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X-RAY AND GAMMA IRRADIATION INDUCED CHROMOSOMAL ABERRATIONS IN PLANT SPECIES AS THE CONSEQUENCE OF INDUCED MUTAGENESIS – AN OVERVIEW

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Abstract

X rays and gamma irradiations create genetic variations by widening the gene pool, and induce gene mutation for generating commercially important products through enhancement of superior qualitative trait(s). Gene mutation is of global significance and successful mutagenesis experiment depends on the sensitivity of the genotype (s) to the administered doses of the mutagen (s). Assessment of LD_{50}, lethality, injury, mitotic and meiotic aberration frequency (key components to determine sensitivity of a species) is prerequisite for determining sub-lethal doses and to monitor successful mutation breeding experiments. Mutagens (ionizing radiations: X rays and gamma rays) inducing chromosomal aberrations can be ascertained from mitotic cells following root tip squash preparation (clumping and stickiness, fragments, rings, polyploid cells, pseudochiasma, bridges with or without fragments, paired fragments, micronuclei, giant cell, chromatin disorganization, among others) as well as from meiotic chromosome preparation of M_1 plant population (plant carrying reciprocal translocation, inversion, desynapsis, etc.). Mutagens induced chromosomal abnormalities reflect upon sterility (pollen and seed sterilities) which in turn plays key role in screening phenotypic macromutants (as it is visible by naked eye) at M_2. Different types of mutagens induced aberrations are discussed.

Key words: Induced mutagenesis, Irradiations, Chromosomal aberrations, Mitosis, Meiosis, Cause and consequence, Cytogenetic lines.

Introduction

Induced mutation by physical mutagens (X-rays and gamma rays) is an important source of creating genetic variations by widening gene pools in a species and significantly associated with crop improvement. There is an upsurge of interest directed towards mutation research using mutagens since Muller, (1927) and Stadler, (1928) (who first artificially increased the rate of mutation following X-irradiation in *Drosophila melanogaster* and in barley respectively). Although spectacular success of chemical mutagens is reported in induced mutagenesis (Konzak *et al.,* 1965), physical mutagens should be included in any mutagenesis experiment (Nilan *et al.,* 1965) as radiations can induce chromosomal engineering by means of chromosome breakages and rearrangements in mitotic and meiotic cells.

Mutation research contributed significantly to global agriculture leading to solve food and nutritional scarcity in several countries of the world (Kharkwal, 2012). Mutagens inducing genetic variations in plant species are well exploited to improve yield (enhancement in raw products and value added products) and yield related traits apart from possessing great relevance in (1) reconstruction of plant ideotypes, (2) incorporation of one or two desirable attributes in otherwise well adapted varieties, (3) upgrading of the protein, (4) getting transgressive variants, (5) developing resistant line against plant pathogens, among others.

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Types of physical mutagens

(A) Ionizing radiations: Energized particles, eject excited outer shell electrons from atoms

(a) Particulate (dense)

i. Beta particles (Radioactive isotopes)

ii. Alpha particles (Radioactive isotopes)

iii. Neutron ray (Nuclear reactor, Cyclotron machine, measured in REPS unit)

(b) Non-particulate (less dense)

i. X-rays – Electromagnetic radiation, broader wavelength, less energized; source X-ray machine

ii. Gamma rays - Electromagnetic radiation, shorter wavelength, highly energized; source ^{60}Cobalt. Both measured in Gray (Gy) scale; 1 kR = 10 Gy

(B) Non-ionizing radiations

UV rays – Ultra Violate rays fall in three sections of pro-visible spectrum namely, UV-A (400-320 nm), UV-B (320-280 nm) and UV-C (280-100 nm). UV radiation of 260 nm cause dimerization of adjacent pyrimidine base specifically induces thymine dimer. Source Mercury vapour lamp). UV irradiations cannot affect sex cells and is rarely used in mutagenesis experiments relating to plant species.

Among all the physical mutagens, X – irradiations and gamma irradiations are widely used in plant species for induced mutagenesis (Ghosh and Datta, 2005; Paul and Datta, 2005; Mukherjee and Datta, 2006; Sutarto *et al.,* 2009; Malek *et al.,* 2012; Mba and Shu, 2012; Salve and More, 2014).

Mode of Action of Physical Mutagens on DNA/ Chromosomes of Plant System

In general, irradiations cause various disturbances (H_2-bond breakage, single strand break, double strand break, base loss, base change, formation of pyrimidine dimmers, DNA cross linkage, DNA-protein cross linkage) in DNA molecule by putting immense external energy in its thermodynamically stabilized state.

The disturbances through radiations may be the consequence of either direct change in the DNA molecule (Lea, 1955) or by indirect effect on the precursor molecules of H_2O_2 and subsequent effect on chromosome by oxidation (Koller, 1953). The radio biochemical event occured by the reaction of free radicals with biological molecules generally takes 10^{-12} to 10^{-6} seconds (IAEA 1970). The free radicals produce unpaired electron resulting in high chemical reactivity. Most of the energy deposited in cells is absorbed initially in water and subsequently oxidizing and reducing reactive hydroxyl radicals. Hydroxyl radicals (OH^-) may diffuse over distances to interact with DNA to cause damages in chromosomes resulting in breakages leading to structural changes and are expressed phenotypically in various ways.

Required Factors for Irradiation Induced Mutagenesis Experiment

Seeds are the most suitable sample to be mutagenized for irradiation induced mutagenesis experiment as it is convenient to use and can be handle appropriately. Assessments of seed sizes and moisture content are needed prior to mutagen(s) treatment. Besides, state of the seeds (dry or pre-soaked) is an important parameter to note. Sample size in the field condition(s) is also a vital attribute for successful mutagenesis experiment. The plant species to be chosen for mutagen doses depend on various factors like chromosome number and sizes, interphase chromosome volume, nature of seeds (as oil seeds need relatively higher doses of treatments than other plant species, as oil acts as protectant) etc. Proper desiccation and storage of the seeds are needed for undergoing mutagen treatments. However, it is better to use freshly harvested seeds.

Classification of Chromosomal Aberrations Due to Physical Mutagen Treatments

Savage, (1976) provided a classification of chromosomal aberration types. Irrespective of mitotic and meiotic cells, mutagens induced chromosomal aberrations are classified into following types:

Exchange

Exchange of chromosomal parts following the occurrence of two lesions which may be asymmetrical or symmetrical.

1. Interchanges – interaction occurring in the arms of homologous or non-homologous chromosomes.
2. Intrachanges – lesions are within one chromosome

a. Inter-arm intrachanges: lesions are in opposite arms with respect to the centromere.

b. Intra-arm intrachanges: lesions are within chromatid. This type is sub-divided into followings

i. Inter-chromatid intrachanges – exchange involving both the chromatids.

ii. Intra-chromatid intrachanges – only one chromatid is involved.

Breaks

Chromosomal or at chromatid level giving rise to acentric fragment(s).

Achromatic lesions

'Gaps', either in sister chromatid (chromatid gap) or in identical position of both chromatid of a chromosome (chromosome gap).

Unconventional aberrations

1. Shattering–affected chromosomes at metaphase

appear as many broken small pieces of varying length. Acentric rings, partial exchange and isochromatid-type structural changes.

2. Pulverization – masses of small, thin fragments or longer fragments of uncoiled chromosomes.
3. Physiological effect (stickiness) – Degradation or depolymerization effect of DNA.
4. Agglomerated mitotic division–affected cells fails to complete division.

Apart from the mentioned types, mutagens induce other abnormalities like nuclear shape deformation, enhancement in cell size and deformity in cell shape, chromatin condensation, fragmentation and attachment with cell membrane, cell fusion and polyploidization, c-mitosis effects (chromatin condensation, bipartite nature and pseudochiasma formation, granulation and fragmentation), spindle aberrations resulting in tri- and multipolarity, laggard formation, multisporic conditions.

Mechanism Related to Aberrations

1. Breakage-and-reunion theory (Classical theory): (Sax, 1938; Catcheside, 1945; Evans, 1962; Lea, 1946)

Double strands breakage by irradiations, followed by the joining of broken ends through the process of non-homologous end joining.

2. Exchange theory: (Revell, 1955; 1959; 1963; 1974)

Mutagens induced double strands break (DSB) which would be sufficient enough to initiate an exchange with an otherwise undamaged part of DNA through the process of homologous exchange at variable sites.

3. Molecular theory: (Chadwick and Leenhouts, 1978; 1981)

DNA double strands break generally have a linear quadratic dose-response curve form and are not directly proportional to doses of treatments (Savage, 1998).

Types of Chromosomal Aberrations in Mitotic and Meiotic Cells

Different types of chromosomal aberrations induced by X-rays and gamma irradiations in mitotic (Fig. 1) and meiotic (Fig. 2) cells are discussed briefly.

Mitotic aberrations: causes and consequences

Mitotic aberrations namely stickiness and clumping of chromosome, diplochromosomes or pseudochiasma, ring(s), fragment(s), bridge(s) with or without fragment(s), micronuclei, giant cell, cellular and nuclear shape deformities (Fig. 1a-o) encountered in plant species following irradiations (X–rays and gamma rays) are discussed briefly with an objective to highlight the effect of mutagens in plant species as well as to demonstrate the configurations for academic perspectives.

Clumping and stickiness of chromosomes is described as 'physiological effect'. Evans, (1962) suggested that sticky behavior of chromosomes is caused by partial dissociation of nucleo-proteins and an alteration in the pattern of organization. Conger, (1961; 1963) suggested that sticky behavior of chromosomes is the consequence of an indirect effect of the mutagens resulting in the formation and release of some chemical substances in the cell, which remain active for quite some time and is capable of producing some kind of nucleo-proteins as done by ionizing radiations. The concept was further supported by the fact that various chemical, both exogenous and endogenous, are known to produce physiological effects identical to those caused by irradiations in actively dividing cells and also in resting cells of dry seeds. Sudhakaran, (1972) suggested that such irregularities are induced by 'mitotic poison' arising from breakdown of micromolecules and macromolecules in the cytoplasm by irradiation, specially enzymes and nucleoproteins. These poisonous chemicals may interfere with the synthesis, state and structure of nucleic acid, thereby inducing physiological effects in the chromosomes during cell division. Clumping and stickiness of chromosomes caused by mutagen treatments are reported in different plant species (Mukherjee and Datta, 2011; Khanna and Sharma, 2013).

Chromosomal fragments occurring in root tip mitotic cells induced irradiations may be due to the change in the molecular constituents of the chromosomes (Sax, 1941). Sharma and Sharma, (1960) suggested that an upset in nucleic acid metabolism due to reverse action of enzyme DNA polymerase (Ahnström and Natarajan, 1966) causes hazard in protein reduplication process leading to chromosomal breakages. Occurrence of paired fragments during anaphase suggests localized breakage in the chromosome possessing sub-terminal constrictions; while, such fragments are reported to be the outcomes of breaks induced at G_1 phase (Sato and Gaul, 1967) and have arisen from chromosomes rather than chromatid (Caldecott and Smith, 1952). Thoday, (1953) describes the occurrence of paired fragments due to isochromatid deletion. Chromatid breaks may result if one of the chromatid restituted after splitting (Evans, 1962). Tarar and Dnyansagar, (1980) opined that multiple and non-paired fragments give an indication of chromatid break.

Diplochromosomes that are observed in mitotic cells due to treatments may possibly be the cause of induce anomalous relational coiling between sister chromatids.

Appearance of ring configurations of chromosomes in somatic metaphase cells in the mutagen treated materials might be the consequence of asymmetric interchanges. Polyploid and aneuploid cells are found to be associated with chromosomal fragments and such complex types can be attributed to breakage associated with spindle disturbances.

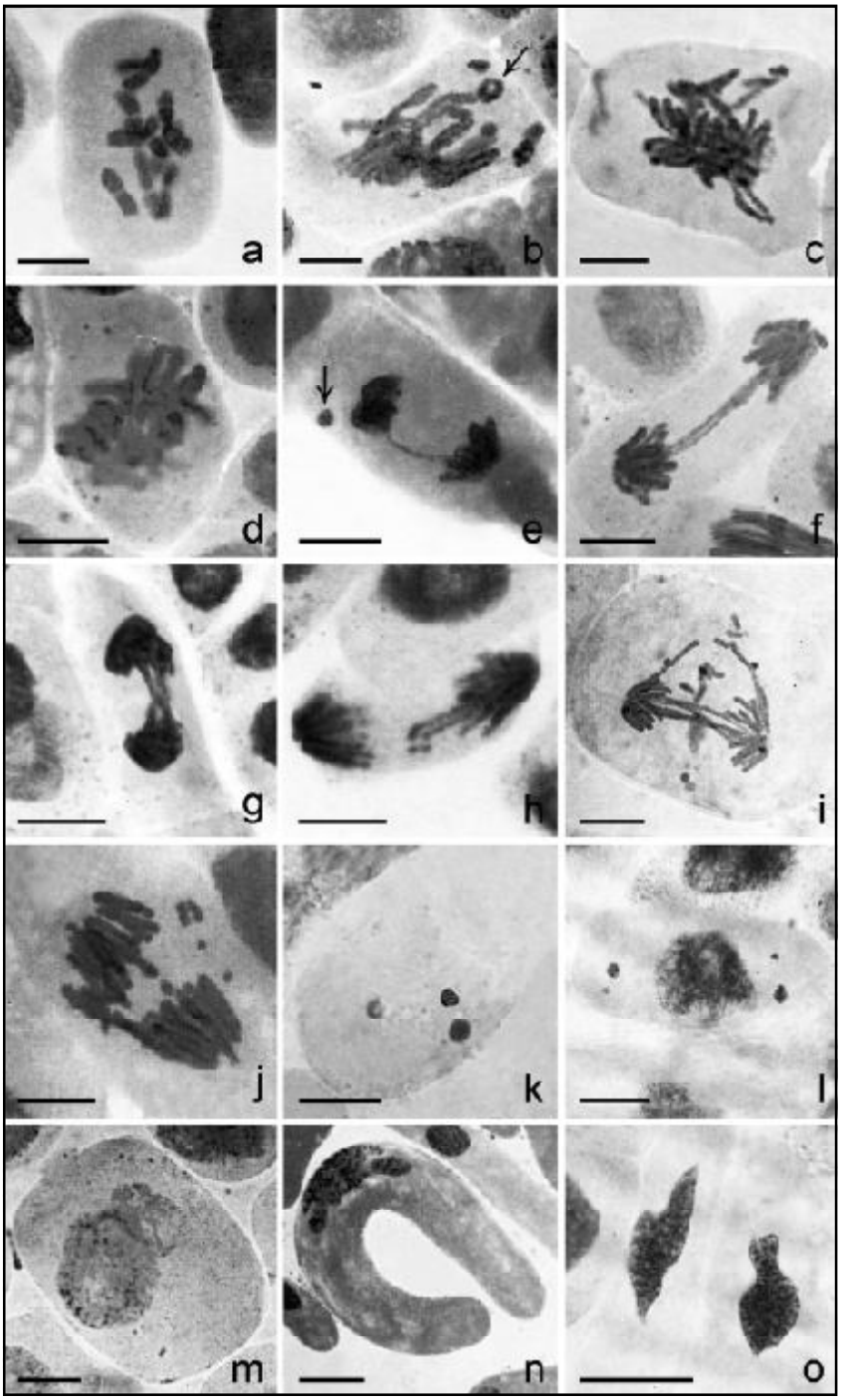

Fig. 1a-o: Mitotic consequences (a-d: metaphase; e-j: anaphase; k-o: resting stage). **(a)** 2n=12 chromosomes, **(b)** Ring (arrow), **(c)** Fragment (arrow), **(d)** Stickiness and clumping of chromosome, **(e)** Single bridge with a fragment, **(f)** Double bridges, **(g)** Criss-cross bridge, **(h)** Incomplete bridge with 2 paired fragments, **(i)** Bridges with fragments, **(j)** Fragments, **(k)** Chromatin fragmentation and agglutination, **(l)** Micronuclei, **(m)** Giant cell, **(n)** Cell shape deformity, **(o)** Nuclear shape deformity. *Scale bar = 10 μm.*

Source of photographs: Mukherjee and Datta (2011), Journal of Plant Development Sciences Vol. 3 & 4: 233–238.

Occurrence of chromosomal bridges gives an indication of chromosome breakage and translocation. The single somatic bridges accompanied with fragment(s) arise when both the chromatids of a chromosome are broken at the same locus followed by lateral fusion (Sax, 1940). They may also arise from chromatid translocation when the chromosomes of mutagen treated materials are bipartite in nature (Kallo, 1972). Double or paired bridges accompanied with fragments are dual dicentric and possibly arisen through asymmetrical interchanges. The formation of paired bridges in the mitotic anaphase has been described as the result of fusion between broken chromosomes rather than broken chromatids (Sax, 1940; Caldecott and Smith, 1952; Sparrow, 1951). Criss-cross and interlocked bridges are also dicentric. Due to anaphasic failure of dicentric chromosomes, criss-cross bridge may occur (Sax, 1940). Absence of fragment with bridge may be due to restitution or the fragments getting entangled or attached with the normal chromatids of chromosomes. The sticky bridges reported due to mutagen treatments result from disturbances at the cyto-chemical level caused by the effect of single and combined effect of mutagens.

Micronuclei occurring in resting cells are the outcome of chromosomal fragmentation at dividing stages. The term 'condensed' and 'non-condensed' have been used by Shaikh and Godward, (1972) to describe the obvious differences in structure, thickness of chromatin materials and stainability between the two groups of micronuclei. Giant cells studied in different treatments of physical mutagens is reported to occur as an outcome of deficiency in nuclear materials followed

by ultimate failure of cell division process (Gray and Scholes, 1951; Tolmach and Marcus, 1960).

Many mitotic cycles lead to meiosis in pollen mother cells (PMCs). Chromosomal aberrations persisting at meiosis affect the viability of gametes and therefore, the fertility of plants resulting to genetic consequences in the form of gene mutations. X- rays and gamma rays can induce several types of chromosomal aberrations namely, formation of quadrivalents – ring or rod, alternate or adjacent, dicentric chromatid bridges with an acentric fragment at anaphase I and of bridges at anaphase II, and univalent in meiotic cells (Fig. 2a-l). These aberrations reflect translocations, inversion (intra-chromosomal gene alterations, desynapsis) among others. Such aberrations are extremely important for cytogenetic analysis in the species. Induced translocation lines are generally screened following pollen fertility-sterility analyses and subsequently confirmed from meiotic study. Reciprocal translocation lines carry one quadrivalent in association to bivalent and univalent in more than 30% of the meiocytes. The quadrivalents are ring (when the break point is sufficient enough to produce chiasma) or of chain (break point is small and chiasma slips out) or both. Ring or chain is either alternate or adjacent in orientation. In a given species the orientation is either random (equal frequency of alternate and adjacent) or directed (more alternate configurations) (Burnham, 1956; Sybenga, 1972). However, both random as well as predominant of adjacent orientation are reported in *Nigella sativa* (Saha and Datta, 2002; Mukherjee and Datta, 2011) and in *N. damascena* (Ghosh and Datta, 2006) suggesting that the entire process is under genetic control. Translocation lines provide significant understanding of gene and chromosome relationship of a species.

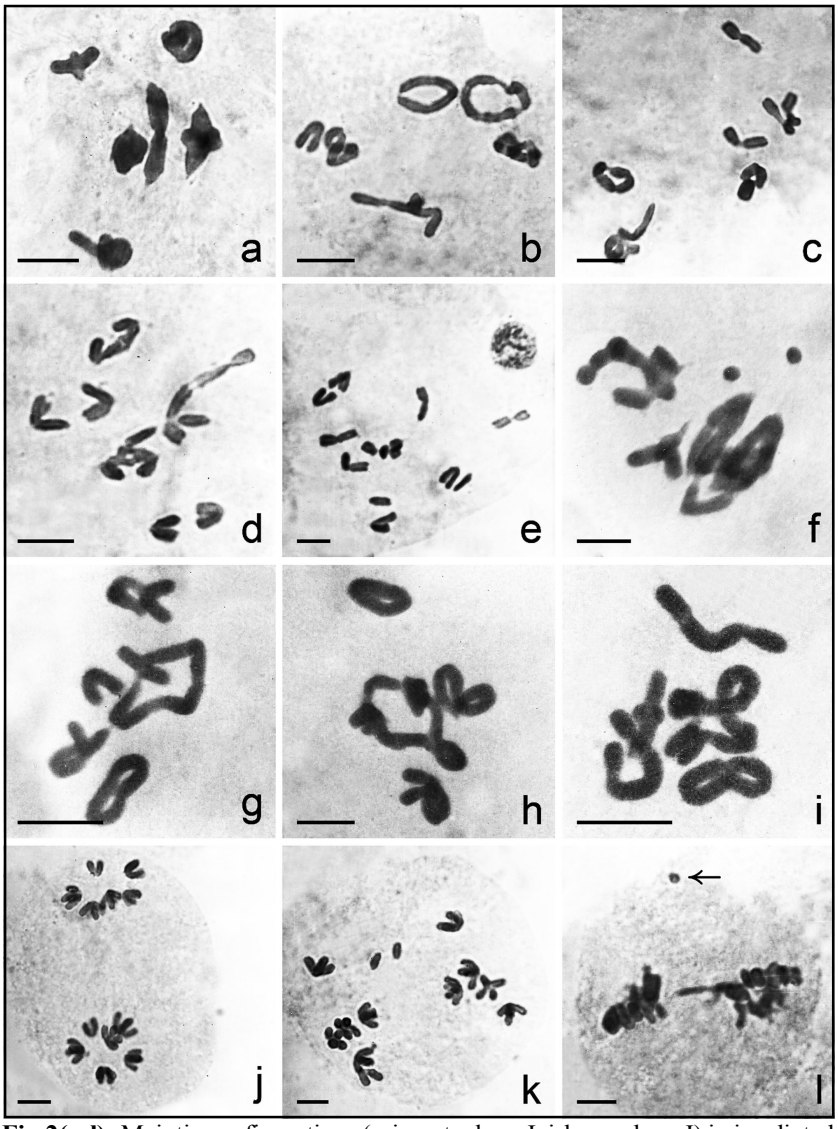

Fig.2(a-l): Meiotic configurations (a-i: metaphase I; j-l: anaphase I) in irradiated cells of a plant species (*Nigella sativa*). **(a-b)** 6II, **(c)** 4II + 4I, **(d)** 2II + 8I, **(e)** 12I, **(f)** 6II + 2 identical sized fragments, **(g-i)** 1IV + 4II, **(j)** 6:6 separation of chromosomes, **(k)** 2 equal sized lagging fragments, **(l)** Bridge with an acentric fragment. *Scale bar = 10 μm.*

Source of photographs: Mukherjee and Datta (2011), Journal of Plant Development Sciences Vol. 3 & 4: 233–238.

Conclusion

Irradiations induced cytological abnormalities cannot be considered as factors that directly affect the genotype in relation to growth and development but can be used as an index of sensitivity of the species to mutagen doses. Meiotic anomalies will be transmitted to following generation and therefore important for genetic consequences. However, assessment of cytological abnormalities is of utmost important as it not only reflects responsiveness of the species but also signifies sub-lethal doses to be monitored for successful mutagenesis experiment. Further, knowledge of chromosomal aberrations occurring in somatic and reproductive cells of plant species provide keen interest among students to conduct cytogenetical studies aiding to crop improvement.

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