Plasmas for plant bio-engineering and in agriculture for resource recovery

N. Puač[1], N. Škoro[1], S. Živković[2], M. Milutinović[2], S. Jevremović[2], O. Jovanović[1], G. Malović[1] and Z.Lj. Petrović[1,3]

[1]*Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia*
[2]*Institute for Biological Research "Siniša Stanković", University of Belgrade, Bul. despota Stefana 142, 11000 Belgrade, Serbia*
[3]*Serbian Academy of Sciences and Arts, Knez Mihailova 35, 11000 Belgrade, Serbia*

Abstract: We have used different low-temperature atmospheric pressure plasmas for direct or indirect treatment of plant tissue. The plasma sources were characterised in detail by using optical emission spectroscopy, mass spectroscopy and electrical probes. The influence of the plasma treatments on the plant cells was investigated through enzyme response, development of calli and process of somatic embryogenesis. Also, the effect of plasma treated water and plasma decontaminated water was followed through the process of seed germination.

Keywords: plasma agriculture, plant calli, plasma decontamination.

1. Introduction

In the last decade the atmospheric pressure plasma systems have been extensively used in biomedical application [1-3]. Their construction and development was governed by the type of application and by the type of biological system that is treated. In parallel another field of plasma applications was growing – plasma agriculture. The first experiments were done with the low pressure nonequilibrium plasmas and primarily in treatments of seeds [4, 5]. Due to the fact that most seeds, plants, plants cells etc. cannot withstand vacuum, the devices that are nowadays mostly used are operating at atmospheric pressure. In such system, for example, the rich plasma chemistry changes the coat of the treated seed resulting in changes in wettability, better water uptake, increased percentage and speed of germination [6-8]. At the same time the pathogens that can be found on the seed coat and can be responsible for low germination percentage or contaminated plant are also destroyed during the plasma treatments. Therefore, unlike the classical methods involving different chemicals, plasma treatments can have a double or even multiple impact on the treated seeds.

As with the mammal cells, the interaction of the chemical species created in plasma and the triggered mechanisms are of the outmost importance. In the world of plants this can be done through investigation of the plasma treatments of the plant calli. In biological research and biotechnology the plant callus (pl. calli) is induced from plant tissue and it forms growing mass of plant parenchyma cells. The influence of the plasma created reactive oxygen and nitrogen species (RONS) and their interaction with calli can be investigated through the response of the cell enzymes. Puač et al. have shown that the interactions of the plasma created RONS with the plant cells can have even a long term influence [9].

Apart from the plasma treatments where plasma is in direct contact with seeds or plant cells like calli, there is a large group of experiments that investigate the influence of plasma treated water (plasma activated water – PAW) on theses specimens. It was shown that PAW used for imbibition of the different types of seeds increases germination percentage and on the molecular level changes the enzyme activity level.

Another very important topic is the possibility of re-usage of the water contaminated by agricultural means (pesticides, fertilization, biological farm waste etc.). Here we will present the results of applications of atmospheric pressure plasmas in direct treatment of plant seeds and calli or treatment of clean or contaminated water.

2. Application of atmospheric pressure plasmas in treatments of plant tissue and water used in agriculture

We have used several types of nonequilibrium plasma systems that operate at atmospheric pressure for direct treatments of plant calli, for treatment of water in order to produce PAW and for treatment of water contaminated with pesticides.

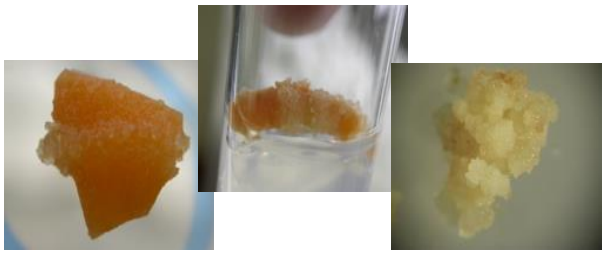

Fig. 1. *Daucus carota* callus

Pin geometry plasma sources used for these applications operate in the range of frequencies from kHz to MHz. These devices were used with helium as working gas and He gas flow was kept constant at 1 slm and 2 slm depending on the application. All plasma systems were characterised in detail by optical emission spectroscopy, mass spectroscopy and by using electrical probes.

The direct plasma treatment of plant calli was used to investigate the plasma cell interactions and to follow the response of the plant tissue several hours and days after the

treatment. Some of the samples that were used were taken from the model plants (like carrot cells – see Fig. 1.) and others from the plants that have an issue that needs to be overcome. For example callus of the yellow irises fall in the second group with its inability to enter the process of somatic embryogenesis (SE).

The direct plasma treatments of calli of *Daucus carota* were performed in order to investigate if the process of SE can be triggered even in the conditions where it is disabled. This means that the ratio of the growth hormones (in particular Auxin) in the culture medium is set to keep out the plant cells from going into SE. The results of such treatments are shown in Fig. 2. We can see that even in the conditions where SE cannot be initiated, plasma treatment triggered this process and the number of formed somatic embryos is higher with longer treatment time.

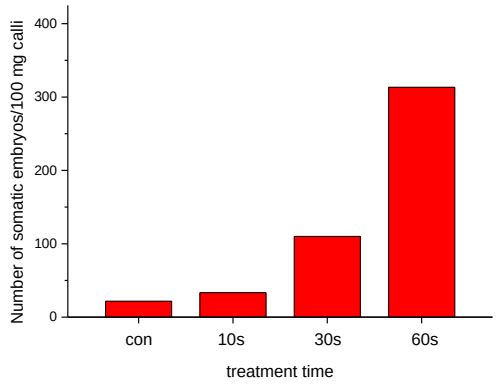

Fig. 2. Number of somatic embryos in *Daucus carota* callus for untreated and treated samples.

In case of water treatment, we have used clean distilled water and water contaminated by pesticides for creation of PAW. Both types of treated samples were then used for imbibition of seeds and investigation of effects on germination percentage and enzyme response. In order to determine the amount of active RONS species the PAW and decontaminated water samples were also characterised in detail by using spectrophotometry, total organic compound, dissolved oxygen, pH and conductivity measurements.

3. Conclusion

Nonequilibrium plasmas have shown a lot of potential in applications in biotechnology and agriculture. We have used several types of atmospheric plasma sources for treatments of plant calli or clean/contaminated water that was used for imbibition of seeds or watering of plants. All the plasma devices were characterised in great detail and optimised depending on the type of the sample and application. In the direct plasma treatment of plant calli it was determined that plasma species interacting with plant tissue can serve as a trigger for process of SE, even when this process is blocked or conditions unfavourable.

When used in treatments of water, RONS created in plasma and deposited in PAW increased the germination percentage of the imbibed seeds. Also, in the case when this water was contaminated by pesticides it was found that plasma treatments reduced harmful effect.

Acknowledgments: Supported by MESTDRS III41011 and ON171037.

4. References

[1] N. Tesla, Journal of Best Plasma Articles, **1**, 2 (1905).
[1] I. Adamovich et al., J. Phys. D. Appl. Phys., **50**, 323001 (2017).
[2] R. Matthes, C. Bender, R Schlüter, I. Koban, R. Bussiahn, S. Reuter, J. Lademann, K.D. Weltmann and A. Kramer, PloS one, **8**, 7, 70462 (2013).
[3] S. Lazović, N. Puač, M. Miletić , D. Pavlica , M. Jovanović , D. Bugarski , S. Mojsilović , D. Maletić, G. Malović, P. Milenković, Z. Petrović, New J Phys, **12**, 8, 083037 (2010).
[4] S. Živković, N. Puač, Z. Giba, D. Grubišić and Z.LJ. Petrović, Seed Science and Technology, **32**, 693-701 (2004).
[5] B. Šerá, V. Straňák, M. Šerý, M. Tichý & P. Špatenka, Plasma Sci. Technol., **10**, 506–511 (2008).
[6] N. Puač, M. Gherardi and M. Shiratani, Plasma Process Polym. e1700174 (2017).
[7] E. Bormashenko, R. Grynyov, Y. Bormashenko and E. Drori, Sci. Rep., **2**, 3–10 (2012).
[8] L. Sivachandiran and A. Khacef, RSC Advances, **7**, 1822–1832 (2017).
[9] N. Puač, S. Živković, N. Selaković, M. Milutinović, J. Boljević, G. Malović and Z. Lj. Petrović, Applied Physics Letters, **104**, 214106 (2014)