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Research Article

Effect of induced mutagenesis in M_1 and M_2 generations of cumin (*Cuminum cyminum* L.)

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Abstract
Cumin is an important spice that has been used for centuries in various cuisines around the world. Two cumin varieties (GC 3 and GC 4) were subjected to mutation to understand macromutational modifications. Ethyl methane sulphonate (EMS) @ 0.6% and gamma rays (190 Gy) significantly reduced germination and plant survival percentage as compared to 0.2% EMS and 130 Gy. Physical mutagens showed higher plant suravival than chemical mutagens. However, chemical mutagen treated plants showed more sterility than physical mutagens. Chlorophyll mutations were confined to rare *albinos* and small number of *xanthas* in the M_2 generation. Morphological changes *viz.,* miniature, loosely branched and thick stem were effective with seed treatment of 0.4% EMS for GC 3 and with 0.6% EMS for GC 4. The estimates of mutagenic efficiency varied with 0.6% EMS and 160 Gy for GC 3, while it varied with 0.6% EMS and 190 Gy for GC 4.

Keywords: Mutation, cumin, EMS, gamma rays Gy, M_1 generation.

INTRODUCTION

India is the largest producer, consumer, and exporter of spices, aptly referred to as the “country of spices”. Zeera, or cumin (*Cuminum cyminum* L.), is a spice crop produced during *rabi season*. It is a member of the Apiaceae family. It is a diploid species with 14 pairs of chromosomes and is cross-pollinated (Eajaz *et al.*, 2019). Cumin is primarily grown in desert and semi-arid regions with dry, relatively chilly climate. The crop can endure dampness or heat, but not intense rain. Infections and aphid attacks are brought on by frost and excessive humidity during the flowering and fruiting stages.

It is mostly grown in India, Egypt, Iran, Pakistan, Israel, Libya, Mexico, and Japan for its flavour, which is attributed to the volatile oil component known as cuminol or cumin aldehyde, which makes about 19.25-27.02 percent of the seeds’ volatile oil (Balakrishna and Pushpakumari, 2001). The average volatile oil level of native collections ranges from 2.6 to 3.5%, whereas that of exotic collections can reach 6.0%. (Sastry and Sharma, 2001). It is employed as a spice for breads, cakes, biscuits, pickles, soups, sausages, and cheese. In addition to flavouring liqueurs and cordials, cumin oil is also utilized as antibacterial, antioxidant and colouring agent.

Since the last three decades, mutation breeding has replaced traditional breeding as a viable alternative with the primary purpose of creating cultivars of strategically vital crops. The effect of induced variation relies on three important factors namely efficiency and effectiveness of mutagens, mutagenic doses and nature of plant material (Usharani and Kumar, 2015). Traditional plant breeders rely only on naturally occurring mutations. There aren't enough spontaneous mutations to use as a basis for systematic breeding. Mutagenic chemicals that hasten the rate of mutation increase the possibility of producing the desired mutant during the breeding phase.

MATERIALS AND METHODS

The present investigation was conducted at Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Dantiwada during *rabi* 2017-18 and 2018-19 and Plasticulture Development Farm, Centre for Natural Resource Management, Sardarkrushinagar Dantiwada Agricultural University, Dantiwada during *rabi* 2019-20 and 2020-21.

The seeds of two varieties of cumin *viz*., GC 3 and GC 4 were treated with mutagens *viz*., 0.2%, 0.4% and 0.6% concentration of ethyl methane sulphonate (EMS) and physical mutagen (gamma rays) *viz*., 130 Gy, 160 Gy and 190 Gy. The EMS was freshly prepared in a phosphate buffer solution of 0.1 M having pH of 7.0. Both types of presoaked seeds in distilled water were immersed in a mutagen solution and incubated for 6 hours with periodic shaking prior to the chemical mutagenesis. The treated seeds were thoroughly washed with running tap water after treatment of six hours. The surface-dried treated seed samples were immediately sown in the field. The two types of control seeds i.e. GC 3 and GC 4 (no mutagens treatment), which had just been presoaked in distilled water were sown alongside the treated seeds as a check. The gamma-treated dry seeds of two cumin varieties were also sown in the field.

The treated seeds and the untreated controls were raised in 4.0 m x 2.5 m plots using a Randomised Block Design with Factorial Concept with three replications. Each plot had 300 seeds that were spaced at 45 cm apart in rows with 10 cm between plants. Germination percentage, plant survival percentage at flowering and pollen sterility percentage were recorded in M_1 generation.

The M_1 generation was taken from a single plant at a time. For the purpose of producing the M_2 generation, the M_1 plants that had a normal appearance were randomly selected and selfed. A total of 30 healthy-looking plants were identified and covered with bages to avoid out crossing before flowering in the M_1 generation. The M_2 seeds were only collected from the M_1 plants that had been bunched up. The M_2 offspring and control were sown (each 2.0 m long) in a 0.9 square metres plot with a single row. Every 30 rows of M_2 progeny were sown along with one line of untreated cumin varieties *viz*., GC 3 and GC 4 as a check. The different types of observations *viz*., germination (%), survival (%), pollen sterility (%), chlorophyll mutation and morphological mutants were recorded during the period of experiment.

RESULTS AND DISCUSSIONS

In addition to selection in cumin, mutation breeding is an effective strategy for generating and selecting for desirable variability in a crop. Physical and chemical mutagens are useful tools for accelerating natural mutation rates, which increases genetic variability and the possibility of producing desired mutants. Macro mutants can be used as a variety or donor for bringing desirable traits into the well adapted cultivars. Knowing which mutagens to utilise is crucial for a systematic mutation breeding effort.

The goal of the current research was to characterise the M_1 and M_2 generations, of two types of cumin, GC 3 and GC 4, by extensive physical and chemical mutagenesis. To produce the M_1 generation, the seeds that had been exposed to radiation and chemical mutagens were directly sowed in the field. With three replications in the field, the M_1 generation of the two types resulting from the six distinct treatments was raised independently in FRBD. However, only observations on pollen sterility, plant survival, and germination were made. Along with the doses of various mutagenesis treatments, the germination percentage of M_1 progenies of the two varieties of cumin had the same trend of germiation. The germination percentage decreased linearly with the increasing doses of both physical and chemical mutagens. According to the analysis of variance, factor B (mutagenic doses) was extremely significant, factor A (varieties) was significant, but interaction of factor A and B (mutagenic doses and varieties) was not significant. Seed treatment of cumin varieties with 0.6% EMS and gamma rays (190 Gy) reduced germination and survival per cent, while their increasing rate had enhanced pollen sterility % (**Table 2**). These findings were corroborated with those of several other researchers, including Singh and Mittal (2003) as well as Verma *et al.* (2017).

The survival rate of M_1 offspring from the two types of cumin generally followed the same pattern as the germination percentage. Treatments with EMS and gamma rays significantly decreased the survival percentage. Highly significant effect of different mutagenic doses on germination (%), survival (%) and pollen sterility (%) as compare to varieties was observed (**Table 1**). The survival of M_1 progenies treated with a three doses of EMS (0.2%, 0.4%, and 0.6%) and physical mutagens (130, 160 and 190 Gy) was decreased with their increasing mutagen dosages (**Table 2**) and such type of study was also carried out by Singh and Mittal (2003) and Verma *et al.* (2017). Among the three EMS doses, LD_{50} value for germination was 0.6%. Such type of results were also found by Krishna and Yadav (2013). Among the three doses of gamma rays, LD_{50} value for germination was 190 Gy. Similar result was also reported by (Verma *et al.*, 2017) for gamma rays (190 Gy).

Table 1. ANOVA for characters in the M_1 generation of cumin

S. No.	Character	Mean sum of square				
		Replications	Treatments			Error
			Factor A (Varieties)	Factor B (Mutagens doses)	Factor A : Factor B	
1	**Degree of freedom**	**2**	**1**	**5**	**5**	**22**
2	Germination (%)	14.18	86.70*	1580.70**	17.35	17.41
3	Survival (%)	26.63	60.57*	2091.22**	36.48*	10.50
4	Pollen sterility (%)	0.52	0.55	55.82**	1.23**	0.16

*,** significant at levels of significance of 0.05% and 0.01%, respectively.

Table 2: Effect of mutagens on mean germination, survival and pollen sterility of different varieties of cumin in M_1 generation

Mutagenic treatments	Germination* (%)		Survival* (%)		Pollen sterility (%)	
	GC 3	GC 4	GC 3	GC 4	GC 3	GC 4
			EMS			
0.2%	78.66	75.77	76.77	74.10	10.20	10.21
0.4%	65.10	66.27	59.44	62.42	11.56	12.28
0.6%	47.22	46.30	35.89	40.10	13.45	14.47
			Gamma rays			
130 Gy	90.10	82.83	88.55	81.89	9.62	8.69
160 Gy	70.55	63.57	67.77	59.78	10.51	10.42
190 Gy	46.33	44.77	39.55	37.74	12.34	13.96
Control	90.21	88.55	89.44	84.55	4.39	3.65
Range	44.77 – 90.21		35.89 – 89.44		3.65 – 14.47	
Mean	68.30		64.14		10.41	
S.Em.±	2.50		1.94		0.23	
C.V. %	6.11		5.05		3.82	
C.D. at 1%	7.00		5.44		0.66	

*The germination and survival data reported are expressed as a percentage compared to the corresponding control.

Mutagenic treatments revealed a gradual increasing trend in pollen sterility percentage from lower to higher doses. The goal of the current experiment was to create mutations that would result in large-scale mutational alterations. Due to their readily recognisable phenotype, macro mutational alterations were simple to detect. In the current study, few changes in chlorophyll but many changes in morphological mutants were discovered. Among the different chlorophyll mutant classes, only a few *xanthas* and a single unusual *albino* were present.

Due to their established mutagenesis potential, gamma rays and alkalyzing chemicals had long been employed to cause mutations in plants. The detrimental effect of mutagen dose on cumin plants was found through pollen sterility. The parameters were also used to identify detrimental effect which had an impact on how effectively efficiency is estimated. When assessing the utility of mutagens, mutagenic efficiency is a superior metric to mutagenic effectiveness.

M_2 offsprings of the two varieties were carefully examined for macromutational alterations. Chlorophyll and morphological variants made up the main mutational changes in the M_2 offspring of both the cumin varieties, and their frequencies are furnished in **Tables 3 and 4**.

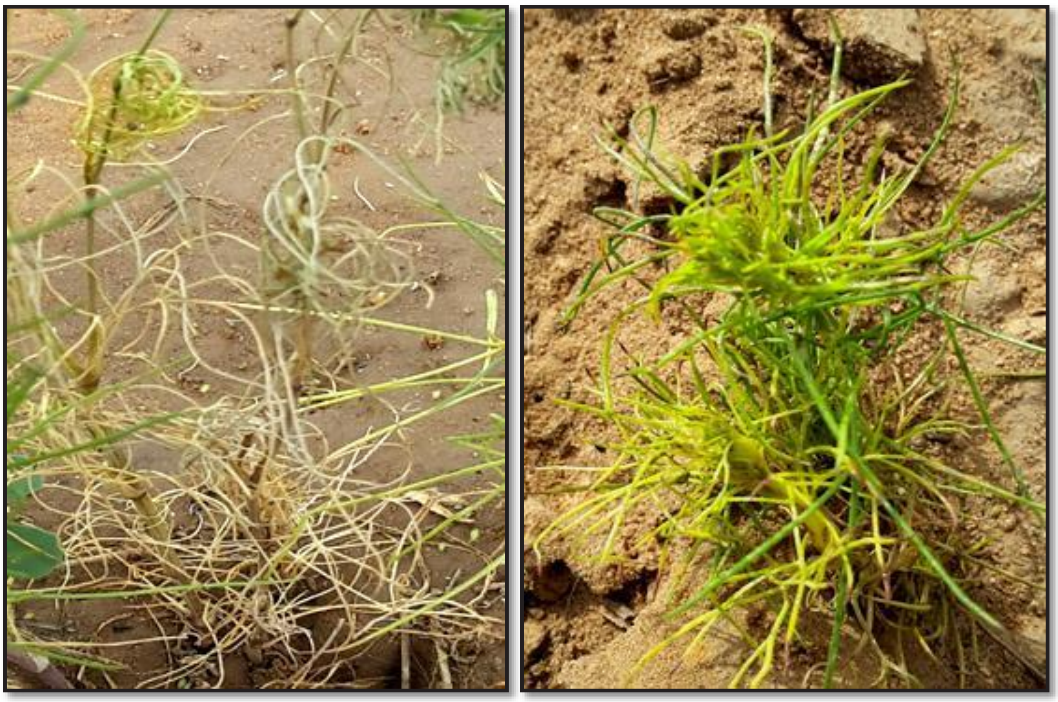
Albinos were rarely observed in the offspring of GC 3 alone, while xantha were considerably more prevalent (up to 13) in the M_2 offspring of the cumin variety GC 3 (**Table 3 and Fig. 1**). This outcome was consistent with the conclusion reached by Parveen *et al.* (2006). The M_2 progenies of both kinds frequently exhibited the same classes of morphological variants, including bushy, tiny,

Table 3. Frequency of morphological and chlorophyll mutants found in M_2 offspring of cumin variety GC 3

Mutagenic treatments	Total number of progenies tested	Total plants observed	Chlorophyll mutants		Morphological mutants						Total number of mutants	No. of progenies representing mutants	Mutation frequency (%) on population basis	Mutation frequency (%) on family basis
			xantha	*albino*	*Bushy*	*miniature*	*loosely branched*	*thick stem*	*deformed*	*drooping type*				
							EMS							
0.2%	30	576	1	-	1	-	1	1	2	-	6	4	1.04	13.33
0.4%	30	544	3	1	-	1	2	4	1	-	12	10	2.20	33.33
0.6%	30	502	4	1	-	2	-	3	1	-	11	11	2.19	36.66
Gamma rays														
130 Gy	30	545	-	1	1	-	1	1	-	1	5	5	0.91	16.66
160 Gy	30	536	2	-	1	1	3	2	1	1	11	8	2.05	26.66
190 Gy	30	496	3	-	2	4	1	2	2	-	14	9	2.82	30.00
Total (excluding control)	180	3199	13	3	5	8	8	13	7	2	59	47	11.21	156.64
Mean													1.87	26.11

Table 4. Frequency of morphological and chlorophyll mutants found in M_2 offspring of cumin variety GC 4

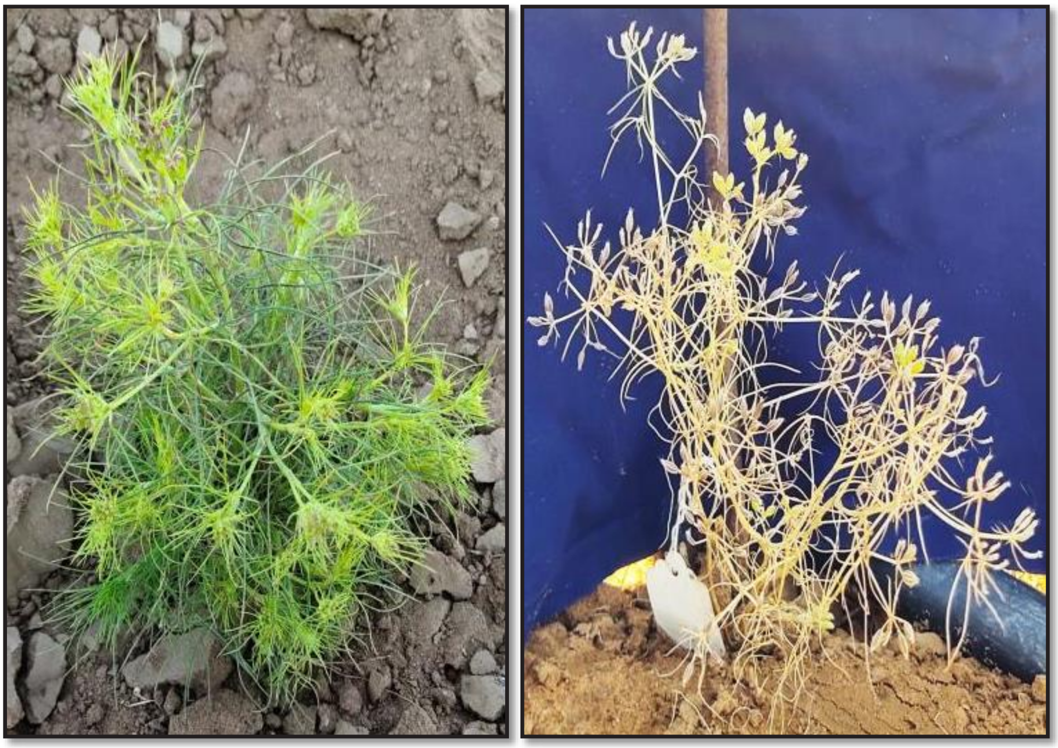
Mutagenic treatments	Total number of progenies tested	Total plants observed	Chlorophyll mutants		Morphological mutants						Total number of mutants	No. of progenies representing mutants	Mutation frequency (%) on population basis	Mutation frequency (%) on family basis
			xantha	*albino*	*bushy*	*miniature*	*loosely branched*	*thick stem*	*deformed*	*drooping type*				
							EMS							
0.2%	30	552	-	1	-	1	2	3	-	1	8	5	1.44	16.66
0.4%	30	538	1	2	1	-	5	7	-	-	16	6	2.97	20.00
0.6%	30	529	2	-	4	3	3	4	1	2	19	12	3.59	40.00
							Gamma rays							
130 Gy	30	567	1		1	1	2	2	1	1	9	6	1.58	20.00
160 Gy	30	527	2	1	-	1	4	5	1	1	15	8	2.84	26.66
190 Gy	30	507	2	1	5	2	2	4	1	1	18	11	3.55	36.66
Total (excluding control)	180	3220	8	5	11	8	18	25	4	6	85	48	15.97	159.98
Mean													2.66	26.66



Albino

Xantha

Fig. 1. Chlorophyll mutants



Bushy

Miniature

Fig. 2. Morphological mutants

Fig. 2. Continued..

loosely branched, thick stem, malformed, and drooping type, though at varying frequencies (**Table 3, 4 and Fig. 2**). However, GC 4 (85) has comparatively more macromutants (chlorophyll and morphological mutants) than GC 3 (59).

For the two varieties of cumin, mutagenic effectiveness and mutagenic efficiency were computed using the results of mutation frequencies on a family basis and pollen sterility (**Table 5 and 6**). A closer examination of physical mutagens (**Table 6**) revealed a linear dependency between the mutagenic effectiveness and mutagenic efficiency in the M_2 generation and biological damage caused by physical mutagens in M_1 generation. The same trend was also found by Selvam *et al.* (2010). For GC 3 offspring, 0.4% EMS had the highest mutagenic efficiency (2.88). Cumin seed of two varieties treated with gamma rays showed more linear effect as compare to chemical mutagens (**Table 5 and Table 6**). This results were in accordance with the finding of Mitra and Bhowmik (1997), Sengupta and Datta (1999), Datta and Sengupta (2002), Mahla and Ramkrishna (2002), Mishra and Singh (2014) and Sarada *et al.* (2015). In the case of GC 4 offspring, it can be observed that the mutagenic efficacy for gamma rays rose with increasing dosages of mutagens, although this tendency was incongruent with EMS treatments. The EMS dose of 0.6% appeared to be the most effective for the variety GC 4 (2.76). The dose of various mutagens did not always correspond to their ability to cause mutations.

Overall, the seed treatment of ethyl methane sulphonate (EMS) @ 0.6% and gamma rays (190 Gy) significantly reduce germination and survival percentage as compare to 0.2% EMS and 130 Gy. Chemical mutagens had a more negative impact on the percentage of plants that survived than physical mutagens did. Physical mutagens did not appear to induce as much sterility as chemical mutagens. Chlorophyll mutations were confined to rare

Table 5. Estimates of the effectiveness and efficiency of mutagenic treatments in cumin variety GC 3

Mutagenic treatments	Percentage of M_2 families segregating for mutations (Me)	Pollen sterility (%)	Mutagenic effectiveness Me/t × c	Mutagenic efficiency Me/S
		EMS		
0.2%	13.33	10.20	11.11	1.31
0.4%	33.33	11.56	13.89	2.88
0.6%	36.66	13.45	10.18	2.73
		Gamma rays		
130 Gy	16.66	9.62	0.13	1.73
160 Gy	26.66	10.51	0.17	2.54
190 Gy	30.00	12.34	0.16	2.43

t ×c represents for the product of treatment time in hours and mutagen concentration, while S indicates for pollen sterility.

Table 6. Estimates of the effectiveness and efficiency of mutagenic treatments in cumin variety GC 4

Mutagenic treatments	Percentage of M_2 families segregating for mutations (Me)	Pollen sterility (%)	Mutagenic effectiveness Me/t × c	Mutagenic efficiency Me/S
		EMS		
0.2%	16.66	10.21	13.88	1.63
0.4%	20.00	12.28	8.33	1.63
0.6%	40.00	14.47	11.11	2.76
		Gamma rays		
130 Gy	20.00	8.69	0.15	2.30
160 Gy	26.66	10.42	0.17	2.56
190 Gy	36.66	13.96	0.19	2.63

t × c represents for the product of treatment time in hours and mutagen concentration, while S indicates for pollen sterility.

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albinos and small number of *xanthas* in the M_2 generation. Morphological changes *viz.,* miniature, loosly branched and thick stem with seed treatment of 0.4% EMS for cumin variety GC 3 and for the GC 4 0.6% EMS effective. Estimates of mutagenic efficiency was more with 0.6% EMS and 160 Gy for GC 3, while it was more with 0.6% EMS and 190 Gy for GC 4.

From the results of the present study, it is concluded that there was a significant variation between the mutants and the control with respect to plant population in M_1 generation due to ethyl methane sulphonate (EMS) and gamma rays. Both the growth characteristics and biological responses decreased with an increasing doses of for all the treatments consisting of EMS and gamma rays. The linear dependency was observed between the viable mutation frequency in M_2 generation and it induced biological damage in M_1 generation. Hence, higher doses of gamma rays and EMS were proved to be efficient in increasing the mutation frequency towards desirable directions. Comparing to gamma rays treatment, the cumin varieties under study, showed a marked reduction due to EMS which could be useful in inducing a wide range of desirable mutations in cumin.

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