INFLUENCE OF SODIUM PHOSPHATE (E 339) ON MITOTIC DIVISION IN *TRIGONELLA FOENUM - GRAECUM L.*

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Abstract: This paper includes the cytogenetic effects induced by sodium phosphate (E 339) 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

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.

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.

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 phosphate (E 339) 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 phosphate is a generic term for the salts of sodium and phosphate. They are:

- Monosodium phosphate (NaH_2PO_4) - used as a laxative and, in combination with other sodium phosphates, as a pH buffer;
- Disodium hydrogen phosphate (Na_2HPO_4) - is a white powder that is highly hygroscopic and water soluble salt. It is therefore used commercially as an anti-caking additive in powdered products;
- Trisodium phosphate (Na_3PO_4) – is a white powder used as cleaning agent, stain remover and degreaser. It is a highly water-soluble ionic salt. Solutions of it dissolved in water have an alkaline pH.

All sodium phosphates may be collectively referred to as **sodium phosphate**, or by E number **E339**, which is used as an emulsifier (to prevent oil separation), an acidity regulator (buffering agent), emulsifier, thickening agent and nutrition enlargement agent. Adding sodium phosphates to food increases the shelf life of the food, maintaining the texture and appearance of the food.

Acceptable daily intake is up to 70 mg/kg body weight.

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 phosphate 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 (9.04%) was registered at a concentration of 1.00%. In this case, the more was the concentration of the solution the small was the proportion of the cells in division (Fig. 1.A.).

In case of 12 hours treatment, the smallest mitotic index (8.05%) was registered at a concentration of 1.00%. In this case, at the middle value of concentration (0.25%) was recorded an increase of the proportion of the cells in division (Fig. 1.B.).

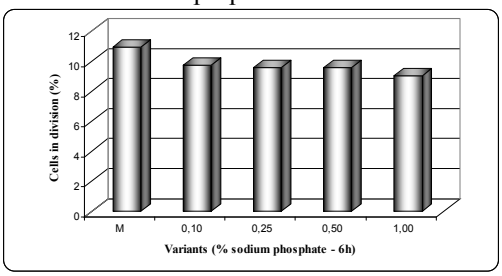

Figure 1.A.

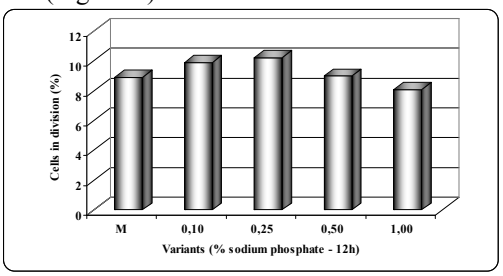

Figure 1.B.

1.A. Mitotic index in fenugreek, after the treatment with sodium phosphate (6 h)
1.B. Mitotic index in fenugreek, after the treatment with sodium phosphate (12 h)

b) Frequency of mitotic phases

After the treatment with sodium phosphate for 6 h, respectively 12 h, the frequency of the mitotic phases is approximately identical in both variants of treatment (Figure 2.). In sodium phosphate treated variants, the higher frequency is for prophases, followed by metaphases, telophases and anaphases.

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

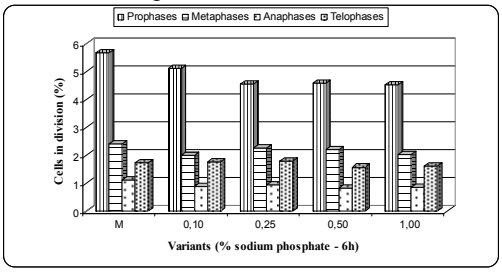

Figure 2.A.

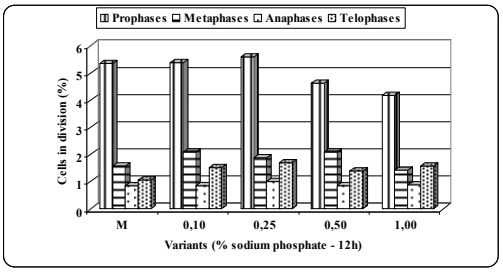

Figure 2.B.

2.A. Phases of mitotic division in fenugreek, after the treatment with sodium phosphate (6 h)
2.A. Phases of mitotic division in fenugreek, after the treatment with sodium phosphate (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.

After the 6 hours treatment with sodium phosphate the larger spectrum of aberrations was recorded in case of 0.10% concentration, followed by a decrease thru 1.00% concentration of the sodium phosphate solution (Figure 3.A.). This observation permit to sustain a reparatory process.

After 12 hours treatment, the relation between concentration of sodium phosphate solutions and frequency of cells with aberrations is inverted, that is most cells with aberrations were recorded at the most concentrated solution (1.00%) (Figure 3.B.).

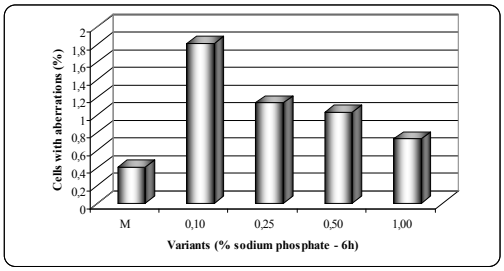

Figure 3.A.

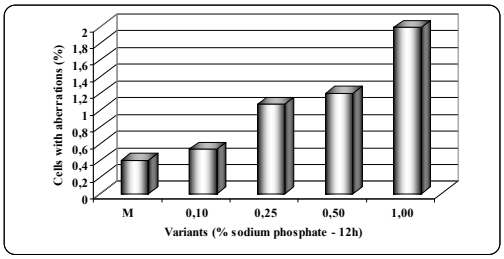

Figure 3.B.

3.A. Frequency of cells with aberrations, after the treatment with sodium phosphate (6 h)
3.B. Frequency of cells with aberrations, after the treatment with sodium phosphate (12 h)

The spectrum of chromosome aberrations identified in mitotic ana-telophases was enough
large: (Figure 4.A., Figure 4.B.)

- ana-telophases with simple and double bridges, lagging chromosomes, expelled chromosomes and C-mitosis in case of 6 h treatment;
- ana-telophases with simple and double bridges, lagging chromosomes, expelled chromosomes and C-mitosis in case of 12 h treatment.

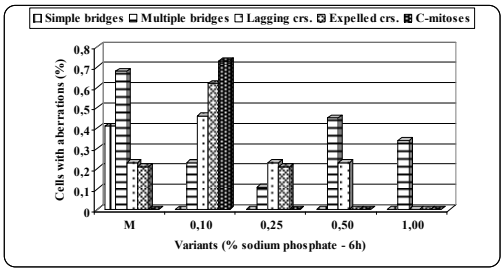

Figure 4.A.

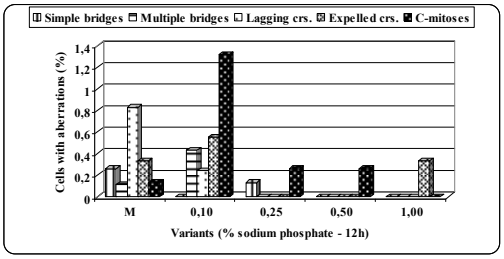

Figure 4.B.

4.A. Types of aberrations, after the treatment with sodium phosphate (6 h)
3.B. Types of aberrations, after the treatment with sodium phosphate (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.

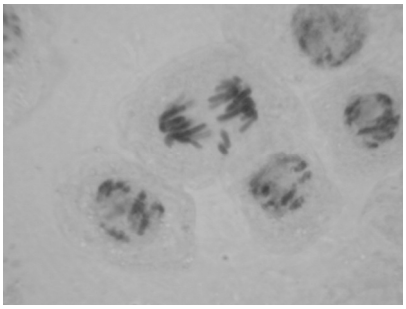

Figure 5. Expelled chromosomes.

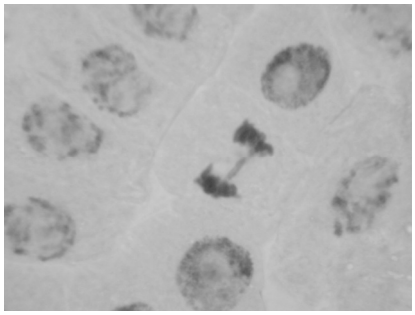

Figure 6. Simple bridge

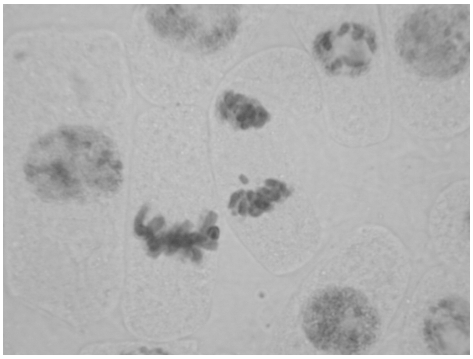

Figure 6. Lagging chromosomes

CONCLUSIONS

Sodium phosphate (E 339) 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 simple and double bridges, lagging chromosomes, expelled chromosomes and C-mitosis.

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