

THE CYTOGENETIC EFFECTS OF THE CAFFEINE TREATMENT ON *HELIANTHUS ANNUUS* L., FAVORIT AND PR64 A83 VARIETIES

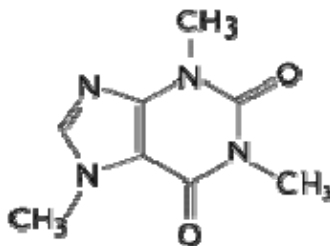
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Abstract: The analysis of some cytogenetic parameters shows that the caffeine application on Favorit and PR64A83 cultivars of sunflower exercises a light mutagen effect on the cells of the root apex. At same time an intensification of the frequency of the ana-telophases with aberration takes place, especially of those with bridges, with retardatar and expelled chromosomes, but also of those with fragments, demonstrating the perturbation action that caffeine exercises on the division axle. The apparition of a great number of ana-telophases with fragments proves that this alkaloid induces ruptures at the chromosomal level (clastogenic effect).

INTRODUCTION

The caffeine is found in a large number of plants, being a product with an extended utilization in the world. From a chemical point of view it can be integrated in the alkaloid group with purinic nucleus which contain a hexagonal cycle of pyrimidine and a pentagonal cycle of carbocondensated imidazole in orto- position. The raw formula is $C_8H_{10}N_4O_2$ having the following chemical structure:



The caffeine belongs to the chemical mutagens which take action in the synthesis S period of the ADN through the blocking of the synthesis of nitrate basis or through their inclusion in the nucleic acids macromolecules as analogous of these bases. From Freeze's view in 1963, the caffeine blocks the synthesis of adenine and guanine, and the research carried out by Tudose and co-workers in 1972-1979, showed its action on mitotic division and on the general development of some cereal and vegetable species. Starting from these reasons, in the paper in discussion, we proposed the investigation of the possible cytogenetic alterations appeared after the caffeine application in different dilutions on the two varieties of *Helianthus annuus* L.

MATERIALS AND METHODS

The biological material is represented by sunflower seeds belonging to two hybrid early cultivars: Favorit and PR64A83 proceeded from the Botanical Garden in Iasi. The germination was ensured in Petri boxes with moistened filter paper at a temperature of $22 \pm 2^{\circ}\text{C}$. After germination when the little roots are between 10-15mm the treatment was made like this:

- the control variant –the seeds and the embryonic roots have been maintained in distilled water for 3 hours.
- the treatment variants were made by placing 25 germinated seeds in solutions of caffeine with the following concentrations: 0.10; 0.25 and 0.50. The dilutions were made in distilled water.

For removing the alkaloid traces, the little roots were maintained for 2 hours in distilled water at the environment temperature. The settlement of the vegetal material was made in absolute ethylic alcohol: glacial acetic acid (3:1) for 20 hours. Until the moment of processing the little roots were kept in refrigerator, in an ethylic alcohol solution of 70%. The microscopic preparations were made using the Squash method (Cimpeanu et al., 2002). For this the little roots were subdued to hydrolysis in 50% HCl (v:v) for 8 minutes and after this were coloured with 10% Carr solution for 24 hours.

For each variant five preparations were analysed and the photos were taken with the Nikon Eclipse 600 microscope, the 100x objective with immersion, with the aid of Nikon Cool Pix 950 digital camera.

RESULTS AND DISCUSSIONS

The reactivity of the two varieties to the caffeine treatment is similar. From figure 1 results that the frequency of the cells being in mitotic division is intensified to all treated variants. For the Favorit cultivar the growth is more considerable with small and medium concentrations.

At the PR64A83 cultivar the mitotic index increases proportionally to the increasing caffeine concentration so that at the maximum variant it grows up to 3.98%, being 1.6 times greater in comparison to the control variant.

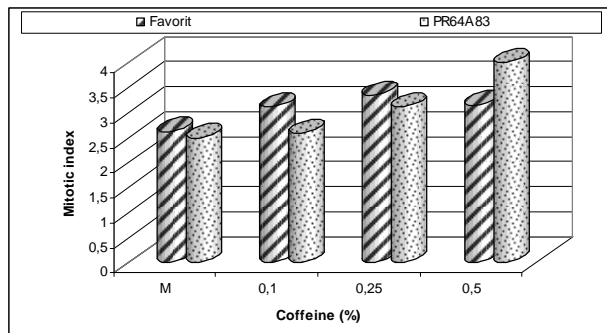


Figure 1. The variation of the mitotic index at Favorit and PR64A83 cultivars after caffeine treatment

Referring to the total number of cells that are in prophase, we observed that they vary from one dilution to another but also from one cultivar to another, even if at the control variant they register similar values.

Thus, at the Favorit cultivar, the frequency of prophase is greater to all the treated variants, the maximum being recorded at 0.25% caffeine where it has the value of 1.60% in comparison with 1.15% to the control variant.

In the case of PR64A83 cultivar the frequency of the cells being in prophase grows proportionally to the alkaloid concentration, the only exception being point out at the 0.1% caffeine variant. The greatest growth rate of the prophases frequency is encountered at the maximum concentration of caffeine, where, this parameter is over 1.7 times greater, in comparison to the control variant (Fig. 2).

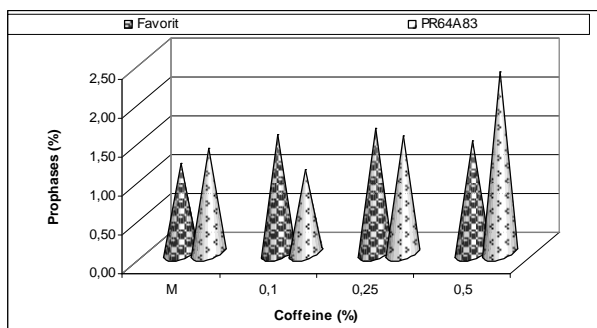


Figure 2. The frequency of the cells in prophases at the Favorit and PR64a83 cultivars, after caffeine treatment

The frequency of cells that are in metaphases presents a growth that is almost linear with the increased concentration of caffeine. If at the control variant this parameter was 0.59% (Favorit cultivar), respectively 0.61% (PR64A83 cultivar), at the maximum concentration that was used it gets to 1.06% in the case of Favorit cultivar, meanwhile in the case of PR64A83 cultivar it gets up to 1.34%, being over 2 times greater than the control variant (Fig. 3).

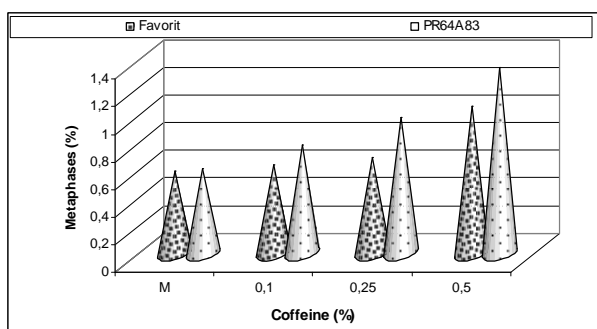


Figure 3. The frequency of the cells in metaphases at the Favorit and PR64A83 cultivars, after caffeine treatment

The figure 4 shows that the number of cells being in anaphases is greater only at small and medium caffeine concentrations, meanwhile to the maximum caffeine variant their frequency is smaller in comparison to the other dilutions and to the control variant. This fact may be due to the perturbations made by the caffeine on the division axle through which the migration of the chromosomes to the poles is inhibited.

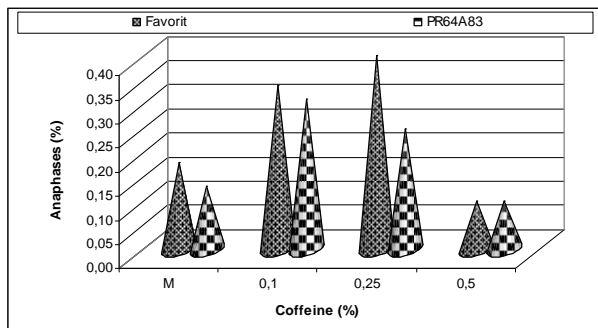


Figure 4. The frequency of the cells in anaphases at the Favorit and PR64A83 cultivars, after caffeine treatment

The cells that are in telophase have been evidenced in smaller numbers at the treated variants. The decreasing of the telophase frequency is made proportional to the increasing of alkaloid concentration. In the case of PR64A83 cultivar, the caffeine variant of 0.5%, the frequency of cells being in telophase is almost 2 times smaller in comparison to the control variant (Fig. 5). This result is in direct relation to the decreasing number of cells being in anaphases recorded with this variant.

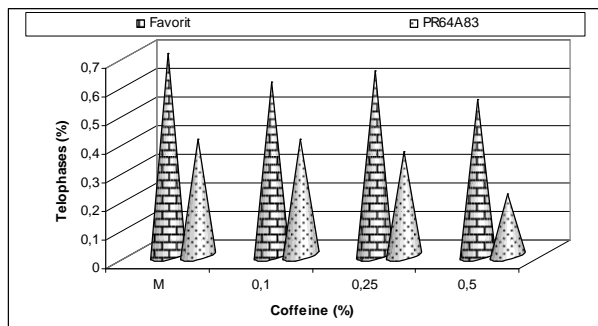


Figure 5. The frequency of cells in telophase at the Favorit and PR64A83 cultivars, after caffeine treatment

Taking into account these results we can say that the intensification of the frequency of cells in division, at the treated variants, is produced mostly by the accumulation of prophase and metaphase, and the anaphases are more frequent only at small and medium concentrations.

Another cytogenetic parameter that was analysed was represented by the frequency establishment and the main types of emphasized aberrations in the mitotic ana-telophase. Analysing figure 6 we see that their number varies from one dilution to another and from one cultivar to another.

At the Favorit cultivar the aberrations frequency is greater at all the treated variants, a maximum being emphasized at maximum caffeine concentration where this parameter is 1.91 times greater in comparison to the control variant.

In the case of PR64A83 cultivar the ana-telophases with aberrations are present in greater number only with small and medium concentrations. At the 0.5% caffeine variant the number of

anaphases with aberrations is close to the value recorded at the control variant. This result is correlated with the fact that at this variant the number of ana-telophases is smaller comparatively to the other analysed variants.

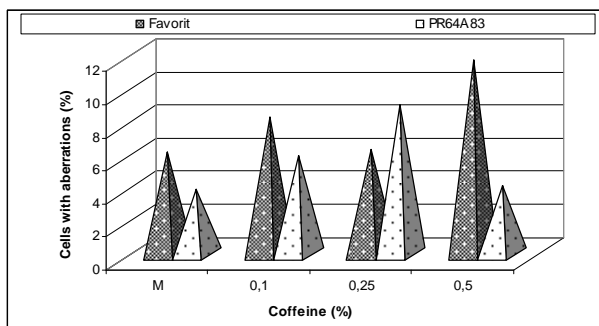


Figure 6. The total frequency of ana-telophases with aberrations

The spectrum of aberrations was large being represented by ana-telophases with simple and multiple bridges, lagging chromosomes, fragments and even by a small number of complex aberrations (ana-telophases with bridges and fragments) (Fig. 9-13).

The clastogenic action of the caffeine may be explained through the appearance of a great number of fragments and the perturbation effect exercised on the axle but also on the kinetochor is materialized by the high frequency of retardatar chromosomes and of ana-telophases with bridges.

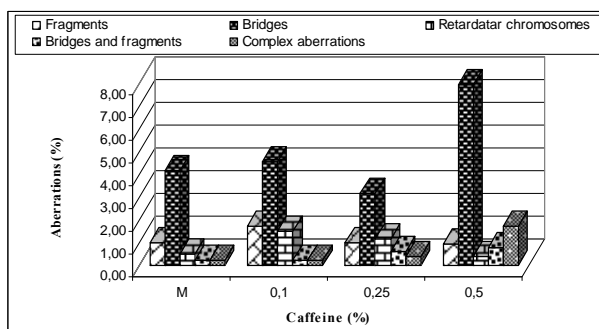


Figure 7. The frequency of aberrations types in ana-telophases, Favorit cultivar

In the case of PR64A83 cultivar the caffeine sensibility is greater because the number and the spectrum of emphasized aberrations are greater in comparison to the control variant. The highest frequency is taken by the bridges followed by the retardatar chromosomes and fragments. The record of these types of aberrations proves, in the case of this cultivar too, the clastogenic and perturbation action of a better functioning of the mitotic spindle exercised by the caffeine.

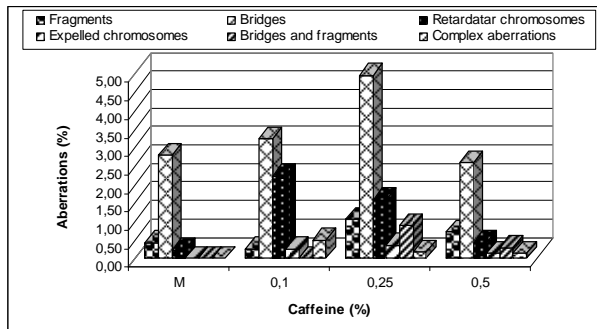


Figure 8. The frequency of the types of aberrations in ana-telophases, at PR64A83 cultivar

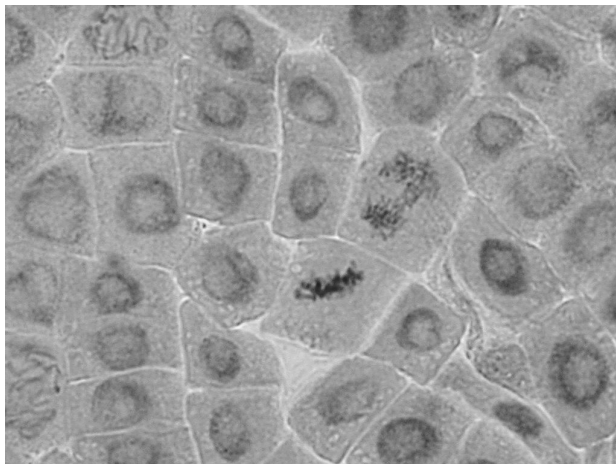


Figure 9. A-T with multiple bridges – Favorit cultivar (0.1% caffeine).

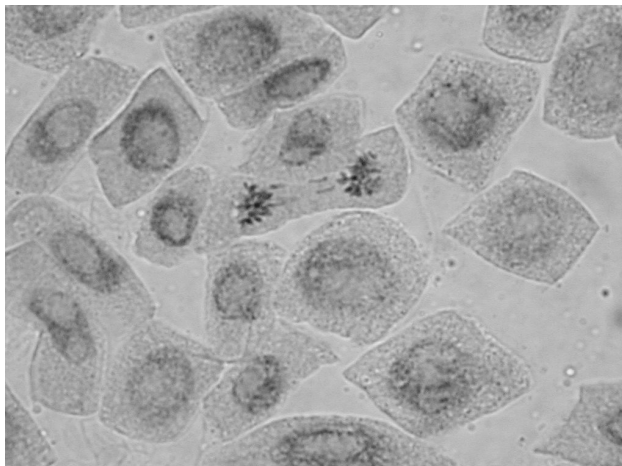


Figure 10. A-T with lagging chromosomes – Favorit cultivar (0.25% caffeine)

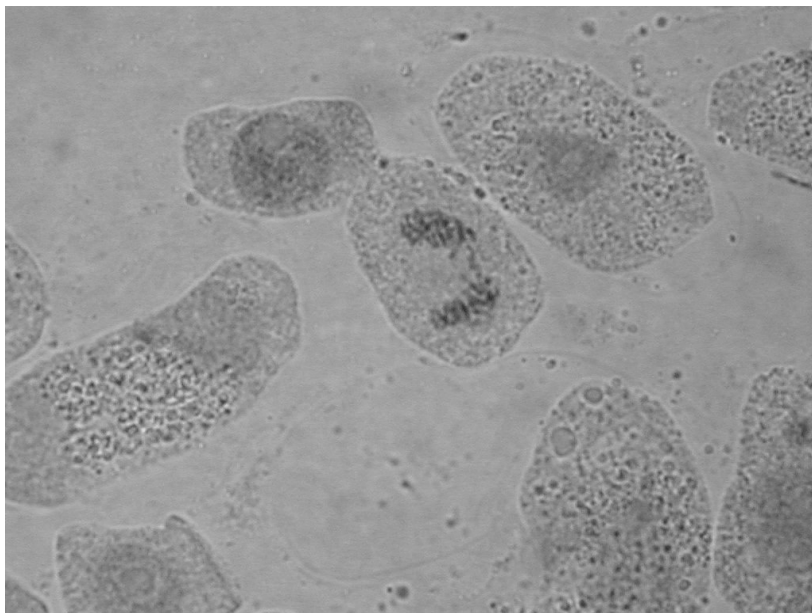


Figure 11. A-T with lagging chromosomes – PR64A83 cultivar (0.1% caffeine)

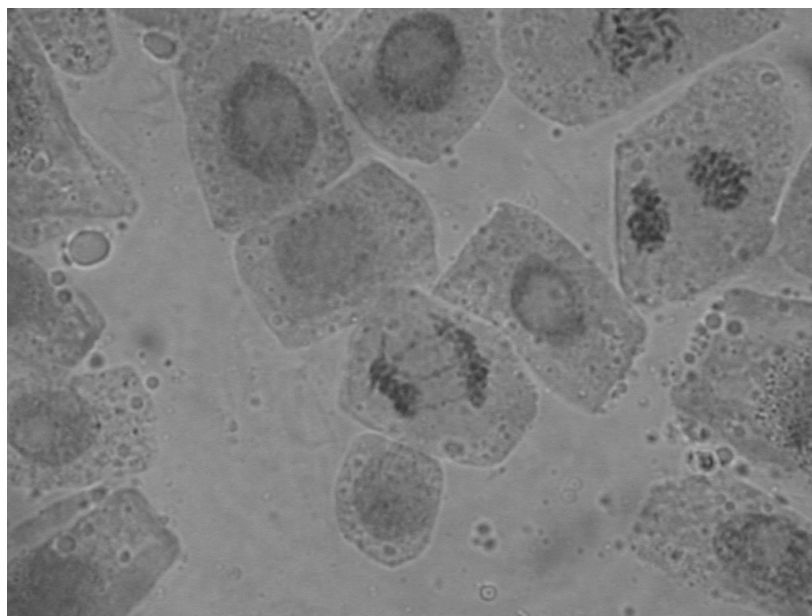


Figure 12. A-T with triple bridge – PR64A83 cultivar (0.25% caffeine)

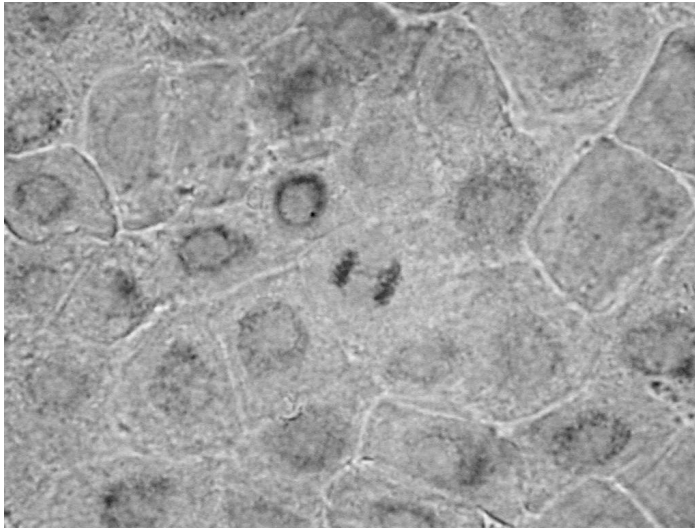


Figure 13. A-T with double bridge – PR64A83 cultivar (0.5% caffeine)

CONCLUSIONS

The caffeine exercises a light mutagen effect on the cells from the apex roots of the Favorit and PR64A83 varieties of sunflower.

The increasing of mitotic index takes places proportionally with the increasing of alkaloid concentration, the only exception being emphasized at Favorit cultivar, 0.5% caffeine variant.

At all the treatment variants the number of ana-telophases with aberrations is superior to that recorded at the control variant, a fact which is current at both varieties.

The spectrum of ana-telophases with aberrations is enough large being represented by simple aberrations, like bridges, lagging and expelled chromosomes, and fragments, as well as by complex aberrations: ana-telophases with bridges and fragments, with bridges and retardatar chromosomes.

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