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BIOLOGICAL EFFECTS INDUCED BY COLD PLASMA TREATMENTS

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The cold plasma type discharges have more applications lately such as air and solutions depollution treatments, surface treatments (surface degreasing using cold plasma in oxygen enriched environment or surface etching using cold plasma in argon enriched environment), combustion improving, biological treatments for bio-decontamination. This work proposes the study of cold plasma treatments on seeds performed in a GlidArc reactor and the effects on the mitotic division.

Keywords: GlidArc, cold plasma, mitotic division, chromosomal aberrations.

1. Introduction

The cold plasma type discharges have various uses lately, among these, mentioning air and solutions depollution treatments, surface treatments, combustion improving, biological treatments for bio-decontamination. The sterilization treatments are useful for materials that can not be decontaminated by classical, thermal methods without changing their chemical or physical properties. These treatments can be performed in the presence of cold plasma or in the post-discharge time frame with encouraging results.

Bio-decontamination treatments performed in humid air environment with atmospheric temperature and pressure conditions, by cold plasma GlidArc type have proven their efficiency. In several minutes of exposure, the treatment induces a reduction of the bacterial population by 10 logarithmic units [2].

Previous work has shown that low-pressure high frequency air plasma treatment of plant seeds stimulates strength and branching of their sprouts and roots within several minutes of processing. An influence of 5.28 MHz electromagnetic field on plant seeds increases their quality, speed and energy of germination [3].

Lately, bio-medical applications for cold plasma treatments are being researched [4].

Cold plasma is a source for very active species such as electrons, positive and negative ions that produce oxidation, reduction and synthesis chemical reactions in the active environment.

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The cold plasma electrochemical reactors can operate in low pressure conditions as well as in high pressure conditions (usually close to atmospheric pressure). For the first option, there are difficulties related to the accessories needed for maintaining the low pressure. These reactors can be used with fluid pumping or with fluid suction (a desirable option for treatments that imply toxic environments).

Cold plasma discharges are produced at high voltages (up to 60 kV) and small currents (up to 1 A). These discharges are characterized by a significant difference between the electrons temperature (high temperature) and the positive ions temperature (close to normal environment temperature) that can be observed in Fig. 1.

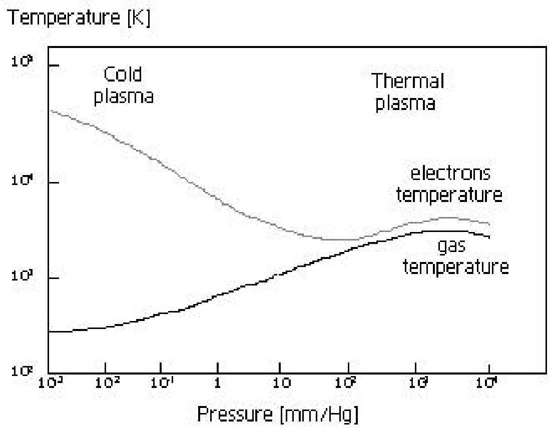

Fig.1. Temperature versus pressure, concerning electrical discharges.

Considering the discharges these use, the cold plasma electrochemical reactors can be classified as: Corona discharge reactors, Dielectric Barrier Discharge (DBD) reactors and gliding discharge (GlidArc) reactors.

Corona discharges are produced at voltages higher than 10 kV and currents smaller than 10^{-5} A.

DBD discharges are characterised by voltages arround 100 kV and currents arround 0.1 A.

Glidarc discarges are usually produced for voltages between 1 and 20 kV, with currents around 1 A.

If the Voltage versus current dependency is taken into account we can observe the areas where each of these discharges evolve, as presented in fig. 2.

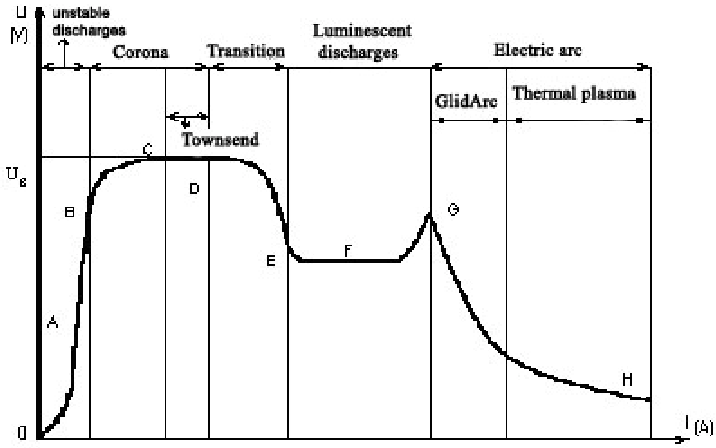

Fig. 2. Voltage + current dependency

2. Experimental Setup

For the treatments, a GlidArc type cold plasma electrochemical reactor has been used. The geometrical configuration of the electrodes is presented in Fig. 3 (1 – electrodes; 2 – insulator; 3 – electrodes). Two main divergent electrodes made of aluminum having a thickness of 3 mm, with a minimum distance between them of 2 mm, are connected to an AUPEM type power converter suitable for producing electrical discharges due to the fast drop outlet characteristic. The AUPEM power converter is connected to the power grid (220 V at 50 Hz frequency).

A humid air flow, axially directed through an injection nozzle of 0.6 mm diameter, is used for maintaining the temperature and ionization degree at levels that correspond to cold plasma parameters in the active zone of the reactor. The active species generated by the electrical discharge are transferred to the gas flow and generate metastable radicals capable of producing useful electrochemical reactions.

The main parameters for GlidArc discharges are roughly presented in table 1.

Table 1.

GlidArc discharge parameters

GlidArc discharge	U [kV]	I [A]	P [atm]	T [K]	J [A/cm^2]	X
	1 – 20	1	1	200	1 – 10^2	< 10^{-2}

The electrical connections for the GlidArc reactor can be observed in Fig.4.

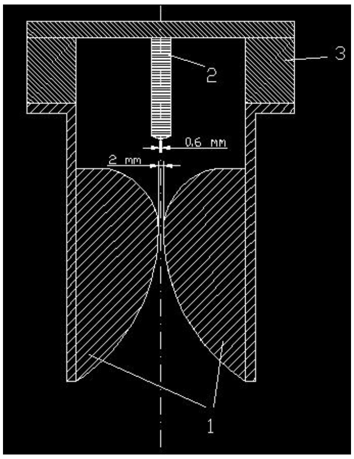

Fig. 3. Geometrical configuration of the electrodes

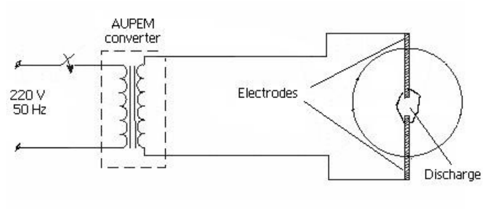

Fig.4. Electrical connections for the GlidArc reactor

The waveforms for the current and voltage are presented in fig. 5.

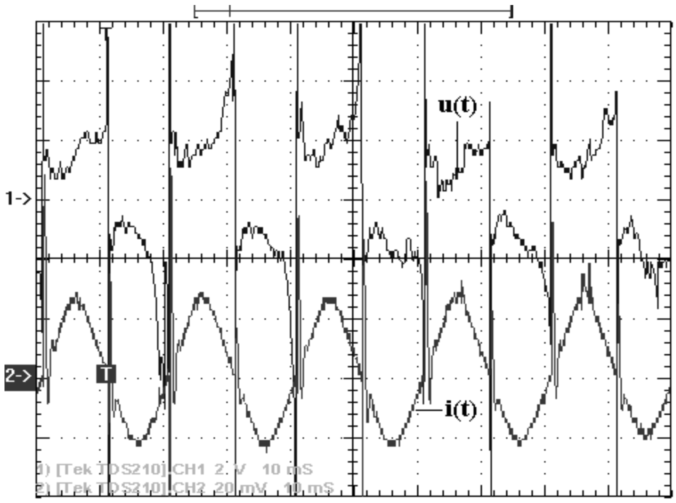

Fig. 5. Current and voltage waveforms

Triticum aestivum L. (wheat) and Lycopersicum esculentum Mill. (tomato) seeds were used for the experiments. The seeds were placed in Petri containers and set to germination in controlled environment at constant temperature. When the roots had a length around 15 to 18 mm (the mitotic index is considered to have maximum values at this size) the seeds were exposed to GlidArc. Experiments were conducted for 5, 2.5 and 1 minute exposure times. One Petri container was used as control sample for comparison purpose for each type of seeds.

The seeds under treatment are placed in a Petri container in the active area of the reactor, at a convenient distance in order to outline the active species influence and to avoid the unwanted thermal effects.

3. Results

Specific responses to cold plasma exposure were induced for each phase of the mitotic division.

For both types of seeds, the treatments led to the decrease of the division rhythm for the mitogen cells in the radicular meristems, directly correlated with the exposure time, as illustrated in Fig. 6 and Fig. 7

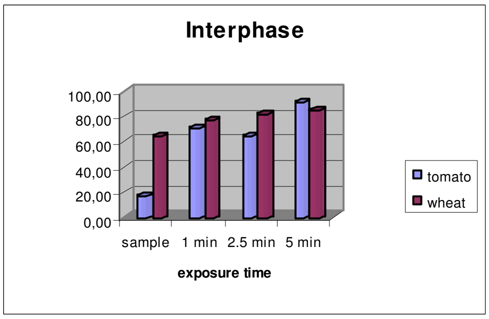

Fig. 6. Percentage of cells in interphase

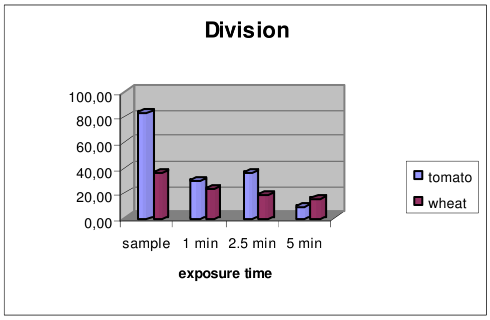

Fig. 7. Percentage of cells in division

The percentage of cells in phases of the mitotic division can be observed in fig. 8.

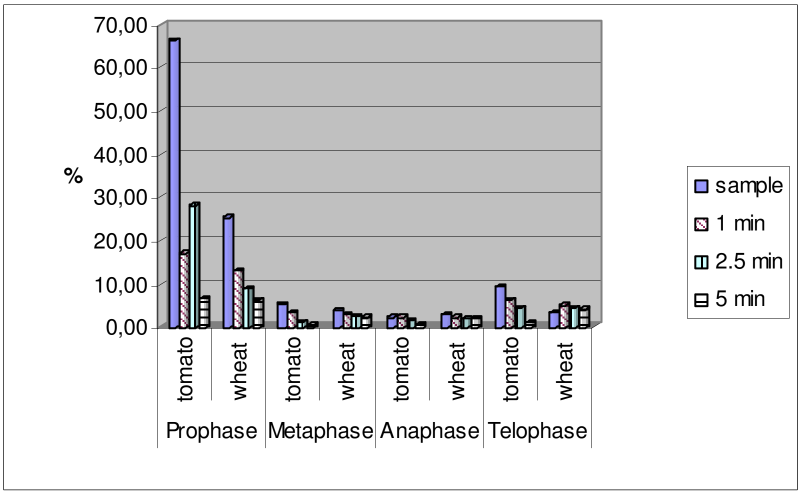

Fig. 8. Percentage of cells in phases of the mitotic division

The reactive species generated by the glidarc caused aberrant metaphases and ana-telophases in the root meristems of Lycopersicum esculentum and of Triticum aestivum L., as depicted in fig. 9.

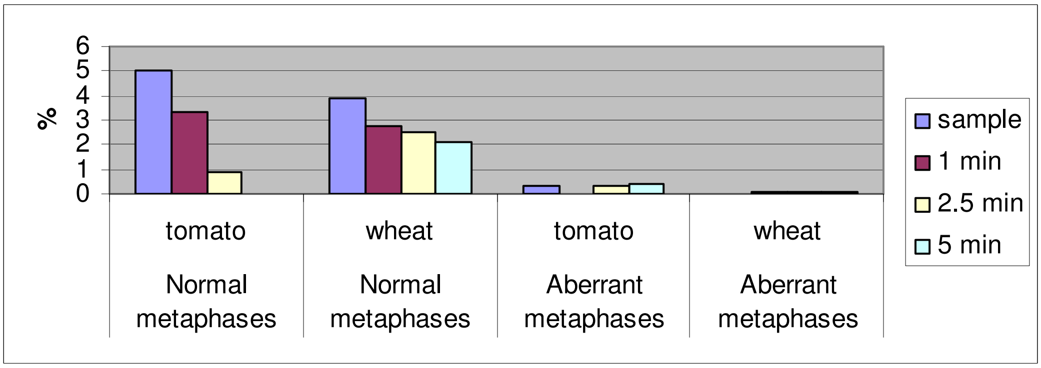

Fig. 9. Precentage of cells presenting aberrant metaphases

The aberrant ana-thelophases were present in all the treated samples, with a percentage that exceeded the control sample, illustrated in fig 10.

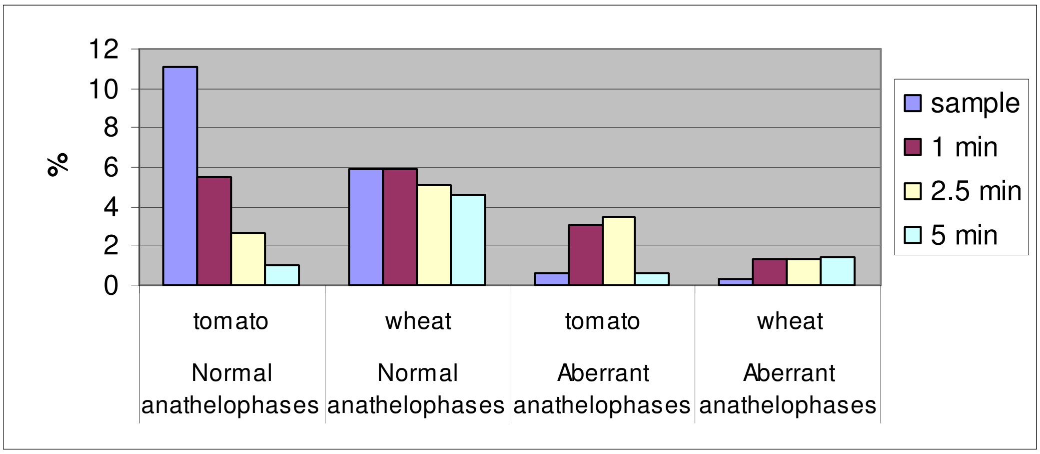

Fig. 10. Precentage of cells presenting aberrant ana-telophases

For tomato seeds, the chromosomal bridges were present in all treated samples, for the 2.5 and 5 minutes exposed seeds, very thick bridges were produced.

The retardary chromosomes and micronuclei were induced in all treated seeds, while the multipolar ana-telophases were present in the seeds treated for shorter exposure times (2.5 and 1 minute). Also, inert nuclei were discovered in the treated samples, not found in the case of wheat seeds.

The types of chromosomal aberrations are presented in fig. 11.

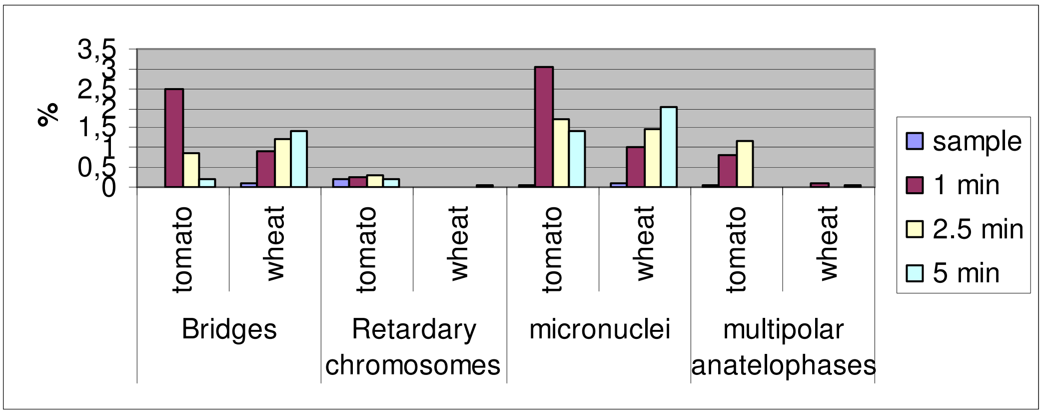

Fig. 11. Types of induced chromosomal aberrations and their occurence

For wheat seeds, the chromosomal bridges are present in all samples, with a rate smaller than 1% for the 1 min exposed sample and larger than 1% for the other treated samples. For the reference sample, the presence of the bridges is not significant (0.1%). The retardary chromosomes are present only for the 5 min treated sample in a percentage of 0.05%. The multi-polar ana-telophases appeared in the samples treated for 1 and 5 minutes with percentages between 0.05% and 0.12%. The occurrence of micronuclei is between 1.02% and 2.04% - the sample treated for 5 min and these are present in all samples. For the control sample, the percentage of micronuclei is 0.1%.

4. Conclusions

The cold plasma GlidArc type treatments have influences on the mitogen cells from radicular meristems of wheat and tomato by decreasing the mitotic index, and on the other side, by inducing chromosomal aberrations. The mitotic index decreases with the increase of the cold plasma exposure time. The cells had different reactions in the four phases of the mitotic division. The identified chromosomal aberrations are in a wide variety, proving the real mutagenic potential of the active species generated by cold plasma. The cold plasma treatments slowed the growth process for the plantlets. These results are well correlated with the mitotic indexes. For all the treated wheat samples the content of pigments higher than for the control sample proves a reaction produced by the plants in response to cold plasma exposure.

The fact that only for the tomato seeds, a five minute exposure time has been lethal indicates a dependency of these effects according to the genotype.

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