INFLUENCE OF SODIUM METABISULPHITE (E 223) ON MITOTIC DIVISION IN *CALENDULA OFFICINALIS L.*

ROMEO - CRISTIAN MARC [1], GABRIELA CĂPRARU [1]

Keywords: mitotic index, chromosome aberrations, sodium metabisulphite (E 223), *Calendula officinalis L.*

Abstract: This paper presents the cytogenetic effects induced by sodium metabisulphite (E 223) (a food additive used as preservative) in meristematic cells of *Calendula officinalis L.* root tips. The treatment has determined the lessening of the mitotic index (comparative with the control variant), until mitotic division total inhibition, as well as a growth frequency of division aberration in anaphase and telophase.

INTRODUCTION

Food additives are substances added to food to preserve flavor or improve its taste and appearance. Some additives have been used for centuries; for example, preserving food by pickling (with vinegar), salting, as with bacon, preserving sweets or using sulfur dioxide as in some wines. With the advent of processed foods in the second half of the 20th century, many more additives have been introduced, of both natural and artificial origin.

One group of additives that can cause problems in sensitive individuals is the sulfiting agents. This group includes several inorganic sulphite additives (E 220-228), including sodium sulphite, potassium bisulphite and metabisulphite containing sulphur dioxide (SO2). These preservatives are used to control microbial growth in fermented beverages and they have been widely used in wines, beers and fruit products for over 2000 years. In sensitive (asthmatic) individuals, sulphites may trigger asthma characterised by breathing difficulties, shortness of breath, wheezing and coughing.

It is used as a food additive, mainly as a preservative and is sometimes identified as E223. As an additive, it may cause allergic reactions, particularly skin irritation e.g. eczema; gastric irritation and asthma. It is not recommended for consumption by children. It is present in many dilutable squashes (i.e. fruit juice concentrates) and in candy bars.

To take in consideration the importance of Pot Marigold (*Calendula officinalis L.*) as medicinal plant and the possible negative effects of food additive use, we proposed to evidence the modifications induced by sodium metabisulphite (E 223) at the level of mitotic cell cycle.

MATERIALS AND METHODS

As biologic material, seeds of *Calendula officinalis L.* (2006 harvest, S.C.D.A. Secuieni, Neamt) 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 mm root length, as follows:

▪ *Control*: seeds with embryonary roots for 3 hours were maintained in distilled water;

▪ *Variants*: the tested solutions (0.10%, 0.25%, 0.50% and 1.00%) were prepared in distilled water. Each variant had 25 seeds.

To remove the sodium metabisulphite solutions, the roots were kept in distilled water, for 2 hours, at room temperature. As fixative, the mixture absolute ethyl 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 analyzed for each variant.** The photos were effectuated at Nikon Eclipse 600 microscope, 100x immersion objective, and Nikon Eclipse 600 digital camera.

Sodium metabisulphite (E 223) is a salt of sulphurous acid, which contain SO_2 up to 65.50%. $Na_2S_2O_5$ is the chemical formula for sodium metabisulphite.

When mixed with water, sodium metabisulfite releases sulfur dioxide (SO_2), a pungent, unpleasant smelling gas that can also cause breathing difficulties in some people. For this reason, sodium metabisulfite has fallen from common use in recent times, with agents such as hydrogen peroxide becoming more popular for effective and odorless sterilization of equipment. Released sulfur dioxide however makes the water a strong reducing agent.

Sodium metabisulphite has 3 presentation forms:

- non-food sodium metabisulphite
- photographic sodium metabisulphite, non-food
- food sodium metabisulphite (E 223)

Sodium metabisulphite is a white, instable powder, which react with the oxygen in order to form sodium sulphate. In acid conditions, the result is sulphurous acid, which react as preservative.

An accepted daily intake is up to 0.7 mg / kg. Despite this, is not recommended intakes at children.

Due to his oxidative effect, sodium metabisulphite can decrease the vitamin composition in food.

RESULTS AND DISCUSSIONS

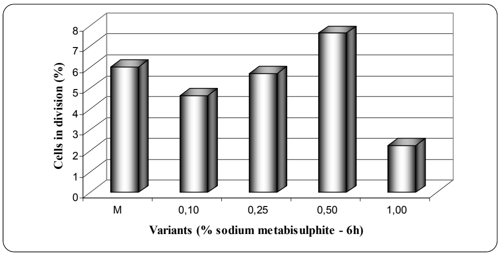
The main analyzed parameters were mitotic index, frequency of mitotic phases, frequency and type of chromosome aberrations.

a) Mitotic index

The increasing concentrations of sodium metabisulphite determined a generally decrease of dividing cell frequency, in root apex of Pot Marigold (*Calendula officinalis L.*).

In case of 6 hours treatment, the smallest mitotic index (2.26%) was registered at a concentration of 1.00%. In this case, the value of mitotic index was approximately 3 times smaller than that of control (6.04%) (Fig. 1.A.). After the 6 hours treatment, it is easily to observe an increase of mitotic index (7.65%) at 0.50% concentration of sodium metabisulphite concentration, which can be explained by a growing stimulating process.

In case of 12 hours treatment, the smallest mitotic index (6.05%) was registered at a concentration of 1.00%. In this case, the value of mitotic index was approximately 1.5 times smaller than that of control (7.12%) (Fig. 1.B.). After the 12 hours treatment, the mitotic index recorded an increase at 0.25% concentration of sodium metabisulphite solution.



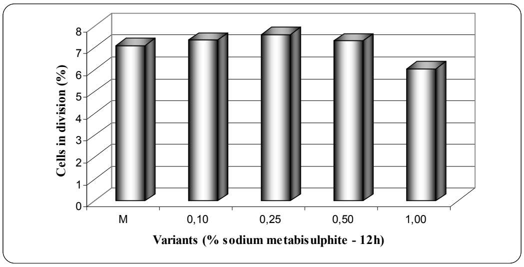


Figure 1.A. Figure 1.B.

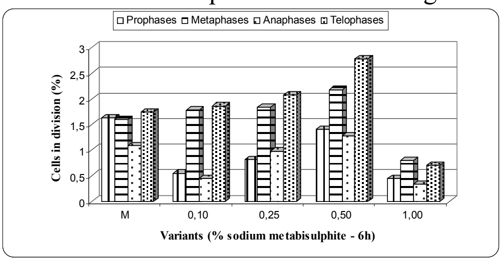
1.A. Mitotic index in Pot Marigold, after the treatment with sodium metabisulphite (6 h)

1.B. Mitotic index in Pot Marigold, after the treatment with sodium metabisulphite (12 h)

b) Frequency of mitotic phases

After the treatment with sodium metabisulphite for 6 h, respectively 12 h, the frequency of the mitotic phases is approximately identical both in case of 6 hours and 12 hours treatment (Figure 2.A. and 2.B.).

An exception was recorded after the treatment for 6 hours with 1.00% concentration, when all 4 division phases recorded a significant decrease (Figure 2.A.).



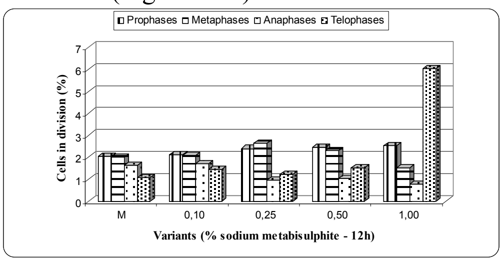


Figure 2.A. Figure 2.B.

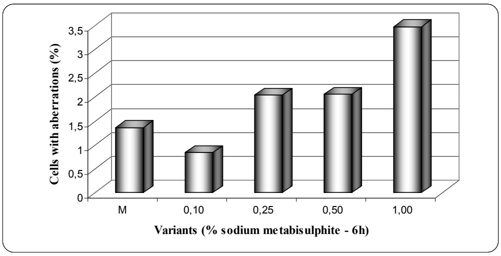
2.A. Phases of mitotic division in Pot Marigold, after the treatment with sodium metabisulphite (6 h)

2.A. Phases of mitotic division in Pot Marigold, after the treatment with sodium metabisulphite (12 h)

c) Frequency and type of chromosome aberrations

As shown in Figure 3, in control, the frequency of aberrant ana-telophases is much reduced, but in treated variants their incidence is significant increased. A direct relationship appears between the frequency of aberrant cells and sodium metabisulphite concentration increase in case of 6 h treatment.

In case of 12 h treatment, the frequency of aberrant ana-telophases is a little bit different relating 0.10% and 0.25% concentrations (frequency of aberrant cells in case of 0.10% and 0.25% concentration is approximately identical as in control) fact that permit us to suppose the existence of repair processes. Instead, at 0.50% and 1.00% concentration (12 h treatment) frequency of aberrant ana-telophases is representative increased (5.03% and respectively 3.86%).



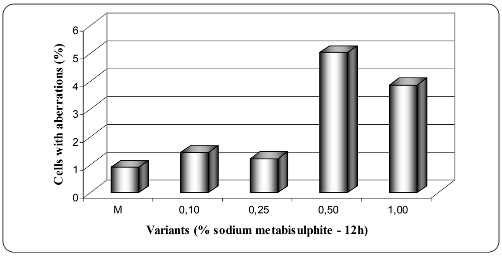


Figure 3.A. Figure 3.B.

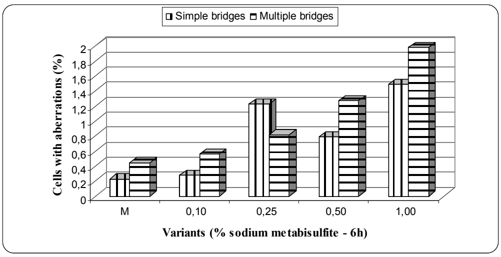
3.A. Frequency of aberrant ana-telophases in Pot Marigold, after the treatment with sodium metabisulphite (6 h)

3.B. Frequency of aberrant ana-telophases in Pot Marigold, after the treatment with sodium metabisulphite (12 h)

The spectrum of chromosome aberrations identified in mitotic ana-telophases was enough large:

▪ ana-telophases with simple bridges and double bridges in case of 6 h treatment;

▪ ana-telophases with simple bridges, double bridges and expelled chromosomes in case of 12 h treatment.



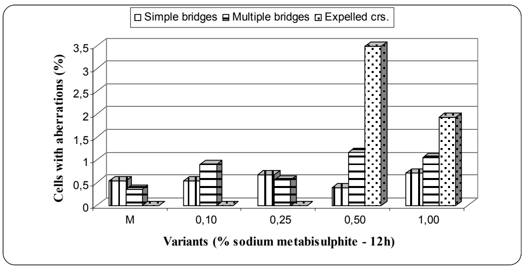


Figure 4.A. Figure 4.B.

4. A. Types of aberrations in Pot Marigold, after the treatment with sodium metabisulphite (6 h)

4. B. Types of aberrations in Pot Marigold, after the treatment with sodium metabisulphite (12 h)

The most frequent aberrations were ana-telophases with bridges. Sodium metabisulphite affects the normal function of mitotic spindle, so that the chromosome migration to the poles is disturbed.

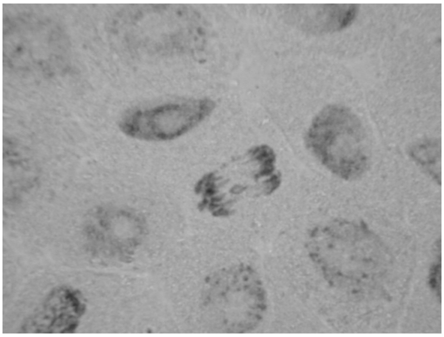


Figure 5. Bridges with expelled chromosomes

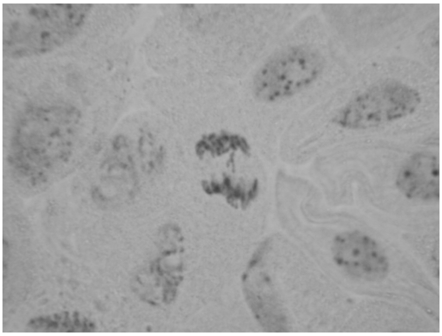


Figure 6. Expelled chromosomes

CONCLUSIONS

Sodium metabisulphite (E 223) increasing concentrations induce a significant reduction of frequency of dividing meristematic cells in Pot Marigold root tips.

The incidence of aberrant cells increases proportional to increase of food additive concentration.

The main aberrations types were ana-telophases with simple bridges, multiple bridges and (at most concentrated solutions – 0.50% and 1.00%) expelled chromosomes.

REFERENCES

Căpraru Gabriela, Băra I.I., Băra C. Iuliana, Cîmpeanu M. Mirela, Maxim Elena, Axente F. Mihaela, 2004. *Analele Universității „Al. I. Cuza"* Iași, (serie nouă) Genetică și Biologie Moleculară. Tomul V: 219-222.

Cîmpeanu M. Mirela, Maniu Marilena, Surugiu C. Iuliana, 2002. *Genetica-metode de studiu*. Editura Corson Iași: 31-36, 127-130.

Florina Mihaela Axente, Gabriela Căpraru, Mirela Mihaela Cîmpeanu, Ion I. Băra, 2006. *Analele Ştiinţifice ale Universităţii „Alexandru Ioan Cuza"*, Secțiunea Genetică şi Biologie Moleculară. Tom VII, 217-220.

http://en.wikipedia.org/wiki/Food_additive

http://en.wikipedia.org/wiki/Sodium_metabisulfite

http://www.eufic.org/article/en/page/BARCHIVE/expid/basics-food-additives/

http://hc-sc.gc.ca/fn-an/legislation/acts-lois/fdr-rad/index_e.html (Food Additive Dictionary, updated April 21, 2006).

http://www.tradezone.com/tradesites/na2s2o5.html

1 – University "Alexandru Ioan Cuza", Iaşi, Faculty of Biology