



Genotoxicity of Methyl Tert-butyl Ether (MTBE) to *Vicia faba* L. Plants

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Authors' contributions

This work was carried out in collaboration between both authors. Author MAI designed the study, and wrote the protocol. Author MMM performed the statistical analysis, managed the analyses of the study, managed the literature searches and wrote the manuscript. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The aim of this study is to investigate the genotoxicity of Methyl Tert-Butyl Ether (MTBE) to *Vicia faba* L. plants.

Study Design: Experiments were carried out to determine the depressive effect in the mitotic process of *Vicia faba* L. when treated with different concentrations of Methyl Tert-Butyl Ether.

Place and Duration of Study: Department of Biotechnology and Biology, Faculty of Science, Taif University, between January 2011 and February 2011.

Methodology: *Vicia faba* L. seeds were germinated in different concentrations of Methyl Tert-Butyl Ether (0.5, 1.0, 5.0, 10 and 15 %). For each concentration, five root tips were transferred to five microscope slides, stained with feulgen technique, covered with cover slip, squashed and observed microscopically. The cytotoxicity and genotoxicity induced by each concentration was compared with the value for the concomitant negative control using t-test.

Results: The trend of the results showed that the higher the concentration of MTBE, the inhibitorier the effect on mitosis with more pronounced chromosomal aberrations. Types of

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abnormalities revealed the induction of spindle disturbance, stickiness, laggards, fragments, bridges and micronuclei which lead to the loss of genetic material.

Conclusion: The induction of sticky chromosomes and micronuclei indicated that the Methyl Tert-Butyl Ether caused abnormal DNA condensation and inactivated the spindles. Because abnormalities of the cell division process results from the genotoxic effects of environmental chemicals, the Methyl Tert-Butyl Ether has the potential to cause aneuploidy in exposed organisms and adverse human health and environmental effects.

Keywords: Genotoxicity; Methyl-Tert-Butyl Ether (MTBE); *Vicia faba* L.; mitotic indices; abnormalities.

1. INTRODUCTION

Mitotic activity (a measurement of actively dividing cells known as the mitotic index), alterations in the mitotic phase, and individual cell aberrations are key parameters by which plant growth may be evaluated.

Methyl tertiary-butyl ether (MTBE) is a colorless, relatively volatile liquid that has found widespread use as an octane enhancing gasoline additive to reduce tailpipe emissions and its use has been discontinued. Several toxicity studies in animals were conducted to examine the acute, subchronic, chronic toxicity, carcinogenicity, reproductive effects, developmental abnormalities and teratogenicity employing various routes of exposure to MTBE. In rats, the primary target organ of inhalation toxicity was the liver in the females, as mentioned by [1]. On the other hand, exposure of Wistar rats to MTBE in the drinking water for two years resulted in several effects, including increased kidney weights, chronic progressive nephropathy in male and female rats and was exacerbated by exposure to MTBE [2]. Another study using different accessions of *Sorghum bicolor* was done to assess the toxicity of crude oil on plant and the results obtained can help in the biological monitoring of soils [3].

Mutagenic activity of chemicals has been analyzed with different plant systems such as *Allium cepa* and *Vicia faba*. With these plant systems, chromosomal aberration assays, mutation assays and cytogenetic tests have been performed [4,5,6,7,8,9,10,11]. Other studies have the same effect as in our study including; some food additives and showed generally increasing of the total percentage of aberrations with increasing concentrations of these chemicals and longer period of treatment on root tips of *Allium cepa* L. Among these abnormalities were stickiness, anaphase bridges, C-mitosis and micronuclei [12]. Heavy metals also gave the same results e.g.; Lead induced mitosis depression and has genotoxic effects, expressed in the occurrence of many chromosomal aberrations in root meristematic cells of wheat seedlings [13]. Moreover, the cytogenetic effects of individual treatment of Cd, Cu and Zn and combined treatments of Cd with Cu and Zn in two cultivars of *Vigna radiata* L. showed that mitotic index in both the cultivars were significantly reduced however, mitotic anomalies were enhanced [14].

Several seed extracts from medicinal plant like *Nigella sativa* L. was toxic on root number and length and reduced the mitotic index of *Allium cepa* [15], while the higher the concentration of the root extracts of *Boerhaavia diffusa* on the root tips of *Crinum jagus*, the more inhibitory the effect on mitosis with more pronounced chromosomal aberrations, the results of this study showed several chromosomal abnormalities including stickiness of

chromosomes, c-metaphase, lagging chromosomes, and sticky bridges [16]. Likewise, leaf extracts from *Lantana camara* and *Ageratum conyzoides* reduced mitotic index and caused mitotic chromosomal aberrations in legumes, *Lathyrus sativus* and *Lens culinaris* [17,18]. However, an herbicide from *Jasminum officinale* f. var. grandiflorum L. caused decreased in the mitotic index in onion root tips with increasing concentrations of the extracts and longer periods of treatment. Furthermore, crude extract produced mitotic abnormalities resulting from its action on chromatin organization and mitotic spindle [19].

Hence, the objective of this study was to investigate the genotoxic effect of MTBE using *Vicia faba* L. seeding through mitotic index and the chromosome aberration assay.

2. MATERIAL AND METHODS

2.1 Plant Material

The experimental plant used in the current study was pure strain of *Vicia faba* L. (Frensh) seeds of equal size and weight about (100gm).

2.2 Methyl Tert-butyl Ether (MTBE)

The high grade chemical MTBE was obtained from ARAMCO Jeddah, Saudi Arabia. DC 20436 and published in federal register 64 F R 5312 of Feb 3-1999.

2.3 Experiment

Seeds were soaked in tap water for 24 hours, and subdivided into six equal groups; one group was germinated in pure water and sampled as control. The other five groups were allowed to germinate in petri dishes watered with different concentrations (0.5, 1.0, 5.0, 10 and 15%) of MTBE.

2.4 Cytological Experiment

Radicals of 1.5-2cm were cut, fixed with Carnoy's fixative for 24hours; repeat washed in water, and then Feulgen squash technique was carried out. Five temporary slides were prepared for each treatment and control. Approximately 1000 cells per slide were examined. Recorded data included: mitotic index; numbers and types of abnormalities.

2.4.1 Mitotic Index determination

The mitotic index was calculated using the following formula:

$$\text{Mitotic Index (MI)} = \frac{\text{Total Dividing Cells (TDC)}}{\text{Total dividing and non dividing Cells (TC)}} \times 100$$

2.4.2 Total percentage of abnormal cells

$$\text{Total Abnormal (TAbn)} = \frac{\text{Total Abnormal Cells (TC}_{\text{Abn}})}{\text{Total Dividing Cells (TDC)}} \times 100$$

2.5 Statistical Analysis

Cytological parameters were statistically analyzed using multiple comparison procedure using t-test. Comparison of any two means was done by least significant difference (LSD) test within the two levels (0.05 & 0.01).

3. RESULTS AND DISCUSSION

3.1 Cytological Study

3.1.1 Mitotic index and frequency of mitotic phases

The mitotic frequency of *Vicia faba* L. meristematic cells were scored after the treatment with MTBE for the selected concentration. Cytological result indicated a progressive depression in mitotic indices after all concentrations as compared to their respective control.

Mitotic index decreased significantly with increased concentrations at the same time period and reached 3.67% after application of 10% as compared to 7.65% for control as reported in Table 1. These results indicated that the treatment with the highest concentration caused total inhibition of cell division or toxicity after 15%.

The decrease in mitotic indices was significant at 0.01 levels and was accompanied by an accumulation in prophase stage which was not dose-independent and documented in Table 2. These results demonstrated that MTBE treatment inhibits cell division and can also inhibits growth and development of *Vicia faba* L. plant. Similar results were reported by [20] and [3] after application of crude oil on *Sorghum bicolor*. The mitotic index in *Vigna radiata* cultivars was significantly reduced under the influence of all treatments of heavy metals: Cd, Cu and Zn. Thus, the heavy metals reaching to the soil contaminate it and are toxic to the plants growing in that area. Another explanation after the use of some medicinal plants as *Nigella sativa* L. which contains antimetabolic constituents that can stop the mitosis in anywhere of the cell cycle, furthermore these constituents probably affects the cytoskeleton or tubulin polymerization or degradation [15]. Similar results were found in the studies carried out to determine the mutagenic effects of chemicals. For example, [21] and [22] thought that chemicals affect DNA synthesis or various enzymes involved in cell energy systems because it is claimed that insufficient oxygen causes a delay in division by inhibiting the metaphase. [23] reported that mitotic inhibition caused by different chemicals can be related with an increase in G2 period. Moreover, similar observation of commercial herbicides like pentachlorophenol, 2, 4-D and butachlor was reported by [24]. Such a reduction in mitotic index suggests that exposure to biorational herbicide like Spanish jasmine extracts led to cell cycle disturbances and decreases in number of cells entering mitotic division [19]. Due to the reduced number of dividing cells, we can postulate that MTBE might have similar effects on cell division of *V. faba* L. and possibly be involved in blocking the DNA or protein synthesis required for normal cell division process [25].

The lowering of Mitotic Index might have been achieved by the inhibition of DNA synthesis at S-phase after the use of some food additives [26] and this inhibition could be due to blocking in G2 preventing the cell from entering mitosis [27,28,29]. [30] reported that the mitotic index and root growth rate of *A. cepa* were considerably decreased after the application of *Aloe vera* gel extracts which have a cytotoxic effect. [31] demonstrated that Glyphosate inhibited

cell division due to mitodepressive and toxicity of herbicide on cell and cell cycle, the same effect was happened after MTBE treatment.

The treatment conducted with MTBE showed an effect on the percentage of the different mitotic stages where the percentage of prophase increased with a corresponding decreased in the percentages of the other stages. The same result obtained by [30] after the use of *Aloe vera* gel extract on *Allium cepa* L. and by [17,18] after using the leaf extracts on grass pea and lentil. This may attributed to the blocking of cell division by MTBE at the end of the prophase stage. In this case, this chemical may be accepted as premetaphase inhibitors. Similar results have been reported after treatment of *A. cepa* root-tip cells with various food additives [32,33,34,9,35]. This prophase accumulation could also occur as a result of disturbance or breakdown of spindle apparatus [36,37].

3.1.2 Percentage and types of abnormalities

Cytological analysis of cells in the course of mitosis revealed a universal increase in the percentage of abnormalities of *V. faba* L. roots after all treatments as compared to their respective control. The progressive increase was dose of exposure dependent reaching its highest value (26.5%) after the use of 10% as compared by its respective control, Table 1, while the highest concentration 15% of MTBE showed toxic effect on the percentage of abnormalities. The chromosomal aberrations noticed in this study indicate that exposure of plants to MTBE treatment can lead to mutation or can act as clastogene, the same result reported by [3] who found that altered chromosomes may possible have altered DNA and gene sequences which will affect the survival and continual existence of *S. bicolor* in crude oil polluted soil. On the other hand, induction of mitotic anomalies under different heavy metal treatments may be due to chromatin agglutination [14,38].

The different concentrations of MTBE produced similar types of chromosomal abnormalities: irregular prophase, spindle disturbance, stickiness bridges, laggards, fragments and micronuclei, Table 3. Numerical and structural changes in chromosomes are attributed to spindle failure leading to endoreduplication, c-mitosis, nuclear fragmentation, multipolar configurations, and lagging chromosomes [39].

Disturbance, Fig. 1 (c, d and f) may be caused by enhanced disturbances of spindle function [40], or inhibition of DNA synthesis at S-phase of cell cycle [14].

In this study, chromosome stickiness was the other major chromosomal aberrations and was recorded in a considerable percentage, Fig. 1 (e, h & j). Chromosome stickiness reflects highly toxic effects, usually of an irreversible type probably leading to death. According to [41], stickiness of chromosomes might have resulted from increased chromosome contraction and condensation or possibly from the depolymerization of DNA. The presence of chromosome stickiness is an indication of MTBE affecting organization of chromatin, and suggests a possible role by which this cytotoxic agent may impact the physical and chemical properties of DNA, protein, or both, ultimately leading to improper folding of chromatin [42,43,19]. Moreover, stickiness of chromosomes may be due to polymerization of chromosomal nucleic acid [44,14]; there is an agreement that stickiness reflects highly toxic and usually irreversible effect that probably leads to cell death [45].

Table 1. Mitotic index and percentage of mitotic abnormalities of *Vicia faba* L. root tip cells treated with Methyl Tert-Butyl Ether (MTBE)

MTBE concentrations (%)	Total No. of cells in 5 slides	Mean no of dividing cells	Mitotic index \pm SD	Mean of abn. cells	% of Mitotic abn \pm SD.
0.0	5210	79.8	7.65 \pm 5.84	5.2	6.50 \pm 1.78
0.5	5050	63.2	6.25** \pm 8.93	10.4	16.45* \pm 4.56
1.0	5110	55.2	5.40 ** \pm 9.95	11.2	20.28** \pm 3.34
5.0	5160	56.8	5.50** \pm 9.12	11.2	19.92** \pm 3.34
10.0	5336	39.2	3.67** \pm 7.15	10.4	26.50** \pm 2.19
15.0	Toxic	Toxic	Toxic	Toxic	Toxic

* significant from control at 0.05 level (T-test). ** significant from control at 0.01 level (T-test).
Abn: abnormality, SD: Standard Deviation, No: number.

Table 2. Frequency and percentage of abnormalities of mitotic phases in *Vicia faba* L. root tips after treatment with Methyl Tert-Butyl Ether (MTBE)

MTBE concentrations (%)	Total no of cells in 5 slides	No. of dividing cells	Prophase		Metaphase		Anaphase-Telophase	
			% of phase \pm SD	% of abn.	% of phase	% of abn.	% of phase	% of abn.
0.0	5210	399	55.38 \pm 3.19	0	19.54	61.5	25.56	38.46
0.5	5050	316	63.29 \pm 10.19	46.15	5.06	0	31.64	53.84
1.0	5110	276	43.47** \pm 8.48	42.85	23.18	21.42	33.30	35.71
5.0	5160	284	53.52* \pm 10.80	28.57	21.12	42.85	23.94	28.57
10.0	5336	196	51.02** \pm 4.89	38.46	20.4	30.76	28.57	30.76
Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic

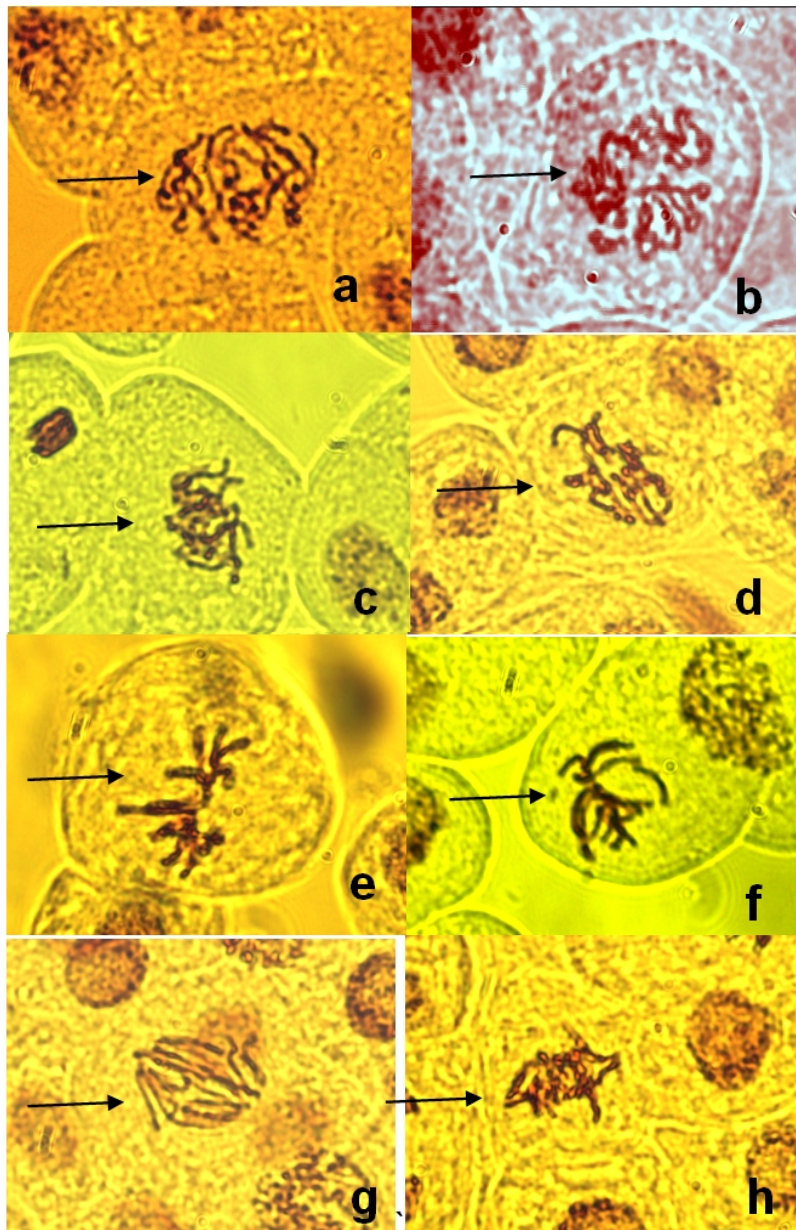
* significant from control at 0.05 level (T-test). ** significant from control at 0.01 level (T-test).
Abn: abnormalities, SD: Standard Deviation, No: number.

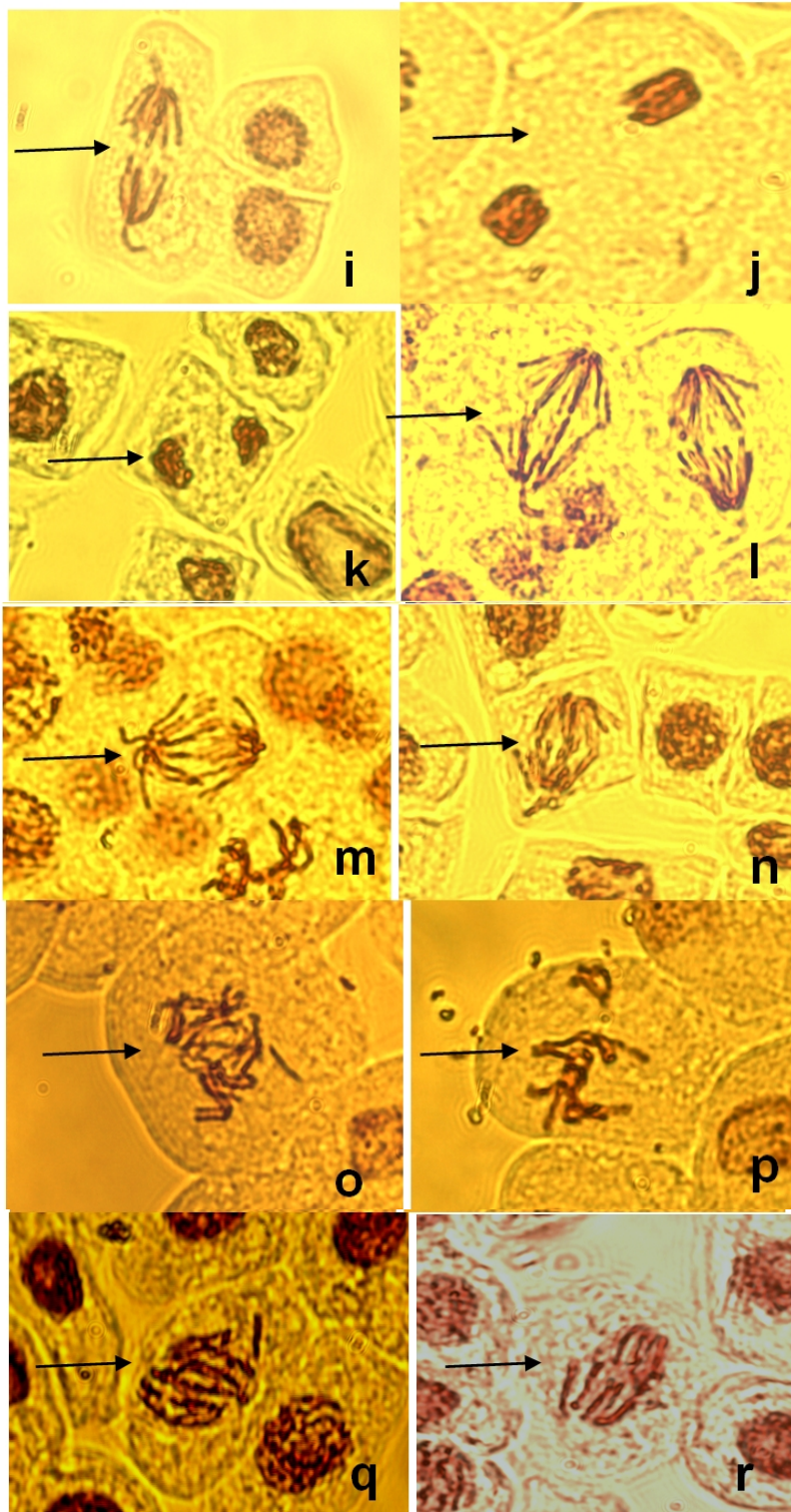
Table 3. Percentage of different types of abnormalities in *Vicia faba* L. roots using Methyl Tert-Butyl Ether (MTBE)

MTBE concentrations (%)	Total % of abn. in 5 slides	Types of abnormalities							
		Disturb.	Irr. Prophase	Stick.	Bridge	Lagg.	Frag.	Micro-nucleus	Interphase Micro-nucleus
0.0	6.5 \pm	61.5	0	23.07	15.38	0	0	0	0
0.5	16.45	23.06	38.46	15.38	7.69	7.69	0	7.69	0.08
1.0	20.28	42.85	42.85	7.14	0	0	7.14	0	0.18
5.0	19.92	42.85	21.42	14.28	7.14	14.28	0	0	0.16
10.0	26.50	30.76	30.76	15.38	7.69	7.69	7.69	0	0.28
Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic

Abn: abnormalities, Disturb: Disturbance, Irr: Irregular, Stick: Stickiness, Lagg: Laggard, Frag: Fragment.

The bridges, Fig. 1 (g, l and l) noticed in the cells are probably formed by breakage and fusion of chromosomes and chromatids [46]. According to [20,3,47], the altered chromosomal structure especially vagrant and bridged chromosome due to crude oil can also lead to improper arrangement of the chromosome strands during meiosis with subsequent effects like aneuploidy and cell death. On the other hand, bridge formation due to chromosomal stickiness or due to chromosomal breakage and reunion stated by [44,14]. [33,35] stated that chromosome bridges may be due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to unequal translocation or inversion of chromosome segments.





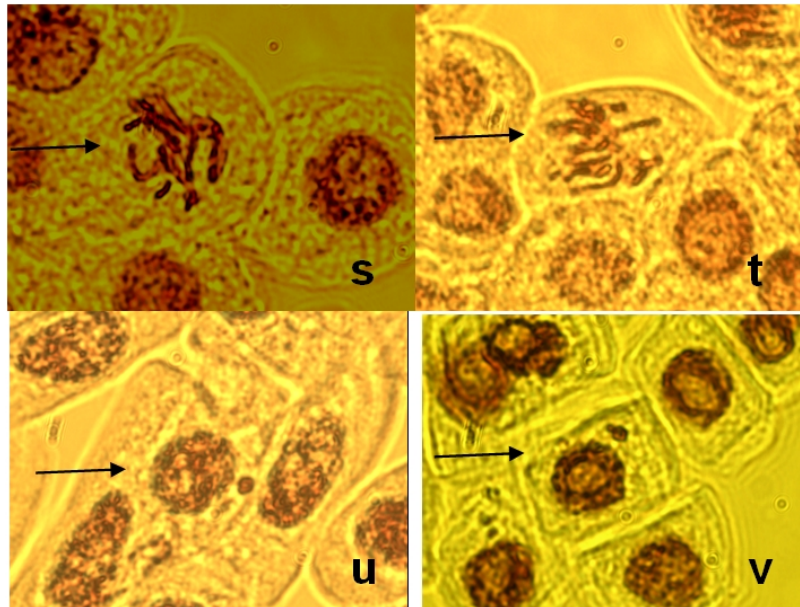


Fig. 1. Different types of chromosome aberrations of *Vicia* root meristems induced by MTBE treatment at different concentrations. (a, b): irregular prophase, (c, d): disturbed metaphase and stickiness, (e, f): disturbed metaphase, (g): disturbed anaphase with laggard and multibridge, (h): early disturbed and sticky anaphase, (i): disturbed and light sticky anaphase, (j, k): severe stickiness in anaphase, (l, m): anaphase with mutibridge, (n): sticky anaphase with mutibridge and laggard, (o, p, q): disturbed metaphase with laggard, (r): sticky and disturbed anaphase with laggard and multibridge, (s, t): sticky and disturbed metaphase with fragments, (u, v): interphase with micronucleus. Magnification is 1000X.

Laggards are a potential source of aneuploidy because they lost the ability to attach by spindle fibers, Fig. 1 (o, p and q); they do not participate to the normal division and cause genetic disequilibriums between daughter cells [13]. [48] stated that the occurrence of chromosome laggards at anaphase was due to the failure of the chromosomes or acentric chromosome fragments to move to either of the pole.

Micronuclei are the results of acentric fragments or lagging chromosomes that fail to incorporate in to either of the daughter nuclei during telophase of the mitotic cells [49,50]. [35] found that micronucleus formation implies loss of genetic materials. Origin of micronuclei from lagging chromosome or form a chromosome fragment was demonstrated by [44,14,18]. Moreover, micronucleus analysis is considered to be one of the most economical, quickest and most effective ways in determining genotoxicity of different chemicals [23,51].

4. CONCLUSION

MTBE treatment caused harmful effects on the root tip cells of *Vicia faba*. The reduction in mitotic activity, the high percentage of chromosome and nuclear irregularities in the meristematic cells of *Vicia faba*, suggests the presence of certain cytotoxic/genotoxic substances in the tested MTBE.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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