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Some observations on the genotoxicity of the yellow food dye in *Allium cepa* meristematic cells

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Elena BONCIU[1]

[1]*Department of Agricultural and Forestry Technologies, Faculty of Agronomy, University of Craiova, Craiova, ROMANIA*
Corresponding author: elena.agro@gmail.com

Abstract. Processed foods, such as coloured commercial sweets, can be dangerous to the health of consumers, especially for children, because they hide many artificial food dyes. These dyestuffs do not improve absolutely the taste of the food, but give them the most extraordinary shades to attract children. The purpose of this paper was to observe, from cytogenetic point of view, the influence of one of the commonly artificial food colours (AFCs) used for the colouring of commercially sweets, namely Yellow food dye (YFD), using as a testing plant the species *Allium cepa* (onion). The meristematic roots of *A. cepa* were exposed for 6 hours at 4 different dye concentrations, namely: 1, 3, 5 and 6%, along with an untreated control. The results obtained showed that, especially at high concentrations, YFD induced both a mitodepresive effect in the *A. cepa* meristematic cells and a significant genotoxic effect by the occurrence of some chromosomal abnormalities such as multipolar anaphases, stickiness and C–mitosis. The cytogenetic effect was more pronounced with the increase of dye concentration. These results suggest caution in the consumption of commercial coloured sweets, especially by children, who are attracted by the beautiful colours.
Keyword: YFD, *A. cepa*, mitodepresive, genotoxicity.

Introduction

Generally, substances with colouring properties were extracted from natural sources, but due to high cost and difficulty in incorporating those in modern western technology of processing food, might have resulted in the shift to using synthetic food colours. Today virtually all dyes commercially available are synthetic substance [BHATTACHARJEE, 2014].

AFCs are conceded to be one of the most difficult problems, which may cause some toxicological effects on human being especially on children, who are attracted more towards coloured food items [BHATTACHARJEE, 2014; FENG *et al.*, 2012; OLIVEIRA *et al.*, 2013, YADAV *et al.*, 2013].

AFCs are substances present in processed foods, but the biggest problem is that they are also found in sweets (muffins, candies, lollipops, cakes, etc.) that are very often consumed by children.

Food dyes are also present in fast–food foods, many consumer associations demanding a ban on being used in food. Such toxins are found in carbonated juices, chips, spices and even some types of cheese. Some researchers have found that AFCs used in the food industry lead to increased hyperactivity to children, also causing behavioural disorders [ARNOLD *et al.*, 2012; STEVENS *et al.*, 2011; McCANN *et al.*, 2007]. On the other hand, other researchers suggest that no evidence supports broad claims that food colouring causes food intolerance and ADHD–like behaviour in children [TOMASKA and BROOKE, 2013]. It is possible that certain food colouring may act as a trigger in those who are genetically predisposed, but the evidence is weak [MILLICHAP and YEE, 2012].

The purpose of this paper was to observe, from cytogenetic point of view, the influence of different concentration of some commercially YFD (purchased from a supermarket) in *A. cepa* meristematic cells. *A. cepa* assay is one of the simplest methods for detecting the degree of alterations in the system subjected to chemical causing damage and to describe the effects of these damages by observing chromosomal aberrations [CABUGA *et al.*, 2017; ROSCULETE *et al.*, 2019; BONCIU *et al.*, 2018].

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Material and methods

The YDF was purchased from a supermarket and the following ingredients were listed on the label: glucose syrup, sugar, water, carrageenan (E407), acetic acid (E260), lactic acid (E270), sodium lactate (E325), curcuma dye (E100), potassium sorbate (E202).

Equal sized bulbs were chosen from a local population of the common onion, *A. cepa* L. (2n =16). The biological material was immersed in glasses with water for 72 hours, time required for the meristematic root's occurrence.

When the meristematic roots reached the length of 15–20 mm, they were immersed in dilutions of various concentrations of YFD (1, 3, 5 and 6 %) for 6 hours, at room temperature.

A number of 5 onion bulbs were used for each treatment variant as well as an untreated control that was immersed in tap water.

At the end of the 6 h exposure, the root tips from control and treated samples were excised and fixed immediately in a mixture of 95% ethanol: acetic acid glacial (3:1) for 90 min, hydrolysed in 1N HCl for 5 min at room temperature and stained for 60 min in Schiff reactive.

After staining, the terminal root tips (1.5 mm) were squashed in 45% acetic carmine.

Statistical analysis was done using MS Excel 2007. The analysis of variance (ANOVA) was used to assess the significant differences between the control variant and each treatment.

The differences between treatment means were compared using the LSD–test at a probability level of 0.05 % subsequent to the ANOVA analysis.

Means and standard deviation were calculated from 3 replicates.

The mitotic index was calculated using the following formula:

$$MI\ (\%) = \frac{\text{Total number of cells in division}}{\text{Total number of analysed cells}} \times 100$$

The index of the chromosomal aberrations (CAb) was also calculated:

$$CAb\ (\%) = \frac{\text{Total number of aberrant cells}}{\text{Total number of cells in division}} \times 100$$

Photomicrographs of cells showing chromosomal aberrations as well as showing mitosis were taken using the Kruss microscope.

Results and discussion

Table 1 shows the results of the influence of YDF at concentration of 1, 3, 5 and 6% after 6 h exposure on the MI and on the frequency of CAb. Thus, the MI recorded values amounted to 27.5 % (Ct), 26.4 % (1 % YDF), 24.1 (3%YDF), 18.6% (5% YDF) and 15.8 % (6 % YDF).

Table 1.

Results of the influence of YDF on the MI and on the frequency of CAb

Concentration (%)	Exposure time (h)	MI±SD (%)	CAb (%)		
			MA	S	CM
Control	–	27.5±2.4	0	0	0
1	6	26.4±3.1	0	1.2	0
3	6	24.1±2.7	1.3	1.5	2.7
5	6	18.6±2.2*	5.8	4.3	3.2
6	6	15.8±2.9**	7.5	5.6	4.7

YDF=Yellow dye food; MI=Mitotic index; CAb=Chromosomal aberrations; SD=Standard deviation; MA=multipolar anaphases; S=stickiness; CM=C–mitosis. Data are expressed as Means ±SD, Significant at P ≤ 0.05 (*), P ≤ 0.01 (**) as compared to control variant

The higher mitodepresive effect was found in the treatment of YDF at the concentration of 6%, when MI was 15.8%, i.e. 42.5% lower mitotic activity compared to control variant.

Changes in mitotic activity show that the YDF depressed cellular proliferation in *A. cepa* root tips. The percentage of MI decreased with increasing concentrations of YDF compared with untreated control, which suggest its cytotoxicity in plant test system (Figure 1). Similar cytotoxic effects were described by other

researches following the exposure of plants meristematic roots to different AFCs [BHATTACHARJEE, 2014; OLIVEIRA *et al.*, 2013].

Generally, the cytotoxicity level of some pollutants can be determined by the decreased rate of the mitotic index [SMAKA–KINCL'S *et al.*, 1996].

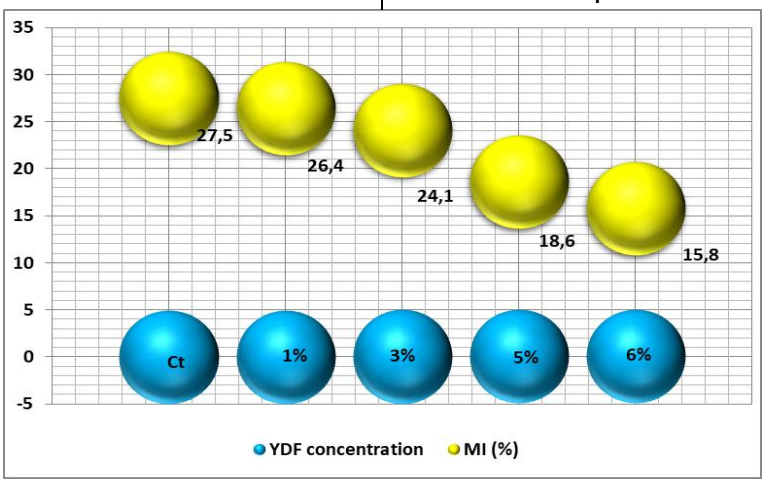

Figure 1. The mitodepresive effect induced by YDF in *A. cepa* meristematic cells

The exposure of biologic material to the tested YDF determined beside MI decrease, a increase of aberrant cells. High rates of Cab were found at 6% concentration followed by 5 and 3% YDF concentration. The CAb consists in multipolar anaphases, stickiness and C–mitosis (Figure 2).

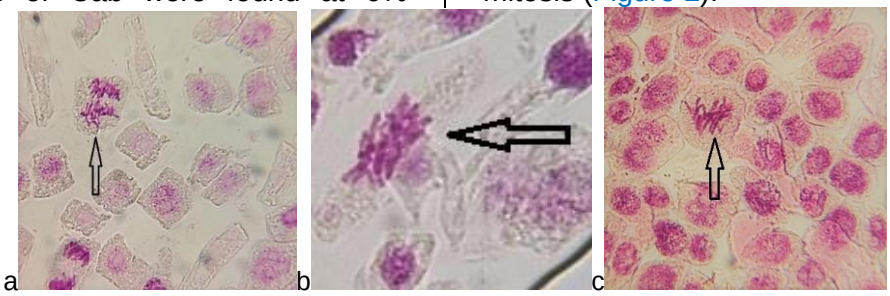

Figure 2. Some CAb identified in *A. cepa* meristematic roots exposure to YDF: multipolar anaphase (a); stickiness (b) and C–metaphase (c)

The number of Cab induced by YDF increased with increasing concentration, especially at 5 and 6%, which represented its mutagenic action in *A. cepa* root tips. Thus, the frequency of multipolar anaphases recorded values between 1.3% (3% YDF), 5.8% (5% YDF) and 7.5% (6% YDF).

At the control variant as well as the 1% YDF treatment variant, has been recorded only one type of chromosomal aberrations, namely stickiness (1.2%).

Also, the frequency of cells with stickiness abnormalities was 1.2% (1% YDF), 1.5% (3% YDF), 4.3% (5% YDF) and 5.6% (6% YDF). Regarding the cells with chromosomal aberrations type C–mitosis, their frequency ranged between 2.7% (3% YDF), 3.2% (5% YDF) and 4.7% (6% YDF). Multipolar anaphases and stickiness were the dominant abnormality induced after treatment, especially at higher concentrations.

Stickiness is an irreversible chromosomal aberration and reflects high toxicity of tested solutions.

Some authors suggested that chromosome stickiness result from inadequate folding of the chromosome fibber into single chromatids [DWIVEDI and KUMAR, 2015].

Compared to control, the stickiness was induced by all concentrations but with a high frequency at the highest concentrations. The presence of multipolar anaphases suggests the

disturbing action of on YDF to normal function of division spindle. Thus, the chromosomes migration to poles of the cells was affected. C–mitosis occurs during the anaphase where one or more detached chromatids is incapable to moving towards the poles.

The results of our study showed that at concentrations of 5 and 6 %, YDF induces a significant cytotoxic and genotoxic effect in *A. cepa* cells. This suggests prudence in the consumption of YDF–coloured foods, as it is generally not known exactly which is the concentration of a food colour agent which has been used in that product.

From this point of view, a healthier alternative for colouring the food in general and sweets in particular is to use natural colorants that can be easily extracted from certain plants. The choice belongs to us. For example, the yellow colour can be obtained from saffron soaked in water and squeezed. The same can be done to get other colours: pink, red: from red beets; purple: from fresh cranberries or red cabbage; green: from spinach, etc. However, the modern science of nutrition and numerous biotechnological findings suggest that the diet is one of the most important environmental factors affecting the health and the quality of life of every person [BUTNARIU *et al.*, 2013]. The results of many researchers suggest safe use of different natural sources for dyes to avoid some health problems for consumers and especially for children [BUTNARIU and GIUCHICI, 2011; BUTNARIU, 2014]. The clear purpose of biotechnological studies is improving the quality of life [ROSCULETE et al., 2018; BONEA and URECHEAN, 2015; BONEA, 2016; BONEA and BONCIU, 2017; BONEA and URECHEAN, 2018; BUTNARIU, 2012; BUTNARIU *et al.*, 2016]. Higher plants provide a useful genetic system for screening and monitoring the potency of environmental damage [DWIVEDI and KUMAR, 2015; ROSCULETE *et al.*, 2019; BONCIU *et al.*, 2018].

Conclusions

The experiment demonstrates the harmful effect of YDF on *A. cepa* meristematic cells, but only at high concentrations of 5 and 6 %. At concentrations of 1 and 3 % these effects were insignificant. These results suggest caution in the consumption of commercial yellow coloured sweets, especially by children, who are attracted by the beautiful colours. A healthier alternative can be represented by the home–made sweets and their colouring with natural herbal extracts. Also, the study demonstrates once again that *A. cepa* it can be a valuable biosensor for in situ screening the cyto–genotoxicity potential of some AFCs.

References

1. Arnold, L.E.; Lofthouse, N.; Hurt, E. Artificial Food Colors and Attention–Deficit/Hyperactivity Symptoms: Conclusions to Dye for. *Neurotherapeutics,* **2012**, 9(3), 599–609.
2. Bhattacharjee, M. Evaluation of mitodepressive effect of Sunset Yellow using *Allium sativum* Assay. *International Journal of Science, Environment and Technology*, **2014**, 3(3), 1120–1130.
3. Bonciu, E.; Firbas, P.; Fontanetti, C.S.; Wusheng, J.; Karaismailoğlu, M.C.; Liu, D.; Menicucci, F.; Pesnya, D.S.; Popescu, A.; Romanovsky, A.V.; Schiff, S.; Ślusarczyk, J.; De Souza, C.P.; Srivastava, A.; Sutan, A.; Papini, A. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. *Caryologia*, **2018**, 71(3), 191–209.
4. Bonea, D. Effect of the aqueous extracts of *Amoracia rusticana* L. on the seed germination and seedling growth of *Zea mays* L. under drought stress. *Annals of the University of Craiova–Agriculture, Montanology, Cadastre Series*, **2016**, XLVI(1), 56–61.
5. Bonea, D.; Urechean, V. Effect of Sweet Marjoram (*Origanum majorana* L.) Cogermination and Aqueous Extracts on Maize (*Zea mays* L.). *Agriculture for Life, Life for Agriculture, Conference Proceedings*, **2018**, 1(1), 11–16.
6. Bonea, D.; Urechean, V. The evaluation of heterosis for Romanian maize germplasm. *Pakistan Journal of Botany*, **2015**, 47(6), 2387–2390.
7. Bonea, D.; Bonciu, E. Cytogenetic effects induced by the fungicide Royal Flo to

maize *(Zea mays L.)*. *Caryologia*, **2017**, 70(3), 195–199.
8. Butnariu, M. Detection of the polyphenolic components in *Ribes nigrum* L. *Annals of Agricultural and Environmental Medicine*, **2014**, 21(1), 11–14.
9. Butnariu, M. An analysis of *Sorghum halepense's* behavior in presence of tropane alkaloids from *Datura stramonium* extracts. *Chemistry central journal,* **2012**, 28;6(1):75.
10. Butnariu, M.; Sarac, I.; Pentea, M.; Samfira, I.; Negrea, A.; Motoc, M.; Buzatu, A.R.; Ciopec, M. Approach for Analyse Stability of Lutein from *Tropaeolum majus*. *Revista de chimie*, **2016,** 67(3), 503–506.
11. Butnariu, M.; Caunii, A. Design management of functional foods for quality of life improvement. *Annals of Agricultural and Environmental Medicine*, **2013**, 20(4), 736–741.
12. Butnariu, M.V.; Giuchici, C.V. The use of some nanoemulsions based on aqueous propolis and lycopene extract in the skin's protective mechanisms against UVA radiation. *Journal of Nanobiotechnology*, **2011**, Volume: 9, Article Number: 3.
13. Cabuga Jr., C.C.; Abelada, J.J.Z.; Presilda, C.J.R.; Havana, H.C. *Allium cepa* test: An evaluation of genotoxicity. *Proceedings of the International Academy of Ecology and Environmental Sciences*, **2017**, 7(1), 12–19.
14. Dwivedi, K.; Kumar, G. Genetic Damage Induced by a Food Coloring Dye (Sunset Yellow) on Meristematic Cells of *Brassica campestris* L. *Journal of environmental and public health*, **2015**, (2):1–5.
15. Feng, J.; Cerniglia, C.E.; Chen, H. Toxicological significance of azo dye metabolism by human intestinal microbiota. *Frontiers in Bioscience (Elite Edition),* **2012**, 1(4), 568–86.
16. McCann, D.; Barrett, A.; Cooper, A. et al. Food additives and hyperactive behavior in 3–year–old and 8/9–year–old children in the community: a randomised, double–blinded, placebo–controlled trial. *Lancet,* **2007**, 370:1560–1567.
17. Millichap, J.G.; Yee, M.M. The diet factor in attention–deficit/hyperactivity disorder. *Pediatrics*, **2012**, 129(2), 330–337.
18. Oliveira, M.V.A.; Alves, D.D.L.; Lima, H.G.de M.; Castro, J.M.; Peron, A.P. Cytotoxicity of erythrosine (E–127), brilliant blue (E–133) and red 40(E–129) food dyes in a plant test system. *Acta Scientiarum. Biological Sciences Maringá*, **2013**, 35(4), 557–562.
19. Rosculete, C.A.; Bonciu, E.; Rosculete, E; Olaru, L.A. Determination of the Environmental Pollution Potential of Some Herbicides by the Assessment of Cytotoxic and Genotoxic Effects on *Allium cepa*. *International Journal of Environmental Research and Public Health*, **2019**, 16(1), 75.
20. Rosculete, E.; Bonciu, E.; Rosculete, C.A.; Teleanu, E. Detection and Quantification of Genetically Modified Soybean in Some Food and Feed Products. A Case Study on Products Available on Romanian Market. *Sustainability,* **2018**, *10(5),* 1325.
21. Smaka–Kincl's, V.; Stegnar, P.; Lovka, M.; Toman, M.J. The evaluation of waste, surface and ground water quality using the *Allium* test procedure. *Mutation Research,* **1996**, 368, 171–179.
22. Stevens, L.; Kuczek, T.; Burgess, J.R.; Hurt, E.A.; Arnold, L.E. Dietary sensitivities and ADHD: 35 years of research. *Clinical pediatrics,* **2011**, 50, 279–293.
23. Tomaska, L.D.; Brooke–Taylor, S. Food Additives–General. In Encyclopedia of Food Safety, Vol 2: Hazards and Diseases. *Eds, Motarjemi Y et al. Academic Press*, **2013**.
24. Yadav, A.; Kumar, A.; Tripathi, A.; Das, M. Sunset yellow FCF, a permitted food dye, alters functional responses of splenocytes at non–cytotoxic dose. *Toxicology Letters,* **2013**, 217(3), 197–204.

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