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Mutagenic efficiency of gamma-rays, ethyl methane sulphonate and its combination on microsperma lentil (*Lens culinaris* Medik)

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ABSTRACT

Seeds of microsperma lentil (*Lens culinaris* Medik) variety Pant L-406 were selected for the mutagenic treatment. Fresh healthy seeds were irradiated with 10kR, 15kR, 20kR, 25kR, and 30kR doses of gamma-rays. Healthy seeds of this variety were presoaked in distilled water and treated with different concentration of ethyl methane sulphonate (0.02M, 0.03M, 0.04M and 0.05M). Half of the irradiated seeds were also treated with 0.03M ethyl methane sulphonate (EMS) for combination treatments. Observations were recorded for percent germination, seedling height, pollen fertility, plant survival and chromosomal abnormalities in M_1 generation. Frequency and spectrum of chlorophyll and viable mutations were scored in M_2 generation. Higher doses of mutagen either alone and / or in combinations severely reduced the percentage of germination, seedling height, pollen fertility and plant survival than their corresponding lower doses. The percent chromosomal abnormalities *viz*. fragments, bridges, laggards and micronuclei were increased with increase in dosage/ concentration of mutagens. Mutagenic efficiency was computed on the basis of seedling injury, lethality and pollen sterility. Lower dose of EMS (0.02M) was found to be most efficient in inducing more chlorophyll and viable mutations. Various types of chlorophyll and viable mutations were identified from all the treatments.

Key words : Lentil, Mutation frequency, Chromosomal aberratious, Mutagenic efficiency.

INTRODUCTION

Lentil is grown as a winter crop all over India either as sole or mixed crop. The species *Lens culinaris* Medik has been divided into two subspecies macrosperma and microsperma on the basis of seed size and cotyledon colour (Barulina, 1930). The seeds of macrosperma are bold with yellow cotyledon colour and seeds of microsperma are small with red cotyledon colour. The choice of mutagen holds great importance in changing the spectrum of mutations in a predictable manner. The physical and chemical mutagens cause three types of effects i.e. physical damage, gene mutation and chromosomal aberration (Swaminathan 1965). Among all the mutagens, the most effective physical and chemical mutagens are x-rays, gamma-rays and EMS respectively. The information available on the relative potency of the frequency of chlorophyll and viable mutations induced by these two major groups of mutagens in microsperma lentil is insufficient. Therefore, a comparative study of the frequency and spectrum of chlorophyll and viable mutations induced by gamma rays, EMS and their combinations was undertaken in microsperma lentil.

MATERIALS AND METHODS

Healthy seeds (100g) of lentil variety Pant L-406 were irradiated with gamma-rays at 10 kR, 15 kR, 20 kR, 25 kR and 30 kR doses (irradiation source was ^{60}Co gamma cell with capacity to release 3000 Ci delivery 7200 r/min.). Half of the irradiated seeds were used for combination treatment with 0.03 M EMS. Separate seed lots of this variety were presoaked in distilled water for 6 hrs. The soaked seeds were treated with 0.02 M, 0.03 M, 0.04 M and 0.05 M EMS for 6 hrs. (pH 7.0).The treated seeds were washed thoroughly in running tap water. The hundred seeds from each treatments along with control were placed in Petri dishes in the laboratory (25 ± 1° C) for taking observations on seed germination and seedling height. Rest of the treated seeds was sown in the field to obtain M_1 generation (2001-02). Observations on seed germination and seedling height were recorded on 8th and 12th day of incubation on treated and untreated (control) seeds. The fifty root tips from primary roots were excised from germinated seeds from each treatment including control. The root tips were fixed in Cornoy's solution (1:3:6, glacial acetic acid: chloroform: absolute alcohol) for 24 hrs for cytological studies. The root tips were boiled in acetocarmine solution (1g in 45% acetic acid) and smear were prepared in acetocarmine solution. The preparation were observed under microscope for cytological analysis (*viz.* laggard, bridges, fragments and micronuclei).The plant survival and pollen fertility were recorded from field grown plants to estimate mutagenic effect in M_1 generation. The mutagenic efficiency was determined as per the formula suggested by Konzak *et al*. (1965). All the M_1 plants were harvested individually and separately treatment-wise and M_2 generation was raised in the field in the next season (2002-03). The M_2 population were screened for both chlorophyll and viable mutations throughout the lifespan and presented as per 1000 M_2 plants.

RESULTS AND DISCUSSION

The doses of gamma-rays, EMS and their combinations cause corresponding decrease in germination per cent and plant survival in this variety (table 1). Lowest germination and plant survivals were found in combination treatment of 0.03 M EMS + 30 kR Gamma rays. Combination treatments in general, permitted low plant survival than all gamma-rays and EMS treatment individually. Similar relationship has been reported in lentil (Sarkar and Sharma, 1989; Gaikwad and Kothekar, 2004) and in other pulses (Waghmare and Mehra, 2001; Sharma *et al.,* 2005). The

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reduction in germination and plant survival in M_1 generation is primarily due to lethal damage because of altered physiological balance caused by mutagenic treatments. Reductions in seedling height increased with increase in dose of mutagens were observed (table 1). Combination treatment causes chromosomal aberrations in cells that affect the enzyme production. The poor enzyme production affects the growth and metabolism in plants of treated population. Due to low production of growth promoting enzymes and growth substances, the seedling height

Table 1: Seed germination, plant survival, seedling height reduction and pollen fertility as percent of control in lentil variety Pant L-406 in M_1 generation.

Treatments	Germination	Plant survival	Seedling height reduction (8^{th} day)	Seedling height reduction (12^{th} day)	Pollen fertility
Control	100 (96.50)	100 (90.00)	00.00 (7.94)	00.00 (10.65)	100 (97.00)
Gamma-rays					
10kR	87.05	89.33	4.91	7.98	80.38
15kR	85.50	76.88	6.96	10.05	78.12
20kR	78.24	76.44	15.49	18.21	76.24
25kR	72.54	71.11	28.68	29.01	72.59
30kR	65.28	62.00	33.97	31.55	69.68
EMS					
0.02M	82.38	83.78	6.42	8.83	81.41
0.03M	78.24	77.77	16.37	18.87	76.20
0.04M	73.06	73.11	24.43	23.94	73.73
0.05M	65.28	70.76	39.29	37.56	70.53
EMS + Gamma-rays					
0.03M+10kR	73.06	64.44	34.01	35.21	72.48
0.03M+15kR	70.13	59.33	34.13	39.99	65.24
0.03M+20kR	63.21	56.69	40.81	44.13	58.91
0.03M+25kR	59.81	52.77	52.68	50.23	53.27
0.03M+30kR	48.19	47.78	59.82	56.68	43.71

treatment with EMS (0.03 M) and gamma-rays 30 kR strongly depressed seedling height. The growth inhibition of seedling, particularly at higher doses is due to gross injury caused at cellular level ,either due to gene mutation, gene controlled physiological process or chromosomal aberrations or both (Tripathi and Dubey 1992a). Reduction in pollen fertility also increased with increase in the doses of mutagenic treatments. Similar findings were also reported by Rao and Suryanarayana (1989) in okra. The causes of pollen sterility may be due to chromosomal aberration (deficiency, duplication), genic mutation and physiological effects. Mutagen induced reduction of reproductive capacity has been assigned to various phenomena and varied causes. The pollen fertility count is a better and dependable parameter to find out the mutagenic effect in M_1 generation.

Higher chromosomal aberrations were found in combination treatment followed by gamma-rays and EMS (table 2). Gamma-rays induced high chromosomal abnormalities than EMS which indicates greater efficiency of gamma-rays for inducing mitotic abnormalities in cells of treated population. Mitotic abnormalities (fragments, bridges, micronuclei and laggards) were numerous at higher doses of gamma-rays. El-Araqi *et al*. (1996) reported chromosomal damaged induced by radiation. Mutagenic reduced (Singh, 1987).

Chlorophyll and viable mutations :

High frequency of chlorophyll mutations were observed in EMS treatment followed by combination and gamma-rays treatments. The frequency of chlorophyll mutations ranged from 4.44 to 14.90 among the treatments (table 3). The lower doses of mutagen induced high frequency of chlorophyll mutations. There were four types of chlorophyll mutations i.e. *albina*, *xantha, chlorina* and *viridis* were observed in various treatments. The frequency of *viridis* mutant was high in combination treatment. *Albina* mutants were found in low frequency (table-3). Similar results were also reported by Waghmare and Mehra (2001) in lathyrus. The chlorophyll mutation frequency decreased with the increasing concentration/dose of mutagen which may be attributed to differences in the molecular mechanism involved in mutagenesis due to treatments with EMS and gamma-rays and also due to specificity of EMS for certain regions of chromosome (Vanirajan *et al*., 1993). The reduction in chlorophyll mutation frequency at higher doses of mutagen has been attributed to the vigour of both diplontic and haplontic selection in the biological material and elimination of mutations.

Table 2 : Chromosomal aberration in root tip cells of lentil variety Pant L-406 in M_1 generation.

Treatments	Metaphase	Anaphase			Telophase	Others	No. of abnormal cells	No. of cells examined	% abnormal cells
	No. of cells having fragments	No. of cells having			No. of cells having micronuclei				
		Bridges	Laggards	Laggards + Bridges					
Control	1	1	2	0	1	0	5	425	1.18
Gamma-rays									
10kR	7	3	1	0	0	0	11	345	3.19
15kR	2	0	7	0	0	0	9	265	3.40
20kR	7	0	5	3	0	0	15	327	4.55
25kR	5	2	4	0	2	1	14	255	5.49
30kR	5	3	4	3	1	0	16	284	5.63
EMS									
0.02M	4	1	0	2	1	1	9	377	2.39
0.03M	7	0	2	0	2	0	11	322	3.42
0.04M	7	1	6	2	2	2	20	384	5.21
0.05M	6	0	5	1	0	0	12	194	6.19
EMS + Gamma-rays									
0.03M+10kR	3	0	6	3	0	1	10	341	3.81
0.03M+15kR	4	1	4	2	0	0	11	262	4.20
0.03M+20kR	5	3	6	2	3	0	19	367	5.18
0.03M+25kR	10	2	5	2	0	2	21	328	6.40
0.03M+30kR	6	4	5	2	2	1	20	274	7.30

Table 3 : Frequency and spectrum of chlorophyll mutations in lentil variety Pant L-406

Treatments	No. of M_2 plants	No. of mutants	Frequency (per 1000 M_2 plants)	Type of chlorophyll mutants			
				Albina	*Xantha*	*Chlorina*	*Viridis*
Control	1882	0	0	0	0	0	0
Gamma-rays							
10kR	1632	14	8.58	1.23	3.68	0.0	3.68
15kR	1715	9	5.24	1.16	0.0	1.16	2.29
20kR	1705	10	5.87	0.59	1.76	0.0	3.52
25kR	1685	8	4.75	1.19	2.38	1.19	0.0
30kR	1545	7	4.53	0.0	1.29	1.29	1.29
EMS							
0.02M	1565	23	14.70	3.19	4.47	1.28	5.74
0.03M	1490	21	14.90	2.01	2.01	6.04	4.02
0.04M	1370	12	08.76	0.73	0.0	2.92	5.09
0.05M	1419	9	6.34	1.41	2.11	0.0	2.82
EMS + Gamma-rays							
0.03M+10kR	1323	19	14.36	1.51	3.02	2.26	7.56
0.03M+15kR	1438	15	10.43	3.47	0.70	0.0	6.26
0.03M+20kR	1342	10	7.45	0.74	4.47	0.74	1.49
0.03M+25kR	1335	8	5.99	0.75	1.50	3.00	0.75
0.03M+30kR	1125	5	4.44	0.0	0.0	0.0	4.44

Table 4 : Frequency and spectrum of viable mutations in lentil variety Pant L-406

Treatments	Total population	No. of viable mutants	Frequency / 1000 M_2 plants	Types of mutants and their frequency/ 1000 M_2 plants															Types of viable mutants
				Stature type					Mutants for duration		Pod mutants		Seed size mutants		Other mutants				
				1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Control	1852	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gamma rays																				
10kR	1662	16	9.63	-	1.81	-	-	-	2.41	-	-	-	2.41	-	-	-	-	-	3.01	3
15kR	1527	12	7.86	-	-	-	1.96	-	-	2.62	1.31	-	-	-	-	1.96	-	-	-	-
20kR	1480	10	6.76	-	-	-	2.03	-	-	2.02	1.35	-	-	-	-	1.35	-	-	-	4
25kR	1342	8	5.96	2.24	-	-	1.49	-	-	-	-	-	-	-	1.49	0.75	-	-	-	4
30kR	1212	7	5.76	-	-	-	-	-	-	-	-	-	-	-	0.83	1.65	0.83	-	2.47	4
EMS																				
0.02M	1392	21	15.09	-	-	-	3.59	-	-	-	2.87	-	2.87	-	2.16	-	-	-	3.59	5
0.03M	1362	18	13.22	2.20	-	-	-	2.94	-	-	-	-	-	-	-	3.67	4.41	-	-	4
0.04M	1222	8	6.92	-	-	1.47	-	-	-	-	-	-	-	-	-	2.94	-	-	1.47	2
0.05M	1170	6	5.13	-	-	1.71	-	-	-	-	-	-	-	-	1.71	-	-	-	1.71	3
Gamma rays + EMS																				
0.03M+10kR	1262	17	13.47	-	2.37	-	1.59	-	-	2.37	-	-	-	3.17	-	2.37	-	-	1.59	4
0.03M+15kR	1160	15	12.93	4.31	-	1.72	-	-	-	1.72	-	-	-	-	2.59	-	-	1.72	0.86	3
0.03M+20kR	1212	9	7.42	-	-	-	-	-	-	3.30	-	-	-	-	-	1.65	-	-	2.48	3
0.03M+25kR	1192	10	8.39	-	-	-	-	2.52	-	2.52	-	-	-	-	-	-	3.36	-	-	3
0.03M+30kR	1112	5	4.50	-	0.90	-	-	1.80	-	-	0.90	0.90	-	-	-	-	-	-	-	4

*1- Dwarf, 2- Semi dwarf, 3- Bushy dwarf, 4- Tall, 5– Spreading, 6- Early, 7- Late, 8- Profuse pod, 9- Constricted pod, 10- Bold seed 11- Small seed, 12- Broad leaf, 13- Small leaf, 14- Narrow leaf, 15- Tendril leaf, 16- Sterile.

Higher frequency of viable mutants was observed in EMS treatment as compared to gamma-rays treatment and their combinations with EMS treatments (table-4). The microsperma lentil showed wide mutation spectrum i.e. for 16 characteristics. Early maturing mutants were found in 10 kR gamma-rays treatment where as tendril leaf and small seed size mutants were observed only in combination treatments of 0.03 M + 25 kR and 0.03 M + 10 kR respectively. Various types of viable mutations *viz. dwarf, bushy, sterile, bold seeded, spreading* and tall were observed in different treatments. Lower doses of EMS (0.02 M & 0.03 M) were effective in inducing more viable mutations as compared to treatments with gamma-rays and its combinations. This finding confirms the popular view that mutagenic efficiency varied with criteria of its estimation. Mutagenic efficiency was computed on the basis of seedling injury, lethality and pollen sterility. Induced sterility, lethality and reduction in germination percentage were more pronounced in higher doses of mutagens as compared to their corresponding lower doses. The efficiency of EMS at lower dose (0.02M) was found to be highest when compared to all other treatments for inducing various types of mutations. Thus, EMS (0.02 M) is most efficient in inducing chlorophyll and viable morphological mutations. However, efficiency of 10 kR gamma-rays was also better in comparison to higher doses of gamma-rays for inducing morphological mutations. Similar findings were also reported earlier by Vanniarajan *et al.* (1993) in black gram.

Table 5: Mutagenic efficiency for chlorophyll and viable mutations in M_2 generation in lentil variety Pant L - 406

Treatment	Germination as % of control	Lethality (L)	Injury		% of pollen sterility (S)	Mutation per 100 M_2 plants (Chl. + Via.)	Efficiency			
			8^{th} day (I_1)	12^{th} day (I_2)			L	I_1	I_2	S
Control	100.00	0	0	0	0	0	0	0	0	0
Gamma rays										
10kR	87.08	10.67	4.91	7.98	19.62	1.84	17.24	37.47	23.06	9.38
15kR	85.50	23.12	6.96	10.05	21.88	1.22	5.28	17.35	12.14	5.58
20kR	78.24	23.56	15.49	18.21	23.76	1.17	4.97	7.55	6.43	4.92
25kR	72.54	28.89	28.68	29.01	27.41	0.95	3.29	3.31	3.27	3.47
30kR	65.28	38.00	33.97	31.55	30.32	0.91	2.39	2.68	2.88	3.00
EMS										
0.02M	82.38	16.22	6.42	8.83	18.59	2.81	17.32	43.77	31.82	15.12
0.03M	78.24	22.23	16.37	18.87	23.80	2.62	11.79	16.00	13.88	11.01
0.04M	73.06	26.89	24.63	23.94	26.27	1.46	5.43	5.98	6.10	5.56
0.05M	65.20	29.33	39.29	37.56	29.47	1.06	3.61	2.70	2.82	3.60
Gamma rays + EMS										
0.03M+10kR	73.06	35.56	34.01	35.21	27.52	2.72	7.64	8.00	7.73	9.88
0.03M+15kR	75.13	40.67	34.14	39.99	34.76	2.09	5.14	6.12	5.94	6.01
0.03M+20kR	63.21	43.31	40.80	44.13	43.09	1.42	3.28	3.48	3.22	3.30
0.03M+25kR	59.81	47.23	52.68	50.23	46.73	1.35	2.86	2.56	2.69	2.89
0.03M+30kR	48.19	52.22	59.82	56.68	56.29	0.89	1.70	1.49	1.57	1.58

EMS induces higher frequency of gene mutations than ionizing radiations (Singh *et al*., 1989). Viable mutation frequency decreased with the increase in doses of the mutagens. Such trends have been reported by other workers (Tripathi and Dubey 1992b) and the decrease could have been attributed to be due to saturation effect leading to an increase in the size of mutated sector involving multiple mutations.

Mutagenic efficiency :

It is evident from the result (table-5) that high mutation frequency was found in EMS treatment followed by combination and gamma-rays treatments. The lower doses showed high mutation frequency as compared to high doses of all the mutagenic treatments. In the present study,

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