

GAMMA RAYS AND ETHYL METHANESULPHONATE INDUCED CYTOTOXICITY IN GREEN GRAM (*VIGNA RADIATA* (L.) WILCZEK)

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ABSTRACT: The cytological studies provide more information regarding the response of a genotype to the particular mutagen and provide chances to select desirable characters. The seeds of green gram var. Vamban₂ were exposed to different dose/conc. of gamma rays (20, 30, 40, 50 and 60 kR) and EMS (10, 20, 30, 40 and 50 mM) to induce mutation. The chromosome of a treated and control plants under mitotic stages were observed. The common chromosomal aberrations are precocious movement, stickiness, bridges, fragments, laggards etc. As increase in the concentration, the frequency of cells showing chromosomal aberrations shows a linear increase up to a certain level. Compared to gamma rays, EMS produced the highest chromosomal aberrations.

KEYWORD: Gamma Rays, EMS, Green Gram, Cytotoxicity, Stickiness, Bridges.

INTRODUCTION

After the discoveries of Muller and Stadler, a large amount of genetic variability has been induced by various mutagens and contributed to modern plant breeding. For the past five decades the induced mutation had played a major role in the development of superior plant varieties are food crops especially cereals and pulses. The genetic variation may create by induced mutation through the physical and chemical mutagens. These mutagens induced various abnormalities resulting new varieties with desired characters. One of the chief advantages of mutation breeding applied to this crop that it can give rise to many different alleles with different degree of trait modifications ([Chopra, 2005](#)).

Cytogenetical investigation is one of the best documented experimental proofs for the elucidation of the mode of speciation on different groups of plants ([Zohary, 1984](#)). Mutation induction can be done on the plants by mutagenic treatment of certain materials of plant reproductive organs such as seeds, stem cuttings, pollen, root rhizome, tissue culture and others. Using gamma irradiation caused different chromosomal aberrations in different plants ([Jayabalan and Rao, 1983](#)). One of the important alkylating agents like EMS has recently received much attention as the most effective mutagenic agent in higher plants known today. Studies revealed that EMS is an

effective mutagen and has been used to induce genetic variability in a number of crop plants ([Kumar and Rai, 2005](#); [Jabeen and Mirza, 2002](#)). Green gram is a protein rich stable food. It contains about 25% protein, which is almost three times than that of cereals. It supplies protein requirements of vegetarian population in our country. Therefore, the present paper deals with the cytological effect of gamma rays and EMS on greengram plants.

MATERIALS AND METHODS

The material used for this cytological study was Vamban variety of green gram. Dry seeds were irradiated from a ⁶⁰Co source at Agricultural University, Coimbatore with doses of 20, 30, 40, 50 and 60 kR gamma rays. Another set of seeds were pre soaked in distilled water for 6 hr and was treated with different concentration of EMS (10, 20, 30, 40 and 50 mM) to 4 hr with constant intermittent shaking. After mutant induction, seeds were thoroughly washed in running water for 10 to 15 times to leach out the residual of chemicals. Untreated seed stock was used as a control.

2.1. Observation Of Chromosomal Aberration

The greengram root tips about 3cm in length were excised, fixed in glacial acetic acid: alcohol (1:3 ratio) solution for 48h. Then root tip squashes were made by using iron alum, haematoxylin squash technique ([Marimuthu and](#)

[Subramanian, 1960](#)). The most frequent chromosomal aberrations were stickiness, precocious movement, bridges followed by laggards was more frequent. Frequency of chromosomal aberration was expressed as the number of cells with chromosomal aberration per 100 scored cells, out of approximately 1000 examined cells taken from 10 separate seedlings for each dose/concentration.

2.2. Statistical Analysis

Statistical analyses were performed ANOVA by using NPROC software package.

RESULT

Cytological analysis with respect to their mitotic behavior is considered to be one of the most dependable indices to estimate the potency of mutagen. The root mitotical studies revealed a wide range of chromosomal aberration such as stickiness, precocious movement, anaphasic laggards, fragments, anaphasic single and multiple bridges. In all the mutagenic treatments, the chromosome bridges and laggards were observed commonly. Even though, in the present study, more chromosomal aberration was observed dose/conc. level in 40 kR gamma rays and 40 and 50 mM EMS (Figure 1).

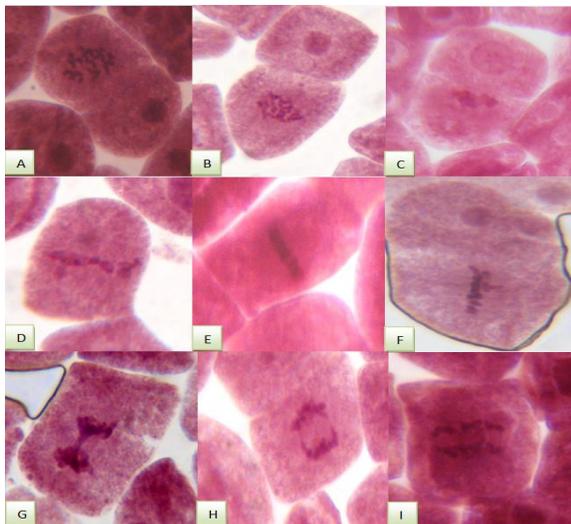


Figure 1: Mutagenic effects of Gamma rays and EMS on greengram ($2n=24$). A and B: Metaphase; C-E: Stickiness; (F) Precocious movement (G) Anaphase with single bridge (H) Anaphase with double bridges and (I) Anaphase with multiple bridges.

DISCUSSION

The inhibition of seedling growth seemed to be well correlated with the amount of chromosomal damage. Different types of chromosomal abnormalities have been observed in different

dose/conc. after treatment with physical and chemical mutagens. Similar studies were reported aberrations caused by mutagen were due to partial or complete failure of spindle mechanism in *Cicer arietinum*, *Trigonella* and *Lathyrus sativus* ([Kumar and Dubey, 1998](#)).

Among the physical and chemical mutagens, maximum abnormalities both structural and behavioural were induced by EMS when compared to gamma rays. Dose dependent increase in frequency of different chromosomal aberrations has been reported in chilli by [Abdul Salam and Thoppil \(2010\)](#). Similar result has also been reported by [Abbasi and Anis \(2002\)](#) in *Trigonella*. The common aberration of stickiness could be due to depolymerization of nucleic acids caused by mutagenic treatment or due to partial dissociation of the nucleoproteins and alterations in their pattern of organisation ([Kumar et al., 2007](#)). In this study, laggard was commonly observed at anaphase and telophase stages at 40 kR gamma rays and 50 mM EMS treatments. [Bhattacharya, \(1974\)](#) attributed the formation of laggards to chromosome spindle inhibition under radiation.

Low frequency of anaphasic bridges was observed with gamma rays (20 kR) and EMS (10 mM) treatments on greengram. It may be due to the effect of mutagens on root tip cells very meager dose/concentration level. But the maximum performance of bridges was noted in higher concentration of mutagens. [Saylor and Smith \(1966\)](#) described that the presence of single and double bridges suggests that fragmentation and rejoining of broken ends of the chromosomes have occurred due to the acute effect of the mutagens. The formation of bridges can be due to the failure of chiasmata in a bivalent to terminalize and the chromosomes get stretched between the poles.

The precocious separation of chromosomes at metaphase was observed mostly at 40 mM EMS when compared to gamma rays. It might result due to the disturbed homology for chromosome pairing or disturbed spindle mechanism. Besides the precocious separation of univalents, the bivalents were also observed to move ahead and seemed as stray chromosome, this may move to one pole resulting into unequal distribution of chromosome or loss of a complete bivalent at metaphase stage ([Khan et al., 2009](#)).

CONCLUSION

In the present investigation, the percentage of abnormal cells increased with increase in dose/concentration under some exceptions of both the physical and chemical mutagens. Among the different dose/concentration of

mutagens, EMS shows more chromosomal abnormalities than the gamma rays.

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