International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **4(1),** 82-85, January **(2015)** *Int. Res. J. Biological Sci.*

Effect of Gamma Radiation on Chromosome at Mitotic Division in Phlox drummondii

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Available online at: www.isca.in, www.isca.me
Received 4th August 2014, revised 22nd November 2014, accepted 1st January 2015

Abstract

The present study was conducted to understanding effects of gamma radiation on mitosis. Seeds of Phlox drummondii were irradiated with five doses viz. 5kR, 10kR, 15 kR, 20kR and 25kR of gamma rays and studied of effects on mitosis. Various type Chromosomal aberrations were displayed like, dicentric, tricentric, translocation, deletion, fragment, ring, minute, bridge and micronucleus in treated root tip mitosis. Chromosomal aberrations were found to be correlated with dose. The maximum frequency of Chromosomal abnormalities was in 25kR.

Keywords: Gamma radiation, chromosomal aberrations, *Phlox drummondii.*

Introduction

Chromosomes are composed of long thin molecules of DNA. When cells are exposed to radiation or carcinogens, DNA sometimes breaks, and the broken ends may rejoin in different patterns from their original arrangement. It may be visualized at mitosis when cells divide[1]. It has been extensively utilized for many years to cause mutations and chromosomal damage for experimental purpose. These can induce a change in the molecular organization of chromosomes and the change may be expressed as a mutation, a break in a chromosome or an alteration in the physiological activity of the cell[2]. The study of radiation induced chromosomal aberrations has been done by several workers as, in *Lathyrus sativum*[3], in *Vicia faba*[4], in *Vigna mungo*[5]. The *Phlox drummondii* is an ornamental plant belonging to the family Polemoneaceae. The chromosome number is 2n =14. The advantages of *Phlox drummondii* is as a test material is easily available, easy to grow, very high frequency of dividing cells per root tip, intensely stained chromosomes[6]. The main object the present study was enhancing the understanding of the doses response for chromosomal aberration and induction of mutation could be to produce a new useful mutant.

Material and Methods

Dry seeds of *Phlox drummondii* were irradiated with gamma rays from Co60 source at the rate of 5, 10, 15, 20 and 25kR at B.A.R.C. Mumbai. After irradiation, seeds with control were germinated on wet filter paper in Petridis. Seeds germinated after four-six days, about 0.5 to 1.0 cm long root tips were cut with the help of razor and fixed in freshly prepared Carnoy's fluid after pretreated with 0.05% colchicines solution for about three hours. Fixed root tips were hydrolyzed in 1N Hydrochloric acid at 60^0C with fifteen minutes, stained in Fulgen solution and squashed in 1% acetocarmine. Slides of control and irradiated root tip were prepared individually by taking single root tip at a time. Slides with well spread cells and clear chromosomes were selected for scoring. Photomicrographs were taken with help of digital camera under oil immersion (X100) and magnified to X1000.

Results and Discussion

The chromosomes are a definite number, shave and size in particular species. It can be easily studied during cell division. In the present study, 14 chromosomes were observed at metaphase in each cell and chromosomes were equally distributed at each pole at anaphase/telophase in control root tips (figures-1,2). The karyotypic study also was done and all the seven pairs of homologous chromosomes are characterized by the presence of subtelocentric chromosomes. The fifth pair is nucleolar in nature with prominent satellites (figure-10). Gamma radiation is an electromagnetical ionizing type of radiation, its effect on biological system is based on the interaction with atoms. The radiation may acts directly on the cellular components, molecules or indirectly on water molecules, causing water derived radicals. Radicals react with nearby molecules in very short time, resulting in breakage of chemical bonds of the effected molecules. The major effects in cells are DNA breaks[7]. Radiation induced chromosomal abnormalities have reported by various workers in different plant species such as in *Capsicum annum*[8], *Chrysanthemum carinatum*[9], *Phlox drummondii*[10-11], *Vicia*[12].

Table-1
Total number of dividing cells and abnormal cell at metaphase in mitosis of *Phlox drummondii*

Dose	No. of analyzed root tips	Total No. of dividing cells	Total No. of abnormal cells	% of abnormal cells
Control	6	602	-	
5kR	-	568	297	52.2%
10kR	-	516	326	63.1%
15kR	-	498	359	72.%
20kR	-	472	367	77.7%
25kR	-	456	368	80.7%

Table-2
Various type of radiation induced chromosomal aberration at metaphase in mitosis of *Phlox drummondii*

Dose	Total No. of abnormalities	Dicentric	Tricentric	Translocation	Ring	Minute	Deletion	Acentric fragment
Control	-	-	-	-	-	-	-	-
5kR	391	126	-	63	32	55	12	103
10kR	498	148	13	78	26	74	23	136
15kR	587	198	19	74	38	85	28	145
20kR	682	212	28	82	41	92	36	191
25kR	696	216	27	79	47	98	26	203

Table-3
Various type of radiation induced chromosomal aberrations at anaphase/telophase in mitosis of *Phlox drummondii*

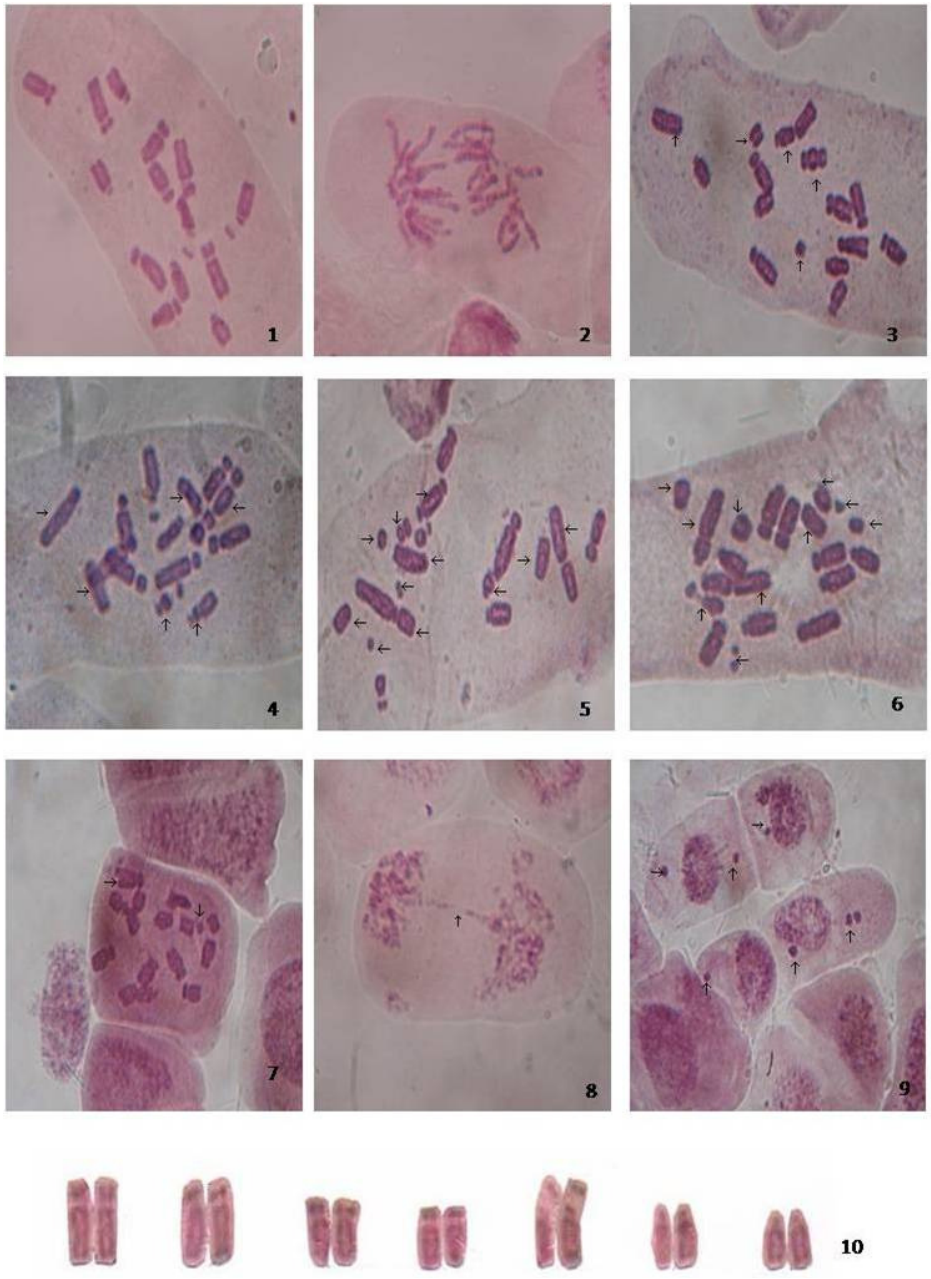
Material	Analyzed cells	Abnormal cells	Number of abnormalities	Bridges	Micronuclei
Control	200	-	-	-	-
5kR	-	64	64	27	37
10kR	-	68	70	18	52
15kR	-	78	89	23	66
20kR	-	110	115	32	83
25kR	-	136	155	56	99

During present investigation five radiation doses such as 5, 10, 15, 20 and 25kR were used to find out various types of chromosomal abnormalities at mitosis. Ten slides were scored in each dose with control. Total number of dividing cells decreased with doses. Maximum number of dividing cells were 568 in 5kR and minimum 456 in 25kR as compared to 602 in control. Total number of abnormal cells and total number of chromosomal abnormalities were increased along with dose. Minimum number of abnormal cells 297 in 5kR and maximum 368 in 25kR were found whenever 100% cell normal in control at metaphase (table-1). The minimum number of chromosomal abnormalities were 391, out of which 126 dicentrics, 63 translocations, 32 rings, 55 double minutes, 12 deletions and 103 acentric fragments were observed in 5kR, 696 out of which 216 dicentrics, 27 tricentrics, 79 translocations, 47 rings, 98 minutes, 26 deletion, 203 acentric fragments were observed in 25kR (table-2, figures-3,7). At the anaphase/telophase, out of 200 cells 64 were abnormal whereas 27 bridges 37 micronuclei in5kR and 136 were abnormal whereas 56 bridges 99 micronuclei 25kR (table-3, figures-8,9). Their modes of origin are discussed below according to Savage[13].

Chromosomes with dicentrics were observed in large number. They arise when there are at least two breaks occurs in each arm of two adjacent chromosomes and if the broken ends lie close to each other not more than 0.2 µm apart, reunion produce a dicentrics chromosome and an acentric fragment. Tricentric arise from four breaks in tree chromosomes. Polymorphism in the size of dicentrics and positions of centromere was observed. In many cells, different combination s of dicentric and tricentric chromosomes were recorded. The separation of dicentric/tricentric at subsequent anaphase may to the formation of bridges[14]. Many bridge such bridge were observed at anaphase in present investigation.

Translocation occurs between larger and shorter arms of the chromosomes and they are produced by two breaks in two chromosomes and the reciprocal rejoining of the broken ends. If a break occurs in each side of the centromere of one chromosomes and centromere is included in the displaced fragment, it forms a centric ring. The remaining arms of the chromosome undergo reunion to give rise to an acentric fragment, which is eventually lost from the nucleus and the result is a deficiency aberration. Chromosomes with acentric ring or a pair of minutes were also found. The reunion of broken pieces can produce two compound acentric fragments.

The size of the deleted portion, gauged from the diameter produced acentric ring. Single minutes encountered in many cells were the formation of interstitial deletion occasionally of intra-arm intrachanges type. Intra chromatid X-type exchanges leads to the deletion of a small ring which may remains in close proximity to the site of removal or may be displaced and are lost in the metaphase spread. One incomplete form produced a minutes plus a terminal deletion. The minutes were always seen to be displaced in the cell as in *Tradescantia*[13]. Formation of micronuclei could be originated from acentric fragments after chromosome breakage or from whole lagging chromosomes. As results many cells had micronuclei in present case.



Figures-1-10

Mitosis stages in control and irradiated root tips of *Phlox drummondii.* **1-2.** Control mitosis. **1.** Metaphase, fourteen chromosomes. **2**. Anaphase. **3-9.** Irradiated mitosis. **3.** Two dicentrics, ring, two acentric fragments. **4.** Tricentric, dicentric, three fragments. **5.** Two dicentrics, translocation, ring, two deletions, two acentric fragments. **6.** Dicentric, translocation, two minutes, ring, two fragments. **7.** Deletion, fragment. **8.** Bridge. **9.** Micronuclei **10.** Karyotype of control, fourteen subtelocentric chromosomes with prominent satellites in fifth pair.

Conclusion

In the present study, chromosomal aberration frequency was increased in mitosis along with the doses of gamma rays might perhaps be due to the interactions of ionizing particles with DNA molecules by excitation either directly or indirectly that ultimately increased aberration frequency. The present investigation would be helpful to standardize the methodology.

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