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Influence of gamma rays and ethyl methane sulphonate on germination and seedling survival in sesame (*Sesamum indicum* L.)

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SUMMARY

An investigation was carried out to study the influence of gamma rays and EMS (Ethyl methane sulphonate) on germination and seedling survival in *Sesamum indicum* L. Two sesame varieties were treated with gamma rays ^{60}Co source with doses of 10,20,30,40 and 50 krad followed by Ethyl methane sulphonate with concentrations of 0.8, 1.0, 1.2, 1.4 and 1.6 per cent. The LD_{50} values based on germination reduction in the M_1 generation were fixed at 30krad and 1.2 per cent for gamma rays and EMS, respectively. The seed germination percentage was reduced more under chemical mutagen than under physical mutagen treatment. Survival rate was less in cardeboriga when compared to SVPR 1, irrespective of mutagens. The percentage of seed germination, shoot length and root length decreased progressively with increasing dose/concentration of both the mutagens in both the varieties. In M_1 both gamma rays and EMS produced deleterious effect on seedling survival.

Key words : Sesame, Seeds, Gamma ray, Ethyl methane sulphonate, Germination and seedling survival

The improvement of a cultivar is usually accomplished by adding one or two desirable attributes to the initial strain and if these desirable characters happened to be introduced by mutagens, it is certainly the simplest means to achieve the breeding objectives (Moe and Han, 1973). The inhibition of seed germination in M_1 generation was the indication of degree of radio sensitivity of different genotypes and the extent of damage caused by the mutagens (Gaul, 1958).During early phase, the seedlings adjust or repair themselves to eliminate the dead and un wanted cells. On the other hand, some of the seedlings were not able to overcome the radiation damage and they die before they put forth any side effects.The present experiment was carried out to study the influence of gamma ray and ethyl methane sulphonate on germination and seedling survival in sesame and to provide scientific basis for sesame mutation breeding.

MATERIALS AND METHODS

Two promising sesame genotypes namely, SVPR 1(ruling popular white seeded type) and Cardeboriga(monostem African type) were treated with the two mutagens *viz.*, gamma rays and EMS. Two hundred well filled dry seeds were sealed in butter paper covers and exposed to 10 to 50 krad doses of gamma rays from ^{60}Co source at Indira Gandhi Centre for Research, Kalpakkam, Tamil Nadu. Another variety of two hundred seeds of each variety, for each treatment were presoaked in distilled water for four hours then treated with different concentrations of EMS ranging from 0.8 to 1.6 per cent for three hours. After the treatment, the seeds were thoroughly washed with tap water ten times.

Laboratory studies:

From the gamma irradiated and chemical treated seeds, 100 seeds were placed in the moist germination paper replicated twice for the purpose of laboratory analysis, respectively. The following observations namely, (i) Seed germination, (ii) Shoot length (iii) Root length and (iv) Vigour index were recorded.

Seed germination:

Germinated seeds were counted from third to seventh day. Emergence of cotyledonary leaf was taken as the indication of germination. Germination percentage was worked out in each treatment in each genotype separately.

Shoot length:

The length of the shoot from the cotyledonary node to the tip of the shoot was measured on ten randomly

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selected seedlings on the seventh day and expressed in cm.

Root length:

The root length from the cotyledonary node to the tip of the primary root was measured on ten randomly selected seedlings and expressed in cm.

Vigour index:

The seedling length from root tip to stem tip was measured on seventh day in ten randomly selected seedling and numerically represented.

Field studies:

The treated seeds were sown in the field along with control in a Randomized Block Design with Four replications by adopting a spacing of 30 cm between rows and 30 cm between plants. All the recommended agronomic practices and plant protection measures were followed uniformly for all the treatments. The following observations were made in ten randomly selected plants in each variety and in each treatment replication wise.

Seed germination:

The total number of seeds germinated on 15th day after sowing was counted and expressed in percentage.

Seedling survival:

The total number of seedlings survived on 30th day after sowing was counted in entire population and expressed in percentage.

RESULTS AND DISCUSSION

Evaluation of the effects of mutagens in M_1 generation is a common procedure in any mutation breeding experiments. Physical and chemical mutagens induce physiological damages (injury), gene mutations (point mutations) and chromosomal mutations (Chromosomal aberrations) in the biological material in M_1 generation (Gaul, 1970). The biological damages caused by the mutagens in M_1 generation could be measured based on seed germination, survival reduction (lethality), plant growth reduction (injury) and fertility reduction (sterility). Gaul (1970) reported that the damage to the biological material as reflected in the above parameters might be considered as an indication of the mutagenic effects. As similar to aforesaid findings, the following different M_1 damages (effects) have been studied in the present investigation with sesame.

Fixation of LD_{50} value:

The observed data on germination percentage and germination reduction are presented in Table 1. As regards

Table 1 : Effect of mutagens on germination at laboratory condition in M_1 generation

Treatments	SVPR 1			Treatments	Cardeboriga		
	Mean	Transformed mean	Per cent reduction		Mean	Transformed mean	Per cent reduction
γ - ray				γ - ray			
V_1T_0	98.00	81.87	-	V_2T_0	96.00	78.55	-
V_1T_1	78.66	62.48*	23.69	V_2T_1	72.00	58.05*	26.10
V_1T_2	67.66	55.34*	32.41	V_2T_2	60.33	50.96*	35.13
V_1T_3	52.00	46.14*	43.65	V_2T_3	49.66	44.80*	42.97
V_1T_4	42.33	40.58*	50.44	V_2T_4	40.66	37.06*	52.82
V_1T_5	41.00	39.81*	51.38	V_2T_5	36.33	36.61*	53.40
EMS				EMS			
V_1T_0	98.00	81.87	-	V_2T_0	969.00	78.55	-
V_1T_6	65.66	54.12*	33.90	V_2T_6	62.33	52.13*	33.64
V_1T_7	58.66	49.98*	38.96	V_2T_7	56.66	48.66*	38.06
V_1T_8	51.33	45.76*	44.11	V_2T_8	50.00	45.00*	42.72
V_1T_9	44.33	41.74*	50.02	V_2T_9	43.66	41.35*	47.36
V_1T_{10}	36.00	36.87*	54.97	V_2T_{10}	42.33	40.58*	48.34

Treatment	Mean	S.E. ±	C.D. (P=0.05)	CV (%)	Treatment	Mean	S.E. ±	C.D. (P=0.05)	CV (%)
γ-rays	54.37	0.47	0.97	14.89	γ-rays	51.0	0.44	0.91	14.76
EMS	51.72	0.19	0.39	12.02	EMS	51.04	0.19	0.39	12.14

* indicates significance of value at P=0.05

gamma rays under laboratory condition, the germination ranged from 41.00 (V_1T_5) to 78.66 per cent (V_1T_1) in SVPR 1 and from 36.33 (V_2T_5) to 72.00 per cent (V_2T_1) in Cardeboriga. The 50 per cent reduction crossed between two treatments V_1T_3 and V_1T_4 in SVPR 1 whereas in Cardeboriga, it occurred between V_2T_2 and V_2T_3. In case of EMS, the germination percentage extended from 36.00 (V_1T_{10}) to 65.66 (V_1T_6) in SVPR 1 and from 42.33 (V_2T_{10}) to 62.33 (V_2T_6) in Cardeboriga. The LD_{50} value occurred in between V_1T_8 and V_1T_9 in SVPR 1, while in Cardeboriga, it was in V_2T_8.

Germination:

In the present study, the percentage of seed germination, shoot length and root length decreased progressively with increasing dose / concentration of both the mutagens in both the varieties. The decrease in germination due to mutagenic treatment observed in the present study was also in conformity with the earlier reports of Anitha Vasline (1998), Radhakrishnan *et al.* (2001), Rizwana Banu (2000) in cowpea, Sheebha *et al*.(2003) in Sesame, Samiullah Khan *et al.* (2004) in blackgram, Janila *et al*.(2007) in castor and Mansah *et al*.(2007) in sesame.

The seed germination percentage was reduced more under chemical mutagen than under physical mutagen treatments. Maximum reduction was recorded at 50 krad of gamma rays in Cardeboriga (58.82 per cent reduction over control) and 1.6 per cent of EMS in SVPR 1 (59.03 per cent reduction over control).The white seeded genotype (SVPR 1) is more sensitive than the black seeded one (Cardeboriga) to EMS. This finding is in agreement with the report of Ganesh kumar *et al*. (2001) in sesame.

In the present study, considerable reduction in shoot and root development was noted. An inverse relationship was exhibited between the mutagenic dosages and the reduction rate of shoot and root development. The influence on shoot and root growth has been related to many factors which may be attributed to chromosomal abnormality with height reduction, reduction in auxin levels, inhibition of auxin synthesis, failure of assimilation mechanism and chromosomal damage cum mitotic inhibition (Riley, 1954). The hampered protein synthesis in the embryonic cells could also prevent passage of cells from G_1 onwards thereby retarding the emergence of root and shoot.

In accordance with the above findings, in the present investigation, at higher dosage of mutagens the seed germination got delayed and the seedlings were shorter which subsequently died in a short period. This might be due to the effect of mutagens by which, affected seedlings after the cotyledonary emergence remained alive only for a particular period of time (Dubinin, 1964).

Vigour index :

The vigour index of the seedlings was reduced due to the treatments of gamma rays and EMS is presented in Table 2 . As regards to gamma rays, the vigour index value ranged from 93.87 (V_1T_5) to 465.55 (V_1T_1) in SVPR 1 and from 63.24 (V_2T_5) to 457.93 (V_2T_1) in Cardeboriga. In case of EMS, the vigour index value

Table 2 : Effect of mutagens on vigour index under laboratory conditions in M_1 generation

Treatments	SVPR 1				Treatment	Cardeboriga			
	Germination Per cent	Shoot length	Root length	Vigour index		Germination Per cent	Shoot length	Root length	Vigour index
γ - ray						γ - ray			
V_1T_0	81.87	7.34	6.25	1112.6	V_2T_0	78.55	6.99	5.99	1019.57
V_1T_1	67.66	3.45	3.43	465.55	V_2T_1	60.33	3.38	4.21	457.93
V_1T_2	52.00	3.82	1.98	301.60	V_2T_2	49.66	2.95	2.25	258.27
V_1T_3	42.33	2.64	2.89	234.10	V_2T_3	40.66	2.45	2.78	212.67
V_1T_4	41.00	1.95	1.65	147.60	V_2T_4	36.33	1.54	1.65	115.90
V_1T_5	36.00	1.37	1.19	93.87	V_2T_5	31.00	1.15	0.89	63.24
EMS						EMS			
V_1T_0	81.87	7.34	6.25	1112.6	V_2T_0	78.55	6.99	5.95	1019.57
V_1T_6	65.66	4.67	6.03	628.43	V_2T_6	62.33	3.86	4.28	507.60
V_1T_7	58.66	3.91	5.40	472.26	V_2T_7	56.66	1.94	3.69	319.41
V_1T_8	51.33	3.32	5.85	301.35	V_2T_8	50.33	0.89	3.91	241.60
V_1T_9	44.33	2.78	4.90	240.29	V_2T_9	43.66	0.75	2.25	131.00
V_1T_{10}	36.00	1.80	4.14	134.64	V_2T_{10}	32.33	0.55	1.36	61.86

extended from 134.64 (V_1T_{10}) to 628.43 (V_1T_6) in SVPR 1 and from 61.86(V_2T_{10}) to 507.60(V_2T_6) in Cardeboriga. An increase in dosage of mutagen led to a gradual reduction in vigour index.

Seedling survival:

The per cent reduction in survival of plants over control on 15th and 30th day is given in Tables 3 and 4, respectively. During early phase, the seedlings could adjust

Table 3 : Effect of mutagens on seedling survival on 15th day in M_1 generation

Treatments	SVPR 1			Treatments	Cardeboriga		
	Mean	Transformed mean	Per cent reduction		Mean	Transformed mean	Per cent reduction
γ - ray				γ - ray			
V_1T_0	97.00	80.02	-	V_2T_0	95.00	77.08	-
V_1T_1	78.33	62.25*	22.21	V_2T_1	74.33	59.56*	22.73
V_1T_2	65.33	53.92*	32.62	V_2T_2	63.65	52.92*	31.35
V_1T_3	52.00	46.14*	42.34	V_2T_3	50.66	45.37*	41.14
V_1T_4	44.33	41.74*	47.84	V_2T_4	42.33	40.58*	47.36
V_1T_5	38.00	38.05*	52.45	V_2T_5	35.00	36.27*	52.95
EMS				EMS			
V_1T_0	97.00	80.02	-	V_2T_0	95.00	77.08	-
V_1T_6	81.66	64.64*	19.23	V_2T_6	79.33	62.95*	18.34
V_1T_7	68.00	55.55*	30.58	V_2T_7	60.00	50.76*	34.15
V_1T_8	51.33	45.76*	42.82	V_2T_8	53.33	46.91*	39.15
V_1T_9	49.33	44.61*	44.26	V_2T_9	46.66	43.08*	44.12
V_1T_{10}	44.00	41.55*	48.08	V_2T_{10}	41.00	39.81*	48.36

Treatment	Mean	S.E. ±	C.D. (P=0.05)	CV (%)	Treatment	Mean	S.E. ±	C.D. (P=0.05)	CV (%)
γ-rays	53.68	1.15	2.36	11.92	γ-rays	51.96	1.16	2.38	12.30
EMS	55.35	2.36	4.84	23.65	EMS	53.43	0.82	1.69	8.82

* indicates significance of value at P=0.05

Table 4 : Effect of mutagens on seedling survival on 30th day in M_1 generation

Treatments	SVPR 1				Treatment	Cardeboriga			
	Germination per cent	Shoot length	Root length	Vigour index		Germination per cent	Shoot length	Root length	Vigour index
γ - ray						γ - ray			
V_1T_0	95.70	78.03	-	1112.6	V_2T_0	89.33	70.93	-	1019.57
V_1T_1	73.00	58.69*	24.79	465.55	V_2T_1	69.33	56.37*	20.53	457.93
V_1T_2	61.33	51.55*	33.94	301.60	V_2T_2	58.66	49.98*	29.54	258.27
V_1T_3	49.33	44.61*	42.83	234.10	V_2T_3	46.66	42.89*	39.53	212.67
V_1T_4	39.66	39.03*	49.98	147.60	V_2T_4	37.00	37.46*	47.19	115.90
V_1T_5	36.06	36.06*	53.79	93.87	V_2T_5	31.60	34.20*	51.78	63.24
EMS						EMS			
V_1T_0	95.71	78.03	-	1112.6	V_2T_0	89.33	70.93	15.73	1019.57
V_1T_6	77.66	61.79*	20.81	628.43	V_2T_6	74.66	59.77*	28.99	507.60
V_1T_7	64.33	53.32*	31.67	472.26	V_2T_7	59.33	50.37*	35.48	319.41
V_1T_8	48.00	44.61*	42.83	301.35	V_2T_8	51.33	45.76*	35.49	241.60
V_1T_9	45.33	42.32*	45.76	240.29	V_2T_9	42.66	40.78*	42.50	131.00
V_1T_{10}	39.33	38.84*	50.22	134.64	V_2T_{10}	38.00	38.05*	46.36	61.86

Treatment	Mean	S.E.±	C.D. (P=0.05)	CV (%)	Treatment	Mean	S.E.±	C.D. (P=0.05)	CV (%)
γ-rays	51.32	0.75	1.54	18.38	γ-rays	48.63	0.82	1.67	9.36
EMS	53.15	0.91	1.86	19.79	EMS	50.94	0.92	1.88	10.13

* indicates significance of values at P=0.05

or repair themselves to eliminate the dead or unwanted cells. On the other hand, some of the seedlings were not able to overcome the radiation damage and hence, they died before they put forth any side effects. This might be due to inhibition of auxin synthesis (Skoog, 1935), lack of assimilatory mechanism, inhibition of mitosis and chromosomal damages (Gunckel and Sparrow, 1961).

An inverse relationship between the dosage of mutagen and survival on 15th and 30th day was observed in both the genotypes. The survival reduction on 15th day ranged from 30.83 in 10 krad to 57.72 cent in 50 krad in gamma rays and from 28.18 in 0.8% to 53.83 per cent in 1.6% in EMS in SVPR 1. In Cardeboriga, this was between 28.17 in 10 krad and 53.83 per cent in 50 krad in gamma rays and between 30.05 in 0.8% to 55.76 per cent in 1.6% in EMS, respectively. More than 50 per cent reduction in survival on 15th and 30th day was observed in higher dosage between 30 to 40 krad of gamma rays and between 1.2 and 1.4 per cent of EMS in both SVPR 1 and Cardeboriga.

Survival rate was less in Cardeboriga when compared to SVPR 1, irrespective of mutagens. The differential sensitivity of genotypes may be attributed to their metabolic processes affected in differential manner, either by mutagen uptake or degradation and sites of action in the embryo (Ahmed John, 1996). Many workers *viz.,* Anitha Vasline (1998), Khin Mark Mar New *et al.* (2001) and Mansah *et al.* (2007) have reported a dose dependent decrease in plant survival in sesame.

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