

# Plasma seeds treatment as a promising technique for seed germination improvement

I. Filatova<sup>1</sup>, V. Azharonok<sup>1</sup>, V. Lushkevich<sup>1</sup>, A. Zhukovsky<sup>2</sup>, G. Gadzhieva<sup>2</sup>, K. Spasić<sup>3</sup>, S. Živković<sup>4</sup>, N. Puač<sup>3</sup>, S. Lazović<sup>3</sup>, G. Malović<sup>3</sup> and Z.Lj.Petrović<sup>3</sup>

<sup>1</sup> Institute of Physics NAS of Belarus, Nezavisimosti Ave. 68, 220072 Minsk, Belarus

<sup>2</sup>Institute of Plant Protection NAS of Belarus, Mira Str. 2, 223011 Priluki, Minsk District, Minsk Oblast, Belarus

<sup>3</sup>Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia

<sup>4</sup>Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia

An influence of RF air plasma pre-sowing treatment on seed germination of some important agricultural plants has been studied. Two plasma systems (plan-parallel and cylindrical) were used for treatment of maize, spring wheat and lupinus seeds. It is shown that the treatments contribute to seeds germination enhancement decrease and their phytosanitary conditions improvement. The modification of seed coat surface structure by plasma irradiation is investigated with scanning electron microscopy and plasma emission spectra are analyzed for possible mechanisms determination of biological effect of plasma treatment.

## 1. Introduction

Cold plasma treatment is widely used for activation and decontamination of surfaces. Owing to the unique plasma features this technique is applicable for modification of a wide range of thermally sensitive materials including biological tissues. Recently it has been applied successfully for treatment of plant seeds [1–7]. Low temperature plasma pre-sowing seeds treatment was shown in some cases to be ecologically safe, cheap and effective method for improvement of seed germination and their resistance to stress and diseases.

At the same time there is a lack of research explaining the mechanism of plasma bio-stimulation. Possible processes were proposed in the literature. Plasma processing subjects the seed surface to UVradiation, charged particles bombardment, radicals and chemically active molecules resulting in the formation of functional groups on the treated surface [3, 4]. Air plasma treatment changes the wetting properties of seeds due to oxidation of their surface that leads to faster germination and greater yields [8], increases the concentration of free radicals in seeds which play an important role in acceleration of the seed metabolism [9]. The possibility of delay or enhancement of germination is demonstrated by plasma-induced coating with thin films containing different macromolecular components [10, 11]. It is obvious, that plasma treatment can have a variety of effects on morphological and sowing characteristics of seeds due to the complexity of plasma interaction with organic materials and living cells [12]. Seeds are an extremely complex system too, and vitally important

biological processes may be affected by the treatment in a number of different ways.

The aim of this paper is to study the efficiency of low-pressure RF air plasma treatment of seeds of some important agricultural crops for improvement of their sowing properties and to try to identify some of the main plasma agents that contribute to enhancement of germination.

## 2. Experimental

Seeds of spring wheat (*Triticum aestivum* L.), blue lupine (*Lupinus angustifolius*) and maize (*Zea mays* L.) were chosen for investigations. Tested species were treated with RF air plasma using two plasma systems. The first one was a planar geometry reactor operating at 5.28 MHz [5]. The electrode system consists of two identical water-cooled copper disks with the diameter of 120 mm placed in a stainless steel vacuum chamber. A supplied full specific RF power  $W$  could be changed in the range from  $0.2 \text{ W/cm}^2$  to  $0.6 \text{ W/cm}^2$  resulting in different treatment conditions. All species were treated at pressure of 500 mTorr. A Petri dish with seeds was put on the grounded (lower) electrode. Duration of the exposure was 2.5, 5, 8 and 10 min. Each Petry dish contained 50 seeds. All treatments for all experimental conditions were replicated four times. Control group was only subjected to vacuum and gas pressure  $P=0.5 \text{ Torr}$  for at least 15 min. The gas temperature did not increase beyond  $310^\circ\text{C}$ .

The second plasma system used for pre-sowing treatment of seeds was a cylindrical CCP reactor operating at 13.56 MHz. In this system, a central, powered electrode is aluminum rod and the grounded

electrode is the wall of the chamber. A detailed description of the system can be found in [6]. The treated samples were placed in Petri dishes and put on a platform that is positioned at the bottom of the chamber. Every Petri dish housed 20 seeds with exception of maize where, due to seed size, only 10 seeds per dish were used. Total amount was 60 seeds per treatment. Spring wheat was treated at powers of 50 W and 100 W at pressure of 500 mTorr, while maize was treated at 300 mTorr with 200 W of applied RF power. Since different time of plasma exposure can cause very different results, this parameter was varied in a wide range (1min, 5min, 7min, 10min and 20 min).

The effectiveness of pre-sowing plasma seed treatments was examined by means of evaluation of the laboratory germination ability and biometric characteristics (mean root and plant length) of treated and control samples. Seeds were grown on a moist filter paper in sterile Petri dishes in a thermostat at 20° C (for wheat and lupine) and 25° C (for maize) under a light-dark regime. The seed germination and the seed infection were estimated after 7 and 10 days incubation for wheat and lupine/maize correspondingly.

Optical emission spectra (OES) were obtained with a Compact Spectrometer S100 "SOLAR LS" in the optical range from 190 to 1100 nm with an average spectral resolution of 1 nm to identify the species present in plasma during the treatment. Surface structure of the treated and untreated seeds was imaged with a high resolution scanning electron microscope (LEO 1455 VP).

### 3. Results and discussion

It has been found that plasma pre-treatments of seeds positively influenced their germination and biometric characteristics of sprouts. The results obtained in planar discharge are shown in Fig. 1. The plasma treatment of seeds with low germination ability (spring wheat, maize) stimulated their germination and the early stages of seedling development, while it did not affect negatively the germination of seeds with high germination ability (lupine). The seed pre-treatments for 2.5 and 5 min were the most effective for all species. The seedling of treated spring wheat was 2.1 cm higher than that in the control group (Fig. 1b). The same result was observed for maize seeds as a result of plasma treatment during 2.5 min. Large seedlings have a higher survival and growth rates than small seedlings that will provide good conditions for plant growth at the later stages of ontogenesis.

Similar results for plasma treatment of spring wheat and maize were obtained in asymmetric CCP

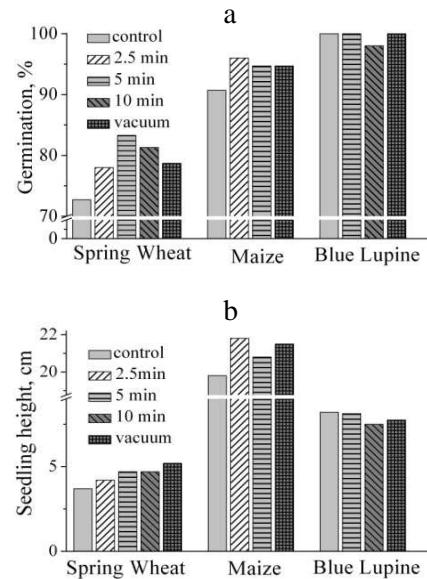


Fig. 1. Germination (a) and seedling height (b) of spring wheat, maize and blue lupine as a result of plasma and vacuum (without plasma) seed pre-treatments ( $W = 0.2 \text{ W/cm}^2$ ,  $P = 500 \text{ mTorr}$ )

discharge. Results for spring wheat seed germination after plasma treatment at 50 and 100W are presented in Fig. 2. For both powers better results are achieved for shorter treatment times. Since the control group germination percentage is quite high the overall increase in germination percentage in treated batches is only few percent. Treatments with longer treatment times then 7 min showed a decrease in germination due to the damage of the seed inflicted by plasma bombardment. For higher power of 100 W and longer times this damage is quite high. In this case the germination percentage was reduced down to 70 %.

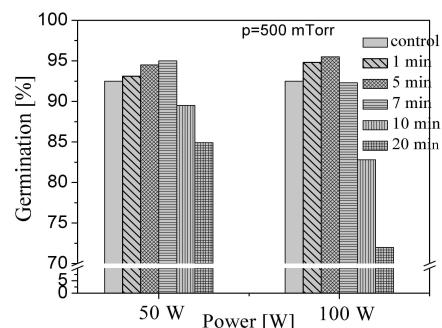


Fig. 2. Germination of spring wheat after plasma treatment in asymmetrical CCP discharge. Control represents untreated seeds. Feeding gas was air and the pressure was  $P = 500 \text{ mTorr}$

Unfortunately most of the seeds used for commercial purpose are infected with different types of fungi (belonging to the genera *Mucor*, *Fusarium*,

*Alternaria* etc.). Therefore, it is important to check if same plasma conditions can be used to decrease the infection of seeds.

The results for infection percentage of spring wheat are shown in Fig. 3 for both plasma systems. We can see that we are able to reduce total infection in treated wheat seeds up to 10 min of treatment. For longer treatment times (20 min) the percentage of infected seeds increases. This is most probably due to the damage of the seed coat caused by treatment. The higher damage of the seed coat further increases seed's susceptibility to infection. Plasma treatment is shown as an effective tool against *Fusarium* spp. that causes the most harmful root disease of wheat worldwide.

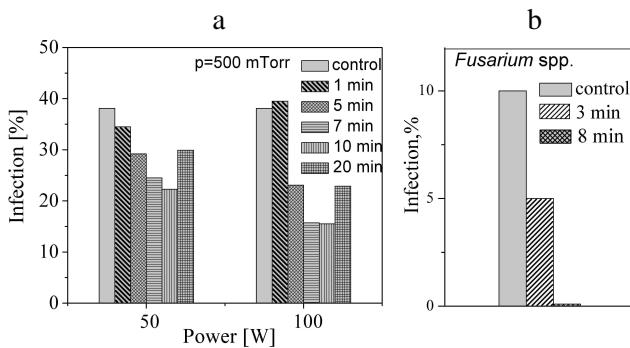


Fig. 3. Infection of spring wheat after plasma treatment in asymmetrical CCP discharge (a) and infection with *Fusarium* spp. after treatment in planar discharge (b). Control represents untreated seeds. Feeding gas was air at a pressure of  $P = 500$  mTorr

Similar results are observed for maize and lupine (see Fig. 4 and 5).

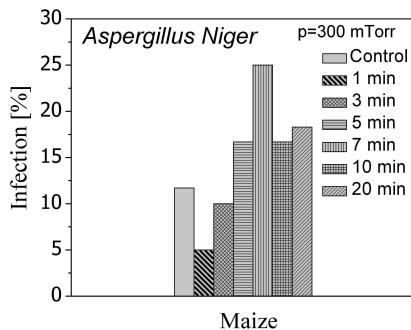


Fig. 4. Infection of maize after plasma treatment in asymmetrical CCP discharge. Control represents untreated seeds. Feeding gas was air at a pressure of  $p = 300$  mTorr. Power given by RF power supply was 200 W

For lower treatment times in asymmetrical CCP discharge we have observed a decrease of the infected seeds percentage, but with an increase in treatment times infection spreads even more than in the control

group. We can conclude that both effects (germination increase & infection decrease) can be accomplished only for lower treatment times for the present setup of the asymmetric CCP. For this pressure/power combination this interval is between 1 and 7 min of treatment. The same treatment durations (between 3 and 8 min) were the most effective against fungi and bacteria for treatment of maize and lupine seeds in planar discharge (see Fig. 5).

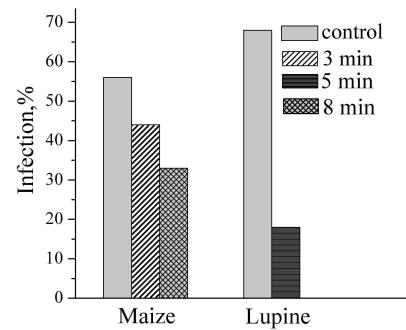


Fig. 5. Level of total infection of maize and lupine after plasma treatment in planar discharge. Control represents untreated seeds. Feeding gas was air at a pressure of  $P = 500$  mTorr

It was revealed from the SEM images of seed coats that the surface structure of seeds changed sharply as a result of plasma treatment (Fig. 6). SEM indicated that the surface sculpture of untreated wheat seeds had a reticulate texture (Fig. 6a). The plasma treated wheat seeds had an eroded surface, with no significant ridges (Fig. 6b). The untreated lupine seed coat was formed by elongated polygonal cells (Fig. 6c). The plasma treated lupine seed coat had a granular surface (Fig. 6d).

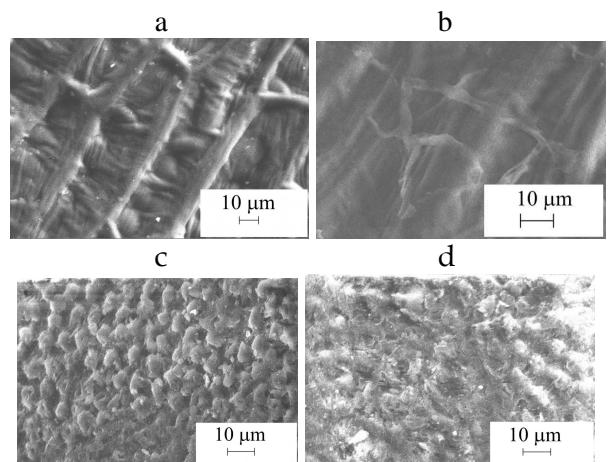


Fig. 6. Scanning electron micrograph (SEM) images of seed coat surface of wheat (a, b) (magnification = 2.00 KX) and blue lupine (c, d) (magnification = 1.00 KX) for non-treated (control) seed (a, c) and plasma treated for 5 minutes (b, d)

No well-defined cristate-papillate structure on the seed surface of lupine was observed after the plasma treatment. Similar results were obtained for another seeds which provided experimental evidence of the seed coat surface etching induced by plasma treatment [2, 3]. Study of the seed coat thicknesses after the plasma processing showed that the treatment removes effectively the very thin lipid layer that makes seeds water-repellent and probably reduces the length (and average molecular weight) of the biopolymer chains that make up the seed coat, thus enabling better water transport through the seed coat improving the germination [13].

In order to characterize the change of seed coat structure and seed germination as a function of plasma treatment conditions the OES spectra of the plasma were analyzed. The difference was observed between spectra generated by plasma without seeds and during their treatment (Fig. 7). The species identified in the spectra are neutral molecular nitrogen N<sub>2</sub> (bands of the first and the second positive systems), ionized molecular nitrogen N<sub>2</sub><sup>+</sup> (bands of the first negative system). When seeds are in the plasma bands of the Angstrom system of the CO molecule appear in the spectrum.

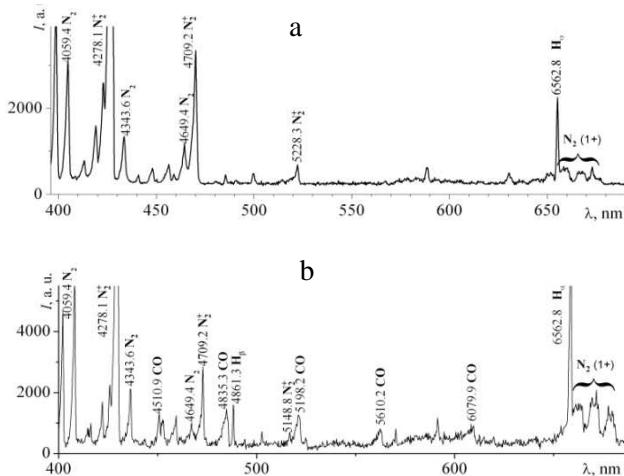


Fig. 7. Emission spectra of RF air plasma: a – pure air, b – under conditions of plasma treatment of wheat seeds

This confirms effectiveness of seed surface etching during the treatment and a possibility of formation of functional groups on treated surface that may play an important role in stimulation of seed germination [4, 5, 7].

#### 4. Summary

This study confirms that the low temperature air plasma pre-treatment of seeds of some important agricultural crops is an effective tool for improvement of germination, shoot and root growth, providing a

good fungicidal and bactericidal effect in the optimal experimental conditions that can vary for different species. SEM investigations of seeds surface have shown a significant change in its texture that is an evidence of surface etching provided by bombardment by charged particles and radicals formed in plasma. Since the seed coat for treated samples are eroded it provides a better water permeability and leads to enhancement of seed germination.

**Acknowledgements.** This work is partly supported by the Belarusian Republican Foundation for Fundamental Research (grant No. F11SRB-015) and by the MES, Serbia, under the contract numbers ON171037 and III41011.

#### 5. References

- [1] A. Dubinov, E. Lazarenko, V. Selemir, IEEE Trans. Plasma Sci. **20** (2000) 180.
- [2] M. Dhayal, S.Y. Lee, S.U. Park, Vacuum. **80**, No.5 (2006) 499.
- [3] B. Šerá, V. Straňák, M. Šerý, M. Tichý, P. Špatenka, Plasma Science and Technology. **10**, No. 4 (2008) 506.
- [4] S. Živković, N. Puač, Z. Giba, D. Grubišić, Z.Lj. Petrović, Seed Science and Technology. **32**, No. 3 (2004) 693.
- [5] I.I. Filatova, V.V. Azharonok, A. Shik et al., in: Plasma for Bio-Decontamination, Medicine and Food Security, ed. by Z. Machala et al., NATO Science for Peace and Security Series A: Chemistry and Biology, Springer (2012) 469.
- [6] N. Puač, Z.Lj. Petrović, S. Živković, Z. Giba, D. Grubišić and A.R. Đorđević, "Low temperature plasma treatment of dry Empress-tree seeds", Plasma Processes and Polymers, Eds. R. d'Agostino, P. Favia, C. Oehr and M.R. Wertheimer, (Whiley: (2005) 193.
- [7] M. Selcuk, L. Oksuz, P. Basaran, Bioresource Technology, **99** (2008) 5104.
- [8] <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3473364/pdf/srep00741.pdf>.
- [9] I.I. Filatova, V.V. Azharonok, N.A. Poklonski, et al., Proc. of the VII Int. Conf. Plasma Physics and Plasma Technology, September 16-21, 2012, Minsk, Belarus, ed. by V.M. Astashinski et al., Kovcheg, Minsk (2012) 734.
- [10] J.C. Volin, F.S. Denes, S.M.T. Park, R.A. Young, Crop Science. **40** (2000) 1706.
- [11] R.A.M. Carvalho, A.T. Carvalho, M.L.P. da Silva et al., Quim. Nova. **28**, No. 6 (2005) 1006.
- [12] B. Šerá, P. Špatenka, M. Šerý et al., Plasma Science, IEEE Transactions on Plasma Science. **38** (2010) 2963.
- [13] [http://www.aff.org.au/Griesser\\_Plasma\\_treatment\\_of\\_%20seeds\\_Final.pdf](http://www.aff.org.au/Griesser_Plasma_treatment_of_%20seeds_Final.pdf).