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Effect of induced physical and chemical mutagenesis on the cytological behaviour of Sorghum (*Sorghum bicolor* (L.) Moench)

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Abstract

Chemical mutagens are used to induce resistance against harmful pathogens in various susceptible crops to improve their yield and quality. A comparative study on the cytological and developmental effects on EMS (Ethyl Methane Sulphonate) and Gamma rays on meiotic features was made. Studies were undertaken in M_3 generation *Sorghum bicolor* (L.) Moench var of CSV-23. The results showed, both the mutagens EMS and Gamma rays induced, various chromosomal aberrations. The common chromosomal aberrations recorded during mitosis included precocious movement, stickiness, bridges, fragments and laggards. The frequency of cells with chromosomal aberrations showed a linear increase up to a certain level with increase in the concentration. 40mM EMS produced higher level of chromosomal aberrations than gamma rays.

Key Words: EMS, Gamma rays, Mitotic aberrations, M_3 generation *Sorghum bicolor*,,

INTRODUCTION

Sorghum(*Sorghum bicolor* (L.)Moench), the second largest cereal crop in India until the Green Revolution, presently occupies the third place in terms of area of cultivation and fourth place in production amongst the food grains. Sorghum has a wide agroecological adaptation, drought tolerance and high production and low input crop and more resistant to pest and disease than other food crops. It has high nutritive value and is a multifunction plant due to its high economic value as a source of food, feed and industrial raw material for bio-fuel. It is generally considered that the hereditary variations induced by irradiation and chemical are two fundamentally different types of changes produced within the induced cells. They include i. breakage and rearrangement of the chromosomes, and ii. modification of the composition of individual genes. Stadler (1926) reported that a large amount of genetic variability had 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 of food crops especially cereals and pulses (Kumar and Tripathi, 2003). The genetic variations created by induced mutation through the physical and chemical mutagens are various abnormalities resulting in new varieties with desired characters (El-Ghamery *et al.*, 2003).

Recent advances in Sorghum improvement are mainly made through spontaneous mutation followed by selection and hybridization. Induced mutations received relatively limited attention. Singer (1975), critically reviewed different aspects of mutagens sensitivity with emphasis on the importance of such factors for the genotype constitution of the genetic material, and type of mutagen, dose and techniques of handling(Rangaswamy, 1973; Marimuthu, 1960). 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 which deals with the effect of physical and chemical mutagens on morphological, biochemical, cytological and yield parameters to improve the crop.

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, Andhra Pradesh. Gamma rays are ionizing radiation with low wave length and high penetrable power which 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 Petri dishes 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

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Figure 1. *Sorghum bicolor* (L.)

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 minutes. 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% 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 with Canada balsam (Bales and Durfee, 1992). Microphotographs were taken from freshly prepared slides using trinocular research phase contrast microscope attached with Digital Olympus Camera.

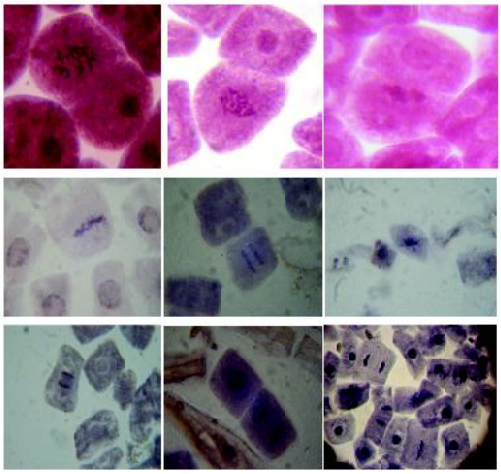
RESULTS AND DISCUSSION

Cytological analysis with respect to their mitotic behaviour 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 desired characters (Raicu and Mixich, 1992; Gurley *et al.*, 1992).

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 *et al.* (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.

Mitosis was perfectly normal in control plants. Maximum aberrations were found when induced with relatively high dose of the mutagen. The chromosomal abnormalities were present in almost all the treatments.

Plate 1. Chromosomal Aberrations in root tip cells of Sorghum



Mitotic abnormalities increased with increasing dosage of gamma rays and EMS. The effect of gamma rays and EMS has been studied on mitotic activities of the root meristem following the standard methods (Permjit and Grover, 1985; Zeerak, 1991) Mitosis was normal in the control plants of *Sorghum bicolor*. The chromosome number of the Sorghum plant was counted as 2n = 20. Lower dosage of mutagen revealed more or less normal pairing similar to that of control as reported by .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 due to polymerization of nucleic acids caused by the irradiation of gamma rays and EMS. Sticky metaphase was found when higher dosages of 30KR and 50mM of gamma rays were treated.

Precocious movements

In the normal chromosome pairing there is no precocious moment of chromosomes. If the homology of chromosome pairing is disturbed or spindle mechanism is disturbed or inactivated, one or few chromosomes move 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 in the metaphase stage at 20KR gamma ray treatment.

LAGGARD CHROMOSOME

The failure of chromosomal moments as a result of spindle fiber discrepancies leads to one or few of the chromosomes lag behind other chromosomes that are moving towards the poles, which results in the formation of laggard chromosome. The laggard chromosomes appeared in the anaphase and also in the telophase

stage. In the anaphase and telophase stages laggards were observed at 40KR of gamma ray and 40mM EMS treatments

Fragments

Chromosomal fragments are formed due to the failure of broken chromosome to recombine. Fragment was observed in the metaphase stage in the plants treated with high dosages i.e., 50mM EMS.

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 formation of fragments at metaphase could be due to the failure of broken chromosome to recombine as reported by Rao and Lakshmi (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 could also be acentric chromosomes formed as a result of inversion. Stickiness was one of the abnormalities found both in mitosis and in meiosis. It occurs due to disturbances of cytochemically balanced reactions caused by 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

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 showed more chromosomal abnormalities than the gamma rays.

Table 1. Chromosomal aberrations caused by different concentrations 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

REFERENCES

Agarwal R., Ansari M. Y. K., *J. Cytol. Genet.* 2 (2001) 129-134. sMuller H. J., *Science* 66 (1927) 84-87. Incomplete SA.Sridevi and L.Mullainathan 2011 genotoxicity effect of ethy methane sulphonate on Root tip cells of chilli(capsicum annumL.) W.J.Agri Sci.7 (4):368-374.

Bhaduri,P.N. and Ghosh,P.N.1954.Chromosome squashes in cereals stain tech.29:269-79.

Bales CE and Durfee GR. Cytological technique, part 1. In: Koss LG, ed. *Diagnostic Cytology and Its Histopathologic Bases.* 4th ed. Philadelphia, PA: Lippincott; 1992:1451-1474.

Chidambaram A., Sundaramoorthy P., Murugan A., Sankar Ganesh K., Baskaran L., *Iran J. Environ. Health Sci. Eng*. 6 (1) (2008) 17-22.

Chowdhury, s., A.K.Datta and S.Maity, 2009. Cytogenetical and agronomical aspects of radiation induced marker trait mutants in Sesame (Sesamum indicum L.), Indian J. Sci and Tech, 2(5):58-61.

D.Arulbalachandran,L.Mullainathan,S.Velu 2009.Genetic Variation in Quantitative Traits of Black gram(Vigna mungo(L.)Hepper)induced by Gamma rays treatment in M_1 generation.J phyto 1(5):308-312.

El-Ghamery,A.A., M.A. El-Kholy and M.A.A ElYousser, 2003. "Evaluation of cytologicaleffects of zn in relation to germination and root growth of *N*.2+ *sativa* L. and *Triticum aestivum* L.". Mutation Res., 537: 29-41.

Gurley AM, Silverman JF, Lassaletta MM,1992; The utility of ancillary studies in paediatric FNA cytology. *Diagn Cytopathol.* 8:137-146.

Kumar, G. and A. Tripathi, 2003. Comparative mitotoxicity and mutagenicity of two heavy metals in *Allium cepa* L. Cytol. Genet. 4:169173.

Marimuthu, K. M. and M. K. Subramaniam. 1960. A haematoxylin squash method for the root tips of *Dolichos lablab* L. Curr. Sci. 29:482493.

Permjit, K. and I. S. Grover. 1985. Cytological effects of some organophosphorus pesticides II meiotic effect.Cytologia 50:199-211.

Rangaswamy M. (1973). Induced mutagenesis in gingelly (*Sesamum indicum* L.) with gamma rays and ethyl methane sulphonate. M.Sc. (Ag). Thesis, *Tamilnadu Agrl. Univ*., Coimbatore.

Raicu, P. and F. Mixich, 1992: Cytogenetic effects of sodium azide encapsulated in liposomes on heteroploides cell cultures. Mutation Research, 283: 215-219.

Rao,N.B. and Lakshmi, N.1980. Gamma ray induced miotic abnormalities n capsicum annumL. Caryo logia 33:509-518.

Singer, B., 1975. The chemical effects of nucleic acid alkylation and their relation to mutagenesis and carcinogenesis. Prog. Nucleic acids Mol. Biol., 15: 219-284.

Stadler,L.,J.(1926) "The variability of crossing-over in maize,"Genetics,11,1-37.

Zeerak, N. A., 1991: Cytological effect of gamma rays and ethyl methane sulphonate in brinjal (Solanum melongena L.) Cytologia, 56: 639-643.