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MEIOTIC IRREGULARITIES INDUCED BY SINGLE AND COMBINED TREATMENTS OF GAMMA RAYS, EMS AND SODIUM AZIDE IN LENTIL

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Mutagen induced chromosomal abnormalities in M2 generation were studied in two varieties of lentil LL-19 and P332. The effect of gamma rays, EMS, sodium azide and their combined treatments were studied on various cytological parameters such as quadrivalents, bivalents, univalents, laggards, bridges/fragments. The mean number of quadrivalents and, rod bivalents were increased in mutagenic treatments. The chiasma frequency and ring bivalents were decreased. Combined treatments showed more chromosomal abnormalities. Pollen sterility was more in plants with higher number of quadrivalents.

Keywords : Induced mutagens; Lentil; Cytological abnormalities.

Introduction

Cytological analysis with respect to either mitotic or meiotic behaviour is considered to be one of the most dependable indeces to estimate the potency of mutagens. Studies on lentil regarding the effect of various mutagens and their combinations on different cytological parameters are not made earlier. The present paper reports the effect of gamma rays, EMS, sodium azide and their combinations on different cytological parameters in two lentil varieties.

Materials and Methods

Two lentil varieties namely LL-19 and P-332 were induced with one physical mutagen-gamma rays and two chemical mutagens EMS and sodium azide. The details of mutagens and their combinations are as follows :

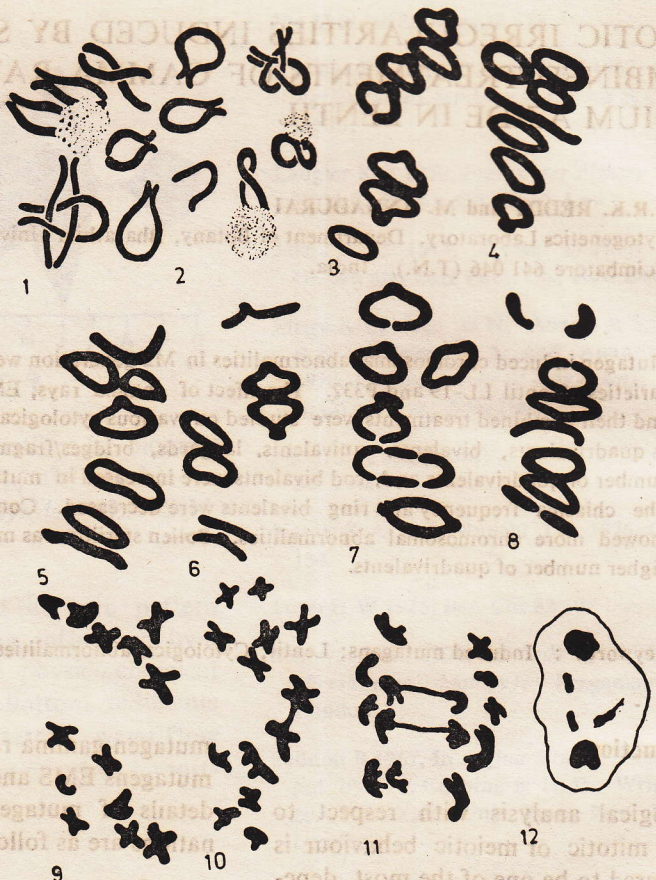
a) Gamma rays—(3 treatments)—20 Kr, 30 Kr, 40 Kr.

b) EMS (0.5%)—(3 treatments)—10h, 12h, 14h

c) Sodium azide—(3 treatments)—1%, 1.5%, 2% (4 hours each treatment)

d) Gamma rays+EMS—(3 treatments)—20 Kr+10h; 30 Kr+12h; 40 Kr+14h.

e) Gamma rays+sodium azide (3 treatments)—20 Kr+1%; 30 Kr+1.5%; 40 Kr+2%.



Figs. 1 12. Various meitic stages in lentil (Camera Lucida diagrams)

1. Diakinensis (control); 2. Diakinensis showing two nucleolus; 3. Metaphase showing seven ring bivalus; 4 Metaphase showing one rod bivalent and six ring bivalents; 5. Metaphase showing two rod bivalents and five ring bivalents; 6. Metaphase showing three rod biva'ents and four ring bivalents; 7. Metaphase showing one quadrivalent and five ring bivalents; 8. Metaphase showing two rod bivalents, two univalents and four ring bivalents; 9. Anaphase (control); 10. Anaphase showing 'late disjunction'; 11. Anaphase showing 'Anaphase bridges' 12. Late Anaphase showing 'laging chromosomes';

One hundred seeds were used for each treatment. Each M_1 plants were harvested separately and were sown as plant to row to raise M_2 generation. Meiotic studies were made on fifty randomly selected plants from each treatment of the two lentil varieties.

For meiotic studies, flower buds were fixed in Carnoy's fluid (6:3:1). Anthers

Table 1 Cytological effects of various mutagenic treatments in M_2 generation in lentil cultivar LL-19

(First row is the mean and second row is range)

Treatment	II		I	Fragments/ bridges	Laggards	Irregular cells (%)	Pollen sterility	Mean X ta/ Cell
	IV	Ring	Rod					
Control	—	5.89 (6–7)	1.11 (0–1)	—	—	—	0.89	12 94
Gamma Rays								
20 Kr	0.28 (0–1)	*3.93 (0–7)	1.51 (0–3)	1.28 (0–2)	0.17 (0–1)	0.96 (0-2)	7.14	39.14	11.68
30 Kr	0.31 (0–1)	*3.28 (0–7)	*1.98 (0–3)	1.43 (0–2)	0.19 (0–1)	1.09 (0–2)	8.26	46.38	*10.31
40 Kr	0.42 (0–1)	*2.82 (0–7)	*2.11 (0–3)	1.65 (0–2)	0.26 (0–1)	1.14 (0–2)	9.14	51.08	*9.28
EMS									
10 h	0.11 (0–1)	4.49 (0–7)	1.71 (0–2)	0.69 (0–2)	0.13 (0–1)	0 64 (0-2)	4.92	29.16	11.73
12 h	0.22 (0–1)	*3.94 (0–7)	1.86 (0–2)	0.98 (0–2)	0.17 (0–1)	0.72 (0–2)	5.11	33.24	*10.69
14 h	0.30 (0–1)	*3.58 (0–7)	*1.98 (0–2)	1.14 (0–2)	0.21 (0–1)	0.81 (0–2)	6.48	39.61	*10.47

Contd.

1	2	3	4	5	6	7	8	9	10
Sodium azide									
1%	0.10	4.96	1.38	0.56	0.11	0.51	3.61	26.29	12.74
	(0–1)	(0–7)	(0–2)	(0–2)	(0–1)	(0–2)			
1.5%	0.15	4.53	1.51	0.81	0.15	0.69	4.08	30.24	12.41
	(0–1)	(0–7)	(0–2)	(0–2)	(0–1)	(0–2)			
2%	0.17	4.21	1.64	0.98	0.18	0.76	5 14	36.09	12.09
	(0–1)	(0–7)	(0–2)	(0–2)	(0–1)	(0–2)			
G R + EMS									
20 Kr + 10 h	0.81	*3.02	1.59	1.58	0.33	1.36	11.06	54.18	*9.23
	(0–1)	(0–7)	(0–3)	(0–2)	(0–1)	(0–2)			
30 Kr + 12 h	0.91	*2.50	*1.88	1.71	0.38	1.48	12.48	61.39	*8.71
	(0–1)	(0–7)	(0–3)	(0–2)	(0–1)	(0–2)			
40 Kr + 14 h	0.98	*2.10	*2.08	1.84	0.41	1.51	13.01	68.46	*8.36
	(0–1)	(0–7)	(0–3)	(0–2)	(0–1)	(0–2)			
G.R. + S.A.									
20 Kr + 1%	0.28	*3.82	1.44	1.46	0.29	1.12	9.58	50.29	11.44
	(0–1)	(0–7)	(0–3)	(0–2)	(0–1)	(0–2)			
30 Kr + 1.5%	0.32	*3.30	1.79	1.59	0.31	1.23	10.41	54.37	*10.63
	(0–1)	(0–7)	(0–3)	(0–2)	(0–1)	(0–2)			
40 Kr + 2%	0.41	*2.82	*2.08	1.73	0.38	1.31	11.18	60.18	9.83
	(0–1)	(0–7)	(0–3)	(0–2)	(0–1)	(0–2)			

* Significant at 5% level

Table 2. Cytological effects of various mutagenic treatments in M_2 generation in lentil cultivar P-332. (First row is the mean and second row is range)

Treatment	II				Fragments/ bridges	Laggards	Irregular cells (%)	Pollen sterility %	Mean Xta/ cell
	IV	Ring	Rod	I					
Control	—	5.92 (6–7)	1.08 (0–1)	—	—	—	—	1.02	12.86
Gamma rays									
20 Kr	0.49 (0–1)	3.98 (0–7)	1.64 (0–3)	0.89 (0–2)	0.19 (0–1)	0.91 (0–2)	8.61	41.19	*10.64
30 Kr	0.55 (0–1)	*3.63 (0–7)	*1.81 (0–3)	1.01 (0–2)	0.23 (0–1)	1.11 (0–2)	9.48	44.61	*9.49
40 Kr	0.61 (0–1)	*3.02 (0–7)	*2.08 (0–3)	1.29 (0–2)	0.28 (0–1)	1.16 (0–2)	10.81	58.33	*9.13
EMS									
10 h	0.23 (0–1)	4.35 (0–7)	1.63 (0–2)	0.79 (0–2)	0.16 (0–1)	0.58 (0–2)	6.39	29.61	*10.71
12 h	0.34 (0–1)	4.07 (0–7)	1.76 (0–2)	0.83 (0–2)	0.18 (0–1)	0.61 (0–2)	7.61	31.47	*10.53
14 h	0.41 (0–1)	*3.74 (0–7)	*1.89 (0–2)	0.96 (0–2)	0.21 (0–1)	0.77 (0–2)	8.59	39.16	*10.14

Contd

1	2	3	4	5	6	7	8	9	10
Sodium Azide									
1%	0.10 (0-1)	4.96 (0-7)	1.46 (0-2)	0 48 (0-2)	0.12 (0-1)	0.47 (0-2)	4.41	18.39	11.84
1.5%	018 (0-1)	4.62 (0-7)	1.59 (0-2)	0.61 (0-2)	0.13 (0-1)	0.59 (0-2)	5.09	29.47	11.59
2%	0.22 (0-1)	4.16 (0-7)	1.76 (0-2)	0.86 (0-2)	0.19 (0-1)	0.63 (0-2)	6.39	36.52	11.23
G.R. + EMS									
20 Kr + 10 h	0.71 (0-1)	*2.63 (0-7)	*1.89 (0-3)	1.77 (0-2)	0.36 (0-1)	1.33 (0-2)	12.68	59.08	*9.49
30 Kr + 12 h	0.76 (0-1)	*2.20 (0-7)	*2.01 (0-3)	1.83 (0-2)	0.39 (0-1)	1.39 (0-2)	13.64	66.14	*9.01
40 Kr + 14 h	0.89 (0-1)	*1.79 (0-7)	*2.41 (0-3)	1.91 (0-2)	0.43 (0-1)	1.48 (0-2)	14.18	71.03	*8.49
G.R.+S.A.									
20 Kr + 1%	0.64 (0-1)	*2.98 (0-7)	1.74 (0-3)	1.64 (0-2)	0.25 (0-1)	1.13 (0-2)	10.18	51.06	11.19
30 Kr + 1.5%	0.68 (0-1)	*2.62 (0-7)	*1.98 (0-3)	1.72 (0-2)	0.27 (0-1)	1.19 (0-2)	12.41	54.29	*10.48
40 Kr + 2%	0.71 (0-1)	*2.35 (0-7)	*2.13 (0-3)	1.81 (0-2)	0.33 (0-1)	1.29 (0-2)	13.02	63.37	*10.16

*=Significant at 5% Level

were squashed in acetocarmine. Data on various chromosomal associations including quadrivalents, univalents, fragments, bridges, laggards and other irregularities were recorded at appropriate stage. 't' test was applied for significance test.

Results and Discussion

The data on several cytological parameters collected from various mutagenic treatments (Table 1–2) (Figs. 1–12) in two varieties of lentil suggest that in both the varieties, the mean number of quadrivalents, rod bivalents univalents, laggards, bridges/fragments were increased. The increase was noticed with increase in dose/duration/ concentration of mutagen. The increase was more in combined mutagenic treatments. Other abnormalities like multiple nucleoli, persistent nucleoli and pollen sterility were also increased with the elevation of mutagenic treatments. Earlier, Ignacimuthu and Babu (1989) recorded a dose dependent increase in meiotic chromosome abnormalities in mung bean. Similarly, Sinha and Godward (1972) observed a linear relationship between mutagenic dose/ concentration and the frequency of various cytological abnormalities in lentil. The present results also show that among indiuidual mutagenic treatments, gamma rays produced higher number of quadrivalents, rod bivalents and other abnormalities. This result thus supports the general hypothesis that physical mutagens produce more cytological abnormalities than chemical mutagens and has been observed in crop plants. Jana *et al.* (1974) noticed synergestic effect for chromosomal abnormalities in lentil. However, in the present study, in combined treatments, only less than additive effect were noticed for various cytological parameters. Among chemical mutagens, EMS produced slightly more abnormalities than sodium azide. However, recently, Roy (1989) recorded equal frequency of chromosomal abnormalities in EMS and sodium azide induced mutagenic population, thereby suggesting a genotypic vartation.

Reduction in chiasma frequency may be attributed to increase in rod bivalents and univalents. Reduction in chromosome pairing has also been attributed to mutations in the genes governing homologous chromosome pairing and / or chromosome structural changes (Gottschalk and Villalobos—Pietrini, 1965; Acharia and Sinha, 1975; Narasinghani and Kumar, 1976).

Plants having higher number of quadrivalents also exhibited higher pollen sterility, therefore, pollen sterility can be taken as a direct criteria for selection of plants having more number of quadrivalents. Such plants subsequently can be used for preparation of translocation tester sets in lentil and location of genes on specific chromosomes.

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