Scientific Research Publishing

Open Journal of Genetics, 2022, 12, 1-10
https://www.scirp.org/journal/ojgen
ISSN Online: 2162-4461
ISSN Print: 2162-4453

Analysis of Genotoxicity Induced by Food Dyes in Root Meristem of *Salvia hispanica* L.

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How to cite this paper: Kumar, G., Pandey, S., Tiwari, N.K. and Pandey, P. (2022) Analysis of Genotoxicity Induced by Food Dyes in Root Meristem of *Salvia hispanica* L. *Open Journal of Genetics*, **12**, 1-10.
https://doi.org/10.4236/ojgen.2022.121001

Received: March 3, 2022
Accepted: March 28, 2022
Published: March 31, 2022

Abstract

Food dyes are chemicals either natural or synthetic that were used by humans to give attractive colors to numerous food products. Colorful food products may cause several health-related issues to us because they may be prepared through synthetic food dyes. The color which is intended to attract people to eat is given to them by the use of some artificial food dyes such as Erythrosine, Allura red, Indigostine, Tartrazine, Sunset yellow, etc. These artificial food dyes are synthetic chemicals that can cause numerous health-related problems if they were taken in by individuals more than the limited amount. Artificial food dyes are becoming very common in use in different ways to provide an attractive and soothing color to food products such as sweets, drinks, candies, etc. The food products we consume with very good and attractive colors are not as good for our health as they appear. Here in this study, we are observing the cytological effect of two different synthetic food dyes (Brilliant blue and Sunset yellow) in the root meristem of *Salvia hispanica* L. (Chia plant) which is a major source of α-linolenic acid, mainly omega 3 fatty acids and dietary fibers found in the seed. For this purpose, germinated seeds of Chia are in the germinator. After the emergence of the young roots, we have given the treatment of the respective dyes of 0.5%, 1%, 1.5% & 2% respectively with a control set. After treatment, the roots were fixed in Carnoy's fixative and preserved in alcohol for further analysis. When the slides of the treated roots were prepared and analyzed we observed that numerous cytological abnormalities such as (stickiness, fragmentation, bridge, laggards, disorientation, etc.) were there in the root cells and the level of abnormalities are increasing with increasing treatment period due to the exposure of them to respective dyes. These results are enough to conclude that the consumption of these dyes is harmful to the health of humans.

Keywords

Cytogenetical Abnormalities, *Salvia hispanica* L., Synthetic Food Dyes

1. Introduction

The coloring of food products is not a very new phenomenon in human civilization. It is an ancient methodology used to enhance the aesthetic value of food products. In ancient times some natural coloring agents are used to give a specific color to food products, but as time passed some synthetic or chemical food dyes are also introduced as colorinagentsnt, taste modulators, and, preservatives. These synthetic food dyes are used for coloring several food products such as sweets, gels, jellies, ice creams, and drinks to enhance their aesthetic value [1].

Azo dyes are compounds characterized by the presence of one or more azo groups (–N=N–) and constitute the most important class of dyes in the textile industry (Kunz *et al.* 2002). The synthetic dyes used for coloring are generally azo compounds having functional azo groups (-N=N-) [2]. Sayed *et al.* [3] suspected that there is a significant correlation exists between n mutagenic effect of an azo group containing food dyes and the triggering of numerous human health problems. The use and intake of food dyes are directly based on acceptable daily intake (ADI), which is based on the results of International Research & recommendations of the Codex Committee on Food Additives and Contaminants (CCFAC) [4].

Based on many studies, ADI concluded that blue followed by yellow, and lastly green, and white color dyes are less harmful even at a high ADI concentration. As these colorants may be harmful, legislative control is increased to restrict the use of harmful color dyes and to monitor the permitted colored food items [5].

Here in this study, we selected two dyes Sunset yellow & Brilliant blue for the experiments. Sunset yellow belongs to Tartrazine and it is an artificial Sunset yellow azo dye, recognized as E102 or C.I. 19140 or FD & C Yellow 5 and used for the coloration of food. It is water-soluble and derived from coal tar [6]. It is most frequently used in coloring soft drinks, flavored chips, sauces, jams, jellies, etc. [7]. In a few countries, it is used as a low-cost alternative to saffron in cooking [8]. According to joint FAO/WHO (1970), Expert Committee on Food Additives established an acceptable daily intake (ADI) for humans of Sunset yellow is 2.5 mg/kg [9]. According to Sasaki *et al.* [10], the effect of dyes is not the same for every individual and it varies according to dose, age, gender, nutritional status, genetic factor, and most importantly time of exposure.

Azo dyes which are more frequently used in numerous edible preparations nowadays could be hazardous due to their adverse effect on living beings, especially in sense of humans. Despite having high esthetic value, the azo dyes could be a serious threat to human health. A few azo dyes are found to be metabolized in the intestine wall and liver producing free aromatic amines which are potentially carcinogenic and mutagenic [11] [12].

Brilliant blue is an organic chemical dye classified under triarylmethane dye [13]. It is used in coloring several food products such as dairy products, sweets in general, and some pharmaceutical and cosmetic products as well. This additive can provoke hyperactivity, allergic reactions, eczema, and asthma, mainly in

children [14]. According to joint FAO/WHO (1970), Expert Committee on Food Additives established an acceptable daily intake (ADI) for humans of brilliant blue is 12.5 mg/kg [15].

Recently the increasing and high uses of synthetic food dyes in several food products have taken the consideration of the scientific community towards itself to assess the benefits as well as harms of these dyes [6]. Concerning this, we also experimented to assess the toxicity level of both dyes, *i.e.* Sunset yellow & Brilliant blue. The experiment is performed on the seeds of *Salvia hispanica* L. commonly known as Chia, which is a plant of the family Lamiaceae and it is an economically and medicinally important plant as its seeds are rich in proteins, fats, dietary fibers, and vitamins [16]. The plant has very a much smaller number of chromosomes *i.e.* 2n = 12 so it is quite easy to study its chromosomal behavior in it.

We had selected a plant species for our experimentation to conclude the cytotoxicity level of these synthetic food dyes as according to Grant (1982), plant bioassays are more sensitive and simple in comparison with animal bioassays and they have been validated in international collaborative studies under the United Nations Environment Program (UNEP), World Health Organization (WHO) and US Environmental Protection Agency (US EPA), and proven to be efficient tests for genotoxic monitoring of environmental pollutants & other chemicals [17] and according to Turkoglu Sifa (2009), the study of the effect of several chemicals on plant mitosis may provide valuable information about possible genotoxicity in mammals and especially in humans [18].

2. Materials & Method

Procurement of seeds: The inbred seeds of *Salvia hispanica* L. were purchased from NutriPlanet Private Limited, Bengaluru-560068, Karnataka, India, and the dyes Sunset yellow and Brilliant blue are acquired from Science Corporation, Prayagraj-211003, UP, India. The rest of the materials and facilities needed for the experimentation were provided by the Naithani Plant Genetics Laboratory, Department of Botany, University of Allahabad-211002, UP, India.

Treatment of germinated seeds through different concentrations of food dyes: First of all, the seeds of *Salvia hispanica* L. were soaked in water for 12 hours, after that soaked seeds were placed in a Petri plate and transferred to the germinator for germination at 25˚C.

Afterward, the germinated seeds were treated with, four different concentrations of dyes prepared *i.e.* 0.5%, 1%, 1.5%, and 2% of Brilliant blue and Sunset yellow. After the solutions of both Sunset yellow & Brilliant blue were prepared, the germinated seeds were kept in all four concentrations for 3 hours with a control set of germinated seeds dipped in water.

***Fixation of roots*:** After three hours of treatment the seeds with their roots were washed with distilled water to remove the excess dyes and then they were fixed in Carnoy's fixative (3:1 Alcohol & Glacial acetic acid respectively) accord-

ing to their respective tubes with different concentrations. After 24 hours they were removed from the fixative and kept in 90% alcohol for further analysis.

Preparation of Mitotic study: The treated roots were first hydrolyzed in 1N HCl for about 40 - 60 seconds in a water bath at 60˚C - 62˚C temperature. After hydrolysis serial washing 4 - 5 times was performed to remove the strains of HCl. Hydrolysis of germinated seeds is a critical step for softening and exhaustion of root tip tissue which accessibility in squash preparation. After washing, they were kept on filter paper to dry them out to remove excess water, after that the roots were kept for staining in 2% acetocarmine for a few minutes. The tips of the roots were cut and placed in the slides and the slides were prepared through the squash technique, post staining. These prepared slides were observed under a Nikon phase-contrast microscope (Eclipse iE200, Japan). The observation is made to calculate the active mitotic index (AMI %) & total abnormality percentage (TAB %) of treated roots and control as well.

Formulae used for calculation: The formula to calculate the active mitotic index (AMI %) and TAB % are as follows:

$$\text{Active mitotic index } (\text{AMI }\%) = \frac{\text{Total number of dividing cells}}{\text{Total number of observed cells}} \times 100$$

$$\text{Total abnormality percentage } (\text{TAB }\%) = \frac{\text{Total number of dividing cells}}{\text{Total number of observed cells}} \times 100$$

Statistical analysis: The statistical analysis was performed using SPSS 16:0 software. One-way analysis of variance (ANOVA) and Duncan's multiple range test ($p < 0.05$) were performed and the graph was plotted using Sigma Plot 10.0 software. The actual mean and standard error were calculated and the data were subjected to analysis.

3. Results & Discussion

The plant *Salvia hispanica* L. is a very famous plant of the family Lamiaceae due to its high nutritional value. The somatic chromosome number of the plant is 2n = 12. In this experimentation, the normal or control seeds grown in distilled water have shown a normal cell cycle with normal metaphase and anaphase stages in it (Figure 1). But the seeds treated with different concentrations of Sunset yellow & Brilliant blue have shown several abnormal stages of the cell cycle (Majorly in Metaphase & Anaphase) including stickiness, fragmentation, bridge, laggards, and disorientation, etc. (Figure 1) The TAB% and AMI of both Brilliant blue & Sunset yellow is represented in Table 1. The AMI of the control set is 12.00 ± 0.37^{a} and it decreased from 11.79 ± 0.38^{a} to 7.41 ± 0.15^{d} and the TAB% increased from 2.18 ± 0.75 to 5.90 ± 0.25 in the case of Sunset yellow while in case of Brilliant blue the AMI decreased from 11.49 ± 0.41^{ab} to 7.34 ± 0.20^{d} and TAB% increased from 2.44 ± 0.17 to 6.17 ± 0.12. Table 1 depicts that in the case of Sunset yellow treatment the level of abnormality (TAB%) is less than the Brilliant blue while AMI is just opposite it.

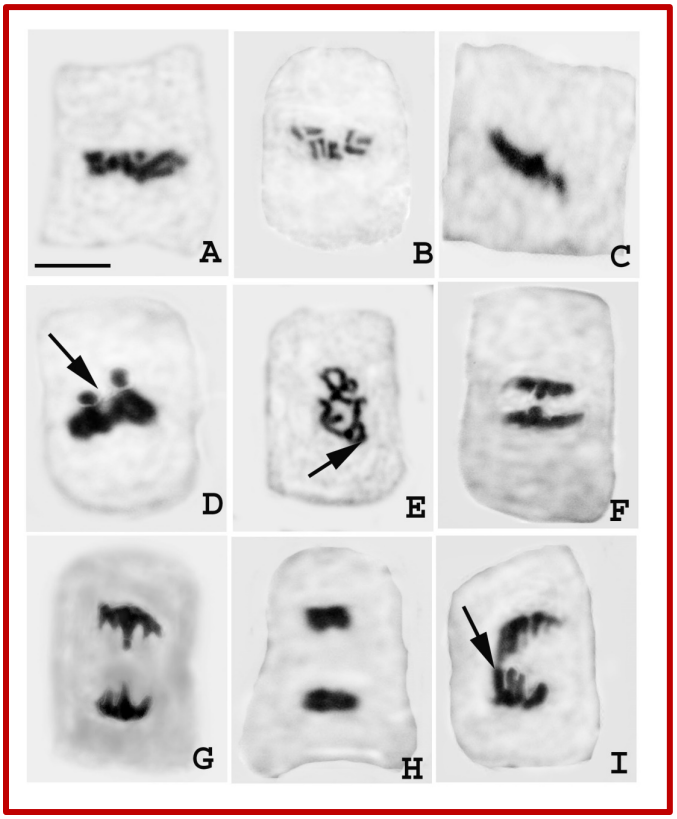

Figure 1. Different types of chromosomal aberrations induced by Food dyes in *Salvia hispanica* L. (A) Normal Metaphase (2n = 12); (B) Scattering at Metaphase; (C) Unorientation at Metaphase; (D) Two precocious chromosomes at Metaphase; (E) Loop formation at Metaphase; (F) Early Anaphase; (G) Normal Anaphase (12:12) separation; (H) Stickiness at Anaphase; (I) Unorientation with bridge formation at Anaphase.

Table 1. A Comparative account of cytological abnormalities induced by Tartrazine and Sunset yellow in root meristems of Chia plants.

Treatment	Concentrations (%)	AMI (%) (Mean ± S.E.)	Metaphasics Abnormalities (%) (Mean ± S.E.)					Anaphasic Abnormalities (%) (Mean ± S.E.)					TAB (%) (Mean ± S.E.)
			Sc	St	Un	Lp	Pr	Sc	St	Br	Pr	Other	-
Sunset yellow	Control	8.28 ± 0.14	-	-	-	-	-	-	-	-	-	-	-
	0.5	7.56 ± 0.30	0.30 ± 0.15	0.14 ± 0.14	0.15 ± 0.15	0.14 ± 0.14	0.29 ± 0.29	0.16 ± 0.16	-	0.29 ± 0.14	-	0.14 ± 0.14	1.49 ± 0.19
	1.0	6.66 ± 0.22	0.14 ± 0.14	0.14 ± 0.14	0.28 ± 0.14	0.15 ± 0.15	0.14 ± 0.14	0.29 ± 0.15	0.42 ± 0.24	0.14 ± 0.14	-	0.15 ± 0.15	1.99 ± 0.15
	1.5	6.09 ± 0.06	0.14 ± 0.14	0.45 ± 0.25	0.14 ± 0.14	0.32 ± 0.32	0.43 ± 0.0	0.16 ± 0.16	0.29 ± 0.14	0.57 ± 0.28	0.14 ± 0.14	0.14 ± 14	2.38 ± 0.14
	2.0	5.39 ± 0.20	0.45 ± 0.26	0.29 ± 0.15	0.29 ± 0.14	-	-	0.14 ± 0.14	0.30 ± 0.15	0.73 ± 0.14	0.44 ± 0.01	0.14 ± 0.14	3.21 ± 0.11
Brilliant blue	Control	8.24 ± 0.31	-	-		-	-	-	-	-	-	-	-
	0.5	7.53 ± 0.12	0.29 ± 0.29	0.28 ± 0.14	0.29 ± 0.29	0.14 ± 0.14	0.43 ± 0.26	-		0.46 ± 0.27	0.14 ± 0.14	_	1.62 ± 0.11
	1.0	6.64 ± 0.21	0.42 ± 0.23	0.44 ± 0.25	0.14 ± 0.13	-	0.43 ± 0.25	0.15 ± 0.14	0.28 ± 0.14	0.15 ± 0.14	0.15 ± 0.14	0.15 ± 14	2.16 ± 0.22
	1.5	5.89 ± 0.29	0.16 ± 0.16	0.59 ± 0.15	0.47 ± 0.28	0.15 ± 0.14	0.15 ± 0.14	0.16 ± 0.16	0.43 ± 0.24	0.60 ± 0.14	0.16 ± 0.16	0.14 ± 0.14	2.89 ± 0.38
	2.0	4.93 ± 0.36	0.46 ± 0.26	0.14 ± 0.14	0.45 ± 0.25	0.44 ± 0.24	0.45 ± 0.26	0.59 ± 0.15	0.15 ± 0.14	0.59 ± 0.13	0.14 ± 0.14	0.30 ± 0.15	3.59 ± 0.30

Note: **Sc**: Scattering; **Pr**: Precocious movement; **St**: Stickiness; **Un**: Un-orientation; **Br**: Bridge formation; **Lg**: Laggard formation; **Fm**: Fragmentations; **Oth**: Others. Means followed by lowercase letters are statistically significant at $p < 0.05$ in Duncan's Multiple Range Test.

The treated root meristems of the roots showed several abnormalities in the case of both the dyes. These abnormalities include both metaphasic (Scattering, unorientation, precocious movement, stickiness, etc.) and anaphasic (Laggards, stickiness, fragmentation, Bridge formation, etc.). The anomalies in both the cases (Brilliant blue & Sunset yellow) are nearly similar but the numbers of cells having the same anomalies are different in both. The numbers of anomalies of the same property are higher in Brilliant Blue while they are lesser in the case of Sunset yellow.

So, based on results obtained in the study both the dyes are harmful and mitodepressive. With the increasing concentration, they have significantly decreased the AMI and increased the TAB% in a dose-dependent manner. The Brilliant blue is more toxic for the cells than Sunset yellow. Both the dyes decreased the mitosis rate in root tips, made the dividing cells prone to anomalous cell divisions, and increased chromosomal aberrations. The data in **Table 1** also depicts that metaphasic abnormalities are more common in comparison to anaphasic abnormalities. The results also ensured that Brilliant Blue is more chromotoxic and mitoinhibitory in comparison to Sunset yellow, the given graph (**Figure 2**) also suggests the same.

The cytological study of the root tips confirms that a higher concentration of food dyes is mitodepressive and chromotoxic to them and this mitodepressive activity may be due to inhibition of DNA replication during the S phase or maybe inhibition of a few other cell cycle regulating proteins such as DNA polymerase, Cdk, and Cyclins, etc. [19] [20].

According to Levan (1938) scattering of chromosomes (**Figure 1(B)**) may be due to spindle dysfunction formed by the loss of microtubules of the spindle fibers, disruption of the spindle fibers leads to the random scattering of the condensed chromosomes in *Allium cepa* L. [21].

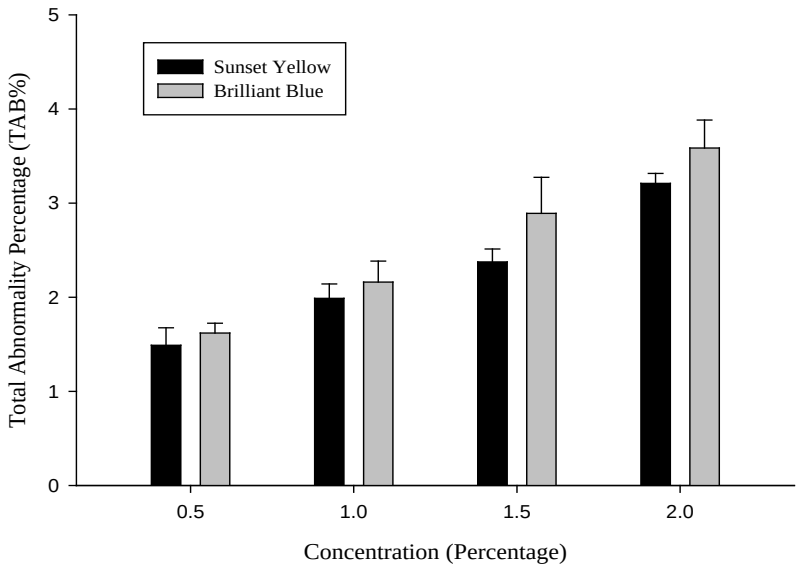

Figure 2. Comparative account of total abnormality percentage of Sunset yellow & Brilliant blue.

An orientation (**Figure 1(C)**) in the cells may be due to the disturbed microtubule orientation or disturbed polarity of the cells.

In this study, the stickiness (**Figure 1(H)**) is observed significantly in root tips treated with both the dyes, according to Gaulden [22] sticky chromosomes may have resulted from defective functioning of one or two types of specific non-histone proteins, involved in the chromosome organization which is needed for chromatid separation and segregation.

Kuras *et al.* [23] concluded that the stickiness of chromosomes in the cells is due to the disturbed balance in the number of histones or other proteins which are responsible for controlling the proper structure of nuclear chromatin. According to another study, the stickiness caused in the cells may be due to the increased chromosomal contraction and condensation [24].

Precocious movement (**Figure 1(D)**) observed in the cells of the treated root tips may be due to early criminalization of the chromosome or due to chemical breaking of the protein moiety of the nucleoprotein backbone [25].

Lagging of chromosome (**Figure 1(C)**) during the anaphase stage of cell division is also a significant anomaly observed in the study and this anomaly in the cells may be due to abnormal spindle formation and depolymerization that's why they failed to carry the chromosomes towards the respective pole [26].

Chromosomal bridges (**Figure 1(I)**) are formed by chromatin fibers that join sister chromatids at metaphase and hold the chromatids together until late anaphase or telophase; if these connections become too strong, chromatids might break at or near the points of connection at anaphase [18]. The azo group dyes present a naphthalene ring connected to a second benzene ring by an azo bond (N=N). Those rings can contain one, two, or three sulfonic groups.

So, the cytotoxicity of these dyes could be related to the azo structure present in the composition of the dyes, which is known to cause mutagenic and carcinogenic effects in the cells. Most of these colorants bind directly to the DNA and cause both structural and numerical anomalies [27]. Bhattacharjee [9] evaluated the mitodepressive effects of Sunset yellow in root tips of *Allium sativum*.

The results related to Sunset yellow and Brilliant Blue dye obtained in the present study indicate that it has antiproliferative activity action and the potential to cause cellular aberrations, which confirms the results obtained by other researchers in other system tests indicating that this food additive has cytotoxic activity [28].

The similar cytotoxic behavior of these food additives was also reported by Donbak *et al.* [29]. Food dyes are envisioned to have paramount effects on living beings because these dyes are components of food ingredients [30].

Kumar & Mishra [31] also concluded that Tartrazine and Sunset yellow are quite harmful to cells as they can disrupt vital functions of the cell and cause numerous cellular anomalies.

4. Conclusion

After the accomplishment and analysis of data obtained through the experimen-

tation, we can conclude that the use of these dyes is quite harmful to human beings and they have enough potential to cause cytological problems as well as a few other problems due to their inhibitory action mechanism. Was undertaken the study that these artificial dyes are potent inhibitors of the cell cycle at a higher concentration as they have significantly reduced the AMI% and enhanced the TAB% of the cells of root apical meristem of our experimental plant *Salvia hispanica* L. By the study, the authors also want to suggest a reduction of the use of these artificial dyes and its products of as well.

Acknowledgements

The authors are extremely thankful to the CSIR-Central Food Technological Research Institute (CFTRI) for providing seeds of *Salvia hispanica* L. and also thankful to all the members of Naithani Plant Genetic Laboratory for cooperation.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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