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Cytotoxic Effects of Chemical Mutagens in Root Tip Cells of *Allium cepa* L.

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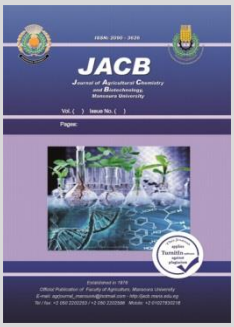

ABSTRACT

One of the excellent plant models for identifying various chromosomal abnormalities is Allium cepa L. In order to create genetic diversity, chemical mutagens such as; Ethyl methanesulfonate (EMS), Ethidium bromide (EtBr) and Sodium azide (SA) were used to mutagenize the plant's germplasm. The present study was carried out to determine how these chemicals affected the Mitotic Index (MI) and phase, chromosomal abnormalities, and frequency of micronuclei in the root tip cells of A. cepa Mutagens, in general, alter the genetic structure of the organisms, which is reflected in changes to a variety of features. Further, the genome's sensitivity was discovered through cytological examination when the three mutagens were used. The chromosomal entity was impacted by a variety of clastogenic and nonclastogenic alterations, as well as mitodepressive effects. The increase in dose/concentration of all the mutagens was shown to increase the frequency of both mitotic aberrant cells and mitotic chromosomal abnormalities in the treated root tip cells. Both chemical mutagens were shown to be less effective than EtBr at disrupting mitotic cell activity and primarily causing clastogenic chromosomal abnormalities. Numerous chromosomal aberrations, including stickiness and clumping of chromosomes with varying frequencies, fragments, laggards, bridges, precocious movements, micronuclei formation at telophases, and multipolar anaphases and telophases, were reported to be present in the mitotic aberrant cells. Anaphases, telophases, disorganised metaphases, desynchronized metaphases, and persisting nucleoli during anaphase were all noted.

Keywords: Allium cepa L, Mutagens, EMS, EtBr, Sodium azide, cytotoxic, sensitivity, chromosomal abnormalities.

INTRODUCTION

One of the most significant vegetable crops in the world is A. cepa L (x=8) is the most common basic chromosomal number. A. cepa L was chosen for this investigation due to its low chromosome count, relatively big size, and responsiveness to cytological treatments. The use of induced mutations in crop genetic improvement is a complementing strategy (Doroftei and Bercu (2013). Numerous studies on the impacts of mutagenic chemicals on living systems have been conducted because of their detrimental influence on genes that may be passed down via succeeding generations (Hilada et al., 2017). Further, chemicals' capacity to impair the function of live cells is known as cytotoxicity.

Mutations originate from the harm that cytotoxic substances cause to the DNA's constituent parts. The A. cepa assay has been extensively used to research the genotoxicity of several mutagens, finding that these substances can induce chromosomal abnormalities in root meristems of A. cepa. It is an effective test for chemical screening and in-situ monitoring for genotoxicity of environmental pollutants (Jyotirmoy Sharma et al., 2018). In addition, the most effective method to change an organism's genetic makeup and produce the desired traits is mutation breeding. In order to understand the nature and function of the genes that serve as the building blocks and the foundation for plant growth and development, mutations are utilized as tools and as a source of data (Adamu and Aliyu 2007). If the mutation isn't auto-corrected and transferred down the germ line, it really causes a gene to lose or gain function, which can be passed down to the next generation. In the first and succeeding generations, these induced mutations changed the genetic architecture of plants, which is reflected at the physiological, morphological, and biochemical levels (Aney and Choudhary 2019). In order to introduce numerous desirable characteristics of agronomic value in a variety of plants, mostly crop plants, mutation breeding has proven to be a useful method (Ali M et al., 2023). Moreover, the most accurate method for assessing the impact and potency of different mutagens is cytological study with regard to the behavior of the chromosomes during mitosis and meiosis. It also offers a hint for determining the sensitivity of different plant genotypes to specific mutagens such as mutagenic chemicals. It is also widely known that many chemical mutagens, such as SA and EMS, have the ability to alter the structural integrity of chromosomes in many plants.

Chemical mutagens typically cause point or gene mutations that result in base pair substitutions and alter protein activities without completely eliminating them. Variations at the morphological and physiological levels may come from the mutagenesis effects (Ali M et al., 2023) that happened at the chromosomal level and changed the genetic architecture of the plant (Aney and Choudhary 2019).

For morphological, physiological, and other yield-attributing features, it aid in the identification and isolation of the desired mutants. Therefore, one of the most reliable criteria for determining the impact of mutagens is the cytological study with reference to chromosomal abnormalities, either in mitosis or meiosis. In contrast, plant cells metabolize the promutagen SA to create a mutagenic agent that is likely Azido Alanine. Thus, an organic molecule

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that enters the nucleus, mingles with DNA, and causes point mutations in the genome facilitates SA mutagenicity. The hydrogen ion content of the treatment solution is the key to the high carcinogenic efficacy of SA, which is an inhibitor of the catalase and peroxidase enzymes, in barley (Nilan et al. 1972). Additionally, it has been shown to result in mutations in Pisum (Sander and Muehlbauer 1977). One of the nucleic acid intercalators, EtBr, binds preferentially to double-stranded DNA (dsDNA). Since intercalators prevent the DNA they bind from being replicated and transcribed, they have been employed as anticancer medications (Hurley, 2002). Because they glow when they attach to DNA, they have also been employed as DNA staining dyes (Stuart and Cole, 2000). For the identification of a particular sequence or the site-specific inhibition of transcription or replication, sequence-specific intercalators have been created (Choudhury and Bierbach, 2005).

Importantly, the Intercalation is the word for the binding mode where EtBr attaches to dsDNA between neighboring base pairs. The angle between base pairs that flank an EtBr molecule reduces by 268 and their distance grows by 0.34 nm. Then, dsDNA is lengthened and unwound by EtBr intercalation. These structural details were acquired from the mobility of the EtBr/dsDNA complex in solution (Wang, 1974) or its crystal structure (Tsai et al., 1975).

During EtBr intercalation, Coury et al. 1996 examined the contour length change of a single dsDNA (Coury et al., 1996). According to Wang (1974) and Waring (1965), the bulk EtBr/dsDNA complex's absorbance, fluorescence, or buoyant densities were used to determine the isotherm of intercalation. EtBr intercalation then causes dsDNA to lengthen and unravel. The mobility in solution (Wang, 1974) or crystal structure (Tsai et al., 1975) of the EtBr/dsDNA complex were used to extract this structural information. In order to determine how a single dsDNA's contour length changed during EtBr intercalation, Coury et al.

According to Wang (1974) and Waring (1965), the bulk density of the EtBr/dsDNA complex was used to determine the isotherm of intercalation. Therefore, the present study was carried out to assessment the cytotoxic effects of three chemical mutagens SA, EtBr and EMS on A. cepa Karyotyping for Chromosomal Abnormalities.

MATERIALS AND METHODS

The Institutional Review Board of the Benha University, Faculty of Agriculture's Genetics and Genetic Engineering department gave its approval to the idea. The A. cepa bulbs used in this investigation were acquired from a neighbourhood shop in Benha, Egypt. It was utilized 100 medium-sized (4 to 5 cm) A. cepa bulbs.

A. cepa L's healthy root tips are treated with saturated aqueous solutions of SA at concentrations of 0.1, 0.2, and 0.4%, EtBr at concentrations of 0.25, 0.50, and 0.75 g/ml, and EMS at concentrations of 0.1, 0.2%, and 0.4% for six hours. The chemical mutagens were diluted with deionized water to create the concentrations. Each Allium bulb was cultivated in a glass bottle that was transparent and held roughly 50 cc of the chemical solution. Five Allium bulbs were used for each concentration, however the Allium bulb with the worst root development was eliminated at harvest time in accordance with procedure (Fiskesjo G., 1985; Odeigah et al., 1997). 1 to 1.5 cm long root tips were removed, fixed in newly made Carnoy's fluid (3:1 absolute alcohol to glacial acetic acid) for 24 hours, and then stored in 70% ethanol. The squashes of the root tips were cleaned carefully with distilled water before being hydrolyzed in 1N HCl for 15 minutes at 60 C to prepare them for mitotic investigations. The root tips with hydrolysis were washed twice in distilled water. The root tips were then stained for five minutes in 1% Acetocarmine and then pressed into a drop of the stain.

Using various grades of butanol acetic acid, the data on mitotic index and mitotic chromosomal aberrations were documented, photographed, and preserved (Darlington and La Cour 1976).

According to the formulas below, mitotic index (MI) and percentage abnormality were determined (Jabee et al. 2008).

(Total number of cells dividing/Total number of cells scored) X 100 is the formula for the mitotic index (MI).

% Abnormality = (Total number of aberrant cells in the root tips of treated seeds/ Total number of cells in division) X 100.

RESULTS AND DISCUSSION

Results

Effect of the chemical mutagens on mitotic division

The effectiveness of the mutagens utilized in the current study on the mitotic cell divisions in various plants has been demonstrated. With an increase in the dose or concentration of the chemical mutagen, fewer dividing cells are reported to occur (Tables 1-3). The mitotic chromosomal aberrations caused by the chemical mutagens in the root tip cells of the bulbs indicated considerable impacts, as shown by the values of MI in terms of number of dividing cells (Table 1) (Figures 1, 2, and 3). The mitotic process was not significantly affected by the lower doses, but was negatively impacted by the larger doses, where it was nearly cut in half as compared to the control (Table 1).

Table 1. Chromosomal aberrations and Micronucleus detection in A. cepa root tip cells exposed to three different concentrations of EMS.

Type of abnormality		Treatments			
		EMS			
		Control	0.1 %	0.2 %	0.4 %
Total number of cells	1174	304	318	285	267
Number of dividing cells	443	178	130	84	81
Percentage of dividing cells		58.55	40.88	29.47	30.33
Abnormalities %		3.37	25.38	60.71	96.29
Anaphase bridges		1 (16.67)	6 (18.18)	13 (25.49)	16 (20.51)
Clumping and stickness		1 (16.67)	4 (12.12)	7 (13.72)	9 (11.53)
Micronucleus		0	0	0	3 (3.84)
Binucleated cells		0	12(4.06)	14 (27.45)	19 (24.35)
Multipolarity		1 (16.67)	2 (6.06)	2 (3.92)	7 (8.97)
Fragment chromosome		1 (16.67)	4 (12.12)	6 (11.76)	9 (11.53)
Ring chromosome		0	0	1 (1.96)	2 (2.56)
Unequal poles		2 (33.33)	7 (21.21)	8 (15.68)	13 (16.67)
Total abnormality		6	33	51	78

Table 2. Chromosomal aberrations and Micronucleus detection in A. cepa root tip cells exposed to three different concentrations of EtBr.

Type of abnormality		Treatments			
		EtBr in µg/ml			
		Control	0.25 %	0.50 %	1.00 %
Total number of cells	1212	376	289	280	267
Number of dividing cells	548	183	158	106	101
Percentage of dividing cells		48.67	54,67	37.85	37.82
Abnormalities %		2.73	25.94	63.20	92.07
Anaphase bridges		1 (20 %)	3 (7.31%)	3 (4.47%)	8 (8.60)
Clumping and stickness		0	6(14.63%)	23(34.32)	32(34.41)
Micronucleus		0	0	0	0
Binucleated cells		3 (60 %)	19(46.34%)	22(32.83)	28(30.11)
Multipolarity		0	8 (19.51%)	11(16.41)	13(13.97)
Fragment chromosome		1 (20 %)	2 (4.88 %)	3 (4.47%)	3 (3.22)
Ring chromosome		0	0	0	0
Unequal poles		0	3 (7.31 %)	5 (7.46%)	9 (9.68)
Total abnormality		5	41	67	93

Table 3. Chromosomal aberrations and Micronucleus detection in A. cepa root tip cells exposed to different concentration of SA.

Type of abnormality		Treatments			
		SA			
		Control	0.1 %	0.2 %	0.4 %
Total number of cells	1114	323	289	262	240
Number of dividing cells	605	163	160	147	135
Percentage of dividing cells		50.46 %	55.36 %	56.10 %	56.25 %
Abnormalities %		3.68 %	41.87 %	51.70 %	54.81 %
Anaphase bridges		2 (33.33)	23(34.32)	21 (27.63)	13 (17.56)
Clumping and stickness		0	1 (1.49)	3 (3.94)	4 (5.40)
Micronucleus		0	0	0	3 (4.05)
Binucleated cells		2 (33.33)	32(47.76)	34 (44.73)	10 (13.51)
Multipolarity		0	4 (5.97)	6 (7.89)	13 (17.56)
Fragment chromosome		1 (16.67)	4 (5.97)	6 (7.89)	18 (24.32)
Ring chromosome		0	1 (1.49)	2 (2.63)	4 (5.40)
Unequal poles		1 (16.67)	2 (2.98)	4 (5.26)	9 (12.16)
Total abnormality		6	67	76	74

In all treatment modalities, the three chemical mutagens demonstrated a mitodepressive impact that was concentration dependent, but the mitotic process was significantly more adversely affected by greater concentrations of the chemical mutagens than by lower concentrations (Tables 1, 2, and 3). Chromosomal and mitotic aberrant cell frequency rose linearly with increasing doses or concentrations of all three mutagens (Tables 1-3) and (Figures 1-3).

All three of the mutagens utilized in this study were found to be cytotoxic and capable of causing a variety of chromosomal abnormalities, both clastogenic (structural) and non-clastogenic (physiological), at varied frequencies. In addition to Chromosome fragments, laggards, single and multiple bridges in metaphases, anaphases, and telophases were the clastogenic abnormalities noted.

Effect of mutagens inducing chromosomal aberrations

Chromosome stickiness and clumping, desynchronized metaphase, disoriented chromosomes at metaphase, anaphase, telophase, etc. were the most prevalent nonclastogenic abnormalities seen. By using specific mutagen doses or concentrations, it was also possible to cause multipolar anaphase, telophase, and the development of micronuclei.

Therefore, all three mutagens were observed to enhance the frequency of chromosomal abnormalities in a colinear manner (Tables 1-3, Figures 1, 2 and 3) compared to the root tip cells of the control bulbs which had no chromosomal abnormalities, but when bulbs were exposed to chemical mutagens, these effects were detrimental to the structural integrity of mitotic chromosomes, and it was discovered that the rate of exposure to chemical mutagens increased the frequency of chromosomal aberrations (Figure 2 and Table 2). With an increase in the concentration of chemical mutagens, a similar trend of an increase in the frequency of chromosomal abnormalities was also observed (Figures 3a and 3b) and (Tables 2 and 3). Comparatively, it was discovered that all SA treatment modalities were more successful at inducing chromosomal abnormalities than various EMS and EtBr treatment modalities (Figures 1a- 1b and figure 2).

Moreover, chromosome aberrations were more frequently caused by the chemical mutagens, which had negative effects (Tables 2, 3, Figures 1 and 2). Numerous clastogenic chromosomal changes, including the formation of bridges (single, double, and multiple), fragments, laggards, and micronuclei (Figures 1 and 2), as well as non-clastogenic chromosome stickiness and clumping at metaphase, anaphase, and telophase (Figure 2), were observed in response to increasing doses of all the mutagens.

The stickiness of chromosomes at metaphase and anaphase, where one or more chromosomes were adhered to the remainder of the chromosome complement, was the most noticeable non-clastogenic chromosomal aberration caused by all mutagens (Figure 2). The frequency of chromosomal stickiness was found to be colinearly increased with the dose/concentration of all the mutagens.

At metaphase, anaphase, and telophase, the genome's chromosomes can cluster together to create a clump (Figures 2 and 3). All of the doses/concentrations of the mutagens utilized, with the exception of the lower (0.0075%) concentration of SA, caused the complete chromosomal complement to cluster.

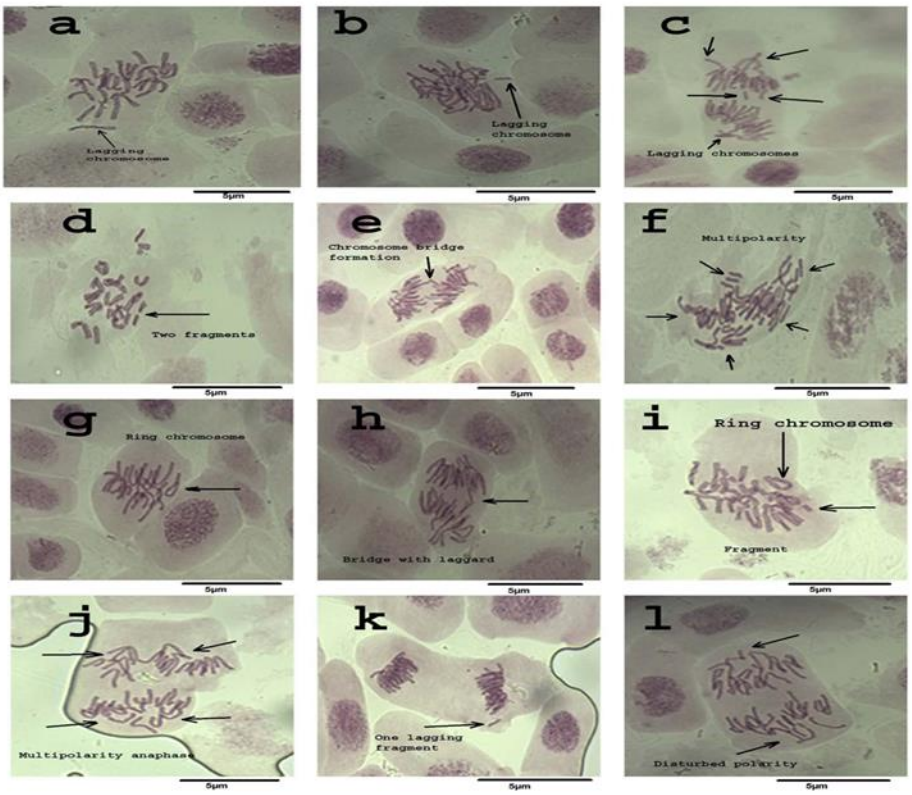

Figure - 1a. Various chromosomal abnormalities observed upon exposure the A. Cepa to SA a, b, c- Lagging chromosomes; d – Two fragments; e- Chromosome bridge formation; f - Multipolarity; g- Ring chromosome; h- Bridge with laggard; i- Ring chromosome and fragment ; j- Multipolarity anaphase; k – One lagging fragment; l- Disturbed polarity.

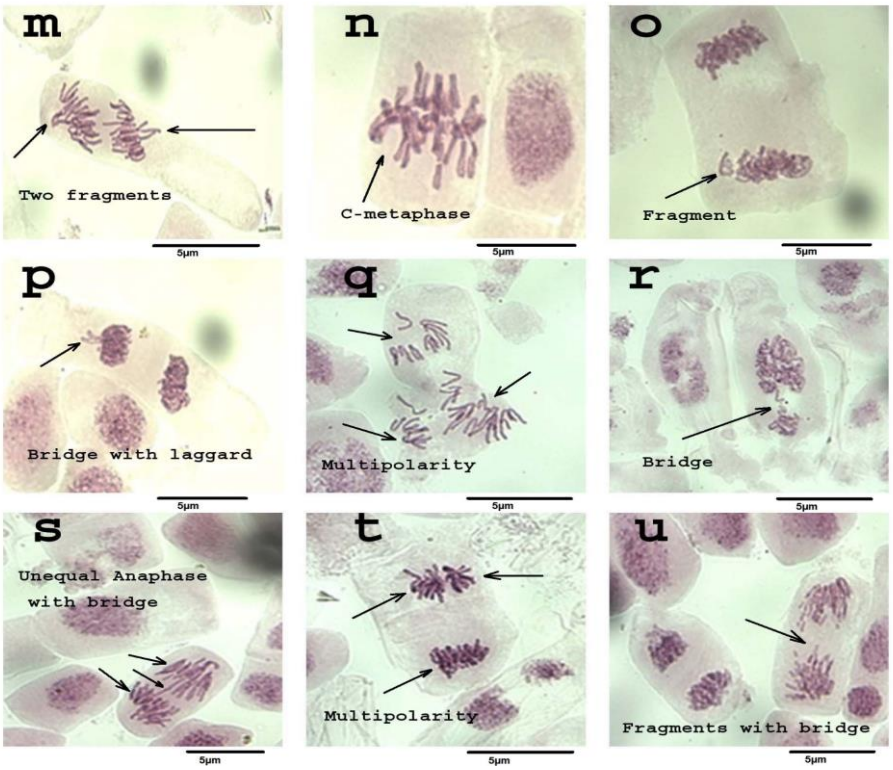

Figure – 1b. Various chromosomal abnormalities observed upon exposure the A. Cepa to SA m - Two fragments; n- C- metaphase; o- Fragment; p- Bridge with laggard; q- Multipolarity ; r- Bridge; s- Unequal anaphase with bridge; t- Multipolarity; u- fragments with bridge.

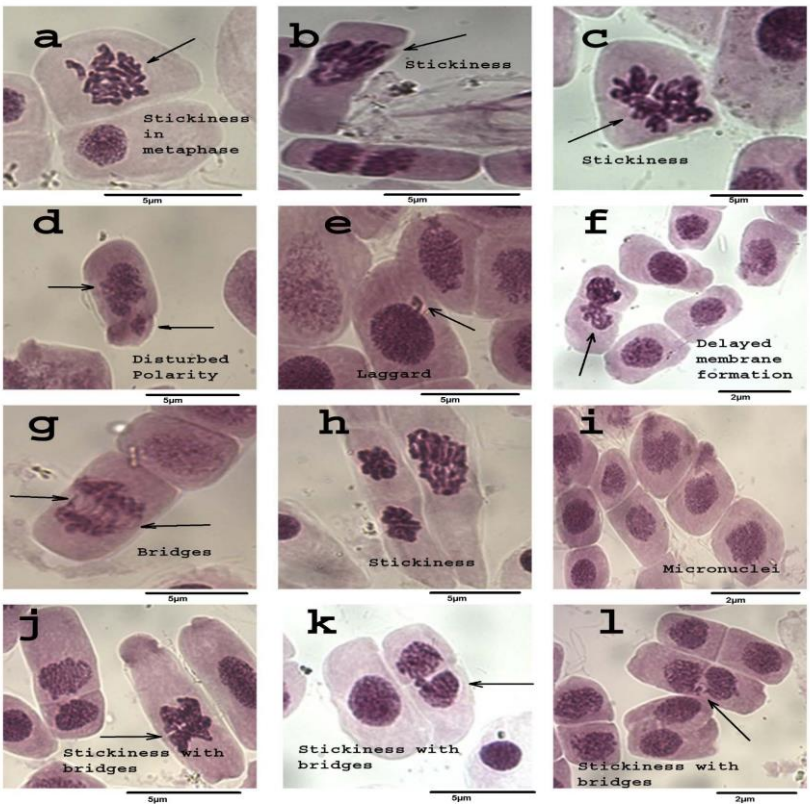

Figure - 2. Various chromosomal abnormalities observed upon exposure the A. Cepa to EtBr a- Stickiness in metaphase; b and h Stickiness; d- Disturbed polarity; e - Laggard; f- Delayed membrane formation; g- Bridge; h- Stickiness; i- Micronuclei; j- k-l- Stickiness with bridge.

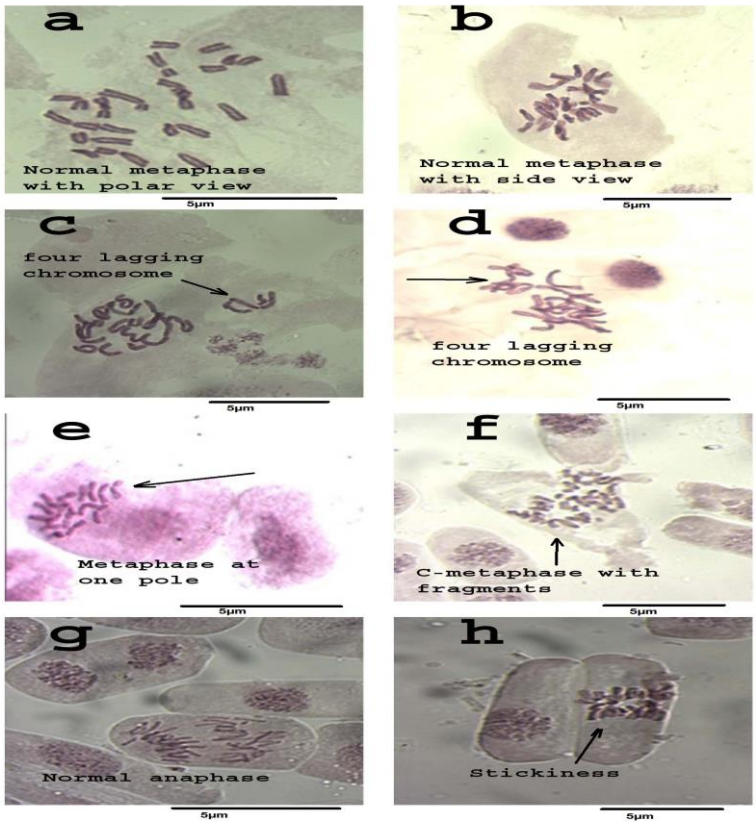

Figure – 3a. Various chromosomal abnormalities observed upon exposure the A. Cepa to EMS a- Normal metaphase with polar view; b - Normal metaphase with side view; c and d - Four lagging chromosome; e - Metaphase at one pole; f- C- metaphase with fragment; g- Normal anaphase; h- Stickiness.

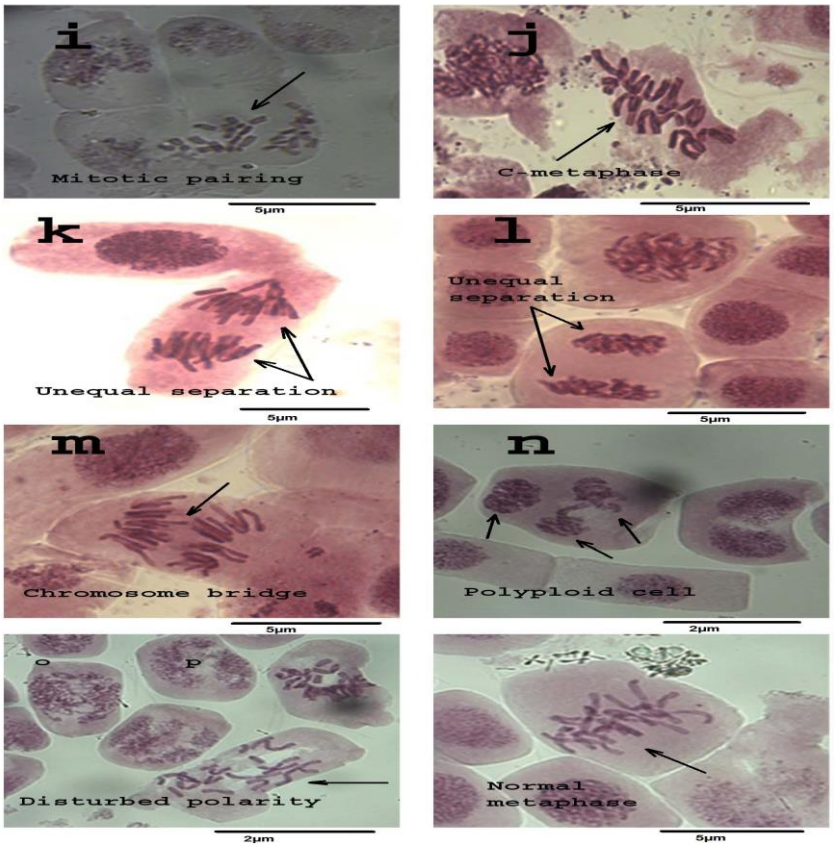

Figure – 3b. Various chromosomal abnormalities observed upon exposure the A. Cepa to EMS i- Mitotic pairing; j- C-metaphase; k and l - Unequal separation ; m - Chromosome bridge; n- polyploidy ; o- Disturbed polarity; p- Normal metaphase.

Chromosome clumping was also seen to increase in frequency with an increase in dose or concentration of all mutagens during metaphase, anaphase, and telophase (Tables 1 and 3). The mutagens utilized in this study also caused the multinuclei to develop, which caused tripolar and multipolar anaphase and telophase to arise. It was observed to be induced by all the doses/ concentrations of the chemical mutagens, in all treatment modes, save lower dosages of 0.50% concentration of EMS. Cells with multinuclei production at telophase were observed to be more with the treatment of SA than that of EMS (Tables 1–3). Other types of chromosomal abnormalities, such as disturbed metaphase caused by chromosome nonsynchronization (Figure 2), disoriented or curved metaphase (Figure 2), disoriented anaphase and telophase (Figures 2), etc., were also induced by a specific dose or concentration of the mutagens used in the current investigation. The nucleolar irregularity caused by the 0.01% SA concentration was observed as a persistent nucleolus during anaphase (Figure 3).

Discussion

The cytological study of the data on the development of abnormal mitotic cells and the prevalence of chromosomal aberrations shows that all mutagens have cytotoxic effects. As, the deleterious effects of mutagens on root tip cells in the plant under study were demonstrated by the dose/concentration dependent increase in mitotic aberrant cells and the chromosomal abnormalities. Further, all mutagens were discovered to have a significant negative impact on the mitotic process and the chromosomal entity. As a result, the increased frequency of mitotic aberrant cells and different chromosomal abnormalities is a sign that all of the mutagens are having a deleterious effect on the plant genome.

Because of the genetic harm was caused by using the mutagens, which causes disruptions in the mitotic and meiotic cells, is crucial because it can be passed from one generation to the next generation (Kumar and Rai 2007).

According to Sarada Mani and Seetharami Reddi (1986), the use of mutagens causes the cell to produce "endogenous poisons" as a result of the breakdown of micro- and macromolecules, particularly enzymes and nucleoproteins in the cytoplasm. Additionally, these mitotic toxins have the potential to produce "metabolic imbalance," which can affect the synthesis, status, and structure of nucleic acids as well as have physiological impacts and alter the structural makeup of chromosomes during cell division. All three chemical mutagens used in the current study had negative effects on mitotic chromosomes, which enhanced the abnormalities of mitotic cells at anaphase, metaphase, and telophase.

Additionally, different clastogenic and non-clastogenic types of chromosomal abnormalities were mainly observed as the detrimental consequence of varied doses/concentrations of all the used mutagens which were reported as chromosomal fragments, laggards, single and multiple bridges, precocious movement, stickiness and clumping of chromosomes as well as persistent nucleolus (Kumar and Dubey (1997).

According to Ricardo and Ando (1998) and Yuan and Zhang (1993), the reactive oxygen-derived radicals produced by chemical mutagens have clastogenic effects on chromosomes. The majority of induced mutations caused by SA result in base pair substitution, particularly GCAT, which changes the amino acids, altering the activities of the proteins. According to the results, which were shown as linear

correlations between the dose rate and the frequency of mitotic aberrant cells, concur with earlier discoveries made by a number of researchers. The occurrence of numerous mitotic chromosomal abnormalities in diverse plants has been observed by Datta and Banerjee (1998), Kumar and Dubey (1997), More and Kothekar (1992), and Verma et al. (2012). Moreover, translocations, lagging chromosomes, bridges, stickiness, and clumping of chromosomes are the most common abnormalities brought on by SA. Additionally, EMS has been shown to cause a variety of chromosomal abnormalities in many plants. According to Akhtar et al. (2012), EMS is more successful than SA at causing chromosomal abnormalities in *Linum usitatissimum*. In the current study, it was discovered that when the mutagens' dose or concentration grew, a greater variety of mitotic chromosomal abnormalities were caused. In general, it has been found that the more mutagen that is present, the more biological harm that result. According to Kumar and Tripathi (2003), an indicator of the potency and effectiveness of a mutagen is the frequency of abnormal cells. Chromosome breaks were brought about by the mutagens used in the current investigation, and these broken chromosomes persisted as acentric fragments seen in aberrant mitotic anaphase, metaphase, and telophase. Further, lagging chromosomes and the production of micronuclei were also more clearly seen in aberrant telophase and anaphase, respectively. The occurrence of lagging chromosomes may be due to the failure to carry the chromosome to the appropriate pole (Tarar and Dnyansagar 1980) and irregular distribution of some of the acentric fragments produced by mutagens those results in the formation of micronuclei.

Moreover, the appearance of fragments may be caused by the failure to reunite with the chromosomes (Kaur and Grover 1985). According to Bhat et al. (2007), chromosomal breakage brought by chemical mutagens on binding to GC-rich regions and making the DNA unstable is what leads to the production of fragments and laggards. Because of the more lagging chromosomes are a result of spindle formation deformity, which also affects chromosomal segregation and the inability of chromosomes to travel towards their respective poles during mitosis. As more lagging chromosomes are seen as a result of improper in foldings of the chromosomes into single chromatid and chromosome, chromatin fibers intertwine and chromosomes become attached to one another by way of sub chromatid bridges, Klasterkii et al. (1976) explained the increased incidence of lagging chromosomes due to the treatment of mutagen. A mutagenesis effect that may lead to the loss of genetic material is the cause of the micronuclei that formed in the current investigation (Aurebach 1976). The lagging chromosomes also contribute to the formation of the micronuclei, in addition to the acentric pieces. The heterochromatic micronuclei, created by acentric chromosomal fragments, and the euchromatic micronuclei, formed by one or more lagging chromosomes, were the two forms of micronuclei that Soren et al. (1981) observed. There have been several theories as to why micronuclei originate and appear at telophases. According to Degrassi and Rizzoni (1982), it may be the result of clastogenic activities in the affected cell, or it may be the outcome of a single chromosome or group of chromosomes establishing a distinct nucleus (Bhat et al. 2007). Levan (1951) claimed that if the amount of chromatin material involved is big enough, the resulting acentric chromosomes, which are seen as lagging chromosomes or fragments during anaphase, may take the form of micronuclei at telophase. Chromosome sticking and clumping were clearly seen in the treated root tip cells in the current investigation at different phases of mitosis. Stickiness owing to a mutation brought on by the sticky (st) recessive gene is referred to as the hereditary stickiness, but stickiness brought on by a mutagen is referred to as induced stickiness. According to McGill et al. (1974), the stickiness may result from chromosomes being improperly clustered at any stage of the cell cycle. Achkar et al. (1989) proposed that breaks in the double strands of DNA and the beginning of the development of intra-chromatid connections during chromosome condensation, which is regulated by histone protein, are the causes of sticky chromosome formation. However, Patil and Bhat (1992) viewed it as a sort of physical adhesion involving mostly the proteinaceous matrix of the chromatin, whereas Klasterkii et al. (1976) attributed it to the tangling of inter-chromosomal chromatin fibers that results in sub chromatid linkage between chromosomes. The genomic complement of one or more chromosomes may become sticky as a result of nucleic acid depolymerization, which prevents DNA synthesis. The partial separation of nucleoproteins and change in their organizational layout are what cause chromosomes to be sticky. While Gaulden (1987) proposed that chemically induced stickiness was caused by the action of mutagens on the failure or improper functioning of one or two types of non-histone proteins leading to improper folding of DNA, Jayabalan and Rao (1987) proposed that stickiness might be caused by disturbances in balanced reactions. The depolymerization of DNA, which ultimately leads in increased fluidity on the surface of chromosomes and adhesion of two or more chromosomes to one another, is what causes chromosomes to be sticky.

Importantly, the clumping of chromosomes observed in the current study may be caused by the complement's entire chromosomes' stickiness. Clumps can occur when metaphase chromosomes swell and thicken, eventually sticking together and forming a clump. Chromosome stickiness and clumping may occur more frequently than expected in the current study as a result of cytochemical disturbances that delay chromosome separation (Sinha and Godward 1972a, b). Chromosomes can reunite and cause separation at anaphase (Badr 1988; Grant 1978). The formation of bridges during anaphase may result from the daughter chromatids' nondisjunction. The fusion of two centromere-bearing chromosomal fragments, which produces dicentric chromosomes, may be the most likely cause of bridge formation in the current investigation.

Additionally, bridge construction ensued from the chromosomes' effective splitting into two chromatids that attempted to travel to the opposite poles. The same results were noted by Kumar and Dubey (1997). Instead of the chromatids, it is assumed that the broken ends of the chromosomes fuse to generate the paired bridges. It is possible that the presence of abnormal metaphase and anaphase, along with the premature movement of one or two chromosomes towards either pole, is caused by spindle dysfunction (Khan et al. 2005), contraction of some spindle fibers during early anaphase, or some combination of these factors (Kumar and Dubey 1997). Prasad (1972) claimed that changes to the gene in charge of the spindle mechanism can disrupt the biochemical chain, which in turn can cause problems with the spindle system and its coordinated process (More and Kothekar 1992). The chromosomal region known as the kinetochore is crucial for

the attachment of the centromeric spindle microtubule. Molecular based studies by various workers (Binarova et al. 2006, Pfarr et al. 1990, Steuer et al. 1990, Walczak et al. 1996, Wordeman and Mitchison 1995, Yao et al. 1997, Yen et al. 1992) have confirmed the role of certain proteins involved in the attachment of spindle fibers with the kinetochore and concluded that the mutations in the genes encoding these proteins led to the disturbed spindle mechanism. The chromosome congression during mitosis is also dependent on the proteins identified as the centromere associated kinesin related microtubule protein E (CENP-E) (Yao et al. 1997). Masoud et al. (2013) contend that mitotic polarity is defined by the microtubule (MT) arrays and that spindle polarity is crucial for plant morphogenesis. The activity of c-tubulin containing complexes is required for the rearrangement of MTs, and the c-tubulin gene was discovered to be lethal (Pastuglia et al. 2003), demonstrating that this protein is absolutely required for microtubule assembly in vivo. When Janski et al. (2012) noticed that the pro-spindle and MTs branching out from the spindle pole to the cortex had altered spindle polarity, they reached the conclusion that down-regulated GIPs were crucial in sustaining these connections. However, nine genes from the MAP65 family of Arabidopsis and from MAP65-1c of Nicotiana tabacum had decreased activity, according to Hussey et al. (2002) and Meng et al. (2010), which explained the deformities in microtubule production. Cycle independent kinases and mitogen-activated protein kinases regulate the activity of these genes by phosphorylating them, which reduces their capacity to bundle MTs. Arabidopsis' defective spindle formation was discovered by Kawamura et al. (2006) to be caused by the temperature-sensitive mutant allele. While Yasuhara and Oe (2011) observed that the RNAi depletion of its tobacco counterpart tobacco MT binding protein 200, known as TMBP200, caused severe abnormalities in bipolar spindle formation that led to the emergence of multinucleated cells with varying sized nuclei. The altered polarity was seen in the current study may be caused by the mutagens' production of mutation, which may have damaged the genes and either caused the depolymerization of microfilaments or disrupted the process regulating spindle formation. According to recent research (Ho et al. 2011, Hotta et al., 2012), spindle microtubule orientation and amplification are regulated by the formin and augmin protein complexes. In *Nicotiana benthamiana*, Lee et al. (2009) located the Rae1 protein dispersed on spindle microtubules and verified its MT-binding capabilities. They also noted that the RNAi-mediated suppression of NbRae1 resulted in a disorganized, unfocused, and disorganized spindle. Furthermore, four Ran genes in the Arabidopsis genome (Vernoud et al. 2003) control the spindle mechanism, and Paya et al. (2002) verified that AtRanGAPs target the spindle, while Chen et al. (2011) detected the abnormal organization of the spindle during mitosis in OsRNA2 knockdown rice lines. The targets of SA are always the metabolic processes. In addition to inhibiting cell division, it also has an impact on the polarity of spindle fibers. Further, in *E. coli*, the SecA gene was displayed resistance to the mutations brought on by SA was found by Fortin et al. in 1990.

It is well known that the chemical mutagens, particularly SA, impair ATP production. ATP is required for the organization and mobility of spindle fibers during cell division. The organization of the spindle fibers of SA-treated cells may have been impacted on how the chromosomes were arranged at the metaphase plate and how they moved during anaphase. Either by the mutagens that may have mutated the genes, causing the depolymerization of microfilaments, or by the mutagens that may have regulated the mechanism of spindle formation.

As a result, all chemical mutagens showed lethal effects when it were used at higher quantities. The results on mitotic activities showed that the mutagenic efficacy increased with the increase in dose/concentration of mutagens, and the clastogenic effect of mutagens on the genome of the study plant was clearly observed in the form of induction of numerous chromosomal abnormalities.

Importantly, it is possible to take use of the genetic variety caused by these mutagens at the genomic level, which caused variations in plant shape, chlorophyll content, sterility, and yield, to improve the agronomic characteristics of the plant. Increased cell membrane permeability (Walles 1967) and physiological seed activation (Roychowdhary and Tah 2013) may be caused by the rise in the frequency of aberrant cells and chromosomal abnormality in both presoaking treatment modes of chemical mutagens.

REFERENCES

Achkar, A. I. W., Sabatier, L. and Dutrillaux, B. (1989). How are sticky chromosomes formed? Ann Genet (32):10–15.

Adamu, A. K., and Aliyu, H. (2007). Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill). Sci World J (2): 9–12.

Akhtar, A., Ansari, M.Y.K., Alka, and Hisamuddin, (2012). Cytological effect of ethyl methanesulphonate and sodium azide in *Linum usitatissimum* L. Int J Plant Animal Environl Sci (2): 70–75.

Ali, M., Abdelkawy, A. M., Darwish, D. B. E., Alatawi, H. A., Alshehri, D., Al-Amrah, H., Soudy, F. A. (2023). Changes in Metabolite Profiling and Expression Levels of Key Genes Involved in the Terpenoid Biosynthesis Pathway in Garden Sage (Salvia officinalis) under the Effect of Hydrazine Hydrate. Metabolites 13 (7): 807.

Aney, A., and Choudhary, A. (2019). Effect of physical and chemical mutagens on mitotic cell division rate (MI) in *Rivinia humilis* L. Int J Life Sci (7): 1–9.

Auerbach, C. (1976). Mutation Research-Problems Results and Perspectives Chapman and Hall London.

Badr, A. (1986). Effect of the S-Trizine herbicide trybytryn on mitosis chromosomes and nucleic acids in root tips of *Vicia faba* L. Cytologia (51): 571–589.

Bhat, T. A., Sharma, M., and Anis, M. (2007). Comparative analysis of mitotic aberrations induced by diethyl sulphate (DES) and sodium azide (SA) in *Vicia faba* L. (Fabaceae). Pak J Biol Sci (10): 783–787.

Binarova, P., Cenklova, V., Prochazkova, J., Doskocilova, A., Volc, J., Vrlik, M., and Bogre, L. (2006). C–tubulin is essential for acentrosomal microtubule nucleation and coordination of late mitotic events in Arabidopsis Plant. Cell Physiol (18): 1199– 1212.

Chen, N., Xu, Y., Wang, X., Du, C., Du, J., Yuan, M., Xu, Z., and Chong, K. (2011). OsRAN2 essential for mitosis enhances cold tolerance in rice by promoting export of intranuclear tubulin and maintaining cell division under cold stress. Plant Cell Environ (34): 52–64.

Choudhury, J. R., and Bierbach, U. (2005). Characterization of the bisintercalative DNA binding mode of a bifunctional platinumacridine agent. Nucleic Acids Res. (33): 5622–5632.

Coury, J. E., McFail- Isom, L., Williams, L. D., and Bottomley, L. A., (1996). A novel assay for drug-DNA binding

mode, affinity, and exclusion number: scanning force microscopy. Proc. Nat Acad. Sci. (93): 12283–12286.

Darlington, C. D., and La Cour, L. F. (1976). The Handling of Chromosomes", 6th ed. George Allen and Unwin Ltd., London.

Datta, S. K., and Banerjee, B. K. (1998). Gamma ray induced somatic mutation in Chrysanthemum. J Nucl Agri Bio (22): 19–27.

Degrassi, F., and Rizzoni, M. (1982). Micronucleus tests in Vicia faba root tips to detect mutagen damage in fresh water pollution. Mut Res (97): 19–33.

Doroftei, E., and Bercu, R. (2013). The cytotoxic effects and mutagen potential induced by sulphites at *Allium cepa* L. Annals of RSCB, XVIII (I).

Fiskesjo¨, G. (1985). The Allium test as a standard in environmental monitoring. Hereditas (102): 99–112.

Fortin, Y., Phoenix, P., and Drapeau, G. R. (1990). Mutations conferring resistance to azide in *Escherichia coli* occur primarily in the secA gene. J Bacteriol (172): 6607–6610.

Gaulden, M. E. (1987). Hypothesis: Some mutagens directly alter specific proteins thus produce chromosome stickiness. Mutagenesis (2): 357–365.

Hilada, N., Jasmin, M., Azra, M., and Kemajl, K. (2013). Chromosomal and Nuclear Alterations in Root Tip Cells of *Allium Cepa L.* Induced by Alprazola. Med Arch. 67(6): 388–392.

Ho, C. M. K., Hotta, T., Kong, Z., Zeng, C., Sun, J., Lee, Y. J., and Liu, B. (2011). Augmin plays a critical role in organizing the spindle and phragmoplast microtubule arrays in Arabidopsis. Plant Cell (23): 2606–2618.

Hotta, T., Kong, Z., Ho, C. M. K., Zeng, C., Horio, T., Fong, S., Vuong, T., Lee, Y. J., and Liu, B. (2012). Characterization of the Arabidopsis augmin complex uncovers its critical function in the assembly of the acentrosomal spindle and phragmoplast microtubule arrays Plant Cell (24): 1494–1509.

Hurley, L. H. (2002). DNA and its associated processes as targets for cancer therapy. Nat. Rev. Cancer (2): 188–200.

Hussey, P. J., Hawkins, T. J., Igarashi, H., Kaloriti, D., and Smertenko, A. (2002). The plant cytoskeleton: recent advances in the study of the plant microtubule associated proteins MAP–65 MAP–190 and the Xenopus MAP215-like protein MOR1. Plant Mol Biol (50): 915–924.

Grant, W. F. (1978). Chromosome aberrations in plants as a monitoring system. Env Health Perspect (27): 37–43.

Jabee, F., Ansari, K. Y. M., and Shahab, D. (2008). Studies on the Effect of Maleic. Hydrazide on Root Tip Cells and Pollen Fertility in *Trigonella foenum-graecum* L. Turk. J. Bot. (32): 337-344.

Janski, N., Masoud, K., Batzenschlager, M., Herzog, E., Evrard, J. L., Houlne, G., Bourge, M., Chaboute, M. E., and Schmit, A. C. (2012). The GCP3-interacting proteins GIP1 and GIP2 are required for c-tubulin complex protein localization spindle integrity and chromosomal stability. Plant Cell (24): 1171–1187.

Jayabalan, N., and Rao, G. R. (1987). Gamma radiation induced cytological abnormalities in *Lycopersicon esculentum*. Mull var Pusa ruby Cytologia (52): 1–4.

Jyotirmoy Sharma, J. D., Rajkumari, W., Ingtipi, R. B., and Shivani, D. (2018). Effect of Chemical Mutagens on Chromosomal behavior of A. cepa. Annals of Plant Sciences (4): 2202- 2204.

Kaur, P., and Grover, I. S. (1985). Cytological effects of some organophosphorous pesticides I Mitotic effects. Cytologia (50): 187–190.

Kawamura, E., Himmelspach, R., Rashbrooke, M., Whittinton, A., Gale, K., Collings, D., and Wasteneys, G. (2006). Microtubule organization 1 regulates structure and function of microtubule arrays during mitosis and cytokinesis in the Arabidopsis root. Plant Physiol (140) 102–114.

Khan, A. H., Bhat, T. A., Parveen, S. and Wani, A. (2005). Cytogenetic effects of methyl methane sulphonate (MMS) on two varieties of chickpea (Cicer arietinum L). J Cytol Genet (6): 97-102.

Klasterkii, I., Natrajan, A. T., and Ramel, C. (1976). An interpretation of the origin of subchramatid aberrations and chromosome stickiness on a category of chromatid aberration. Hereditas (83): 153–162.

Kumar, S., and Dubey, D. K. (1997) .The mitotic consequence of gamma rays EMS and dES in *Lathyrus sativus*. J Cytol Genet (32): 75–80.

Kumar, G., and Rai, P. (2007). Comparative genotoxic potential of mercury and cadmium in soybean. Turkish J Biol (31): 13–18.

Kumar, G., and Tripathi, A. (2003). Comparative mitotoxicity and mutagenicity of two heavy metals in *Allium cepa* L. J Cytol Genet 4 (NS), 169–173.

Lee, J. Y., Lee, H. S., Wi, S. J., Park, Y. K., Schmit, A.C., and Pai, H. S. (2009), Dual functions of Nicotiana benthamiana Rae1 in interphase and mitosis. Plant (59): 278–291.Hyun-Sook

Levan, A. (1951). Localised chromosome breakage in *Vicia faba*. Hereditas (30): 382–388.

Meng, Q., Du, J., Li, J., Lu, X., Zeng, X., Yuan, M., and Mao, T. (2010). Tobacco microtubule-associated protein MAP65-1c bundles and stabilizes microtubules. Plant Mol Biol (74): 537–347.

Masoud, K., Herzog, E., Chaboute, M. E., and Schmit, A. C. (2013). Microtubule nucleation and establishment of the mitotic spindle in vascular plant cells. Plant (75): 245– 257.

Mcgill, M., Pathak, S., and Hsu, T. (1974). Effects of ethidium bromide on mitosis and chromosomes: A possible material basis for chromosome stickiness. Chromosoma (47): 157–167.

More, A.D., and Kothekar, V. S. (1992). Induced chromosomal aberrations in alfalfa In Chennaveeraiah M S (ed) Glimpses of Cytogenetics in India 3 Society of Cytologists and Geneticists pp101–104.

Nilan, R. A. (1972). Mutagenic specificity in flowering plants. Facts and prospects. In Induced Mutations and Plant Improvement (Proc. meeting Buenos Aires, FAOIIAEA, Nov. 1970). IAEA* Vienna. P. 141-'51.

Odeigah, P. G. C., Nurudeen, O., and Amund, O. O. (1997). Genotoxicity of oil field wastewater in Nigeria. Hereditas (126): 161–7.

Pakrashi, S., Jain, N., Dalai, S., Jayakumar, J., Chandrasekaran, P.T., Raichur, A. M., Chandrasekaran, N., Mukherjee, A. (2014). In vivo genotoxicity assessment of titanium dioxide nanoparticles by Allium cepa root tip assay at high exposure concentrations. PLoS One 9 (2):e87789.

Pastuglia, A. M., Azimzadeh, J., Camilleri, C., and Bouchez, D. (2003). Forward and reverse genetics in Arabidopsis: isolation of cytoskeletal mutants. Cell Biol Int (27): 249–250.

Patil, B. C., and Bhat, G. I. (1992). A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in *Clitoria ternatea* L. Cytologia (57): 259–264.

Paya Resch K., Frohmeyer, H., Fejes, E., Nagy, F., and Nick, P. (2002). Plant RanGAPs are localized at the nuclear envelope in interphase and associated with microtubules in mitotic cells. Plant J (30): 699–709.

Pfarr, C. M., Coue, M., Grissom, P. M., Hays, T. S., Porter, M. E., and Mcintosh, J. R. (1990). Cytoplasmic dynein is localized to kinetochores during mitosis. Nature (Lond) (345): 263–265.

Prasad, M. U. R. (1972). A comparison of the mutagenic effectiveness and efficiency of gamma rays EMS and NG. Indian J Genet (32): 360–367.

Ricardo, M., and Ando, A. (1998). Effects of gamma radiation and sodium azide on quantitative characters in rice (Oryza sativa L). Genet Mol Biol (21): 244–251.

Roychowdhury, R. and Tah, J. (2013). Mutagenesis-A Potential Approach for Crop Improvement in Hakeem K R (ed). Crop Improvement pp 149–187.

Sander, C., and Muehlbauer, F. J. (1977). Mutagenic effects of sodium azide and gamma irradiation in Pisum Environ. Exp. Bot. (17): 43-47.

Sarada Mani, N., and Seetharami Reddi, T. V. V. (1986). Radiosensitivity of parents and hybrids of grain Sorghum. Res J Pl Environ (2): 21–25.

Sharma, J., Rajkumari, J.D., Ingtipi, W., Boro, R., and Das, S. (2018). Effect of Chemical Mutagens on Chromosomal behavior of *Allium cepa* L. Annals of Plant Sciences 7 (4): 2202-2204.

Sinha, S. S. N., and Godward, M. B. F. (1972a). Radiation studies in Lens culinaris Meiosis: abnormalities induced due to gamma radiation and its consequences. Cytologia (37): 685–695.

Sinha, S. S. N., and Godward, M. B. E. (1972b). Radiation studies in Lens culinaris. Ind J Genet (32): 331–339.

Soren, V., Sparchez, C., Cracium, C., and Uray, Z. (1981). Observation on micronuclei ultrastructure within broad bean (*Vicia faba*) meristem after gamma-ray radiation. Caryologia (46): 381–386.

Steuer, E. R., Wordeman, L., Schroer, T. A., and Sheetz, M. P. (1990). Localization of cytoplasmic dynein to mitotic spindles and kinetochores. Nature (Lond) (345): 266–268.

Stuart, K. R., and Cole, E.S. (2000). Nuclear and cytoskeletal fluorescence microscopy techniques. Methods Cell Biol. (62): 291–311.

Tarar, J. L., and Dnyansagar, V. R. (1980). Comparison of ethyl methanesulphonate and radiation induced meiotic abnormalities in *Turnera ulmifolia* Linn var angustifolia Wild. Cytologia (45): 221–231.

Tsai, C.C., Jain, S.C., and Sobell, H.M. (1975). X-ray crystallographic visualization of drug-nucleic acid intercalative binding: structure of an ethidium-dinucleoside monophosphate crystalline complex, ethidium: 5-iodouridylyl (30 -50) adenosine. Proc. Natl Acad. Sci. USA, (72): 628–632.

Verma, A. K., Singh, R. R., and Singh, S. (2012). Cytogenetic effect of EMS on root meristem cells of Catharanthus roseus L G Don var Nirmal. Int J Pharm Bio Sci (2): 20–24.

Vernoud, V., Horton, A., Yang, Z., and Nielsen, E. (2003). Analysis of the small GTPase gene superfamily of Arabidopsis. Plant Physiol (131): 1191–1208.

Walczak, C. E., Mitchison, T. J., and Desai, A. (1996). XKCM1 a Xenopus kinesin related protein that regulates microtubule dynamics during mitotic spindle assembly. Cell (84): 37–47.

Walles, S. (1967). Uptake of EMS into embryo of barley. Hereditas (58): 95–102.

Wang, J.C. (1974). The degree of unwinding of the DNA helix by ethidium. I. Titration of twisted PM2 DNA molecules in alkaline cesium chloride density gradients. J. Mol. Biol. (89): 783–801.

Waring, M. J. (1965) Complex formation between ethidium bromide and nucleic acids. J. Mol. Biol. (13): 269–282.

Wordeman, L., and Mitchison, T. J. (1995). Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. J Cell Biol (128): 95–104.

Yao, X., Anderson, K. L., and Cleveland, D. W. (1997). The microtubule dependent motor centromere–associated protein E (CENP-E) is an integral component of kinetochore corona fibers that link centromeres to spindle microtubules. J Cell Biol (139): 435–447.

Yasuhara, H., and Oe, Y. (2011). TMBP200 a XMAP215 homologue of tobacco BY–2 cells has an essential role in plant mitosis. Protoplasma (248): 493–502.

Yen, T. J., Li, G., Schaar, B. T., Szilak, I., and Cleveland, D. W. (1992). CENP-E is a putative kinetochore motor that accumulates just before mitosis. Nature (359): 536–539.

Yuan, H. Y., and Zhang, Z. (1993). Effects of free radicals and temperature on sister chromatid exchanges in Hordeum vulgare L. Acta Botanica Sinica (35): 20–26.

تأثيرات السمية الخلوية لثلاثة مطفرات كيميائية على خلايا القمم النامية لجذور البصل

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الملخص

يعتبر البصل (*Allium cepa L*) واحدا من أفضل النباتات لتحديد الشواذ الكروموسومية المختلفة. من أجل خلق التنوع الوراثي ، واستخدمت المطفرات الكيميائية (إيثيل ميثان سلفونات ، و الإيثيديوم بروميد ، والصوديوم الأزيد) لاستحداث الطفرات وكان الهدف هو تحديد كيف تؤثر هذه المواد الكيميائية على معدل ومرحلة الانقسام بالإضافة الى التشوهات الكروموسومية ، وتواتر النوى الدقيقة في خلايا القمم النامية لجذور البصل. بشكل عام ، بشكل عام ، يغير التركيب الوراثي للكائنات الحية ، والتي تنعكس في التغييرات إلى مجموعة متنوعة من الميزات. تم اكتشاف حساسية الجينوم لتأثيرات جميع الطفرات الثلاثة المستخدمة من خلال الفحص الخلوي. تأثر الكيان الكروموسومات بمجموعة متنوعة من التغيرات الصبغية وغير الكريمة ، وكذلك تأثيرات mitodepressed. تبين أن الزيادة في الجرعة/تركيز جميع الطفرات تزيد من تواتر كل من الخلايا الشاذة الانقسامية وتشوهات الكروموسومات الانقسامية في خلايا طرف الجذر المعالجة. تبين أن كل من الطفرات الكيميائية أقل فعالية من بروميد الإيثيديوم في تعطيل نشاط الخلايا الانقسامية وتسبب في المقام الأول تشوهات الكروموسومات الصبغية. تم الإبلاغ عن أن العديد من الانحرافات الكروموسومية ، بما في ذلك اللقاء وتكتل الكروموسومات ذات الترددات المختلفة ، والشظايا ، والجسور ، والحركات المبكرة ، وتكوين النوى النوى في الخلايا التيصحون ، والأنفاز المتعددة القطب والخلايا telophases ، موجودة في الخلايا الجذابة. ولوحظت جميعها من الحوافز ، والطرازات ، والميتافاز غير المنظمة ، والميتافازات المتزامنة ، والنواة المستمرة أثناء الطو