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Gamma Radiation Induced Chromosomal Aberrations at Mitosis in *Allium cepa* L.

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Abstract: ***Mutagenic effectiveness of gamma rays on Allium cepa was studied. The seeds were treated with five doses (viz. 5kR, 10kR, 15 kR, 20kR and 25kR) of gamma rays. Chromosomal abnormalities were observed. Chromosomal aberrations like, dicentric, tricentric, ring, minute, deletion, fragment, laggard, bridge and micronuclei were noticed in treated root tip mitosis. The chromosomal abnormalities were increased with increasing doses. The chromosomal abnormalities were dependent on the radiation doses.***

Keywords: Gamma radiation, Chromosomal aberrations *Allium cepa* L.

1. Introduction

Gamma rays belong to ionizing radiation, most energetic, more penetrating than other types of radiation such as alpha and beta rays (Kovacs & Keresztes, 2002). The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Borzouei *et al.*, 2010). Radicals react with nearby molecules in very short time, resulting in breakage of chemical bonds of the effected molecules. The major effect in cells is DNA breaks, broken ends of DNA may rejoin in different patterns from their original arrangement. It may be visualized at mitosis when cells divide. It is one of the oldest, simplest and least expensive methods for studying the induction of chromosomal aberrations utilizes plant root tips as experimental material (Kihlman, 1975). Root tips from several plant species have been used for the study of induced chromosomal aberrations by the several workers asAndersson & Kihlman (1987) in *Vicia faba*, Verma & Raina (1980) in *Phlox drummondii* and Verma & Chandel *(*1994) in *Chrysanthemum carinatum. Allium cepa* belong to the family Liliaceae, although it is also listed by some authors in their own family the Alliaceae (Ormsby & Pottinger 2009). In the present study *Allium cepa* has been selected for its chromosome low in number (2n = 16), relatively large in size and susceptible to cytological manipulations is very essay (Mercykutty & Stephen 1980). The main object of the present study was enhancing the understanding of the doses response for chromosomal aberrations and induction of mutation to produce new and useful mutant.

2. Materials and Methods

Seeds of *Allium cepa* were irradiated with different doses like 5, 10, 15, 20 and 25kR of gamma rays. Seeds germinated after 2-3 days, root tips about 0.5 to 1.0 cm length were collected and pretreated with 0.02% colchicine for about 3 hours at room temperature (25 ± 5^0C). Washed root tips were fixed in freshly prepared Carnoy's fluid (absolute alcohol: acetic acid in 3:1 ratio) for at least 24 hours. Fixed root tips were taken out and were washed twice with water and hydrolyzed in 1N HCl at 60 ^{0}C for 10 to 15 minutes. After hydrolysis, root tips were washed and transferred to Leucobasic fuchsine (fulgent stain) for 1 hour in dark. After 1 hour, stained root tips were taken out and washed with water and squashed in 1% acetocarmine. Slides with well spread cells and clear chromosomes were selected for photomicrography. The photomicrographs were taken with help of digital camera under oil immersion and magnified to X1000. Savage's (1975) classification was used for categorizing various types of chromosomal aberrations.

3. Results

Seeds were germination: It was relatively low as compare to control (82%), ranged from 42.5% in 25 kR to 48% in 5 kR.

Mitotic study in Control: Mitosis was studied in the root tips. The chromosomes (2n=16) were observed at metaphase (Fig. 1) and these were equally distributed (16:16) towards each pole at anaphase/telophase.

Mitotic study in irradiated Seeds: Mitosis was studied in root tips, in which various types of chromosomal aberrations at metaphase were observed. Hundred cells were analyzed from each dose for scoring which showed considerable chromosome number with clear metaphases. At metaphase, out of 100 cells, minimum number of abnormal cells 52 in 5kR and maximum 100 cells in 25kR were found. Minimum number of abnormalities were 192 in which 62 dicentrics, 1 tricentric, 12 acentric rings, 8 centric rings, 19 minutes, 16 deletions and 74 acentric fragments were observed in 5kR. Maximum number of abnormalities were 443 in which 174 dicentrics, 14 tricentrics, 28 acentric rings, 19 centric rings, 51 minutes, 12 deletions and 145 acentric fragments were observed in 25kR (Table-1, Figs. 2-9). The most frequent abnormalities were seen at metaphase and at this stage the predominant type of abnormality was dicentric.

At anaphase/telophase, of the 50 cells, obtained minimum 28 cells were found abnormal in 5 kR and maximum 41 in 25 kR. Minimum numbers of abnormalities were 30 in which 6 laggards, 2 bridges and 22 micronuclei were observed in 5kR and maximum numbers of abnormalities 62 in which 7 laggards, 6 bridges and 49 micronuclei were observed (Table-2, Figs. 10-12).

4. Discussion

Chromosomes are composed of long thin molecules of DNA. When cells are exposed to radiation or carcinogens (chemicals), 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. It has been extensively utilized for many years to cause mutations and chromosomal damage for experimental purpose. The study of induced gamma radiation chromosomal aberrations has been done by several workers as, in *Allium* (Evan, 1962, Evan and Savage 1963; Bhatta and Sakya 2008), *Allium* and *Vicia* (Pillai *et al*., 1997), in *Capsicum annum(* Dhamayanthi & Reddy 2000) in *Lathyrus sativus* (Tripathi& Girjesh 2010); *Phlox drummondii* (Verma & Raina, 1980; Verma *et al*., 1998); *Vicia faba* and *Phlox drummondii* (Pillai and Verma 1992) and *Vigna mungo* (Goyal & Khan 2009).

Ionizing radiation induces different types of structural chromosome change depending on the cell cycle. If the cells are irradiated during and after DNA synthesis (S or G2), aberrations are of the chromatid type. However, if the cells are irradiated before DNA synthesis (GI), aberrations are of the chromosomal type (both chromatids are involved in the site of aberrations (Evans & Savage, 1963). In the present investigation, root tips, obtained from irradiated seeds (5, 10, 15, 20 and 25kR) were analyzed to find out different types of chromosomal abnormalities such as dicentrics, tricentrics, fragments, rings, minutes, bridges and micronuclei at mitosis in *Allium cepa*. Their modes of origin are discussed below according to Savage (1975).

1. Chromosome Type:

a) Asymmetrical inter-arm interchanges: Chromosomes with dicentrics were occasionally observed in *Allium cepa.* 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, tricentric arise from 4 breaks in three chromosomes. The region of broken pieces can produce 2 compound acentric fragments in tricentric. The separation of dicentrics/tricentrics may lead to the formation of bridges at subsequent anaphase/telophase. Many such bridges were observed at anaphase/telophase. Dicentric is commonly observed in *Allium cepa*.

b) Asymmetrical inter-arm intrachanges: Chromosomes were complete type discernible by the presence of centric ring of varying sizes and a compound fragment. 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 in the present study.

c) Asymmetrical intra-arm interchanges: The change is symbolized by the presence of acentric ring or pair of minutes. The acentric ring might have formed as a result of interstitial deletion followed by rejoining of deleted fragment. The minutes could be the result of the interstitial deletion of the chromosomes. The minutes, which are without centromere often, undergo non-disjunction at mitosis. The present investigation observed double minutes which are subsequently converted into micronuclei at anaphase/telophase.

2. Chromatid Type:

a) Intra-arm intrachanges: 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 minute plus a terminal deletion. The minutes were always seen to be displaced in the cell. The micronuclei formation, induced by gamma ray could originate from acentric fragments after chromosome breakage or from whole lagging chromosomes. (Kwasniewska *et al.* 2011), therefore many cells had micronuclei at mitosis.

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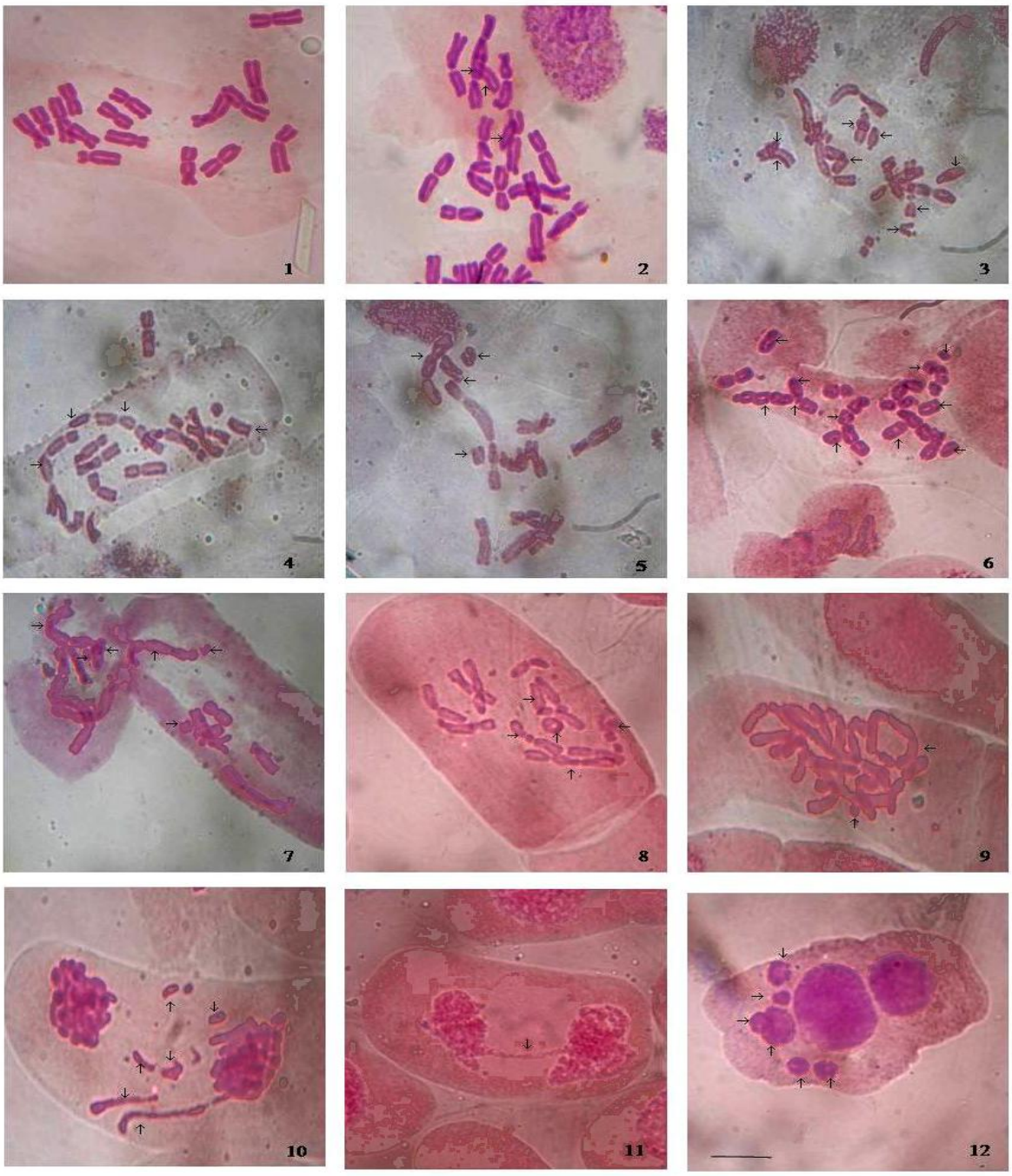

Figure 1-12: Mitotic division in control and irradiated ***Allium cepa* L. 1.** Mitosis in Control ***Allium cepa,*** Metaphase; 16 chromosomes. **2-12.** Mitosis in irradiated. **2.** 1 Dicentric, 2 Fragment. **3.** 1 Dicentrics, 3 Deletions, 4 Fragments. **4.** 1 Tricentric, 1 Dicentric, 3 Fragments. **5.** 1 Tricentric, 1 Dicentric, 1 Acentric ring, 2 Fragments. **6.** 2 Tricentrics, 1 Deletion, 1

Acentric Ring, 1 Minute, 6 Fragments. **7**. 2 Dicentrics, 2 Minutes, 2 Fragments. **8.** 2 Dicentrics, 1 Centric ring, 2 Fragments. **9.** 1 Dicentric ring, 1 Minute. **10.** Laggards. **11.** Bridge, **12.** Micronuclei (Note: Scale bar =4µm).

Table-1: Various type of radiation induced chromosomal aberrations at root tip mitosis in *Allium cepa*

Material	Analyzed cells	Abnormal cells	Number of abnormalities	Dicentrics	Tricentrics	Acentric rings	Centric rings	Minutes	Deletions	Acentric fragments
Control	100	-	-	-	-	-	-	-	-	-
5kR	,,	52	192	62	1	12	8	19	16	74
10kR	,,	66	278	105	2	19	6	16	12	118
15kR	,,	92	385	128	2	28	12	70	19	126
20kR	,,	98	392	124	5	22	17	69	20	135
25kR	,,	100	443	174	14	28	19	51	12	145

Table 2: Various type of radiation induced chromosomal aberrations at post mitosis in *Allium cepa*

Material	Analyzed cells	Abnormal cells	Number of abnormalities	Laggards	Bridges	Micronuclei
Control	50	-	-	-	-	-
5kR	-	28	30	6	2	22
10kR	-	30	37	5	8	24
15kR	-	36	46	9	1	36
20kR	-	39	56	10	3	43
25kR	-	41	62	7	6	49