CHROMOSOMAL ABERRATIONS INDUCED BY SEPARATE AND COMBINED APPLICATION OF GAMMA RAYS AND NITROSO METHYL UREA IN KHESARI (*LATHYRUS SATIVUS)* VAR. P – 24

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Abstract: The paper reports the results of application of 5kr, 10kr and 15kr of gamma rays and/ or 0.02% of Nitroso Methyl Urea (NMU) on Khesari (*Lathyrus sativus*) var. P-24. The seeds of *Lathyrus* were treated with 5 kr, 10 kr and 15 kr doses of gamma rays and 0.02% NMU independently and in combination. Effect of these mutagens was revealed in the form of chromosomal aberrations as stickiness of chromosomes, scattered of chromosomes, ring formation at metaphase, multi polarity, formation of bridges, laggards at anaphase. It was observed that the rate of cell division was affected and measured in term of mitotic index which was decreased with the increase of doses of gamma rays. The combined treatment of physical and chemical mutagens was more effective than independent doses of both mutagens. The higher doses of gamma rays showed deleterious effect when applied separately as well as combined with NMU.

Keywords: *Lathyrus sativus*, gamma rays, NMU, mitosis, chromosomal aberrations.

Introduction

Grass pea (*Lathyrus sativus*) is an important pulse crop being a better source of protein among the pulses. Historically, grass pea has been a daily food for millions in Asia and Africa. The diploid chromosomes number has been reported 2n = 14. Creation of new genetic variability can be achieved through mutagenesis, besides some of the induced mutations may even prove to be useful for direct cultivation. Mutation is caused by physical as well as chemical mutagens in plants. These mutagens are being used in genetic improvement program of different plant species. Simultaneous treatment of different mutagens could be used to increase mutation frequency (Wani, 2009). The physical and chemical mutagens are known to produce chromosomal aberration (Kumar and Dubey, 1998). Mutagenic changes in grass pea are involving chromosomal anomalies, chlorophyll deficiencies and different types of phenotypic modifications (Prasad and Das, 1980; Rybinski, 2003). The present study reports the effects of gamma rays as a physical mutagen and Nitroso Methyl Urea (0.02%) as a chemical mutagen. Eight different seed treatments were used of gamma rays and NMU. The observations were based on different chromosomal changes in grass pea.

Material and method:

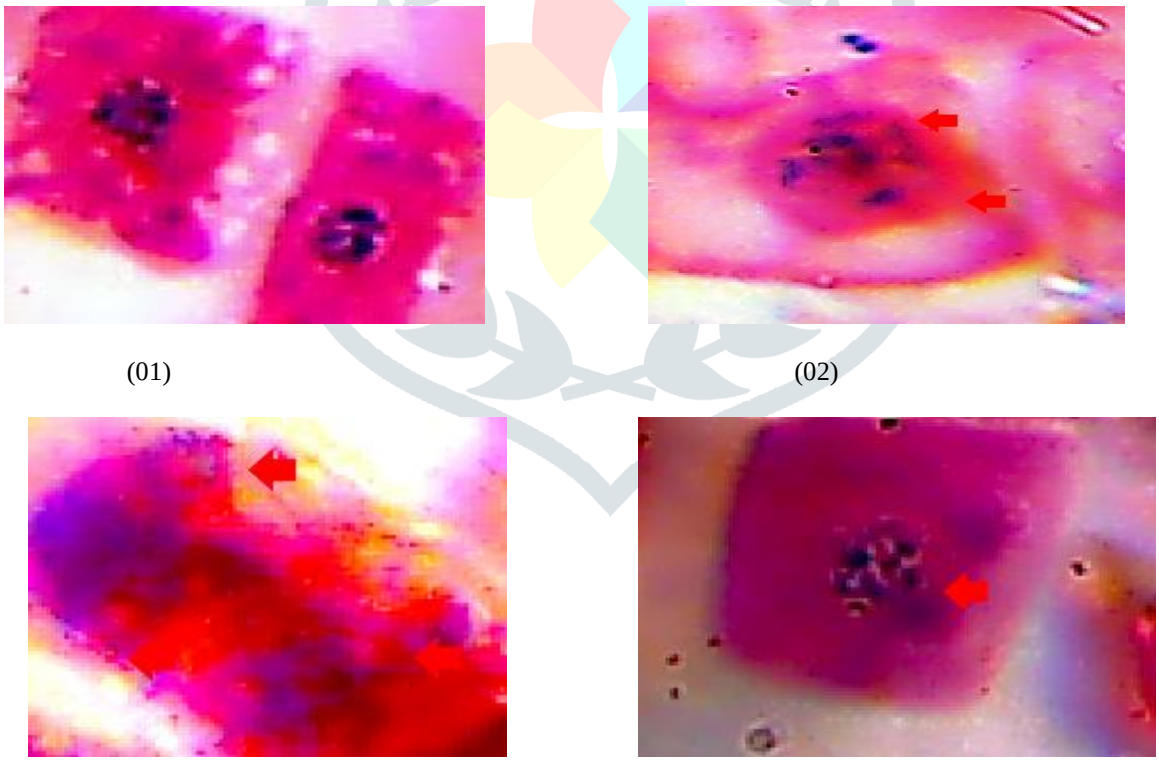
Dry and dormant seeds of grass pea var. P-24 were subjected to gamma irradiations at Nuclear Research Lab IARI New Delhi. Three different dosage of gamma rays 5kr, 10kr and 15kr were applied. 150 seeds from each radiation treatment and 150 fresh untreated seeds were soaked in 0.02% NMU solution for six hours. A sample of untreated seed was also used as a control. Thus there were eight treatment combinations including the untreated control. Fifty seeds from each of the above treatments were sown in Petri dishes in the laboratory. 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. Ten slides of each treatment were observed under phase contrast photographic microscope and ten counts of each slide were scored to cover maximum surface area of the slide for computing mitotic index. Different types of abnormalities were observed in metaphase and anaphase. Scattered chromosomes, sticky of chromosomes, un-oriented chromosomes, and change in polarity were observed in metaphase. Chromatin Bridge, multi polarity, disturbed and un-oriented chromosomes were observed in anaphase.

Result and Discussion –

Observation of effect of gamma rays and NMU on chromosomes during mitosis was made from the study of metaphase and subsequent stages. Well spread normal metaphase showing fourteen (2n = 14) chromosomes and normal anaphase showing 14:14 chromosomes separation could be easily obtained in the control root tip. The normal and abnormal dividing cells of, metaphase, anaphase and telophase were counted for the first generation. The mitotic index was decreased in all the treatments as compared to the control. The maximum mitotic index (MI) 7.38 was observed in control while higher mitotic index 6.23 was observed in both 5 KR and 0.02% NMU treatments. The minimum MI 3.94 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 21 and 27 were found in 10 KR + NMU and 15 KR + NMU treatments while minimum number of abnormal cells 6 was recorded in 5 KR gamma rays treated cells (table 1). 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 (Bhat *et al.*, 2007; Dhanvel *et al*,. 2008). 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 observations 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 (fig. 1 to 4). 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.



(01) (02)

(03) (04)

Figure 1-4: Different type of chromosomal aberrations induced by gamma rays and NMU.

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	-	-	2	1	1	1	3	4
2	Scattered metaphase	-	2	2	3	1	3	2	2
3	Change in polarity	-	1	1	2	1	1	2	2
4	Un oriented metaphase	-	-	2	2	2	2	3	3
5	Ring formation at anaphase	-	-	-	1	1	2	1	3
6	Chromatin bridge	-	-	1	1	-	1	2	1
7	Multi polarity	-	1	-	1	-	1	2	2
8	Laggard	-	-	2	2	1	1	3	4
9	Bi nucleate	-	1	-	-	-	1	1	2
10	Multi nucleate	-	1	-	1	-	1	2	3
Total		-	6	10	14	7	14	21	27

Table 2. Mitotic abnormalities observed during different stages in *Lathyrus sativus.*

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 Mitotic Index was reduced in all the treatments 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). In the present study fragmentation at metaphase as well as at anaphase is one of the most common aberration observed in most of the treatment sets. Fragmentation leads to deletions, insertions that can alter the genetic architecture of the plant. Bridge formation could be chromosomal stickiness and stickiness considered to be a type of physical adhesion. Due to easy identification, the use of bridges and fragments as indicators of the occurrence of chromosomal variations had been found to be an efficient method and favored the counting of a great numbers of cells. This criterion has been reported as being useful in detecting abnormalities in seeds stored for long periods of time (Hang A. et al, `994).

Separate and combined implementation of physical and chemical mutagens showed significant effects on dividing 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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