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Original Research Paper

Determination of lethal dose, effect of EMS and gamma rays on germination and seedling parameters in Coriander (*Coriandrum sativum*) var. CO (CR) 4

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ABSTRACT

Mutation breeding is a powerful tool to enrich variation particularly for attributes of economic importance in the crops like coriander where hybridization is difficult due to presence of complex umbels. An experiment was conducted to estimate the lethal dose of the chemical mutagen EMS and physical mutagen gamma rays in Coriander var. CO (CR) 4. Gamma dose ranging from 50 to 300 Gy and EMS treatments from 0.2 to 0.5% were tried to determine LD_{50} value under both laboratory and field conditions. Fifty per cent lethality had been observed at 200 Gy and 0.3% in gamma ray and EMS treatments, respectively, based on probit analysis. The relationship between mutagenic dose and germination percentage was inversely proportional. In M_0 generation, under field condition inverse relationship was observed between dose of mutagen. All the mutagens significantly affected the germination and seedling growth. The study revealed that germination percentage, root length, shoot length and vigour index decreased with increase in dose/concentration of the mutagens. Higher gamma rays and EMS doses had negative effect on the morphological characteristics and growth parameters of the seedlings derived from mutagen treated seeds. Lower treatments of these mutagens have influenced less biological damage and would be suitable for inducing desirable mutations in coriander. Therefore, consistent dose of gamma rays and EMS can be tested in other varieties or lines of coriander to generate variability for novel selection.

Keywords: Coriander, EMS, gamma ray, mutation, probit

INTRODUCTION

Seed spice is one of the important groups of crops cultivated in India for their large domestic consumption and bright export potential. Coriander (*Coriandrum sativum* L.) is an important spice and annual aromatic herb that belongs to the family Apiaceae. Coriander seeds and leaves are used as common food flavouring agent and as herbal medicine. India is the largest producer, consumer and exporter of coriander with a greater share in the world export market. Rajasthan is the major producer (60%) of coriander in India, followed by Madhya Pradesh, Andhra Pradesh, Karnataka, Tamil Nadu and Odisha.

Crop improvement through hybridization in coriander is very difficult due to the small size of its flowers and the limited availability of resistant sources. Coriander (2n=22) is an andromonoecious crop, where the stigma remains receptive for five days after anthesis. Pollination and artificial crossing in umbelliferous species, especially coriander, is a laborious process because of the small size of the flower buds. Given its importance, there is a need to improve coriander varieties to achieve better yields. Therefore, mutation breeding can be an efficient supplement for crop improvement.

Mutation breeding is a powerful tool to enrich variation particularly for attributes of economical importance in crops like coriander, where hybridization is difficult due to the presence of complex umbels. The spontaneous mutation frequency is low, typically ranging 10^5 to 10^8. Therefore, we need to induce mutations using physical and chemical mutagens. Physical and chemical mutagens cause physiological damages (injury), gene mutations (point mutations) and chromosomal aberrations in the biological material in the M_1 generation (Gaul, 1970). The present study was conducted to know the response of coriander seeds to gamma rays and EMS based on germination and biological parameters with the main aim of identifying appropriate dose or concentration of these mutagens for induction of desirable mutations.

MATERIALS AND METHODS

A coriander variety CO (CR) 4, was selected from the germplasm pool maintained at the Department of Spices and Plantation Crops at Horticultural College and Research Institute, TNAU, Coimbatore. The dry seeds of uniform size were exposed to different doses (50, 100, 150, 200, 250 and 300 Gy) of gamma rays at Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University, Coimbatore. Another set of pre-soaked seeds were treated with EMS solution (0.2%, 0.3%, 0.4% and 0.5%). Soaked untreated seeds were used as control.

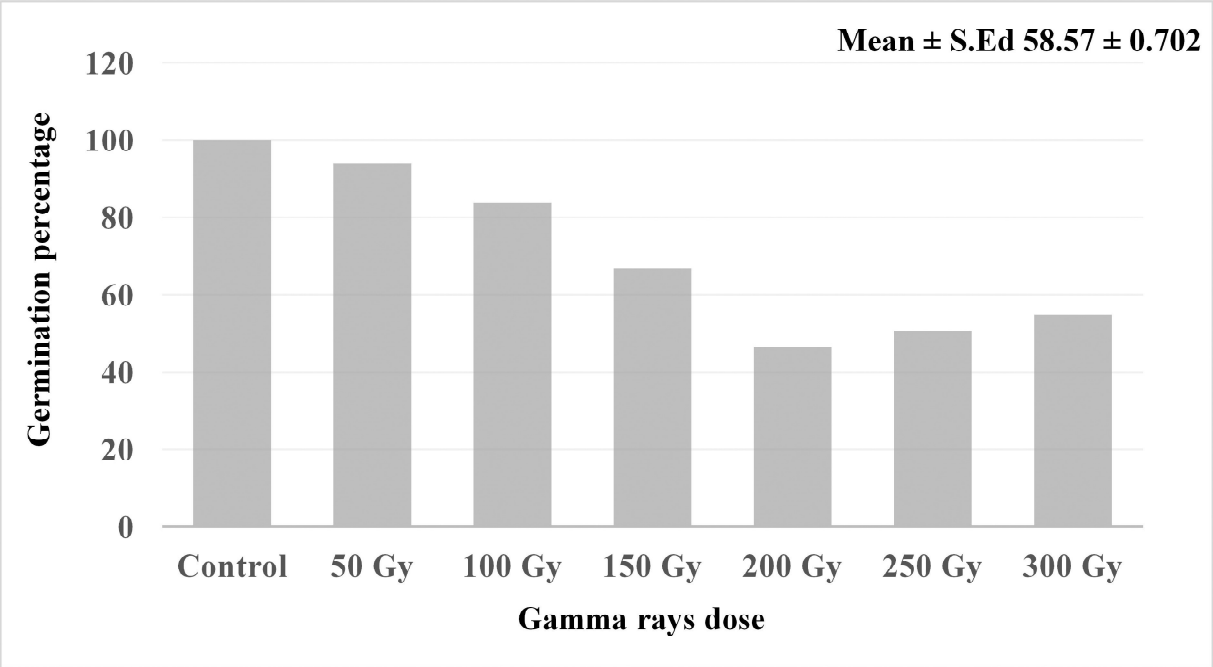
A preliminary study was carried out to determine the lethal dose (LD_{50}) for germination andsurvival with gamma ray irradiation and EMS treatments involving the selected variety under two different conditions: controlled laboratory conditions and field conditions. A total of twenty irradiated seeds per replication was sown in germination paper using the roll towel method with three replications per treatment under a temperature of 28±1R^{O}C. These were kept in a germination room to assess the LD_{50} value. The mutagen treated seeds were sown in field the day after treatment. A total of 550 seeds were sown in the field for each replication under a randomized block design with three replications, spaced 30 cm between rows and 10 cm between plants. The recommended agronomic practices and plant protection measures were followed uniformly for all the treatments. Germination % (11 DAS), survival per centage, shoot length, root length and seedling vigour were observed under laboratory condition and compared with the control. Observations on germination percentage (14 DAS), survival percentage (30 DAS), plant height, number of primary branches and number of secondary branches were recorded under field condition and compared with control. Pollen sterility was tested for each treatment by using a one per cent acetocarmine solution and examined under stereomicroscope. Stained and well filled grains were counted as fertile, while the non-stained and shrunken grains were treated as sterile. Pollen count was made from ten randomly selected plants of each treatment and expressed in per cent. Probit analysis was carried out to determine the lethal dose (LD_{50}) of gamma rays under lab and fieldconditions (Finney & Stevens, 1948).

RESULTS AND DISCUSSION

Coriander variety CO (CR) 4 was chosen to study the effect of different doses of EMS and gamma ray to determine LD_{50}values. LD_{50} for EMS and gamma ray were determined using probit analysis based on their germination rates (Fig 1 & 2). The optimum dose is the dosage that causes maximum of mutation with the minimum damage to the plant. In the present investigation, the LD_{50} value for EMS is 0.3% and for gamma irradiation is 200 Gy under both laboratory and field conditions (Table 1).

In this study, germination was reduced with an increased dose of mutagens in all treatments (Table 2). More reduction of germination percentage was observed at higher doses indicating dose dependency. Maximum reduction was observed under field conditions than laboratory condition indicating the sensitivity of the mutagens to environmental conditions. This indicates that the germination response is not only dose of the mutagen, genotype 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. The relationship between mutagenic dose and germination percentage was inversely proportional. Similar results were recorded by Singh et al. (1992), Saradaet al. (2015) and Prashant et al. (2020) in coriander. 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 (Chowdhury & Tah, 2011).

The mean shoot length, root length and seedling vigour were recorded under laboratory condition (Table 3). Consistent relationship was observed between the mutagenic doses and mean in the shoot length among all gamma ray treatments. Whereas, the EMS treatments had the trend of shoot length reduction when the dose level increases except 0.5% in EMS treatment.Gradual decrease in root length was observed with increase in concentration of the mutagenic doses. Root length per cent of control was recorded highest in 50 Gy (87.50). High per cent of control was observed at 0.2% (89.45). Overall mean per cent of root length was recorded high in EMS treatments with the mean percentage of 6.07 than



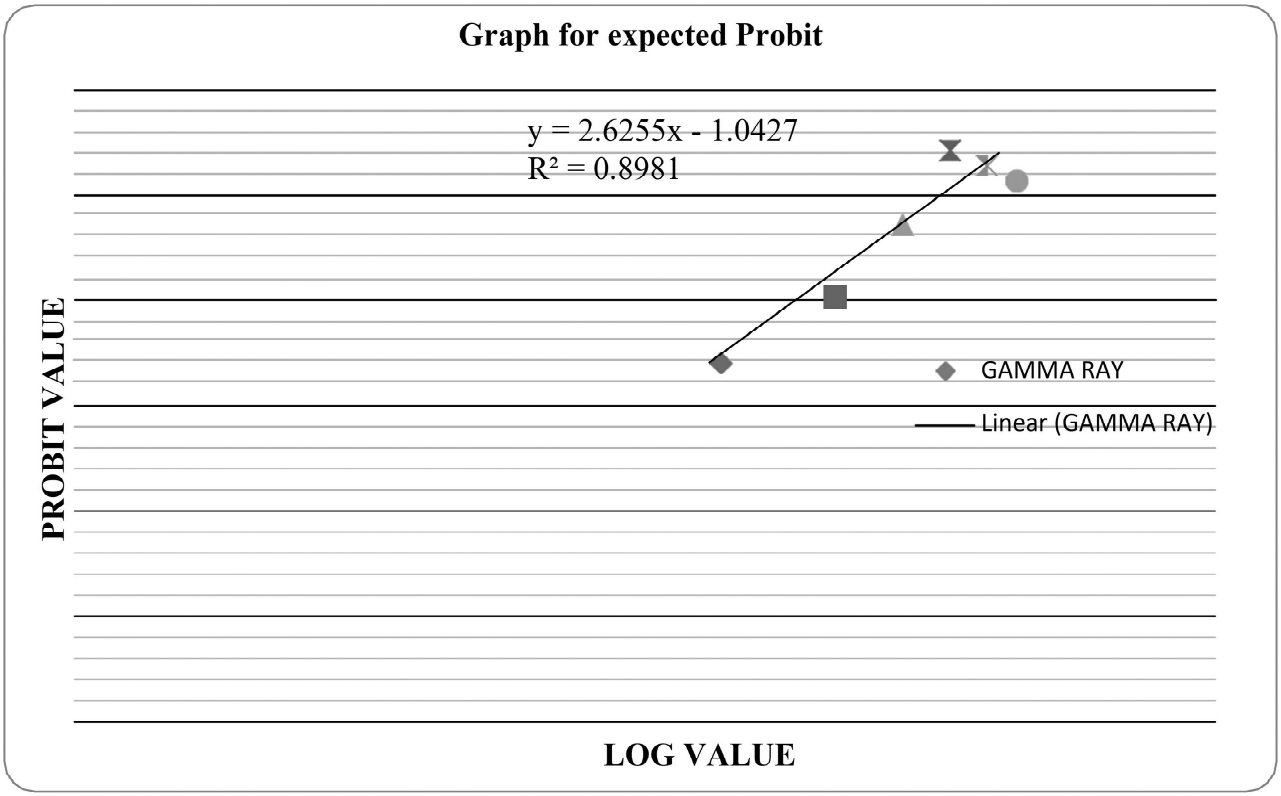
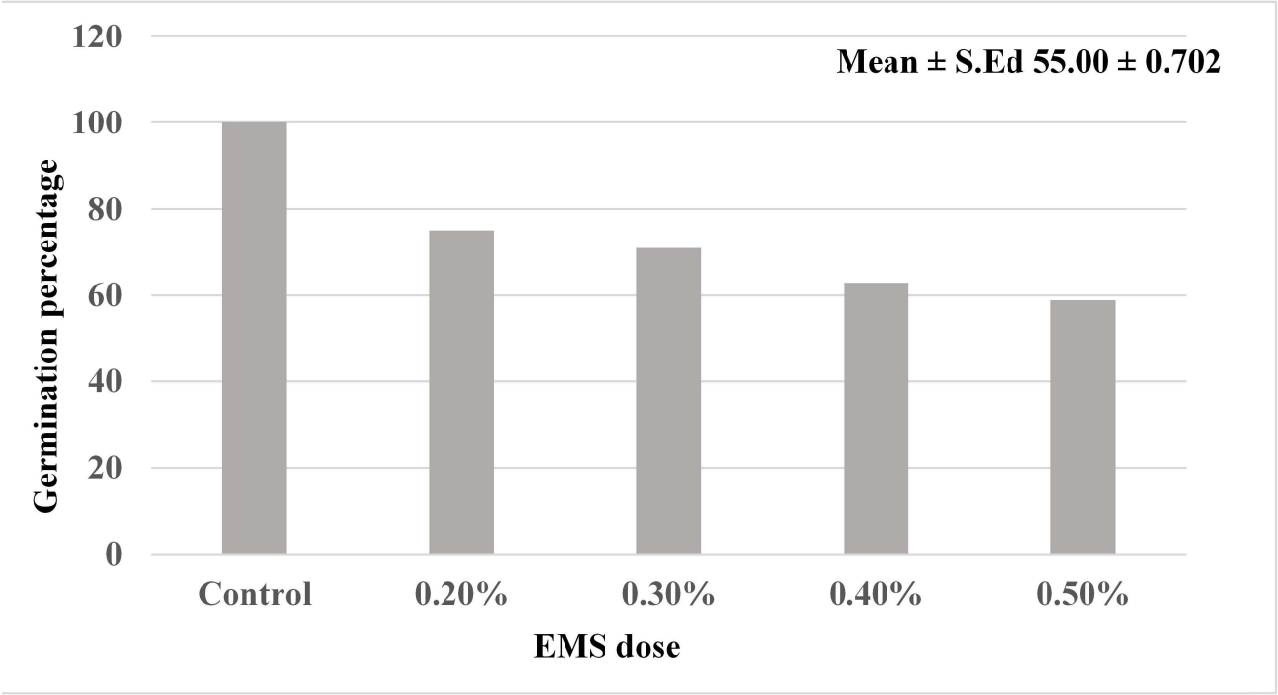


Fig. 1 : Effect of gamma irradiation on seed germination of Coriander var. CO (CR) 4



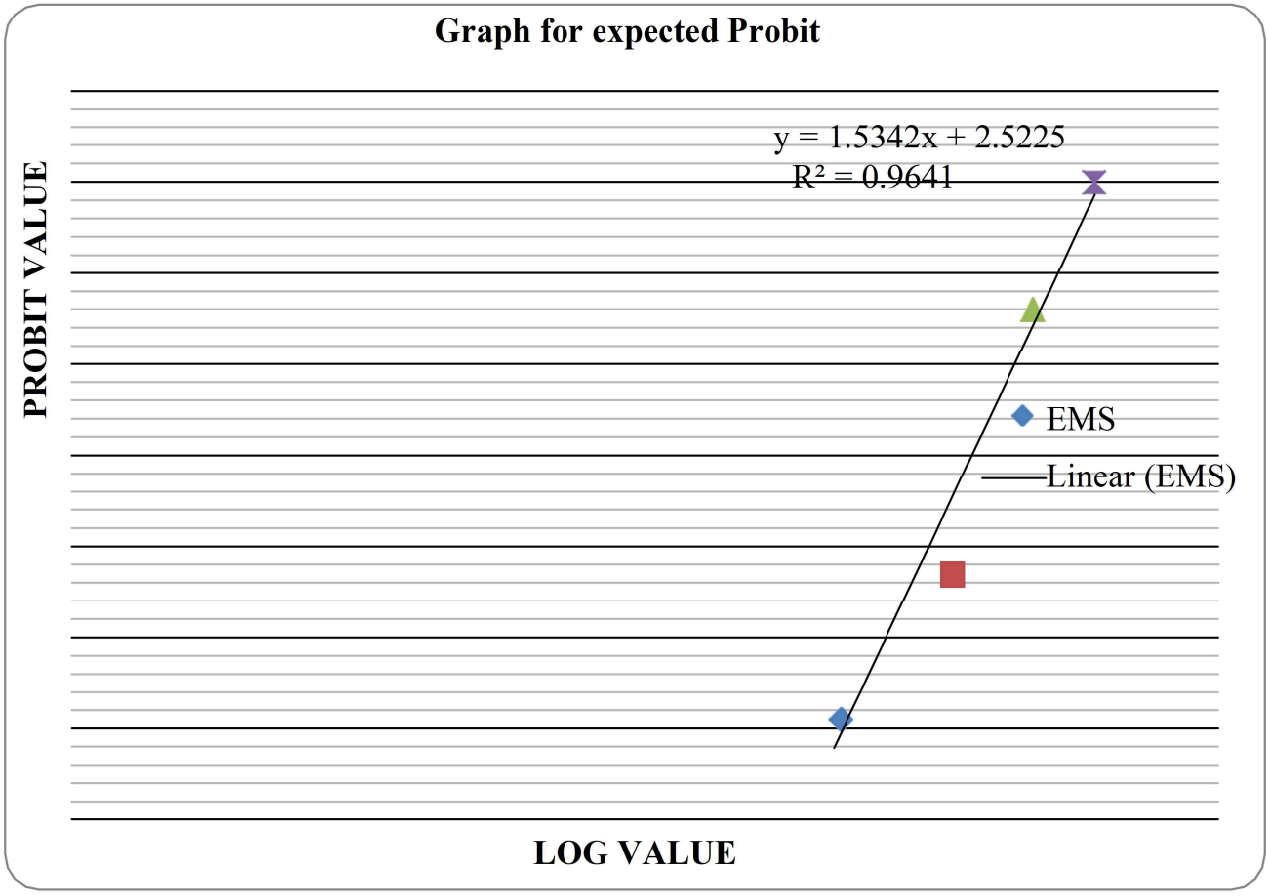


Fig. 2 : Effect of EMS on seed germination of Coriander var. CO (CR) 4

Table 1 : Determination of lethal doses by Gamma ray and EMS in Coriander var. CO (CR) 4: a probit analysis

Dose	Log value for dose of mutagen	Total number of plants	No. of plants dead	Mortality (%)	Corrected mortality (%)	Probit
Gamma rays						
50 Gy	1.70	20	3	15	5.6	3.41
100 Gy	2.00	20	5	25	16.7	4.03
150 Gy	2.18	20	9	45	38.9	4..72
200 Gy	2.30	20	14	70	66.7	5.43
250 Gy	2.40	20	13	65	61.1	5.28
300 Gy	2.48	20	12	60	55.6	5.14
Control	0	20	2	0	0	0
EMS						
0.2%	1.21	20	7	35	27.8	4.41
0.3%	1.38	20	8	40	33.3	4.57
0.4%	1.51	20	10	50	44.4	4.86
0.5%	1.60	20	11	55	50	5.00
Control	-	20	2	10	0	0

Table 2 : Effect of mutagens on germination of Coriander var. CO (CR) 4 in M_0 generation under laboratory and field condition

Treatment	Laboratory condition		Field condition	
	Germination (%)	Germination (%) over control	Germination (%)	Germination (%) over control
Gamma rays				
Control	90.00 (71.57)	100	93.76 (75.41)	100
50 Gy	85.00 (67.21)	93.92	76.77 (61.18)	81.13
100 Gy	75.00 (60.00)	83.84	73.29 (58.88)	78.07
150 Gy	55.00 (47.87)	66.89	72.08 (58.10)	77.04
200 Gy	30.00 (33.21)	46.41	48.42 (44.09)	58.47
250 Gy	35.00 (36.27)	50.68	27.64 (31.71)	42.06
300 Gy	40.00 (39.23)	54.82	20.48 (26.90)	35.68
Mean	58.57	-	53.11	-
SEd	0.702	-	0.718	-
CD (0.05%)	1.464*	-	1.49*	-
CV (%)	42.31	-	46.36	-
EMS				
0.2%	65.00 (53.73)	75.08	53.78 (47.16)	62.54
0.3%	60.00 (50.77)	70.94	48.88 (44.35)	58.82
0.4%	50.00 (45.00)	62.88	45.94 (42.67)	56.58
0.5%	45.00 (42.13)	58.87	34.83 (36.16)	47.96
Mean	55.00	-	45.85	-
SEd	0.702	-	0.718	-
CD (0.05%)	1.464*	-	1.49*	-
CV (%)	16.59	-	17.51	-

* Significant at 5% level; values in parenthesis are arc sign transformed

gamma ray treatments. Per cent reduction in seedling vigour ranged from 18.12 (50 Gy) to 74.87 (200 Gy) and was recorded highest at 300 Gy treatment. Inconsistent relationship between the dose of the mutagen and the per cent reduction was observed. Higher dose of EMS treatments recorded more reduction in vigour at 0.5%. The decreased seedling vigour with the increased mutagenic treatments may be attributed to an increase in physiological damage, variation in auxin level (Goud & Nayar, 1968), changes in the specific activity of few enzymes (Cherry et al., 1962) and physiological injury induced in the seeds and seedlings (Ignacimuthu & Babu, 1988).

Reduction on shoot and root length was invariably observed in all mutagenic treatments. The reduction was more conspicuous at higher doses and vice-versa indicating again dose dependency reduction in these parameters. In general, the shoot and root growth decreased with increased concentration of mutagens. According to Casarett (1968), the inhibition of root length can be attributed to the amount of radiation scattered from the region of elongation to the meristem. These findings are in close agreement with the reports of Jyothsna et al. (2022) and Patel et al. (2017) in Fenugreek, Verma et al. (2017a) in Cumin and Verma et al. (2017b) in Fennel.

Gaul (1970) reported that the biological damage caused by mutagens in the M_1 generation, affecting seed germination and plant survival, may be considered as an indication of mutagenic effects. In this investigation, the impact of different doses of EMS and Gamma rays on biological parameters such as survival percentage, plant height (35 DAS), number of primary branches, number of secondary branches, pollen fertility and seed fertility at maturity under field condition were recorded and presented in Table 4.

Table 3 : Effect of mutagens on seedling traits of Coriander var. CO (CR) 4 in M_0 generation under laboratory condition

Treatment	Shoot length (cm)	Shoot length (%) over control	Root length (cm)	Root length (%) over control	Seedling vigour	Seedling vigour (%) over control
Gamma rays						
Control	7.53	100	7.61	100	1362.35	100
50 Gy	6.46	85.85	6.66	87.50	1115.49	81.88
100 Gy	6.06	80.52	6.38	83.79	933.30	68.51
150 Gy	5.97	79.26	6.25	82.17	672.02	49.33
200 Gy	5.85	77.69	5.57	73.14	342.37	25.13
250 Gy	5.26	69.90	5.41	71.03	373.38	27.41
300 Gy	5.04	66.98	3.74	49.10	351.10	25.77
Mean	5.77	-	5.67	-	631.28	-
SEd	0.126	-	0.134	-	14.49	-
CD (0.05%)	0.264*	-	0.280*	-	30.238*	-
CV (%)	13.62	-	20.43	-	55.87	-
EMS						
0.2%	6.60	87.74	6.81	89.45	871.64	663.98
0.3%	6.47	85.97	6.03	79.29	750.24	55.07
0.4%	4.65	61.84	5.89	77.41	527.43	38.71
0.5%	5.53	73.44	5.56	73.02	498.84	36.62
Mean	5.81	-	6.07	-	662.04	-
SEd	0.126	-	0.134	-	14.49	-
CD (0.05%)	0.264*	-	0.280*	-	30.238*	-
CV (%)	15.65	-	8.72	-	27.08	-

* Significant at 5% level

Table 4 : Impact of gamma ray and EMS on biological parameters of Coriander var. CO (CR) 4 in M_0 & M_1 generation

Treatment	M_0 generation								M_1 generation			
	Survival percentage	Survival (%) over control	Plant height (cm)	Plant height (%) over control	No. of primary branches	No. of primary branches (%) over control	No. of secondary branches	No. of secondary branches (%) over control	Pollen sterility (%)	Pollen sterility (%) over control	Seed Fertility (%)	Seed Fertility (%) over control
Gamma rays												
Control	92.48 (74.08)	100	49.20	100	11.00	100	6.60	100	24.13 (29.42)	100	73.06 (58.73)	100
50 Gy	73.93 (59.30)	80.04	47.26	96.06	9.10	82.73	6.25	94.70	-	-	-	-
100 Gy	71.00 (57.42)	77.50	45.38	92.24	8.80	80.00	6.92	104.85	22.58 (28.37)	96.43	70.08 (56.84)	96.78
150 Gy	70.65 (57.20)	77.21	43.40	88.21	7.67	69.73	6.53	98.94	29.27 (32.75)	111.33	68.99 (56.16)	95.63
200 Gy	44.34 (41.75)	56.36	37.80	76.83	6.53	59.36	6.88	104.24	30.90 (33.77)	114.79	71.32 (57.62)	98.11
250 Gy	35.20 (36.39)	49.12	31.03	63.07	6.25	56.82	6.47	98.03	35.06 (36.31)	123.41	69.72 (56.62)	96.40
300 Gy	21.41 (27.56)	37.20	23.36	47.48	6.11	55.55	6.23	94.39	39.68 (39.04)	132.71	70.68 (57.22)	97.42
Mean	52.76	-	38.03	-	7.41	-	6.55	-	31.50	-	70.16	-
SEd	0.994	-	0.917	-	0.170	-	0.123	-	0.437	-	0.786	-
CD (0.05%)	2.07*	-	1.914*	-	0.356*	-	0.259*	-	0.919*	-	1.652*	-
CV (%)	43.15	-	23.91	-	22.87	-	4.16	-	21.39	-	2.02	-
EMS												
0.2%	52.43 (46.39)	62.62	39.12	79.51	8.13	73.91	6.29	95.30	25.28 (30.18)	102.60	71.33 (57.62)	98.12
0.3%	46.13 (42.78)	57.75	34.97	71.08	7.60	69.09	5.41	81.97	30.00 (33.21)	112.88	71.29 (57.60)	98.08
0.4%	43.55 (41.29)	55.74	29.74	60.45	6.33	57.55	5.45	82.58	35.38 (36.50)	124.06	68.25 (55.70)	94.85
0.5%	32.63 (34.84)	47.02	26.83	54.53	6.23	56.64	6.07	91.97	35.53 (36.59)	124.36	62.49 (52.23)	88.94
Mean	43.69	-	32.66	-	7.07	-	5.81	-	31.55	-	68.34	-
SEd	0.994	-	0.917	-	0.170	-	0.123	-	0.437	-	0.786	-
CD (0.05%)	2.07*	-	1.914*	-	0.356*	-	0.259*	-	0.919*	-	1.652*	-
CV (%)	18.90	-	16.72	-	13.30	-	7.62	-	15.55	-	6.08	-

* Significant at 5% level; values in parenthesis are *arc sign* transformed

The percentage of survival mean ranged from 21.41 (300 Gy) to 73.93 (50 Gy) with high per cent of control in 50 Gy (80.04). In EMS treated plants, the mean survival percentage ranged from 32.63 (0.5%) to 52.43 (0.2%) with high per cent of control at 0.2% (62.62). Gamma treatment, showed highest per cent of survival compared to EMS with theoverall mean percentage of 52.76 per cent. All treatments resulted in linear reduction of survival percentage. This clearly indicated that both the mutagens caused significant influenceon survival that displayed a dose dependent negative linear relationship between dose and survival percentage. The decline in survival percent may be due to absorption of ionizing radiation in biological materials, acting directly on critical targets in the cell (Kovács & Keresztes, 2002). These observations were supported by Mishra & Haq (2014) in Isabgol (*Plantago ovate*, Forsk).

A linear reduction in plant height was observed with increase in dose of the gamma rays mean from 23.36 (300 Gy) to 47.26 (50 Gy). Same trend of reduction as of gamma rays was observed in EMS with mean ranging from 26.83 (0.5%) to 39.12 (0.2%). The number of primary branches in gamma treatments showed a reduction of about 17.27 (50 Gy) to 44.45 (300 Gy). In EMS treated plants per cent reduction ranged from 26.09 (0.2%) to 43.36 (0.5%). Increase of 4.84 and 4.24 per cent of secondary branches in gamma treatments were observed in 100 Gy and 200 Gy, respectively. The per cent mean for secondary branches ranged from 5.41 (0.3%) to 6.29 (0.2%) in EMS treatments (Table 4). The reduction in seedling height could be due to effect in physiological processes. The frequency of seedling height and seedling injury decreased with increasing dose was reported by Bashir et al., (2013) in *Trigonella foenumgraecum* and in *Coriandrum sativum* L. (Salve & More, 2014). Present study depicted those seeds exposed to higher doses (300 Gy) produced the shortest plants with reduced root length compared to the other doses. This reduction could be attributed to reduce in mitotic activity in meristematic tissues and reduced moisture content in seeds (Khalil et al., 1986). Similarly, Norfadzrin et al. (2007) also reported that higher gamma ray doses (600 and 800 Gy) had negative effect on the morphological characteristics of tomato and okra seedlings derived from irradiated seeds.

The effect of mutagens on pollen sterility in the M_1 generation presented in Table 4. Pollen sterility reduction due to gamma ray treatment ranged from 132.71 per cent (300 Gy) to 96.43 per cent (100 Gy), while, EMS treatment ranged from 124.36 per cent (0.5%) to 102.60 per cent (0.2%). The percentage of seed fertility over the controlvaried from 98.11 (200 Gy) to 95.63 (150Gy) in gamma treatments, and from 98.12 (0.2%) to 88.94 (0.5%) in EMS treatments. Mutagen induced sterility in the M_1 generation is mainly caused by small or minute deficiencies. According to Gaul & Mittelstensceid (1960) such mutagen induced M_1 sterility is transferred into later generations. Kumar & Mani (1997) suggested that failure of homologous pairing during meiosis could be the main cause of high pollen sterility. Sinha & Godward (1972) described those chromosomal aberrations, particularly, translocations may be responsible for the reduction in pollen sterility. The seed fertility ratio was found to be higher in control rather than the treated one. In contrast, Rao & Suvartha (2006) observed that the highest seed fertility ratio was found in the irradiated plants of tomato.

Seedling injury represented by reduction in shoot length, root length, plant height is broadly used as an index of determining biological effects of various physical mutagens in M_1 generation, which may be due to the metabolic processes affected at embryonic level. Sparrow (1961), while, working on cytological effect of radiation, concluded that the decrease in vegetative growth is a result of radiation induced cytological changes like chromosomal damages, inhibited mitotic division, degeneration of nuclei and cell enlargement etc.

CONCLUSION

The optimum dosage for mutagenizing coriander variety CO (CR) 4 for the optimum recovery of viable mutants, is 0.3% for EMS and 200 Gy for gamma rays. These doses would be suitable, for producing viable mutants and maintaining population for mutation breeding.

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