ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of Mutagens on Cytological studies in Soybean (*Glycine max* (L.) Merr.)

[1]Pavadai P., S. Gnanamurthy[2] and D. Dhanavel[2]

1. P.G Research Department of Botany, Periyar Government Arts College, Cuddalore, 607 001. TamilNadu, India

2. Department of Botany, Annamalai University, Annamalainagar, 608 002. TamilNadu and India.

Manuscript Info

Manuscript History:

Received: 12 August 2013
Final Accepted: 25 August 2013
Published Online: September 2013

Key words:
Soybean, mutagens, Chromosome aberrations and laggards.

Abstract

Effect of mutagenesis on Soybean (*Glycine max* (L.) Merr.), plants of four generation *viz*., M_1, M_2, M_3 and M_4. The two types of mutagens like physical and chemical such as physical mutagens namely Gamma rays and chemical mutagens namely, ethyl methane sulphonate (EMS), di-ethyl sulphate (DES) and colchicines (COH). The mutagenic treatments for various doses separately. The cytological studies such as chromosomal number was observed and recorded for photographed. The maximum changes of chromosome were observed in gamma rays (Physical mutagen) and colchicines (Chemical mutagen) than the other mutagens. The length and shape of chromosome for varied in treated plants than the untreated plants.

Introduction

Soybean (*Glycine max* (L.) Merr) is a very important food material and high nutrition value than the other crops. The protein content was first place followed by other crops and oil production for the second place for the other oil crop plants such as ground nut, mustard and palm seed. The mutagenesis was quickly treated in gene level so the important of crops production. The FAO/IAEA was first news released the data base for most of mutant varieties of Soybean.

Mutation breeding supplement conventional plant breeding as a source of increasing variability and could confer specific improvement without significantly altering its acceptable phenotype (Ojomo *et al.*, 1979). Mutagen induced anomaly of the chromosome is the primary basis of genetic change; therefore, investigations on the mechanism of chromosome breakage, type of aberrations, and their genetic consequence form an integral part of most mutation studies (Zeerak, 1992). Induced mutagenesis has been recognized as the most efficient method for induction of morphological and genetical variabilities in plants especially in those with limited genetic variabilities, because in plants the gene replacement experiments through homologus recombination with introduced DNA sequences have met with limited success.

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 various genotypes to a particular mutagen and provide greater chances for the selection of desired characters.

A chromosome anomaly, abnormality or aberration reflects an atypical number of chromosomes or a structural abnormality in one or more chromosomes. A karyotype refers to a full set of chromosomes from an individual which can be compared to a normal karyotype for the species via genetic testing. A chromosome anomaly may be detected or confirmed in this manner. Chromosome anomalies usually occur when there is an error in cell divisions following mitosis. There are many types of chromosome anomalies. They can be organized into two basic groups, numerical and structural anomalies.

Root mitotic studies revealed a wide range of chromosomal aberration such as nullisome, anaphasic bridge with laggard, anaphasic multiple bridges and laggards, anaphasic bridge, late anaphase, clubbing of chromosome and precocious movement of chromosomes. The chromosome studied was observed in treated plants such as both physical and chemical treatments. The 40% chromosome aberration was high for M_1 and M_2 generation than the M_3 and M_4 generation. The

chromosome laggard was observed for all treatments than the other chromosome aberration.

Materials and Methods

The dry seeds of soybean variety CO-1 were subjected to both physical and chemical mutagens. The mutagens namely, gamma rays, EMS, DES and COH were given at six different dose/concentrations. The data were recorded till four generations. The plants were selected according to randomized block design (RBD) method. The seeds were treated with gamma rays (10, 20, 30, 40, 50 and 60KR), EMS (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%), DES (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%) and COH (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%). The physical treatments were induced at sugarcane breeding institute (ICAR), Coimbatore. The chemically treated seeds were presoaked in distilled water for 6 hrs to ensure complete hydration of the seeds.

The seeds were treated with solution of EMS, DES and COH for duration of 6 hrs. After, the seeds were thoroughly washed in running tap water for 8 to 10 times. The treated seeds were sown in the field along with the control in a randomized block design with three replications. M_2, M_3, and M_4 generation were used in ten randomly selected in the M_1, M_2, M_3, and M_4 generation plants.

The treated and untreated (control) seeds were germinated in most vermiculite. Three or four days old actively growing root tips were thoroughly washed in tap water and pre treated in 0.002% Hydroxyquinolin at 4 to 10°C for 3 hours. The pre treated root tips were then washed in distilled water and stored in acetic alcohol for further study. Root tip squashes were made following the Iron alum haematoxylin squash technique (Marimuthu and Subramanian, 1962). From the mitotic squash slides, the metaphase stage and chromosomal aberrations of the cell division were found and microphotographed for chromosome analysis. Each treatment was repeated at least three times as described above.

Results and Discussion

Mitotic studies

The physical mutagens like gamma rays and chemical mutagens like ethyl methane sulphonate, Di-ethyl sulphonate and colchicines induced many mitotic abnormalities. The observation was photographed in plate I&II.

Physical mutagen

The physical mutagens namely, gamma rays were used in 10, 20, 30, 40, 50 and 60KR ranges. The somatic chromosome were well scattered and the constriction of chromosomes were clearly seen. The somatic chromosome 2n=40 apart from normal division, nullisomic 2n=38 and anaphasic laggards were also observed.

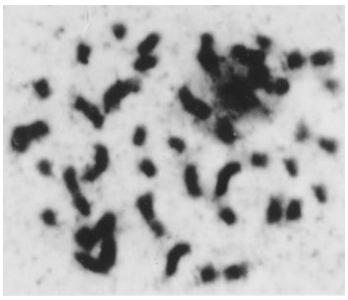
Chemical mutagens

The chemical mutagens namely, EMS (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%). DES (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%) and COH (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%) treatment of the Soybean seedlings. Among the concentration of chemical mutagens some of the cytological behavior like normal metaphase (2n=40), anaphasic laggards, bridge, late anaphase, precocious movement of chromosome and clumping of chromosome were observed in the present study.

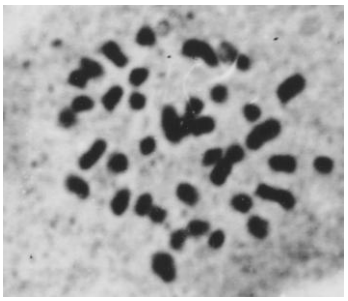
The soybean basic chromosome number is 2n = 20 and with diploid chromosome number 2n = 40 (Sen and Vidyabhusan, 1960). The detailed chromosome studies were carried out understand it cytology (Biswas, 1977; Ahmad *et al.*, 1983). In the present study somatic chromosome was carried out with effect of mutagens. The metaphase chromosome number was 2n=40 in control. Whereas, 50kR of gamma rays treatment showed 2n=38 (Nullisomic) and 0.04% of COH showed 2n=80 (tetraploid) chromosomes (Plate-I). The numerical variation of somatic chromosomes of 2n complement was revealed mutagenic effect in the genome. The tetraploid chromosome number showed 2n=80 was due to the absence of well organized spindle in metaphase (Dusane *et al.*, 1991) caused by effect of COH. Chromosomal rearrangements are one of the most frequently produced classes of mutation that result from the action of physical and chemical mutagenic agents (Gecheff, 1996)

Whereas, chromosomal aberrations such as anaphasic bridge with laggards, multiple bridges, laggards, late anaphase, precocious movement of chromosomes and clumping of chromosomes were observed in present study (Plate-I&II). Similar observations were reported by many workers in wheat (Alam *et al*., 1980), black gram (Bandyopadhyay and Bose, 1983), sunflower (Elangovan and Selvaraj, 1995), chickpea (Sharma and Kumar, 2004; Gania, 2005), maize (Kumar and Kumar Rai, 2007) and chilli (Kumar and Gupta, 2009).

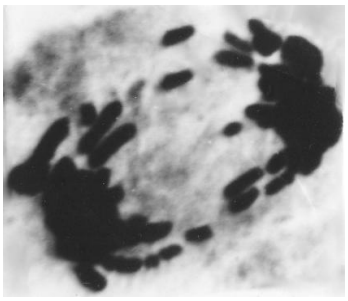
PLATE-I



Metaphase 2n=40 (control)



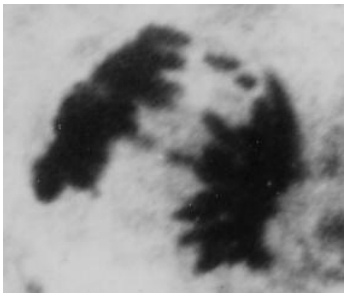
Nullisomic 2n=38 (50 kR gamma rays)



Anaphasic bridge with laggard (60 kR gamma rays)

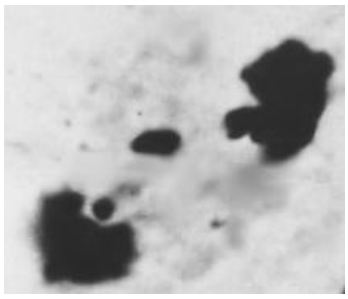
Anaphasic multiple bridges and laggards (0.5%EMS)

Anaphasic laggard (0.5% EMS)

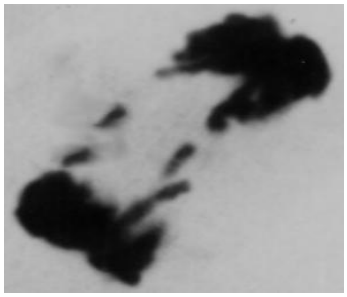


Anaphasic bridge (0.6% EMS)

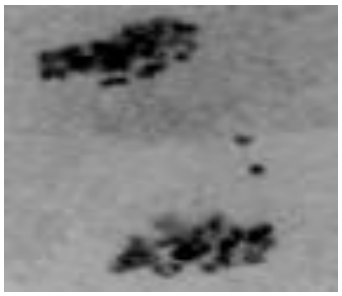
PLATE-II



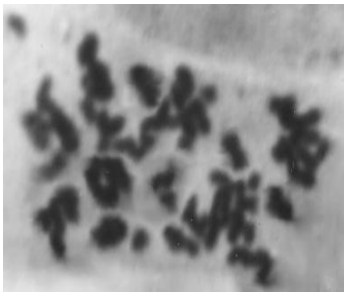
Anaphasic laggard (0.05% DES)



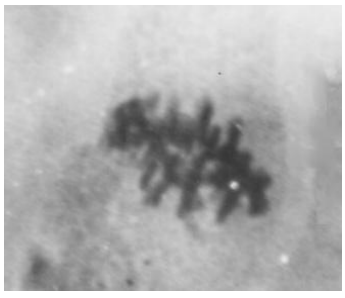
Anaphasic bridge with laggard (0.05% DES)



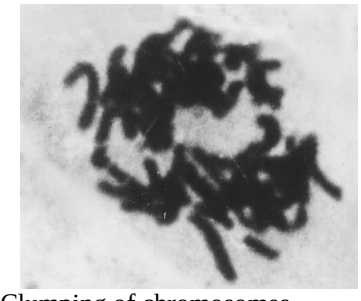
Late anaphase (0.06% DES)



Metaphase 2n=80 (0.04% COH)



Precocious movement of Chromosome (0.05% COH)



Clumping of chromosomes (0.05% COH)

Chromosomal bridge may be formed due to the breakage and fusion of chromosomes. The bridge formation cab be due to the general stickiness of chromosome of metaphase stage (Ahmad and Yasmin, 1992). Bridges and laggards with or without fragments were found both at anaphase and telophase, bridges without fragments were found in lower concentration while as bridges with fragments were found at higher concentrations of the mutagens, both single and double bridges were found but the multiple bridges were not also rare. Multiple bridges were mostly found at anaphase and the spindle bridges at telophase (Bhat *et al.,* 2007).

Precocious movement of chromosomes seems to be a manifestation of improper spindle functioning. The presence of spindle and multiple bridges may be due to the occurrence of dicentric chromosomes formed as a result of breakage fusion bridges cycles (Fluminhan and Kameya, 1997).

The inhibition of seedling growth seemed to be well correlated with the amount of chromosomal damage. The EMS was found to react with the genetic material by alkylating DNA bases and phosphate groups (Thengane, 1984). The radio sensitivity was related to nuclear volume and interphase chromosome volume (Constantin and Love, 1967). Chromosomal and extra chromosomal materials were reported as the primary site of damage in the irradiated seed (Inoue *et al.,* 1975).

Conclusion

In the present study, the aberrations caused by mutagens were due to partial or complete failure of spindle mechanism. The percentage of abnormal cells increased with an increase in the dose/concentration of both physical (gamma rays) and chemical (EMS, DES and COH) mutagens. The maximum changes of chromosome were observed in gamma rays (Physical mutagens) and colchicines (Chemical mutagens) than the other mutagens.

References

Ahmad, Q.N., E.J. Britten and D.E. Byth. 1983. A quantitative method of karyotypic analysis applied to the soybean, *Glycine max*. *Cytologia,* 48: 879-892.

Ahmad, S. and R. Yasmin. 1992. Effect of methyl parathion and tri mitox on the mitosis of *Allium cepa*, *Cytologia*, 57: 155-160.

Alam, S., M.N. Amin and M.R. Khan. 1980. Cytological effects of insecticide (Carbicron-100) and gamma radiation on common wheat. *Bangladesh J. Bot.*, 9: 147-156.

Bandyopadhyay, B. and S. Bose. 1983. Mitotic irregularities induced by single, pre- and post-irradiation treatments with ethylmethane sulphonate, hydroxylamine and ethylene imine in *Phaseolus aureus* Roxb. and *Phaseolus mungo* (L.). *Cytologia,* 48: 13-19.

Bhat, T.A., M. Sharma. and M. Anis. 2007. Comparative analysis of mitotic aberrations induced by Di-ethyl sulphate (DES) and Sodium Azide (SA) in *Vicia faba* L. (Fabaceae). *Pak. J. Bio. Sci*, 10(5): 783-787.

Biswas, A.K., 1977. Karyotype in three cultivated varieties of *Glycine max* (L.) Merr. *Curr. Sci.,* 46: 195-196.

Constantin, M.J. and J.E. Love. 1967. Seedling responses of *Vigna sinensis* (L.) Savi to gamma, neutron irradiation. *Rad. Bot.,* 4: 497-506.

Dusane, A.V., C.K. John and R.J. Thengane, 1991. Colchicine induced tetraploidy in *Cajanus cajan* (L.) Millsp. var. ICPL 87. *J. Cytol. Genet.* 26: 39-47.

Elangovan, M. and R. Selvaraj, 1995. Effect of chemical mutagen on germination and seedling characters of sunflower genotypes. In: Proc. Natl. Symp. on Relevance of Biotechnology in Industry, 4-5, Cochin, India.

Fluminhan, A. and T. Kameya, 1997. Involvement of Knob hetero chromatin in mitotic abnormalities in germinating aged seeds of maize. *Genome,* 40: 91-98.

Gania, F.A., A.H. Khan, T.B. Bhat, S. Parveen and N.A. Wani, 2005. Cytogenetic effects of methylmethane sulphonate (MMS) on two varieties of chickpea (*Cicer arietinum* L.). *J. Cytol. Genet.* 6(2): 97-102.

Gecheff, K.I. 1996. Production and identification of new structural chromosome mutations in barley (*Horteum Vulgare* L.). *Theor.Appl.genet*. 92: 777-781.

Inoue, M., H. Masegawa and S. Hori, 1975. Physiological and biochemical changes in gamma irradiated rice. *Rad. Bot.,* 15: 387-395.

Kumar, G & P. Kumar Rai, 2007. EMS induced karyomorphological variations in maize (*Zea mays* L.) inbreds, *Turk. J. Biol*. 31: 187-195.

Kumar, G. and P. Gupta, 2009. Induced karyomorphological variations in three phenodeviants of *Capsicum annum L. Turk. J. Biol*, 33: 123-128.

Marimuthu, K.M. and M.K. Subramaniam, 1960. An iron alum haematoxylin squash schedule

for the root tips of *Dolichos lablab* L. *Curr. Sci.*, 29: 482-483.

Ojomo, A.O., O. Omueti, J.A. Raji and O. Omueti, 1979. Studies in induced mutation in cowpea, 5. The variation in protein content following ionizing radiation, *Nig. J. Appl. Sci*. 21: 61-64.

Sen, N.K. and R.V. Vidyabhusan, 1960. Tetraploid soybean. *Euphytica*, 9: 317-322.

Sharma, V. and G. Kumar, 2004. Meiotic studies in two cultivars of *Cicer arietinum* L. after EMS treatment. *Cytologia*, 69(3): 243-248.

Thengane, R.J., 1984. Influence of respiratory inhibitors on ethylmethane sulphonate induced mutagenic effects in *Hordeum vulgare* L. *Cytol.*, 49: 333-344.

Zeerak, N.A. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in brinjal (*Solanum melongena* L.), *J.nuc. Agr. Bio*. 21: 84-87.

****