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Plasma Seeds: Cold Plasma Accelerates *Phaseolus Vulgaris* Seed Imbibition, Germination, and Speed of Seedling Growth

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ABSTRACT: Cold atmospheric pressure plasma (CP) can play an important role in agriculture, medicine, chemistry, and biophysical applications. Generated by cold atmospheric pressure He-plasma jet (CPJ), reactive oxygen and nitrogen species (RONS), UV-Vis photons, and high-frequency strong electromagnetic fields with amplitudes of a few kV can interact with *Phaseolus vulgaris* seeds and plants. Here we show that CPJ accelerates seed imbibition, germination, and radicle growing rates. CPJ induces roughness, corrugation, and poration in *Phaseolus vulgaris* seed coats. It produces hydrophilization of seed coats and improves the wetting properties of seed surfaces. Magnetic resonance imaging studies show the acceleration of water uptake in *P. vulgaris* seeds so treated. Understanding the mechanisms of cold plasma interactions with seeds and plants should promote plasma-based technology for plant development control, increased yields and growth rates, and plant protection from pathogens. Our work offers new insight into mechanisms that trigger water transport and absorbance, seed germination, and activation of metabolism by cold plasmas.

KEY WORDS: cold plasma, germination, imbibition, plasma seeds, reactive nitrogen species, reactive oxygen species

ABBREVIATIONS: AFM, atomic force microscope; **CP**, cold atmospheric pressure plasma; **CPJ**, cold atmospheric pressure He plasma jet; **MRI**, magnetic resonance imaging; **RNS**, reactive nitrogen species; **RONS**, reactive oxygen and nitrogen species; **ROS**, reactive oxygen species; **SEM**, scanning electron microscopy; **θ**, young contact angle; θ_A, apparent contact angle

I. INTRODUCTION

Cold atmospheric-pressure plasma (CP) stimulates the growth and development of plants at the first stages of ontogenesis by activating seed metabolism, increasing seed resistance to stress factors, and enhancing fungicidal and bactericidal protection.[1–4] The productivity of agricultural plants increases 10%–70% after even short-duration CP treatment.[5]

CP is a partly ionized gas consisting of charged particles (ions, electrons) and neutral particles (atoms, molecules), as well as photons.[6–9] CP in air can produce reactive oxygen and nitrogen species (RONS) including N, N_2O, NO_x, N_2O_5, HNO_3, HNO_2, HNO, H, H_2, HO_2, H_2O_2, OH, O, and O_3. These CP products and a few kV high-frequency electrical fields lead to the activation of surface-modifying electroporation,[7] hydrophilization,[7,10–12] and corrugation. The mechanism responsible for the effect of plasma on the speed of seed germination and plant growth is still debated.[7] This is because seeds are extremely complex biologically and the effect of plasma on them can occur due to stimulation by a high-frequency strong electromagnetic field, UV-Vis radiation, and chemical reactions between RONS and seed components.

In the last three decades, many applications of CP in agriculture and plant biology have been discovered, and its application in agriculture for the treatment of seeds, plants, and fruits is now called plasma agriculture.[2] Plasma can stimulate the growth and development of plants at the first stages of ontogenesis by activating seed metabolism, increasing resistance to stress factors, and enhancing fungicidal and bactericidal protection. According to the literature, plasma can have both stimulating and depressing effects on the processes of seed germination.[5,7,12–14]

Plasma properties, power, composition, and working gas pressure, have a significant effect on the reaction of seeds when exposed to plasma. A significant increase in the duration of exposure can create side effects such as DNA and protein damage.[15,16] Another important factor to take into consideration is seed type since seeds of different plant species react differently to plasma exposure. One of the most important factors in plasma treatment is duration. Filipov et al.[5] suggested treating seeds of leguminous crops, cabbage, squash, beet, cucumber, tomato, carrot, calendula, marigold, lentil, pumpkin, barley, dock, Swedish turnip, soya, vetch, pea, bean, wheat, and corn with cold plasma for 15–32 s; seeds of pepper, eggplant, parsley, aster, lobelia, potato, chrysanthemum, phlox, and harebell with gas plasma for 20–40 s; and dill, hops, celery, magnolia vine, digitalis, Melissa, tobacco, and lignified plants with gas plasma for 25–45 s. Many authors suggest different optimal durations. CP treatment can lead to a decrease in the apparent contact angle of water at the seed coats and to an increase in water imbibition through the testa, independent of the micropyle effect.[7,10,11]

It is known that reactive oxygen species (ROS) participate in plant development by acting as signaling molecules for cell proliferation and differentiation, programmed cell death, seed germination, gravitropism, root hair growth, pollen tube development, and senescence.[13,14,17–20] However, the specific mechanisms responsible for the regulatory action of ROS at various developmental stages remain largely unknown. RONS produced by CP in atmospheric air appear to be the primary cause of oxidation, modification, and destruction of some proteins and peroxidation of lipids in plants and seeds. Some are known to be signaling molecules, which control plant development as well as plant cell death.

CP has been shown to disinfect and sterilize seed surfaces via RONS and UV radiation,[1,21,22] which can increase the percentage of germinated seeds. They can also increase the seed germination rate and speed up plant growth.[7]

Typical seed germination is triggered by absorption of water, which leads to activation of metabolism, ion channels, and other processes.[23] It is also known that CP can modify the wettability of plant seeds such as lentil, bean, and wheat.[10,11] Hydrophilization of seed coats induced by plasma is important, and so it is the focus of this paper.

CP has been used in biology and medicine for transdermal drug delivery due to the poration of human skin.[24–26] Poration is the formation of pores or a pattern of pores in a surface. The cause may be that the magnitude of the electric fields generated by a plasma jet exceeds the threshold for electroporation.

The effects of electrical fields on vegetation have been the subject of research since the 18th century.[27–34] When applied to seed surfaces, CP may induce electroporation and/or corrugation of the dormant seed to improve germination and water imbibition due to penetration of electric fields and RONS.[7] Plasma can stimulate the growth and development of plants at the first stages of ontogenesis by activating seed metabolism, increasing resistance to stress factors, and enhancing fungicidal and bactericidal protection. Treatment with gas plasma shows positive results in almost all climatic zones under various growing conditions.

Here, we studied the effects of cold atmospheric pressure He plasma jet (CPJ) on the surface characteristics, imbibition, and germination of *Phaseolus vulgaris*, cv. dragon's tongue bush-bean seeds. The objective of this research was to determine how plasma modifies the seed surface to encourage water absorption and thus fast germination and growth. *P. vulgaris* seeds were selected because of their sufficiently large surface area and economic importance in the agriculture and food industries.

II. MATERIALS AND METHODS

A. Seeds

Phaseolus vulgaris, cv. dragon's tongue bush-bean seeds were obtained from Catbird Seat Garden Center (Madison, Alabama) and Johnny's Selected Seeds (Winslow, Maine). All experiments were performed on healthy specimens. The temperature of the air was 21°C. The humidity in the laboratory was kept at 40–43%. The length of the dormant seed was 1.5 cm (mean 1.47, median 1.50, std. dev. 0.12, std. err. 0.03, $n = 20$).

B. Ozone Tests

Ozone test strips were purchased from Macherey-Nagel (Dueren, Germany). They were used to determine ozone concentration in the air under the plasma jet.

C. Plasma Jets

The cold plasma jets used in our experiments have an annular geometry using a pair of nested quartz tubes. Ultra-pure helium gas flows between the tubes in the annular space. The outer quartz tube has an outer diameter of 6 mm and an inner diameter of 4 mm and is open at both ends. The inner tube has an outer diameter of 3 mm and an inner diameter of 2 mm and is closed at one end. The tubes are held inside a standard plastic compression Tee tube fitting (Swagelok NY-400-3, Swagelok, Solon, Ohio) with the gas line at the perpendicular leg of the tee. This creates a sealed volume with the only outlet being the annular space between the inner and outer quartz tube. The experimental setup has been described elsewhere.[7–9]

The entire plasma jet source is placed in a metal enclosure to reduce electromagnetic interference. A 1-mm-diameter tungsten pin is inserted into the inner tube while a stainless-steel ring is placed around the outer tube. A high-voltage coaxial power connector provides shielded power delivery. The central pin carries the high-voltage signal from the pulsed dc power source to the tungsten pin. The grounded shield wire of the coaxial connector and line are connected to the metal enclosure around the plasma source. The ring is grounded via contact with the metal enclosure. This design prevents direct contact between the plasma generated in the annulus and either electrode, preventing seed-damaging arcing.

In this study, the pulsed dc power source was driven at 9 kV, 16 mA, 6-kHz frequency and 1-μs pulsed width. The power source consisted of a Matsusada AU-10P60 10-kV dc power supply (Matsuada, Shiga, Japan), an IXYS PVX-4110 pulsed generator, and a SRS DG-645 delay generator. The pulse had a square shape with a slight slope during the rising edge. The current trace had two distinct current pulses at the rising and failing fronts of the voltage. The ultra-pure helium gas was metered with MKS digital mass flow controllers. The helium flow rate was set at 2.0 L/min. The gas was ionized by the strong electric field between the electrodes and formed a plasma jet approximately 3 cm long as measured from the exit of the outer tube.

Although a plasma jet looks continuous to the naked eye (Fig. 1), it actually consists of fast ionization waves (plasma bullets) propagating along the noble gas channel with speeds of a few hundred meters per second. Electrons inside the fast ionization wave have energies of a few electron volts and are capable of producing ionization and nonequilibrium chemical reactions at room temperature. The reactive species are generated by these electrons in areas where mixing of noble gas with air takes place. The charged species (electrons and ions) recombine quickly beyond the plume (the glow plasma region), but the neutral species with longer life spans can propagate beyond the plasma plume and interact with seeds. The charged species were not explicitly measured here, but some comparisons can be made with similar works in the literature. Van Gessel et. al. measured electron density in a cold plasma jet using laser-induced scattering and showed that density decreased by an order of magnitude from the tube exit to 6 mm downstream.[35] Xu and Doyle measured electron density in an RF atmospheric plasma jet with Langmuir probes and found that it decreased from one to three orders of magnitude approximately 7 mm downstream of the exit.[36] It is important to point out that direct comparison between different atmospheric plasma jets is difficult, as geometry, power, and gas flow and composition make significant differences in the resulting plasma properties.

D. Atomic Force Microscope

The surface of the *P. vulgaris* seed was analyzed using the Pico-Plus atomic force microscope (AFM) from Molecular Imaging (now Agilent Technologies). The sample seed

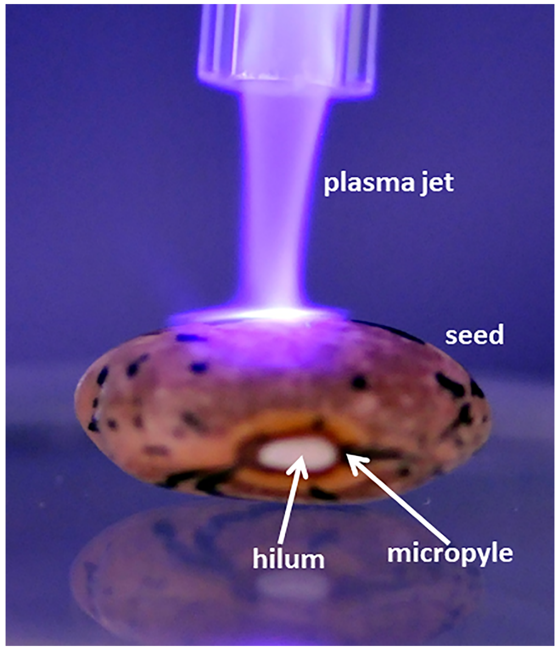

FIG. 1: Dormant dragon's tongue bush bean (*Phaseolus vulgaris*) seed in contact with CPJ. The hilum is a scar left by the stalk which attached the ovule to the ovary wall before it became a seed. The hilum on a bean seed is called the eye. The micropyle is a tiny pore in the testa that lies on the opposite of the tip of the radicle. The micropyle is covered by the caruncle.

was firmly fixed on the AFM table with no possible movement. The AFM creates a three-dimensional representation of the sample surface by monitoring the force of interaction between the sample and a cantilever probe. The seed was placed on top of a microscope cover glass (22 × 22 mm), and topographical images were obtained using the contact mode of the AFM. The 3D topographical images of the *P. vulgaris* seeds were then analyzed. Gwyddion-2.51 software (http://gwyddion.net/) was used for AFM data visualization and analysis.

E. Magnetic Resonance Imaging

The seeds were imaged using a 9.4T system (BioSpec, Bruker BioSpin GmbH, Ettlingen, Germany) equipped with a surface coil as the receiver. Axial T2-weighted images were acquired with a T2-weighted fast spin echo sequence (rapid acquisition with relaxation enhancement). The parameters were as follows: TR = 2,500 ms, TE = 36 ms, rare factor = 8, FOV = 20 × 20 mm, matrix size = 200 × 200, 10 slices, slice thickness = 1 mm, 120 signals averaged, total acquisition time 2 h 5 min for each state (open or closed). After water incubation, the surfaces of imbibed seed coats were gently dried with soft tissues and placed in dry Petri dishes before placement in the MRI machine.

All experimental results were reproduced at least 25 times. AFM and MRI measurements were taken at least 5 times.

III. RESULTS

A. CPJ Treatments: Electroporation of Seed Coats

Plasma produces reactive oxygen and nitrogen species, many of which are unstable and have a very short life span. The most common and relatively stable products are HNO_3, H_2O_2, O_3, N_2O, and NO_x. To determine the types of product from the plasma which may affect the seeds, commercial ozone test strips were used. The ozone test strips, when placed under the cold plasma jet at a distance of 1 cm for 10 min, showed production of ozone by the He plasma jet in a concentration of 150–210 $\mu g/m^3$.

CPJ treatment of *P. vulgaris* dormant seeds can induce poration, corrugation, and hydrophilization of the seed surface. The roughness of a seed surface can affect its wetting. The greater the roughness, the more the surface properties causing water attraction or repulsion. Such an effect can be explained by the fact that at a contact angle $\theta < 90°$ for a smooth surface, water penetrates into the surface cavities. This improves the wetting of a rough seed surface. If $\theta > 90°$, water does not penetrate, which lessens the wetting of a rough surface. CPJ treatment can reduce the apparent contact angle of the seed coat surface,[10,11] as shown in Figs. 2–5. According to Bormashenko et al., the apparent contact angle for untreated bean seeds is 98 ± 2° and decreases to 53 ± 1.5°

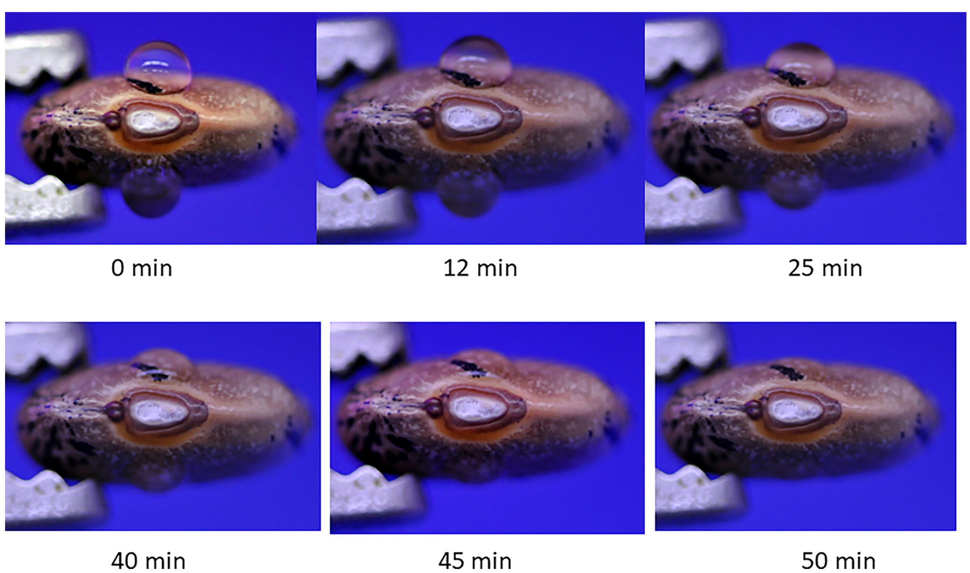

FIG. 2: Kinetics of 10-μL water drop penetration of a dormant dragon's tongue bush-bean seed coat from the top and from the bottom of a seed was not treated with CPJ

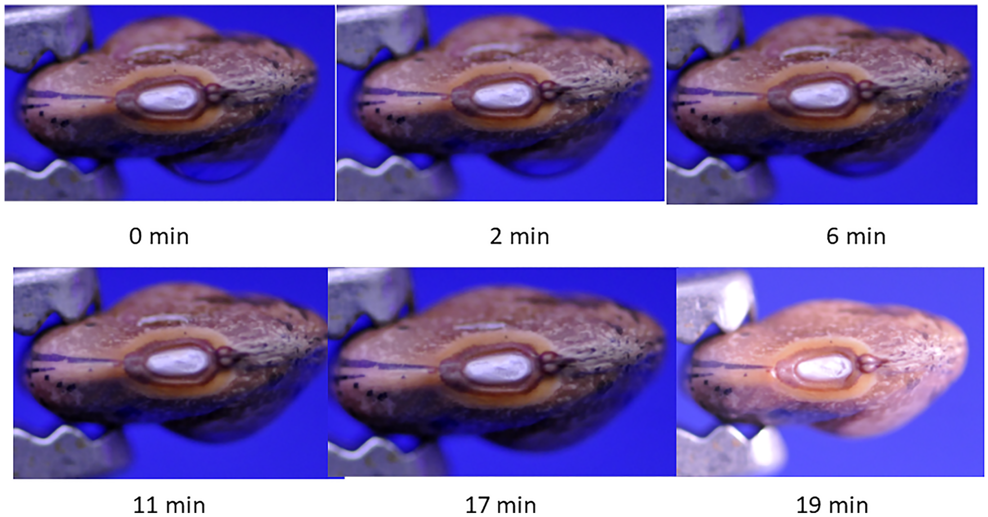

FIG. 3: Kinetics of 10-μL water drop penetration of a dormant dragon's tongue bush-bean seed coat from the top and from the bottom of the seed, which was treated with CPJ for 15 min on each side

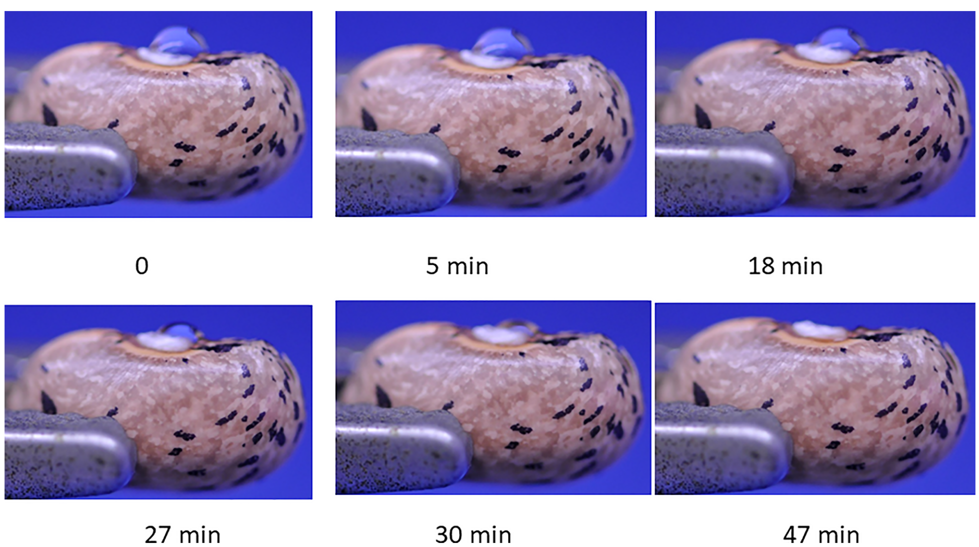

FIG. 4: Kinetics of 10-μL water drop penetration of a hilum of a dormant dragon's tongue bush-bean seed coat not treated with CPJ

for seeds coats treated with cold atmospheric pressure plasma. We found the apparent contact angle of the seed coat surface treated by CPJ not to be constant but to decrease with time (Figs. 3 and 5).

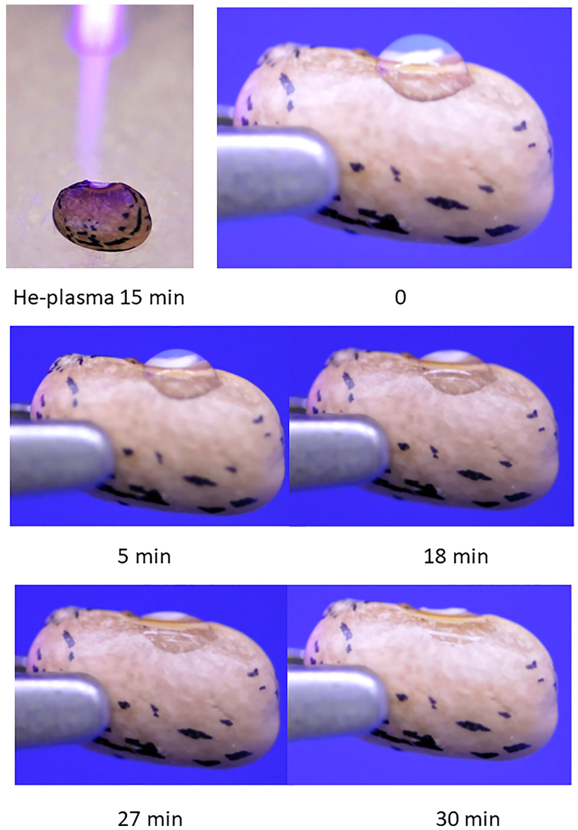

FIG. 5: Kinetics of 10-μL water drop penetration of a hilum of a dormant dragon's tongue bush-bean seed coat treated with CPJ for 15 min

Roughness makes a hydrophilic surface more hydrophilic, and the roughness of a hydrophobic surface makes it more hydrophobic. On real seed surfaces, which are usually rough, one must distinguish between the Young contact angle (θ) and the apparent contact angle (θ_A). In the case of a rough surface, the Young contact angle is measured between the tangent and the water–air interface and between the tangent and the local ideal smooth seed surface. The apparent contact angle is measured between the tangent and the water–air interface and the macroscopic seed surface.

Both sides of the *P. vulgaris* dormant seed were treated by CPJ for 1 or 15 min per side in direct-contact mode. Formation of hydrophilic pores in the seed coat was detected with water drop experiments as shown in Figs. 2–4. Drops of pure water on the top and bottom of preliminary treated by plasma surfaces of dormant seeds (Fig. 3) penetrated in 19 min (Fig. 3). The speed of water penetration through a seed coat increases with increasing duration of plasma treatment: 0 (Fig. 2) < 1 min < 15 min (Fig. 3). Because of the balance of gravitational and capillary forces, a water drop on the bottom of a seed does not fall under gravitational force. Penetration of aqueous drops through the coat of a dormant seed is induced by the difference in water potentials outside and inside the seed.

Formation of hydrophilic pores and surface defects in coats of *P. vulgaris* seeds, induced by CPJ, can facilitate water transport during imbibition and germination. The images shown in Figs. 2 and 3 give the impression that water droplet absorption without CPJ leaves the seed homogeneously swelled (Fig. 2) while the plasma-treated seed shows bulges (Fig. 3). Redox interactions of RONS, generated by plasma, with organic compounds at the seed cover surface can induce formation of hydrophilic compounds and groups. Hydrophilization of seed coats and a decrease in the apparent contact angle of water drops at the surface of seed covers take place during absorption (Figs. 2 and 3).

A pulsed electric field used to generate the plasma creates pores in seed coats for the acceleration of water and RONS absorption. An estimate of the size of pores or defects required to create hydrophilic surfaces sufficient to hold water droplets under a seed, as shown in Fig. 3, can be made via phase equilibrium energy analysis. When the three phases—solid seed coat, liquid water, and gaseous air—meet, the system as a whole tends to minimize the total value of surface energy. The behavior of such a system depends upon the ratio between surface energy values at all three interfaces: seed–water, seed–air, and water–air. If the energy of the interaction between water and solid is greater than between water and air, the aqueous phase will tend to occupy more surface area, thereby replacing the air molecules in the same volume. The equilibrium state, corresponding to a minimum of the system's total surface energy, is achieved when the energy gain due to replacement of air by water is compensated by the expenditure of energy due to the increasing surface area of liquid.[37,38]

Due to the difference in water potential inside and outside the seed coat, water can penetrate the coat due to osmosis. We estimated the minimal radius of a pore needed to keep a drop of water on the underside surface of a *P. vulgaris* seed. The gravitational force F acting on the ideal drop is

$$F = mg \tag{1}$$

where m = mass of drop; and g = acceleration due to gravity. According to Tate's law, the force acting on the drop is also equal to

$$F = 2\pi r\gamma \tag{2}$$

where γ = interfacial tension; and r = pore radius. Osmotic force can be estimated from

$$F = iRT\pi r^2 \sum\nolimits_k C_k \tag{3}$$

and the balance of the three forces can be written as

$$RT\pi r^2 \sum\nolimits_k iC_k + 2\pi r\gamma - mg = 0 \tag{4}$$

where $R = 8.3145\ m^3\ Pa\ mol^{-1}K^{-1}$; $T = 293.15$ K; $m = 10^{-5}$ kg; $g = 9.8\ m/s^2$; and C = concentration of ions in mole/m^3; the surface tension of water is equal to 0.072 N/m. Concentration of ions and water can be found in literature.[17,39–41] The solution of Eq. (4) gives the pore radius as ~ 1.7 μm.

Formation of surface defects and hydrophilic pores in the seed coat will accelerate water transport, imbibition, and germination of *P. vulgaris* seeds. Energization of ions in CP sheaths and boundaries can also lead to surface modifications.

The micropyle helps in absorbing water at germination. The CPJ effect of a micropyle and a hilum in water penetration is shown in Figs. 4 and 5. A water drop penetrates a seed about two times faster if treated with a plasma jet (Figs. 4 and 5).

B. AFM: Strong Seed Coat Corrugation and Surface Defects

AFM was used to characterize seed surfaces before and after CPJ treatment. Some authors have studied the surfaces of CPJ-treated seeds with scanning electron microscopy (SEM). The benefit of AFM over SEM is that it readily produces a 3D topographical map without damage to living tissue, allowing potential planting of the same sample. AFM topographic images of one surface of a coated dormant *P. vulgaris* seed before and after CPJ are shown in Fig. 6. Dormant seeds have a flat surface with some roughness of the seed cover (Fig. 6A). CPJ produces very significant changes in this topography: strong coat corrugation, surface defects, and possible poration. As shown in Fig. 6, the roughness amplitude after a 1-min treatment was 2.4 and after 15 min was 4.8 μm.

Such results show that CPJ can improve water imbibition (Figs. 3 and 4). AFM images show that CPJ can produce poration, corrugation, and hydrophilization of seed coats. However, the question remains whether poration is due to high-frequency strong electromagnetic field electroporation, UV-Vis radiation, or redox interactions with reactive oxygen and nitrogen species. Also, photons produced by plasma can improve germination.[7,17] To separate the factors involved, control experiments were carried out using AFM (Fig. 7). UV light and RONS can be blocked by placing the seeds inside a closed Petri dish (Fig. 7C). Placing the seeds inside a quartz cuvette allows the UV light to reach the seed (Fig. 7B). In either a closed Petri dish or a quartz cuvette, the electromagnetic field still reaches its target.

Electroporation by a high-frequency electromagnetic field, redox hydrophilization by RONS, and activation by UV light can accelerate the germination of seeds independently (Fig. 7). When in direct contact in the plasma jet, the components combine synergistically for even faster germination. Similar effects of cold plasma on biotissue were found by Lackmann et al.[22] Photons and particles emitted from CP inactivated bacteria and biomolecules independently and synergistically.[22]

AFM is a powerful tool that can measure the topography of very small surfaces with great accuracy. AFM images of the surface topography of *P. vulgaris* seeds are commonly displayed as a pseudocolor plot representing relative surface heights. However, as the colors are autoscaled for each image, they are not wholly useful for cross-comparison. Thus, Figs. 6–8 show the exact scales on the z-axis in μm.

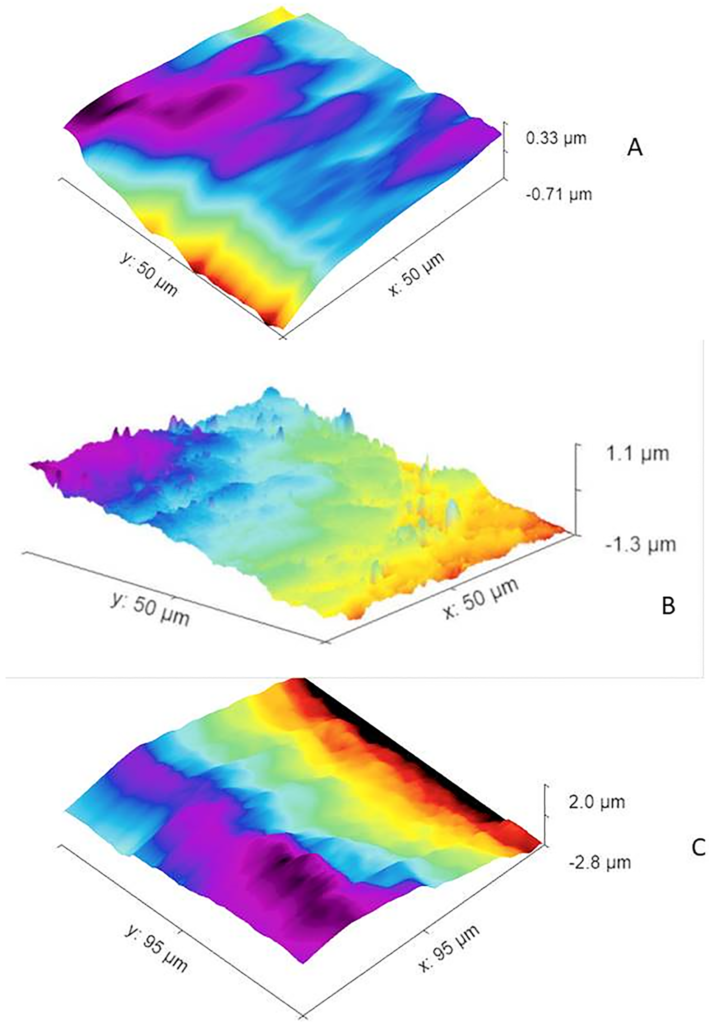

FIG. 6: AFM topographic imaging of the surface of a dormant dragon's tongue bush bean seed (A), after treatment with CPJ during 1 min (B) and 15 min (C). The surface heights are shown on the *z*-axis scale and the color plots in each image correspond to their respective height scales.

Treatment of the hilum, micropyle, and seed coat with CPJ (Fig. 8B) induces surface defects in the hilum (Fig. 8A) and seed coat (Fig. 8C).

C. Magnetic Resonance Imaging of Seed Imbibition

Noninvasive magnetic resonance imaging (MRI) allowed us to obtain spatial representations of water distribution in seeds. Figure 9 shows water in both dormant seeds (Fig. 9A) and seeds imbibed for 7 h (Fig. 9B and C). According to the literature, dormant

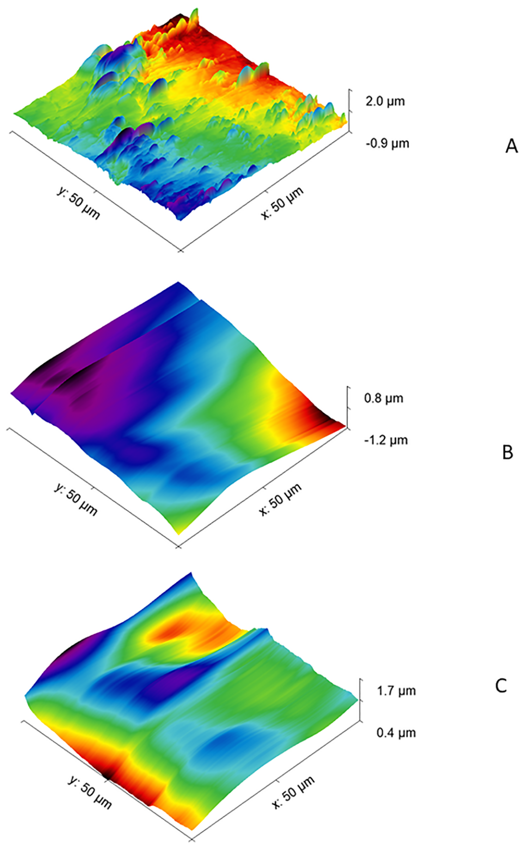

FIG. 7: AFM topographic image of a surface of a dormant dragon's tongue bush-bean seed (A) after treatment with CPJ for 5 min. (B) A seed was located inside a closed quartz cuvette or (C) a Petri glass dish. The surface heights are shown on the *z*-axis scale; the color plots in each image correspond to their respective height scales.

seeds contain 5–18% moisture.[39] Figure 9A shows that the dormant seeds have a tiny inherent water content (a small gray spot in the center). The imbibed seeds in Fig. 9B were not treated, whereas the imbibed seeds in Fig. 9C were treated for 1 min. Water content increased in the CPJ imbibed seeds, likely due to plasma-induced electroporation and corrugation of seed coats, which enhances water penetration. MRI studies show this enhancement seeds exposed to CPJ. Treatment before imbibition clearly increases aqueous content (Fig. 9).

D. Germination

CPJ accelerates seed germination and radicle development (Fig. 10). The radicle is the embryonic root which develops into the primary root and is usually the first part of the embryo to push its way out of the seed during germination. We found germination

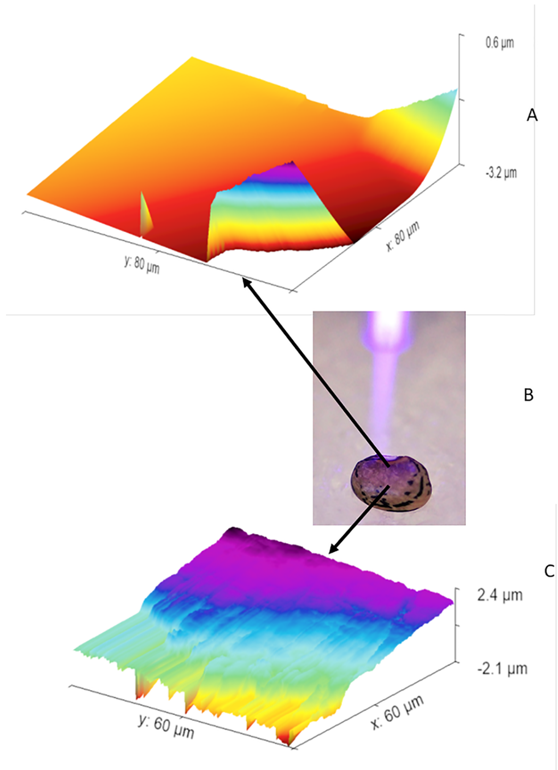

FIG. 8: AFM topographic imaging of a surface of a dormant dragon's tongue bush bean seed after direct treatment of a hilum (A) and a coat (C) with a low temperature atmospheric pressure CPJ (B) for 15 min. The surface heights are shown on the *z*-axis scale and the color plots in each image correspond to their respective height scales.

efficiency and radicle length to be in increasing order: untreated < CPJ in direct contact for 1 min per seed side < CPJ in direct contact for 15 min per side (Fig. 10, Table 1). Germination was accelerated by either direct contact or indirect contact with a 1-cm gap between the plasma jet tip and the seed (Table 1). Here, CPJ increased radicle length 1.5–2.5 times (Table 1).

The effect of CP on seedling growth is shown in Fig. 11 and Table 2. The greatest effect was with 1-min exposure. Plasma treatment induces structural changes on the surface of seeds that improve wettability, which can increase the speed of germination. Changes in seed surface properties after plasma treatment, such as poration, hydrophilization, and corrugation, can intensify the transport of oxygen and water through the seed coat.

E. Side Effects of CPJ: Surface Damage

Direct treatment of dragon's tongue bush-bean leaves by CPJ can induce visible side effects if the treatment time is sufficiently long (Fig. 12). Here, application of CPJ to

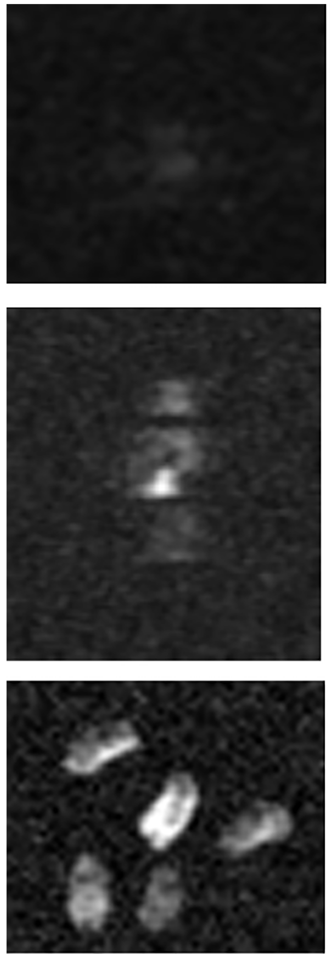

FIG. 9: MRI of dormant (A) and 7-h imbibed (B and C) dragon's tongue bush-bean seeds. (B) Untreated seeds. (C) Seeds with each side treated with 1-min CPJ. Water appear as white regions.

the surface of a leaf for 1 min or less did not produce visible leaf damage (Fig. 12A). Damage can be induced by RONS, the concentration of which increases with length of CPJ exposure. Prolonged exposure can cause side effects due to redox reaction, tissue peroxidation, and acidification by HNO_3 and HNO_2 (Figs. 12 and 13). UV photons and RONS can also cause genotoxic effects.[15,16]

IV. DISCUSSION

One possible explanation for the improvement in germination and growth of plant seeds when exposed to CPJ is that plasma induces structural changes on the seed surface. Germination is strongly influenced by chemical redox reactions on the surface initiated

FIG. 10: Germination of imbibed dragon's tongue bush-bean seeds 2 days after 24-h incubation in water: (A) untreated and (B) 0.5-min, (C) 1-min, (D) 5-min, and (E) 15-min direct-contact CPJ per side

by plasma exposure. Plasma treatment of seed coats improves seed surface wettability (Figs. 3 and 5), which in turn increases germination speed (Fig. 10).

CPJ treatment increased seed coat roughness (Figs. 6 and 8) and hydrophilic properties, and induced poration (Figs. 3–5).

The initial absorption of water by seeds is primarily a result of hydration and osmosis. Water permeation across the coat can occur by partition or through pores and surface defects.[33,37,38,42] Partition transport through a thick coat can be extremely slow; in contrast, fast water transport occurs through hydrophilic pores and surface defects.

TABLE 1: Radicle length of imbibed seeds 2 days after 24-h water incubation following direct-contact CPJ

Treatment	Radicle length, (mm; *n* = 20)				
	Mean	**Median**	**Std. Dev.**	**Std. Err.**	**95%/99% Conf. Int.**
A	10.40	10.00	3.44	1.09	2.46/3.53
B	15.40	15.00	3.99	0.89	1.87/2.55
C	16.30	17.50	4.24	1.34	3.04/4.37
D	21.00	21.00	0.86	0.19	0.40/0.55
E	24.95	25.00	0.51	0.11	0.24//0.33

A = no treatment; B = 0.5 min; C = 1 min; D = 5 min; E = 15 min (all durations per seed side). Mean, median, standard deviation, standard error, and confidence intervals for results shown in Fig. 10.

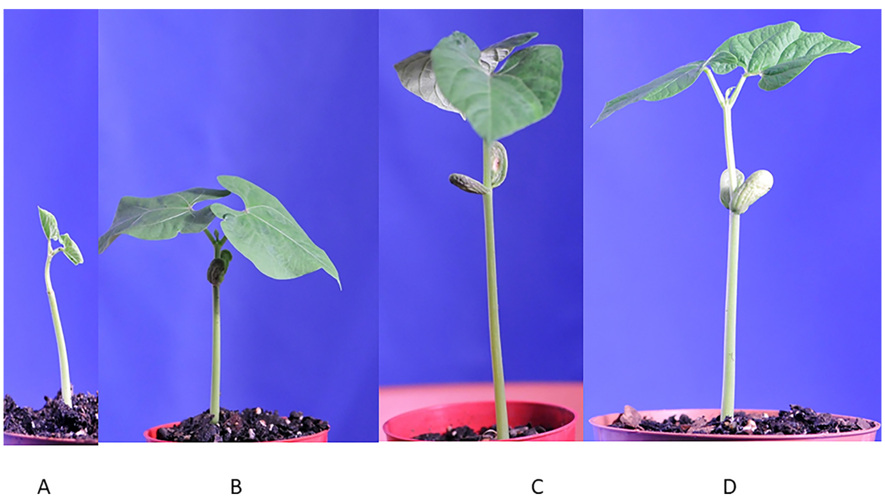

FIG. 11: Seedlings after 7-day growth: (A) untreated and (B) 0.5-min, (C) 1-min, and (D) 15-min CPJ

Chemical redox reactions between plasma-induced RONS and seed coats can produce hydrophilic seed surfaces and generate hydrophilic pores (Figs. 3 and 5). Mechanisms of interaction of many reactive oxygen and nitrogen species with biotissue are still discussed.[12–14,20,43]

The effect of plasma composition or working gas pressure is important for agricultural applications, and is the subject of ongoing research. Understanding the mechanisms of CP interactions with seeds and plants should promote plasma-based technology for plant development control, increased yield, faster growth rates, and protection from pathogens. Our work offers new insight into mechanisms that trigger water transport and absorbance, seed germination, and activation of metabolism by cold plasmas. Fig. 13 is a schematic of CPJ-seed interaction.

TABLE 2: Seedling length after 7-day growth following CPJ treatment of different durations

Treatment	Plant length (cm; *n* = 20)				
	Mean	**Median**	**Std. Dev.**	**Std. Err.**	**95%/99% Conf. Int.**
A	7.60	7.50	2.76	0.87	1.97/2.83
B	8.40	8.50	0.97	0.31	0.69/0.99
C	15.60	16.50	1.90	0.60	1.36/1.95
D	15.85	17.00	1.90	0.42	0.89/1/22

A = 0 min; B = 0.5 min; C = 1 min; D = 15 min. Mean, median, standard deviation, standard error, and confidence intervals for results shown in Fig. 11.

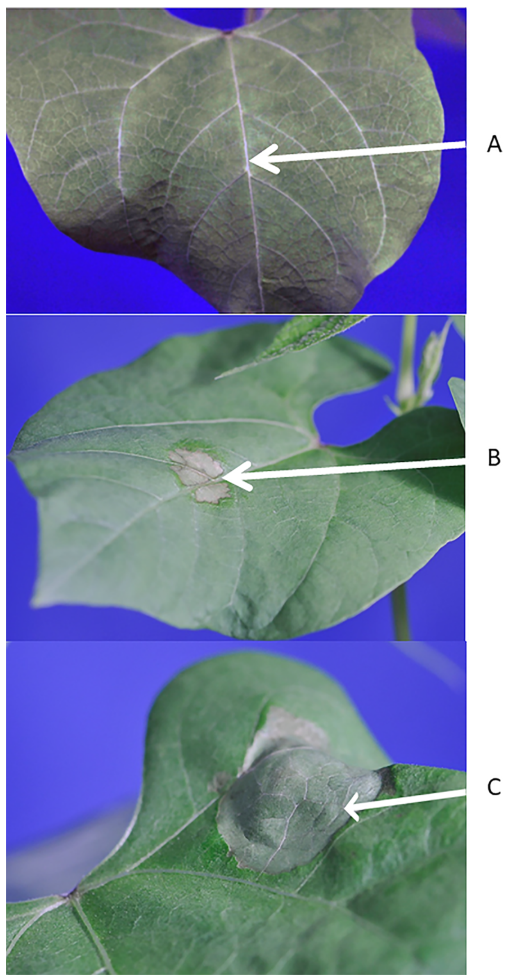

FIG. 12: Side effects immediately after direct CPJ treatment of dragon tongue bush-bean leaves for (A) 1 min, (B) 10 min, and (C) 20 min

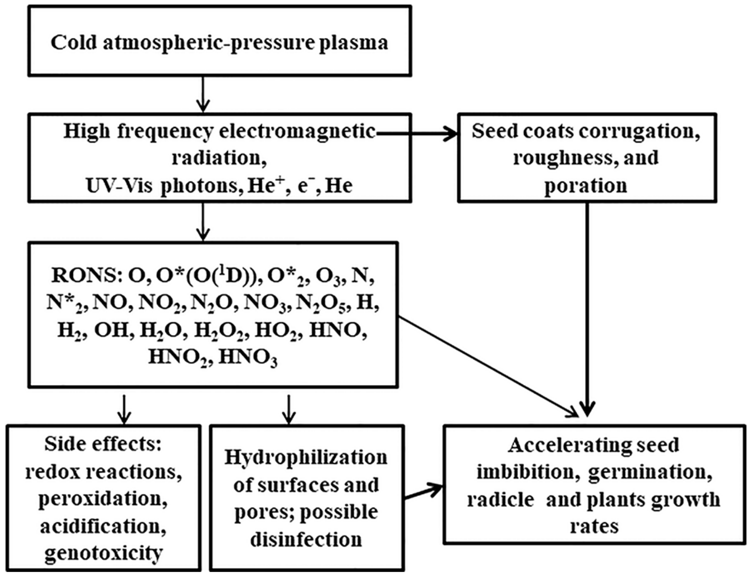

FIG. 13: CPJ interactions with seeds

V. CONCLUSION

Plasma-processing parameters, such as treatment duration, power, composition, and working gas pressure have a significant effect on seeds exposed to plasma. Changes in the seed surface due to poration, hydrophilization, and corrugation of testa can intensify the transport of oxygen and water through the seed coat and accelerate seed imbibition and germination as well as seedling growth (Fig. 11, Table 2). Plasma-generated reactive oxygen and nitrogen species and high-frequency strong electromagnetic field penetration into seed coats accelerate seed germination and seedling growth as well. Cold atmospheric pressure plasma treatment can increase the wettability of seed surfaces. AFM measurement shows that CPJ induces poration and strong corrugation of seed coats.

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