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ORIGINAL PAPER

Interaction of Cold Atmospheric Pressure Plasma with Soybean Seeds: Effect on Germination and DNA, Seed Surface Characteristics and Plasma Diagnostics

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Abstract
This study investigates the relation between cold atmospheric pressure plasma (CAPP) effect on seeds and the plasma parameters. As a source of CAPP, the diffuse coplanar surface barrier discharge (DCSBD) generated in nitrogen, ambient air, and oxygen at atmospheric pressure was used. Results of germination and the level of DNA damage of soybean seeds (*Glycine max* L.) treated in plasma and plasma gaseous products showed that the most advantageous is the use of ambient air plasma treatment. The water contact angle (WCA) of samples treated directly in plasma was significantly smaller than that of samples treated with gaseous products. Using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), we did not observe significant changes between the spectra of individual samples, what indicates that there is no damage on the surface of the samples during the treatment, only the binding of polar groups. The method of optical emission spectroscopy (OES) and Fourier transform infrared spectroscopy (FTIR) were used to study the plasma parameters. The most radiant system observed in ambient air and nitrogen plasma is the second positive system of nitrogen $N_2\left(C^3\prod_u \rightarrow B^3\prod_g\right)$. FTIR measurements showed the presence of reactive oxygen and nitrogen species O_3, NO_2, N_2O, NO, HNO_2 depending on the working gas. Finally, it can be assumed that the positive effect of plasma on the seed is caused not only by the individual components of the plasma, but also by their synergistic effect, while the ratio of the individual active particles as well as the plasma exposure to the seeds are important.

Keywords Cold atmospheric pressure plasma · Soybean seeds · Germination · DNA damage · Seed surface diagnostics · Plasma diagnostics

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Introduction

Cold plasma (CP) generally represents a good alternative way for treatment of thermosensitive materials such as biological seeds to improve their properties. CP has different effects on seeds such as a disinfecting and sterilizing effect [1, 2]; improvement of germination, growth parameters and metabolism of plant seeds [3, 4]. Biological materials consist of living cells; therefore, it is important to study the interaction of plasma with seeds from many aspects.

Currently, interest in research in the field of plasma applications in agriculture is growing rapidly, and many new publications are appearing. Since this work is focused on the study of the effect of cold atmospheric pressure plasma (CAPP) on soybean seeds, we provide an overview (Table 1) of the selected articles dealing with the influence of CAPP on legume seeds. The overview is aimed at improving germination, level of DNA damage of seeds, seed surface diagnostics and plasma diagnostics. To achieve a positive effect of plasma on the biological properties of seeds, there are several important parameters such as plasma exposure to the seed, the uniformity of its processing or the use of a suitable working gas and plasma source, as well as the properties of the generated plasma, especially its homogeneity and diffusivity. The results of the studies [5, 6] show that CAPP generated in ambient air is the most advantageous alternative for improving the properties of plant seeds. The plasma generated in the air contains several reactive oxygen and nitrogen species (RONS), which play a significant role in the interaction with the biomaterial. In addition to the beneficial effect of plasma generated in the air on the biological properties of seeds, the simplicity of plasma processing is also an advantage. No vacuum equipment or special working gas is required.

To achieve several positive effects on the seed, the plasma treatment conditions must be selected with respect to all the desired plasma effects. Therefore, an important part of the research is the monitoring of all essential properties of seeds and plasma parameters. However, it is necessary to ensure that other properties are not negatively affected, which would significantly reduce the quality of the seed or the effectiveness of using plasma treatment in achieving the set goal. An important parameter studied from a food safety point of view is the level of possible DNA damage.

In addition to studying the effects of plasma on seed properties, seed and plasma diagnostics are also important. Physical processes, the influence of plasma components on the surface of the processed material can be studied by various physical surface diagnostic methods that characterize the surface of the material in detail. Changes in surface energy are analysed by measuring the water contact angle (WCA), the surface free energy (SFE), Fourier transform infrared spectroscopy with attenuated total reflectance (ATR-FTIR) or X-ray photoelectron spectroscopy (XPS) to study the chemical groups present on the surface of the material and scanning electron microscopy (SEM) or atomic microscopy forces (AFM) to observe changes in the morphology of the surface of the studied material [7, 8].

The obtained results from the surface diagnostics should correlate with the characteristics of the plasma; therefore, plasma diagnostics is an important part. Plasma diagnostics is essential for better understanding the mechanism of plasma impact on the seed surface and for controlling the safety of the plasma treated seeds. In general, diagnostic methods can be divided into electrical and optical diagnostics and corpuscular analysis [15]. The electrical method of plasma study is the measurement of volt-ampere characteristics, time development of current and voltage waveforms or frequency, which allows estimating the power supplied to the plasma. Optical diagnostics provides information about plasma

Table 1 Summary of the studies dealing with atmospheric-pressure plasma effects on legume seeds

Plasma source	Seed type	Conditions of plasma treatment	Aim of study	Surface seeds diagnostics	Plasma Diagnostics	Ref
VDBD	Soybean Glycine max	(O_2, N_2) + air P = 65, 85 W t = 1, 2, 3 min	Seed quality (i.e. Germination), Plant growth, Lipid peroxidation, Antioxidant activities, Biometric parameters, Agronomic traits	–	–	[9]
SDBD	Pea Pisum sativum	Air t = 1, 2, 3, 5, 10 min	Germination, Growth, Photosynthetic efficiency, Flavonol glycoside profile	–	OES (190–850 nm), Fiberglass encased optic thermocouple	[10]
SMD	Chickpea Cicer arietinum	t = 0.5, 1, 2, 3, 4, 5 min	Microbial analysis, Membrane Permeability, Moisture Content, Germination	Digital microscopy	OES (270–400 nm), UV absorption spectrometry, Chemiluminescence NO_x analyzer	[11]
DCSBD	Pea Pisum sativum	Air P = 370 W t = 60, 120, 180, 600 s	Water uptake, Germination, Growth, Endogenous Phytohormones Content	SEM	–	[4]
DCSBD	Pea Pisum sativum	air, O_2, N_2 P = 400 W t = 60, 180, 300 s	Water uptake, Germination, Growth, Soluble proteins content, Enzyme activities, Genotoxic effect	SEM, ATR-FTIR	–	[5]
DCSBD	Soybean Glycine max	air, N_2, O_2 P = 400 W t = 30, 60, 90, 120 s	Water uptake, Germination, Growth, Soluble proteins content, Enzyme activities, Genotoxic effect	–	–	[6]
Plasma jet	Mung bean Vigna radiata	Ar P ~ 1.2 W t = 1, 2, 4, 6, 8, 10, 15 min	Germination, Growth, Endogenous Hormone Regulation	SEM, WCA	Electrical diagnostics (voltage and current), OES (270–900 nm), Electrochemical sensor for NO	[12]

Table 1 (continued)

Plasma source	Seed type	Conditions of plasma treatment	Aim of study	Surface seeds diagnostics	Plasma Diagnostics	Ref
DBD plasma pencil	Mung bean Vigna radiata	He t = 2, 4 min	Decontamination, Germination, Growth	TEM, CLSM	OES (200–880 nm)	[13]
Plasma nozzle	Pea Pisum sativum	Air t = 30, 60 s	Microbial analysis, Germination, Growth	–	–	[14]

DBD—dielectric barrier discharge, VDBD—volume dielectric barrier discharge, SDBD—surface dielectric barrier discharge, SMD—surface micro-discharge, DCSBD—diffuse coplanar surface barrier discharge, P—input power, t—plasma exposure time, SEM—scanning electron microscopy, ATR-FTIR—attenuated total reflectance FTIR spectroscopy, WCA—water contact angle, TEM—transmission electron microscopy, CLSM—confocal laser scanning microscopy, OES—optical emission spectroscopy

composition, temperatures and velocities or densities of various types of particles obtained by detecting radiation emitted from the plasma or radiation absorbed by the plasma. Electrically charged particles (ions) can be detected and analysed by the corpuscular method based on the energetic or mass separation of particles under the influence of an electric or magnetic field. The choice of a suitable diagnostic method depends mainly on the plasma source and the working gas. As it is still not completely clear what mechanisms are responsible for the positive effects of plasma on the seeds, this process needs to be thoroughly investigated. During the interaction of the plasma with the seed surface, several physical, chemical and biochemical factors are applied that participate in this process [16]. The surface of the seed is a natural polymer that varies depending on the seed type, and the plasma is a mixture of various types of charged or neutral particles and radiation, so the interaction of the plasma with the seed surface is a very complex problem [17].

The physical factors that play a role in the interaction of plasma with the seed surface are heat, UV radiation and electromagnetic fields or mechanical disturbances that can induce further changes following this initial interaction. The results of the research so far [18–23] point to the hypothesis that the heat generated during the plasma burning does not significantly influence the characteristics of the seeds by itself. However, to a certain extent, it can contribute to improving the permeability of the seed coat. Manifesting the effect of UV radiation on seeds requires longer irradiation than the time of plasma treatment, therefore it is assumed that UV radiation in plasma does not directly significantly affect the properties of the seed [16]. Electric field positively affects germination [24, 25], but also causes oxidative stress [26]. The magnetic field has a positive effect on the probability of germination or growth parameters and can also change the redox state of plants, probably due to an increase in hydrogen peroxide (H_2O_2) concentration in the seed, by changing photosynthesis or alleviating drought stress or increasing mineral content [27, 28]. Electromagnetic fields as well as UV radiation can also participate in the formation of RONS in the plasma, which participate in the interaction of the plasma with the seed [16]. Mechanical damage to the surface can lead to improved water absorption and subsequently to improved germination, but it is not yet clear whether the increased permeability occurs only due to mechanical changes on the seed surface, or whether it is a combination of mechanical and chemical mechanisms or a purely chemical effect [16]. The question is also whether the increased water absorption by the seed depends on the plasma treatment, the type of seed or both factors. At the detailed study of the seed surface, it was found that the lipid layers are subject to chemical oxidation [9, 29], the efficiency of which may depend on the concentration of individual types of reactive particles in the plasma. Oxygenic particles in the plasma highly oxidized the outer layers of quinoa seeds, and nitrogen species can be adsorbed [30]. Better absorption of nutrients (e.g. potassium) into the interior of the seed when in contact with water was also demonstrated after seed plasma treatment [31]. Thus, an important factor in plasma treatment is also the choice of a suitable working gas, which plays a role in the composition of the plasma. To improve germination, the presence of oxygen is necessary, and for better plant growth, the most effective gases are oxygen, nitric oxide, and nitrogen [32]. From the previous research on the effects of plasma on seeds, it appears that the main factor for achieving a positive effect of plasma treatment is the setting of optimal plasma parameters as well as the properties of plasma-treated seeds.

Despite the large number of works devoted to the given issue, it is still not clear enough how the mechanism of plasma effect on plant seed takes place. Therefore, our work is focused on investigating the connections between plasma parameters and effect of plasma treatment on seeds. Diffuse coplanar surface barrier discharge (DCSBD) was used as a source of CAP plasma, and soybean seeds as the object of plasma interaction. Plasma

effect on seeds depends on many factors as a plasma source, working gas, type of seed or plasma treatment time. In general, ambient air as a working gas is the most advantageous. In our experiments, ambient air, and oxygen and nitrogen as the main compounds of ambient air, were used as working gases for the better understanding the plasma-seed interaction mechanism in air plasma. Not only the plasma effect on the soybean seeds was studied, but also the effect of plasma gaseous products, without direct contact with active plasma area on seeds. Seed surface characteristics (WCA, ATR-FTIR spectra) as well as plasma parameters (composition of plasma gaseous products and plasma radiation, temperatures) were investigated.

Materials and Methods

Plasma Source

CAPP used in our experiments for treatment of different materials was generated by DCSBD discharge. The basis of the construction of the discharge is a pair of comb-shaped parallel electrodes located in one plane under the surface of the dielectric (Al_2O_3 with a purity of 96%) (Fig. 1a) [33, 34]. The electrodes are powered by high voltage (peak-to-peak 20 kV) with a frequency of ~ 15 kHz (HV generator VF 700, Lifetech, CZ) and isolated and cooled by dielectric oil flowing system. Thanks to the cooling of the electrodes, it is possible to ensure continuous burning of the discharge at room temperature and at the same time supply the electrodes with a high input power of up to 400 W. Typical voltage and current waveforms of a DCSBD plasma source burning in ambient air at an input power of 400 W and a frequency of 15 kHz,

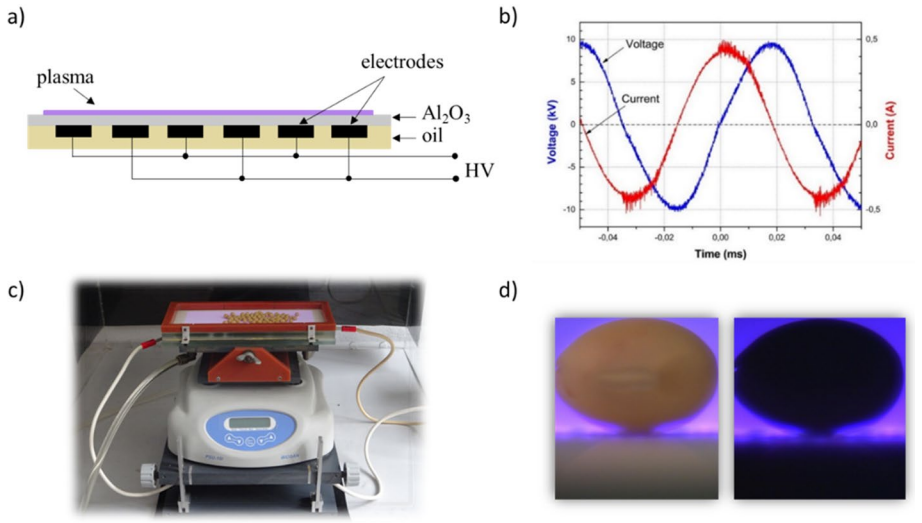


Fig. 1 **a** Scheme of DCSBD discharge configuration with electrodes situated below the surface of the dielectric, **b** Typical voltage and current waveforms of DCSBD plasma generated in ambient air at an input power of 400 W and a frequency of 15 kHz, **c** Soybean seeds putting in plasma of DCSBD, **d** Soybean seed in DCSBD plasma without the cover (left) and with the cover (right)

measured with a Rogowsky ring (Pearson Electronic, model 4100) using two Tektronix P6015A high-voltage probes, recorded with a Tektronix TDS 2024B oscilloscope, are shown in Fig. 1b [35].

The DCSBD discharge formation mechanism is very complex and is influenced by several parameters such as electrode geometry, permittivity of the dielectric and coefficient of secondary emission of electrons from the dielectric, power supply, etc. [34]. The plasma covers the dielectric surface with a macroscopically homogeneous thin layer and is composed of many microdischarges. The active area of the plasma on the dielectric surface is (80×200) mm^2. Individual microdischarges are formed in the shape of the letter H and move rapidly along the surface of the dielectric along the electrodes. The formation of microdischarges in terms of size and intensity also depends on the used working gas. The homogeneity of the plasma increases with increasing supply voltage with the diffuse part of the discharge glowing more compared to the filamentary part of the discharge. The effective thickness of the plasma layer in air was measured using a CCD camera and estimated to be approximately 0.3 mm [33] and the volume plasma energy density to be on the order of 100 W/cm^{-3}.

Plasma Treatment, Germination and DNA Damage

Soybean seeds (*Glycine max* L.) cv. Nížina, obtained from the Central Agricultural Inspection and Testing Institute in Bratislava, were treated in DCSBD plasma generated at atmospheric pressure in oxygen (O), ambient air (A) and nitrogen (N) at different exposure times (30, 60, 90, 120 s) at the input power of 400 W.

After treating the seeds in plasma (see Fig. 1c), germination and a degree of DNA damage of plasma treated soybean seeds were evaluated. Total germination (germinated seeds with at least 5 mm long seminal root), full germination 1 and 2 (germinated seeds with seminal roots and at least 5 mm long hypocotyls) were calculated according to the formulas:

$$\text{total germination } (\%) = \frac{\text{number of germinated seeds}}{\text{number of seeds}}.100\%$$

$$\text{full germination } 1(\%) = \frac{\text{number of full germinated seeds}}{\text{number of seeds}}.100\%$$

$$\text{full germination } 2(\%) = \frac{\text{number of full germinated seeds}}{\text{number of germinated seeds}}.100\%$$

and the level of DNA damage was determined by Comet Assay [6]. The plasma seed treatment procedure and after-treatment experiments were described in more detail in the work [6]. The biological parameters were statistically evaluated using the ANOVA, where statistical significance was assigned to individual data using a one-step multiple comparison of the averages of the 95% LSD test. The statistical significance of the results in the graphs is indicated by different letters. When assessing the effect of plasma seed treatment, we compare not only changes in individual parameters based on the data, but also based on the statistical significance compared to the control.

Comparison of Plasma Treatment and Plasma Gaseous Products Treatment

An additional experiment represents the comparison of germination of plasma treated and DCSBD plasma gaseous products treated soybean seeds, without plasma radiation. Soybean seeds were bought from the local supermarket. Seeds were treated in the plasma generated in air, by the means described upper in the part about the plasma treatment, and in the gaseous products generated by air DCSBD plasma. Treatment of the seeds in plasma gaseous products were realized behind the discharge in a small, closed dose with the input and the output of the plasma gaseous products (Fig. 2).

Ambient air from the compressor was carrying into the glass cover with the plasma at the flow rate of 3 L/min. Subsequently, the plasma gaseous products flowed through the closed dose with soybean seeds. The dose with seeds was moved manually for homogeneous treatments of the seeds. 30 seeds per one treatment was used and each variant was repeated three times with exposure times 20 and 30 s.

Seed Surface Diagnostics

Water Contact Angle

Changes in wettability of soybean seeds were evaluated by the measurement of the WCA. Drop Shape Analysis DSA30 (Krüss GmbH) connected to a computer with appropriate software for calculating the WCA was used. The measurements were taken immediately after the seed plasma treatment with exposures of 0, 60 and 120 s. A drop of distilled water with a volume of 2 µl was put onto the seeds surface using automatic system and consequently WCA was determined using the software for measurement the contact angle on curved surfaces. The resulting WCA were determined as the average of 10 measurements corresponding to measurements on 10 samples of soybean seeds with statistical evaluation.

ATR-FTIR Chemical changes on seeds surface caused by the plasma treatment were determined using attenuated total reflectance—FTIR spectroscopy (ATR-FTIR). For these surface diagnostics, the spectrometer Bruker Optics Vector 22 with the additional device Pike MIRacle™ with the diamond crystal was used. Measurements were performed in the range (4000–500) cm^{-1} using the spectrometer resolution of 4 cm^{-1} after the plasma treatment with exposures of 0, 60 and 120 s.

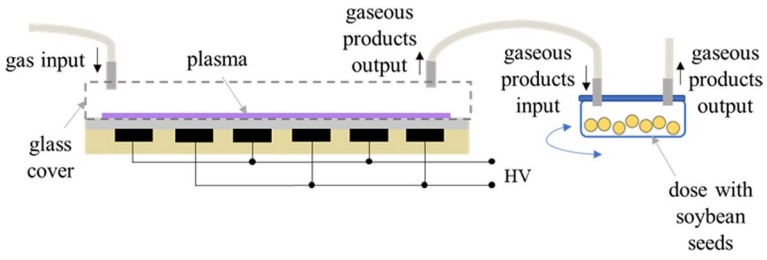


Fig. 2 Plasma gaseous products treatment of soybean seeds behind the DCSBD discharge

Plasma Diagnostics

Optical Emission Spectroscopy

The optical emission spectra of the DCSBD plasma were measured using the experimental set-up illustrated in Fig. 3. Plasma was generated at the input power of 400 W inside the reactor chamber, through which the working gas (nitrogen, ambient air or oxygen) was flowing with a flow rate of 3 L/min. The radiation from the plasma passed through the quartz window on the top of the reactor chamber, the lens (f = 8 cm) and the aperture (hole with a diameter of 0.7 cm) into the optical fibre (Avantes FC-UV200-2-SR, F1000 UV–VIS SR) connected to the spectrometer. The distance of the lens from the ceramic surface and the fibre from the lens was ~ 8 cm. The aperture between the lens and the fibre was placed at a distance of ~ 4 cm from the lens. For spectrum measurements, two types of spectrometers (AvaSpec-2048 TEC (Thermo-Electric-Cooled) with the range of ~ (300–400) nm and the resolution of 20 px/nm and StellarNet EP 2000 with the range of ~ (200–1100) nm and the resolution of 2 px/nm) were used. The radiation from the plasma was collected from the plasma volume in the shape of a cone with a circular base on the dielectric surface with a diameter of ~ 1.5 cm.

From the measured optical emission spectra, we subsequently determined systems emitted in the plasma and calculated vibrational and rotational temperatures of the plasma. To estimate the vibrational temperatures, the program Spectrum Analyzer 1.8 [36], which calculates the temperatures from the relative intensities of the peaks, was used. The rotational temperatures were determined by comparing the shape of the peaks in the measured spectrum with the peaks of the simulated spectra in the Specair 3.0 program [37].

Fourier Transform Infrared Spectroscopy

The composition of plasma gaseous products and its concentrations were determined using FTIR spectroscopy according to the experimental set-up in Fig. 4. As in the case of optical emission spectroscopy, DCSBD plasma was generated at the input power of 400 W inside the reactor chamber, through which the working gas (nitrogen, ambient air or oxygen) was flowing with a flow rate of 3 L/min. Plasma gaseous products were detected at the reactor outlet. The plasma gaseous products from the reactor were carried out through an approximately 50 cm long connecting tube into a glass cuvette located in a Bruker Optics Vector 22 FTIR spectrometer. The cuvette with a length of 10 cm and a volume of 31 cm^3 was equipped with germanium windows through which the infrared beam passed in the FTIR

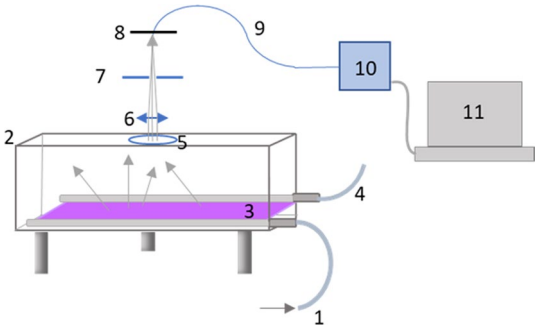


Fig. 3 Experimental apparatus for optical emission spectroscopy of DCSBD plasma: 1—gas input, 2—chamber, 3—plasma, 4—gas output, 5—quartz glass, 6—lens, 7—aperture, 8—attachment of the fibre, 9—optical fibre, 10—spectrometer, 11—computer

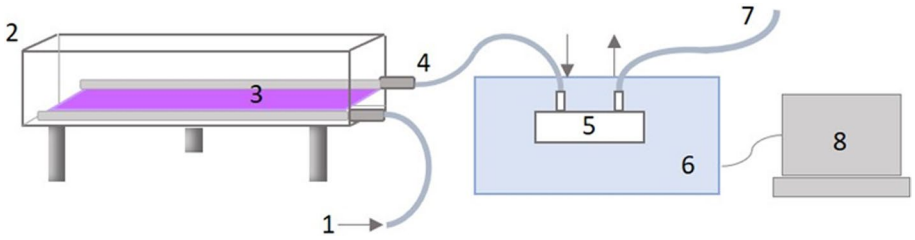


Fig. 4 Experimental apparatus for FTIR spectroscopy of gaseous products of DCSBD plasma: 1—gas input, 2—chamber, 3—plasma, 4—plasma gaseous product output into the cuvette, 5—cuvette, 6—spectrometer, 7—gas output from the cuvette, 8—computer.

spectrometer. Measurements were performed in the range (4000–500) cm^{-1} using the spectrometer resolution of 2 cm^{-1}. The molecules present in gaseous products of plasma were identified from the measured FTIR spectra.

Results and Discussion

Germination and DNA Damage

An example of 5-day old soybean seedlings (after 5 days from the beginning of cultivation) is illustrated in Fig. 5. Since in the case of nitrogen plasma (variants 90 and 120 s) the seedlings germinated very poorly, they are not included in the picture.

The results of testing the germination percentage of soybean seeds (Fig. 6) after plasma treatment showed the greatest increase in the N60 and O30 variants. Long exposure to nitrogen and oxygen plasma (variants N90, N120 and O120) negatively affected the germination percentage of soybean seeds (in N120 variant, the decrease in germination was

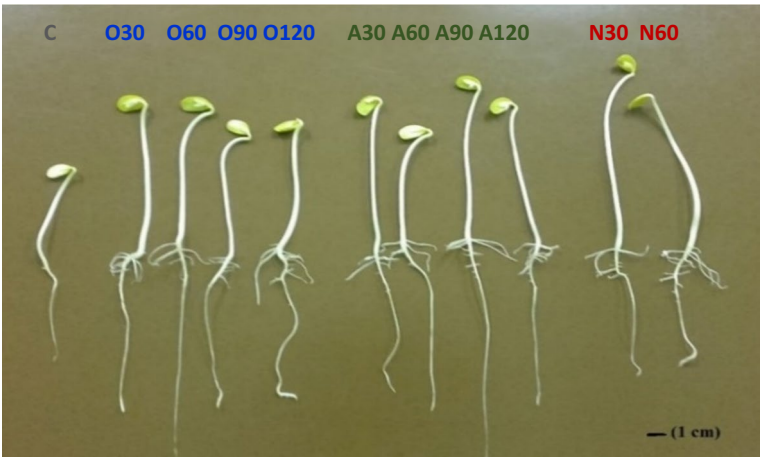


Fig. 5 Five-day-old soybean seedlings grown from seeds treated in nitrogen (N30, N60), ambient air (A30, A60, A90, A120) and oxygen (O30, O60, O90, O120) DCSBD plasma at different treatment times (30, 60, 90, 120 s) and at input power of 400 W. C—control (soybean seedling without plasma treatment)

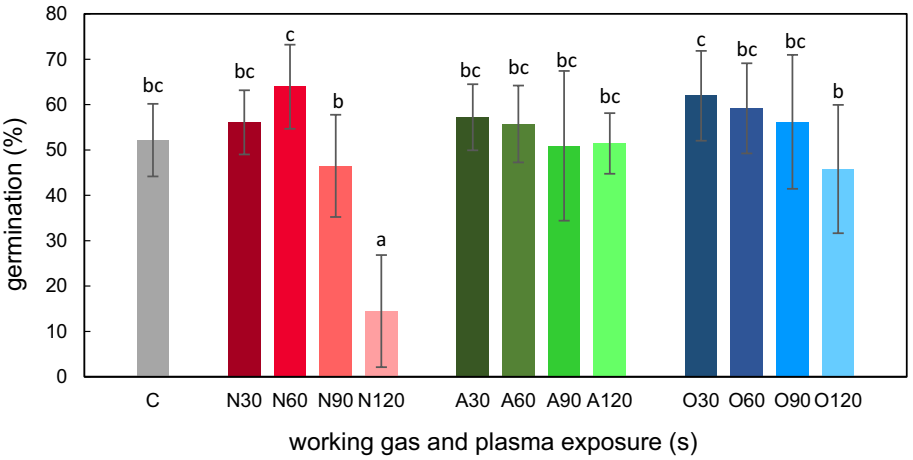


Fig. 6 Germination of soybean seeds before and after plasma treatment depending on the working gas and duration of plasma treatment. C—control (germination of soybean seeds without a plasma treatment). N30, N60, N90, N120—germination of soybean seeds treated in nitrogen plasma at 30, 60, 90, 120 s. A30, A60, A90, A120—germination of soybean seeds treated in ambient air plasma at 30, 60, 90, 120 s. O30, O60, O90, O120—germination of soybean seeds treated in oxygen plasma at 30, 60, 90, 120 s

almost threefold). A slight increase was observed in air plasma (variants A30, A60), oxygen plasma (O60, O90) and nitrogen plasma (variant N30), but these positive changes in germination were not statistically significant compared to the untreated control (C). More detail results about plasma regulation of soybean seeds germination it can be found in the work of Švubová et al. [6].

Study of the DNA damage (Fig. 7) showed a different degree of DNA damage of soybean sprouts depending on the plasma treatment conditions. The soybeans without plasma treatment were used as a negative control (NC) and soybeans treated with zeocin (a radiomimetic that creates breaks in DNA) as a positive control (PC). In the case of plasma treatment in air, DNA damage was at the level of NC at A30 and slightly increased at A60, A90 and A120. Seed treatment in oxygen plasma caused DNA damage at the level of air plasma except for O60, where DNA damage was higher. Nitrogen plasma caused the greatest DNA damage at both N30 and N60. DNA damage detected by the alkaline comet test is a primary DNA damage in the form of single- and double-strand breaks [38]. Typically, DNA breaks can be repaired relatively quickly by triggering repair mechanisms in the plant as a spontaneous response to its damage [39]. However, under excessive stress, repair mechanisms of plant cells are saturated and primary DNA damage persists and can lead to cell death [40]. Based on this, nitrogen plasma likely induced such severe stress that resulted in higher levels of DNA damage in soybean sprouts and probably led to inhibited germination of the N120 variant (Fig. 6). Nonetheless, the DNA damage detected by the comet assay could be repaired and do not have any lasting adverse effects on the plant organism, as was observed in the variants treated with air or oxygen plasma and at lower exposures of nitrogen plasma. Similar results were obtained in previous studies on pea [5, 41], barley [42] and soybean [6] seeds in which lower plasma exposures that induce primary DNA damage have no or even beneficial effects on seed germination. The higher levels of DNA damage in the N30 and N60 variants were likely due to the higher dose of UV radiation and specific amounts of reactive particles in nitrogen plasma [5] that could

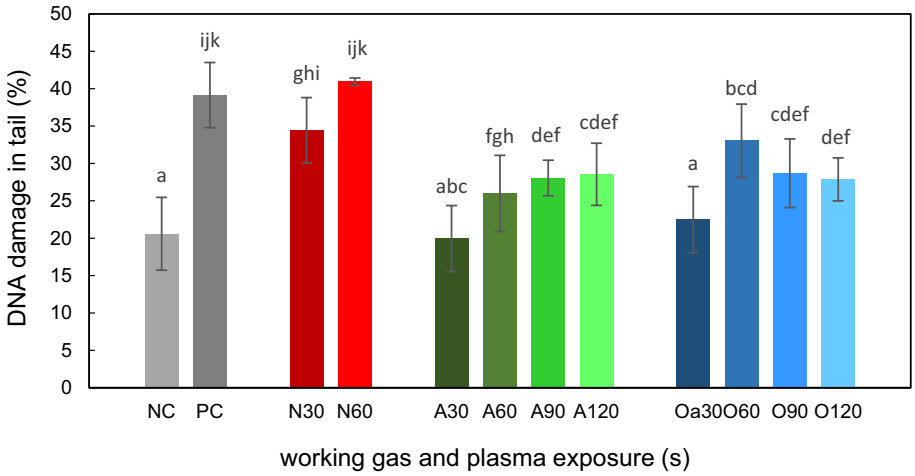


Fig. 7 Degree of DNA damage of three-day-old soybean seedlings before and after plasma treatment depending on the working gas and duration of plasma treatment. NC—negative control (degree of DNA damage of soybean seedlings without plasma treatment), PC—positive control (degree of DNA damage of soybean seedlings treated with zeocin). N30, N60—degree of DNA damage of soybean seeds treated in nitrogen plasma at 30 and 60 s. A30, A60, A90, A120—degree of DNA damage of soybean seeds treated in air plasma at 30, 60, 90, 120 s. O30, O60, O90, O120—degree of DNA damage of soybean seeds treated in oxygen plasma at 30, 60, 90, 120 s

attack DNA strands, leading to the formation of DNA breaks [43] detectable in the comet assay. Kyzek et al. [44] found that plasma treatment of seeds can even reduce DNA damage caused by zeocin. Thus, organisms are probably able to handle even the slightly higher levels of DNA damage detected by the comet assay.

Germination of Plasma Treated and Plasma Gaseous Products Treated Soybean Seeds

In Fig. 8, we demonstrate the results of an additional experiment where we evaluated the effect of plasma gaseous products (PGP) and plasma (P) treatment in ambient air (A) on soybean seeds germination. Our results showed that plasma gaseous products and plasma treatment in exposition 20 s appear to slightly stimulate total soybean seed germination. After recalculating the full germination 1 (full germinated seeds/number of seeds) and 2 (full germinated seeds/number of germinated seeds), we can conclude that variants P A20 and PGP A20 had a markedly positive (increase about 20%) effect on the monitored parameters compared to the untreated control (C).

Seed Surface Diagnostics

The WCA measurements on soybean seeds surface showed a significant reduction of contact angles values in case of plasma treatment compared to the reference sample (sample with the treatment time 0 s) (Fig. 9a). The values of WCA for different working gases were similar. Slight changes were also noticed after the plasma gaseous products treatment of soybean seeds (Fig. 9b). While the WCA after the nitrogen plasma gaseous

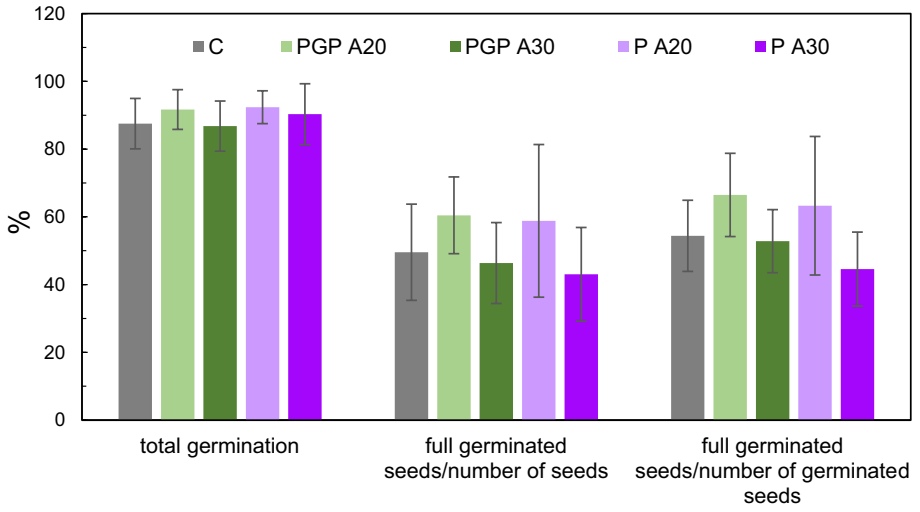


Fig. 8 Germination of soybean seeds: C—germination of untreated seeds; P A20, P A30—germination of seeds treated in atmospheric pressure ambient air plasma at 400 W with the exposure of 20 and 30 s; PGP A20, PGP A30—germination of seeds treated in gaseous products of atmospheric pressure ambient air plasma at 400 W with the exposure of 20 and 30 s.

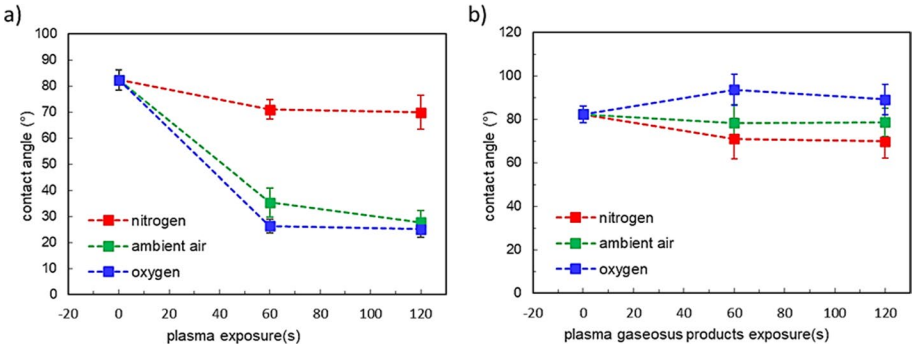


Fig. 9 Water Contact angles of soybean seeds: **a** treated in DCSBD plasma generated in nitrogen, ambient air and oxygen at 400 W, **b** treated in gaseous products of DCSBD plasma generated in nitrogen, ambient air and oxygen at 400 W

products treatment slightly decreased, the oxygen plasma gaseous products slightly improved the values of WCA. The WCA of the soybean seeds samples treated in the gaseous products of the ambient air plasma varies at the level of the control sample. Decreasing the contact angle is a consequence of increasing hydrophilicity of the sample surface and in contrast increasing contact angle reflects increasing hydrophobicity. Improved hydrophilicity leads to higher water absorption by the seed, which can result in improved seed germination and growth parameters, however, at higher plasma doses, water absorption is increased to such an extent that can lead to inhibition of germination or suffocation of the embryo [6].

Fig. 10 ATR-FTIR spectra from the soybean seeds surface of untreated seeds, plasma treated, and plasma gaseous products treated soybean seeds. Working gas is **a** nitrogen, **b** ambient air, **c** oxygen. Ref. belongs to the sample without the treatment

The chemical groups present on the soybean seed surface were identified using ATR-FTIR method. The ATR-FTIR spectra (Fig. 10) showed the presence of compounds typical for legumes: polysaccharides characterised by the bonds C–O, C–C, C–OH and glycosidic bond ((1200–800) cm^{-1}), lipids represented by the C-H bonds ((3000–2800) cm^{-1} and (1460–1400) cm^{-1}) and proteins (amid I—1640 cm^{-1}, amid II—1530 cm^{-1}, amid III—1235 cm^{-1}; N–H—3280 cm^{-1}) [45–49]. Peak in the region of (3500–3000) cm^{-1} belongs to the valency vibrations of O–H [45, 46]. Deformation vibrations of bound water is shown at 1640 cm^{-1} [45, 50]. In the spectra of the samples after plasma treatment or plasma gaseous products treatment, any changes, that would indicate the destruction of the basic chemical bonds belonging to the components forming the soybean seed coat, were not observed.

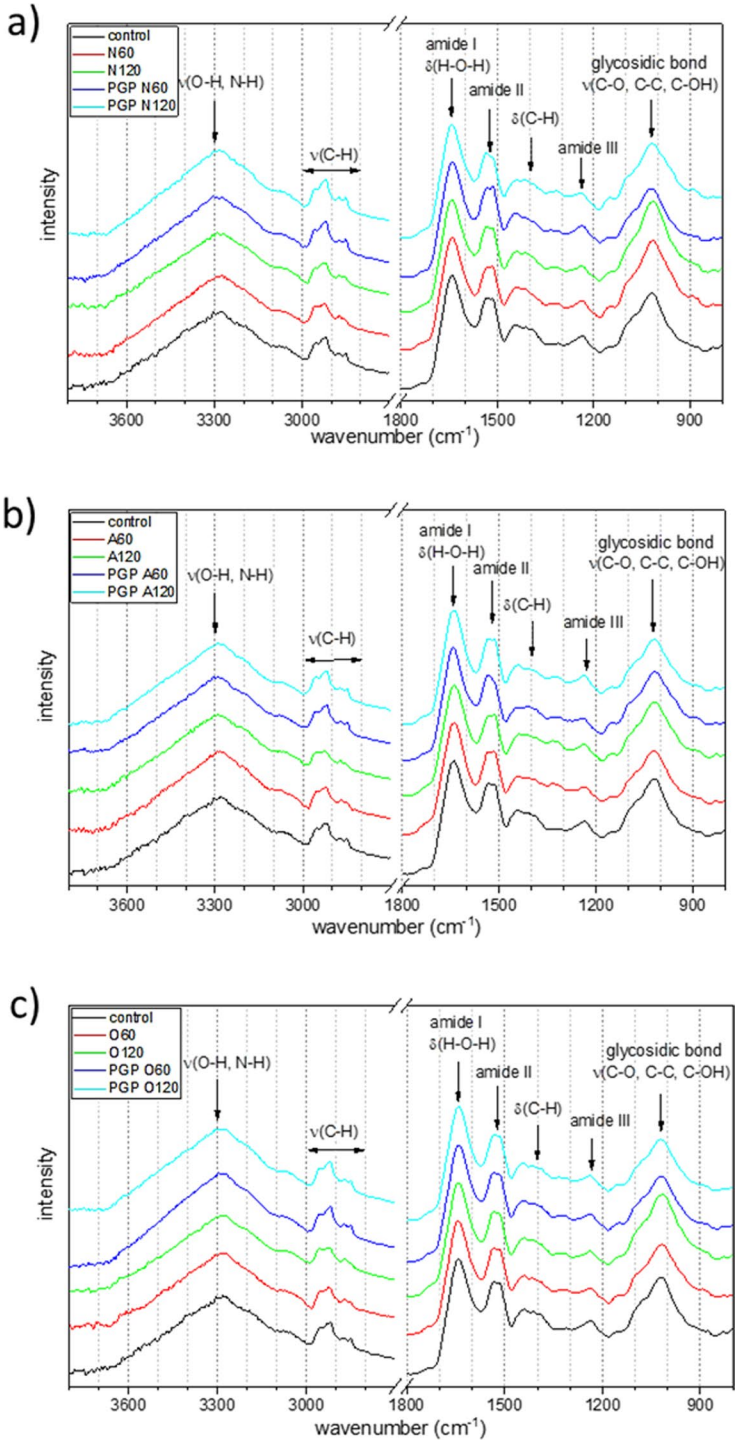
Plasma Diagnostics

Optical emission spectroscopy measurements of DCSBD plasma showed that plasma radiation is dominant in UV–VIS range of wavelength ~ (300–400) nm. As we can see in Fig. 11, the presence of individual radiative systems in the plasma depends on the working gas used in plasma generation. The most intensive system observed in DCSBD plasma generated in nitrogen and ambient air is the second positive system of nitrogen $N_2\left(C_u^3 \rightarrow B_g^3\right)$. Molecular bands of $NO\left(A^2\Sigma^+ \rightarrow X^2\right)$ system, the first negative system of nitrogen $N_2^+(B^2\Sigma_u^+ \rightarrow X^2\Sigma_g^+)$ or the first positive system of nitrogen $N_2\left(B_g^3 \rightarrow A^3\Sigma_u^+\right)$ were also observed. Optical emission spectra also showed the presence of atomic nitrogen N in nitrogen plasma and atomic oxygen O in oxygen plasma. Vibrational (T_{vib}) and rotational (T_{rot}) temperatures calculated from the second positive system of nitrogen N_2(C-B) are shown in the Table 2. Significant difference between T_{vib} and T_{rot} reflects the non-equilibrium character of the DCSBD plasma.

By measuring the FTIR spectra of gaseous products of the DCSBD discharge plasma at the outlet of the reactor, various RONS such as O_3, N_2O, NO_2, NO, and HNO_2 were detected. The presence of individual molecules in the plasma depends on the working gas (Fig. 12). Gaseous product of oxygen plasma was ozone molecule observed with the maximum of the peaks at 3055 cm^{-1}, 2122 cm^{-1}, 1124 cm^{-1}, 1053 cm^{-1} and 716 cm^{-1}. Ambient air plasma gaseous products were composed from nitrogen dioxide NO_2 (2918 cm^{-1}, 1628 cm^{-1}), nitrous oxide N_2O (2236 cm^{-1}, 1298 cm^{-1}), nitric oxide NO (around 1875 cm^{-1}) and nitrous acid HNO_2 (1698 cm^{-1}, 1264 cm^{-1}, 852 cm^{-1}, 791 cm^{-1}). FTIR measurements of plasma gaseous products of nitrogen plasma did not show the presence of any molecules. To improve germination, the presence of oxygen is necessary, and for better plant growth, the most effective gases are oxygen, nitric oxide and nitrogen [32].

Most studies show that RONS present in plasma, whose formation and concentration depend on the working gas used, are the dominant factors responsible for the positive effect of plasma on plant growth [16]. However, it is not clear whether this effect is caused by reactive oxygen species (ROS), reactive nitrogen species (RNS) or a synergistic effect of several plasma components.

Nitrogen plasma is characterized by a high dose of UV radiation, but it can also contain other reactive particles in low concentrations, which are not detectable by the used


a)
control
N60
N120
PGP N60
PGP N120
ν(O-H, N-H)
ν(C-H)
amide I
δ(H-O-H)
amide II
δ(C-H)
amide III
glycosidic bond
ν(C-O, C-C, C-OH)
intensity
3600
3300
3000
1800
1500
1200
900
wavenumber (cm-1)
b)
control
A60
A120
PGP A60
PGP A120
ν(O-H, N-H)
ν(C-H)
amide I
δ(H-O-H)
amide II
δ(C-H)
amide III
glycosidic bond
ν(C-O, C-C, C-OH)
intensity
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control
O60
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ν(O-H, N-H)
ν(C-H)
amide I
δ(H-O-H)
amide II
δ(C-H)
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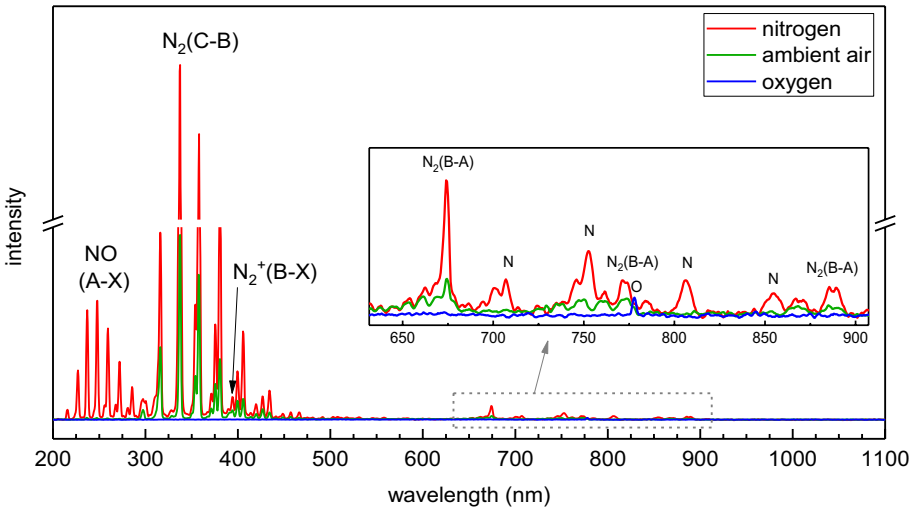

Fig. 11 OES spectra of DCSBD plasma generated in nitrogen, ambient air and oxygen at an input power of 400 W

Table 2 Vibrational (T_{vib}) and rotational (T_{rot}) temperatures of DCSBD plasma generated in nitrogen, ambient air and oxygen at the input power of 400 W

Working gas	T_{vib} (K)	T_{rot} (K)
Nitrogen	2150 ± 55	380 ± 30 K
Ambient air	2610 ± 225	385 ± 30 K
Oxygen	–	–

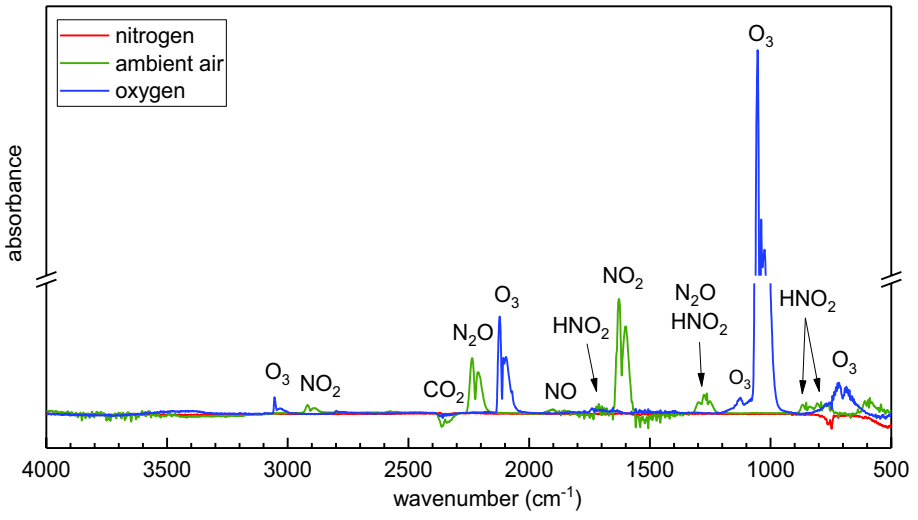

Fig. 12 FTIR spectra of gaseous products of DCSBD plasma generated in nitrogen, ambient air and oxygen at an input power of 400 W

methods. From the comparison with the results of soybean germination, it can be assumed that UV radiation can have a positive effect on the seed, however in a high dose (longer plasma exposure time) it has a negative effect. Since the DNA damage of soybean increases with the increasing amount of nitrogen in the working gas, it can be assumed that it is caused by UV radiation, since its intensity increases with the increasing proportion of nitrogen. This negative effect of UV radiation as well as reactive oxygen species on DNA is also confirmed by the work [5]. As it was more deeply investigated in the work [5], a longer time of plasma treatment of pea seeds (180 and 300 s) in pure nitrogen plasma with the most intense UV radiation leads to higher production of ROS ($^{\cdot}O_2^-$ and H_2O_2) inside the pea seed and thus causes oxidative stress and subsequently DNA damage.

A large amount of ozone, which is highly reactive and has strong oxidizing effects, was observed in the plasma generated in oxygen. In connection with the results of soybean germination, we could conclude that ozone improves these properties positively with shorter plasma treatment times (especially 30 s, but also 60 and 90 s), however negatively with longer exposure of the plasma to the seed (120 s). As it is illustrated by Fig. 9b, exposure to gaseous products of oxygen, i.e. mainly ozone, only a slight increase in value of WCA was shown which correspond to an improvement in the hydrophobicity of the seed surface. Therefore, in oxygen plasma the improvement of germination is not related to the improvement of surface hydrophilicity, but it would be caused probably by the penetrating of ozone and other reactive gaseous products into the seeds and influencing the biochemical processes.

Plasma generated in ambient air mainly contains nitrogen oxides NO_2 and N_2O as well as UV radiation. Since the air plasma does not cause a negative effect on germination at the exposure times we used, it can be assumed that the intensity and duration of the UV radiation does not cause deterioration of the seed properties in this case. On the other hand, in addition to UV radiation, RONS generated in the plasma can also contribute to the improvement of germination.

Conclusion

This work was aimed on better understanding the mechanism of the plasma interaction with soybean seeds surface. The study was focused on the plasma effect on germination of soybean seeds and the level of DNA damage. Results showed that the germination is the best in the case of O30 and N60 and then A30, O60 and N30. DNA damage is the lowest in A30. Finally, we can state that the most advantageous is the using of ambient air plasma treatment what confirms our previous studies. For better understanding the plasma effect on seeds, it is important to study plasma compounds and its effect on seeds. Therefore, the experiments where the soybean seeds were treated only in plasma gaseous products without UV radiation were realized. It can be concluded that plasma as well as plasma gaseous products applicated in short (20 s −30 s) interval had a positive effect on the full seed germination. The WCA of soybean seeds treated directly in plasma was significantly smaller than that of samples treated with gaseous products. Thus, we can state that plasma gaseous products are not directly responsible for increasing hydrophilicity caused by plasma treatment. ATR-FTIR measurement indicates that there is no damage on the surface of the samples during the treatment, only the binding of polar groups. Using OES, we identified that the most radiant system observed in ambient air and nitrogen plasma is the second positive system of nitrogen $N_2\left(C^3\prod_u \rightarrow B^3\prod_g\right)$ which corresponds the most with UVA region

((315–400) nm). Significant difference between T_{vib} and T_{rot} reflects the non-equilibrium character of the DCSBD plasma. FTIR measurement showed the presence of RONS (mainly O_3, NO_2, N_2O, NO, HNO_2) in the plasma, which play a role in plasma interaction process with seeds.

Finally, from the results, it can be assumed that the positive effect of plasma on the seed is not caused only by the individual components of the plasma, but also by their synergistic effect, while the ratio of the individual active compounds as well as the duration of the action are important. Of course, it should be considered that the differences in the effect of plasma on the surface of different types of plant seeds are also affected by the type of seed, its size, hardness, and other surface properties.

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Author contributions Conceptualization [A.Z.], Methodology [J.T., Ľ.S., E.G.], Experiments and analyses [J.T., Ľ.S., Ľ.H.-Č., A.Z.], Writing—original draft preparation [J.T.], Writing—review and editing [J.T., Ľ.S., Ľ.H.-Č., S.K., R.Š., A.Z.], Funding acquisition [A.Z., E.G.]

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Data availability The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interests I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

References

1. Scholtz V, Pazlarova J, Souskova H et al (2015) Nonthermal plasma—A tool for decontamination and disinfection. Biotechnol Adv 33:1108–1119. https://doi.org/10.1016/j.biotechadv.2015.01.002
2. Laroussi M (2005) Low temperature plasma-based sterilization: Overview and state-of-the-art. Plasma Process Polym 2:391–400. https://doi.org/10.1002/ppap.200400078

3. Šerá B, Scholtz V, Jirešová J et al (2021) Effects of non-thermal plasma treatment on seed germination and early growth of leguminous plants—a review. Plants 10:1–15. https://doi.org/10.3390/plants10081616
4. Stolárik T, Henselová M, Martinka M et al (2015) Effect of low-temperature plasma on the structure of seeds, growth and metabolism of endogenous phytohormones in pea (Pisum sativum L.). Plasma Chem Plasma Process 35:659–676. https://doi.org/10.1007/s11090-015-9627-8
5. Švubová R, Kyzek S, Medvecká V, et al. (2020) Novel insight at the effect of cold atmospheric pressure plasma on the activity of enzymes essential for the germination of Pea (Pisum sativum L. Cv. Prophet) Seeds. Plasma Chem Plasma Process. https://doi.org/10.1007/s11090-020-10089-9
6. Švubová R, Slováková Ľ, Holubová Ľ et al (2021) Evaluation of the impact of cold atmospheric pressure plasma on soybean seed germination. Plants 10:1–17
7. Štěpánová V, Slavíček P, Kelar J et al (2018) Atmospheric pressure plasma treatment of agricultural seeds of cucumber (Cucumis sativus L.) and pepper (Capsicum annuum L.) with effect on reduction of diseases and germination improvement. Plasma Process Polym 15:1–9. https://doi.org/10.1002/ppap.201700076
8. Volkov AG, Hairston JS, Patel D et al (2019) Cold plasma poration and corrugation of pumpkin seed coats. Bioelectrochemistry 128:175–185. https://doi.org/10.1016/j.bioelechem.2019.04.012
9. Perez-Piz MC, Prevosto L, Grijalba PE et al (2019) Improvement of growth and yield of soybean plants through the application of non-thermal plasmas to seeds with different health status. Heliyon 5:1495. https://doi.org/10.1016/j.heliyon.2019
10. Bußler S, Herppich WB, Neugart S et al (2015) Impact of cold atmospheric pressure plasma on physiology and flavonol glycoside profile of peas (Pisum sativum 'Salamanca'). Food Res Int 76:132–141. https://doi.org/10.1016/j.foodres.2015.03.045
11. Mitra A, Li YF, Klämpfl TG et al (2014) Inactivation of surface-borne microorganisms and increased germination of seed specimen by cold atmospheric plasma. Food Bioprocess Technol. https://doi.org/10.1007/s11947-013-1126-4
12. Quynh T, Le X, Nguyen LN et al (2022) Effects of cold plasma treatment on physical modification and endogenous hormone regulation in enhancing seed germination and radicle growth of mung bean. Appl Sci 12:1–12
13. Motyka-Pomagruk A, Dzimitrowicz A, Orlowski J et al (2021) Implementation of a non-thermal atmospheric pressure plasma for eradication of plant pathogens from a surface of economically important seeds. Int J Mol Sci 22:1–20. https://doi.org/10.3390/ijms22179256
14. Khatami S, Ahmadinia A (2018) Increased germination and growth rates of pea and Zucchini seed by FSG plasma. J Theor Appl Phys 12:33–38. https://doi.org/10.1007/s40094-018-0280-5
15. Ishikawa K (2016) Plasma Diagnostics. In: Cold Plasma in Food and Agriculture: Fundamentals and Applications. Elsevier Inc., pp 117–141
16. Waskow A, Howling A, Furno I (2021) Mechanisms of plasma-seed treatments as a potential seed processing technology. Front Phys 9:1–23. https://doi.org/10.3389/fphy.2021.617345
17. Bartis EAJ, Knoll AJ, Luan P et al (2016) On the interaction of cold atmospheric pressure plasma with surfaces of bio-molecules and model polymers. Plasma Chem Plasma Process 36:121–149. https://doi.org/10.1007/s11090-015-9673-2
18. Lotfy K (2017) Effects of cold atmospheric plasma jet treatment on the seed germination and enhancement growth of watermelon. Open J Appl Sci 07:705–719. https://doi.org/10.4236/ojapps.2017.712050
19. Mildažienė V, Aleknavičiūtė V, Žūkienė R et al (2019) Treatment of common sunflower (Helianthus annus L.) seeds with radio-frequency electromagnetic field and cold plasma induces changes in seed phytohormone balance, seedling development and leaf protein expression. Sci Rep 9:1–12. https://doi.org/10.1038/s41598-019-42893-5
20. Kitazaki S, Sarinont T, Koga K et al (2014) Plasma induced long-term growth enhancement of Raphanus sativus L. using combinatorial atmospheric air dielectric barrier discharge plasmas. Curr Appl Phys. https://doi.org/10.1016/j.cap.2013.11.056
21. Iranbakhsh A, Ardebili NO, Ardebili ZO et al (2018) Non-thermal plasma induced expression of heat shock factor A4A and improved wheat (Triticum aestivum L.) growth and resistance against salt stress. Plasma Chem Plasma Process 38:29–44. https://doi.org/10.1007/s11090-017-9861-3
22. Al-Whaibi MH (2011) Plant heat-shock proteins: a mini review. J King Saud Univ - Sci 23:139–150. https://doi.org/10.1016/j.jksus.2010.06.022
23. Kobayashi M, Wang Y, Kumagai S et al. (2019) Effects of cold atmospheric plasma irradiation on Arabidopsis seedlings. Jpn J Appl Phys 59(SA), SAAB09. https://doi.org/10.7567/1347-4065/ab4e7b

24. Nian W, Guo J, Zhang J, Fang J (2013) Effects of nanosecond pulsed electric field exposure on seed of Saxaul (Haloxylon ammodendron). In: 2013 Abstracts IEEE International Conference on Plasma Science (ICOPS). p 1
25. Dymek K, Dejmek P, Panarese V et al (2012) Effect of pulsed electric field on the germination of barley seeds. LWT - Food Sci Technol 47:161–166. https://doi.org/10.1016/j.lwt.2011.12.019
26. Teissie J, Golzio M, Rols MP (2005) Mechanisms of cell membrane electropermeabilization: a minireview of our present (lack of ?) knowledge. Biochim Biophys Acta - Gen Subj 1724:270–280. https://doi.org/10.1016/j.bbagen.2005.05.006
27. Maffei ME (2014) Magnetic field effects on plant growth, development, and evolution. Front Plant Sci 5:1–15. https://doi.org/10.3389/fpls.2014.00445
28. Bilalis DJ, Katsenios N, Efthimiadou A et al (2012) Investigation of pulsed electroagnetic field as a novel organic pre-sowing method on germination and initial growth stages of cotton. Electromagn Biol Med 31:143–150. https://doi.org/10.3109/15368378.2011.624660
29. da Silva ARM, Farias ML, da Silva DLS et al (2017) Using atmospheric plasma to increase wettability, imbibition and germination of physically dormant seeds of Mimosa Caesalpiniafolia. Colloids Surf B Biointer 157:280–285. https://doi.org/10.1016/j.colsurfb.2017.05.063
30. Gómez-Ramírez A, López-Santos C, Cantos M et al (2017) Surface chemistry and germination improvement of Quinoa seeds subjected to plasma activation. Sci Rep. https://doi.org/10.1038/s41598-017-06164-5
31. Cakmak I (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J Plant Nutr Soil Sci 168:521–530. https://doi.org/10.1002/jpln.200420485
32. Sarinont T, Amano T, Attri P et al (2016) Effects of plasma irradiation using various feeding gases on growth of Raphanus sativus L. Arch Biochem Biophys 605:129–140. https://doi.org/10.1016/j.abb.2016.03.024
33. Šimor M, Ráhel' J, Vojtek P, et al (2002) Atmospheric-pressure diffuse coplanar surface discharge for surface treatments. Appl Phys Lett 81:2716–2718. https://doi.org/10.1063/1.1513185
34. Černák M, Černáková L, Hudec I et al (2009) Diffuse Coplanar Surface Barrier Discharge and its applications for in-line processing of low-added-value materials. Eur Phys J Appl Phys 47:22806. https://doi.org/10.1051/epjap/2009131
35. Šerá B, Šerý M, Zahoranová A, Tomeková J (2021) Germination improvement of three pine species (Pinus) after diffuse coplanar surface barrier discharge plasma treatment. Plasma Chem Plasma Process 41:211–226. https://doi.org/10.1007/s11090-020-10128-5
36. Navrátil Z, Trunec D, Šmíd R, Lazar L (2006) A software for optical emission spectroscopy-problem formulation and application to plasma diagnostics. Czechoslov J Phys 56:944–951. https://doi.org/10.1007/s10582-006-0308-y
37. Laux CO (2002) Radiation and Nonequilibrium Collisional-Radiative Models. In: D. Fletcher, J.-M. Charbonnier, G.S.R. Sarma and TM (ed) Physico-Chemical Modeling of High Enthalpy and Plasma Flows, von Karman. Rhode-Saint-Genèse, Belgium
38. Collins AR (2004) The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 26:249–261. https://doi.org/10.1385/MB:26:3:249
39. Menke M, Chen IP, Angelis KJ, Schubert I (2001) DNA damage and repair in Arabidopsis thaliana as measured by the comet assay after treatment with different classes of genotoxins. Mutat Res - Genet Toxicol Environ Mutagen 493:87–93. https://doi.org/10.1016/S1383-5718(01)00165-6
40. Szurman-Zubrzycka M, Jędrzejek P, Szarejko I (2023) How do plants cope with DNA damage? a concise review on the ddr pathway in plants. Int J Mol Sci 24: https://doi.org/10.3390/ijms24032404
41. Švubová R, Válková N, Bathoova M et al (2021) Enhanced in situ activity of peroxidases and lignification of root tissues after exposure to non-thermal plasma increases the resistance of pea seedlings. Plasma Chem Plasma Process 41:903–922. https://doi.org/10.1007/s11090-021-10160-z
42. Pet'ková M, Švubová R, Kyzek S, et al (2021) The effects of cold atmospheric pressure plasma on germination parameters, enzyme activities and induction of dna damage in barley. Int J Mol Sci 22:1–16. https://doi.org/10.3390/ijms22062833
43. Chatterjee N, Walker GC (2017) Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen 58:235–263
44. Kyzek S, Holubová Ľ, Medvecká V et al (2019) Cold atmospheric pressure plasma can induce adaptive response in pea seeds. Plasma Chem Plasma Process 39:475–486. https://doi.org/10.1007/s11090-018-9951-x
45. Plans M, Simó J, Casañas F et al (2013) Characterization of common beans (Phaseolus vulgaris L.) by infrared spectroscopy: Comparison of MIR, FT-NIR and dispersive NIR using portable and benchtop instruments. Food Res Int 54:1643–1651. https://doi.org/10.1016/j.foodres.2013.09.003

46. El-Bahy GMS (2005) FTIR and Raman spectroscopic study of Fenugreek (Trigonella foenum graecum L.) seeds. J Appl Spectrosc 72:111–116
47. Kuhnen S, Ogliari JB, Dias PF et al (2010) ATR-FTIR spectroscopy and chemometric analysis applied to discrimination of landrace maize flours produced in southern Brazil. Int J Food Sci Technol 45:1673–1681. https://doi.org/10.1111/j.1365-2621.2010.02313.x
48. Vasques CT, Domenech SC, Severgnini VLS et al (2007) Effect of thermal treatment on the stability and structure of maize starch cast films. Starch/Staerke 59:161–170. https://doi.org/10.1002/star.200600500
49. Durak T, Depciuch J (2020) Effect of plant sample preparation and measuring methods on ATR-FTIR spectra results. Environ Exp Bot 169:103915. https://doi.org/10.1016/j.envexpbot.2019.103915
50. Wolkers WF, Oliver AE, Tablin F, Crowe JH (2004) A Fourier-transform infrared spectroscopy study of sugar glasses. Carbohydr Res 339:1077–1085. https://doi.org/10.1016/j.carres.2004.01.016