INFLUENCE OF SODIUM METABISULPHITE (E 223) ON MITOTIC DIVISION IN *TRIGONELLA FOENUM - GRAECUM L.*

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Abstract: This paper includes the cytogenetic effects induced by sodium metabisulphite (E 223) food additive in meristematic cells of *Trigonella foenum - graecum L.* root tips. The increase of food additive concentration determined the decrease of mitotic index, while the frequency and the type of chromosome aberrations are much greater in treated variants, comparatively with control.

INTRODUCTION

Food additives have been used by mankind for centuries. Salt, sugar and vinegar were among the first and used to preserve foods. In the past 30 years, however, with the advent of processed foods, there has been a massive explosion in the chemical adulteration of foods with additives. Considerable controversy has been associated with the potential threats and possible benefits of food additives.

Most food additives are considered safe. However, some are known to be carcinogenic or toxic. Hyperactivity in children, allergies, asthma, and migraines are often associated with adverse reactions to food additives.

To take in consideration the importance of fenugreek (*Trigonella foenum - graecum 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 *Trigonella foenum - graecum 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.

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 decrease of dividing cell frequency, in root apex of fenugreek (*Trigonella foenum - graecum L.*).

In case of 6 hours treatment, the smallest mitotic index (6.49%) was registered at a concentration of 0.50%. In this case, the value of mitotic index was approximately 2 times smaller than that of control (10.96%) (Fig. 1.A.).

In case of 12 hours treatment, the smallest mitotic index (4.17%) was registered at a concentration of 0.50%. In this case, the value of mitotic index was approximately 2 times smaller than that of control (8.87%) (Fig. 1.B.).

It is very important to observe the total lack of germinated seeds in case of treatment with sodium metabisulphite in maximum concentration, namely 1.00%.

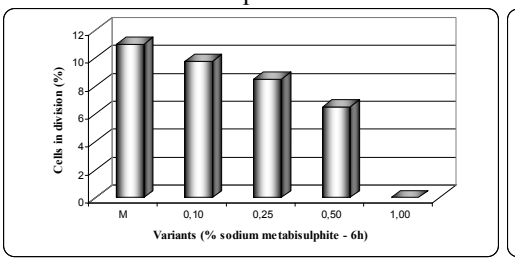

Figure 1.A.

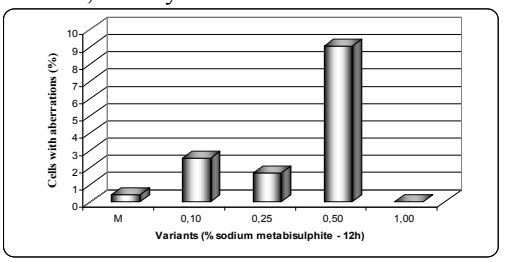

Figure 1.B.

1.A. Mitotic index in fenugreek, after the treatment with sodium metabisulphite (6 h)
1.B. Mitotic index in fenugreek, 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 in both times of treatment (Figure 2.). In sodium metabisulphite treated variants, the higher frequency is for prophases, followed by metaphases, telophases and anaphases.

The decrease of mitotic index, after sodium metabisulphite treatment, was realized by the decrease of all four division phases, therefore the food additive affects the good development of whole mitotic process.

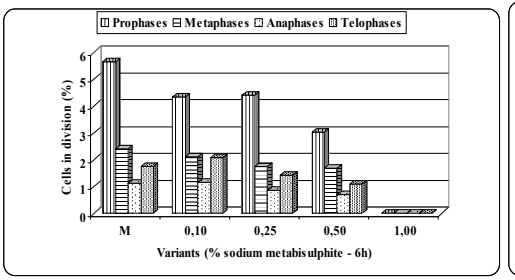

Figure 2.A.

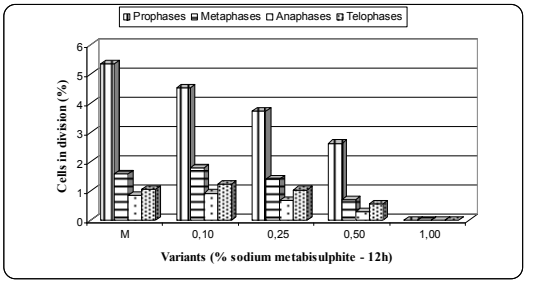

Figure 2.B.

2.A. Phases of mitotic division in fenugreek, after the treatment with sodium metabisulphite (6 h)
2.A. Phases of mitotic division in fenugreek, 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 0.25% concentration is approximately half reduced than in 0.10% concentration case) fact that permit us to suppose the existence of repair processes.

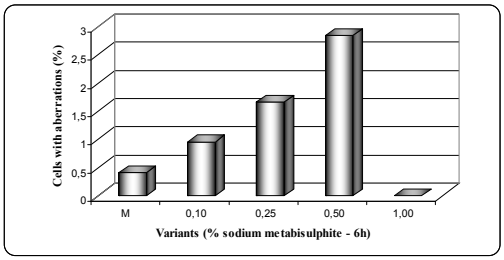

Figure 3.A.

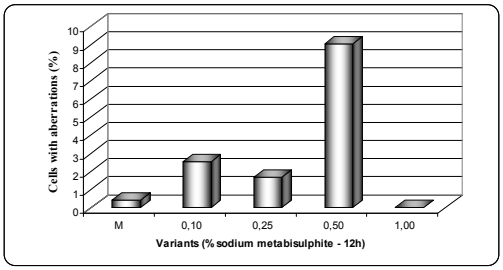

Figure 3.B.

3.A. Frequency of aberrant ana-telophases in fenugreek, after the treatment with sodium metabisulphite (6 h)
3.B. Frequency of aberrant ana-telophases in fenugreek, 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 and double bridges and C-mitosis in case of 6 h treatment;
- ana-telophases with simple and double bridges, tripolar and quadruple ana-telophases, expelled chromosomes, C-mitosis and micronuclei in case of 12 h treatment.

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.

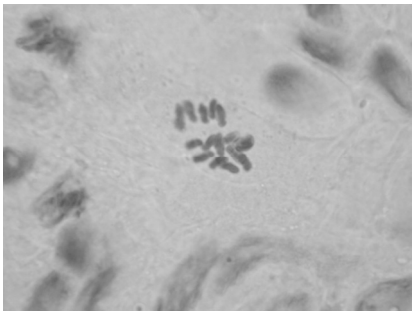

Figure 4. C-mitosis.

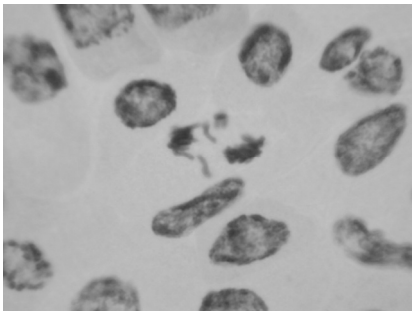

Figure 5. Tripolar ana-telophase

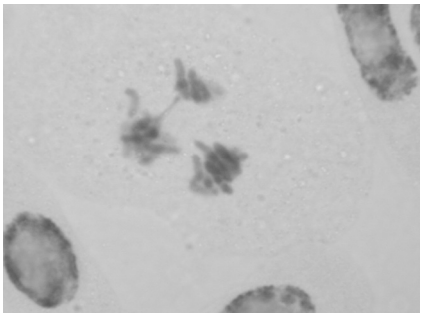

Figure 6. Tripolar ana-telophase with simple bridge in *Trigonella foenum graecum L.*

CONCLUSIONS

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

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

The main aberrations types were ana-telophases with bridges, multipolar ana-telophases, retardatary chromosomes and fragments, and interphases with micronucleuses.

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