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INDUCED PHYSICAL AND CHEMICAL MUTAGENESIS AND ITS EFFECT IN CYTOLOGICAL BEHAVIOR OF SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

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ABSTRACT

The present investigation provides a comparative account of cytological and developmental effects on EMS (Ethyl Methane Sulphonate) and Gamma rays on meiotic features. Chemical mutagens have become important tools in crop improvement. These mutagens are being used to produce resistance in various susceptible crops to improve their yield and quality traits against harmful pathogens. There are several mutagens available for crop improvement and each mutagens has its important role as positive or negative effect on crops. Studies undertaken in M_3 generation. Variety of CSV-23 Sorghum *bicolor* (L.) Moench showed that both the mutagens EMS and Gamma rays various chromosomal aberrations. 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.in 40mM EMS produced the highest chromosomal aberrations.

KEYWORDS: *Sorghum bicolor*, EMS, Gamma rays, Mitotic aberrations, M_3 generation.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench), the second largest grain crop in India until the Green Revolution, Presently occupies the third place in term of area sown and fourth place in

production amongst the food grains. Sorghum is a typically an annual, but some cultivars perennial. Sorghum has a wide agroecological adaptation, drought tolerance, high production, low input crop and more resistant to pest and disease than other food crops. Meanwhile Sorghum also has high nutritive value so that it is very good to be used as alternative food and animal feed sources. Inspite rapid decline in Sorghum area in Asia due to the competition, for another remunerative crop, Sorghum grain production levels have not declined at the same rate of owing to adaptation, of hybrid. Sorghum is a multifunction plant due to its high economic value as a source of food, feed and Industrial raw material of Bio-fuel. The present study is to investigate the effect of mutagens on Morphological, Biochemical, Cytological, Molecular and yield parameters to improve the crop induced by Physical and chemical mutation. A. Sridevi and L. Mullainathan 2011.

It is generally considered that the hereditary variations induced by irradiation and chemical are to two fundamentally different types of change produced within the induced cells.(1) breakage and rearrangement of the chromosomes and (2) modification of the composition of individual genes. the possibility that the induced gene mutations may be due to mechanical changes analogous to those causing the grosser chromosomal aberration is rejected chiefly on the basis of the induced reverse mutations in drosophila which have been convincingly demonstrated by Muller 1927 and Bhaduri, P.N., Ghosh, P.N.1954 and others. Agarwal R., 2001 after the comprehensive experimental and theoretical analysis of this question, concludes that the induced point mutations are changes in this chemical composition of the genes, and that they probably are “endless in their eventual possibilities”.

After the discoveries of Stadler, L., J. (1926), 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. Kumar, G. and A. Tripathi, 2003. 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. El-Ghamery, A.A., et al 2003.

Recent advances in Sorghum improvement are mainly due to spontaneous mutation followed by selection and hybridization. Induced mutations received relatively limited attention. Singer, B., 1975 A critical reviews of different aspects of mutagens sensitivity, considering the importance of such factors as genotype constitution of the material. Rangaswamy M,

1973 and Marimuthu, K. M 1960 Type of mutagen and dose, techniques of handling. The material and treatment procedures to maximize the induction of mutation together with the scope of induced mutation in sorghum improvement constitute the subject matter of this paper.

MATERIALS AND METHODS

The dry and dormant viable seeds of Sorghum (Sorghum bicolor) Var-CSV-23 were obtained from Directorate of Sorghum Research (DSR), Rajendiranagar, Hyderabad, and Andhra Pradesh. Gamma rays are ionizing radiation having low wave length with high penetrable power; interact with atoms or molecules to produce free radicals in the cells. The radicals can damage or modify important components of plant cells. The source of gamma rays is $Cobalt^{60}$, one of the labeled metals, which emit gamma rays. The Sorghum seeds were treated with (20, 30, and 40KR) of gamma rays. The untreated seeds were used as control. The seeds were germinated on moist filter paper in petridishes at 25±2 °C in BOD (Biological oxygen demand) incubator under dark conditions in Genetics and mutation breeding research laboratory, Department of Botany, Annamalai University till the emergence of radicals. The gamma rays irradiated seeds were also sown in pots for morphological studies. The root tips were collected in the morning time between 7.30 to 8.30 a.m, root tips of appropriate length (0.5-1.0 cm) were excised and after pretreatment, the root tips were washed 4-5 times in distilled water, carefully dried by absorbing the moisture and subsequently fixed in 1:3 glacial acetic acid- absolute ethanol mixture. The root tips were stored in 10 % ethanol at 10°C in a refrigerator for long term use, whenever needed. The root tips of Sorghum were taken from 1:3 Acetic-Alcohols and thoroughly washed in distilled water for 2 or 3 times. The root tips were hydrolyzed in 1N HCL for 8-10 min and the tips were kept at 60 °C in water bath, followed by washing with 3 changes of distilled water and then in iron alum for 5-10 min. Finally the root tips were stained in hematoxylin stain for 2-3 hours and then washed in distilled water. Then the root tips were transferred to 45% percentage acetic acid solution few seconds. The root tips were kept in a clear side and a cover slip was mounted over it, and later it was squashed. The cover slip was mounted by Canada balsam. Bales CE and Durfee GR 1992.Microphotographs was taken from freshly prepared slides using Trinocular Research Phase Contrast Microscope attached with Digital Olympus Camera.

RESULTS AND DISCUSSION

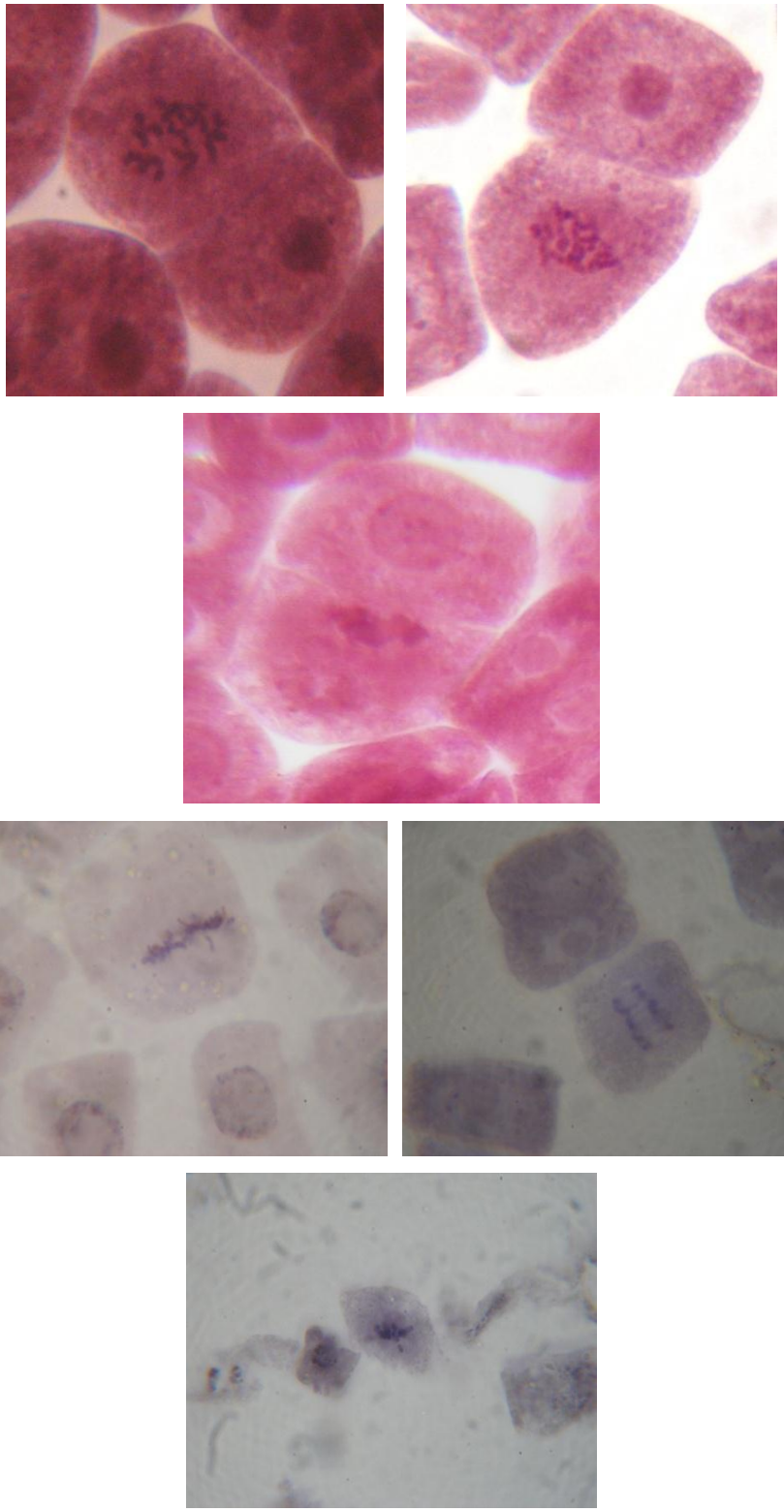
Cytological analysis with respect to their mitotic behavior is considered to be one of the most dependable indices to estimate the potency of mutagen. Cytological studies provide information regarding the response of sorghum genotype to particular greater chances for the selection of desire characters. Raicu, P.1992 and Gurley AM, et al 1992.

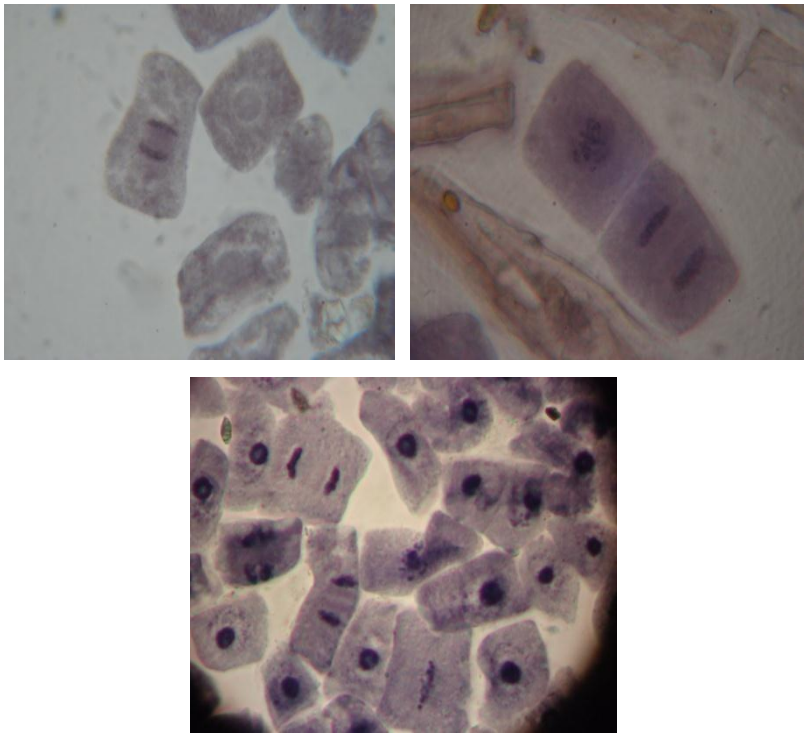
Photo 1. *Sorghum bicolor* (L.)

The root mitotical studies revealed a wide range of chromosomal aberration such as stickiness, precocious movement, anaphasic laggards, fragments, anaphasic single and multiple bridges. Chowdhury, S.et all.2009.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 30kR gamma rays and 40 and 50mm EMS.

PLATE-1

Chromosomal Aberrations in root tip cells of Sorghum.





Mitosis was perfectly normal in control plants. The maximum aberrations were found at higher dose of the mutagen. The chromosomal abnormalities were present in almost all the treatments. Mitotic abnormalities increased along with increasing dosage of gamma rays and EMS. The effect of gamma rays and EMS has been studied on mitotic activities of the root meristems. Permjit, K. and I. S. Grover. 1985 and Zeerak, N. A., 1991 Mitosis was normal in the control plants of *Sorghum bicolor*. The chromosome number of the Sorghum plant counted as 2n = 20. Lower dosage of mutagen revealed more or less normal pairing likes that of control. D. Arulbalachandran et al 2009 However, a consistent increase in the frequency of various types of chromosomal abnormalities was observed with increasing dose of gamma rays and EMS treatments.

CHROMOSOMAL ABERRATIONS

Stickiness

Stickiness of chromosomes was caused due to the polymerization of nucleic acids Caused by the irradiation of gamma rays and EMS. Sticky metaphase was present in higher dosages 30KR and 50mM of gamma rays.

Precocious movements

In the normal chromosome pairing there is no precocious moment of chromosomes Occurs. If the homology of chromosome pairing is disturbed or spindle mechanism is disturbed or

inactivated, one or few chromosome moves towards the pole from the equatorial metaphase stage. These types of precocious moment occur due to the effect of gamma rays. Precocious movement of chromosome was dominant at metaphase stage at 20KR of gamma ray treatments.

Laggard Chromosome

The failure of chromosomal moments as a result of spindle fiber discrepancies one or few of the chromosome lags behind the other chromosomes moving towards the poles and lead to the formation of laggard chromosome. The lagging chromosomes appeared in the anaphase and also in the telophase stage. In the anaphase and telophase stage laggards was observed at 40KR of gamma ray and 40mM EMS treatments.

Fragments

Chromosomal fragment occurs due to the failure of broken chromosome to recombine. Fragment was observed in the metaphase stage of higher dosages 50mM EMS Treatments.

Chromosomal Bridge

In the normal separation of chromosomes the chromosomes separated equally. Chromosomal bridges occur due to sister chromatid exchange followed by delayed or failure of their separation at later stages of anaphase and telophase chromosome. Thick and sticky bridge appeared due to stickiness of chromosomes. The dominant telophasic and anaphasic bridge was observed in 40KR of gamma ray treatment.

The fragments at metaphase may be due to the failure of broken chromosome to recombine. Rao, N.B. and Lakshmi, N.1980 The Fragments might have arisen due to the stickiness of chromosome and the consequent failure of the arrival of chromatids at the poles. Fragments may also be acentric chromosomes formed as a result of inversion Agarwal, 2001. Stickiness was one of the abnormalities found both in mitosis and in meiosis. It occurs due to disturbances of cytochemically balanced reactions by the effect of alkylating agents Chidambaram *et al.,* 2008. Bridges and laggards with or without fragments were found both at anaphase and telophase, bridges without fragments were found at higher dosages of mutagen.

Chromosomal aberration of Sorghum root tip cells treated with different conc. of gamma rays and EMS.

Mutagenic Treatments	Number of Cells	Total number of aberration	%
Gamma Rays			
20Kr	28	9	32.14
30Kr	30	11	36.66
40kr	34	7	20.58
EMS			
30mM	34	12	35.29
40mM	37	15	40.54
50mM	29	6	20.68

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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