RESEARCH ARTICLE

Separate and Combined Mutagenic Effect of Gamma Rays and Nitroso Methyl Urea in Chickpea (*Cicer arietinum*) var. RSG- 963.

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ABSTRACT

Comparative mutagenic effectiveness and efficiency of different dosage of gamma rays, NMU and their combined treatment were studied in the genotype of chickpea (Cicer arietinum) var. RSG – 963. Seeds of chickpea were treated with 5KR, 10KR and 15KR doses of gamma rays and 0.02% NMU independently and in combination. Gamma irradiation and NMU induced various chromosomal aberrations as scattered chromosomes, ring formation, stickiness at metaphase, formation of bridges, laggards, multi polarity at anaphase. It was revealed that the rate of cell division was affected and measured in term of mitotic index which decreased with the increase of doses of gamma rays. The combined treatment of physical and chemical mutagens was more effective in comparison to independent treatment. The higher doses of gamma rays and combined doses of gamma rays and NMU treatment revealed deleterious as there was less germination percentage observed in M_1 generation.

Keywords: *Cicer arietinum*, gamma rays, NMU, mitosis, chromosomal aberrations.

INTRODUCTION

Cicer arietinum is a legume crop which belongs to family Leguminosea. The diploid chromosomes number has been reported 2n = 16 (Ahmad, 2000). Physical and chemical mutagens are being used in genetic improvement program of different plant species. These are used to induce the genetic variability in *lens culinaris* (Kumar *et al,* 2003). Ahmad and Chen (2000) reported the difficulties to recognize most of the chromosomes by differences in length due to small size of Cicer chromosome. The findings of chromosomal aberration provide the data for estimation of differential mutagenic sensitivity (Prasad and Das, 1980). Different mutagens could be used in combination treatment to increase mutation frequency (Wani, 2009). The physical and chemical mutagens are known to produce chromosomal aberration (Kumar and Dubey, 1998). Mutagenic breeding is playing an important role to increase the genetic variability for qualitative traits in various crops such

as *Vigna (*Kozgar *et al.,* 2011). Cytological 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. The chromosomes in *Cicer* species have been reported rather small in size and therefore, they required intense staining of the root tips with stain for cytological study (Ohri and Pal, 1991).

MATERIALS AND METHODS

The seeds of *Cicer arietinum* var. RSG- 963 were collected from National Seed Corporation Bharatpur, Rajasthan. Dry, dormant and healthy seeds of chickpea were subjected to Co^{60} gamma irradiation dosage of 5KR, 10KR and 15KR at the Nuclear Research Lab, IARI New Delhi. A part of seeds from each irradiation treatment and a sample unirradiated seeds were soaked in 0.02% Nitroso Methyl Urea (NMU) for six hours. A sample of untreated seed was also used as a control. Thus there were eight treatment combinations including the control. Healthy and actively growing root tips of 15 days old seedlings of each treatment and untreated control were collected. The root tips were excised in the morning 8.30 am to 9.30 am.

Squash Method

Root tips of 2 to 3 mm long were collected in fixed acetic acid and alcohol (1: 3) for 24 hours and stored in 70% ethyl alcohol under low temperature. Before the cytological studies root tips were hydrolyzed by heating in 1N HCL for 3 to 5 minutes. Two percent (2%) Aceto orcein was used to stain the chromosomes in the present study. Eight slides of each treatment were observed under phase contrast photographic microscope and eight counts of each slide were scored to cover maximum surface area of the slide for computing mitotic index. Various abnormalities were observed in metaphase and anaphase. The abnormalities observed in metaphase were sticky of chromosomes, scattered chromosomes, change in polarity, disturbed and un-oriented chromosomes and ring formation. The abnormalities observed in anaphase were Chromatin Bridge, multi polarity and change in polarity, disturbed and un-oriented chromosomes; while the abnormalities observed in telophase were micro nucleus formation, bi or tri nucleate cell in all the treatments except control.

Mitotic Index

MI is computed in term of percentage frequency of dividing cells and mitotic index was calculated by scoring the total dividing cells out of total cells scored.

$$\text{Mitotic Index} = \frac{\text{Total no. of dividing cells}}{\text{Total no. of cells scored}} \text{ X100}$$

Standard Deviation and Standard Error –

The data obtained was statically analyzed as per procedure given by Panse and Sukhatme (1978)

$$(\mu)\bar{x} = \frac{\Sigma^{-} x_1}{n}$$

Where $\bar{x}\ (\mu)$ is mean, $\Sigma^{-} x_1$ is sum of 'i' observations, 'n' is number of observations.

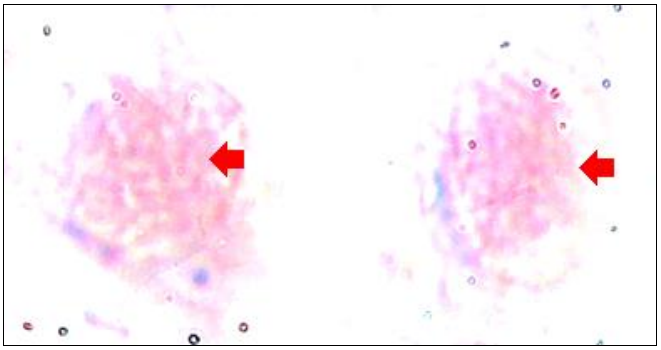
The standard deviation for mitotic index and standard error of mean were calculated for each treatment of M_1 generation.

RESULTS AND DISCUSSION

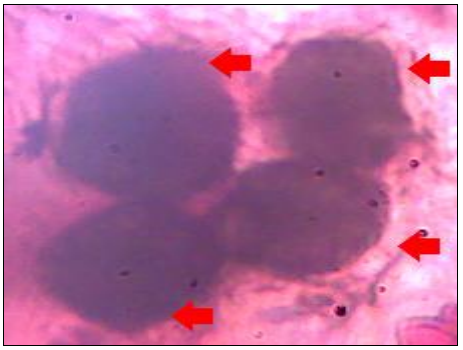
The total count of cells scored to compute the mitotic index and standard deviation for all the treatments. The normal and abnormal dividing cells of prophase, metaphase, anaphase and telophase were counted (table 1) for the first generation. The mitotic index was decreased in all the treatments as compared to the control. The maximum mitotic index (MI) 7.85 was observed in control while higher mitotic index 6.74 was observed in both 5 KR and 0.02% NMU treatments. The minimum MI 4.08 was observed in 15KR + NMU treatment. The total number of abnormal cells was increased with the increase in the doses of mutagens due to which Mitotic Index (MI) was decreased. The maximum abnormal cells 22 and 27 were found in 10 KR + NMU and 15 KR + NMU treatments while minimum number of abnormal cells 9 was recorded in 5 KR treatment of gamma rays (table 2). The maximum chromosomal aberrations were observed at metaphase and anaphase stages. Aberrations increased along with the increasing concentration of the mutagens have been reported by Khan *et al.,* (2009) in *Cichorium intybus L.* The varied degree of effectiveness and efficiency varied between different mutagens and also between varieties has been reported in the chickpea by Wani (2009). The similar differences in mutagenic response have also been reported by many workers (Kharkwal, 1998; Bhat *et al.,* 2007; Dhanvel *et al,.* 2008).

Table 1: Mitotic Index and Standard Deviation of the dividing cells in mitotic cell division of M_1 generation in *Cicer arietinum.*

S. No.	Treatment	Mitotic Index Mean ± Standard deviation	Total cells scored Mean	Dividing cells Mean (Normal + Aberrant)	Prophase Mean (Normal + Aberrant)	Metaphase Mean (Normal + Aberrant)	Anaphase Mean (Normal + Aberrant)	Telophase Mean (Normal + Aberrant)
1	Control	7.85(±0.2)	1290	100	27	30	26	17
2	5 KR	6.74 (±0.02)	1018	69	26	18	16	9
3	10 KR	6.18 (±0.05)	1034	65	24	21	11	8
4	15 KR	5.08(±0.07)	1125	57	21	15	11	10
5	0.02% NMU	6.53(±0.02)	1025	67	25	19	14	9
6	5 KR + NMU	4.75(±0.05)	1022	48	14	15	11	8
7	10 KR + NMU	4.38(±0.05)	1169	52	15	16	14	8
8	15 KR + NMU	4.08(±0.03)	1001	41	10	12	13	6

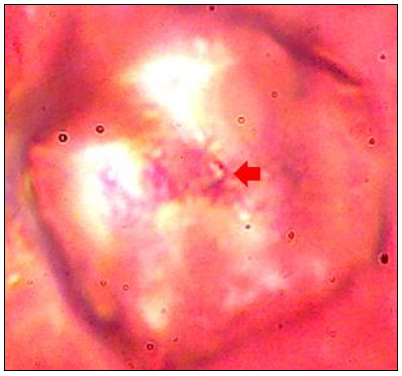


1. Two Polyploid cells (5KR)

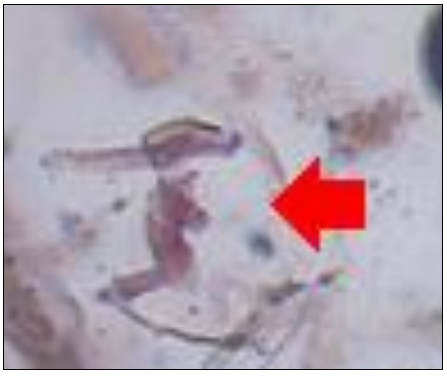


2. A multinucleate cell (5KR)

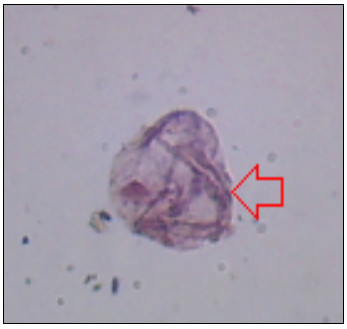
3. Ring formation at anaphase (10KR)



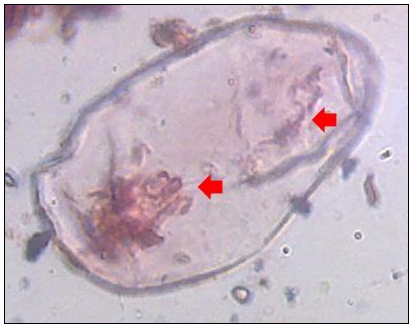
4. Scattered chromosome (10KR)



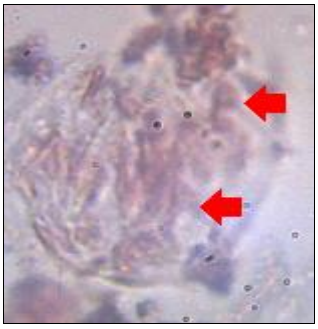
5. Stickiness of chromosomes at metaphase (15KR+NMU)



6.Unoriented chromosomes (15KR+NMU)



7. Unequal distribution of chromosomes at telophase (15KR+NMU)



8. Anaphasic chromatin bridge (15KR+NMU)

Table 2. Various mitotic abnormalities in M_1 generation of *Cicer arietinum.*

S. No.	Mitotic Irregularity	Control	5 KR	10 KR	15 KR	0.02% NMU	5 KR + NMU	10 KR + NMU	15 KR + NMU
1	Sticky at metaphase	-	1	2	1	2	2	3	4
2	Scattered metaphase	-	2	2	3	1	3	2	2
3	Change in polarity	-	-	1	2	-	1	3	2
4	Un oriented metaphase	-	1	2	1	2	2	3	3
5	Ring formation at anaphase	-	-	1	2	1	2	1	3
6	Chromatin bridge	-	-	1	2	2	1	2	1
7	Multi polarity	-	1	-	1	1	1	2	2
8	Laggard	-	2	2	2	1	2	3	4
9	Bi nucleate	-	1	-	1	-	1	1	2
10	Tri nucleate	-	-	-	1	-	-	-	1
11	Multi nucleate	-	1	1	1	-	1	2	3
Total		-	9	12	17	10	16	22	27

Combined treatments of different mutagens increase the mutation frequency and alter the mutation spectrum (Wani, 2009). Maximum abnormal dividing cells have been reported in chickpea when combined treatment of EMS and gamma rays was applied by Wani and Anis, 2008. The observation scored in the present study represents gamma rays and NMU induced various types of qualitative and quantitative chromosomal aberration comprising scattered chromosomes, ring formation, stickiness at metaphase, chromatin bridges, laggard and multi polarity at anaphase (figure 1to 8) . The Mitotic Index was decreased with the increase in doses of gamma rays combined with 0.02% NMU. The number of cells with various anomalies has been scored at different stages of mitosis (Kumar and Dubey, 1997). Mehandjiev (2005) reported that combined treatments of physical and chemical mutagens induced a wider range of mutation spectrum, which is of great significance to the experimental mutagenesis. In the present study, all the treatments applied reduce the Mitotic Index in comparison to control. Similar observations of mitotic aberrations have been reported by different workers (Vandana and Dubey, 1992; Kumar and Dubey, 1997). The percentage of abnormalities as an Index of effectiveness of individual mutagen and the combined treatment has been reported to be most effective (Kumar *et al.,* 2003).

CONCLUSION

Separate and combined implementation of physical and chemical mutagens showed significant effects on dividing cells. Increase in doses of mutagens invariably increased the frequency of abnormal division in the cells. However, the frequency of the particular abnormality did not show dose dependent relation. The combined treatment of gamma rays and 0.02% nitroso methyl urea (NMU) showed more potent effects as compared to independent uses of both the mutagens.

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