Cytologia 57: 213–216, 1992

Cytological Effects of Different Mutagens in Lentil (*Lens culinaris* Medik)

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Accepted November 14, 1991

Cytogenetic studies are important for obtaining information regarding the role and effect of various mutagens and elucidating the response of various genotypes to a particular mutagen. Although there are a number of reports available in different crops on the effect of different mutagens, no studies were undertaken in lentil in this regard. The present paper deals with effect of gamma rays, EMS and sodium azide and their combinations on various cytological parameters in one lentil variety.

Materials and methods

Seeds of lentil variety PL-639 were subjected with 20, 30, 40 KR (1 KR=2.58×10^{-4}C/kg) of gamma rays; three different durations 10, 12, 14 h of 0.5% EMS (at 25±1°C with pH 4, adjusted with citric phosphate buffer); three different concentrations of sodium azide–1, 1.5, 2%–4 hr each treatment (at 25±1°C with pH 3, adjusted with citric phosphate buffer); combination treatments involving gamma rays+EMS (20 KR+10 h; 30 KR+12 h; 40 KR+14 h) and gamma rays+sodium azide (20 KR+1%; 30 KR+1.5%; 40 KR+2%).

One hundred seeds were used for each treatment. Each M_1 plant was harvested seperately and grown as plant to row to raise M_2 generation. Meiotic studies were conducted on fifty randomly selected plants from each treatment. For meiotic studies, young unopened flower buds were fixed in freshly prepared Carnoy's fluid (6: 3: 1) (absolute alcohol: chloroform: acetic acid) having a few drops of saturated solution of ferric chloride. Flower buds were fixed in the forenoon, from 8.00 am to 9.00 am. Squashes were made in 2% acetocarmine. The data on the following parameters were taken at appropriate stage.

i) Quadrivalents, bivalents, univalents at metaphase I.
ii) Fragments/bridges, laggards at anaphase I.
iii) Cells (%) showing other chromosomal irregularities.
iv) Pollen sterility.

Standard 't' test was applied to test the means of mutagenic population with that of control.

Results and discussion

Meiotic studies were made in mutagenic populations of one lentil variety PL-639. The data on chromosomal association such as quadrivalents, bivalents, univalents, fragments, bridges, laggards and other cytological irregularities are presented in Table 1 (Figs. 1–12). The mean quadrivalents were induced in mutagenic treatments and are increased with the elevation of mutagenic level. Combined treatments produced more number of quadrivalents than the individual treatments. Similarly, the mean values for rod bivalents, univalents, fragments/bridges, laggards, and other irregular cells particularly the cells showing two and more number of nucleoli. Earlier, dose dependent increase in meiotic abnormalities were

recorded in mung been (Ignacimuthu and Babu 1989) and a linear relationship between dose/duration/concentration of various mutagens and the frequency of different cytological abnormalities were recorded in lentil (Sinha and Godward 1972). The reduction in chiasma frequency in the present study can be attributed to increase in rod bivalent and univalents. Reduction in chromosome pairing has also been attributed to mutations in the genes governing homolo-

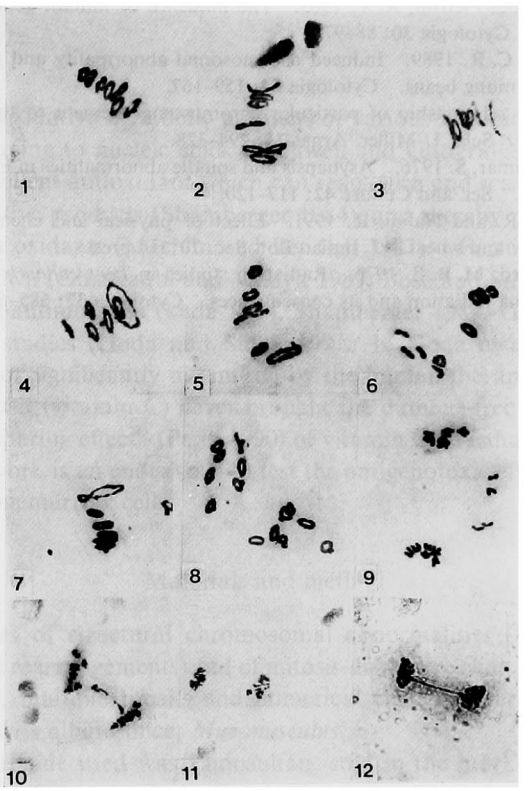
Table 1. Cytological effects of various mutagenic treatments in M_2 generation in lentil cultivar PL-639 (First row is the mean and second row is range)

Treatment	IV	II		I	Fragments/ bridges	Laggards	Irregular cells (%)	Pollent sterility (%)	Mean Xta/cells
		Ring	Rod						
Control	—	6.27 (0–7)	0.73 (0–1)	—	—	—	—	—	13.02
Gamma rays									
20 KR	0.41 (0–1)	*3.78 (0–7)	*1.85 (0–3)	0.96 (0–2)	0.23 (0–1)	0.88 (0–2)	9.43	41.02	*10.69
30 KR	0.52 (0–1)	*3.26 (0–7)	*2.14 (0–3)	1.08 (0–2)	0.29 (0–1)	0.98 (0–2)	10.12	45.61	*9.28
40 KR	0.61 (0–1)	*2.67 (0–7)	*2.39 (0–3)	1.33 (0–2)	0.31 (0–1)	1.13 (0–2)	11.26	49.73	*8.98
EMS									
10 h	0.20 (0–1)	4.55 (0–7)	*1.43 (0–2)	0.82 (0–2)	0.13 (0–1)	0.48 (0–2)	6.21	23.61	11.69
12 h	0.24 (0–1)	4.29 (0–7)	*1.56 (0–2)	0.91 (0–2)	0.19 (0–1)	0.53 (0–2)	8.43	29.11	11.51
14 h	0.31 (0–1)	*3.96 (0–7)	*1.71 (0–2)	1.02 (0–2)	0.21 (0–1)	0.61 (0–2)	9.56	33.46	11.39
Sodium azide									
1%	0.10 (0–1)	4.78 (0–7)	*1.45 (0–2)	0.67 (0–2)	0.11 (0–1)	0.39 (0–2)	3.73	18.29	12.71
1.5%	0.15 (0–1)	4.43 (0–7)	*1.61 (0–2)	0.81 (0–2)	0.15 (0–1)	0.47 (0–2)	4.69	24.76	12.47
2%	0.18 (0–1)	4.11 (0–7)	*1.79 (0–2)	0.92 (0–2)	0.17 (0–1)	0.59 (0–2)	6.38	27.61	12.16
G. R.+ESM									
20 KR+10 h	0.79 (0–1)	*2.67 (0–7)	*1.86 (0–3)	1.68 (0–2)	0.38 (0–1)	1.36 (0–2)	11.48	54.12	*9.83
30 KR+12 h	0.84 (0–1)	*2.11 (0–7)	*2.26 (0–3)	1.79 (0–2)	0.43 (0–1)	0.41 (0–2)	12.16	61.08	*9.41
40 KR+14 h	0.91 (0–1)	*1.84 (0–7)	*2.41 (0–3)	1.84 (0–2)	0.51 (0–1)	1.49 (0–2)	13.32	66.13	*8.28
G. R.+S. A.									
20 KR+1%	0.51 (0–1)	*3.15 (0–7)	*1.79 (0–3)	1.55 (0–2)	0.29 (0–1)	1.21 (0–2)	9.46	47.40	11.39
30 KR+1.5%	0.63 (0–1)	*2.51 (0–7)	*2.18 (0–3)	1.86 (0–2)	0.31 (0–1)	1.27 (0–2)	11.39	49.29	*11.12
40 KR+2%	0.69 (0–1)	*2.10 (0–7)	*2.40 (0–3)	1.81 (0–2)	0.39 (0–1)	1.33 (0–2)	12.37	55.36	*11.09

* =Significant at 5% level

gous chromosome pairing and/or chromosomal structural changes (Gottschalk and Villalobos-Pietrini 1965, Acharia and Sinha 1975, Narasinghani and Kumar 1976, Reddy *et al.* 1991). In the present study, among individual mutagenic treatments, gamma rays produced to higher number of quadrivalents, rod bivalents, univalents and other cytological abnormalities. This result was supports the general hypothesis that physical mutagens produce more cytological

abnormalities over chemical mutagens. Considerable number of abnormalities in chemical mutagen treated populations also revealed that they are capable of inducing mutations for cytological characters. Multinucleolate condition has been explained to a particular genotypic change suppressing the organising capacity of nucleolar chromosome and induce the formation of adventitious nucleoli (McClintock 1934). Increase in pollen sterility in plants with more number of quadrivalents, could be profitably used to isolate large number of plants showing quadrivalents, which in turn useful for preparation of translocation tester tests.



Figs. 1–12. Various meiotic stages in lentil. 1, Metaphase showing seven ring bivalents. 2, Metaphase showing one rod bivalent and six ring bivalents. 3, Metaphase showing three ring bivalents and four rod bivalents. 4, Metaphase showing five ring bivalents and one quadrivalent. 5, Metaphase showing one rod bivalent, one quadrivalent and four ring bivalents. 6, Metaphase showing one chain quadrivalent and five ring bivalents. 7, Metaphase showing one quadrivalent (alternative disjunction) and five ring bivalents. 8, Metaphase showing two univalents and six ring bivalents. 9, Anaphase showing dividing univalents. 10, Anphase showing late disjunction. 11, Anaphase showing lagging chromosomes. 12, Anaphase showing 'anaphase bridge'. (×1000)

Summary

The effect of gamma rays, EMS, sodium azide and their combination on various cytological parameters in M_2 generation were studied in lentil variety PL-639. The mean values of quadrivalents, rod bivalents, univalents, fragments/bridges, cytological abnormal cells and pollen sterility were increased in mutagenic treated population, while the chiasma frequency was decreased. Combined treatments showed additive effect, EMS produced slightly more abnormalities over sodium azide. Pollen sterility could be used as a parameter for selection

plants having more number of quadrivalents.

Key words: *Lens culinaris*, induced mutagens, cytological abnormalities.

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