INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Mutagenic sensitivity of Gamma rays, EMS and their combinations on germination and seedling vigour in Coriander (*Coriandrum sativum* L)

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ABSTRACT

Coriander is an important seed spice crop used in culinary purpose both for leaf and in powder and spice mixtures. Creation of variability through mutagenesis is the only available option in view of its small flower size. Hence, the present study was under taken with an objective to know the sensitivity of the crop to physical and chemical mutagens. This study was performed by exposing the seeds of four varieties of coriander (*Coriandrum sativum* L.) to gamma rays (5, 10 and 15Kr), ethyl methane sulphonate [EMS] at 0.2, 0.3 and 0.4% concentrations and combination of gamma rays (5Kr) and EMS (0.2, 0.3 and 0.4%) at Horticultural Research Station, Lam. The observations were made on seed germination both under field and laboratory conditions, root and shoot length, seedling growth at 20 and 30 days after sowing. All the mutagens significantly affected the germination and seedling growth. The study revealed that germination percentage, seedling height, shoot length and number of leaves decreased with increase in dose/concentration of the mutagens. Among the different mutagens, gamma rays were more effective in reducing germination and growth of seedlings as compared to EMS and combination treatments. Lower treatments of all the three mutagens have influenced less biological damage and would be suitable for inducing desirable mutations in coriander.

Key words: coriander, EMS, gamma rays, germination, Injury, mutagen, pollen fertility,

INTRODUCTION

Coriander is one of the most widely used annual herb grown for leaf and grain throughout the country. The young plants are used in curries for decoration and seeds are used as a spice and as herbal medicine. Mutation breeding is a powerful tool to enrich variation particularly for attributes of economical importance in the crops like coriander where hybridization is difficult. Physical and chemical mutagens induce physiological damages (injury), gene mutations (point mutations) and chromosomal aberrations in the biological material in M_1 generation (Gaul, 1970[12]). Gamma rays, an energetic form of electromagnetic radiations are known to be the most popular mutagens for their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Ghosal, 2002[6]). Ethyl methane sulphonate (EMS), a chemical mutagen of the alkylating group has been reported to be the most effective and powerful mutagen and usually causes high frequency of gene mutations and low frequency of chromosome aberrations in plants (Khatri *et al.*, 2005[19]). In mutation breeding studies, it is important to determine a suitable dose/concentration of mutagen for a crop plant which can be employed for inducing maximum variability through point mutations. Seed germination, seedling growth are some of the commonly used criteria for studying mutagenic sensitivity in plants (Lal *et al.*, 2009[21]; Sangle *et al.*, 2011[26]).The present study was conducted to know the response of coriander seeds to gamma rays, EMS and their combinations based on germination and seedling vigour with the main aim of identifying appropriate dose/conc. of these mutagens for induction of desirable mutations.

MATERIALS AND METHODS

The material used for this study consists of four varieties of coriander viz Sindhu, sadhana, swathi and Sudha. The dry seeds of uniform size were exposed to different doses (5, 10, and 15 Kr) of gamma rays at post harvest technology center, Rajendranagar. Another set of presoaked seeds were treated with EMS solution (0.2, 0.3 and 0.4%EMS). For combination treatments, seeds irradiated with 5Kr were treated with mutagen solution (0.2%, 0.3% and 0.4%EMS). Thus the present study was conducted with four varieties with ten treatments in factorial RBD replicated thrice. The dried seeds of the genotypes treated with mutagens were sown in the field directly and in laboratory using paper towel technique. The data on root and shoot length was recorded from 15 randomly selected seedlings on 15th day after sowing from seeds germinated under laboratory conditions. Seed germination was recorded on alternate days from 15th day after sowing in the field. The data on shoot length and number of leaves were recorded from ten randomly selected seedlings at 20 and 30 days after sowing.

RESULTS AND DISCUSSION:

I) EFFECT OF MUTAGENS ON SEED GERMINATION IN LABORATORY AND FIELD:

The data pertaining to percentage of germination in laboratory and field as influenced by different mutagenic treatments in four varieties of coriander compared to control (in percentage) was calculated genotype wise and is presented in Table 1 and Fig.1 respectively. In the present study, a steady decrease in germination was observed with the increase in dose of different mutagens indicating that higher doses had adverse effect. Among the different mutagenic treatments, germination in laboratory ranged from 70.93 (10Kr) to 90.31 (5Kr gamma rays +0.2% EMS) in Swathi, 65.81(15Kr) to 79.78(0.2% EMS) in Sudha, 68.0 (0.4%EMS) to 94.91(5Kr) in Sadhana and 69.85(15Kr) to 83.45 (5Kr) in Sindhu. Among the varieties, variety Sudha was most severely affected in the germination than all the genotypes. (Figure.1). Among the different treatments, the reduction in germination percentage was more at combination treatments. General toxicity due to mutagens is an established fact. Dose dependent reduction in germination, observed in the present study is also in agreement with previous reports in several umbelliferous crops like coriander (Salve and more(2014)[28],Sikdar et al 2013[30], Singh 1991[32], Bhavanisingh *et al*., 1992[1], Vedamuttu *et al*., 1989[33]), in fenugreek (Yadav 1992[34]), and in cumin (Koli 1997[20]) and in fennel (Mahla and Ramakrishna, 2002[22]). The decrease in germination with increase in dose might be due to lethal combination of mutant genes which do not allow germination of the seeds. In some case the lethal effects become 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)[16]. The decrease in seed germination induced by mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009[18]; Chowdhury and Tah, 2011)[8].

Similar trend of reduction in percentage of germination was observed under field conditions (Table 1 and Fig 1). The germination percentage in field ranged from 64.13(15Kr) to 86.15 (0.2%EMS) in Swathi, 58.77(15Kr) to 78.1(0.2%EMS) in Sudha, where as in Sadhana it is 70.55(15Kr) to 86.61 (5Kr), 68.19(15Kr) to 86.45 (5Kr) in Sindhu varieties. Among the four varieties germination percentage was less in Sudha. With regard to mutagenic treatment, 15Kr gamma rays severely affected the germination. However, all the treatments recorded lower per cent of germination over control. Similar results of decrease in germination with mutagenesis was observed in cluster bean (Shinde 2013)[29], cow pea (Galkwad, 2013)[10], in *Withamnia somnifera* (Bhosala and Mose 2013)[2], in tomato (Sikdar *et al* 2013)[30]. The reduction in germination was more under field conditions when compared to laboratory. This indicates that the germination response is not only dose, mutagen and genotype dependent but also affected by the prevailing environmental conditions as evidenced by greater reduction of germination under field conditions. This may be due to the fact that seed germination in the laboratory requires a little strength for radical and plumule for eruption and it had little hurdles, while the seedling emergence in the field requires more strength for radical and plumule to erupt out of the soil which could have been damaged by mutagenic treatments.

III) EFFECT OF MUTAGENS ON SHOOT AND ROOT LENGTH:

A) SHOOT LENGTH:

The vigour of seedling as influenced by different mutagenic treatments was studied by measuring shoot and root length at 15 days after sowing in the laboratory was presented in Table-2. In general, the shoot and root growth decreased with increase in concentration of mutagens. The shoot length and root length were more affected in Swathi variety followed by Sindhu, Sadhana and Sudha (Figure 3). The shoot length ranged from 3.47 cm (5Kr Gamma Rays+0.4%EMS) to 6.1 cm (5Kr Gamma rays) in

Swathi variety (6.42cm in control), whereas it is from 4.41 cm (15Kr Gamma Rays) to 8.7 (0.3%EMS) in Sudha (8.59 cm in control), 4.55 cm (15Kr Gamma rays) to 6.4 cm (0.2%EMS) in Sadhana (7.47cm in control), and it is from 3.9 cm (15Kr Gamma Rays) to 7.45 cm (0.2%EMS) in Sindhu Varieties(7.70cm in control). The EMS treatment (0.3%) recorded increased shoot length (8.7 cm) than control in Sudha variety (8.59 cm in control). In Swathi variety, sudden increase in shoot length was observed at 0.3%concentration (5.57cm) than lower concentration *i.e*. at 0.2%EMS (5.03cm) and again there is decrease in shoot length at 0.4%EMS. The stimulatory effect may be attributed to the increase in growth promoters, the sudden increase in metabolic status of seeds at certain levels of dosage of the mutagens. Among the treatments, 15Kr recorded significantly lowest shoot length (4.35cm) followed by 5Kr+0.4%EMS (4.82cm) and 0.2% EMS recorded maximum shoot length (6.83cm). These findings are in close agreement with the earlier reports in cluster bean (Shinde 2013)[29], cow pea (Galkwad, 2013)[10], in *withamnia somnifera* (Bhosala and Mose 2014)[3], in tomato (Sikdar *et al* 2013)[30]. The differential response of genotypes to mutagens may be due to the specificity of the genotype and physiological maturity of seed material.

B) ROOT LENGTH:

The root length ranged from 4.36 cm (5Kr gamma rays + 0.2%EMS) to 6.24 cm (5 Kr Gamma Rays+0.3%EMS) in Swathi variety (6.60cm in control), whereas it is from 5.28 cm (5Kr Gamma Rays+0.4%EMS) to 6.74 cm (0.2%EMS) in Sudha (8.20cm in control), 3.87 cm (5Kr Gamma Rays+0.4%EMS) to 6.23 cm (5 Kr Gamma Rays) in Sadhana (7.80 cm in control), and it is 4.5 cm (15Kr Gamma Rays) to 5.66 cm (5Kr GammaRays+0.2%EMS) in Sindhu Variety (7.71cm in control).In Swathi variety, similar to shoot length, sudden increase in root length was observed at 5Kr + 0.3%EMS concentration (6.24cm). Among the treatments, significantly maximum root length was recorded by 0.2%EMS (5.93cm) followed by 5Kr (5.74 cm) and root length was affected in 15Kr (4.86cm) followed by 5Kr +0.4%EMS. Gamma rays treatment and combination treatments of 5 Kr Gamma rays with EMS resulted in more reduction of shoot length compared to EMS treatments. Prakash and Shambhulingappa, 2000[22] in rice bean, Sheeba *et al*., 2003[27] in sesamum also observed reduction in length of roots and shoots due to mutagenic treatments. The decrease in seedling vigour with the increase in mutagenic treatments may be attributed to an increase in physiological damage, variation in auxin level (Goud and Nayar, 1968)[13], change in the specific activity of few enzymes (Cherry et al., 1962)[7] and physiological injury induced in the seeds and seedlings (Ignacimuthu and Babu, 1988)[16]. Evans and Sparrow (1961)[9] suggested that the chromosomal damage and inhibition of cell division are the chief causes of reduced seedling growth. Blixt (1970)[4] opined that the inhibition in seedling growth might be due to the gross injury caused at cellular level either due to gene controlled biochemical processes or acute chromosomal aberrations or both.

III) EFFECT OF MUTAGENS ON GROWTH OF SEEDLINGS:

Seedling height:

The effect of different mutagenic treatments on seedling height and number of leaves at 20 and 30 days after sowing in field are shown in Tables 3 and 4 and in figure 4, 5 and 6 respectively. The shoot length at 20 days after sowing ranged from 6.90 cm (5Kr Gamma Rays + 0.4% EMS) to 10.66 cm (0.2% EMS) in Swathi variety, whereas it is from 9.37 cm (15Kr Gamma Rays) to12.41 (0.2%EMS) cm in Sudha, 8.77 cm (15Kr Gamma rays) to12.87 cm (0.3%EMS) in Sadhana, 8.1 cm (15Kr Gamma rays) to 12.47 cm (0.2%EMS) in Sindhu. Among the treatments, 0.2% EMS recorded significantly maximum seedling height (12.03 cm) whereas; 15Kr gamma rays decreased the plant height (8.58 cm).

Among the different treatments under study, gamma rays decreased the plant height. The range of plant height at 30 days after sowing was 9.09 cm (15Kr Gamma Rays) to 19.31 cm (5Kr + 0.3 % EMS) in Swathi variety, 12.88 cm (5Kr + 0.3% EMS) to 20.31 cm (0.2 % EMS) in Sudha, 15.43 cm (15Kr) to 18.74 cm (5Kr Gamma Rays + 0.2% EMS) in Sadhana (20.35 cm in control), and 12.37 cm (15Kr Gamma rays) to 15.78 cm (0.2%EMS) in Sindhu. At 30 Days after sowing also, 15Kr gamma rays decreased plant height (12.57cm) where as, EMS at 0.2% concentration recorded more seedling height (18.09cm). EMS at 0.2% concentration recorded more plant height (20.31cm) in Sudha than control (20.0cm). Similar results of decrease in plant growth with mutagenesis was observed in cluster bean (Shinde 2013)[29], cow pea (Galkwad, 2013)[10], in withamnia somnifera (Bhosala and Mose 2014)[3], in tomato (Sikdar *et al* 2013)[30].

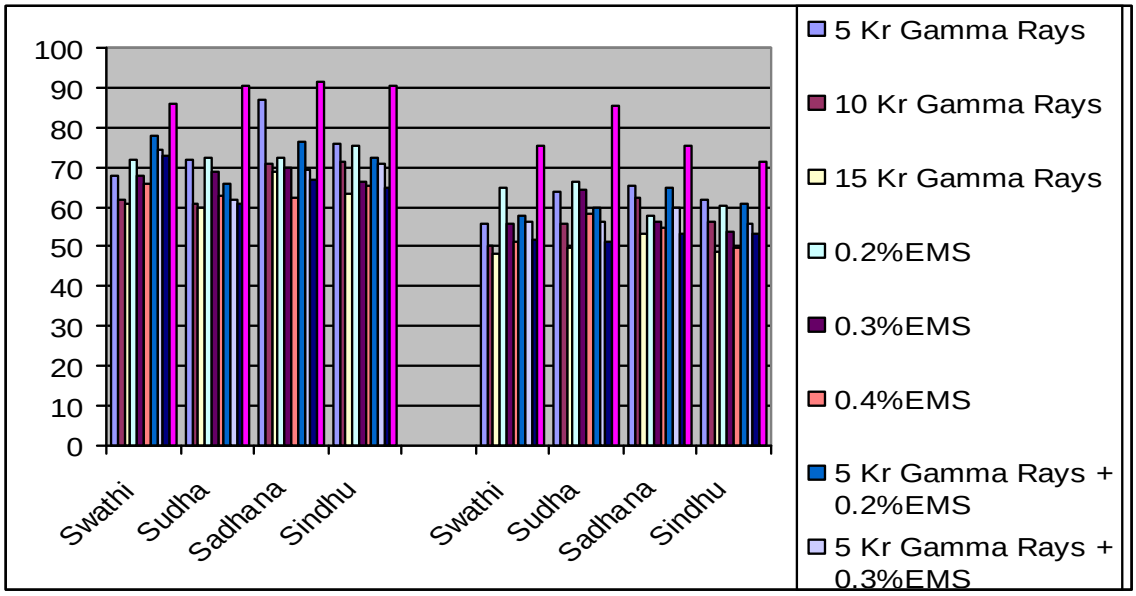

Fig1
Effect of mutagens on germination in laboratory and field

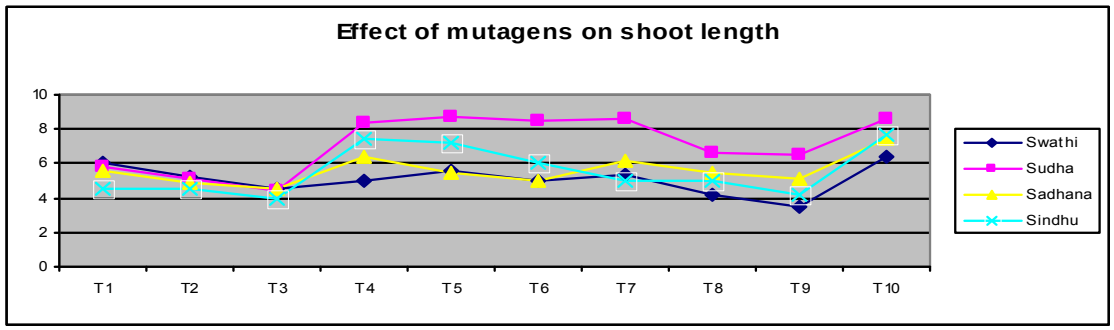

Fig 2
Effect of mutagens on shoot length

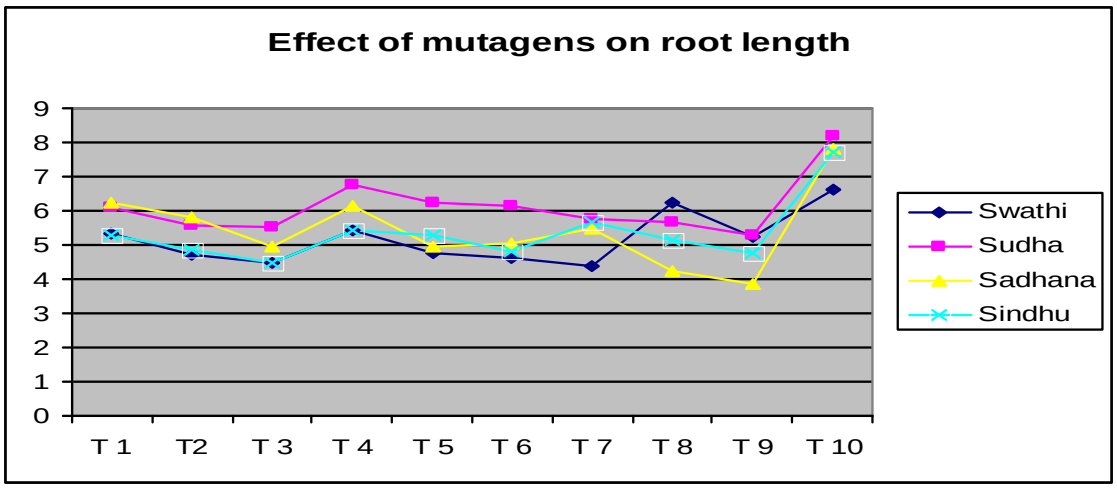

Fig3
Effect of mutagens on root length

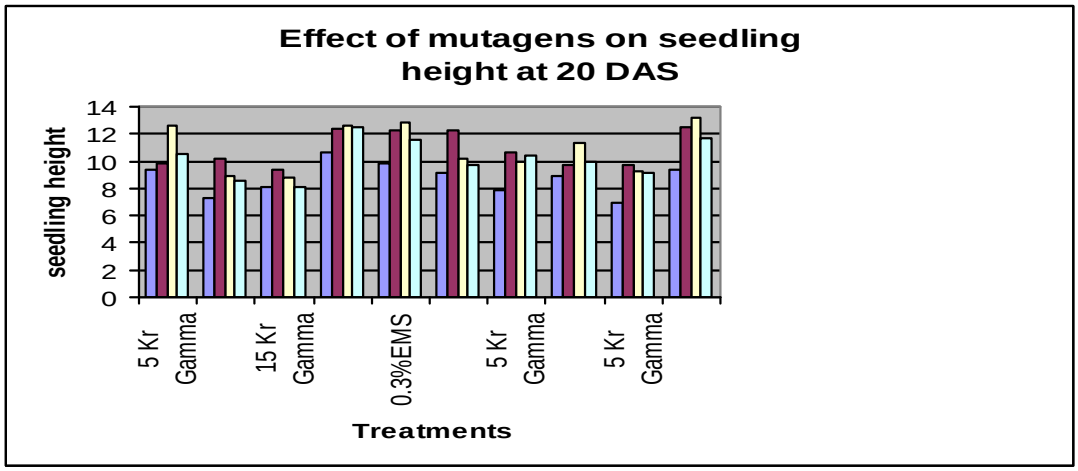

Fig 4
Effect of mutagens on seedling height at 20 days after sowing

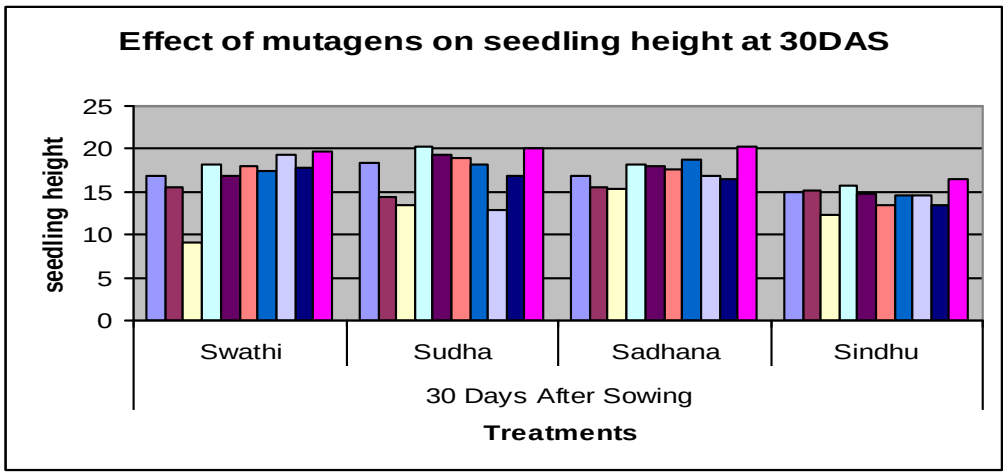

Fig 5
Effect of mutagens on seedling height at 30 days after sowing

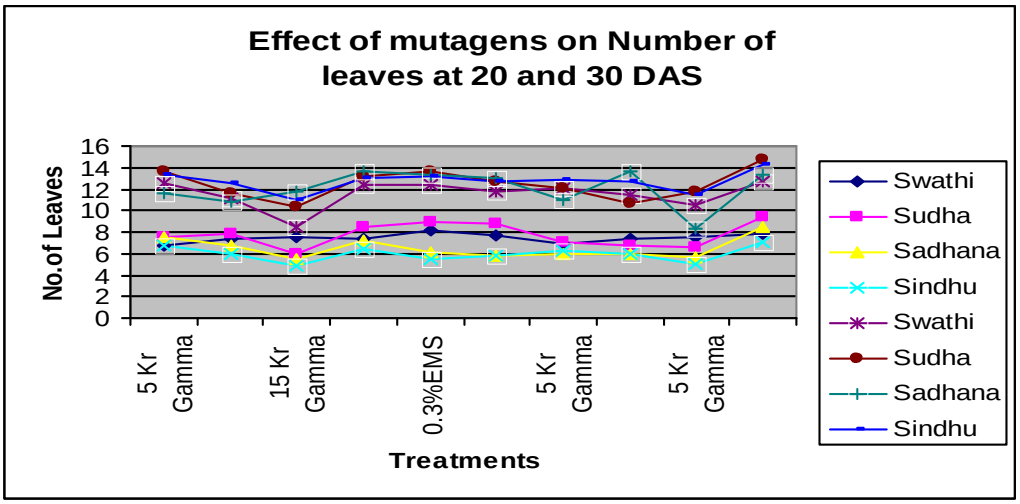

Fig 6
Effect of mutagens on number of leaves at 20 and 30 days after sowing

Table 1
Effect of mutagens on seed germination (per cent) in M_1 generation in four varieties of coriander (*Coriandrum sativum* L) in laboratory

Treatment	Coriander Varieties				Treatment mean	Coriander Varieties				Treatment mean
	Swathi	Sudha	Sadhana	Sindhu		Swathi	Sudha	Sadhana	Sindhu	
	Germination % In Lab					Germination % In Field				
5 Kr Gamma Rays	67.67	72	87	75.67	75.58	56	63.88	65.13	61.87	61.72
10 Kr Gamma Rays	62	61	70.67	71.33	66.25	50.5	55.59	62.2	56.43	56.18
15 Kr Gamma Rays	61	59.67	68.67	63.33	63.17	48.33	49.99	53.05	48.8	50.93
0.2 % EMS	72	72.33	72.33	75.33	73	64.93	66.54	58.03	60.13	62.81
0.3 % EMS	67.67	68.67	69.67	66.33	68.08	55.98	64.52	56.47	53.77	57.69
0.4 % EMS	66	63	62.33	65.33	64.17	51.5	58.18	54.73	49.57	53.5
5 Kr + 0.2 % EMS	77.67	65.67	76.33	72.33	73	58.01	59.78	64.63	61.03	60.9
5 Kr + 0.3 % EMS	74.23	62	69.33	70.67	68.58	56.07	56.34	59.63	55.84	56.97
5 Kr + 0.4 % EMS	73	60.67	67	64.67	66.33	51.97	51.35	53.1	53.43	52.46
CONTROL	86	90.67	91.67	90.67	89.75	75.37	85.2	75.2	71.57	77
Variety mean	**70.72**	**67.57**	**73.5**	**71.57**		**56.87**	**61.21**	**60.22**	**57.77**	
	SEm±.±	CD at 5%	CV(%)			SEm±.±	CD at 5%	CV(%)		
Variety	0.97	2.75	7.56			1.12	3.15	10.39		
Treatments	1.54	4.35				1.77	4.98			
VT	3.08	8.69				3.54	9.97			

Table 2
Effect of mutagens on shoot and root length in M_1 generation in four varieties of coriander (*Coriandrum sativum* L) under laboratory conditions

Treatment	Shoot length (cm)				Treatment mean	Root length (cm)				Treatment mean
	Swathi	Sudha	Sadhana	Sindhu		Swathi	Sudha	Sadhana	Sindhu	
5 Kr Gamma Rays	6.1	5.78	5.6	4.59	5.52	5.35	6.11	6.23	5.28	5.74
10 Kr Gamma Rays	5.23	5.13	4.83	4.48	4.92	4.73	5.57	5.8	4.85	5.24
15 Kr Gamma Rays	4.53	4.41	4.55	3.9	4.35	4.48	5.52	4.93	4.5	4.86
0.2 % EMS	5.03	8.41	6.4	7.45	6.83	5.43	6.74	6.13	5.43	5.93
0.3 % EMS	5.57	8.7	5.5	7.18	6.74	4.77	6.23	4.93	5.27	5.3
0.4 % EMS	5	8.51	5.04	6.06	6.15	4.6	6.14	5.04	4.83	5.15
5 Kr Gamma Rays + 0.2 % EMS	5.3	8.55	6.18	5.02	6.27	4.36	5.75	5.46	5.66	5.31
5 Kr Gamma Rays + 0.3 % EMS	4.2	6.64	5.44	4.97	5.31	6.24	5.67	4.26	5.16	5.33
5 Kr Gamma Rays + 0.4 % EMS	3.47	6.5	5.13	4.16	4.82	5.25	5.28	3.87	4.74	4.78
CONTROL	6.42	8.59	7.47	7.7	7.54	6.6	8.2	7.8	7.71	7.58
Variety mean	**5.09**	**7.12**	**5.61**	**5.55**		**5.18**	**6.12**	**5.44**	**5.34**	
SEm±	SEm.±	CD at 5%	CV (%)			SEm ±	CD at 5%	CV (%)		
Variety	0.06	0.18	6.95			0.04	0.11	3.99		
Treatments	0.1	0.28				0.06	0.18			
VT	0.2	0.56				0.13	0.36			

Table 3
Effect of mutagens on seedling height in M_1 generation in four varieties of coriander (C*oriandrum sativum* L) under Field conditions

Treatment	Seedling height of coriander varieties					Seedling height of coriander varieties				
	20 Days After Sowing					30 Days After Sowing				
	Swathi	Sudha	Sadhana	Sindhu	Treatment Mean	Swathi	Sudha	Sadhana	Sindhu	Treatment Mean
5 Kr Gamma Rays	9.40	9.83	12.62	10.57	10.61	16.77	18.41	16.92	14.88	16.75
10 Kr Gamma Rays	7.33	10.17	8.93	8.57	8.75	15.51	14.42	15.47	15.20	15.15
15 Kr Gamma Rays	8.07	9.37	8.77	8.10	8.58	9.09	13.37	15.43	12.37	12.57
0.2% EMS	10.66	12.41	12.60	12.47	12.03	18.14	20.31	18.12	15.78	18.09
0.3% EMS	9.85	12.24	12.87	11.53	11.62	16.95	19.40	18.06	14.70	17.28
0.4% EMS	9.18	12.27	10.13	9.67	10.31	17.91	18.89	17.68	13.48	16.99
5 Kr + 0.2% EMS	7.85	10.63	9.97	10.40	9.71	17.38	18.22	18.74	14.62	17.24
5 Kr + 0.3%EMS	8.95	9.69	11.30	9.97	9.98	19.31	12.88	16.81	14.59	15.90
5 Kr + 0.4%EMS	6.90	9.71	9.23	9.13	8.74	17.71	16.95	16.55	13.51	16.18
Control	9.33	12.50	13.17	11.63	11.66	19.78	20.00	20.35	16.51	19.16
Variety mean	**8.75**	**10.88**	**10.96**	**10.20**		**16.85**	**17.28**	**17.41**	**14.56**	
	SEm.±	CD at 5%	CV (%)			SEm.±	CD at 5%	CV (%)		
VAR	0.15	0.65	12.41			0.24	0.91	10.67		
TR	0.23	1.03				0.37	1.43			
VT	0.46	2.06				0.75	.87			

Table 4
Effect of mutagens on Number of leaves in M_1 generation in four varieties of coriander (*Coriandrum sativum* L) under Field conditions

Treatment	**Number of leaves in coriander varieties**					**Number of leaves in coriander varieties**				
	20 Days After Sowing					**30 Days After Sowing**				
	Swathi	Sudha	Sadhana	Sindhu	Treatment Mean	Swathi	Sudha	Sadhana	Sindhu	Treatment Mean
5 Kr Gamma Rays	6.77	7.50	7.57	6.77	**7.15**	12.60	13.63	11.53	13.27	**12.76**
10 Kr Gamma Rays	7.37	7.90	6.73	5.93	**6.98**	11.20	11.63	10.80	12.60	**11.56**
15 Kr Gamma Rays	7.54	6.00	5.53	4.87	**5.99**	8.47	10.37	11.80	10.97	**10.40**
0.2% EMS	7.43	8.50	7.27	6.37	**7.39**	12.33	13.20	13.60	12.97	**13.03**
0.3% EMS	8.17	8.97	6.13	5.50	**7.19**	12.40	13.67	13.33	13.10	**13.13**
0.4% EMS	7.70	8.83	5.77	5.77	**7.02**	11.73	12.70	13.07	12.77	**12.57**
5 Kr + 0.2% EMS	6.90	7.13	6.03	6.20	**6.57**	12.07	12.07	10.93	12.90	**11.99**
5 Kr + 0.3%EMS	7.43	6.73	5.93	5.90	**6.50**	11.47	10.73	13.67	12.73	**12.15**
5 Kr + 0.4%EMS	7.53	6.63	5.70	5.00	**6.22**	10.47	11.80	8.33	11.40	**10.50**
Control	7.83	9.47	8.53	7.13	**8.24**	12.73	14.77	13.37	14.23	**13.78**
Variety mean	**7.47**	**7.77**	**6.52**	**5.94**		**11.55**	**12.46**	**12.04**	**12.69**	
SEm±	SEm.±	CD at 5%	CV (%)			SEm .±	CD at 5%	CV (%)		
Variety (V)	0.15	0.41	11.60			0.24	0.67	10.65		
Treatment (T)	0.23	0.65				0.37	1.05			
V x T	0.46	1.30				0.75	2.11			

Number of Leaves:

The number of leaves at 20 days after sowing ranged from 6.77 (5Kr Gamma Rays) to 8.17 (0.3% EMS) in Swathi variety, whereas it is from 6.00 (15 Kr Gamma Rays) to 8.97 (0.3%EMS) in Sudha, 5.53 (15Kr Gamma rays) to 7.57 (5Kr Gamma Rays) in Sadhana, 4.87 (15Kr Gamma rays) to 6.77 (5Kr Gamma Rays) in Sindhu. Among the treatments 0.2%EMS had recorded more number of leaves (7.39) and 15Kr gamma rays recorded significantly lower number of leaves (5.99). Among the varieties, significantly more number of leaves were recorded in Sudha (9.47). In varieties Swathi and Sudha, sudden increase in number of leaves was observed at 0.3%EMS concentration (8.17 and 8.97 respectively) than lower concentration.

Significant differences were observed among the treatments with respect to number of leaves at 30 days after sowing. Among the different treatments under study, gamma rays decreased the number of leaves. The number of leaves at 30 days after sowing ranged from 8.47 (15 Kr Gamma Rays) to 12.6 (5Kr Gamma Rays) in Swathi variety, in Sudha it is from 10.37 (15Kr Gamma Rays) to 13.67 (0.3% EMS), 8.33 (5Kr Gamma Rays+ 0.4% EMS) to 13.67 (5Kr Gamma Rays+ 0.3% EMS) in Sadhana, 10.97 (15Kr Gamma rays) to 13.27 (5Kr Gamma Rays) in Sindhu. Among the mutagens, gamma rays reduced the number of leaves (10.4), whereas EMS had less effect for this character (13.13).In variety Sudha, sudden increase in number of leaves was observed at 0.3%EMS concentration (13.67).Similar trend was observed in Swathi and Sindhu also with Ethyl Methane Sulphonate treatments The inhibition of seedling growth due to mutagenic treatments, might be due to the auxin destruction or due to inhibition of auxin synthesis as reported by Gardon (1954)[11], due to inhibition of mitosis (Gunckel, 1957)[15] at growing points, may be due to destruction of auxin at the site of growing point (Smith and Kersten, 1942)[31]. The differential response of varieties to different mutagenic treatments with variation in seedling growth may be attributed to specific genetic differences (Budrik, 1956)[5], cytoplasmic differences (Reddy and Smith 1983)[25], levels of differentiation and development of embryo (Rahman and Sariano, 1972)[24] at the time of mutagenic treatment.

CONCLUSION

The present study indicated that, in general, the reduction in seed germination and seedling growth was more at the higher doses/concentration levels, indicating the greater sensitivity of coriander because of more physiological disturbances at higher concentration and it explains that, the growth variations could be caused by different mutagenic treatments at various doses. However, the lower treatments of these mutagens used in the present study can be successfully utilized for enhancing genetic variability.

ACKNOWLEDGEMENTS

The authors are thankful to the ANGRAU for providing facility of gamma rays and for providing facilities for research.

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