ESTIMATION OF CITOGENETIC EFFECTS ON CAFFEINE TRATMENT IN DILL ROOT MERISTEMS

ELENA MAXIM[1], GABRIELA CĂPRARU[2], FLORINA MIHAELA AXENTE[3]

Keywords: mitotic index, chromosome aberration, caffeine, *Anethum graveolens* L.

Abstract: The analysis of some cytogenetic parameters shows that the caffeine application on *Anethum graveolens* L. species induced a light mutagen effect on the cells of the root apex. At the same time is an accumulation of cells with aberrations in ana-telophases, especially ana-telophase with lagging and expulsed chromosomes but also in metaphases (metaaphases with expulsed chromosomes, C-metaphases and picnotic chromosomes) demonstrating the perturbations of mitotic spindle function after treatment with caffeine.

INTRODUCTION

Caffeine, as well as other purine derivatives, can be incorporated in DNA macromolecule. In the next DNA replication, the initial nitrogenous base will be changed by a base analogue, because of some incorporation errors. To be mutagens and, therefore, incorporated in DNA macromolecule, it is required to action during DNA active synthesis. Caffeine is a base analogue of adenine, and in fact can sometimes be incorporated into a growing DNA chain, instead of adenine. Caffeine is considered by some authors as a weak mutagen, for this reason.

The mechanism of caffeine action is not completely deciphered. It is possible that the methylated oxypurines (including caffeine) act as solubilizing agents and form molecular complexes, because of the existence of a close relationship between the solubilizing power of a chemical and its ability to produce chromosome aberrations. It seems, also, that these substances interact with DNA and alter its physical properties, in particular the denaturation temperature, fact that determines the increase of spontaneous rate of mutations.

Because of lack of some identical or similar results on the mutagenic effect of caffeine in different biological systems, the mutagenic potential of caffeine and certain phenoxy-methyl-xanthine compounds is controversial and discussible. Kaul and Zutshi (1973) and Hernández et al. (1986) sustain the idea that these compounds induce chromosome aberrations, centric breakages, or sub-chromatid exchanges, and Sax and Sax (1966) highlight the radiomimetic effect of caffeine from tea, coffee and Coca Cola on plant chromosomes. Caffeine produces mutations in bacteria, fungi, and insects, and induces chromosome aberrations in both onion root tips and human tissue culture cells (Sax and Sax 1966). Itoyama and Bicudo (2000) sustain that this compound has an inhibitive effect on DNA repair, stopping the repair of cytogenetic lesions, but does not determine an increase of micronuclei number, in the first ascendant phase of caffeine concentration curve.

Raicu and Stoian (1967) evidenced a great number of chromosome aberrations (bridges, lagging chromosomes, tetraploid cells) and a modified dynamics of cell cycle (prolongation), in *Vicia faba* L., under the impact of treatments with purine derivatives (adenine, adenosine, 3'-adenylic acid, xanthine, hypoxanthine), at 0.1–0.3% concentrations, while Tudose and Filimon (1972–1973) established the mutagenic effect of caffeine in wheat, where this purine derivative determined the increase of dividing cells and of chromosome aberration frequency in mitotic ana-telophases. In relation to mutagenicity, even the results for the same organism in literature are occasionally antagonistic (Laranja et al. 2003).

In our laboratory, genetical studies about influence of caffeine on some crop plants reveal decrease of mitotic index and accumulation of aberration cells (Roşu et al., 2006; Vlad Rusen et al., 2007).

MATERIALS AND METHODS

Biological material: dill seed, harvest from 2007. Germination was ensured in Petri dishes covered with distillated water moisturized filter paper, in thermostat at 22^0C. At 5-10mm root length it was applied the following treatment:

- the control dish – little embryonic roots were kept, for three hours, in distillated water, at room temperature;
- the variants of treatment in which were used three dilutions of caffeine (0,1%; 0,25%, 0,5% and 1%) the germinated seeds were maintained for three hours, at room temperature.

After this step all variants are kept in distillated water, for two hours. As fixative, the mixture absolute ethyl alcohol : glacial acetic acid, was used for 12 hours. For storage use 70% ethyl alcohol solution and the vials were placed in refrigerator conditions.

The microscopic preparations were obtained by squash method (Cîmpeanu et al., 2002) and analyzed by used light microscope 40x objective. The photos were realized at 100x in immersion with Cool Pix Nikon digital camera, 1600x1200 dpi resolution. Five preparations per variant were scored and ten microscopic fields per slide to estimate mitotic index and chromosome aberrations.

RESULTS AND DISCUSSIONS

Mitotic index, frequency and types of chromosomal aberrations in *Anethum graveolens* L. root meristem cells after caffeine treatment were studied.

Total frequency of division cells is little low in 0.1% and 0.5% caffeine, but increase at 0.25% and 1% variants. The most significantly mitogen effect is at 0.25% variant, mitotic index being 1.48 fold higher than control (Fig. 1).

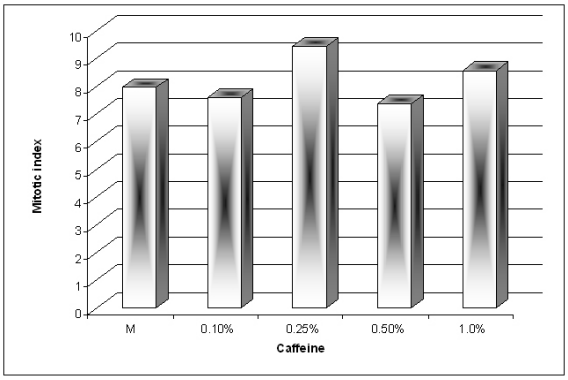

Fig. 1. Mitotic index in the dill root apex after caffeine treatment

The establishment of the frequency of the four mitosis phases can also offer data about the mechanism by which the xenobiotic factor induces modifications in cell division.

The exposure of biological material to the caffeine treatments, in above mentioned concentrations and comparatively to control, determined variations of division phases as follows: prophase cells percentage has an insignificant decrease at minimum tested concentration (0.10%), while for the other three concentrations a more or less obvious increase was registered. Maximum prophases accumulation was noted at 0.25% caffeine, variant at which mitotic index registered also the biggest value. Frequency of the other mitosis phases (metaphases, anaphases, telophases) not significantly differs comparatively to control (Fig. 2). This behavior proves that in caffeine treatments, like other substances, the mitodepressive or mitogen action is exerted in early phases of cell cycle, when the chromosomes have a high degree of despiralisation.

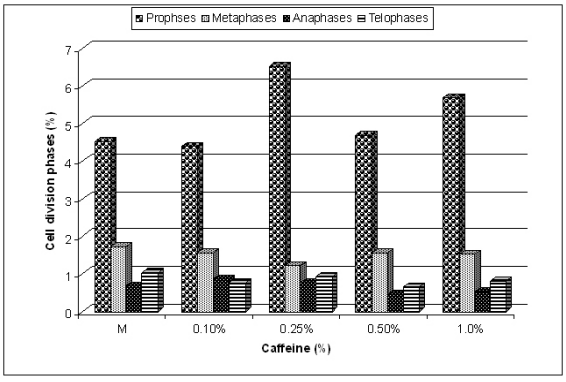

Fig. 2. Mitotic phases frequency in the dill root apex after caffeine treatment

Establishing the frequency and types of aberrations that may arise from the action of the external agent (the spontaneous or induced) plays a particular role in the cytogenetic determinations. In our case, application of caffeine on the dill root meristem induces an increase in the number of cells with aberrations. Significantly accumulation of the chromosomal aberration is at 0.1% caffeine where this parameter is two fold higher (12.90%) than control (5.42%). Decreased of this parameter at higher concentrations (0.50% and 1.0% caffeine) may be correlated both with reduction of anaphase and telophase number and reparatory and adaptive phenomena.

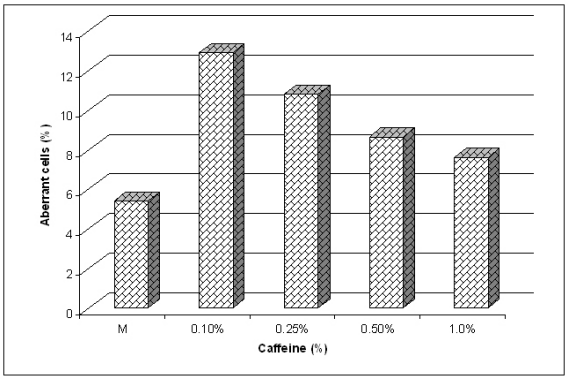

Fig.3. Aberration cells frequency in the dill root apex after caffeine treatment

The different types of aberrations were found, mainly frequent being: ana-telophases with lagging and/or expulsed chromosomes, metaphases with single and multiple chromosomes expulsed, C-metaphases and picnotic chromosomes (Fig. 4-8). In the last case we can suggest that action of caffeine is similar to colchicines inducing hierpcondensation of chromosomes (picnosos) and destruction of achromatic apparatus (C-metaphases).

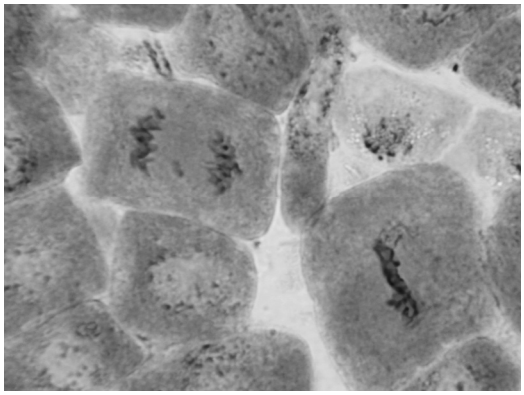

Fig. 4. A-T with lagging chromosome

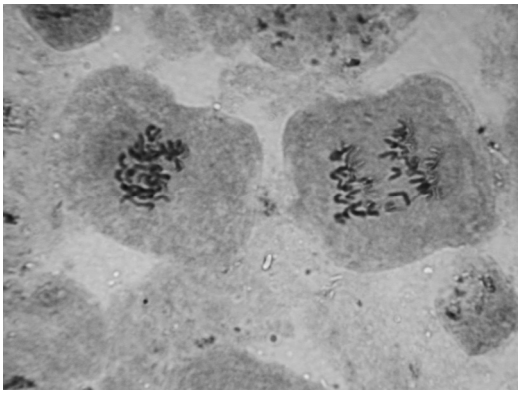

Fig. 5. A-T with lagging and expulsed chromosomes

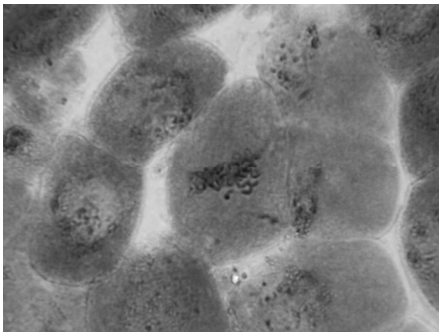

Fig. 6. Metaphse with expulsed chromosomes

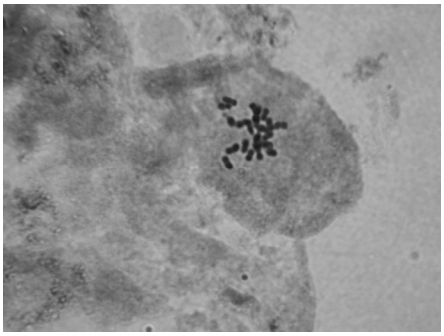

Fig. 7. C-metaphase

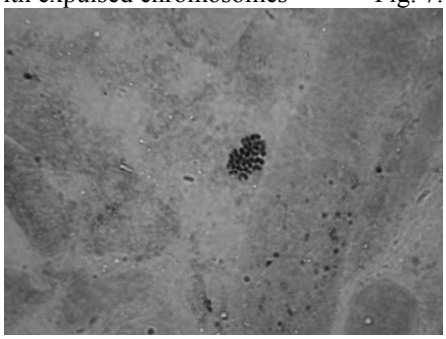

Fig. 8 Picnotic chromosomes

CONCLUSIONS

Caffeine treatment induced a few diminishing of mitotic index at 0.10% and 0.50% variant, and an increase at 0.25 and 1% variants.

Higher or lower mitotic index is correlated with a high or less number of cells in prophase.

The numbers and types of aberration cells increase comparatively to control. The highest frequency of them is at 0.1% caffeine.

Chromosomal aberration were identified both ana-telopase and metaphase.

REFERENCES

Itoyama, M.M., Bicudo, H.E.M.C., 2000. *Genet. Mol. Biol*., 23 (1): 105-107.
Hepler, P.K., Bonsignore, C.L., 1990. *Protoplasma*, 157 (1–3): 189-192.
Hernández P., Mingo R., González-Fernández A., López-Sáez J.F., 1986. *Mutat Res*., 164 (5): 327-33
Kaul B.L., Zutshi U., 1973.*Cytobios* 7 (28): 261-4.
Laranja, A.T., Manzatto, A.J. Bicudo H.E.M.C. 2003. *Genet. Mol. Biol*., 26 (4): 419-429.
Raicu, P., Stoian, V. 1967. *Caryologia*, 20 (4): 317-322.
Roşu Elena, Ioana Ieremie, Felicia Vlad Rusen, Ioan I. Băra, 2006. *An. Şt. Univ "Al. I Cuza" Iasi*, Secţiunea II a. Genet. şi Biol. Molec., VII (159-163).
Singh C., Kaul B.L., 1985. *Mutat. Res*. 144 (4): 239-42.
Tudose, I., Filimon, M. 1972–1973. *Lucrările Staţiunii "Stejarul", III. Ecologie Terestră şi Genetică*, 317-324.
Sax, K., Sax, H.J. 1966. *Proc. Natl. Acad. Sci.* U.S.A., 55 (6): 1431-1435.
Vlad Rusen Morela Felicia, Căpararu Gabriela, Cîmpeanu Mirela Mihaela, Ioana Anca Ieremie, Elena Cristina Roşu, I. Băra, 2007. *An. Şt. Univ "Al. I Cuza"* Iasi, Secţiunea II a. Genet. şi Biol. Molec., VII (1): 235-242.
[1] "Mihai Eminescu" School, Vaslui
[2] Biological Research Institute, Iaşi
[3.]"Alexandru Ioan Cuza" University, Iaşi