CITOGENETIC EFFECTS INDUCED BY SODIUM NITRITE ON MITOTIC DIVISION AT *TRITICUM AESTIVUM* L.

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The paper presents the influence of sodium nitrite on the cell division at Triticum aestivum L., Rubin variety. The treatments with sodium nitrite was used in three concentrations: 5%, 1% and 0.1%. The time of action of the respectively solutions was 4 hours and 2 hours, six experimental variants have resulted. The treatments actioned of on wheat radicular meristems, were expressed by chromosomial mutations, particular in ana-telophases, but in metaphases. The types of chromosomial aberrations in wheat radicular meristems, induced by sodium nitrite are very varied: bridges, fragments, complex multipolar ana-telophases, micronuclei. The rate of this types of chromosomial aberrations was differentiated depending on the concentration function and time of action of respective chemical agent. Beside of these types of chromosomial aberrations appear metaphases with picnotic chromosomes, chromatin bridges very thick known with denomination cytomixiy, also picnotic nuclei in high rate, genetically inert. Last types of cromosomial aberrations are considered special effects of sodium nitrite. Moreover, sodium nitrite has a inhibitory effect on mitotic division of Triticum aestivum, diminish the mitotical index, in correlation with the concentration and time of action by sodium nitrite The cells reacted differently in each phase of mitotic division to the action of the chemical agent. The experiment proved that sodium nitrite known as a polluting agent and a food additive has a real mutagenic potential and inhibitory upon vegetable mitotical cells.

***Key words**: root meristem, cell, nucleus, mitotic division, chromosomial aberration.*

Sodium nitrite is used in food industry as food additive, known as E250. The concentration of sodium nitrite the European Union allows is 0.6%. This salt is pink-coloured and it is used for processing meat and in fish cans, in order to preserve the colour of these products and to prevent the multiplication of *Clostridium botulinum*.

Recent research of nutritionists has shown that sodium nitrite is toxic for mammals. Added to the products of processed meat, sodium nitrite is a real carcinogenic N- nitrosamines by the reaction of sodium nitrite with amino acids in

the presence of heat in an acidic environment [4, 6]. Therefore, sodium nitrite is responsible for the stomach, pancreas and colon cancer. It is also a main cause of migraines and lung diseases [2, 3, 4, 6]. Associated to thiosulfate, it may be used as antidote in cyanide intoxications [1]. The goal of this paper was to investigate the influence of sodium nitrite on wheat meristematic cells. Such investigations were also carried out on onion meristematic cells [5].

MATERIAL AND METHOD

The biological material used in the experiment, was represented by seeds of *Triticum aestivum* L., *Rubin* variety. The seeds were put to germination in lab conditions. When the roots reached 15 -17 mm in length, they were treated with sodium nitrite – $NaNO_2$.

Sodium nitrite was used in the form of watery solutions in three concentrations: 5%, 1%, 0.1%. The time of action of the respective solutions on the radicular meristems was differentiated as follows: 5%, 1% and 0.1% solutions acted for 4 hours and 2 hours.

Taking into account the concentration and the time of action of the solutions 6 variants have resulted. Besides these eight experimental variants, there was also used a control plot and in this case no treatments were applied to the radicular meristems.

For further cytogenetic investigations, the treated and non-treated roots (control) were fixed in Carnoy fixing solution for 24 hours at 4°C then hydrolised with HCl and coloured with the basic colouring matter Carr. The radicular meristem was displayed using squash technique. 15 preparations and 10 microscopical fields/preparation were examined for all the variants and control. The microscopical examination was carried out using the optic microscope Hund Wetzlar. The microphotographies were made with the camera from the endowment of the microscope.

RESULTS AND DISCUSSIONS

Analysis of the mitotic index

The sodium nitrite treatments had a higher inhibiting effect, especially at the concentration of 5% *(fig. 1, 2)*.

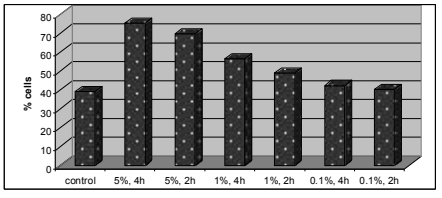

Figure 1 **Proportion of cells in interphase**

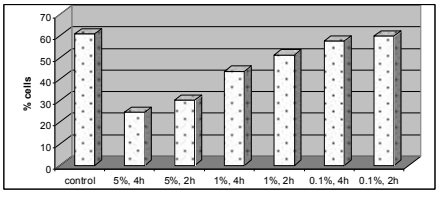

Figure 2 **Proportion of cells in division**

In the other four phases of the mitotic division, cells reacted differently. Thus, in prophase, the cell proportion was below the control level (29.11% cells) in all the tested variants *(fig. 3)*. In metaphase, the lowest cell proportions were found at the variants with 5% (2.4-4.6% cells) and 1% concentrations (7.9-8.75% cells), while at the 0.1% concentration (12.7-12.9% cells), the cells proportion has exceeded the control (10.32% cells) (*fig. 4)*. In anaphase, the lowest cells

proportion was found at the variants with 5% concentration (0.72-1.26% cells). The 1% concentration, 2 hours action time, and 0.1% have resulted in higher percent levels (6.73-7.9% cells) than the control (5.55% cells) *(fig. 5)*. In telophase, the cell proportion was diminished over the control (16.04% cells) in all the experimental cases, especially at 5% concentration (2.01-3.5% cells) *(fig. 6)*.

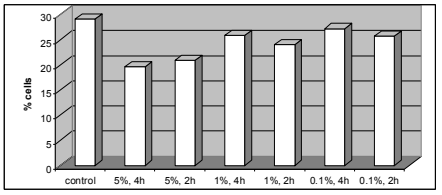

Figure 3 **Proportion of cells in prophase**

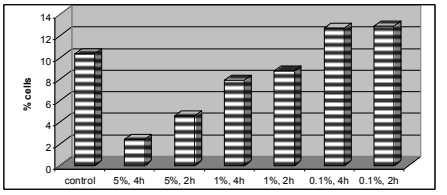

Figure 4 **Proportion of cells in metaphase**

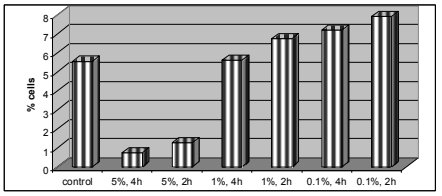

Figure 5 **Proportion of cells in anaphase**

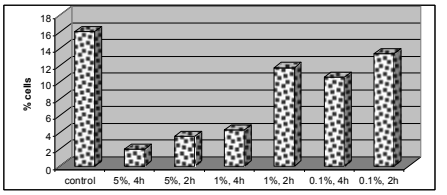

Figure 6 **Proportion of cells in telophase**

Analysis of cells proportion in aberrant metaphase and ana-telophase

The effect of sodium nitrite on wheat meristematic cells is found not only as inhibitor but also as mutagen, by inducing the chromosome mutations, which are easy to detect by optical microscopy. High rates of aberrant metaphases and ana-telophases were found at 1% and 0.1% concentrations *(fig. 7, 8)*. The aberrant metaphases consist in picnotic chromosomes.

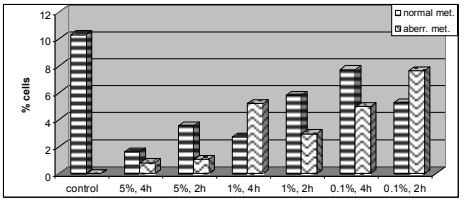

Figure 7 **Proportion of cells in normal and aberrant metaphases**

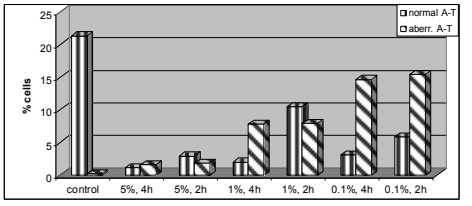

Figure 8 **Proportion of cells in normal and aberrant ana-telophases**

Analysis of chromosomial aberration types

In ana-telophases, five types of chromosome aberrations appeared, which frequency differs according to the concentration and action time of sodium nitrite *(fig. 9)*. Thus, in 5% concentration variants, only chromosome bridges and micronuclei were found, while at 1% and 0.1% concentrations, which allowed the presence of more ana-telophases, the range of chromosome aberrations is higher.

The chromosome bridges are found at all the variants, in subunitary proportions at 5%, and supraunitary ones at 1% and 0.1% concentrations. The highest frequencies of bridge cells (14.69% and 15.42%) were found at 0.1% concentration. The bridges induced by sodium nitrite were of different types:

simple, double, continuous, seldom interrupted, thin, thick and very thick; the last one, which is responsible of cytomixy appeared at 1% and 0.1% concentrations.

The chromosome fragments appeared only at 0.1% concentration, with a frequency of 0.7-1.2%.

The associations between bridges and fragments were found only at the variants with 0.1% concentration.

The multipolar ana-telophases were induced by 1% and 0.1% concentrations, at proportions between 7.5% and 9.1%. Most of them consisted in asymmetrical telophases. At the 0.1% concentration, complex multipolar ana-telophases were pointed out.

Micronuclei, associated to interphasic and telophasic nuclei, were found at all the variants in supraunitary proportions (1.67-3.44%). Their frequency is in direct correlation with the increase in the concentration and in the action time of the chemical agent.

At the control, only bridges in subunitary proportions (0.17%) appeared spontaneously.

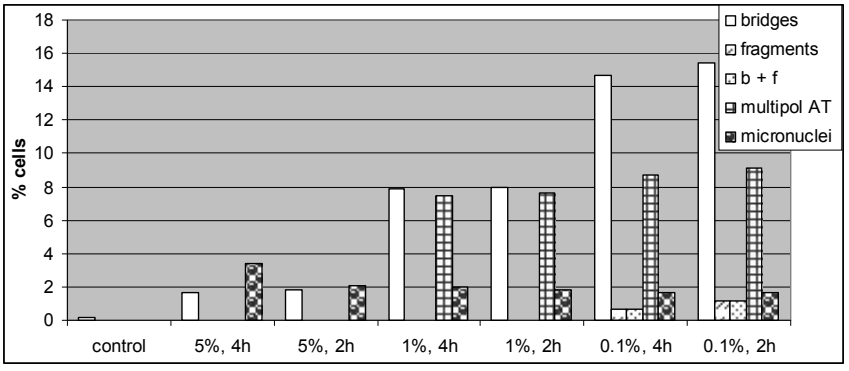

Figure 9 **Proportion of chromosomial aberration types**

Next to the shown aberration types, sodium nitrite has also induced the formation of picnotic nuclei, which are genetically inert, at all the variants, which frequency (4.7-7.7%) is in direct relation with the concentration and the action time of the chemical agent (*fig. 10)*.

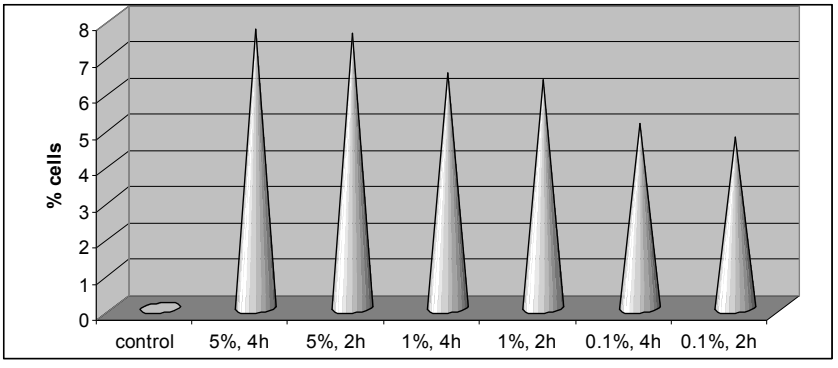

Figure 10 **Proportion of picnotic nuclei in root meristem at wheat, treated with sodium nitrite**

The limit differences of effects of sodium nitrite at *Triticum aestivum* is represented in *table 1.*

Table 1

The influence of sodium nitrite upon mitotic division in root meristems at *Triticum aestivum* L.

Variant	Aberrant metaphases		Aberrant ana-telophases	
	Average value (%)	Significance of difference	Average value (%)	Significance of difference
Control	0.00	-	0.20	-
5%, 4 hours	0.8	-	1.62	**
5%, 2 hours	1.05	*	1.83	**
1%, 4 hours	5.20	***	7.85	***
1%, 2 hours	2.95	***	7.95	***
0.1%, 4 hours	4.99	***	14.69	***
0.1%, 2 hours	7.65	***	15.42	***
DL 5% = 0.900 DL 1% = 1.263 DL 0.1% = 1.784			DL 5% = 0.931 DL 1% = 1.307 DL 0.1% = 1.845	

Different aspects of chromosomial aberrations induced by sodium nitrite at *Triticum aestivum* are presented in *figures 11-16.*

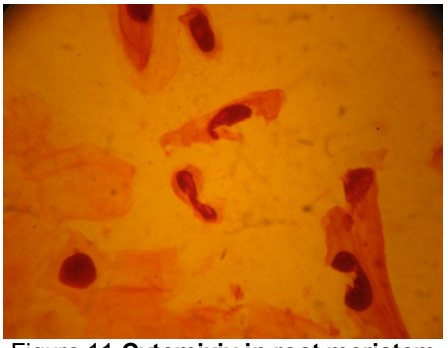

Figure 11 **Cytomixiy in root meristem at wheat treated with sodium nitrite 1%, 4 hours (1000X)**

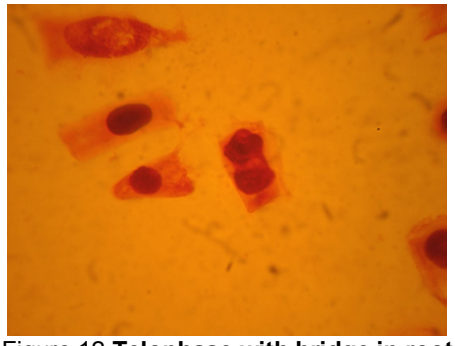

Figure 12 **Telophase with bridge in root meristem at wheat treated with sodium nitrite 5%, 2 hours (1000X)**

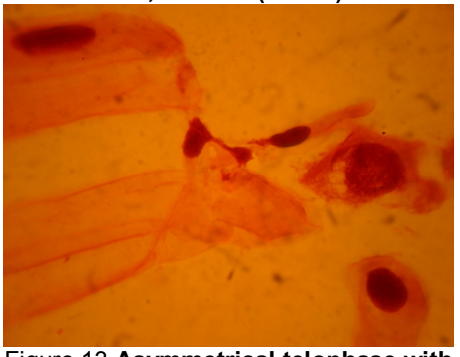

Figure 13 **Asymmetrical telophase with bridge in root meristem at wheat treated with sodium nitrite 5%, 4 hours (1000X)**

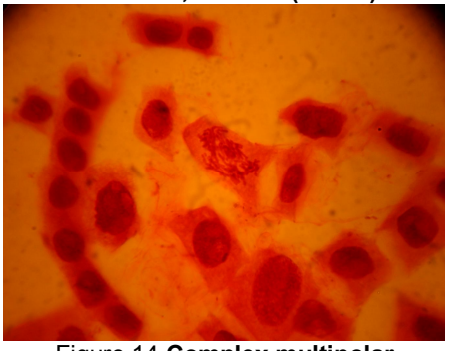

Figure 14 **Complex multipolar anaphase in root meristem at wheat treated with sodium nitrite 0.1%, 4 hours (1000X)**

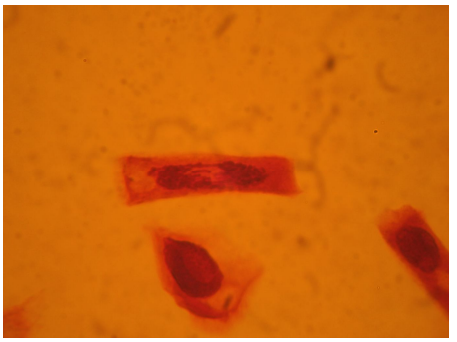

Figure 15 **Anaphase with bridges in root meristem at wheat treated with sodium nitrite 1%, 2 hours (1000X)**

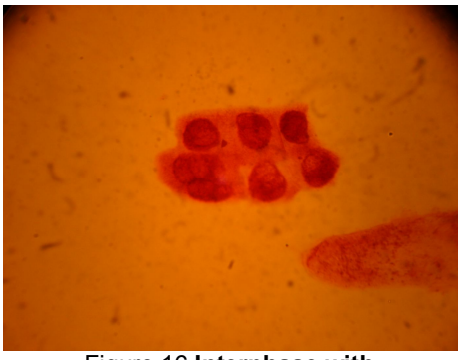

Figure 16 **Interphase with micronucleus in root meristem at wheat treated with sodium nitrite 5%, 4 hours (1000X)**

CONCLUSIONS

1. Sodium nitrite, known as a carcinogenic food additive, has an inhibiting effect on *Triticum aestivum* mitogene cells.

2. Sodium nitrite has a mutagen potential on plant cells, which is shown by chromosome aberrations induced in ana-telophases: chromosome bridges, chromosome fragments, associations between bridges and fragments, multipolar ana-telophases and micronuclei.

3. Cytomixy and asymmetrical telophases might be considered as characteristics of the effect of sodium nitrite.

4. Picnotic nuclei, like picnotic chromosomes from metaphases, are other features of the effect of sodium nitrite.

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