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Genotoxicity Induced by Food Coloring Dyes on Meristematic Cells (Root Tips) of Allium Cepa

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ABSTRACT

Food color has a great impact on food consumption and production. Many companies, restaurants and markets use the color perception theory to increase their sales. Recent studies have shown the negative impact of the food colors. So we analyzed the effect of synthetic food colors like orange red, lemon yellow, kesar yellow and apple green on actively dividing root tip cells of Allium cepa. Four different dyes were administered for the treatment of actively dividing root tip cells for 7-day duration along with control. Mitotic analysis clearly revealed the dye induced endpoint deviation like reduction in the frequency of normal divisions in a dose dependent manner. Mitotic divisions in the control sets were found to be normal dye has induced several chromosomal aberrations (genotoxic effect) at various stages of cell cycle such as stickiness of chromosomes, micronuclei formation, precocious migration of chromosome, unorientation, forward movement of chromosome, laggards, and Chromatin Bridge. Among all, stickiness of chromosomes was present in the highest frequency followed by partial genome elimination as micronuclei. The present study suggests that extensive use of synthetic dye should be forbidden due to genotoxic and cytotoxic impacts on living cells. Thus, there is an urgent need to assess potential hazardous effects of these food colors on other test systems like human and nonhuman biota for better scrutiny.

KEYWORDS: *Genotoxicity, Mitotic divisions, Chromosomal aberrations, Unorientation, Chromatin Bridge, Cytotoxicity*

Introduction

Food color is any dye or substance which impact color when added to food. It is used both in commercial and domestic cooking. It also makes food more attractive, appealing, appetizing and informative. Hence people prefer foods decorated with food colors, though artificial food coloring makes food more appealing and attractive but they also contain plenty of chemicals which are not safe for us.

Many food colors haven't been tested enough to determine the long-term dangers. Some studies have shown association to certain types of diseases such as cancers, adrenal failure, bladder failure, allergies etc. The need for avoidance of food color is required because they only cause good perception to food but do not have any nutritive value.

Preservatives, it only makes food attractive as visual aspect is considered to be an important factor for the selection of products. Hence we made an attempt to study four food colors used in common used. They are Orange red (a blend of sunset yellow and carmoisine), Lemon yellow (tartrazine), Kesar yellow (a blend of tartrazine and sunset yellow) and Apple green(a blend of tartrazine and brilliant blue CFC). Allium cepa root tips have been used as test plant to study the cytotoxic and genotoxic effects of food dyes.

Materials and Methods

Food Dyes

The different food colors which are used commonly in household were collected for this experiment. The various food colors which have been used in this experiment are as follows: Orange red (a blend of sunset yellow and carmoisine), Lemon yellow (tartrazine), Kesar yellow (a blend of tartrazine and sunset yellow), Apple green(a blend of tartrazine and brilliant blue CFC) .

Experimental plant

Allium cepa is the experimental plant which was employed. To test the effect of food dyes , the root system of Allium cepa was treated with four food dyes. Dried onion bulbs were not used in this experiment.

Procedure

Sixteen Onion bulbs were allowed to germinate(3 in each dye and 3 in water) in the 250ml beakers with different food colors(100mg)added to 100ml distilled water at room temperature until the roots reached a length of 4-6cm for 1 week. These roots were collected for the squash preparation.

Squash Preparation

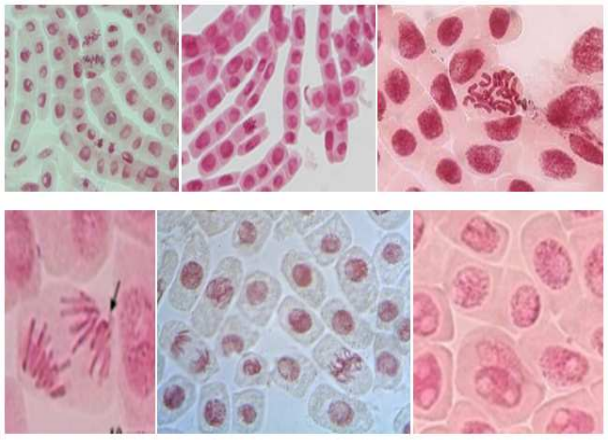
The roots which were collected from the sixteen onion bulbs that have been treated with water and food dyes were analyzed. In the next step, few roots which had average size of 5cm were then dipped in the fixative Carnoy's solution

3:1(ethanol: acetic acid) for some time to allow the fixation of the cells. After cell fixation, the roots were hydrolyzed in HCl-Ethanol(1:1) solution in order to break the cell wall. Then the root tip was cut with the blade and squashed by tapping with the spatula. The roots were further stained by treating of the onion root tips with 2% acetocaramine. The root tip was placed on glass slide and a drop of acetocaramine stain is added again once the squashing is done. The slide is then covered with a cover slip .Excess of stain was removed by the use of blotting paper and the slide was exposed to flame for a while until it was warm. After the squash preparation, these cells were then observed under the compound microscope and for better magnification; oil immersion was applied to the slide. The different stages of mitotic division were then examined under the compound microscope.

Results and Discussions

Allium cepa exhibits species level genomic constitution. Present assessment showed the normal course of mitotic division in the control set, that is, alignment of 16 chromosomes at metaphase and segregation of chromosomes into 16:16 at anaphase. In untreated meristematic cells (root tips) was registered with no chromosomal manifestations. On the other hand, treated sets displayed the considerable range of irregularities during mitosis that were found to be distributed in almost all phases of division, that is, metaphase, anaphase, and telophase.

As a consequence of irregular mitosis, several aberrations were recorded, namely, precocious movement of chromosome, unorientation, C-mitosis, forward movement of chromosome, micronuclei formation at prophase and telophase, chromatin bridge, and stickiness of chromosomes, at metaphase and diagonal anaphase. Among all the aberrations observed, stickiness was registered to be the highest followed by micronuclei formation. Moreover some other abnormalities have also been recorded such as binucleate cell, unequal separation, and fragmentation.



In general, chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material and most often are permanent in nature. Further investigations showed the dominance of micronuclei after stickiness. Occurrence of micronuclei as aberration might be the results of acentric fragments or lagging chromosomes that fail to incorporate into either of the daughter nuclei during telophase of the mitotic cells. Thus, the micronuclei formation at telophase is attributed to genetic loss through genome elimination of chromosomes. Such genome loss plays a significant role in the production of aneuploids when occurring in germinal cells. Several hypotheses have been suggested in an attempt to explain the phenomenon, including inactivation of chromosomes by nuclease, formation of multi polar spindles, asynchrony in nucleoprotein synthesis, genome ratios, spatial separation of genomes, and suppression of centromere function in the eliminated chromosomes, asynchronous cell cycle phases, and asynchronous mitotic and meiotic rhythms. However, more precise explanation is still lacking.

Table 1 presents the occurrence characteristics of normal and disturbed phases of cell cycle during mitotic cell cycles

Table 1

Treatment with	Sample	Prophase	Anaphase	Meta phase	Telophase	Other observations
water	sample 1	+	+	+	+	Cells were normal
	sample 2	+	+	+	+	
	sample 3	+	+	+	+	
red	sample 1	+	-	-	-	Cells found had no nucleus, broken DNA strands, distorted nucleus.
	sample 2	+	-	-	+	
	sample 3	-	-	-	-	
yellow	sample 1	+	-	+	-	Cells found had been elongated, had many nucleus,had blots on nucleus had holes in cell.
	sample 2	+	-	-	-	
	sample 3	-	-	-	-	
orange	sample 1	-	-	+	-	Cells had holes in them, micronuclei were found, DNA was found out side of cells and inside cells.
	sample 2	-	+	-	-	
	sample 3	-	-	-	-	
green	sample 1	-	+	-	-	Cells were shrunk, had blots all over, cells had chromosomes in them Cellwall had holes.
	sample 2	+	+	-	-	
	sample 3	+	-	-	-	

Conclusion

Our study has shown that Food colors show severe cytotoxicity in terms of cell death. So, our present finding clearly depicts the genotoxic and cytotoxic impact of different food colors on actively dividing root tip cells of Allium cepa. This investigation is also in agreement with several previous studies in the literature suggesting that there is an urgent need to assess potential hazardous effects of these food colors on other test systems like human and nonhuman biota for better scrutiny

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