Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Singh, V. (2024). An account of chromosomal damage in PMCs of stripe rust infected barley. *Caryologia* 77(1): 13-19. doi: 10.36253/caryologia-2430

Received: December 31, 2023

Accepted: May 26, 2024

Published: July 8, 2024

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
VS: 0000-0002-4510-7974

An account of chromosomal damage in PMCs of stripe rust infected barley

Vivek Singh

Department of Botany, Shri Jai Narain PG College, Lucknow, UP-226001, India
Email: viveksingh_2@yahoo.com

Abstract. A study, on the effects of natural mild fungal infection on meiosis of Barley Pollen Mother Cells (PMCs), was done in order to analyze the chromosomal damage elicited by the pathogenic conditions. A pattern similar to common mutagens, of reduction in mitotic index and chiasma frequency, as well as, production of various aberrations that demonstrate chromosomal damage, was observed. The most common abnormalities were un-orientation and other spindle related aberrations, as well as stickiness and clumping of chromosomes. The disease induced a reduction in pollen viability as compared to the control plants. The results were compared with those of a high dose of a known mutagen ie gamma rays in order to draw commonalities between the two conditions.

Keywords: anomalies, Barley, chromosomes, fungal mycotoxins, gamma rays, *Puccinia*.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is among the most important cereal crops in the world. In India, it is used for the purposes of animal feed, flour making and for malting and brewing purposes (Selvakumar et al., 2014; Singh et al., 2019). Barley is a low input crop and has much better adaptability when compared to wheat (Verma et al., 2012). Barley is naturally inbred and provides a very good genetic material for study of mutagenesis using various agents like radiations, chemicals or combinations of both. The response of this crop in transferring the mutations from one generation to another is exceptionally good which makes it a preferred choice of material for mutagenic studies.

This important crop suffers from various diseases causing great reduction in yield and grain quality. One such disease is the stripe rust caused by *Puccinia striiformis* f. sp. *hordei* (Psh). This disease is common in various countries of South Asia, East Africa, and Central and North America. *P.striiformis* f. sp. *hordei* is a macrocyclic rust having two hosts, primary host being Barley. The most damaging spore, in this fungus, is the uredospore which follows multiple asexual cycles to spread the disease. The primary symptoms include yellow/orange pustules (uredosorus) lined linearly along midribs. The diseased plants are shorter, less vigorous and have a poor root

system. The photosynthesis is affected as a result of the dark pustules reducing the green area. Grains are poorly filled and many florets show abortion. It affects both quantity and quality of grain production (Luthra and Chopra, 1990; Roelfs and Huerta-Espino, 1994).

All exogenous agents capable of producing chromosome aberrations (CAs), i.e., clastogens, are mutagens, and most are also carcinogens. For that reason, cytogenetic damage has long been a favored surrogate endpoint for assessment of carcinogenic and mutagenic potential. One very important conclusion from ionizing radiation (IR) mutagenesis studies in cells of higher organisms has been that large-scale genomic structural changes generally dominate the spectrum of new mutations, as compared with point mutations or other small intragenic changes. Granted, the spectrum of mutations can differ vastly, depending on the mutagenic agent, but in the present study, our emphasis will be on mutagenic events resulting from large-scale structural changes to the host chromosomes genome caused by fungal pathogenesis. These include deletions, insertions, inversions, and translocations, any of which can disrupt genes, alter the control of gene expression, or even result in expression of new fusion sequences. IR is virtually unique regarding its efficiency for producing prompt DNA double-strand breaks (DSBs) randomly throughout the genome, which is the prerequisite lesion for the development of these structural rearrangements (Cornforth et al, 2021). An analogy of the action of IR with fungal toxin will thus enable us to understand the mechanism by which pathogen brings about chromosomal damage and heritable changes in host.

It can be speculated that together with physiological effects, the pathogens, like fungus, might have some cytogenetic effects on the host plant. Though a number of studies attributing the effect of fungal toxins on inhibition of various enzymes and interference with physiological processes are available the effect at cellular and genetic level has not been explored. The level of DNA damage after treatment with fungal metabolites would be related to the ability of the host to survive and reproduce after infection. Impaired activity of antioxidant defense and DNA repair contribute to the DNA damage by free radicals. A few workers have observed induction of chromosomal anomalies by fungal infections eg *Aspergillus* on Cotton (El-Naghy, 1992), *Fusarium* on wheat and maize (Helmey, 2003), *Fusarium* on Maize (El-Daisty, 2009) etc. Various studies have evaluated the effects of radiations, chemicals, pesticides, plant metabolites etc on the genetic material for the purpose of mutagenesis but very few studies are available which show the impact of microbial toxins up to the Chromosomal/DNA level (Kaur et al, 2018). Therefore, it was planned to study the meiosis in fungus infected plants and compare it with a known mutagen ie Gamma radiations, in order to evaluate the chromotoxic potential of fungal toxins.

MATERIALS AND METHODS

Hordeum vugare variety K10 of barley was used for the study. Naturally infected plants were monitored for morphological parameters and young ears collected at the time of flowering. At the flowering time, ie about 50 days after planting, floral buds were collected and fixed in Farmer's fixative (3:1 absolute ethanol-acetic acid) for 24 h. They were then transferred to 70% alcohol and stored at 4°C. Cytological investigations were done using 1% acetocarmine squash technique. Anaphase and Metaphase stages were considered as active division. All chromosomal abnormalities were screened and recorded under the respective stages of cell division where they occurred. Pollen viability was estimated by Acetocarmine stain method where deeply stained pollen grains were considered viable, while non-stained ones were considered non-viable. Similarly, gamma irradiated sets of half of LD_{50} ie 25 kR were screened for comparison of all parameters. Suitable controls were also maintained and all sets given exactly similar environmental conditions.

RESULTS AND DISCUSSION

The control buds showed perfect bivalents at metaphase I and a separation of 7:7 at anaphase I. Metaphase II and Anaphase II were also perfectly normal in controls. However the fungal infected sets showed various types of abnormalities (Fig. 1). The total abnormality percentage was moderate. Common Metaphase anomalies included stickiness, clumping, precocious movement, fragmentation, multivalent formation, univalents, secondary association, unorientation etc. The Anaphase was also marked with different types of chromosomal anomalies like stickiness, laggards, bridges, unequal separation, multipolarity and micronuclei. Table 1 presents a list of anomalies induced by fungi as well as those induced by gamma rays, on the meiosis of barley.

Meiotic anomalies have been reported by a number of workers in a variety of crops following mutagenic treatments eg Wani & Anis, 2008 (Gamma rays on *Cicer*), Pakorn etal, 2009 (Gamma rays on *Anubias*), Motilal etal, 2012 (EMS on *Astercantha*), Akhtar, 2014

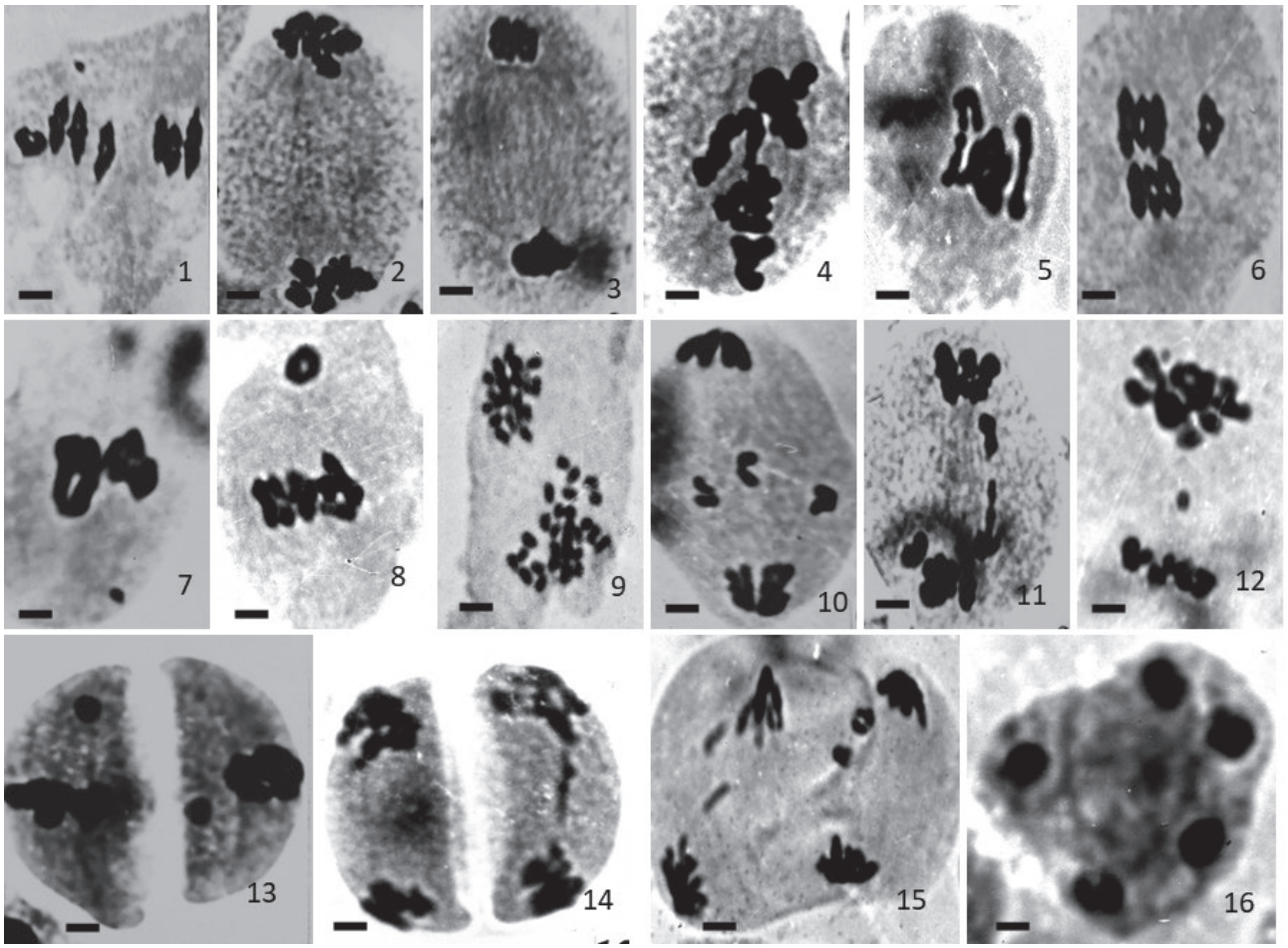

Figure 1. Cytological anomalies induced by chromosomal damage in Barley; 1- Normal Metaphase I, 2 - Normal Anaphase I showing 7:7 separation, 3 – Normal Telophase I, 4 – Stickiness and secondary association of bivalents at Metaphase I, 5 – Multivalents, 6 – Unorientation at Metaphase I, 7 – Clumped multivalents, 8 – Precocious movement, 9 – Fragmentation, 10 – Laggards at Anaphase I, 11 – Chromosome bridge, 12 – Unequal separation and laggard at Anaphase I, 13 – Precocious movement at Metaphase II, 14 – Bridge at Anaphase II, 15 – Laggards at Anaphase II, 16 – Multipolarity at Telophase II. [Scale Bar 1 cm = 4μ].

(Gamma rays and EMS on *Solanum*), Asare etal, 2017 (Gamma rays on *Abelmoschus*), Gnankambary etal, 2019 (Gamma rays on *Vigna*), Chen etal, 2020 (EMS on *Arachis*), Rashid etal, 2021 (Stress on *Trillium*), Liu et al, 2022 (Natural factors on *Elymus*), Turkoglu etal, 2023 (Sodium Azide on *Triticum*) etc. However there are only a few studies which suggest that fungal toxins may also induce chromosomal anomalies. Agar and Alpsoy (2005) studied aflatoxin G_1 (AFG_1) induced chromosomal aberrations in *Vicia faba* and *Zea mays*. Their results showed that 0.1, 0.2, 0.4 ppm concentrations of aflatoxin G_1 could induce gradient based chromosomal aberrations.

Since, they point towards the instability of the genome, chromosomal damages and aberrations have often been regarded as the index of cytotoxic potential of a mutagen. As far as radiations and chemical mutagens are concerned, an increase in cytological anomalies is the obvious manifestation. But similar effects induced by mycotoxins, is an interesting aspect of this study.

Different authors have given various explanations for occurrence of different anomalies. In general the metaphase abnormalities are related to spindle dysfunction e.g. scattering, unorientation and precocious movement of chromosomes. An alteration in genes governing spindle formation may lead to a loss of directive influence on chromosome arrangement and movement leading to consequent dysfunctional anomalies. The current observations in fungal treatment are in concurrence with studies of Styer and Horace (1984). They treated maize roots with solutions of moniliformin (a metabolite of *Fusarium moniliforme* Sheldon). They mentioned that higher concentration caused a disruption of the spindle apparatus.

Table 1. A comparison of cytological abnormalities induced by gamma rays and fungal infection in meiosis of barley.

Treatments	CF/biv ± SE	Metaphase I/II abnormalities (%)								Anaphase I/II abnormalities (%)						Telophase I/II abn Cytoki-nesis (%)				abn (%)	TAb (%) ± SE
		Lm	Do	Pc	Mv	Uv	Fg	St	Cl	Sa	Lg	Br	Us	Ns	St	Mp	Lg	Br	Mn	Tr	Pa		
Control	1.66 ± 0.05	0.07	0.13																			0.20 ± 0.11
Gamma Rays (20 kR)	1.48 ± 0.06	0.61	0.67	0.33	0.27	0.20		2.03	0.54	1.55	1.01	0.88		0.82	1.21		0.40	0.10	0.81	0.11		11.54 ± 0.23
Fungal infected	1.30 ± 0.10	0.95	0.36	0.68	0.31	0.29	0.27	1.26	0.82	0.22	1.97	0.77	0.54		1.04	0.47	0.54		1.02		0.41	11.92 ± 0.18

Lm=Late movement of bivalents; Do=Disturbed orientation of chromosomes; Pc=Precocious movement of chromosomes; Mv=Multivalent formation; Uv= Univalent formation; Fg=Fragmentation of chromosomes; St=Stickiness of chromosomes; Cl=Clumping of chromosomes: Sa=Secondary association of bivalents; Lg=Lagging chromosomes; Br=Bridge formation between poles; Us=Unequal separation of chromosomes at anaphase; Ns=Non synchronous disjunction; Mp=Multipolarity; Mn=Micronuclei; Tr=Triads; Pa=Polyads; TAb=Total Abnormality; SE=Standard Error of Mean.

The presence of univalents and multivalents at metaphase has been reported in different mutagenic studies. Multivalent formation could be attributed to irregular pairing and breakage followed by translocations and inversions. The predominance of ring or chain multivalents is dependent upon the length of interchanged segments and position of interchange. Stray bivalents at metaphase I and II are usually caused by spindle disfunction (Bhat et al., 2007b). The observed precocious chromosomes migration to the poles may be resulted from univalent chromosomes at the end of prophase I or precocious chiasma terminalization at diakinesis or metaphase I. Precocious migration of univalents to the poles is found to be a very common abnormality among plants which have been treated with mutagens (Consolaro et al., 1996). Secondary associations can result from modified chromosomes arrangement due to the duplication, interchanges or stickiness (Kumar and Singh 2003).

Chromosome stickiness has been reported to be a result of partial dissociation of the nucleoprotein and alteration in their pattern of organization (Evans 1962). Mc Gill et al (1974) and Klasterska et al (1976) suggested stickiness due to improper folding of chromosome fibre. Jayabalan and Rao (1987) reported stickiness in meiosis as due to the disturbances in cytochemically-balanced reactions by secondary effects of radiations.

Fragmentations or chromosome shattering observed in present study has also been reported by Cremer and Cremer (1986), Albanese (1982), Cremer et al (1981) as effects of radiation alone or in combination with chemicals. These may be due to damaged mechanisms of DNA repair caused by radiations (Periera, 1995).

Laggards were one of the most common Anaphasic abnormalities characterized by delayed movement of some chromosomes during Anaphasic separations. These have been reported by a number of workers and may be due to delayed terminalization, stickiness of chromosomes ends or because of failure of chromosome movement (Permjit and Grover 1985, Jayabalan and Rao 1987, Sohair 1989). These laggards may move randomly to any pole and give rise to unequal separation of chromosomes or they may form a pole by aggregating together and causing multipolarity. These may just clump together while remaining away from daughter nuclei at each pole and form micronuclei at Telophase.

Bridges are also a very common chromosome damage indicator. These are caused by paracentric inversion, which lead to formation of a dicentric bridge joining two poles (Swanson, 1988). The bridges may also be formed by stickiness between separating chromosomes. During separation these bridges break randomly and give rise to unbalanced poles having unequal chromatin volume.

Disturbances in spindle formation in meiosis II leads to formation of three or more than four poles at Ana/Telophase II. Subsequent wall formation gives rise to triads or polyads instead of normal isobilateral tetrads.

Changes in the surface proteins of PMC walls, lead to clumping of PMCs and sometimes gives rise to cytoplasmic channels allowing transmigration of chromatin. This is known as cytomixis and it is a powerful agent in causing polyplodization and increase in chromosome numbers within PMCs. Changes in cytoplasmic viscosity may also lead to shrinkage of PMCs, which was evident in a few PMCs.

The chiasma frequency showed a decrease in fungi infected plants. Presence of greater number of univalents might be responsible for a consequent decrease in the chiasma frequency although it may get balanced somewhat by a simultaneous increase in multivalents. Greater occurrence of rod bivalents might also cause a decrease in chiasma frequency. Some authors like Raghuvanshi and Singh (1974) have reported a decrease in chiasma frequency with increase in dose of treatment while some others like Prasad and Godward (1969) had observed an opposite trend. The reduction observed here is common to most radiation and chemical mutagenic treatments and has been demonstrated by workers like Sinha and Mahapatra (1969) in *Zea,* Sinha and Roy (1976) in *Phaseolus* and Lal and Srinivasachar (1979) in *Pennisetum*.

A high degree of pollen sterility, in gamma treatment as well as fungi infected sets, is a result of increase in the chromosomal abnormalities, which give rise to pollen with varying degrees of chromosomal imbalance. Pollen sterility has been attributed to stickiness that leads to irregular segregation and improper fragmentation of chromosomes. Such unbalanced pollen grains are very often non-viable and unable to fertilize the ovules. This in turn causes adverse impact on seed setting.

A comparison of the chromosomal anomalies present in Fungi infected plants with those present in conventional mutagens like gamma irradiated or chemical treated plants reveals a great level of similarity. When we compare the results obtained with by fungal pathogens with those elicited by other mutagens, we get striking similarities which indicates similar mode of action. Kumar and Yadav (2010) reported almost similar chromosomal anomalies induced in *Sesamum indicum* (L.) by EMS (Ethyl Methane Sulphonate) which is an alkylating agent. Singh et.al (2019) and Nilan et.al (1964) also found identical chromosomal damage was reported by use of radiations. Studies suggest that even some non-conventional agents like Catalase and Lipase enzymes have elicited reduction in germination and survival of plants (Ananthaswamy et.al; 1971). However, if fungal pathogen induced mutations are considered, there was a clear predominance of physiological abnormalities like stickiness and clumping over clastogenic ones like fragmentation or micronuclei. Such anomalies lead to high degree of gamete sterility and bring the plant into a growth disadvantage. As a result high degree of lethality is induced even at low infections.

It seems that the reduction in active mitotic division occurs due interference of chemicals in the G1 cell cycle which suppresses DNA synthesis as reported by Mohandas and Grant (1972) in several higher plants. There are many studies that compare the chromosomal abnormalities induced by the chemical, physical mutagens and the combination of both like those of Sree Ramalu (1973), Mehra and Mann (1974), Kumar and Singh (2002), Alam et al(2022) etc. However, the progress in the effective and efficient use of mutagens is hindered by complex interplay of many physical and chemical factors that determine the ultimate yield of mutations (Konzak et al 1975). According to Wilson (2019) and Jeong (2014) ionizing radiations can stimulate ROS production through nitric oxide synthase (NO) pathway. Interaction of NO molecule with superoxide radical (O_2^-) to produce peroynitrite ($ONOO^-$). Peroxynitrite is a powerful oxidant radical reacts with DNA bases, amino acids and lipids. NADPH oxidase is also been reported to cause production of ROS. When the ROS encounter biological organisms, they cause damage to biomolecules such as DNA, RNA and proteins in living cells.

It is evident that fungal toxins either themselves act as mutagens or induce formations of certain chemicals in the host which causes chromosome damage. DNA damage during plant interactions with virulent pathogens is largely under-described, and whether DNA damage arises during responses activated by core plant defense mediators such as salicylic acid, jasmonic acid or activated microbe-associated molecular pattern (MAMP) receptors also is not known (Song and Bent 2014). The present study calls for a calibration study on chromosomal damage by mycotoxins which can have intergenerational effects. It also brings into forefront that fungal diseases can have manifestations that are not only physiological but may also be genetical. Damaged genes may bring about mutations, at least some of which may show some degree of inheritance. A deeper study in this area is required.

REFERENCES

1. Agar, G. and L. Alpsoy (2005). Antagonistic effect of selenium against aflatoxin G1 toxicity. Botanical Bulletin of Academia Sinica 46:301-305.

2. Akhtar, N. (2014). Effect of physical and chemical mutagens on morphological behavior of tomato (*Solanum lycopersicum*) cv. "Rio grande" under heat stress conditions. Plant Breeding and Seed Sci. 70: 69-79.
3. Alam Q., Khan M.A. and Azaz Ahmad Z.R. (2022). Gamma Irradiation Induced Chromosome Aberrations in Meiotic Cells of Bread Wheat (*Triticum aestivum* L.). Int. J. Plant and Soil Sc. 34(5):81-89.
4. Albanese, R. (1982). The use of fertilized mouse eggs in detecting potential clastogens. Mutat. Res. 97:315-326.
5. Ananthaswamy H. N., Vakil U.K., Sreenivasan A. (1971). Biochemical and physiological changes in gamma-irradiated wheat during germination. Radiation Botany 11:1-12
6. Asare, A. T., Mensah, F., Acheampong, S., Asare-Bediako, E., and Armah, J. (2017). Effects of gamma irradiation on agromorphological characteristics of okra (*Abelmoschus esculentus* L. Moench.). Adv. Agric. 2017(3):1–7.
7. Bhat, T. A.; M. Sharma and M. Anis (2007b). Comparative Analysis of Meiotic Aberrations Induced by Diethylsulphate and Sodium Azide in Broad Bean (*Vicia faba* L.). Asian J. Plant Sciences, 6 (7):1051-1057.
8. Chen, T., Huang, L., Wang, M., Huang, Y., Zeng, R., Wang, X. (2020). Ethyl methyl sulfonate-induced mutagenesis and its effects on peanut agronomic, yield and quality traits. Agronomy 10(5):655-667.
9. Consolaro, M. E. L. and Pagliarini, M. S. (1996). Spontaneous Chromosome Stickiness in Microsporocytes of *Centella asiatica* (L.) Urban (Umbelliferae), Cytologia 61:57-61.
10. Cornforth M.N., Bedford J.S. and Bailey S.M. (2021). Radiation on Chromosomes: Sizing up the Damage. Cytogenet Genome Res 161(6-7):328–351.
11. Cremer, C. and Cremer, T. (1986). Induction of chromosome shattering by UV light and Caffeine: The influence of different distributions of photolesions. Mut. Res. 163:33-40.
12. Cremer, C., Cremer, T., Zorn, C. and Zimer, J. (1981). Induction of chromosome shattering by UV irradiation and caffeine: Comparison of whole-cell and partial cell radiation. Mut. Res. 84:331-348.
13. El-Diasty, Z. M., Zaied, K.A., Kosba, Z.A., Kash, K. S. and Abd El-Azez, M. H. (2009). Meiotic chromosomal anomalies resulting from fungal infection of maize compared with those resulting from gamma irradiation. J. Agric. Sci. Mansoura Univ., 34(9):9233-9248.
14. El-Naghy, M.A., Mazen, M.B. and Fadl-Allah, E.M. (1991). Studies on the fungus flora and aflatoxin production of cotton seeds in Egypt. Medical Journal of Islamic World Academy of Sciences. 4(2):141-145.
15. Evans, H. J. (1962). Chromosomal aberration induced ionizing radiations. Int. Rev. Cytol. 13:221-232
16. Gnankambary, K., Batieno, T. B. J., Sawadogo, N., Sawadogo, M., Yonli, D., and Ouédraogo, J. T. (2019). Assessment of radio-sensitivity for three cowpea genotypes to gamma irradiation. Int. J. Genet. Mol. Biol. 11(2):29-33.
17. Helmey, R. K. (2003). Cytological studies of some Fusarium toxins on growth, cell ultrastructure and electrophoretic profile of seed proteins in wheat and maize. Ph. D Thesis, Botany Department, Faculty of Science, Minia University, Minia, Egypt.
18. Jayabalan, N. and Rao, G. R. (1987). Gamma radiation induced cytological abnormalities in *Lycopersicon esculentum* Mill var. Pusa Ruby. Cytologia 52:1-4.
19. Jeong K. S. (2014). Effects of temperature-dependent optical properties on the fluence rate and temperature of biological tissue during low-level laser therapy. Lasers in Medical Science 29(2):637-644.
20. Kaur M., Chadha P., Kaur S., Kaur A., Kaur K., Yadav A. K., and Kaur R. (2018). Schizophyllum commune induced genotoxic and cytotoxic effects in Spodoptera litura. Scientific Reports 8:4693.
21. Klasterska, I., Natarajn, A.T. and Ramel. C. (1976). An Interpretation of the origin of sub-chromatid aberrations and chromosome stickiness as a category of chromatid aberration. Hereditas 83:153-162.
22. Konzak C.F., Niknejad M., Wickham I. and Donaldson E. (1975). Mutagenic interaction of sodium azide on mutations induced in barley seeds treated with diethyl sulfate or *N*-methyl-*N*'-nitrosourea. Mut. Res. 30(1):55-61.
23. Kumar G. and Yadav. R.S. (2010). EMS induced genomic disorders in sesame (Sesamum indicum l.) Rom. J. Biol. – Plant Biol. 55(2):97-104.
24. Kumar, G and Singh, V. (2003). Comparative analysis of meiotic abnormalities induced by gamma rays and EMS in barley. J. Ind. Bot. Soc. 82:19-22.
25. Liu B., Tao X., and Dou Q (2022). Meiotic chromosomal abnormality detected in heterozygotes of *Elymus nutans*. Front. Plant Sci 13:3-9.
26. Luthra, J. K. And Chopra, V. L. (1990). "Genetics of stripe rust resistance in barley", Ind. J. Genet. Plant Breeding 50(4):390-395.
27. Mc Gill, M., Pathak, S. and Hsu, T.C. (1974). Effect of Ethidium bromide on mitosis and chromosomes: A possible amatorial basis for chromosomal stickiness. Chromosoma 47:157-168.
28. Mehra, P. N. and Mann, A. C. (1974) Cytological effect of chemical mutagens on Pterotheca falconeria

1. Monofunctional alkylating agent. Nucleus 17:167-182
29. Mohandas, T and Grant, W. F. (1972). Cytogenetic effects of 2,4-D and amitrole in relation to nuclear volume and DNA content in some higher plants. - Can. J . Genet. Cytol. 14:773-783.
30. Motilal B., Jogeshwar, P., Mishra, R. R. and Rath, S. P. (2012). Analysis of EMS induced in vitro mutants of *Asteracantha longifolia* L. Nees using RAPD markers. Indian J. Biotechnology 11:39-47.
31. Nilan. R. A., Konzac, C. F., Heiner, R. E. and Froese-Gertzen, E. E. (1964) Chemical Mutagenesis in barley. Proc. In: Int Barley Genetics Symp.Washington pp. 35-54.
32. Pakorn, T., Taychasinpitak, T. Jompuk C. and Jompuk. P. (2009). Effects of Acute and Chronic Gamma irradiations on in vitro culture of *Anubias congensis*. J. Nat. Sci. 43:449-457.
33. Pereira, C. M., Taschetto, O. M., Defani-Scoarize, M. A. and Pagliarini, M. S. (1995). Spontaneous Chromosome Fragmentation in Maize Microsporocytes. Cytologia 60:297-301.
34. Permijit, K. and Grover, I. S. (1985). Cytological effects of some organophosphorus pesticides II. Meiotic effects. Cytologia 50:199-211.
35. Prasad, A. B. and Godward, M. B. E. (1969). Comparison of the developmental response of diploid and tetraploid *Phalaris* following irradiation. Rad. Bot 9:167-173.
36. Raghuvanshi, S.S. and Singh, A.K. (1974). Studies on effect of gamma rays on *Trigonella foenum graecum* L. Cytologia 39:473-482.
37. Rashid, K., Rashid, S., Ganie, A. H., Nawchoo, I. A., Khuroo, A. A. (2021). Meiotic Studies, Pollen Fertility and Seed Set of *Trillium govanianum*, an Endangered Endemic Plant Species of the Himalaya. Cytologia 86:245-249.
38. Roelfs, A.P. and Huerta-Espino, J. (1994). Seedling resistance in *Hordeum vulgare* to barley stripe rust from Texas. Plant Dis 78:1046-1049.
39. Selvakumar, R., Meeta, Madhu, Shekhawat, P.S., Verma, R.P.S. and Sharma, I. (2014). Management of stripe rust of barley using fungicides. Indian Phytopath. 67(2):138-142.
40. Singh, B., Mehta, S., Aggarwal, S. K. and Tiwari, M. (2019). Barley, Disease Resistance, and Molecular Breeding Approaches. In book: Disease Resistance in Crop Plants. Edition I, Springer, Cham; pp. 261-299.
41. Sinha S.K. & Mohapatra B.K., (1969). Compensatory chiasma formation in maize. Cytologia 34:523–527.
42. Sinha, S. S. N. & Roy. H. (1976). Distribution of chiasmata between and within nuclei in irradiated and non- irradiated population in Phaseolus sp. J. Cytol. Genet. 11:7-9.
43. Sohair, E. Antoinette, H. and Atif, H. (1989) Cytogenetic effects of herbicide Garlon 4 on root mitosis of *Allium cepa*. Cytologia 54:465-472.
44. Song, J. and Andrew, A. F. (2014). Microbial Pathogens Trigger Host DNA Double-Strand Breaks Whose Abundance Is Reduced by Plant Defense Responses. Plos Pathogens 10(6):1-16.
45. Sree Ramalu, K. (1973). Mutagenic effects of gamma rays, chemical mutagens and Combined treatments in Sorghum. P. Pfanzucitg. 68:287-293.
46. Srinivasachar H.R. (1979). Induction of segmental interchanges in pearl millet (Pennisetum typhoides). Theoretical and Applied Genetics 54(1):27-34.
47. Styer, C. H. and. Horace, G. C. (1984). Effects of Moniliformin on Mitosis in Maize (*Zea mays* L.). Plant and Cell Physiology 25(6):1077-1082.
48. Swanson, C. P. (1988). Variation: Nature and Consequences of Altered Chromosomal Structure. Cytogenetics, 2nd ed., Prentice Hall of India.
49. Turkoglu, A., Haliloglu, K., Tosun, M., Szulc, P., Demirel, F., Eren, B., Bujak, H., Karagoz, H., Selvet, M., Uzkan, G. and Niedbala, G. (2023). Sodium Azide as a Chemical Mutagen in Wheat (*Triticum aestivum* L.): Patterns of the Genetic and Epigenetic Effects with iPBS and CRED-iPBS Techniques. Agriculture 13(6):1242-1259.
50. Verma, R.P., Sharma, R.K. and Mishra, B. (2012). Barley Cultivars released in India. Names, Parentages, Origins and adaptations, Research Bulletin No. 29. Directorate of Wheat Research, Karnal.
51. Wani, A., and Anis, M. (2008). Gamma ray- and EMS-induced bold-seeded high-yielding mutants in chickpea (Cicer arietinum L.). T. J. Biol. 32:161-166.
52. Wilson A, Menon V, Khan Z, Alam A, Litovchick L, Yakovlev V. (2019). Nitric oxide-donor/PARP-inhibitor combination: A new approach for sensitization to ionizing radiation. Redox Biology 24:101-169.