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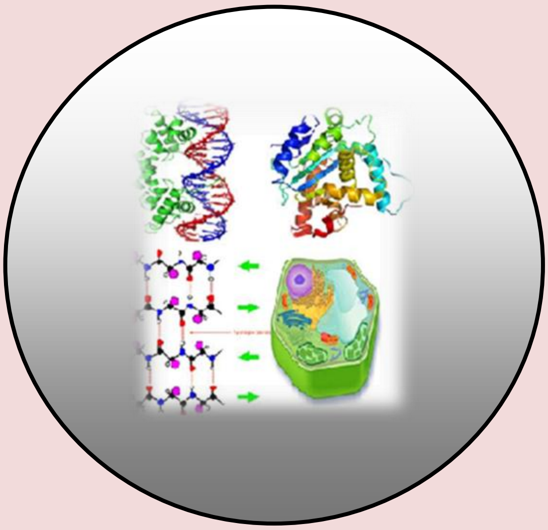
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 36 (1) 2019 Pages No. 337-341



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 36, No. 1, 337-341, 2019
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 36/01/1921/2019

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

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RESEARCH PAPER

Received: 14/05/2019 Revised: 10/06/2019 Accepted: 11/06/2019

Variation in Chiasma Frequency: A Comparison of Effect of Gamma Rays and EMS on Pearl Millet

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ABSTRACT

The present study takes a deep insight into one of the lesser studied effects of mutagens on the meiosis of Pearl Millet (Pennisetum glaucum L. (R.Br)) ie. variation in Chiasma Frequency. Both the mutagens viz. Gamma rays and Ehtyl Methane Sulphonate (EMS), proved to be potent enough in reducing the Chiasma Frequency per Pollen Mother Cell to varying degrees in comparison to Controls. However, Gamma rays had greater effect on this aspect as compared to EMS. The present study looks into similar works on other plants and tries to bring out the probable causes, significance, and mechanism etc. for the observations. The process of formation of chiasma, genetic relationship and probable mode of action of mutagens has been discussed in detail.
Keywords: Chiasma frequency, EMS, Gamma rays, mutation, Pearl millet and Variation.

INTRODUCTION

According to Wikipedia, a chiasma (or chiasmata) is the point of contact, the physical link, between two (non-sister) chromatids belonging to homologous chromosomes. At a given chiasma, an exchange of genetic material can occur between both chromatids, which is called a chromosomal crossover during meiosis (Andersen and Sekelsky, 2010). In meiosis, absence of a chiasma generally results in improper chromosomal segregation and aneuploidy (Fledel-Alon et al, 2009) The phenomenon of chiasmata formation was discovered and described in 1909 by Frans Alfons Janssens, a Professor at the University of Leuven in Belgium (Carlson, 2004, Schwartz, 2008).

When each tetrad, which is composed of two pairs of sister chromatids, begins to split, the only points of contact are at the chiasmata. The chiasmata become visible during the diplotene stage of prophase I of meiosis, but the actual "crossing-overs" of genetic material are thought to occur during the previous pachytene stage.

Sister chromatids also form chiasmata between each other (also known as a chi structure), but because their genetic material is identical, it does not cause any noticeable change in the resulting daughter cells.

Practically, Chiasma Frequency is the number of points of attachment between all the bivalents in a PMC. However, Chiasma frequency is also considered as the calculation of the level of genetic recombination of a population. It is calculated by formula
CF = 2 X Fr

Where, CF is the Chiasma Frequency, Fr is recombination frequency.

Recombination frequency is given by
Fr = $\frac{\mathrm{N\ X\ 100}}{\mathrm{Np}}$
Where, N is the number of recombinants, Np is the total number of progeny.
Natural genetic variation in chiasma frequencies is not uncommon and has also been documented for a wide range of eukaryotic species (Simchem and Stamberg 1969; Brooks 1988). Similarly, natural genetic variation has been found in chiasma frequencies within and among many species of insects, mammals, and plants (Sybenga 1975; Jones 1987). Some mutation experiments for inducing high and low chiasma number have also been successful.

It has been well established that Chiasma formation and frequency is controlled by a very large number of genes (Gottschalk and Klein 1976) and further provides the organism a mechanism of adaptation to new habitats (Sun and Rees 1964). Mutagenic agents like Gamma rays and alkylating agents bring about changes in these gene sequences, thereby reducing or enhancing the Chiasma frequency. The present study has been undertaken to assess/compare the effects of gamma rays and Ethyl Methane Sulphonate (EMS) on chiasma frequency in *Pennisetum glaucum.*

MATERIALS AND METHODS

For the study, selfed seeds of var Mainpuri were obtained from CSAU, Kanpur. One set of seeds were irradiated in a Co^{60} source at NBRI, Lucknow with exposures of 0.10 kGy, 0.20 kGy, 0.30kGy and 0.40kGy. In another set of seeds, treatment was done with EMS prepared in sodium phosphate buffer solution of 7.0 pH. The concentrations of EMS used were: 10 mM, 20 mM, 30 mM, 40 mM for 5h. Prior to treatment seeds were soaked in distilled water for 8 hours. The treated seeds were washed in running water for half an hour. They were then sown in the field along with controls. The plants were grown, under uniform experimental conditions in the botanical garden of Shri Jai Narain PG College, Lucknow. For each concentration, three samples, each with 100 seeds, were taken along with controls.

At the time of flowering, buds from very young cobs were fixed at a particular time in modified Carnoy's fluid. The fixed buds were marked and stored in 70% Ethanol at $4^{O}C$ for study. Meiosis was studied using Acetocarmine squashes of pollen mother cells (Bhaduri and Gosh 1954). The PMCs at diakinesis and metaphase-I, were taken into consideration for estimation and comparing the frequency of chiasmata in treated as well as in control plants. In each treatment, five hundred cells, per stage, at random, were analyzed.

RESULTS AND DISCUSSION

In *P.glaucum,* the bivalents are usually rings with two terminal chiasmata. In the present investigation, in addition to normal ring bivalents, rod bivalents, univalents and multivalents were observed. The chiasma frequency estimated in controls as well as in treated plants is presented in Table 1.

In treated plants, as compared to controls, there was a marked reduction in chiasma frequency. CF/PMC showed a decrease from 12.46 in Controls to a minimum of 8.61 in 0.40kGy Gamma radiation treated set. As compared to EMS, Gamma rays proved to be more potent in reduction of the chiasma frequency.

Besides reducing the Chiasma Frequency, radiation treatments induced a higher frequency of translocations and univalents than the chemical mutagenic treatments which was in concurrence with results of Konzak et al. 1961; Sree Ramulu 1973; Jayabalan and Rao 1987; Kumar & Singh 2003. In similar studies by Bennett and Rees (1970) in rye and Singh (1974) in *Delphinium* it was observed that the chiasma frequency showed an increase after mutagenic treatments. On the other hand, Goud (1967) in wheat, Prasad and Gonward (1969) in *Phalaris*, Sree Ramulu (1973) in *Sorghum*, Sadanandam and Subash (1984) in *Capsicum* reported reduction in chiasma frequency due to mutagen induced structural changes. Jain and Basak (1965) in *Delphinium* and Sinha and Mahapatra (1969) in *Zea* found that mutagens induced no reduction in chiasma frequency despite the occurrence of univalents and this has been explained as due to compensatory mechanism by which the remaining bivalents formed more chiasmata than usual.

Table 1. Chiasma Frequencies of various treated sets.

Treatment		CF / PMC $\pm$ SE	CF / Biv $\pm$ SE	Relative CF/PMC as % of Control
CONTROL	-	12.46 $\pm$ 0.03	1.78 $\pm$ 0.01	100.0
GAMMA RAYS	0.10 kGy	11.34 $\pm$ 0.09	1.62 $\pm$ 0.04	91.17
	0.20 kGy	10.99 $\pm$ 0.07	1.57 $\pm$0.05	88.20
	0.30 kGy	9.10 $\pm$ 0.12	1.30 $\pm$ 0.05	73.03
	0.40 kGy	8.61 $\pm$ 0.07	1.23 $\pm$ 0.13	69.10
EMS	10mM	11.62 $\pm$ 0.07	1.66 $\pm$ 0.05	93.25
	20mM	10.43 $\pm$ 0.11	1.49 $\pm$ 0.08	83.71
	30mM	10.15 $\pm$ 0.10	1.45 $\pm$ 0.11	81.46
	40mM	9.45 $\pm$ 0.11	1.35 $\pm$ 0.21	75.84

CF/PMC = Chiasma Frequency / Pollen Mother Cell, CF/Biv =Chiasma Frequency / Bivalent, SE = Standard Error

According to Lawrence (1961 a and b), decrease in chiasma frequency due to mutagenic treatment, might be a result of inference at two stages of division viz. DNA synthesis stage and chiasma formation stage. If mutagen acts on DNA synthesis then it may delay DNA synthesis or disturb chromosome coiling or pachytene pairing. However if the mutagen acts during chiasma formation, then it may disturb the steps of chiasma formation, thereby reducing their frequency.

It may also be considered that rapid terminalization of chiasmata in bivalents might also reduce frequency. The role of mutated genes in creating the change in chiasma formation has been reported in desynaptic mutants by Gottschalk and Villaobas-Petrini (1965) in peas, Kitada and Omura (1983) in rice, Sadanandam et al. (1981) in *Capsicum* and Kumar and Singh (2002) in *Hordeum*. DNA and proteins have an important role on crossing over. Alternatively, the effect may be through certain proteins which have a specific role in chiasma formation (Stern and Hotta 1969).

CONCLUSION

In the present investigation, physical mutagen ie. Gamma rays proved to more potent in reducing CF/PMC than chemical mutagen ie. EMS. The reduction in chiasma frequency may be attributed to the nature, potency and mode of action of mutagens and also to the underlying factors such as complex structural changes or changes in nature of genes responsible for chiasma formation. The low chiasma frequency at high doses may be attributed either to failure of complete pairing, or to the change in nature of gene controlling chiasma formation as an effect of the mutagen. Together with reduction in Chiasma Frequency, other abnormalities related to chiasmata like univalent, multivalents, translocations etc are also common effects of mutagenic treatments.

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