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Caffeine induced genotoxic effects in *Phaseolus vulgaris* L. and *Raphanus sativus* L.

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ABSTRACT: The cytogenetic effects induced by two caffeine concentrations (0.1%, 0.5%) in root meristematic cells of plants belonging to two species of economic importance *Phaseolus vulgaris* and *Raphanus sativus* are described in this article. Mitotic index, frequency and type of ana-telophase chromosome aberrations, as well as the frequency and categories of metaphase abnormalities were comparatively analyzed for the two species. The results showed that caffeine has genotoxic potential; it induces important alterations at the level on genetic material. The maximum tested concentration (0.5% caffeine) provided the most complex pattern of ana-telophase aberrations, especially in radish. *R. sativus* genotypes presented a higher sensibility to the caffeine action.

Key words: caffeine, chromosome aberrations, genotoxicity, *Phaseolus vulgaris*, *Raphanus sativus*

Abbreviations: PI - prophase index; MI - metaphase index; AI - anaphase index; TI - telophase index; TC - total analyzed cells (dividing and non-dividing); A-T – ana-telophases; TDC - total dividing cells; $A\text{-}T_{abr}\%$ - percentage of ana-telophase aberrations; $M_{abn}\%$ - percentage of metaphase abnormalities.

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INTRODUCTION

The bean, *Phaseolus vulgaris* L. (Leguminosae) (2n=22) is a highly polymorphic species; annual herb with special economical features. Beans are a high nutritive, relatively low-cost protein food. They are an important and inexpensive source of protein, dietary fibre and starch for a large part of the world's population, mainly in developing countries. For example, in Mexico, Brazil, Burundi, Rwanda, Kenya, Tanzania, the beans are the primary source of protein in human diets. This plant contains important levels of protein, lipids, carbohydrates, minerals (Ca, P, Fe, and Zn), vitamins (thiamine, riboflavin, nicotinic acid, ascorbic acid), crude fibre (5-7 g/100 g, depending on bean variety). Common bean represents one of the best non-meat sources of iron, providing 23–30% of daily recommended levels of this element from a single serving (Shimelis & Rakshit 2005). The bean medicinal uses are multiple. Initially, it was utilized as folk remedy for a variety of ailments, especially coughing due to consumption or bronchitis. Later on, plant parts were administered as adjuvant in diabetes or as kidney antiseptic, but also they were used in the treatment of acne, eczema, burns, cardiac troubles, rheumatism, arthritis and sciatica, because of emollient, carminative and depurative properties of this leguminous plant. Recently, its use has focused on its ability to block starch absorption.

The radish, *Raphanus sativus* L. (Cruciferae) (2n=18) is an herbaceous vegetable, cultivated especially for its edible roots which in fresh state are used in food. Root contains 5-10% dry matter, 0.8-4.0 % sugars, 0.8-1.3 % proteins, amino acids, vitamins C, B_1, carotene, essential oils, and glycosides giving specific pungency**.** Raphanin is a radish component with antibacterial and antifungal properties.

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The cultivated radish is used in the treatment of troubles caused by various intestinal parasites, though the part of the plant used is not specified. The leaves, seeds and old roots are used in the treatment of asthma, while the juice of the fresh leaves is diuretic and laxative. The seeds are carminative, diuretic, expectorant, laxative and stomachic, and the root has antiscorbutic, antispasmodic, astringent, cholagogue, digestive and diuretic effects. It is crushed and used as a poultice for burns, bruises and smelly feet.

Due to the great economic value of common bean and cultivated radish, it is necessary to enlarge the genotype and phenotype diversity, to optimize some desired traits and to obtain new valuable genotypes by various ways, including chemical mutagenesis. For this reason, the objective of the present study is to analyze the effects induced by a chemical stressor with mutagenic potential: caffeine. The approach of the study of cytogenetic effects induced by caffeine is also justified by the presence of this substance in tea and in coffee, the most widely consumed beverages, caffeine being suspected as having a potential genetic risk in human (Sax & Sax 1966).

MATERIAL AND METHODS

Seeds of common bean (*P. vulgaris*) and cultivated radish (*R. sativus*) were subjected to caffeine treatment for 3 hours. Two caffeine concentrations were tested: 0.1% and 0.5%. From chemical point of view, caffeine ($C_8H_{10}N_4O_2$) (molecular weight=194.19 g/mol) is a purine alkaloid, from methyl-xanthine chemicals' group, together with theophylline and theobromine.

Seed germination took place in Petri dishes, on moist filter paper, in dark. The fixation of root tips (10-15 mm) was done for about 24 hours in ethylic alcohol/acetic acid, 3:1 mixture, at room temperature. After 10 min of hydrolysis in 50% HCl, the plant material was stained in modified charbol fuchsin solution (Gamborg & Wetter 1975). For each variant, five slides were prepared according squash method, in 45% glacial acetic acid, and 10 microscopic fields were microscopically analyzed on every slide. A Nikon Eclipse 600 light microscope was used for this analysis. Photos were taken with a Nikon Cool Pix 950 digital camera, at 1600x1200 dpi resolution.

The different phases of mitosis were counted to calculate the mitotic index (MI) and phase indices, as following:

Mitotic Index = TDC x100/TC
PI% = prophase cells x 100/TDC
MI% = metaphase cells x 100/TDC
AI% = anaphase cells x 100/TDC
TC% = telophase cells x 100/TDC, where

TC = total cells (dividing and non-dividing), and TDC = total dividing cells.

The percentages of ana-telophase aberrations (A-T_{abr}%) and metaphase abnormalities (M_{abn}%) were also calculated:

A-T_{abr} %= A-T_{abr} x 100/TDC
M_{abn} %= M_{abn} x 100/TDC

Table 1. Behaviour of cytogenetic parameters in root tip meristems of *Phaseolus vulgaris* L. and *Raphanus sativus* L. seedlings, after caffeine treatment

Species	Variant	Total cells*	Dividing cells*	Mitotic index* (%)	Indices of mitotic phases*					A-T_{abr} (%)						M_{abn} (%)	
										types						types	
					PI%	MI%	AI%	TI%	Total	bridges	expulsed	laggards	multipolar	complex	Total	C-metaphases	expulsed
Phaseolus vulgaris L.	Control	1894±192.1	94.8±5.3	5.11±0.3	42.03±3.2	27.39±2.6	19.63±1.8	10.92±1.3	7.38	2.95	1.47	1.05	0.41	0.82	8.22	2.32	5.90
	0.1%	1832±194.3	116.4±17	6.83±1.1	36.54±3.2	30.34±3	21.84±1.1	11.25±0.7	7.56	3.95	1.54	0.51	0.17	0.68	10.48	6.02	4.46
	0.5%	2161.8±103	72.6±12.6	3.29±0.4	43.58±5.5	23.42±4.1	22.63±1.9	10.34±0.6	3.58	0.82	1.10	0.27	0.00	1.37	7.16	4.13	3.02
Raphanus sativus L.	Control	1115±182.8	56.2±11	5.00±0.6	50.09±3.8	24.15±3.3	14.71±1.6	11.02±2.1	4.27	1.78	0.71	1.06	0.35	0.35	6.04	1.06	4.26
	0.1%	999±70.1	45.8±5.1	4.83±0.7	44.45±3.1	24.88±1.8	17.74±3.4	12.89±0.7	6.11	1.31	1.31	1.31	0.87	1.31	9.06	3.05	5.23
	0.5%	993.2±104	54.2±9.3	5.30±0.4	41.45±4.2	28.09±2.3	20.06±2.3	10.35±2.9	14.02	5.16	2.58	1.47	0.73	2.16	8.85	2.21	5.53

*mean ± standard error (x±Sx)

RESULTS AND DISCUSSION

Caffeine is a purine derivative and like to other analogues of nitrogenous bases it can be incorporated in DNA macromolecule generating grave troubles. At a new replication, the initial nitrogenous base will be substituted by an analogue base, because of the incorporation errors. Being an analogue of adenine, caffeine can sometimes be incorporated into a growing DNA chain, instead of this nitrogenous base.

The studies on the effects produced by caffeine in various biological systems not led to identical results, some of these being even contradictory. In relation to mutagenicity, even the results for the same organism in literature are occasionally antagonistic (Laranja *et al.* 2003). Some authors evidenced the genotoxic and mutagenic potential of caffeine in bacteria, fungi, insects, plants and in human tissue culture cells (Sax & Sax 1966; Raicu & Stoian 1967; Tudose & Filimon 1972–1973; Kaul & Zutshi 1973; Hernandez *et al.* 1986). Sax & Sax (1966) also noted the radiomimetic effect of caffeine from tea, coffee and Coca Cola on plant chromosomes. Itoyama & Bicudo (2000) sustained that in *Drosophila* sp. caffeine has an inhibitive effect on DNA repair, but it does not determine an increase of micronuclei number.

Regarding the caffeine mechanism action, it is possible that this compound acts as solubilizing factor and form molecular complexes thus favouring the generation of chromosome aberrations. Also, this substance can interact with DNA and alter some of its physical properties (denaturation temperature, for example), fact that determines a higher rate of spontaneous mutations.

Mitotic index. Concerning the behaviour of mitotic index (Tab. 1, Fig. 1) the individuals belonging to the two examined species displayed different profiles of this cytogenetic parameter. The caffeine-induced effects were more marked for bean mitotic index. So, for *P. vulgaris*, the minimum tested concentration (0.1% caffeine) determined the stimulation of mitosis with 33%, comparatively with control, while 0.5% caffeine exerted an inhibitory effect, materialized in the decrease of dividing cells with approximately 35%.

In *R. sativus* roots, the amplitude of caffeine effects was reduced, the average values of mitotic index being close to those of control (4.83% for 0.1% caffeine, and 5.3% for 0.5% caffeine, compared to 5.00% for control). The mitosis phases showed in all variants the following decreasing order: prophase>metaphase>anaphase>telophase.

Chromosome aberrations in mitotic ana-telophases. In *P. vulgaris*, at 0.1% caffeine, we have not found significant differences compared to control (Tab. 1; Fig. 2). In 0.5% caffeine, the number of ana-telophase aberrations produced by caffeine was low (in this variant, the number of dividing cells and mitotic index registered the lowest level), but it must be noted that the complex aberrations (multipolar ana-telophases with bridges, ana-telophases with lagging and expulsed chromosomes and chromosome etc.) represented the major part of ana-telophase aberrations. They have more severe repercussions at genetic level and on subsequent plant growth and development.

In *R. sativus*, in both variants higher levels of ana-telophase aberrations were registered than in control (6.11% for 0.1% caffeine, and 14.02% for 0.5% caffeine,

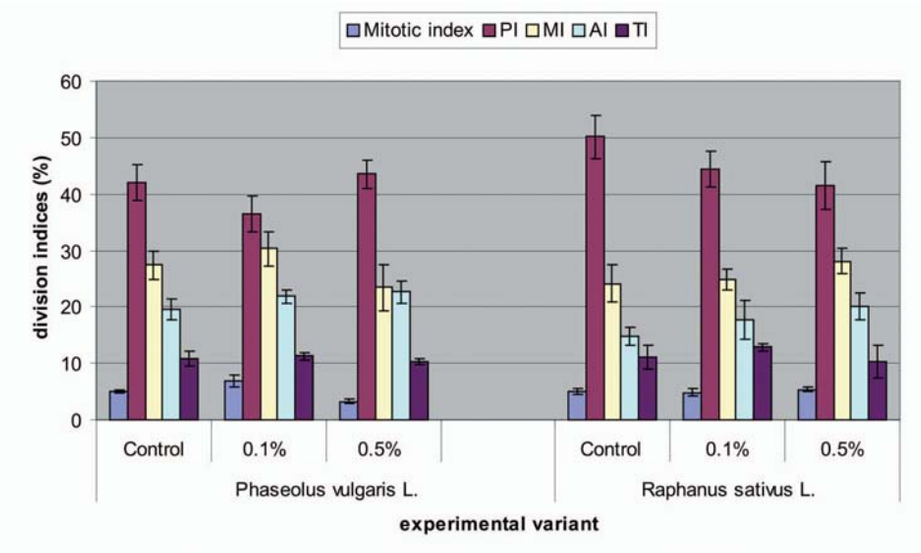

Fig. 1. Mitotic index and frequency of division phases in root tip meristems of *Phaseolus vulgaris* L. and *Raphanus sativus* L. seedlings, after caffeine treatment

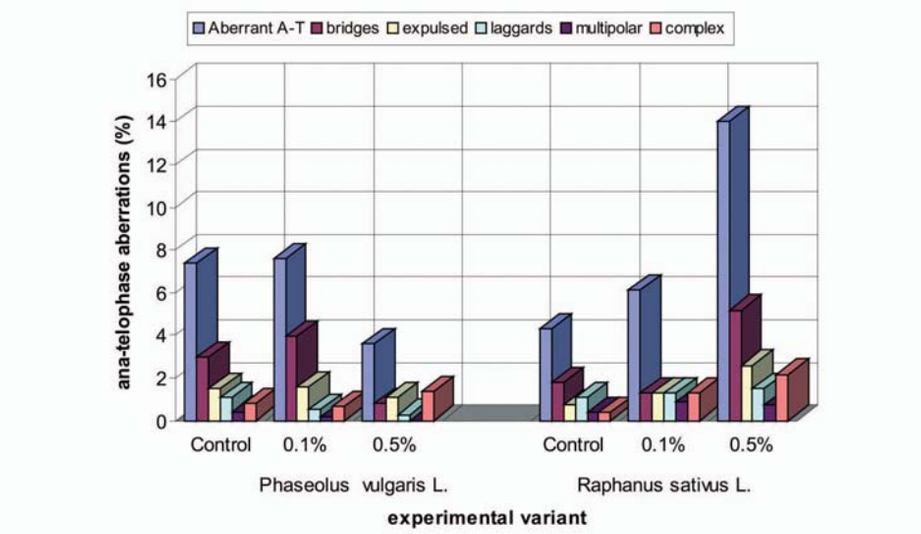

Fig. 2. Frequecy of ana-telophases with chromosome aberrations and of aberration types, in root tip meristems of *Phaseolus vulgaris* L. and *Raphanus sativus* L. seedlings, after caffeine treatment

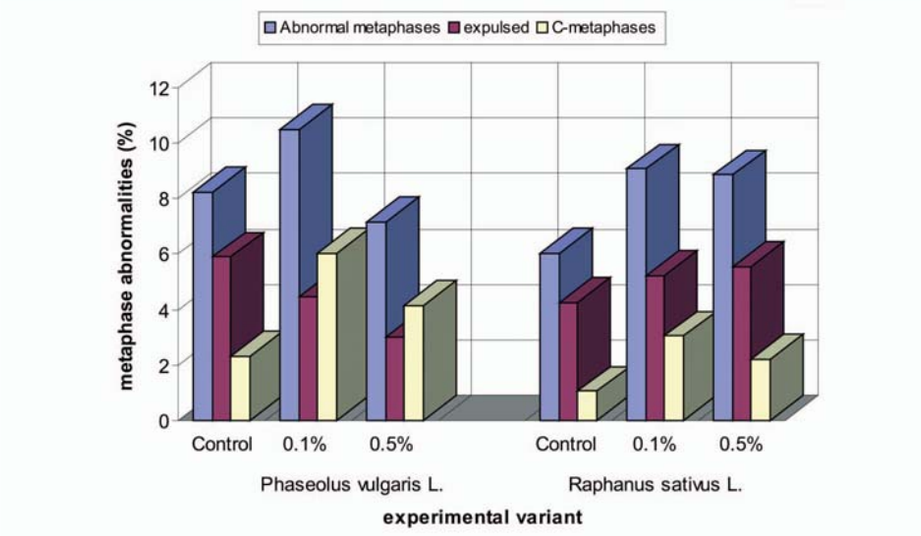

Fig. 3. Frequency and types of metaphase abnormalities, in root tip meristems of *Phaseolus vulgaris* L. and *Raphanus sativus* L. seedlings, after caffeine treatment

comparative to control value 4.27%). The radish seed treatment with 0.5% caffeine determined the most complex profile of chromosome aberrations and of other types of mitosis troubles. For example, out of main categories of chromosome aberrations, the presence of ana-telophases with polar deviations, binucleate cells, zones with chromatin lysis, and of polyploid cells (2n=4x=36) was evidenced. Binucleate cells were also present in bean at the same caffeine concentration (0.5%). Another situation observed in caffeine treated radish material is the chromosome stickiness. This chromosome state can be the result of reaction of caffeine with DNA, fact causing DNA-DNA or DNA-protein cross linking (Amin 2002). Bennett (1977) considered that sticky chromosomes might also be the result of incomplete replication of chromosomes by defective enzymes. According to Zellnig *et al.* (2000), sticky chromosomes are indicatives of a highly toxic usually irreversible effect, leading to cell death.

Generally, the categories of chromosome aberrations established the next decreasing order: bridges, expulsed chromosomes from ana-telophase groups, laggards, and multipolar ana-telophases. The bridges are the result of the fusion of two chromosomes which possibly supported terminal deletions, these aberrations being visible between the two chromatidic groups separated to the cell poles. Lagging chromosomes lost their ability to attach by spindle

fibres; they do not participate to the normal division and cause genetic disequilibrium between daughter cell. A significant increase of bridges (5.16%), lagging chromosomes (1.47%) and ana-telophases with expulsed chromosomes (2.58%) was registered in radish, at 0.5% caffeine concentration, comparatively with control.

The metaphases with abnormal configurations. (Tab. 1, Fig. 3), mainly represented by metaphases with expulsed chromosomes from equatorial plate and C-metaphases (configurations similar to those colchicine-treated) showed higher levels in 0.1% caffeine treated bean and in the two radish variants, comparatively with control. The cells with C-metaphases surpassed the control values, both in bean and radish, in all caffeine treated variants. This comfirms the noxious effect of this substance on spindle fibres and the perturbation of the chromosome moving to the cell poles. In radish metaphases, the expulsed chromosomes increased as caffeine concentration increased.

Our previous studies on effects induced by caffeine and some phenoxy-methyl-xanthinic derivatives on hemp and onion nuclear genetic material (Truță *et al*. 2000, 2009) are generally in accordance with the results presented in literature, in the sense of the confirmation of mutagenic effect of caffeine, by altering the chromosome structure in investigated plants. The amplitude of modifications was in relation with the genotype and the tested concentration. The results of the present study confirmed the genotoxic potential of this chemical compound in other two species of economic interest *P. vulgaris* and *R. sativus* Radish genotypes generally manifested a higher sensibility to the caffeine action materialized in the increased number and the severity of metaphase and ana-telophase aberrations.

CONCLUSIONS

Concerning the influence on mitosis division, caffeine had more pronounced effects in bean; it was stimulant at low concentration and inhibitive at high concentration. Caffeine proved genotoxic potential for the tested biological material; it induced important alterations at genetic level. The amplitude of caffeine effects was in relation to caffeine concentration and studied genotypes. The maximum tested concentration (0.5% caffeine) induced the most complex pattern of ana-telophase aberrations, especially in radish. *R. sativus* genotypes showed a higher sensibility to the caffeine action.

REFERENCES

Amin AW. 2002. Cytotoxicity testing of sewage water treatment using *Allium cepa* chromosome aberration assay. *Pakistan J. Biol. Sci.* **5**: 184–188.

Bennett MD. 1977. Heterochromatin, aberrant endosperm nuclei and grain shriveling in wheat-rye genotypes. *Heredity* **39**: 411-418.

Gamborg OL & Wetter LR. 1975. Plant tissue culture methods. National Research Council, Saskatoon, Canada

Hernandez P, Mingo R, Gonzalez-Fernandez A & Lopez-Saez JF. 1986. Relationship of chromosomal damage induced by caffeine to growth temperature and ATP level in proliferating cells. *Mutat. Res.* **164**: 327-333.

Itoyama MM & Bicudo HEMC. 2000. Effect of stannous chloride combined with caffeine on fecundity of *Drosophila prosaltans*. *Genet. Molec. Biol.* **23**: 105-107.

Kaul BL & Zutshi U. 1973. On the production of chromosome breakage in *Vicia faba* by caffeine. *Cytobios* **7**: 261-264.

Laranja AT, Manzatto AJ & Bicudo HEMC. 2003. Effects of caffeine and used coffee grounds on biological features of *Aedes aegypti* (*Diptera*, *Culicidae*) and their possible use in alternative control. *Genet. Molec. Biol.* **26**: 419-429.

Raicu P & Stoian V. 1967. The influence of some purine derivatives on the chromosomes and on the mitotic cycle in *Vicia faba*. *Caryologia* **20**(4): 317-322

Sax K & Sax HJ. 1966. Radiomimetic beverages, drugs, and mutagens. *Proc. Natl. Acad. Sci. U.S.A.* **55**: 1431-1435.

Shimelis EA & Rakshit SK. 2005. Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *J. Food Sci. Technol.* **38**: 331-338.

Truță E, Băra II, Maniu M & Tudose C. 2000. The effects induced by some 8-(4R)-phenoxy-methyl-xanthinic compounds on cytogenetical parameters in *Allium cepa* L. *An. Ști. Univ."Al. I. Cuza" Iași, Sect. 2.a, Genet. Biol. Molec.* **1**: 43-49.

Truță E, Surdu S, Olteanu Z, Zamfirache MM & Oprică L. 2009. Cytogenetic effects induced by caffeine in *Cannabis sativus* (hemp) root meristems. In: Ivanova D. (ed.), Plant, fungal and habitat diversity investigation and conservation. Proceedings of IV Balkan Botanical Congress, Sofia, 20–26 June 2006, Institute of Botany, Sofia pp. 77–81

Tudose I & Filimon M. 1972–1973. Observații cu privire la influența cofeinei asupra frecvenței celulelor în diviziune mitotică și a aberațiilor cromosomiale din rădăcinițele de grâu (*Triticum aestivum* L.)(2n=42). *Lucr. Staț. "Stejarul", Ecol. Terestră Genet*. **3**: 317-324 (in Romanian).

Zellnig G, Tausz M, Pešec B, Grill D & Müller M. 2000. Effects of glutathione on thiol redox systems, chromosomal aberrations, and the ultrastructure of meristematic root cells of *Picea abies* (L.) Karst. *Protoplasma* **212**: 227-235.

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REZIME

Genotoksični efekat kafeina na vrste *Phaseolus communis* L. i *Raphanus sativa* L.

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U radu se opisuju citogenetički efekti izazvani različitim koncentracijama kafeina (0,1% i 0,5 %) na ćelije korenskog meristema vrsta *Phaseolus communis* i *Raphanus sativa*. Kod ove dve vrste upoređivani su mitotički indeks, učestalost i tip hromosomskih ana-telofaznih aberacija. Rezultati su pokazali genotoksičan potencijal kafeina, koji dovodi do važnih promena genetičkog materijala. Maksimalna testirana koncentracija kofeina (0,5%) je izazvala kompleksne ana-telofazne aberacije, posebno u slučaju rotkvice. Genotipovi *R. sativus* su pokazali veću osetljivost prema kafeinu u poredjenju sa *P. communis*.

Ključne reči: kafein, jromozomske aberacije, genotoksičnost, *Phaseolus vulgaris*, *Raphanus sativus*