DISCUSSION

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The discussion in the present study is basically concerned with the effect of chemical mutagens (6-AminoPurine, Methyl Methane Sulphonate, 2, 4-Diclorophenoxy acetic acid & Cadmium Nitrate) on various phenotypic and chromosomal characters of the plant under consideration i.e. *Cichorium intybus* L. the probable and possible reasons as well as explanations regarding the mutagenic effects of the chemicals on the plant have been discussed in detail.

**A. SEED GERMINATION AND BIOLOGICAL DAMAGE:**

Germination of seeds after breaking dormancy period is a process of resumption of active metabolism manifested in visible growth. Inhibition of seed germination after treatment of seeds with different concentrations of physical and chemical mutagens is a convenient technique for studying the effects of mutagens in plants. It was inversely proportional to the concentrations of mutagens in the present study. Seed germination was highly affected by Cd(NO$_3$)$_2$ followed by MMS, 6-AP and 2, 4-D. The toxic effect of mutagens on seed germination has also been observed previously by many workers in different plants such as, *Nigella sativa* (Mitra & Bhowmik 1998), *Vigna radiata* (Khan et al. 1998), *Plantago ovata* (Lal & Sharma 2000), *Vicia faba* (Agarwal & Ansari 2001) *Trigonella foenum graecum* (Siddiqui et al. 2008). Reduced germination has also been reported in other plants following heavy metal (lead) treatment in *Capsicum annuum* L (Kumar & Gupta 2008).

Resultantly the seed germination was inhibited parallel to the increasing concentrations of mutagens and anti parallel to the germination percentage. Moreover Cd(NO$_3$)$_2$ exhibited more inhibitory effect on germination. Several explanations have been given by different workers regarding the inhibition in seed germination. Krishna et al. (1984) suggested that inhibition in germination may be due to interaction between mutagen
and the seed cell system. It may also be due to toxicity of mutagens followed by mutational changes at genic or chromosomal level, because the reduction in germination corresponds with the increasing chromosomal aberrations. Falque (1994) considered that occurrence of seeds without completely developed embryos may be one of the reasons for reduction in germination. Endogenous growth regulators play an important role in seed germination and there exists a balance between promoters and inhibitors and any disturbance in this balance results in reduction in seed germination (Aman 1986). According to Khanet et al. 2007, reduction in seed germination in mutagenic treatment may be due to chromosomal deletion, delay or inhibition of physiological and biological processes necessary for seed germination. Enzyme activity (Kurobane et al. 1979), hormonal imbalance (Chrispeeds & Varner 1976) and inhibition of mitotic process (Ananthaswamy 1971) may also add to inhibition process. Inhibition in seed germination following heavy metal treatment may be due to disturbance of physiological process and induction of chromosomal aberrations leading to mitotic arrest and cell death (Sree Ramulu 1972; Datta & Biswas 1985).

Plant survival was the highest in 2, 4-D and least in heavy metal Cd(NO\(_3\))\(_2\). It was directly related to germination and inversely to the inhibition percentage. Since 2, 4-D and 6-AP caused less inhibition therefore survival was higher in these mutagens. Reduction in plant survival with increasing doses of mutagens as reported in chicory is in support with earlier findings in Triticale by EMS and gamma rays treatment (Reddy & Gupta 1989, Edwin & Reddy 1993), Triticum by industrial chemical agents (Kalia et al. 2001), Capsicum annuum by Caffeine (Kumar & Tripathi 2004), Nigella and Triticum by heavy metal treatment (El Ghamery et al 2003).

Reduction in survival following mutagenic treatment may be due to various factors such as cytogenetic damage and physiological disturbances (Sato & Gaul 1967), changes in the metabolic activity of cells (Natarajan & Shivashankar 1965) disturbances in balance
between promoters and inhibitors of growth regulators (Meherchandani 1975). Thus biological damage was higher in higher concentrations of mutagens.

**B. FREQUENCY OF VARIATIONS/Mutations:**

In the present study, frequency of variations was found to increase with the increasing concentrations of mutagens. Growth hormone 2, 4-D was found to be more responsive for induction of variations followed by base analogue (6-AP), alkylating agent (MMS) and heavy metal Cd(NO₃)₂. Earlier reports by Jabeen (2002) in *Cicer arietinum*, Solanki & Sharma (2002) in *Lens culinaris* also showed a similar concentration-dependent increase in the frequency of mutations. However the frequencies of mutations in M₂ obtained after selfing the M₁ variants were comparatively lower and finally the frequency of mutants in M₃ were the lowest, possibly due to ceasing toxic effects of mutagens in successive generations and possible segregation of recessive genes.

**C. POLLEN FERTILITY/STERILITY:**

Pollen fertility decreased with the increasing concentrations of 6-AP, MMS, 2, 4-D & Cd(NO₃)₂ with a simultaneous increase in pollen sterility. The negative effect of mutagens on pollen fertility was highest in Cd(NO₃)₂ followed by MMS. Similar adverse effect on pollen fertility has also been reported in many plants such as in mungbean (Ignacimuthu & Babu 1992), lentil (Reddy & Annadurai 1992), chilli (Dhamyanti & Reddy 2000), cowpea (Singh et al. 2006), cotton (Sheidai & Dezfolian 2008), chicory (Khan et al. 2009a) etc.

Increase in pollen sterility may be due to cumulative effects of various meiotic aberrations as well as physiological and genetic damages induced probably by the chromosomes through the formation of antimetabolic agents in cell or due to irregular disjunction of chromosomes at anaphase, resulting from the formation of multivalents.
(Larik, 1975). Bhat et al. (2005) reported that sterility may be attributed to vast array of meiotic aberrations that were induced by chemical mutagens leading to aberrant pollen grains. According to Srivastava and Kapoor (2008) the spindle related aberrations like tripolarity, multipolarity and non orientation may cause the formation of unbalanced and sterile gametes affecting the plant fertility. Chromosomal and cytoplasmic anomalies were manifested in the form of defected microspores which in turn lowered down the pollen fertility. Thus it appears that gene mutations or in other words cytological undetectable cryptic structural changes may also contribute to some extent (Girija & Dhanavel 2009). The actual reason of sterility caused by these mutagens may be a gene mutation or probably invisible deficiencies.

The present study has shown that pollen grains produced as a result of mutagenic treatments allow the production of chromosome-altered plants, mostly without any loss of vigour, which facilitate the perpetuation of the induced chromosome variations. The pollen sterility was much less in M₃ generation as compared to M₁ and M₂ indicating that some sort of recovery mechanism have operated in the intervening period. These findings were in support with earlier observations of Viccini & Carvalho (2002) Ansari & Ali (2009), Khan et al. (2009b).

D. VARIATIONS IN Cotyledonary AND Vegetative leaves morphology:

Variations in shape, size, orientation of cotyledonary and vegetative leaves were the common effects of the mutagens. The frequency of these variations was more in M₁ than the mutations in M₂ and M₃, because most of the variations that occurred as adopted characters/variants in M₁ were eliminated in further generations and the recessive mutations appeared along with few homozygous dominant mutations. The variations observed in leaf morphology were
unequal size, curved margin, notching, cup shaped chimeras, cotyledonary leaves etc. Similar variations in leaf morphology have been induced by many authors using physical and chemical mutagens, such as in *Trigonella* (Jain & Agarwal 1993), faba bean (Yasin 1996), mungbean (Gaur & Gour 2003; Soehendi *et al.* 2006; Begum *et al.* 2008), chickpea (Srinivasan *et al.* 2008), *Cichorium* (Khan *et al.* 2010).

The production of abnormal leaf types in treated populations may be attributed to the disturbances of internal growth regulators like IAA induced by the action of mutagens or may be sequel to primary or secondary effects of free radicals caused by the mutagens (Lea 1955). Blixt (1972) considered that leaf aberrations seemed to be due to actual mutation process. According to Dubinin (1976) several enzymes are involved in mutation, these enzymes participate in the whole process at various stages and many of the potential lesions are converted into mutations as a result of enzymatic activity. Bifurcated deshaped leaves as observed in the present study have also been reported by Ansari & Ali (2009) in faba bean. They suggested that occurrence of these mutants may be correlated with the increasing meiotic anomalies at higher doses of mutagens. The disturbances in metabolic activities due to mutagenic treatments may also be one of the important factors responsible for such anomalies in plants.

**E. PLANT HEIGHT:**

Average height of plants generally decreased with the increasing concentrations of mutagen, but it was found to increase in lower concentrations of 2, 4-D insignificantly over control in M₁ as well as in successive generations. The overall retarding effect on height in M₁, M₂ & M₃ generations was caused by Cd(NO₃)₂ and MMS, 2, 4-D being the least damaging. Higher CVs in Cd(NO₃)₂ treated populations showed more variability in height. Reduction in plant height followed by mutagenic treatments was also observed by many workers such as Jain &

There have been many theories put forward by many workers regarding reduction in plant height. According to Ansari & Siddiqui (1995), injury caused to the meristematic cells may be responsible for reduction in growth. Salam (1990) concluded that reduction in seedling growth may be due to the gross injury caused at cellular level, either due to gene controlled biochemical process and/or acute chromosomal aberrations. Krishna *et al.* (1984) hold the opinion that reduction in growth might be due to auxin destruction, changes in ascorbic acid content and other physiological and biochemical disturbances. According to Tabassum (2002) the chromosomes carrying various genes responsible for the life process and expression are one of the most sensitive elements and the damage to any part of these vital and tiny elements are bound to go a long way to bring about several morphological and growth abnormalities in the plant or plant organs. Kumar & Tripathi (2008) are also of the opinion that the reduction in plant height can be attributed to chromosomal abnormalities after the treatment of mutagenic chemical. Moreover chromosomal breakage during mitotic division and inhibition of DNA synthesis, have also been implicated as the cause of reduced plant growth because chromosomal aberrations and height reduction in higher concentration of mutagens were positively correlated.

Moreover the average height in 20ppm 2, 4-D in M₀ generation increased insignificantly over control. Similarly in M₂ generation the lowest concentration of 6-AP showed an insignificant increase in height. The selfing in M₁ and M₂ generations and segregation of desirable genes exhibited improved result in M₃ generation in which taller and high yielding mutants had been obtained. Similar enhancing effect has also been reported by Anis & Wani (1997) in *Trigonella* by caffeine, Sawan *et al.* (2000) in *Gossypium* by
kinetin. Jabeen (2002) in Cicer by DES. Moreover the reduction in height sometimes proves
to be better because some semi dwarf mutants selected in M₃ were high yielding, providing
better chances for selection.

**F. YIELD:**

A crop plant can be improved in productivity and adaptation to environment only when
genetic variability for the specific trait is available in the treated population. Although the
yield decreased in most of the treatments of 6-AP, MMS, 2, 4-D and Cd(NO₃)₂ in M₁
generation but lower concentrations of 2, 4-D and 6-AP exhibited stimulatory effect on yield
giving rise to some positive mutants in M₂ which persisted in M₃ generation. Moreover in
20ppm 2, 4-D the seeds per plant and 1000 seeds weight increased significantly (at 5%) over
control. Such type of mutants have also been reported by Anis & wani (1997) in Trigonella,
in Cicer, Khursheed et al. (2009) in Helianthus. The reason behind the increased yield in
lower concentrations may be attributed to enhancing effect of 2, 4-D (Audus 1961). The
1000 seeds weight and average seeds weight per plant also increased over control due to
slight increase in seeds size. Moreover the main reason for the increase in number of seeds
per plant was due to increased number of branches and flowers over control in lower
concentrations of mutagens.

In present study the yield generally decreased with the increasing concentrations of
all mutagens. The decrease in yield as observed in Cichorium has also been reported by
many workers, such as Temple (1990) in Lycopersicon esculentum, Kumar et al. (1993) in

Reduction in yield might have occurred due to disturbances in meiosis which affected the frequency of normal microspore to a greater extent and megaspore to a lesser extent and hence the fruit set was directly affected. The reason for reduction in yield at lower concentrations of MMS and Cd(NO$_3$)$_2$ may be due to their highly genotoxic nature, which might have resulted in physiological disturbances, chromosomal damage, failure or restricted pairing, delay in DNA synthesis and/or disturbed spindle formation and high pollen sterility. However, pollen sterility appeared to be more responsible, because the yield was found to decrease under the condition of high pollen sterility (Lakshmi et al. 1988). Decrease in yield is also indirectly related to decrease in plant height, number of branches and number of flowers per head due to the toxic effects of mutagens.

G. PROTEIN CONTENT:

Meagre amount of work has been recorded for induction of variations in protein content in chicory. Protein estimation of selected mutants in M$_3$ generation showed considerable variations in protein content. In control seeds it was estimated to be 5.34 mg per ml (in 0.05M phosphate buffer). It was higher in 2, 4-D induced mutants followed by some mutants in other mutagens. In some of the mutants there was decrease in protein content as compared to control, confirming the fact that mutations may occur in both positive and negative directions. Moreover protein content of seeds treated with Cd(NO$_3$)$_2$ were more adversely affected as the protein content was generally lower than control. It has also been reported that protein production is directly linked with the quality of seeds, better the quality of seed more is the protein production. Therefore, in the case of increased protein content the seed quality also increased. It may also be due to interaction of genes and environment (Singh et al.
1990). Similar increase as well as decrease in protein content in some mutants have also been demonstrated by Shaikh et al. (1982) in chickpea using gamma rays, Ignacimuthu & Babu (1989) in Vigna radiata and Vigna mungo using EMS and gamma rays as mutagens. Induction of substantial variations in seed protein content was also reported in earlier studies by Quednan & Wolff (1978) in pea and Singh et al. (1983) in chickpea.

H. ROOT VARIATIONS:

The variations in root biomass have been induced by chemical mutagens in the present investigation. Since the roots of the plants are medicinally and economically important, the effort was made to increase the root biomass also. In all mutagens except the lowest concentrations of 2, 4-D, the root length and biomass (fresh and dry root weight) was found to decrease in their increasing concentrations in M1 generation. The reason for decrease in root length may be due to the presence of some mitotic inhibitors in meristmatic regions. Contrastingly in 2, 4-D the increase in length and biomass may be due to increase in the length and diameter of main root, number and size of lateral roots. Moreover 2, 4-D in low concentration acts as a growth regulator, thus affecting the root length and biomass. Abeles (1968) reported that 2, 4-D increased ethylene production and suggested that certain morphological changes may be due to enhanced production of ethylene.

Moreover, the increase in root length and root weight was recorded in 6-AP, MMS and Cd(NO$_3$)$_2$ in M3 generation. The increase was equal to or slightly higher than control, the reason may be due to the ceasing toxic effect of mutagens, recovery, as well as DNA damage repair mechanism. The increased root biomass has also been reported in Vigna by hypoxanthine treatment (Mahna et al. 1993) and in Cichorium by DMS (Khan et al. 2010).
I. MUTAGENIC EFFECTIVENESS AND EFFICIENCY:

Mutagenic effectiveness is an index of the response of a genotype to the increasing concentrations/doses of the mutagens in $M_2$ generation. The selection of effective and efficient mutagen is very essential to recover a high frequency and spectrum of desirable mutations (Solanki and Sharma 1994).

In the present experiment the mutagenic effectiveness decreased with the increasing concentrations of mutagens. The order of mutagens based on effectiveness was 2, 4-D $>$ Cd(NO$_3$)$_2$ $>$ 6-AP $>$ MMS. Moreover mutation rate was not proportional to the increase in concentrations of mutagens, rather it was found to be inversely proportional. Similar results were obtained by Mitra & Bhowmik (1999) in Lens culinaris, Waghmare & Mehra (2001) in Vigna radiata, Sharma et al. (2005) in Vigna mungo (L) Hepper. Kharakwal (2001) considered that chemical mutagens showed higher effectiveness than physical mutagens.

Efficiency of a mutagenic agent is of complex nature, as it depends not only on the reactivity of the agent with the material and on its applicability to the biological system but also on the degree to which physiological damage (M$_p$/I), and sterility (M$_p$/S) are induced in addition to mutation. Since sterility depends directly on chromosomal behaviour, the chromosomal aberrations are also responsible for mutagenic efficiency. According to Girija & Dhanavel (2009) it gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagenic agent.

In Cichorium the mutagenic efficiency with respect to inhibition and sterility also decreased with increasing concentrations of mutagens. The efficiency with regard to inhibition was the highest in 6-AP, followed by 2, 4-D, MMS and Cd(NO$_3$)$_2$ and with respect to sterility the order was 2, 4-D $>$ 6-AP $>$ MMS $>$ Cd(NO$_3$)$_2$. The decline in mutagenic
effectiveness at higher concentration was due to elimination of highly affected seedlings or plants at an early stage. Higher efficiency of a mutagen indicates relatively less biological damage in relation to mutation induced (Solanki & Sharma 1994). This may be taken as an established fact for almost all situations.

**J. MEIOTIC STUDIES:**

Induction of chromosome alterations represents an effective method for analysis of genotoxic potential of a mutagen. In the present study the changes in chromosomal behaviour induced via chemical mutagens have been extensively studied in order to assess the spectrum of chromosomal damage caused by these mutagens and their effect on morphological characters as well. Cytological studies also provide informations regarding the response of various genotypes to a particular mutagen and provide greater chances for the selection of desired characters.

(i) **UNIVALENTS:**

The univalents occurred uniformly at diakinesis and their frequency generally increased with the increasing concentrations of mutagens. The frequencies were more in Cd(NO$_3$)$_2$ followed by MMS, 6-AP and 2, 4-D in M$_1$ and that these were more in M$_1$ and that these were more in M$_1$ than M$_2$ generation.

The reason behind the formation of univalents may be due to induced heterology in some of the homologous chromosomes or as a result of desynapsis or asynapsis. According to Kaul & Nirmala (1993) & Reddi et al. (1999), where univalency is complete the asynapsis is presumed and where it is partial desynapsis is inferred. Occurrence of univalents might also be due to the induction of structural changes in chromosomes (Zeerak 1992), failure of pairing or slipping off of the chiasmata followed by failure of chromosome movement.
(Singh 1992), disturbed normal pairing of homologus chromosomes (Siddique & Ansari 2005), failure of chromosome pairing leading to desynapsis at metaphase-I (Kumar et al. 2006), absence of crossing over at pachytene (Kumar & Rai 2007b), due to precocious chiasmata terminalisation (Sidhu 2008) etc.

The reduction in the frequency of univalents in $M_2$ generation was due to the fact that desynapsis or synapsis did not occur due to ceasing effect of mutagens leading to normal pairing among bivalents or due to repair mechanism in case of DNA damage.

(ii) MULTIVALENTS:

Multivalents occurred both at prophase and metaphase stages but their frequency was higher at prophase. Although it was dose dependent but did not occur in lowest concentration of 6-AP, MMS and 2, 4-D. The frequency of multivalents in $M_1$ generation was highest in almost all concentrations of Cd(NO$_3$)$_2$ followed by MMS and other mutagens while in $M_2$ it was the highest in 2, 4-D followed by MMS, 6-AP and Cd(NO$_3$)$_2$. The MMS showed most consistent result for the induction of mutagenicity.


The formation of multivalents may be attributed to the irregular pairing and breakage followed by translocation and inversion (Vandana et al. 1996), abnormal pairing and disjunction of bivalents (Siddiqui & Ansari 2005), irregular breakage followed by translocation and inversion (Zeerak 1992), non or irregular pairing of chromosomes due to translocation (Katiyar 1978).
(iii) CHIASMATA FREQUENCY:

Formation of chiasmata results in homologous pairing of chromosomes and controls degree of recombination. Variation in chiasmata can be considered as a means for generating new forms of recombination which influences variability within natural populations in an adaptive way (Rees & Dale 1974, Sheidai et al. 2006). Therefore, the attempts were aimed to induce variations in chiasmata per cell and per bivalent.

Chiasmata frequency at diakinesis was comparatively higher than metaphase in control as well as treated populations. The frequencies per cell and per bivalent at both stages were inversely proportional to the increasing concentrations of all mutagens. The maximum adverse effect on chiasmata was observed in Cd(NO\(_3\))\(_2\). Apparently it was caused due to increasing frequency of univalents, rod bivalents and multivalent but particularly due to increased heterology induced by mutagens which directly affected the crossing over.

Reduction in chiasmata frequency have also been reported by Reddy et al. (1991) in barley and wheat, Reddy & Annadurai (1992) in lentil, Khan et al. (1998) and Haroun & Al Shehri (2001) in Vicia faba, Khan et al. (2009b) in Cichorium, Khan & Tyagi (2009) in Glycine max etc.

Reduction in chiasmata may be attributed to the mutations in the genes governing homologous pairing. Probably it may be the cryptic structural changes in the chromosomes forming genetic differences and restricting the pairing with other homologous ones as suggested by Anis & Wani (1997). Sadanandam & Subhash (1985) attributed the reduction in chiasmata, to the nature and potency of mutagens and also to the underlying factors such as complex structural changes or the change in nature of gene responsible for chiasmata formation. It might also be attributed to the failure of complete pairing.
(iv) PRECOCIOUS MOVEMENT:

Precocious movement of chromosomes was observed at metaphase I and II stages. Besides the precocious separation of chromosomes of univalents, the bivalents were also observed to move ahead and seemed as stray chromosomes at metaphase. Their frequency was positively correlated with the concentrations of mutagens and was comparatively more in M₁ generation and highest in Cd(NO₃)₂ followed by others. The frequency decreased significantly in M₂. As in the present study its occurrence has been reported previously by many workers such as Pagliarini & Pereira (1992) in *Pilocarpus pennatifolius*. Lem., Defani-Scoarize et al. (1995) in *Zea mays*, Kumar & Rai (2007b) in *Glycine max*, Malik & Shrivastava (2007) in *Carthamus*. Khan et al. (2007) in *Vicia faba*.

Precocious movement of chromosomes might have occurred due to disturbed homology for chromosome pairing, disturbed spindle mechanism or inactivation of spindle mechanism (Agarwal & Ansari 2001). It may also be ascribed to the presence of chromosome breakage. Some of the univalents disjuncted early and presumably this happened due to genic differences. Such chromosomal divergences in the form of precocious movement is pointed towards structural differentiation of homologous pair.

(v) STICKINESS:

Stickiness among the chromosomes occurred at metaphase and anaphase I and II stages. Chromosome stickiness is characterised by the clustering of chromosomes and their number involved in comprising the stickiness varied from two to whole chromosome complement with the PMCs and failed to disjunct individually.

Stickiness was a common meiotic abnormality reported by various workers such as Pagliarini *et al.* (2000), Jabee & Ansari (2005). Khan & Aslam (2006), Chidambaram et
al. (2008). According to Tarar & Danyansagar (1980) the stickiness might arise due to depolymerisation of nucleic acid caused by mutagenic treatment. Myers et al. (1992) suggested that stickiness occurred due to improper folding of chromosome fibres and as a result there is intermingling of fibres, consequently the chromosomes become attached to each other by means of subchromatid bridges. It could also be due to result of partial dissociation and altered pattern of organization of nucleoproteins. Rao & Lakshmi (1980) hold the view that stickiness could be due to disturbances of cytochemically balanced reactions by the effects of alkylating agents. According to Chidambaram et al. (2008) stickiness appears as a result of disturbances in the nucleic acid metabolism in the cell. It may also cause movement of whole bivalent towards one pole at anaphase due to the non-disjunction of homologus chromosomes.

(vi) STRAY CHROMOSOMES, UNSYNCHRONISED MOVEMENT & UNEQUAL SEPARATION OF CHROMOSOMES:

Stray chromosomes at metaphase I & II stages occurred in all concentrations of 2, 4-D and Cd(NO₃)₂ and higher concentrations of other mutagens. They occurred uniformly in M₂ but in lower frequencies as a result of spindle dysfunction and clumping of chromosomes. According to Khan et al. (2009a), along with the precocious separation of univalents, the bivalents were also observed to move ahead and seemed as stray chromosome, this may move to one pole resulting into unequal distribution of chromosome or loss of a complete bivalent at later stage.

Unsynchronised movement of chromosomes occurred only in higher concentrations of mutagens and with less frequency. Maximum frequency was noted in Cd(NO₃)₂. It might have occurred due to early or delayed separation of univalents and bivalents or movement of one chromosome of a quadrivalent to one pole and remaining three to the other. It might have
also occurred due to possibility of failure of a bivalent to disjoin and inability to move synchronously as a whole to one of the poles. The unsynchronised movement of bivalents, univalents, laggards might have also occurred due to the discrepancies in spindle formation. Its presence has also been reported by Venkateswarlu et al. (1988) in Catharanthus and Khan et al. (1998) in faba bean.

Unequal separation of chromosome was found in higher concentration of mutagens at anaphase and telophase I/II stages owing to disturbances in spindle mechanism. Cadmium was found to induce unequal separation in more number of cells. Its presence has been also reported by Venkateswarlu et al. (1988) in Catharanthus, Tarar & Dnyansagar (1980) in Turnera, Ahmad (1993) in Cicer arietinum, Kaul & Nirmala (1993) in Pisum, Singh (2002) in Hordeum vulgare, Ansari & Ali (2009) in Vicia faba etc. Unequal separation might have resulted due to disturbed spindle mechanism and stickiness.

(viii) LAGGARDS:

Laggards were present in almost all treatments except in the lowest concentrations of mutagens but their frequency decreased in the subsequent generations. Occurrence of laggards in the present study has also been reported previously by many workers such as Singh et al. (1999) in Vigna radiata, Zeerak (1992) in Lycopersicon, Iqbal & Dutta (2007) in Withania somnifera L. Dun, Khan et al. (2009b) in Cichorium intybus L. Siddiqui et al. (1982) in Helianthus annuus L. etc. Delayed terminalisation and/or failure of chromosomal movement, following spindle fibre discrepancies have lead to lagging chromosomes in Cichorium intybus L. The fragments which appeared on the breakage of bridges, as a result of spindle fibres functioning to pull the chromosome towards poles, formed laggards (Kumar & Gupta 2009). Asynaptic condition which results in abnormal meosis in later stages may also lead to laggard formation (Sjodin 1970).
(vii) BRIDGES:

Bridges occurred both at anaphase I & II and telophase I & II stages. The frequency was generally higher at anaphase than telophase and increased with the increasing concentrations of all mutagens in M₁ generation. The lower frequency at telophase was due to the fact that the bridges broke during separation of the chromosomes at telophase. In M₂ generation the pattern was same but their frequencies were comparatively lower. The bridges may be due to sister chromatid exchange followed by delayed or failure of their separation at later stages. This might have resulted in unequal division of chromosomes and hence sterile microspores.


Singh & Khanna (1988) suggested that anaphase bridges may be formed due to unequal exchange or dicentric chromosomes. The occurrence of breaks at the same locus and their lateral fusion leads to the formation of dicentric chromosomes, which are pulled equally to both the poles at anaphase and a bridge is formed. El-Ghamery et al. (2000) hold the opinion that the presence of single and multiple bridges may be due to the occurrence of dicentric chromosomes formed as a result of breakage and reunion of these chromosomes.

Agarwal & Ansari (2001) suggested that chromosomal stickiness, subsequent failure of anaphase separation and unequal translocation or inversion of chromosome segments are the main reasons for the presence of chromosomal bridges during cell division. Bridges might have occurred as a result of delayed terminalisation, stickiness of chromosome ends, failure of chromosome movement (Das & Roy 1989), unequal separation of chromosomes (Iqbal & Datta, 2007), late terminalisation (Bipasha & Shella 1992).
**Kumar & Gupta (2009)** reported that gene mutation or direct action of mutagen on the target protein responsible for chiasmata terminalisation during diakinesis at meiosis-I cause some structural defects in the protein which lead to their improper functioning thus resulting into bridges.

(ix) **MULTINUCLEATE CONDITION:**

Multinucleate condition (more than four nuclei) at telophase II stages were found in low frequency only at higher concentrations of all mutagens but cadmium was more effective to induce the same. Moreover their frequency decreased in subsequent generations. Occurrence of micronuclei was also reported by Reddi & Rao (2000) in rice, Dryanova & Dimitrova (2000) in *Triticale* callus, Singh (2002) in *Hordeum vulgare*, Utsunomiya *et al.* (2002) in *Zea mays*, etc.

There is a correlation between the occurrence of laggards due to failure of spindle formation and the formation of micronuclei at telophase. The laggards and non-oriented chromosomes when fail to reach the poles in time to be included in the main telophase nucleus, form micronuclei leading to multinucleate condition (Reddy & Rao 1982, Utsunomiya *et al.* 2002). This may also occur due to reorganisation of the chromosomes between prometaphase and anaphase II (Pozzobon & Schifino-Wittmann 2006) and coalescence or aggregation of chromatin materials into masses of various number and size (Kabir & Alam 1986).

**CONCLUSION:**

In the present study cytomorphological variations observed were an outcome of physio–biochemical disturbances induced at genic level by the action of mutagens along with their interactions with environment.
Several positive and negative mutants were screened in M$_3$ generation. 6-AP showed linear concentration effect on different aspects of chicory, such as seed germination, growth and yield etc. 2, 4-D showed enhancing effect on these aspects in lower concentrations, but its effect in higher concentrations was similar to 6-AP and induced more variations leading to screening of mutants in M$_2$ and M$_3$ generations. In addition giant and high yielding mutants have been isolated in M$_3$ generation. Most of the effects of MMS and Cd(NO$_3$)$_2$ were deleterious as they caused maximum chromosomal abnormalities. However in M$_3$ generation the deleterious effect was minimized to a much extent possibly due to operation of some recovery mechanism and/or elimination of highly affected seedlings and plants. The morphological variations were more in 2, 4-D treatments. Inspite of their deleterious effects some positive mutants were established in M$_3$ generation viz. Giant and high yielding mutants with bigger leaves, white flower mutants, bold seeded mutants etc. Most consistent results were obtained in MMS and 2, 4-D treatments.

Maximum frequency of chromosomal aberrations were induced by Cd(NO$_3$)$_2$ at cytological level. The chromosomal abnormalities as a whole were dose dependent and increased along with the increasing concentrations of mutagens. The occurrence of many cytological irregularities clearly indicates that the mutagens have genotoxical effect and the mutants which survived after going through these stages were genic mutants.

The above results thus suggest that cytomorphological diversities induced in the chicory via mutagenic application can be used for selection of better mutants with desirable characters, because more the variations greater will be the chances for the selection of better qualitative and quantitative characters and better chances for the adaptation of these mutants.