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Genotoxicity of Bisphenol A on Root Meristem Cells of *Allium cepa*: A Cytogenetic Approach

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Abstract: The present study was carried out to evaluate the genotoxic effect of a plasticizer, Bisphenol A (BPA). Cytogenetic damaging effect of BPA was examined through chromosomal aberrations (CA) on the root meristem cells of *Allium cepa*. In order to identify the genotoxicity effect, root meristem cells were exposed to BPA at 0.005%, 0.010%, 0.015% and 0.020% for five days. Cells in the interphase and undergoing division were examined to assess the presence of Chromosomal Aberration (CA), Mitotic Index (MI), Mitotic Aberration (MA) and Micronucleus (MN) formation. At a lower concentration, i.e. 0.005% BPA induce mutagenetic alteration in the roots of *A. cepa*. At a higher concentration, i.e., 0.020% BPA inhibits root growth and Mitotic Index (MI). These findings are of concern, since cell damage may be transmitted to subsequent generation, possibly affecting the organism as a whole. If the damage results in cell death, the development of the organism may be affected which could lead to its death. The results are discussed in the paper.

Key words: Bisphenol A, *Allium cepa*, mitotic aberration, chromosomal aberration, mitotic index, plasticizers.

Introduction

Many studies have shown that air, water, soil and food are frequently contaminated with mutagens and cytogenetic compounds, which can reach humans and increase environmental cyototoxic hazards. For this reason, the monitoring of genotoxic compounds in the environment has become an important objective of public health, with the intention of avoiding or minimizing direct and indirect human exposure to these toxic substances (Feretti et al., 2007). Number of potentially mutagenic chemicals have been studied mainly because they can cause damage and inheritable changes in the genetic material (Vogel, 1982). Many studies have shown that environment is frequently contaminated with mutagens and carcinogens and therefore, monitoring of genotoxic compounds has became an important object of public health (Feretti et al., 2007).

Genotoxicity assays are used specifically to evaluate the genotoxicity potential of environmental and industrial effluent sample (Cotelle et al., 1999; Grover and Kaur, 1999). The meristematic mitotic cells of plant roots are appropriate indicator cells for the detection of clastogenicity of environmental pollutants, especially for monitoring of water and soil contaminants. Several endpoints can be monitored in these fast-dividing cells, such as chromosome aberrations, sister chromatid exchanges, and micronuclei. Micronucleus formation is the most frequently used, the most effective, and the simplest indicator of DNA damage (Hala et al., 2007). In order to find hazardous, harmful effect of a substance in different concentration and time of exposure a variety of tests have been employed, such as, cytogenetic test, chromosomal aberration test, comet assay, etc. These tests are commonly used to evaluate the effect of toxic and mutagenic substance in the environment (Carta et al.,

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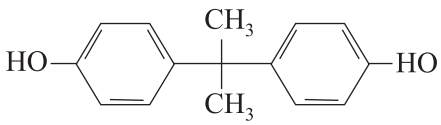
2008). Among the genotoxicity plant tests the *Allium cepa* assay has been considered as an effective test plant to indicate the presence of the mutagenic chemicals as well as commonly used in many laboratories due to its kinetic characteristics of proliferation and chromosomes suitable for these type of study ($2n$ = 16 large chromosomes). *A. cepa* root aberration (AL–RAA) and micronuclei (AL–MCN) tests are widely employed to evaluate genotoxicity of many chemical compounds and environmental pollutants (Feretti et al., 2007).

Bisphenol A (2,2-(4,4-dihydroxydiphenyl)propane), (BPA) is widely used as a starting material for the synthesis of polycarbonate plastics, epoxy resins, polyesters and coatings which have extensive applications in the food packaging industry. Bisphenol A is potentially toxic to most organisms at a low concentration. Bisphenol A has been shown to exert estrogenic effects in animals, but its effects in plants were not known. Although plasticizers are widely used in plastic production as a monomer, but we do not have enough information about their genotoxicity effect in plants (Palani Kumar and Panneerselvam, 2007). Therefore, the genotoxicity potential of the Bisphenol A in the mitotic cells of *Allium cepa* was carried out to determine the effect of BPA on cell division and chromosomal aberration (CA).

Material and Methodology

Bisphenol A (BPA) (2,2-(4,4 dihydroxydiphenyl) propane) is widely used as a starting material for the synthesis of polycarbonate plastics, epoxy resins, polyesters and coatings which have extensive applications in the food packaging industry. The CAS number of Bisphenol A is 80-05-07. Melting point of BPA is 153–157ºC and solubility of BPA in water is 123–300 mg/l at 25ºC. The chemical formula is $C_{16}H_{18}O_2$ and molecular weight is 228.29, which the BPA has obtained from Sigma Aldrich, USA. Since the genotoxic effects of BPA in human and animal were reported, genotoxic activity of BPA has been extensively investigated in plant using *Allium cepa* as a model plant. In the present study, the root tips of *Allium cepa* ($2n$ = 16) are used to test the cytogenetic effect of Bisphenol A (BPA).



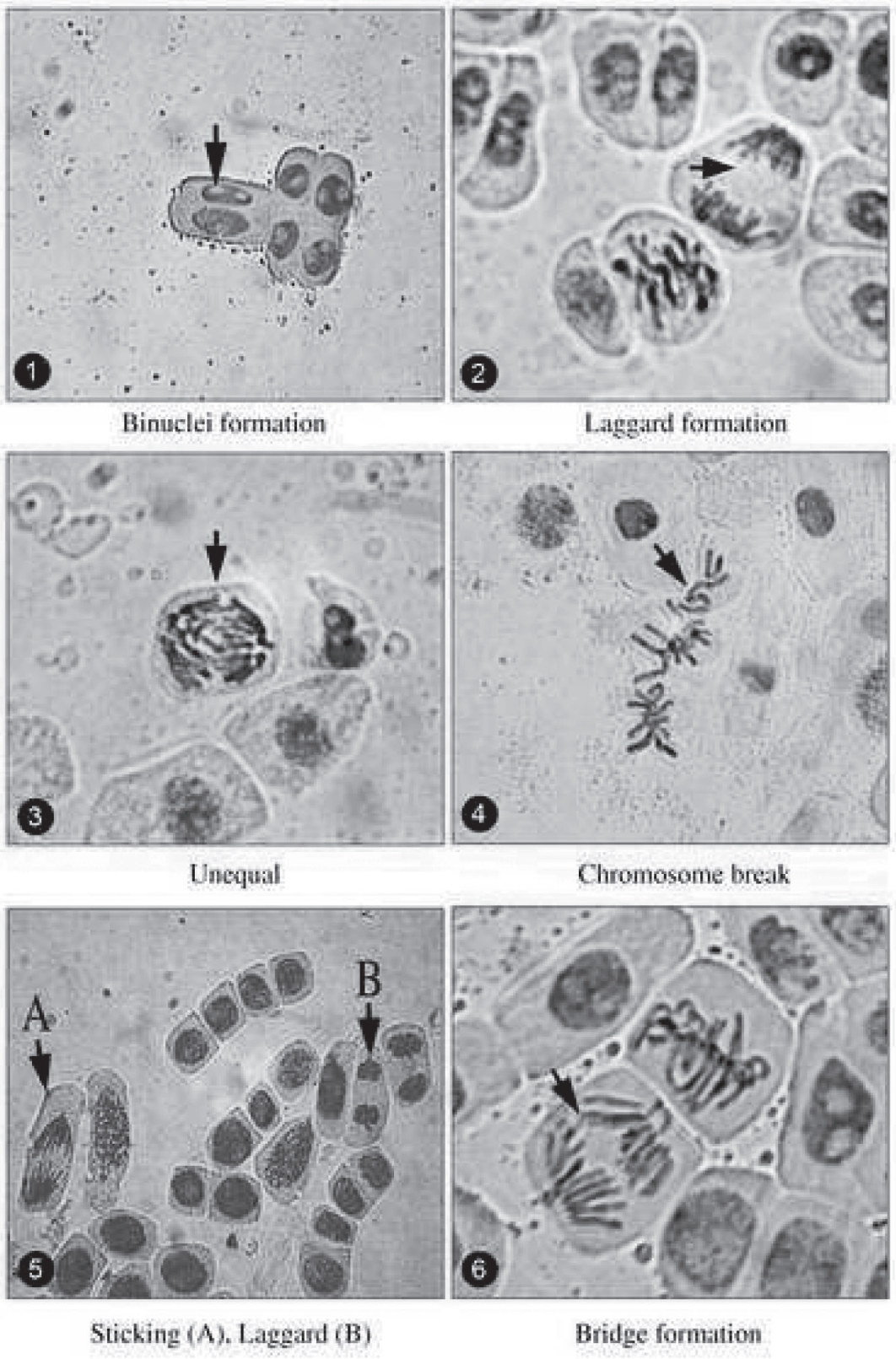
Bisphenol A (2,2-(4,4 dihydroxydiphenyl)propane)

To study the cytotoxic effect five different concentrations were prepared including negative control in deionised water as 0.005%, 0.010%, 0.015%, 0.020%. Five tubes of each concentration of BPA were used. The allium test was carried out as described by Fiskesjo (1985) and last modified by Rank and Nelsen (1993). Commercial onion bulbs were obtained from the local market. Before use, the loose outer scales were carefully removed and dry bottom plates were scraped away without destroying the root primordial. For each concentration five onions were set up and allowed to produce roots. *A. cepa* bulbs were treated with test solution for five days. After completion of five days exposure period few root tips (approximate length 1.5 cm) are sampled for cytogenetic study. Sampled roots were fixed in Carnoy fixative in 3:1 proportion. The root meristems were used for test of chromosomal and nuclear aberration.

Slides were prepared by carefully squashing the material. Meristem previously fixed in carnoy solution (3:1) were washed with distilled water. The material was then hydrolysed in 1N HCl at 60ºC for 5 to 7 minutes. Again material was properly washed 3–4 times with distilled water and immersed in 45% acetic acid for five minutes and stained by Orcen reagent. Stained root tips were observed and analysed under inverted microscope for cytogenetic effect. The selected portions were photographed for study.

Results

Three cytogenetic endpoints from *A. cepa* root cells were verified to evaluate cytogenetic potential of BPA. One parameter of cytotoxicity to assess the mitotic indexes (MI) and two parameters of genetoxicity, i.e., chromsomal abberation (CA) and mitotic abberation (MA) were carried out. Cytogenetic analysis indicates that BPA caused strong genotoxic effect in mitotic cell. To obtain MI approximately 1500 cells (300 cells for each of five slides) were observed for each concentration. Table 1 shows MI for examined cells from different concentration and negative control. The control exhibited the highest MI, while cells exposed to concentration at 0.020% had the lowest average. Table 2 shows the frequency of CA and MA observed with exposure at different concentrations. All concentrations of BPA induce CA in concentration dependent manner though significant frequencies of aberrations were observed. Chromosomal break and fragments were found to be frequent aberrations. BPA also induced MA significantly and dose dependently. Laggards (Figure 2), chromosomal



Figures 1–6: Chromosomal aberrations at interphase and different mitotic stages of *Allium cepa* root meristem cells exposed to different concentrations of Bisphenol A showing: (1) Binuclei formation, (2) Laggard formation, (3) Unequal, (4) Chromosomal Break, (5) Sticking and Laggard formation and (6) Bridge formation.

Table 1: Inhabitation of mitotic index in the root meristem cell of *A. cepa* exposed to BPA for five days

Concentration of BPA in %	*Mitotic Index (MI)*
Negative control	14.92±5.52
0.005	12.20±6.92
0.010	8.14±2.01
0.015	5.02±6.92
0.020	3.45±5.42

Table 2: Chromosome and mitotic aberration in the root meristem cell of *A. cepa* exposed to BPA for five days

Concentrations in %	*Chromosomal aberration (CA)[a] %*		*Mitotic aberration (MA)[b] %*
	% Break	*% Fragments*	
Negative control	0.4	0.3	0.52
0.005	1.9	1.2	10.24
0.010	2.7	1.9	22.74
0.015	5.9	2.7	39.94
0.020	7.2	3.2	52.47

Data obtained from *a* = 200–300 mitotic phase cell, *b* = 400–600 dividing cell and % includes sticking, laggard, bridges, unequal, etc.

stickiness (Figure 5), Bridge (Figure 6) were found to be major MA.

Discussion

The *Allium* test has often been used for the determination of cytotoxic or genotoxic effect of various substances (Grant, 1982; Samaka et al., 1996). It is considered to be a standard procedure for quick testing and detection of toxicity and pollution level in the environment. Result of the *Allium* test may indicate the presence of certain cytotoxic/genotoxic or mutagenic substance in the environment, which represents the direct or indirect risks for all living organisms (El-Shahaby et al., 2003). Findings of the present study reflecting the utility of root meristem cells of *A. cepa* for monitoring of the cytoxicity level of test compound can be determined based on the increase or decrease in the MI (Seth et al., 2008) which can be used as a parameter of cytoxicity in studies of bio monitoring. MI lower than the negative control may indicate that growth and development of exposed organisms have been affected by the compound. The frequencies of chromosomal aberration increase with increasing concentration (Palani Kumar and Paneerselvam, 2007). The difference between concentrations have been significant, when compared with negative control. The most frequent aberrations are chromosomal break, bridge formation, stickiness formation and laggarding chromosome formation.

Inhibition of mitotic activities is often used for tracing cytotoxic substances. An MI decrease below the negative control causes lethal effects on test organisms, while a decrease below 50% (cytotoxic limit value) usually has sublethal effects. With regard to the MI values in Table 1, it is clear that there is reduction in mitotic activity in the root tips of *A. cepa*.

The data presented in Table 2 shows the cytogenic effect of BPA. The number of abnormal cells and aberrations are dependent. The frequency of CA increase with increase in concentration. Same thing happens with the MA. Plant system has a verity of well defined genetic end points and includes decrease in MI, chromosomal aberration, etc. BPA has decreased MI in the treatment group compared with the negative control at all concentrations.

The higher sensitivity of the chromosomal aberration test in *A. cepa* can be explained by the fact that chromosomal aberrations are more closely associated with DNA damage (bridges, laggards), whereas the induction of micronuclei in root meristems of *A. cepa* is the manifestation of chromosome damage and disturbance of the mitotic process. The micronucleus is formed by a new membrane developing around the chromatin matter that failed to move to either pole during the anaphase of mitosis. Such chromatin matter arises either from anomalous disjunction of chromosomes due to spindle abnormalities or from breakage of chromosomes resulting in the formation of acentric fragments, dicentric chromosomes and chromatin bridges. Therefore, the aberration in chromosome suggests that the BPA may have been either spindle inhibitor or a clastogens.

The marked reduction in mitotic activity was registered with increase in concentration from 0.005% to 0.020%. Among the tests carried out with *A. cepa* chromosomal aberration provides important information and may be considered an effective test to investigate the genotoxicity potential of Bisphenol A. Genotoxicity effect of BPA in both end point-like CA/MA are possible.

Significant and dose-dependent inhibition of root growth and MI, as observed in the present study, suggests that the exposure of BPA prevent cells to enter into cell division, which indicates the cytotoxic potential of BPA. The interaction of BPA with the proteins essential for cell cycle progression may be the cause of inhibition of MI.

Reduction in mitotic activity could be due to inhibition of DNA synthesis (Schneiderman et al., 1971; Sudhakar et al., 2001) or blocking in the G2 phase of cell cycle

preventing the cell from entering mitosis (Van't Hof, 1968). Beu et al. (1976) have also shown that exposure of root tips of *V. faba* to high concentrations of the herbicide paraquat has led to inhibition of DNA synthesis. This suggests that BPA may cause inhibition of DNA synthesis (Palani Kumar and Paneerselvam, 2007). Sifa (2005) reported 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 chromosomal segments. Chemicals that induce chromosome breakage are known as clastogens and their action on chromosomes is generally regarded to involve an action on DNA (Grant, 1978; Chauhan et al., 1990). Thus the induction of chromosome breaks by BPA may be independent of its effect on the amount of DNA. Our findings are in agreement with earlier studies which reported inhibition of MI (Seth et al., 2008; El-Shahaby, 2003; Carta et al., 2008; Fiskesjo, 1985; Grover and Kaur, 1999).

In conclusion, as has been stated Bisphenol A has harmful effect on the meristem cells of *A. cepa.* BPA is found at below detectable level in the environmental matrices can have its effect on plants as well as on animal system and, therefore, this type of work can give a first alert of an environmental hazard and a large scale monitoring work using the plant bioassay can give idea to protect ecosystem including human being.

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Contents