Pak. J. Bot., 51(3): 887-894, 2019 DOI: http://dx.doi.org/10.30848/PJB2019-3(48)

DETECTION OF CYTOGENETIC AND GENOTOXIC EFFECTS OF GAMMA RADIATION ON M_1 GENERATION OF THREE VARIETIES OF *TRITICUM AESTIVUM* L.

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Abstract

The effects of ^{60}Cobalt (^{60}Co) gamma radiation on cell division and chromosomal structure in M_1 generations of three varieties (NKU Lider, Bezostaja and GK Beke's) of *Triticum aestivum* L. genotypes were determined in this study. To understand and compare the tolerance of on three bread wheat varieties to gamma radiation (100, 200 and 300Gy), the frequency of mitotic index, phase indices and genotoxicity rate were scored and statistically interpreted under irradiated and unirradiated conditions, respectively. In parallel with the increasing radiation dose, mean mitotic index rate decreased in *NKU Lider* and *GK Beke's* genotypes. 100Gy ^{60}Co gamma radiation application of *NKU Lider* was the most powerful genotype to increase mitotic activity as compared to other genotypes. However; depending on increasing dose of gamma radiation, cell division decreased in all studied genotypes except 200Gy application of *Bezostaja* genotype. Moreover, genotoxicity index of M_1 generation in *NKU Lider* genotype was decreased in all studied radiation doses with the increasing dose of ^{60}Co. Furthermore, three different variety of *T. aestivum* L. seeds treated with different doses of ^{60}Co gamma radiation showed many aberrant chromosomes such as disorderly prophase, stickiness, uncoiling chromosomes, disrupted equatorial plate, fragment, micronucleus, alignment anaphase, fault polarization, anaphase and telophase bridges, lagging chromosomes and stickiness in all mitotic phases. Consequently, the results suggest that gamma radiation effects are specific to the radiation dose and species, and even show different responses in different varieties of the same species.

Key words: Bread wheat, Chromosome aberration, ^{60}Co, mitotic activity, Mutagenesis.

Introduction

Concerns about food security are increased depending on the raised world population. To feed the world population might be possible if the food production increase double by 2050. However, this aim is associated with many climate-based comprehensive challenges. Plant breeders take effort to change genetic structures of the plants to improve preferred food by humans. Although classical breeding techniques have presented knowledge of genetic structure of plants, modern breeding such as genetic engineering and mutation breeding give much more information than traditional breeders. Mutation breeding may be more helpful and faster to reach the aim of breeding program than conventional breeding. If conventional breeding does not work mutation breeding can be an appropriate option to improve new characters (Van Harten, 1998; Ahloowalia & Maluszynski, 2001). Mutation breeding can be applicable to breed a specific character. Moreover, it is also effective to create a novel character which is not associated with the parents of the plant. The aim of the breeding programs is to reveal disappeared or suppressed characters during evolution. Instead of waiting for the occurrence of natural mutation breeding studies focus on creating physical or chemical mutations via mutagens to improve plant structure. Enhancing world food security is the main aim for mutation breeding that to increase crop production (Kharkwal & Shu, 2010) using physical mutagens such as X-rays and gamma rays are very common applications to develop new food crop varieties.

Application of gamma radiation is in use commonly in agriculture, industry and medicine. Gamma rays have many different types of advantages all applied areas and it is used to create new mutant crops in agriculture (Ali *et al.,* 2015). Especially in wheat, it is a need to develop new varieties short height. To have this character makes the plant tolerant to strong wind and prevent losses of grain yield. It is also not clearly identified how gamma radiation influence grain quality. Mashev *et al*., (1995) implied that higher radiation doses could be effective to develop more rich plants with regards to protein and essential amino acids. Din *et al*., (2003) revealed that effects of higher doses of gamma radiation (30 and 35krad) created some aberrations on plant structure such as one tiller with two ears attached with each and/or prevalence of sterile ears etc. Gamma rays belong to the ionizing electromagnetic group of radiations and are highly penetrating because of the low linear energy transfer. This situation makes various foods more hygienic and minimize losses against microbial contaminants and harmful insects (Farkas, 1998). Because of applied dose dependency, low doses of gamma rays could have fewer side effects while higher doses could have more detrimental effects on various cell organelles and biochemical components of the plant (Begum & Dasgupta, 2011; Heidarieh *et al.,* 2012). Various doses of gamma radiation is a type of physical mutagenesis that it induce cellular uptake of water via reactive oxygen species (ROS), hydroxyl radical (.OH), ionized water (H_2O^{+2}) and the reactive nitrogen species (RNS) (Nurmansyah *et al.,* 2018; Reisz *et al.,* 2014). That's why this process effects the structure of DNA and mitosis (Singh, 1983). Depending on radiation dose, different types of chromosome aberrations such as stickiness, laggards, fragments, bridges, fault polarization and micronuclei can be shown in nuclei (Beadle, 1932; Sakin *et al.,* 2005; Verma & Khah, 2016).

The main goal of the study is determination of cytogenetic and genotoxic variability of three bread wheat varieties and comparison of the tolerance to different doses of gamma-rays for mitotic potential of three different genotypes.

Materials and Methods

Plant materials: Three bread wheat (*Triticum aestivum* var. *aestivum* L.) varieties, Bezostaja (tall, mid-early, awnless, superior in flour quality for bread making, but inferior in lodging resistance and yield capacity), GK Beke's (mid-tall, mid-early, awned, superior in flour quality for bread making, but inferior in rust resistance and yield capacity) and NKU Lider (mid-tall, mid-late, awned and good in flour quality for bread making and yield capacity) were used as experimental material.

Gamma radiation: The moisture contents of the seeds of wheat genotypes used in this study were 13.1% for Bezostaja, 13.5% for GK Beke's and 13.6% for NKU Lider. The grains for each genotype were divided into four groups (A, B, C, D), each of which contained 2000 grains. Group A was kept unradiated (0 Gray (Gy); control), while the other groups were radiated with 100Gy (B), 200Gy (C) and 300Gy (D) gamma rays. The gamma radiation doses were chosen depending on the most effective dose on wheat mutation breeding literature (Borzouei *et al.*, 2010; Aly *et al.*, 2018).

Gamma treatment was obtained from ^{60}Co, Ob-Servo Sanguis Co-60 Research Irradiator with isotope model, while the dose rate was 2.190 kGy h^{-1} in 2016-17 growing season in Turkish Atomic Energy Authority, Saraykoy Nuclear Research and Training Center, Ankara, Turkey. The unit for the absorbed dose of radiation energy is the gray, which is equivalent to 1 J Kg^{-1} and 100 rads.

After the application of radiation doses, the experiment was set up with using total 12 M_0 combination seeds together with the unirradiated (0Gy; Control) seeds in the Department of Field Crops at Namık Kemal University during the growing season of 2016 and 2017. The experiment was carried out in the randomized complete block design (RCBD) with 3 replicates. Nitrogen and P_20_5 at 140 and 70 kg ha^{-1}, respectively, were incorporated into the soil as compound fertilizer (20-20-0) before sowing, urea during tillering and ammonium nitrate before heading. The crop was kept free of weeds by hand hoeing when necessary. The remain seeds were sown in the greenhouse in order to confirm the work. The plants were harvested with hand on July 07, 2017.

Cytogenetical analysis: 10 uniform sized of each three-bread wheat variety (NKU Lider, Bezostoja and GK Beke's) seeds were put into petri dishes covered with two sheets filter papers moistened with 20 ml of distilled water (0Gy) and different ^{60}Co radiation (100, 200, 300Gy). Seeds were germinated in incubator under 24°C. After the root tips were 1–1.5 cm long, they were cut off, pre-treated with iced water for 24 hours. Then, root tips were fixed for 24 h in Carnoy solution [(ethanol (99%): glacial acetic acid (3:1)] and then stored in 70% ethanol at 4°C until required. Root tips were hydrolysed with 5 N HCl for 35 min and stained in Feulgen at least 1 h and then squashed in a drop of 45% acetic-acid on slides. Three roots were examined for each radiation of ^{60}Co and control groups for three slides. Mitotic index, phase indices (*I*) of dividing cells and chromosome aberrations was scored by analysing at least 3,000 cells per treatment (1.000 per slide). The mitotic index was calculated through the number of divided cells/total number of cells (Sehgal *et al.*, 2006). To compare the cell division in more detailed, we calculated the indices (*I*) of the separate phases — prophase (I_P), metaphase (I_M), anaphase (I_A) and telophase (I_T). According to Ivanova *et al*., (2003), the phase indices were examined by the formula:

$$I_{phase} = \frac{\text{The cell number of respective phase}}{\text{the total number of divided cells}}$$

To express the cytotoxicity and genotoxicity the chromosomal aberrations were determined by scoring the number of aberrant cells / total number of divided cells. The abnormal chromosomes were observed at 100X objective on Olympus CX- 41 research microscope and they were photographed with C–5060 WZ camera.

Statistical analysis: Data from unirradiated (0Gy, Control) and radiated with different doses of ^{60}Co (100, 200, 300Gy) were compared using ANOVA analysis with MiniTab 17 (MiniTab 2010) and SPSS 22 (Anon. 2013) software. Differences between each group were evaluated using non-parametric Kruskal–Wallis Analysis and Duncan's multiple range test at a level of significance $p \leq 0.05$ (Duncan, 1955).

Results

Effects of ^{60}Co gamma radiation on cell division: The effects of different doses (100, 200, 300Gy) of ^{60}Co gamma radiation on mitotic index and mitotic phases in the root tip cells of three bread wheat varieties (Bezostaja, GK Beke's, NKU Lider) was shown in Table 1. The effects of gamma radiation on mitotic activity were significant ($p<0.05$) in all studied parameters. Mitotic index rates in all control groups of M_1 generations of three bread wheats showed the highest mitotic activity in *NKU- Lider* (0.23±0.02) and the lowest (0.10±0.02) in *Bezostaja*. Mean mitotic index value was approximately 50% lower than *NKU Lider* genotype in control groups of *GK Beke's* (0.13±0.01) and *Bezostaja* (0.10±0.02) genotypes. In parallel with increasing radiation doses mean mitotic index decreased in all studied genotypes except *Bezostaja. NKU Lider* was the most successful genotype about mitotic activity as compared to other genotypes of control group ($p<0.005$). 100Gy gamma radiation of ^{60}Co increased the mitotic activity compared to control group in *NKU-Lider* genotype as 61%. However, 200 and 300Gy radiation showed a negative effect on mitotic proliferation in that genotype. Mitotic index values significantly reduced in NKU Lider genotype in higher than 100Gy gamma radiation doses (Table 1) ($p<0.05$). The only 200Gy 60Co gamma radiation application stimulated cell division in Bezostaja genotype. Therefore, it was observed that *Bezostaja* was

the most resistant genotype to higher gamma radiation dose among all varieties. While the control group of *Bezostaja* showed 0.10 mitotic index value, 200Gy of gamma radiation increased the cell division with the rate of 50 percent. Also, a very little increase was found on 300Gy (0.11) in all radiation doses of *Bezostaja*. *GK Beke's* 100Gy (0.21) radiation is also very powerful among all genotypes after *NKU Lider* 100Gy gamma radiation to increase cell division.

The highest dose (300Gy) of ^{60}Co gamma radiation on the meristematic root tips of *NKU Lider* and *GK Beke's* genotypes were significantly ($p<0.05$) lower than the mitotic index of their control group. The most increased cell division rates were scored in 100Gy for both genotypes. However, mitotic inhibition was determined significantly depending on the increasing dose application in all genotypes except Bezostaja ($p<0.05$). The highest rate of prophase indices (*Ip*) was determined in the control group of *NKU Lider* (0.86) and *Bezostaja* 200Gy (0.73). However, prophase indices of *NKU Lider* genotype showed no increase compared to its control group (Table 1). In addition, *GK Beke*'s metaphase indices (I_M) showed the highest value (0.38) for all radiation doses and control groups in three bread wheat. There is a statistically significant difference was observed in anaphase and telophase indices between the all varieties ($p<0.05$) (Table 1). It was also observed that telophase indice (I_T) value showed the highest rate in *GK Beke*'s 100Gy radiation among all doses and their control groups of three different types of bread wheat barley.

Genotoxic effects of ^{60}Co gamma radiation on chromosome structure: The unradiated (control) group which was not exposed to ^{60}Co gamma radiation and germinated in distilled water showed normal mitotic cells (Table 2, Fig. 1) and regular formation of mitotic chromosome number (2n=6x=42). However, the seeds of three varieties exposed to gamma radiations (100, 200, 300Gy) was exhibited with different types of chromosome aberration indices in different phases of mitosis (Table 2, Fig. 2). Genotoxicity index (I_G) in the applied dose of ^{60}Co was significantly ($p<0.05$) higher than control group. Although genotoxicity. It was determined that *NKU Lider* genotype is very successful to inhibit potential detrimental effects of high dose gamma radiation on chromosome behaviour. However, *Bezostaja* and *GK Beke's* genotypes could not showed any regenerative impact on chromosome aberrations. Genotoxicity index was completely increased depending on rising radiation dose (Table 2). In general, 300Gy gamma radiation dose showed highest genotoxicity index in all genotypes except *NKU Lider*.

After the application of various doses of ^{60}Co gamma radiation to the seeds belongs to each genotype, they were monitored by microscopic observations such as disorderly prophase, stickiness, uncoiling chromosomes, disrupted equatorial plate, fragment, micronucleus, alignment anaphase, fault polarization, anaphase and telophase bridges, lagging chromosomes and stickiness for all phases (Table 2, Fig. 2). There was not noticed aberrant chromosomes in control groups of three bread wheat variety (Fig. 1). The highest phase aberration value (0.16) in 300Gy radiation dose of *Bezostaja* variety was observed in disorderly prophase (Fig. 2a-d) and bridges in anaphase and telophase in *GK Beke's* variety under the 100Gy radiation (0.07). All the chromosomal aberrations except fragment and alignment anaphase was statistically significant ($p<0.05$). The most commonly observed chromosomal aberration types were disorderly prophase (Fig. a-d), uncoiling chromosomes (Fig. 2i, j) and disrupted equatorial plate (Fig. 2e, f), bridges in anaphase and telophase (Fig. 2q, r) and lagging chromosomes (Fig. 2k, l, q, s, t, u, v). Fragment (Fig. 2m), micronucleus (Fig. 2n, o), alignment anaphase (Fig. 2u) and fault polarization (Fig. 2v) was less when compared other aberrations. The gamma radiations at 300Gy induced more genotoxic than the 100 and 200Gy dose in mitotic cells of each genotype.

Table 1. Mitotic index and phase indices are shown against concentration increase in induced gamma radiation (100, 200 and 300Gy) and uninduced (0Gy) groups of three varieties of *T. aestivum* L.

Treatments	Mitotic index (MI)	Prophase indices (I_p)	Metaphase indices (I_M)	Anaphase indices (I_A)	Telophase indices (I_T)
NKU Lider-0	* 0.23 ± 0.02^{cd}	0.86 ± 0.02^{c}	0.08 ± 0.02^{ab}	0.03 ± 0.01^{c}	0.01 ± 0.01^{c}
NKU Lider-100	0.29 ± 0.06^{d}	0.63 ± 0.06^{ab}	0.18 ± 0.04^{bc}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
NKU Lider-200	0.19 ± 0.03^{abc}	0.65 ± 0.02^{ab}	0.26 ± 0.02^{cd}	0.01 ± 0.01^{a}	0.01 ± 0.00^{a}
NKU Lider-300	0.17 ± 0.02^{abc}	0.52 ± 0.05^{a}	0.36 ± 0.04^{d}	0.01 ± 0.01^{ab}	0.02 ± 0.01^{ab}
Bezostaja-0	0.10 ± 0.02^{ab}	0.64 ± 0.09^{ab}	0.08 ± 0.04^{ab}	0.02 ± 0.01^{ab}	0.01 ± 0.00^{ab}
Bezostaja-100	0.08 ± 0.01^{a}	0.48 ± 0.04^{a}	0.36 ± 0.05^{d}	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}
Bezostaja-200	0.15 ± 0.04^{abc}	0.73 ± 0.02^{bc}	0.04 ± 0.02^{a}	0.01 ± 0.01^{a}	0.02 ± 0.00^{a}
Bezostaja-300	0.11 ± 0.01^{ab}	0.48 ± 0.10^{a}	0.32 ± 0.03^{cd}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}
GK Beke's-0	0.13 ± 0.01^{abc}	0.50 ± 0.01^{a}	0.38 ± 0.01^{d}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}
GK Beke's-100	0.21 ± 0.06^{bcd}	0.58 ± 0.10^{ab}	0.30 ± 0.10^{cd}	0.01 ± 0.01^{ab}	0.04 ± 0.01^{ab}
GK Beke's-200	0.10 ± 0.01^{a}	0.63 ± 0.02^{ab}	0.20 ± 0.04^{bc}	0.02 ± 0.01^{bc}	0.02 ± 0.02^{bc}
GK Beke's-300	0.08 ± 0.01^{a}	0.54 ± 0.03^{ab}	0.25 ± 0.02^{cd}	0.01 ± 0.01^{ab}	0.02 ± 0.01^{ab}

* Values with insignificant difference ($P < 0.05$) for each column are indicated with same letters (means ± SD)

Table 2. Genotoxicity index (I_G) and mutation rates of chromosome aberration are shown against concentration increase in induced gamma radiation (100, 200 and 300Gy) and uninduced (0Gy) groups of three varieties of *T. aestivum* L.

Treatment	Genotoxicity index (I_G)	Disorderly prophase	Stickiness	Uncoiling chromosome	Disrupted equatorial plate	Fragment	Micronucleus	Alignment anaphase	Fault polarization	Anaphase /Telophase bridge	Lagging chromosome
NKU Lider-0	* 0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
NKU Lider-100	0.19±0.02^{abcd}	0.13±0.01^{bcd}	0.01±0.01^{a}	0.01±0.01^{ab}	0.01±0.01^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{ab}
NKU Lider-200	0.14±0.02^{bcd}	0.09±0.01^{abcd}	0.00±0.00^{a}	0.02±0.01^{ab}	0.02±0.01^{ab}	0.01±0.01^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{ab}
NKU Lider-300	0.09±0.01^{e}	0.04±0.01^{ab}	0.00±0.00^{a}	0.01±0.01^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.02±0.01^{b}	0.01±0.01^{ab}	0.00±0.00^{a}
Bezostaja-0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Bezostaja-100	0.17±0.02^{abc}	0.11±0.02^{bcd}	0.00±0.00^{a}	0.01±0.01^{ab}	0.03±0.02^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.01±0.01^{ab}	0.01±0.01^{ab}
Bezostaja-200	0.21±0.05^{abcd}	0.14±0.05^{cd}	0.00±0.00^{a}	0.02±0.01^{ab}	0.02±0.00^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.01±0.01^{ab}	0.01±0.01^{ab}	0.01±0.01^{ab}
Bezostaja-300	0.22±0.05^{cde}	0.16±0.08^{d}	0.06±0.00^{b}	0.01±0.01^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.02±0.01^{a}	0.00±0.00^{a}	0.01±0.02^{b}	0.01±0.01^{ab}
GK Beke's-0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
GK Beke's-100	0.13±0.01^{de}	0.06±0.01^{abc}	0.00±0.00^{a}	0.02±0.01^{a}	0.02±0.01^{ab}	0.01±0.01^{a}	0.01±0.01^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.07±0.05^{b}	0.01±0.01^{ab}
GK Beke's-200	0.16±0.02^{de}	0.10±0.01^{bcd}	0.00±0.00^{a}	0.00±0.01^{a}	0.03±0.01^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.01±0.01^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.03±0.01^{b}
GK Beke's-300	0.19±0.03^{de}	0.11±0.02^{bcd}	0.00±0.00^{a}	0.03±0.02^{b}	0.03±0.00^{b}	0.01±0.01^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.01±0.01^{ab}	0.00±0.00^{a}

*Values with insignificant difference ($p<0.05$) for each column are indicated with same letters (means ± SD)

Fig. 1. Normal mitotic chromosome structure in the meristematic root tip cells of uninduced (0Gy) gamma radiation in three varieties of *T. aestivum* L. prophase (a), metaphase 2n=6x=42 (b), anaphase (c), telophase (d). Mitotic cells scored by analyzing at least 3,000 cells per treatment (1.000 per slide). Chromosome structure were obtained by 100X objective on Olympus CX- 41 research microscope and they were photographed with C–5060 WZ camera. Scale bar: 10 µm

Discussion

To produce better crop varieties via selecting and combining various desired features, plant breeders use genetic variability in crop plants as a traditional resource. Natural variation can be generated by spontaneous mutations which occur at extremely low frequency. Therefore, to generate novel variation, mutation breeders apply different chemical or physical mutagens to enhance mutation frequency. Improvement in either one or more economic traits and their quality features can be obtained with the help of exogenous mutations within the shortest possible time. Crop improvement programs accept induced mutations as a common approach, thus speeding up the breeding program considerably (D'Souza, 2014). Physical mutagens are most used mutation breeding technique among plant breeders that it leads break on DNA double strand.

Gamma rays are the most energetic form of electromagnetic radiation (Kovacs & Keresztes, 2002). As the main source of gamma radiation, ^{60}Co and ^{137}Cs used for induced mutation (Brown & Caligari, 2008). The gamma rays which interact with atoms and molecules to produce free radicals in cells are ionized forms of radiation. These free radicals could be detrimental in plants cells by changing cell division, chromosome structure, cellular structure and metabolism causing morphological, anatomical, physiological and biochemical damages depending on the radiation levels (Kim *et al.,* 2004; Wi *et al.,* 2005). Cytogenetic monitoring is the most effective method to understand species specific effects on DNA level. It allows the researchers to estimate the cytogenetic changes via mutagenesis. Mitotic index is a cytogenetic test which measures the proliferation of mitotic cells (M phase) in the cell cycle and its inhibition could be scored as cellular death (Rojas *et al.,* 1993; Gadano *et al.,* 2002). Cytogenetic and genotoxic effects of ^{60}Co gamma radiation were observed in three bread wheat varieties by determining mitotic activity and the number of chromosomal aberrations such as disorderly prophase, stickiness, uncoiling chromosomes, disrupted equatorial plate, fragment, micronucleus, alignment anaphase, fault polarization, anaphase and

telophase bridges and lagging chromosomes. Depending on the increase of gamma radiation, significant inhibition was found in mitotic activity between all groups except *Bezostaja*. Likewise, Ananthaswamy *et al*., (1971) irradiated wheat seeds with ^{60}Co gamma rays from 20 to 200krad dose levels and observed an inhibition on seedling growth by 50-62 percent at low doses from 20 to 40 krad at higher doses. Also, Evans & Hof (1975) have determined that gamma rays treated to different plant species caused the disruption of the mitosis frequency. The mitotic activity (MI) of *NKU Lider* and *GK Beke's* varieties applied 100Gy radiation was significantly increased with the range of 26 and 61%, respectively as compared to control group. The highest dose of *NKU Lider* and *GK Beke's* genotypes showed a significant inhibition (27, 38.5%, respectively) Kim *et al*., (2000) revealed that from high dose to low dose of gamma radiation caused inhibition or stimulation of germination, seedling growth, and different types of biological responses (Rojas *et al.,* 1993). Also, the decrease of mitotic activity in high radiation doses of ^{60}Co can be explained that increased application dose may have a lethal impact or can block mitotic phases and inhibit G_2/M phase (Preuss & Britt, 2003; Comai & Henikoff, 2006). Likewise, Eroglu *et al*., (2007) indicated that gamma rays reduced cell division in parallel to rising radiation dose in barley seeds. However, in this study, *Bezostaja* seeds which was applied lowest dose (100Gy) showed the same cytotoxic limit value as the highest doses of other two bread wheat genotypes as well. So, the results of this genotype imply that *Bezostaja* is more tolerant to ^{60}Co gamma radiation than *NKU Lider* and *GK Beke's* genotypes.

Chromosomal aberrations have long been used as an important biomarker of living organisms' exposure to gamma ray radiation and genotoxic chemicals. Changes in structure and numerical aberrations which occur spontaneously correlated with troubles in growth and development depending internal and external factors. Spontaneous chromosome aberration rates are about 0.6% in plant cells which are first-tier assay systems for the detection of possible genetic damage by environmental chemicals (Grant, 1978; Rakhmatullina & Sanamyan, 2007; Nikolova *et al.,* 2015). Numerous cytogenetic studies have reported that mutagenic disorders on plant cell metabolism result from possible structural or numerical abnormalities on chromosomes (Gadano *et al.,* 2002; Swierenga *et al.,* 1991; Bolwell & Wojtaszek, 1997; Nazarenko & Izhboldin, 2017). One of the most important effect of seeds treated to higher gamma ray dose through inhibition of cell division in the meristematic cells is impaired mitosis (Nurmansyah *et al.,* 2018). In general, an increasing spectrum of aberrations on chromosomes was determined depending on the increasing intensity of gamma radiations. In this study, the sensitivity of the genotypes to ^{60}Co gamma radiation was statistically significant and different. However, this study indicated that application of gamma radiation to the seeds caused an increase on chromosome aberrations. ^{60}Co gamma radiation on *NKU Lider* bread wheat variety seeds significantly reduced chromosome aberration frequency in M_1 generations. This could be due to the reduction of the mutagenic effect as well as DNA repair mechanism (Curtis, 2012) in *NKU Lider* genotype. In the present study, genotoxicity index of Bezostaja and GK Beke's bread wheat varieties was determined that frequency of chromosome aberration increased in parallel with the ^{60}Co radiation dose uptake. Although there had been found many similar results which implied that increasing genotoxicity dependent to increasing radiation dose (Nurmansyah *et al.,* 2018; Fenech, 2000; Kiong *et al.,* 2008; Dona *et al.,* 2013) but there were few studies in the opposite direction with that results (Malla, 2011).

Disorderly prophase (63%) was the most common aberration. However, disrupted equatorial plate (11%), uncoiling chromosomes (9%), bridges in anaphase and telophase (8%), lagging chromosomes (6%) and stickiness (5%) are significant to see the detrimental effects on mitotic cells of three bread wheat variety.^{60}Co gamma radiation side effects were dose dependent, where a low dose has fewer side effects in contrast to a high dose of application in all studied varieties except *NKU Lider* genotype. Interestingly the genotype *NKU Lider* had the lowest frequency of chromosome aberration in the highest radiation dose (300Gy). The gamma radiations at 300Gy induced had higher aberrant chromosomes than the 100 and 200Gy doses in abnormal mitotic cells of *Bezostaja* and *GK Beke's* genotypes. The number of fragmentation, alignment anaphase, fault polarization and micronucleus were less than other aberrations. Moreover, the variation of genotypic sensitivity can be explained with the difference of genetic makeup among the genotypes of a species (Khursheed *et al.,* 2015). The decreasing frequency of chromosome aberration in parallel with the dose increase can be explained as higher genetic resistivity and stability toward the effect of gamma radiation in *NKU Lider* genotype.

DNA molecules form genes and thousands of genes compose a chromosome studying genetic damage from ionizing radiation in terms of gross structural damage to chromosomes is often easiest. Structural changes induced in chromosomes by radiation include single breaks, multiple breaks and chromosome stickiness or clumping (Passmore, 2016). Tarar & Dnyansagar (1980) implied that lagging chromosome occurs when a chromosome is improperly positioned, in relation to other chromosomes depending on the treatment to radiations, the spindle fibres may fail to carry the respective chromosome to the polar regions. Liman *et al*., (2012) reported that occurrence of lagging chromosomes was due to the failure of the chromosome or acentric chromosome fragments to move to either of the pole at anaphase stage. However, Verma & Khah (2016) revealed that the most frequent aberration was chromosomal stickiness in gamma irradiated M_1 generations of *T. aestivum*. Gaulden (1987) explained that sticky chromosomes is characterized by losing the function of one or two types of specific nonhistone protein which control chromatid separation and segregation. To expose the genetic material to a mutagenic agent is caused by functional and structural mutation of these kind of proteins (Turkoglu *et al.,* 2007). Many researchers implied that stickiness means high toxicity of the agent and it causes inappropriate protein-protein interaction (Nwakanma & Okoli, 2010). Also, the stickiness led to the disturbance in the enzyme system that it might be decrease in the rate of cell division (Mahakhode & Somkuwar, 2013). Micronucleus, fragmentation, fault polarization and alignment anaphase were observed the lowest aberration frequency in all chromosomal rearrangements of all varieties. Grant (1978) implied that micronuclei generally resulted from fragments or lagging chromosomes which fail to incorporate in the either of the daughter nuclei during telophase of the mitotic cells (Krishna & Hayashi, 2000).

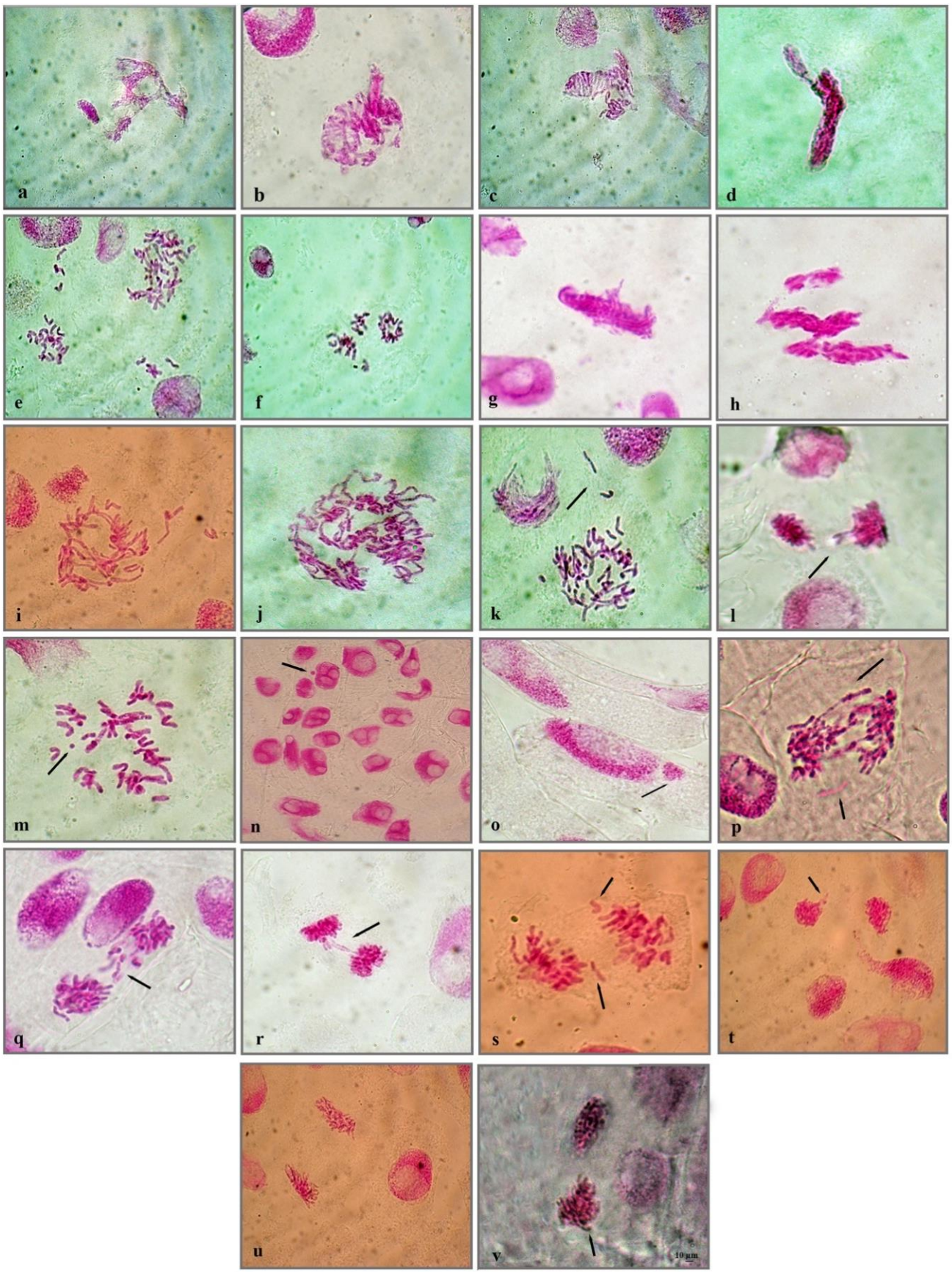

Fig. 2. Mitotic chromosome aberrations in the meristematic root tip cells of induced gamma radiation in three varieties of *T. aestivum* L. The aberrant cells were counted in total slide approximately 1000 cells per treatment by 100X objective on Olympus CX- 41 research microscope and photographed with C–5060 WZ camera. (a-d) disorderly prophase, (e, f) disrupted equatorial plate, (g, h) stickiness in metaphase, (i, j) uncoiling chromosomes at metaphase (k) lagging chromosomes at metaphase (l) lagging chromosomes at telophase (m) fragment at metaphase (n, o) micronucleus (p) lagging chromosomes and bridge at anaphase (q) lagging chromosomes and alignment telophase (r) alignment telophase and bridge at telophase (s) lagging chromosomes and alignment at anaphase (t) l (u, v) fault polarization and lagging chromosome (→ represent aberrant chromosome). Scale bar: 10 µm

Acknowledgements

The authors acknowledged that gamma treatment was performed in Turkish Atomic Energy Authority Saraykoy Nuclear Research and Training Center. The seeds were planted in Department of Field Crops at Namık Kemal University. The cytogenetic and statistical analysis was performed in Department of Molecular Biology and Genetics at Mehmet Akif Ersoy University. However, this research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Ahloowalia, B.S. and M. Maluszynski. 2001. Induced mutations – A new paradigm in plant breeding. *Euphyt*., 118: 167-173

Ali, H., Z. Ghori, S. Sheikh and A. Gul. 2015. Effects of gamma radiation on crop Production. *In:* Hakeem, K.R. (Eds.) *Crop production and global environmental Issues*. Springer International Publishing, Switzerland, pp. 27-78.

Aly, A.A., R.W. Maraei and S. Ayadi. 2018. Some biochemical changes in two Egyptian bread wheat cultivars in response to gamma irradiation and salt stress. *Bulg. J. Agric. Sci*., 24(1): 50-59.

Ananthaswamy, H.N., V.K. Vaksil and A. Sreenivasan. 1971. Biochemical and physiological changes in gamma irradiated wheat during germination. *Radiat Bot.,* 11: 1-12.

Beadle, G.W. 1932. A gene for sticky chromosomes in *Zea mays*. *Ind Abst Vererb.,* 63: 195-217.

Begum, T. and T. Dasgupta. 2011. Effect of mutagens on character association in sesame (*Sesamum indicum* L.). *Pak. J. Bot.,* 43: 243-251.

Bolwell, G. and P. Wojtaszek. 1997. Mechanisms for the generation of reactive oxygen species in plant defence – a broad perspective. *Physiol. Mol. Plant Pathol.,* 51(6): 347-366.

Brown, J. and P.D.S. Caligari. 2008. *An Introduction to Plant Breeding*. 1st End. Blackwell Publishing Ltd, UK.

Borzouei, A., M. Kafi, H.H. Khazaei, B. Naseriyan and A. Majdabadi. 2010. Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings. *Pak. J. Bot.,* 42(4): 2281-2290.

Comai, L. and S. Henikoff. 2006. TILLING: practical single-nucleotide mutation discovery. *Plant J.,* 45(4): 684-694.

Curtis, M. 2012. DNA repair pathways and genes in plant. In: *Plant mutation breeding and biotechnology*, Shu Q, Forster, BP, Nakagawa H., pp. 57-70, Rome, Italy.

D'Souza, S.F. 2014. Radiation technology in agriculture. *J. Crop Weed*. 10(2): 1-3.

Din, R., Q.K. Ahmedand S. Jehan. 2003. Studies for days taken to earing initiation and earing completion in M_1 generation of different wheat genotypes irradiated with various doses of gamma radiation. *Asian J. Plant Sci.,* 2: 894-896.

Dona, M., L. Ventura, A. Macovei, M. Confalonieri, M. Savio, A. Giovannini, D. Carbonera and A. Balestrazzia. 2013. Gamma irradiation with different dose rates induces different DNA damage responses in Petunia x hybrid cells. *J. Plant Physiol.,* 170(8): 780-787.

Duncan, D.B. 1955. Multiple Range and Multiple F Tests. Biometrics. 11: 1.

Eroglu, Y., H. Eroğlu and A. Ilbas, 2007. gamma ray reduces mitotic index in embryonic roots of *Hordeum vulgare* L. *Adv. Biol. Res.,* 1. 1(2): 26-28.

Evans, L.S. and J. Hof. 1975. Dose rate, mitotic cycle duration, and sensitivity of cell transitions from G1 → S and G2 → M to protracted gamma radiation in root meristems. *Radiat. Res.,* 64(2): 331-343.

Farkas, J. 1998. Irradiation as a method for decontaminating food: A review. *Int. J. Food Microbiol.,* 44: 189-204.

Fenech, M. 2000. The *In vitro* micronucleus technique. *Mut. Res.,* 455:81-95.

Gadano, A., A. Gurni, P. Lopez, G. Ferraro and M. Carballo. 2002. *In vitro* genotoxic evaluation of the medicinal plant *Chenopodium ambrosioides* L., *J. Ethnopharmacol.,* 81: 11-16.

Gaulden, M.E. 1987. Hypothesis: Some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. *Mutagenesis,* 2: 357-365.

Grant, W.F. 1978. Chromosomal aberrations in plants as a monitoring system. *Environ. Health Perspect.,* 27: 27-43.

Heidarieh, M., A. Borzouei, S. Rajabifar, F. Ziaie and S. Shafiei. 2012. Effects of gamma irradiation on antioxidant activity of Ergosan. *Iran J. Radiat. Res.,* 9: 245-249.

Anonymous. 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Ivanova, E., T. Staikova, I. Velcheva and K. Kostadinovn. 2003. Somatostatic effect of heavy metal contaminated waters in the region of the town of Panagjurishte, *Bulgaria. J. Envir. Protect Sci*., 4(2): 284-287.

Kharkwal, M.C. and Q.Y. Shu. 2010. The role of induced mutations in world food security. *In:* Era, Shu Q.Y. (Eds.) *Induced Plant Mutations in the Genomics*. Rome, pp. 33-38.

Khursheed, S., R.A. Laskar, A. Raina, R. Amin and R. Khan. 2015. Comparative analysis of cytological abnormalities induced in *Vicia faba* L. genotypes using physical and chemical mutagenesis. *Chrom. Sci.,* 18: 47-51.

Kim, J.H., M.H. Baek, B.Y. Chung, S.G. Wi and J.S. Kim. 2004. Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. *J. Plant Biol.,* 47(4): 314-321.

Kim, J.S., E.K. Lee, M.H. Back, D.H. Kim and Y.B. Lee. 2000. Influence of Low dose radiation on the physiology of germinative seed of vegetable crops. *Korean J. Env. Agric.,* 19: 58-61.

Kiong, A.L.P., A.G. Lai, S. Hussein and A.R. Harun. 2008. Physiological responses of *Orthosiphon stamineus* plantlets to gamma irradiation. *Amer. Eurasian J. Sustain. Agri.,* 2: 135-149.

Kovacs, E. and A. Keresztes. 2002. Effect of gamma and UV-B/C radiation on plant cell. *Micron.,* 33: 199-210

Krishna, G. and M. Hayashi. 2000. *In vitro* rodent micronucleus assay: protocol, conduct and data interpretation. *Mutat. Res.,* 455: 155-166.

Liman, R., U.G. Gokce, G. Akyil, Y. Eren and M. Konuk. 2012. Evaluation of genotoxic and mutagenic effects of aqueous extract from aerial parts of *Linaria genistifolia* subsp. *genistifolia*. *Revista Brasileira de Farmacognosia*, 22: 541-548.

Mahakhode, R.H. and S.R. Somkuwar. 2013. Mitotic abnormalities induced by glyphosate in *Psoralea corylifolia* L. *Int. J. Curr. Pharm. Res.,* 5: 46-48.

Malla, B. 2011. Biological effects of low dose radiation from the cobalt-60 source at as, Norway and of natural background radiation at the thorium-rich area in telemark, Norway, studies with the model plant *Arabidiopsis thaliana*. [Norway]: Department of Plant and Environmental Sciences, Norwegian University of Life Sciences. pp. 32.

Mashev, N., G. Vassilev and K. Ivanov. 1995. A study of N-allyl N-2 pyridyl thiourea and gamma radiation treatment on growth and quality of peas and wheat. *Bulg. J. Plant Physiol.,* 21(4): 56-63.

Minitab 17. 2010. Statistical Software [Computer software] by Minitab Inc.

Nazarenko, M. and O. Izhboldin. 2017. Chromosomal rearrangements caused by gamma-irradiation in winter wheat cells. *Biosys. Diver.,* 25(1): 25-28.

Nikolova, I., M. Georgieva, K. Kruppa, M. Molnor-Long, L. Liu, V. Manova and L. Stoilov. 2015. Cytogenetic effects in barley root apical meristem after exposure of dry seeds to lithium ion beams. Genet. *Plant Physiol.,* 5: 3-9.

Nurmansyah, S., S. Alghamdi, M. Hussein, M.M. Farooq, 2018. Morphological and chromosomal abnormalities in gamma radiation-induced mutagenized faba bean genotypes. *Int. J. Radiat. Biol.,* 94(2): 174-185.

Nwakanma, N.M.C. and B.E. Okoli. 2010. Cytological effects of the root extracts of *Boerhaavia diffusa* on root tips of *Crinum jagus*. *Eurasia J. Biosci.,* 4: 105-111.

Passmore, G.C. 2016. *Burnout in Radiation Therapy: Examining The Six Leading Influences.* University of New England, Portland, US.

Preuss, S.B. and A.B. Britt. 2003. A DNA-damage-induced cell cycle checkpoint in Arabidopsis. *Genet.,* 164: 323-334.

Rakhmatullina, E.M. and M.F. Sanamyan. 2007. Estimation of efficiency of seed irradiation by thermal neutrons for inducing chromosomal aberration in M2 of cotton *Gossypium hirsutum* L. *Russ. J. Genet.,* 43(5): 518-524.

Reisz, J.A., N. Bansal, J. Qian, W. Zhao and C.M. Furdui. 2014. Effects of ionizing radiation on biological molecules—mechanisms of damage and emerging methods of detection. *Antioxid Redox Signal.,* 11: 260-292.

Rojas, E., L.A. Herrera, M. Sordo, M.E. Gonsebatt, R. Montero, R. Rodriguez and P. Ostrosky-Wegman. 1993. Mitotic index and cell proliferation kinetics for the identification of antineoplastic activity. *Anti-Cancer Drugs,* 4: 637-640.

Sakin, A., M. Yildirim and S. Gikmen. 2005. Determining some yield and quality characteristics of mutants induced from a durum wheat (*Triticum durum* Desf.) Cultivar. *Turk. J. Agric. For.,* 29: 61-67.

Sehgal, R., S. Roy and V.L. Kumar. 2006. Evaluation of cytotoxic potential of latex of *Calotropis procera* and Podophyllotoxin in *Allium cepa*. *Biocell.,* 30(1): 9-13.

Singh, I. 1983. Induction of reverse mutation and mitotic gene conversion by some metal compounds in *Saccharomyces cerevisiae*. *Mutat. Res.,* 117: 149-152.

Swierenga, S.H.H., J.A. Heddle, E.A. Sigal, J.P.W. Gilman, R.L. Brillinger, G.R. Douglas and E.R. Nestmann. 1991. Recommended protocols based on a survey of current practice in genotoxicity testing laboratories. IV. Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures. *Mutat. Res.,* 246: 301-322.

Tarar, J.L. and V.R. Dnyansagar. 1980. Comparison of ethyl methane sulphonateand radiation induced meiotic abnormalities in *Turnera ulmifolia* Linn. var. *Angustifolia* Wild. *Cytologia,* 45: 221-231.

Turkoglu, A., M.E. Duru, N. Mercan, I. Kivrak and K. Gezer. 2007. Antioxidant and Antimicrobial Activities of *Laetiporus sulphureus* (Bull.) Murill. *Food Chem.,* 101: 267-273.

Van Harten, A.M. 1998. *Mutation Breeding: Theory and Practical Applications.* Cambridge University Press, New York, US.

Verma, R.C. and M.A. Khah. 2016. Assessment of gamma rays induced cytotoxicity in common wheat (*Triticum aestivum* L.). *Cytologia,* 81(1): 41-45.

Wi, S.G., B.Y. Chung, J.H. Kim, M.H. Baek, D.H. Yang, J.W. Lee and J.S. Kim. 2005. Ultrastructural changes of cell organelles in *Arabidopsis* stem after gamma irradiation. *J. Plant Biol.,* 48(2): 195-200.

(Received for publication 12 April 2018)