applied sciences

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Influence of a Helium–Nitrogen RF Plasma Jet on Onion Seed Germination

Agnieszka Starek-Wójcicka [1], Agnieszka Sagan [1,*], Piotr Terebun [2], Michał Kwiatkowski [2], Piotr Kiczorowski [1] and Joanna Pawlat [2,*]

1 Department of Biological Bases of Food and Feed Technologies, University of Life Sciences in Lublin, Akademicka 13, 20-612 Lublin, Poland; agnieszka.starek@up.lublin.pl (A.S.-W.); piotr.kiczorowski@up.lublin.pl (P.K.)

2 Chair of Electrical Engineering and Electrotechnologies, Lublin University of Technology, Nadbystrzycka 38a, 20-618 Lublin, Poland; p.terebun@pollub.pl (P.T.); m.kwiatkowski@pollub.pl (M.K.)

* Correspondence: agnieszka.sagan@up.lublin.pl (A.S.); j.pawlat@pollub.pl (J.P.)

Received: 18 November 2020; Accepted: 14 December 2020; Published: 16 December 2020

Abstract: This paper presents an experiment using a radio frequency atmospheric pressure plasma jet to generate cold plasma for pre-sowing stimulation of Wolska onion seeds. Impact of the He + N_2 afterglow plasma on germination was investigated. Eight groups of seeds characterized by different exposure times (2, 5, 10, 15, 60, 120, 240, and 480 s) and distance from the electrodes (20 mm and 50 mm) were used. Pre-sowing plasma stimulation of the seeds improved the germination capacity and germination energy for all tested groups, relative to control. The impact of radio frequency plasma on the onion seed germination parameters was statistically significant. The highest germination parameters were obtained for seeds stimulated for 240 s at a distance of 50 mm. No significant differences in physical and morphological properties of onion seeds were found.

Keywords: atmospheric pressure plasma; physical and morphological properties; onion seed germination; environment-friendly technology

1. Introduction

Germination comprises a number of processes occurring inside the seed and leading to the activation of the embryo. Fully mature seeds germinate at a specific rate and energy in favorable environmental conditions, i.e., appropriate temperature, access to oxygen and water, and light radiation on the soil surface. Not in all regions of the world can such optimal conditions be ensured [1,2].

Sowing seeds with weaker germinability produces many unused spaces in the ground, uneven seedling emergence, and uneven distribution of plants on the soil surface. Already in the initial period, emerging plants differ significantly in the level of growth and development, which is one of the basic causes of the stronger competition between plants and deterioration of the crop structure. Given sustainable agriculture recommendations, the global desire to limit the excessive use of chemical compounds in plant production, and the growing acreage of organic or integrated plant cultivation, the agricultural sector seeks environmentally friendly technologies [3–6] for improving germination.

The current methods for increasing plant production are based on the use of various physical factors for stimulation of plants and, especially, their seeds, i.e., laser radiation, constant and variable magnetic and electric field, ionizing radiation, microwave radiation, and ultrasounds. These factors influence the physiological and biochemical reactions in seeds; thus, they may accelerate the germination [7–10].

Cold plasma is an alternative and relatively new technology for increasing plant yields, which is consistent with the pro-ecological approach to the natural environment. It is a fast, economical, and pollution-free seed stimulation method relevant to the developmental and physiological processes in the plants. The treatment effect depends on both the discharge parameters and the type of plant, but many authors indicate the effects of reactive oxygen and nitrogen species (RONS). They can be generated through discharge in the working gas and ambient air or in the secondary reactions with the treated object. The amount and type of particles reaching the surface depends, among others, on values of the working gas flow and distance, where among the species reaching the farthest (NO, NO_2, N_2O, HNO_3, H_2O_2, and O_3), the largest amount of particles are less reactive [11–13]. The species for which the concentration is almost proportional to the treatment time is H_2O_2 [14], which can be transported to the interior of the cells via aquaporins [15,16]. Oxidative stress and pores created by the electric field may also affect the permeability of the other RONS particles, particularly hydrophobic NO, NO_2, O_2, and O_3, however, the mechanisms responsible for changes of plant physiology are not fully understood [15]. In addition to changes in the cell itself, plasma can also reduce the bacterial bearing rate of seeds or change the seed coat structure, increasing the permeability of the seed's coat and stimulating seed germination [17–22].

Onion (*Allium cepa* L.) is one of the most commonly consumed vegetables worldwide. However, it has specific climate and soil requirements that may impede cultivation, which subsequently leads to poor establishment and yield reduction. It reacts very strongly to the length of the day (in short-day conditions, onion varieties produce only leaves instead of bulbs). Additionally, with its shallow root system, onion is sensitive to water shortage in soil at every stage of growth. However, excessive rainfall at the end of the growing season can delay the onion ripening and deteriorate the quality and storage stability of the product. Furthermore, onion requires very carefully prepared field conditions, especially when cultivated from seeds sown directly into soil [23–26]. Partial reports focused on helium–air plasma impact on onion were presented during a conference [27,28]. The highest germination rate of 80% was achieved for 240 s treatment, in comparison to 52% for the control. The aim of the current study was to investigate the effects of radiofrequency plasma treatment on physical and morphological properties, and further improvement of onion seeds' germination rate using a different gas mixture: He + N_2.

2. Materials and Methods

2.1. Cold Plasma Treatment

Tested material were Wolska seeds (*Allium cepa* L.) purchased at Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa PNOS, Ożarów Mazowiecki (Poland). They were exposed to afterglow plasma gas. Plasma was generated in the radio frequency jet reactor (Figure 1). The plasma jet was excited by an AG 1021 RF generator (T&C Power Conversion, Rochester, NY, USA) with integrated amplifier and meter of forward and reflected power. In order to increase the voltage between electrodes (sinusoidal signal of 890 V RMS), an air transformer (1:12 winding ratio) was used with parallel connected capacitor for impedance matching. Load power was set to 45 W (forward power 47 W) with frequency of 14.16 MHz. Plasma was generated in the discharge gap between an external tubular-shaped electrode made of stainless steel (14 mm diameter) and internal tungsten rod-type electrode (5 mm diameter) [13]. Substrate gas was a mixture of He + N_2 (ratio 3:2) at total flow rate of 710 L/h. For the gas dosing, glass tube variable area flow meters (Zakłady Automatyki "ROTAMETR", Gliwice, Poland) were used. One layer of seeds was placed on the movable strainer located in the glass container (60 mm in diameter and 60 mm length). The distance between the nozzle's outlet and surface of the sample was 20 mm (for treatment times up to 15 s) or 50 mm. The energy density inside the RF plasma reactor was quite high, ranging to 230 kJ/m^3. It is difficult to calculate directly the power transferred to the seeds as they were placed at selected distance from the nozzle, in the afterglow area. Direct gross power calculation result was around 7 W forwarded power per 1 g of seeds.

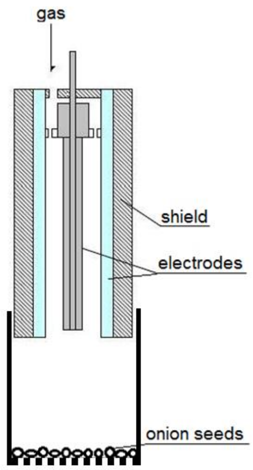

Figure 1. Schema of experimental set up with radio frequency atmospheric pressure plasma jet.

2.2. Physical and Morphological Properties

A DT-847U meter and type K thermocouple (Yu Ching Technology Co., Ltd., Taipei, Taiwan) were used for the measurement of the seeds' surface just after selected plasma treatment time. The moisture of the onion seeds was determined using an SLN15 STD (Wodzislaw Śląski, Poland) laboratory dryer with forced air circulation at 105 °C to constant weight.

Seed compressive strength was determined using a Zwick Roell BT1-FRO.5TN.D14 (Ulm, Germany) testing machine. The head travel speed was 10 mm/min. The test was carried out with the use of a head with maximum pressure force F = 500 N. Samples of individual randomly selected seeds (from each experimental group) were crushed to 50% of height and the compressive force values were recorded. The result was reported as the arithmetic mean of 100 replications.

The morphological properties of seeds were observed with a KEYENCE VHX-5000 digital microscope (Osaka, Japan). Images of the samples were taken immediately after plasma treatment.

2.3. Germination Energy and Capacity

Seed germination was conducted in accordance with the International Seed Testing Association ISTA [29] recommendations of 2017. For substrate preparation, between paper (BP) germination (between paper—the seeds are germinated between two layers of filter paper) was used. Immediately after processing, plasma-treated seeds and control seeds were placed in five rows of 20 seeds on each of the five moistened filter papers. In the experiment, a total of 500 seeds in each combination were examined. The seeds were covered with paper and then rolled into a roll and placed in a foil bag. The experiment on seed germination was carried out in a climatic chamber Memmert GmbH+Co.KG company, type CTC256 (Schwabach, Germany) at temperature T = 20 ± 1 °C. Adequate moisture level was ensured by periodically moistening the filter paper. The number of sprouts was determined every 24 h. The fraction of germinated seeds (number of sprouts) after 6 days of germination was defined as germination energy, G_{EN}, while the fraction of germinated seeds after 12 days of germination was defined as germination capacity—G_C. Both germination energy and germination capacity were expressed as a fraction of the germinated seeds, G, after a certain time, t, and calculated from the following equation [29]:

$$G = \frac{n}{n_T} \cdot 100\% \quad (1)$$

where:

n—the number of seeds germinated at time t,

n_T—the total number of sown seeds.

2.4. Statistical Analysis

StatSoft Polska STATISTICA 10.0 program was used for statistical analysis of measurement results. ANOVA one-way analysis of variance was used to analyze statistical differences between groups. The significance of differences between mean values was determined using Tukey's test at a significance level $\alpha \leq 0.05$.

3. Results and Discussion

Knowledge of the physical and morphological properties of seeds helps to adapt the cultivation, harvesting, and processing technologies to benefit from the yielding potential of plants and to minimize losses associated with seed harvesting, storage, and processing.

Ambient air temperature was 23 °C and relative humidity was 41%. The maximum recorded temperatures of the seeds exposed to plasma are presented in Table 1. For the distance from the torch to the seeds of 20 mm, the temperature slightly increased with the treatment time. For the longest treatment time of 15 s, it was below 36 °C. For the distance enlarged up to 50 mm, increasing of treatment time resulted in significant rise of temperature of the sample, even up to 57.2 °C for 480 s, measured at the surface of the seeds.

Table 1. Selected physical parameters of onion seeds.

Time of Stimulation [s]; Distance between Nozzle's Outlet and Tested Material [mm]	Maximum Seed Temperature after Cold Plasma Stimulation [°C]	Moisture Content [%]	Compressive Force [N]
	Control—25.8	Control—9.18 ± 0.05 ^{abc}	Control—33.46 ± 1.07 ^{ac}
2; 20	27.5	9.09 ± 0.03 ^{abc}	34.34 ± 1.06 ^{ab}
5; 20	29.3	9.11 ± 0.16 ^{abc}	33.72 ± 0.66 ^{abc}
10; 20	33.7	8.92 ± 0.28 ^{ac}	34.02 ± 1.55 ^{abc}
15; 20	35.2	8.83 ± 0.06 ^{c}	37.09 ± 1.64 ^{b}
60; 50	47.2	9.15 ± 0.09 ^{abc}	34.07 ± 0.76 ^{ab}
120; 50	52.6	9.23 ± 0.04 ^{ab}	31.69 ± 1.55 ^{ac}
240; 50	54.0	9.22 ± 0.08 ^{ab}	30.20 ± 1.31 ^{cd}
480; 50	57.2	9.45 ± 0.13 ^{b}	27.44 ± 1.12 ^{d}

± standard deviation; ^{abcd}-average values in the column marked with the same letter are not statistically significantly different ($p < 0.05$).

Onion seed samples subjected to the stimulation with cold plasma produced with the use of a radio frequency generator exhibited a moisture level in the range from 8.83% to 9.45%, and these values were not statistically significantly different from those in the control sample—9.18% (Table 1).

Statistically significant differences were noted only between the control sample (33.46 N) and seeds treated for 15 s at the short distance (37.09 N) and for 480 s at the 50-mm distance between the reactor nozzle and the sample (27.44 N). The data presented in Table 1 also indicate that the stimulation of onion seeds with the plasma was not the cause of the changes in the value of the compressive force.

The morphological structure of the seeds before and after plasma treatment is presented in Figure 2.

Changes in the surface structure are quite subtle and hardly noticeable. Drastic and harmful alterations, such as fragmentation of the external layer, were not observed, even with the longest treatment time. However, some slight differences in the seed coat cell surface could indicate the change in the cell turgor pressure.

An important trait of seed material is its ability to germinate quickly and to produce healthy sprouts in a specified time. The dynamics of the plasma-treated onion seeds germination process is presented in Figure 3 and Table 2.

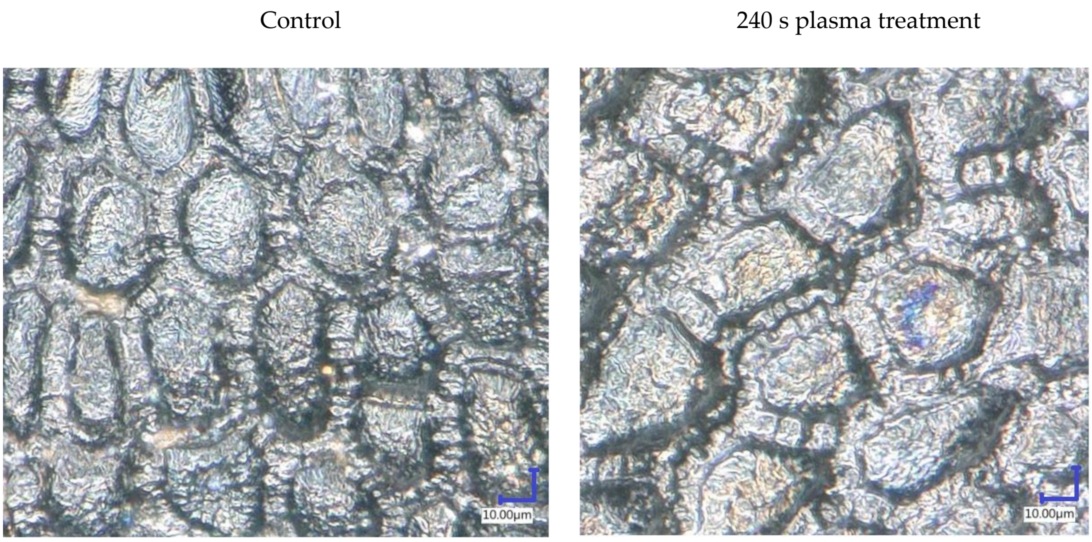

Figure 2. Comparison of the seeds' surface before and after plasma treatment, 1000 magnification.

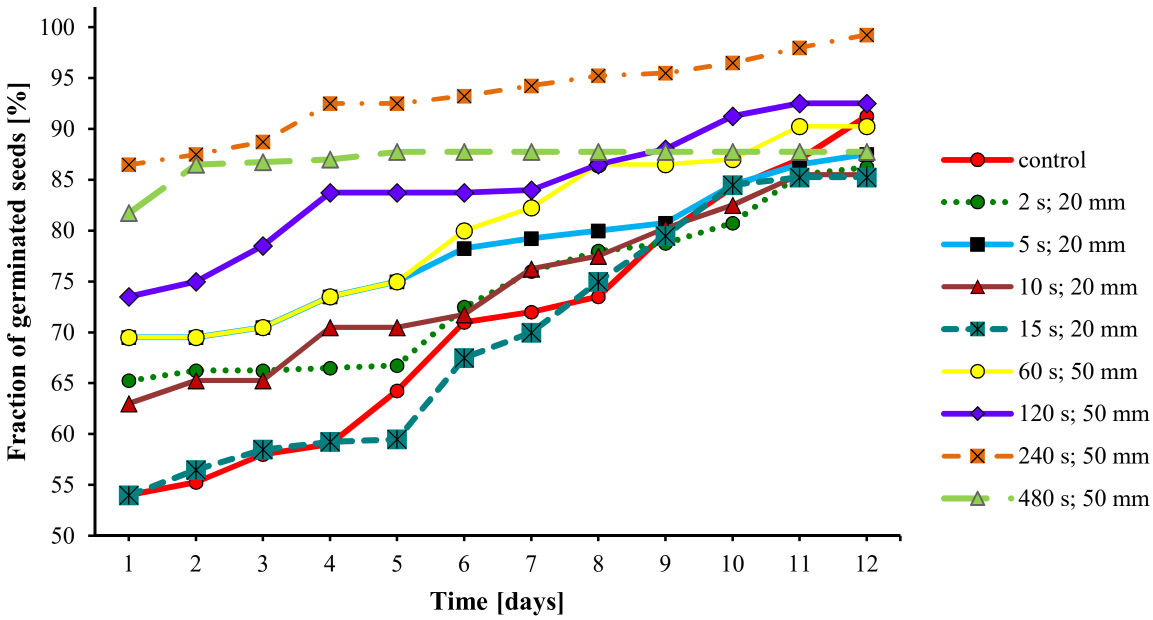

Figure 3. Fraction of germinated onion seeds after pre-sowing plasma treatment. (SD mean: control—2.44 2 s; 20 mm—2.02 5 s; 20 mm—1.86 10 s; 20 mm—1.38 15 s; 20 mm—1.98 60 s; 50 mm—2.02 120 s; 50 mm—1.95 240 s; 50 mm—1.57 480 s; 50 mm—2.32).

The exposure of onion seeds to cold plasma was found to exert an effect on energy and germination. The stimulation of the seeds with cold plasma for 60–480 s at a 50-mm distance between the electrode and the seeds yielded higher values of the germination parameters than the shorter stimulation (2–15 s) with the shorter distance. At the distance of 50 mm, there was a statistically significant increase in the germination energy value in all samples, in comparison with the control. The germination rate increased upon cold plasma exposure for 240 s. It was also greater than in the case of the helium and air gas mixture for the same tested treatment times [28]. The shorter seed stimulation with the lower distance reduced the germinability parameter. The highest energy and germination capacity were achieved by seeds stimulated for 240 s with cold plasma. In comparison with the control sample, the germination energy and germination capacity increased by 22% and 8%, respectively.

Table 2. The results on germination energy and germination capacity parameters of Wolska onion seeds after pre-sowing treatment with cold plasma at different exposure times and distance between electrode and tested material.

Time of Stimulation [s]; Distance between Nozzle's Outlet and Tested Material [mm]	Germination Energy G_{EN} [%] Control—71.0 ± 0.82 [a]	Germination Capacity G_C [%] Control—91.25 ± 0.96 [bc]
2; 20	72.5 ± 1.7 [a]	86.3 ± 2.6 [ab]
5; 20	78.3 ± 2.2 [b]	87.5 ± 2.7 [abc]
10; 20	71.8 ± 2.5 [a]	85.5 ± 3.0 [a]
15; 20	67.5 ± 2.1 [a]	85.3 ± 3.9 [a]
60; 50	80.0 ± 1.4 [bc]	90.3 ± 0.9 [abc]
120; 50	83.8 ± 3.1 [cd]	92.5 ± 0.5 [c]
240; 50	93.3 ± 1.8 [e]	99.3 ± 0.8 [d]
480; 50	87.8 ± 1.1 [d]	87.8 ± 1.1 [abc]

± standard deviation; [abcd]-average values in the column marked with the same letter are not statistically significantly different ($p < 0.05$).

In accordance with presented data, scientific literature provides reports on the application and positive effects of cold plasma on crop seeds. Zhou et al. [30] reported that tomato seedling growth was improved by atmospheric pressure plasma treatment. The bloom times, the height, the caulis, the extent of the plants, and the average weight, length, and diameter of each fruit were increased distinctly. The tomato yields of plasma-treated plants were larger than the untreated samples. Jiafeng et al. [31] and Šerá et al. [32] concluded that plasma treatment significantly improved the wheat germination rate. Cold plasma gives the possibility of thermal damage avoidance and has a positive impact on the seed germination and plant yield of *Oryza sativa* [33], *Glycine max.* L. [17] and *Lavatera thuringiaca* L. [20]. Under the influence of discharge plasma generated in dielectric barrier discharge, Zahoranová et al. [34] noted an improvement in the germination parameters and growth of maize seeds (*Zea mays* L.). The obtained results show an increase in wettability, resulting in a better water uptake and in the enhancement of growth parameters. Plasma source provides significant technical advantages and application potential for seed surface finishing without the use of hazardous chemicals.

4. Conclusions

With the growth of environmental awareness, special emphasis is placed on limiting the role of pesticides, fertilizers, and other agents required for plant growth, which can accumulate in the soil. On the other hand, harmful residue-free, high-quality crops are demanded.

The germination energy and capacity were increased by cold plasma treatment. The 240 s cold plasma treatment, when the distance of the reactor nozzle from the sample was 50 mm, produced the highest stimulatory effect among the different tested treatment conditions.

In summary, cold plasma treatments have the potential to enhance the germination of onion and could be used in future production. More studies are needed to elucidate the mechanisms that promote the effects of cold plasma treatment on onion seed germination.

Author Contributions: Conceptualization: A.S.-W., J.P., M.K., and P.T.; methodology: A.S., J.P., and P.K.; investigation: A.S.-W., J.P., M.K., and P.T.; data curation: A.S.-W., J.P., and A.S.; writing—original draft preparation: A.S.-W. and J.P.; writing—review and editing: A.S.-W., A.S., P.T., J.P.; visualization: J.P., M.K., A.S.-W., and A.S.; supervision: J.P. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by Polish-Slovak Bilateral Cooperation Programme (PlasmaBioAgro) PPN/BIL/2018/1/00065+SK-PL-18-0090, LUT research found, COST Action PlAgri CA19110 and CEEPUS CIII-AT-0063.

Conflicts of Interest: The authors declare no conflict of interest.

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