THE INFLUENCE OF COLD PLASMA PRODUCED BY GLIDARC WITHOUT WATER VAPOR, UPON THE CELLS DIVISION IN *TRITICUM AESTIVUM* L.

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Abstract

This paper presents the influence of cold plasma on mitotic division in *Triticum aestivum* L. Cold plasma is a fourth state of matter existing in the universe. Cold plasma electrodes was produced by a GlidArc, in the absence of water vapor. This study aimed to highlight the role of water vapor in the cold plasma upon the cells mitotic. Wheat seeds were exposed by cold plasma in four time: 2 minutes, 5 minutes, 20 minutes and 40 minutes, resulting in four experimental variants, which were compared with a control. Seeds treated with cold plasma were put to germinate and root meristems were used for cytogenetic analysis. Cytogenetic analysis had aimed to establish the mitotic index and any possible chromosomal aberrations. Cold plasma without water vapor has a inhibitory effect on mitotic division in root meristems of wheat, reducing the value of mitotic index in direct correlation with proportional action during cold plasma. Cells reacted differently in each mitotic phase in cold plasma action: cell proportions metaphases and anaphases are reduced compared to the control, compared with prophase and telophase cells. Mitotic index was affected very significantly in all experimental cases. Chromosomal aberrations induced by cold plasma in the absence of the water vapors, were insignificant in frequency. In experimental variants with 20 minutes and 40 minutes, they were not induced. This phenomenon is explained by the absence of water vapor during the performance of cold plasma treatment. The present study highlights the major role of this water vapor during production of cold plasma on cell mitogen. Water vapor potentiates the mutagenic effect of cold plasma.

Key words: cell, cold plasma, mitotic division, mitotic index

Cold plasma is the fourth state of existence of matter in the universe. Experimentally it can be produced by special reactors. Cold plasma has found wide application in the biomedical field, due to its highly effective sterilization of surfaces. In this way you can eliminate the traditional chemicals which use significant impact on the environment. So can be promote the organic farming (Röder O. et al., 2009).

Cold plasma is widely used for decontamination of surfaces. This method has been successfully applied in agriculture and biomedicine for seed quality improvement and pathogenic micro-organisms inactivation (Kylián O. et al, 2006; Selcuk M. et al, 2008).

Also, cold plasma is used to stimulate seeds germination (Dubinov A.E., et al., 2000; Živković S. et al., 2004; Carvalho R. et al., 2005; Sera B. et al., 2008; Filatova I. et al., 2010; Sera B. et al., 2010). If the vine, cold plasma growth-stimulating effects of grapes (Dardeniz A. et al., 2006).

The effects induced of cold plasma is due to oxygen atoms species and molecules presented in air plasma. Should be noted that the composition of air plasma depends by discharge on the environment (Burlică R. and Locke B., 2007; Brisset J. L. et al., 2008; Burlică R., 2009).

The purpose of this study is the influence of cold plasma produced in an environment without water vapor discharge, upon the cell division from *Triticum aestivum*. Similar studies have been performed, except that the cold plasma was produced in an average discharge of water vapor (Pădureanu S. et al., 2009).

MATERIALS AND METHODS

Cold plasma was produced by a reactor discharge GlidArc. Download medium consisted of blowing air without water vapor.

Seeds of *Triticum aestivum* L., cv. Boema, fresh (from the previous year of this experiment) were exposed in cold plasma without water vapor for 2, 5, 20 and 40 minutes respectively. In each Petri dish were 100 seeds.

The samples of treated seeds were placed into glass Petri dishes (8 cm in diameter) and the distance between the electrodes and the bottom of Petri dish was 15 cm. The temperature of discharge, measured simply by thermistor, was about 55^0C.

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Cold plasma composition produced by the two electrodes (in the absence of water vapor) was to: O_2, N_2, NO (Burlică R. et al., 2009).

As a result four experimental variants which have been compared with the control ones. After plasma treatment the wheat seeds were putted to germinate in laboratory conditions, using Petri dishes on filter paper.

For further cytogenetic investigations, treated and non-treated embryonic roots (control) were fixed in Carnoy's fixing solution for 24 hours at 4°C, then were hydrolysed with HCl and coloured with the Carr basic colouring. The root meristem was displayed by using the squash technique. A number of 20 preparations and 10 microscopic fields/preparation were examined for all variants and for control. Number of cells analysed for control was 6559, for variant 2 minutes was 10549, for variant 5 minutes was 10171, for variant 20 minutes was 6503 and for variant 40 minutes was 6216. The microscopic examination was carried out using the Hund Wetzlar microscope. Microphotographs were taken with the microscope camera.

The dynamic of the growth in length of the embryonic roots and the sheets of the plantlets was monitored during the first phenophase.

Measurements of plantlets were processed statistically using the method limit differences.

The main analysed parameters were: mitotic index, frequency of aberrant mitotic phases, frequency and type of chromosome aberrations, growth dynamics of plantlets.

The dynamics of mitotic index

The exposure to cold plasma determined significant modifications of the mitotic index (*fig. 1*). Proporția de celule în diviziune a diminuat în toate cazurile experimentale. La variantele cu timpul de expunere de 2 și 5 minute, indicele mitotic are valori apropiate, însă sub nivelul martorului. La variantele cu 20 și 40 minute expunere la plasma rece, indicele mitotic a scăzut accentuat față de martor.

The proportion of dividing cells decreased in all experimental cases. At the variants with time of exposure of 2 and 5 minutes, mitotic index has values close to but below the control. At variants with 20 and 40 minutes exposure to cold plasma, the mitotic index dropped very obvious compared to control.

Statistical analysis of mitotic index in root meristems of wheat from seeds treated with cold plasma without water vapor demonstrates that without exception experimental mitotic index decreased very significantly compared to control *(tab.1).*

RESULTS AND DISCUSSIONS

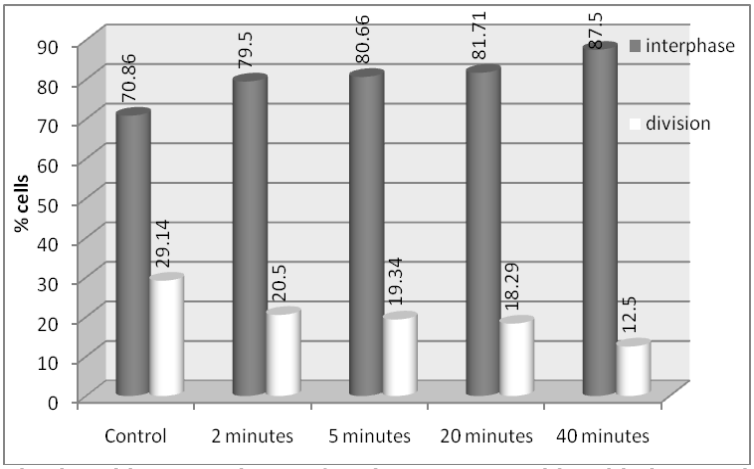

Figure 1 **Mitotic index in *Triticum aestivum*, after the treatment with cold plasma without water vapor**

Table 1

Differences found after the treatment with cold plasma without water vapor upon mitotic division in *Triticum aestivum*

variant	Mitotic index	
	average value (%)	significance of difference
control	29.14	-
2 minutes	20.50	000
5 minutes	19.34	000
20 minutes	18.29	000
40 minutes	12.50	000
DL 5% = 1.04412 DL 1% = 1.51872 DL 0.1% = 2.27808		

Detailed analysis of cells response in each phase of mitotic division, as a result of cold plasma treatment, shows the following situation:

Thus, in prophase, the cell proportion was below the control level in all the tested variants (*fig. 2*). The smallest proportion of prophase cells was recorded in variant with 40 minutes exposure time.

The metaphases registered a percentual decrease in comparison with the control, especially in variant with 40 minutes exposure time (*fig. 3*). By comparison on constat that between variants with 2 and 5 minutes of exposure to cold plasma, the differences are insignificant for prophase and metaphase.

In anaphase, the lowest cells proportion was found at the variant with 40 minutes of exposure to cold plasma (*fig. 4*). In this case, there were only 0.9% cells in anaphase.

In telophase, the situation is different from the other phases of the division analyzed so far. The highest percentage of cells was recorded by variant with 2 minutes (4.31%), percentage which exceeded even the control (4.27). In variants with 5 and 20 minutes, the percentages of cells in telophase has very close (4.06%, 4.05% respectively), while the variant with 40 minutes exposure time, the percentage of cells is higher (4.17%) (*fig. 5*).

A global analysis of response of meristematic cells of wheat to cold plasma treatments without water vapor can be seen in *figure 6.* It can be concluded that most affected were anaphases cells, especially in the variant with 40 minutes of cold plasma treatment.

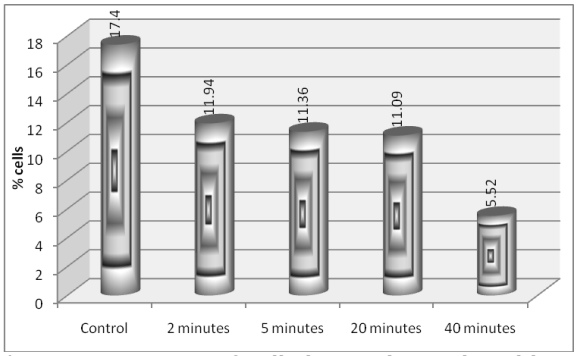

Figure 2 **Frequency of cells in prophases in *Triticum aestivum*, after the treatment with cold plasma without water vapor**

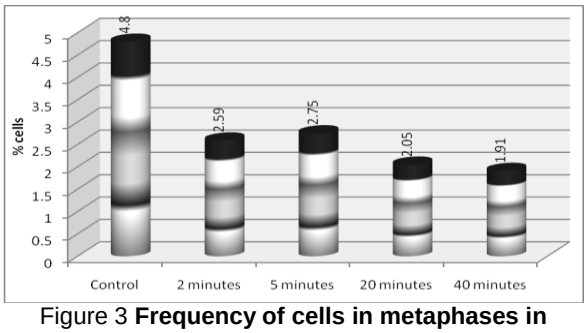

Figure 3 **Frequency of cells in metaphases in *Triticum aestivum*, after the treatment with cold plasma without water vapor**

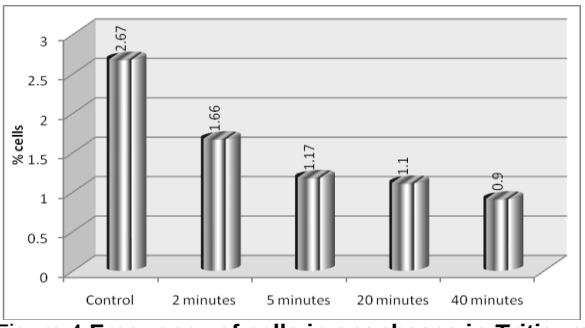

Figure 4 **Frequency of cells in anaphases in *Triticum aestivum*, after the treatment with cold plasma without water vapor**

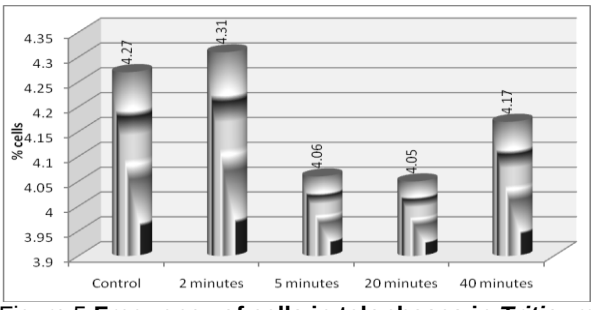

Figure 5 **Frequency of cells in telophases in *Triticum aestivum*, after the treatment with cold plasma without water vapor**

Frequency of aberrant mitotic phases

In parallel to analyze the mitotic index were recorded and chromosomal aberrations. Interestingly is that chromosomal aberrations were almost absent. At variants with 2 and 5 minutes, frequency of chromosomal aberrations was insignificant (0.06%, 0.07% respectively), while at the variants with 20 and 40 minutes, they were completely absent (*fig. 7*).

These chromosomal aberrations occurred only in anat-telophase. All the metaphases were normal.

Other studies have shown that after cold plasma treatment, but produced an environment discharge of water vapor, resulting high frequency in chromosomal aberration at the same genotype of wheat (Pădureanu S. et al, 2009).

Fequency and type of chromosome aberrations

Chromosomal aberrations manifested in ana-telophase consisted by chromosome bridges of single or multiple, thin, whole or broken, and in retardatari chromosomes (*fig. 8, 9*). These chromosomal aberrations were induced only at variants with 2 and 5 minutes exposure of cold plasma.

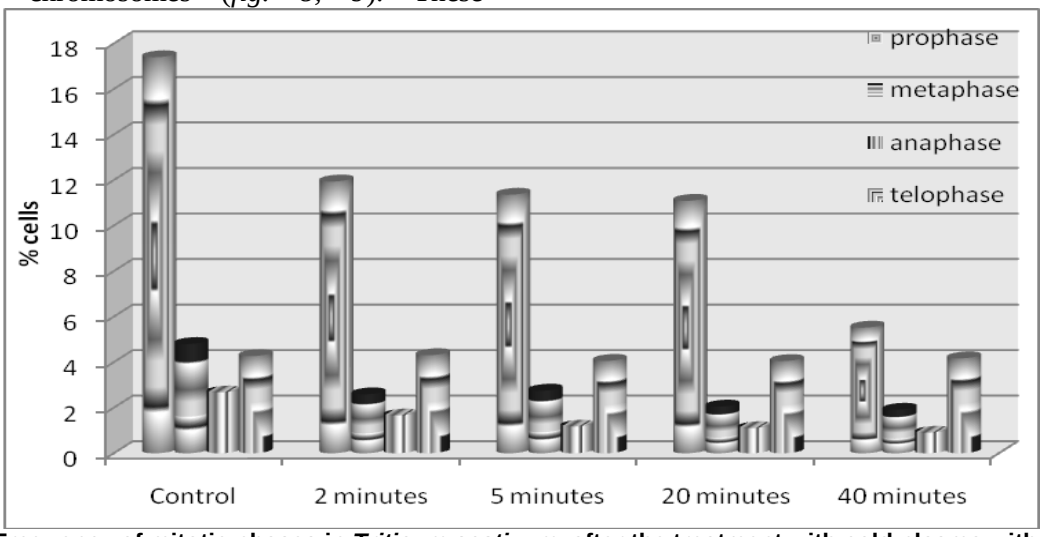

Figure 6 **Frequency of mitotic phases in *Triticum aestivum*, after the treatment with cold plasma without water vapor**

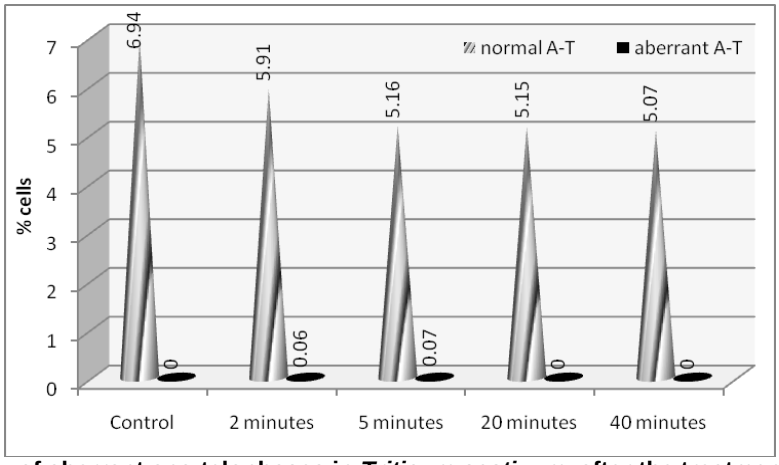

Figure 7 **Frequency of aberrant ana-telophases in *Triticum aestivum*, after the treatment with cold plasma without water vapor**

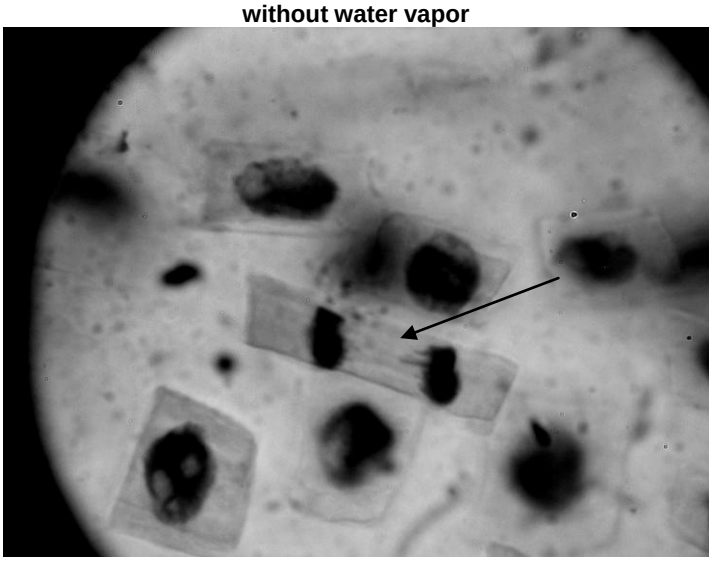

Figure 8 **Telophase with broken multiple bridges in root meristem at wheat, treated with cold plasma without water vapor, 2 minutes (1000X) (Original)**

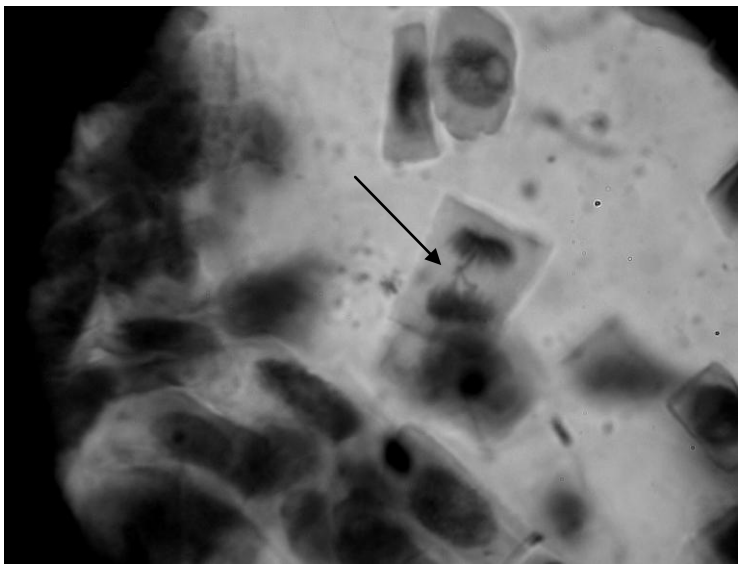

Figure 9 **Anaphase with bridge and chromosome retardatar in root meristem at wheat, treated with cold plasma without water vapor, 5 minutes (1000X) (Original)**

The growth dynamics of plantlets

To make a correlation with the mitotic index value of root meristems was determined and the dynamic growth in length of plantlets. To this end have been measured the embryonic roots and the stalks from the third day after the onset of germination, until the tenth day.

The dynamic growth of length at embryonic roots and stalks is plotted in *figures 10* and *11*. In all cases, the dynamic of growth in length of plantles has aspect sigmoidal.

Differences in growth of plantles of wheat after 10 days of germination are very significant (in negative sens) by comparison to the control (*tab. 2*).

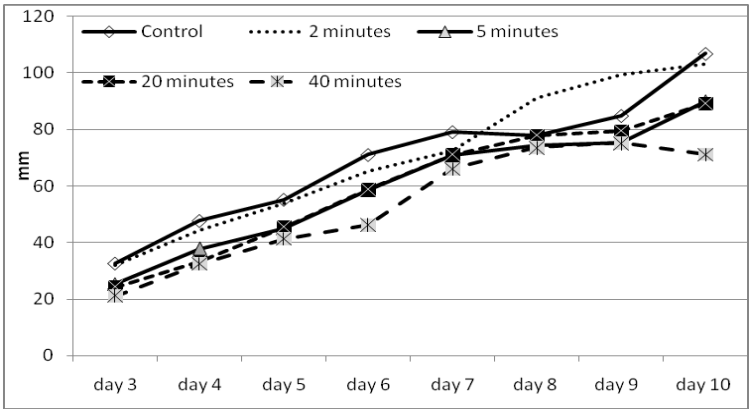

Figure 10 **The dynamics of growth of embryonic roots of wheat, after the treatment with cold plasma without water vapor**

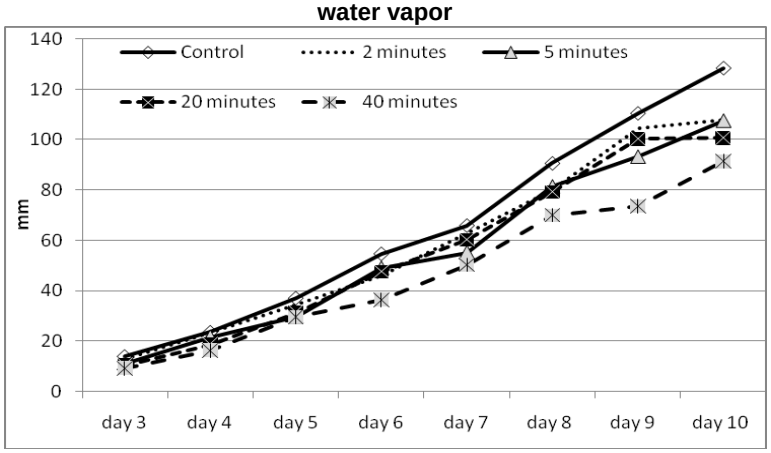

Figure 11 **The dynamics of growth of stalk of wheat, after the treatment with cold plasma without water vapor**

Table 2

Differences found after the treatment with cold plasma without water vapor upon plantlets of *Triticum aestivum*

variant	length of the embryonic root after 10 days (mm)		length of the lenght of stalk after 10 days (mm)	
	average value (mm)	significance of difference	average value (mm)	significance of difference
control	106.83	-	128.43	-
2 minutes	103.00	000	107.83	000
5 minutes	90.00	000	107.67	000
20 minutes	89.17	000	100.83	000
40 minutes	71.17	000	91.50	000
	DL 5% = 1.48071 DL 1% = 2.15376 DL 0.1% = 3.23064		DL 5% = 0.33264 DL 1% = 0.48384 DL 0.1% = 0.72576	

CONCLUSIONS

This experiment showed the effect of cold plasma discharge produced in the environment without water vapor, upon the mitogen cells in the root meristem of wheat.

Thus, it was shown that cold plasma in the absence of water vapor has an effect especially on the mitotic index, that it significantly reduces by comparison with control. This aspect is reflected in increased the plantlets..

Without water vapor, cold plasma can not induce chromosomal aberrations than sporadic, insignificant.

Different effects produced by cold plasma without water vapor, compared with cold plasma with water vapor, upon the cells mitogen, can be explained by the active chemical species and reaction products in the respectively environment of the type of cold plasma.

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