagens, the seeds were presoaked in distilled water for ten hours to activate the torpid embryo, to make cell membranes more permeable for the chemical mutagen to diffuse into the biological material thereby facilitating the uptake of the chemical mutagen (Ashadevi et al., 2019). Different doses of gamma rays used in the present study were 5, 10 and 15Kr and that of EMS were 0.2, 0.3 and 0.4% respectively along with concentrations of 5Kr gamma rays with 0.2, 0.3 and 0.4% EMS. After the treatment, the seeds are thoroughly washed to remove the mutagen residues from the seed surface. The washed seeds are immediately sown to raise the M₁ population. The crop was raised with the recommended package of practices. Seeds of the M₁ plants were harvested individually to rise M₂ generation. The mutation frequency (Mf) is the magnitude of mutated plants in the total population obtained as a result of exposure to the mutagenic agent (Ashadevi et al., 2019).

$$\text{Mutation frequency} = \frac{\text{Number of mutated seedlings}}{\text{Total number of M}_2 \text{ seedlings}} \times 100$$

The mutagenic effectiveness is a measure of the frequentness of mutations induced by a unit of a mutagen (Konzak et al., 1965) or the number of mutations invoked per unit dose of mutagen in unit time (Konzak et al., 1964; Khan and Wani, 2006). It is calculated as per the formula suggested by Konzak et al. (1965).

$$\text{Mutagenic effectiveness (\%)} = M/tc \text{ or } M/kr$$

$$\text{Mutagenic efficiency (\%)} = M/L \text{ or } M/I \text{ or } M/S$$

Where,

M = Percentage of M₂ plants segregating for chlorophyll mutations.

t = Period of treatment with chemical mutagens in hours.

c = Concentration of the chemical mutagens in percentage.

Kr = Kilorad (dose of physical mutagens in Kilorad)

L = Percentage of lethality or survival reduction of seedlings.

S = Percentage of pollen sterility in M₁ plants and

I = Percentage of injury or reduction on seedling height

3. Results and discussion

The mutagenic effectiveness and efficiency are pivotal for the mutation breeding experiments. Both are observed in M₂ generation. Table 1 exhibits the mutagenic effectiveness of different mutagenic treatments.

3.1. Mutagenic effectiveness

The Mutagenic effectiveness is the induction of mutation by unit dose of mutagen (Gy or Concentration x Time). In gamma rays, a drop in mutagenic effectiveness was observed at 10Kr followed by increase with higher concentration. In gamma rays, maximum mutagenic effectiveness was observed at lower dose i.e., 5Kr (8.51). The mutagenic effectiveness ranged from 6.5 to 8.51. With regard to EMS, mutagenic effectiveness decreased at 10Kr and again increased at higher doses. Mutagenic effectiveness due to EMS ranged from 42.09 to 49.55. Mutagenic effectiveness decreased at 5Kr+0.3%EMS and again marginal increase was observed at 5Kr+0.4%EMS. Mutagenic effectiveness due to combination treatments ranged from 12.17(5kr+0.3% EMS) to 18.80(5kr+0.2% EMS) in Sindhu. Mutagenic effectiveness among different mutagenic treatments was high at EMS followed by combination treatments and gamma rays. The most effective mutagen was found to be EMS which exhibited high values for mutagenic effectiveness compared to Gamma rays and combination doses(Fig.1). Konzak et al.,(1965) reported that the, objective of any mutation studies should be to induce low biological damage with high frequency of point mutations. Similar results of maximum mutation rate with EMS was reported by Girija and Dhanavel (2009), Dhanvel et al., (2008) and Wani (2009), Anju Pathania and Sood 2007 in chickpea.

3.1.1. Mutation frequency

There is gradual increase in mutation frequency with increase in concentration of mutagen. The mutagenic frequency ranged from 0.43 to 1.23 in gamma rays, 0.4 to 0.78 in Ems and 0.75 to 1.14 in combination treatments. As the combination of mutagens is concerned, the gamma ray dose, EMS concentration and the treatment duration found to have a role in

the induction of high frequency of useful mutation; an increase in the above resulted decrease in the mutagenic efficiency of the mutagen combination, indicating that the increase in mutation frequency was not proportional to the increase in the mutagen doses (Khursheed et al., 2018).

3.1.2. Mutagenic efficiency

Mutagenic efficiency is an estimate of biological effects such as reduction in survival percentage, reduction in seedling height and pollen sterility. Khan (1981) stated that variation in effectiveness and efficiency of mutagens show genotypic response which is of genetic origin and depends upon the physical and chemical properties of the mutagens. Mutagenic effectiveness and efficiency are two important factors to study a mutagen. According to Patil and Rane (2015) the effectiveness and efficiency gives an idea to evaluate a mutagen. The determination of these factors is necessary in plant breeding programs. The effectiveness and efficiency are studied so that the mutagens that cause genetic variability can be assessed for the selection of genotypes which can be used for the improvement of crops in plant breeding programs. The mutagenic efficiencies for different treatments were estimated on the basis of plant lethality (M/L i.e., per cent reduction in number of plants survived at maturity in M_1), seedling injury (M/I i.e., per cent reduction in seedling height) and pollen sterility (M/S) in relation to M_2 plants segregating for chlorophyll mutations by using the formulae suggested by Kozak et al., (1965). The values of mutagenic efficiency varied depending on the criteria used to estimate them.

The mutagenic efficiency based on survival injury, sterility showed differential response to different mutagens in coriander variety Sindhu. Mutagenic efficiency of lethality varied from 1.78 to 4.0 for gamma rays, 1.69 to 2.42 for EMS, and 2.4 to 3.66 for combination treatments in Sindhu. In the present investigation, the biological damage in the M_1 population is studied through lethality was increasing at higher doses indicating that the higher doses would be detrimental to the plant growth development. (Table1 and fig.2). This shows that the combined usage of mutagen at optimum dosage would be advantageous in generating a high frequency of useful mutations. (Jyothsna et al., 2022) There is increase in mutagenic efficiency due to sterility with increase in dose of mutagen and reduction in mutagenic efficiency was observed due to injury. The mutagenic efficiency for sterility showed positive trend with the dose of mutagen. Mutagenic efficiency due to sterility ranged from 2.59 to 5.18, 2.41 to 3.22, and 3.22 to 4.09 for gamma rays, EMS and combination treatments respectively. Mutagenic efficiency due to injury for gamma rays ranged from 4.32 to 8.20, for EMS 3.83 to 8.96, and for combination treatments it ranged from 6.27 to 6.57. It is evident that the EMS is most efficient mutagen followed by gamma rays and combination when the efficiencies were measured on M/L, gamma rays were the most efficient mutagen when the efficiencies were measured on M/I and as M/S.

Mutagenic efficiency increased with the increasing dose of mutagens. It may be due to the fact that injury, lethality and sterility increases with increase in the mutagen concentration than actual mutations (Kharkwal 1998). Efficient mutagens and their treatments are indispensable for the cost effective use of the mutagen as a tool for the induction of mutations and their direct and indirect utilization in successful breeding programme. Apparently the low efficiency observed with gamma rays might be due to their ability to induce more chromosomal breaks resulting in phenotypic expression by elimination of mutant genes. Pampapathi (1986) in brinjal opined that EMS with its ability to induce point mutations more frequently at lower doses was found to be the most effective and efficient mutagen.

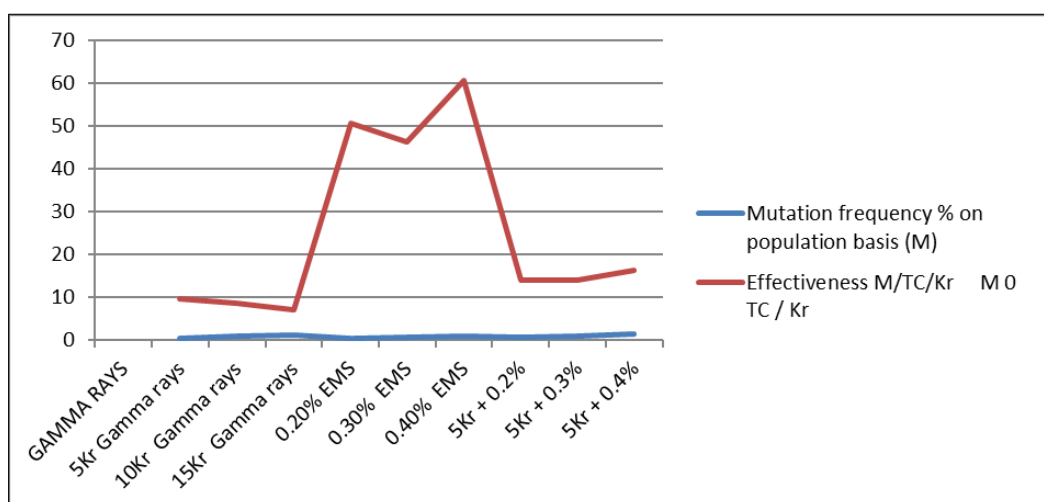


Figure 1 Effect of mutagens on mutagen frequency and effectiveness

Various reports on effectiveness and efficiency of different mutagens are reported earlier by Koli and Ramkrishna (2002) in fenugreek, Velu et al. (2007) in cluster bean, Girija and Dhanavel (2009) in cowpea and Sharma et al. (2009) in garden pea. The above results state that the lower doses are more efficient than the higher doses. The higher mutagenic efficiency at lower doses might be because of the lower levels of biological damage (Bhosle and Kothekar, 2010). This was in similarity with the findings of Dhanavel et al. (2008); Dube et al. (2011); Khursheed et al. (2016).

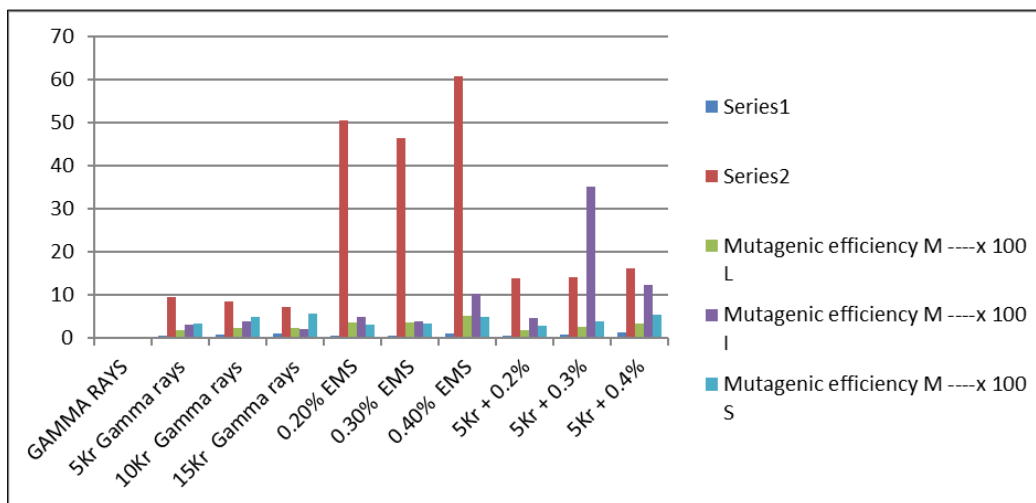


Figure 2 Effect of mutagens on mutagen efficiency

Table 1 Mutagenic effectiveness and efficiency in the M2 generation of coriander (*Coriandrum Sativum* L) in variety Sindhu

Treatment Dose in Kr/conc	Mutation frequency % on population basis (M)	Effectiveness M/TC/Kr	Mutagenic efficiency		
		M -----x100 TC / Kr	M ----x 100 L	M ---- x100 I	M ----x 100 S
GAMMA RAYS					
5Kr Gamma rays	0.43	8.51	1.78	4.32	2.59
10Kr Gamma rays	0.65	6.50	2.36	8.20	3.15
15Kr Gamma rays	1.23	8.17	4.00	4.89	5.18
Mean	0.77	7.73	2.71	5.80	3.64
EMS					
0.20% EMS	0.40	49.55	1.69	8.96	2.41
0.30% EMS	0.51	42.09	1.94	4.60	2.37
0.40% EMS	0.70	43.84	2.42	3.83	3.22
Mean	0.53	45.16	2.02	5.80	2.67
COMBINATIONS					
5Kr + 0.2% EMS	0.75	18.80	2.97	6.57	3.79
5Kr + 0.3% EMS	0.73	12.17	2.40	6.27	3.22

5Kr + 0.4% EMS	1.14	14.22	3.66	6.27	4.09
Mean	0.87	15.06	3.01	6.37	3.70
Control	-	-	-	-	-

M = Mutation Frequency on M2 plant basis; T = Period of treatment with chemical mutagens in hours; C = Concentration of the chemical mutagens in percentage; Kr = Kilorad (Dose of physical mutagen in kilorad); L = Percentage of lethality or survival reduction of seedlings; I = Percentage of injury or reduction in seedling height; S = Percentage of pollen sterility in M1 plants.

4. Conclusion

The present investigation was carried out to study the plant response as mutagenic effectiveness and efficiency of coriander cv. Sindhu. Among the gamma rays, EMS and their combinations, EMS treatments were found to be more effective in mutagenicity. The combined treatments with both physical and chemical mutagens are found highly efficient with low biological damage. The effectiveness and efficiency of the mutagens were found to be increasing with an increase in the mutagen dose. The moderate doses are found more effective and efficient than the higher doses. The determination of mutational frequency, effectiveness and efficiency enables appropriate decisions on the mutagenic doses for the future works. The study has to be continued further for selection of promising mutants for desirable traits and development of high yielding varieties after multi location trials.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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