CYTOGENETIC EFFECTS INDUCED BY 4-CHLOROHYDRATE-BROMO-6-METHYL-3-DIMETHYLAMINO-3-CHROMANONE IN *OCIMUM BASILICUM* L. SPECIES

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Keywords: 4-chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone, chromosome aberrations, *Ocimum basilicum* L.

Abstract: In this study, a series of modifications issued at the material genetic level of meristematic cells of root tips of *Ocimum basilicum* L. are presented, as consequence of the treatment with 4-chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone. The 1/10000 dilution induces the increase of frequency of mitotic dividing cells. The cells with chromosome aberrations are in greater number in treated variants, comparatively with control. The aberration spectrum is enough large and comprises: ana-telophases with bridges, lagging chromosomes, expelled chromosomes, multipolar ana-telophases, as well as binucleate cells and interphases with micronucleuses.

INTRODUCTION

4-Chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone (abbreviated, Z_2) is a compound synthesized by Mr. Alexandru Caşcaval, from Faculty of Chemistry, University „Al. I. Cuza” from Iassy, having the following chemical structure:

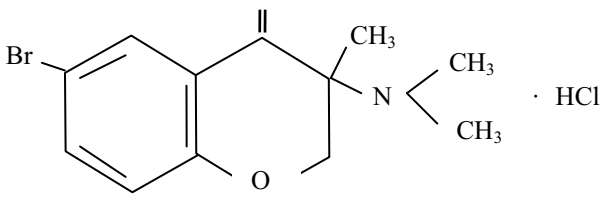

Figure 1. The chemical structure of 4-chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone

4-Chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone belongs to a large and important group of chromanones, compounds which display a remarkable domain of biochemical and pharmacological actions. Thus, Lee et al. (2005) evaluated the antioxidant effects of a series of new synthesized derivatives, respectively 6-hydroxy-7-methoxy-4-chromanone- and chroman-2-carboxamides, establishing that the chroman-2-carboxamide compounds exhibited more potent inhibition of lipid peroxidation (process induced by free radicals with the major species of reactive oxygen species), in rat brain homogenates. Therefore, they have protective effects against oxidative damages. Another research group (Boege et al., 1996) confirmed the effectiveness of a compound carrying two phenolic substituents at the chromanone structure, as topoisomerase I inhibitor and, possibly, as substance with anti-tumor activity. Extracts (including 6-methyl-4-chromanone) from *Aegle marmelos* Correa are able to inhibit the *in vitro* proliferation of human tumor cell lines (Lampronti et al., 2003), while fusarochromanone, a flavinoid produced by the mold, *Fusarium equiseti*, has a potent anticancer activity (Morrison et al., 2002; Wuthier et al., 2002). Cottiglia et al. (2004) evidenced antibacterial activity of chromanone acids from *Calophyllum brasiliense*, and some sythesized 2-heteroaryl-4-chromanones shown fungicidal activity (Yang et al., 2002).

Some chromanone synthesized derivatives were evaluated for *in vitro* antiviral activities against human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) (Xu et al., 1998; Kostova et al., 2005) and it was established they are inhibitors of HIV-1 reverse transcriptase (RT), also exhibiting activity against a variety of viruses selected for resistance to other HIV-1 non-nucleoside RT inhibitors. Gabrys et al (2001) established the feeding deterrent activity of precocenes, their synthetic analogues, and some related compounds, like 4-chromanols and 3-chromanone, to storage pests and aphids.

Considering the medicinal plant importance, as well as the necessity to test on plant and animal cells the effects of new chemically synthesized substances, we have as aim the analysis of some cytogenetical parameters in cells of root apexes of sweet basil, after exposure to 4-chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone.

MATERIALS AND METHODS

As biologic material, seeds of *Ocimum basilicum* L.(2003 harvest, furnished by I.C.C.P.T. Fundulea) were used. The germination was assured in Petri dishes, on moistened filter paper, at 22 ± 2^0C. The treatment was performed at a 10-15 root length, as follows:

- control: seeds with embryonary roots for 3 hours were maintained in distilled water;
- variants: the tested solutions (0.0001%; 0.001%; 0.01% 4-chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone) were prepared in distilled water. Each variant had 25 seeds.

To remove the mutagen, the roots were kept in distilled water, for 2 hours, at room temperature. As fixative, the mixture absolute ethylic alcohol : glacial acetic acid, 3:1, was used, for 20 hours. The roots are kept in 70% ethyl alcohol, before making preparations. The microscopic preparations were realized by squash method (Cîmpeanu et al., 2002). For this, the roots are subjected to hydrolysis in 50% HCl (v/v), for 8 minutes. The Carr solution (10%) was used as staining reactive.

Five preparations were analysed for each variant. The photos were effectuated at Nikon Eclipse 600 microscope, 100x immersion objective, and Nikon Eclipse 600 digital camera.

RESULTS AND DISCUSSIONS

a) Mitotic index

As shown in Fig. 1, the exposure to 4-chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone (Z_2) determined significant modifications of the mitotic index. At minimum used concentration, the frequency of dividing cells is much superior to the control and to the other variants. Thus, the mitotic activity of this variant is two times greater comparatively with control. The medium and maximum concentrations of tested chromanone induced the mitotic index decrease, the value of this parameter being, in respective variants, almost identical (4.9%, respectively 4.8%).

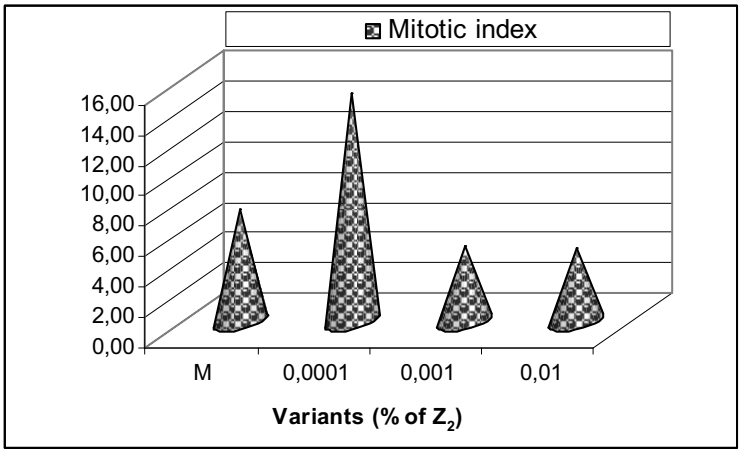

Figure 1. Mitotic index in *Ocimum basilicum* L., after the treatment with Z_2

b) Frequency of mitosis phases

In all analysed variants, predominant are the prophases, followed by metaphases, anaphases, and telophases. In 0.0001% Z_2 variant, the increase of mitotic index was the result of totalizing of

all cells found in the four phases of mitosis. For the other dilutions, a diminishing of dividing cells was observed. The prophases registered a percentual decrease, while the other mitotic phases have values closed to those registered for control (Figure 2).

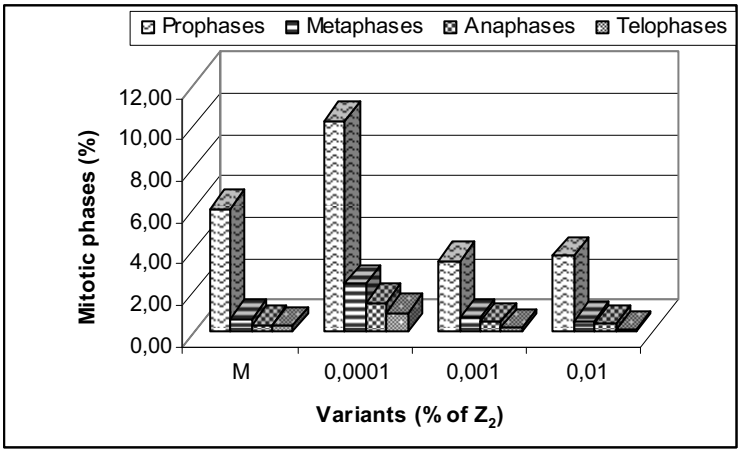

Figure 2. Mitotic phases in *Ocimum basilicum* L., after the treatment with Z_2

c) Frequency and type of chromosome aberrations

By analysing Figure 3, it is obvious that exposure of biologic material to the tested synthetic substance determined a high increase of aberrant cells. If for control, this index was 0.8%, in the treated variants the value of this index was comprised between 11.5% (0.01% Z_2) and 15.9% (0.0001% Z_2). The incidence of chromosome aberrations is 19 times greater than for control. The fact that at maximum tested concentration, the percentage of cells with chromosome aberrations is much smaller than in the other dilutions may be an expression of existence of some repair or adaptive processes, by which the plant try to neutralize the noxious action of the tested substance.

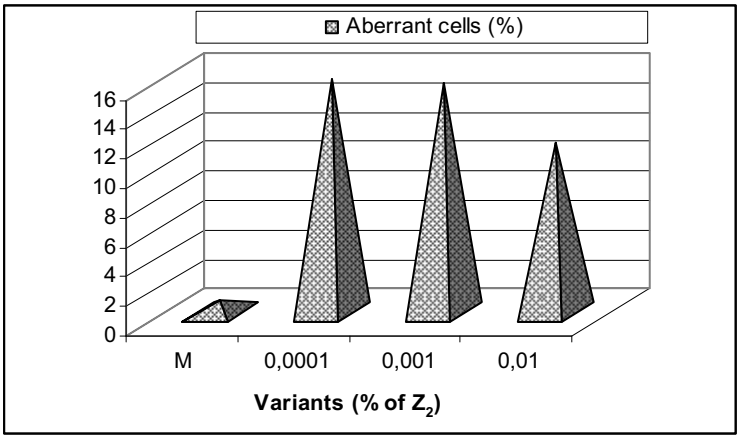

Figure 3. Frequency of aberrant ana-telophases in *Ocimum basilicum* L., after the treatment with Z_2

The spectrum of aberrations identified in mitotic ana-telophases was enough large and was represented by ana-telophases with simple, double, triple, even multiple bridges; lagging chromosomes; fragments, as well as a reduced number of complex aberrations (ana-telophases

with bridges and fragments, ana-telophases with bridges and lagging chromosomes). In interphases, micronucleuses and binucleate cells were evidenced (Fig. 4-6). Ana-telophases with bridges were the most frequent aberrations. The results permit us to conclude that 4-chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone, at small concentrations, has a mitogen effect. The presence of bridges, lagging and/or expelled chromosomes is a proof of disturbing action of tested compound on good function of division spindle, the chromosome migration to poles being strongly affected. The possible clastogenic action of tested substance is presumed from the presence of ana-telophasic fragments and of interphases with micronucleuses.

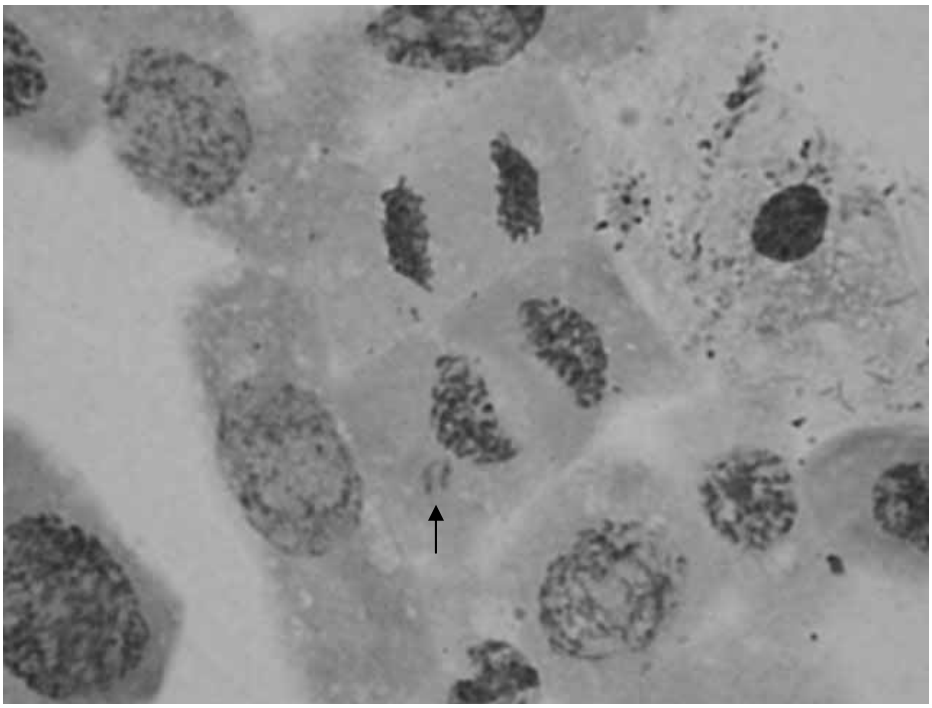

Fig. 4. Ana-telophase with two expelled chromosomes (0.001% Z_2)

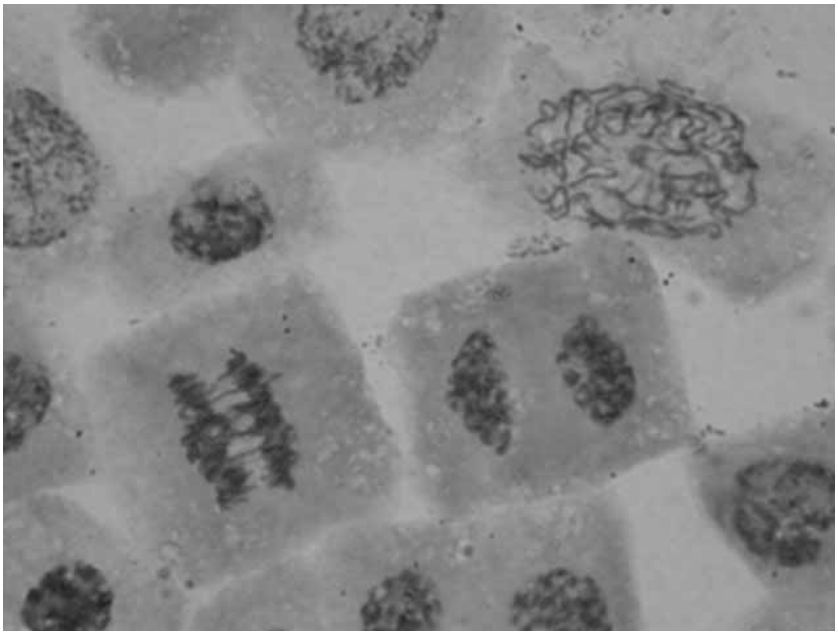

Fig. 5 Ana-telophase with multiple bridges (0.001% Z_2)

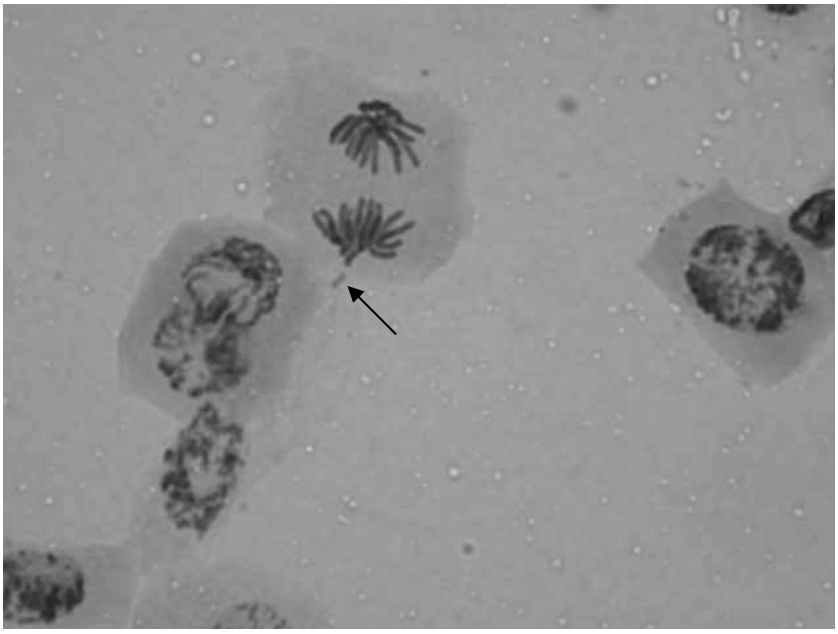

Fig. 6. Ana-telophase with expelled fragment (0.01% Z_2)

CONCLUSIONS

The minimum concentration of 4-chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone stimulates the mitotic activity in the cells from *Ocimum basilicum* L. root apexes.

The mitotic index significantly decreases in 0.001% and 0.01% Z_2 variants.

The frequency of aberrant cells is much greater in treated variants, comparatively with control.

The main types of identified aberrations were: ana-telophases with bridges, lagging chromosomes, and fragments, binucleate cells, and interphases with micronucleuses.

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