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Cold plasma poration and corrugation of pumpkin seed coats

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ABSTRACT

The treatment of seeds and plants by electrically generated cold atmospheric pressure plasma can accelerate seed germination and radicle growing rates. The plasma generated reactive oxygen and nitrogen species, UV photons, and high frequency electromagnetic fields can penetrate into seed coats and modify their surface properties. Atomic force microscope data shows that cold helium or argon plasma induces strong corrugation of pumpkin seed coats, produces pores and surface defects. These structural deformations and poration enhance water uptake by seeds during the imbibing process, accelerate seeds germination, and increase seed growth. The cold atmospheric pressure plasmas treatment of pumpkin seeds also decreases the apparent contact angle between a water drop and the seed surface, thereby improving the wetting properties of seeds surfaces. Magnetic resonance imaging studies show acceleration of water uptake in pumpkin seeds exposed to a cold plasma jet. Reactive nitrogen and oxygen species, high frequency electromagnetic fields and photons emitted by the plasma jets accelerate germination of pumpkin seeds both independently and synergistically. These results show that cold plasma can be used in agriculture for acceleration of seed germination, increasing growth of plants seedlings, poration and corrugation of the bio-tissue surfaces.

1. Introduction

Cold atmospheric-pressure plasma (CAPP) is a partly ionized gas operated at atmospheric pressures, consisting of charged particles (electrons, ions) and neutral particles (atoms, molecules) as well as photons. CAPPs typically have low gas temperatures around 300 K, but high electron temperatures or energies of several electron-volts (1 electron-volt = 11,600 K). CAPPs produce various atomic or molecular species if interacting with molecular gases such as air. For example, CAPP in air can produce reactive oxygen and nitrogen species (RONS) including NO_x, OH, O, and O_3. These CAPP products lead to the activation of surface modifying processes [1–4]. There has been an increased number of applications of CAPP in agriculture and plant biology in the last two decades, so much that the application of cold plasma in agriculture for treatments of seeds, plants and fruits is now called plasma agriculture [5–14].

It is known that reactive oxygen species (ROS) participate in plant developmental processes by acting as signaling molecules for cell proliferation and differentiation, programmed cell death, seed germination, gravitropism, root hair growth, pollen tube development, and senescence [15–17]. However, the specific mechanisms responsible for the regulatory action of ROS at various plant developmental stages remain largely unknown. RONS produced by CAPP in atmospheric air appear to be the primary cause of activation of phytosensors and phytoactuators in plants and seeds [18–20]. Some of these RONS are known to be signaling molecules, which control plants' developmental processes [21–23] as well as plant cell death [24].

For seeds, which are the focus of this study, CAPP have been shown to disinfect and sterilize the surfaces of seeds by RONS and UV radiation, which can increase the percentage of germinated seeds [3,7,25]. The plasma can also increase the seed germination rate and speed-up plant growth. Typical seed germination is triggered by absorption of water which leads to activation of metabolism, ion channels, and other processes [26–28]. It is also known CAPP can modify the wettability of plant seeds such as lentils, beans, and wheat [29]. As seed germination is dependent on water absorption, the effect of plasma on the surface characteristics and structure, thus wettability, of the seeds are important and the focus of this paper.

In this work, we studied the effect of CAPP on the surface characteristics of pumpkin seeds. The objective was to determine how plasma modifies the seed surface to encourage water absorption and thus germination and growth. We selected pumpkin seeds because of their large surface area and economic importance in agriculture and medicine [30]. The pumpkin plant has been used for its medicinal and nutritious

Abbreviations: AFM, Atomic force microscope; *CAPP*, Cold atmospheric pressure plasma; *MRI*, Magnetic resonance imaging; *RNS*, Reactive nitrogen species; *RONS*, Reactive oxygen and nitrogen species; *ROS*, Reactive oxygen species.

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benefits for centuries. Pumpkin seeds have beneficial effects on prostate glands, blood glucose level, cholesterol, learning disabilities, immunity, liver, bladder, parasite inhibition, depression and cholesterol [30,31]. Electrophysiology and electrical signaling in pumpkin plants were analyzed by Vodeneev et al. [32]. Work has been done to study the enzymes and voltage gated ion channels in germinating pumpkin seeds [26–28,33]. In *Cucurbita pepo* L., cv. Cinderella imbibed seeds, Volkov et al. [28] found the presence of resistors with memory known as memristors. The analysis was based on cyclic voltammetry where a memristor should manifest itself as a nonlinear two-terminal electrical element, which exhibits a pinched hysteresis loop on a current-voltage plane for any bipolar cyclic voltage input signal. Dormant pumpkin seeds have very high electrical resistance without memristive properties [28]. The dormant state helps pumpkin seeds to conserve energy. Seed dormancy is caused by endogenous characteristics of the embryo. The electrostimulation by bipolar sinusoidal or triangular periodic waves induces electrical responses in imbibed pumpkin seeds with fingerprints of memristors. Tetraethylammonium chloride, an inhibitor of voltage gated K^+ channels, transforms a memristor to a resistor in imbibed pumpkin seeds. There is an electrophysiological difference between imbibed and dormant pumpkin seeds: absorption of water by pumpkin seeds activates voltage gated K^+-channels and decreases electrical resistance inside seeds. The discovery of memristors in pumpkin seeds created a new direction in the understanding of electrophysiological phenomena in germinated seeds.

CAPP has also been used in medicine for transdermal drug delivery due to poration of the human skin [34–37]. Poration is the formation of pores in a surface, or a pattern of such pores. The cause may be that the magnitude of the electric fields generated by plasma could exceed the threshold for electroporation. When applied to seed surfaces, CAPP may also induce poration and/or corrugation of the dormant pumpkin seed to improve germination and water imbibition due to intracellular penetration of electric fields and RONS.

2. Materials and methods

2.1. Seeds

Pumpkin *Cucurbita pepo* L., cv. Cinderella, *Cucurbita maxima* L., cv. Jarrahdale and *Cucurbita maxima* L. cv. Warty Goblin seeds were received from Catbird Seat Garden Center (Madison, Alabama, USA). A few hundred seeds were removed from pumpkins, rinsed, and dried for seven days. All experiments were performed on healthy specimens. The humidity in the laboratory was kept at 40–43%. The germination of imbibed pumpkin (*Cucurbita pepo* L., cv. Cinderella) seeds was 96% (Mean 96.0%, Median 100.00%, Std. Dev. 19.69%, Std. Err.1.97%, 95% Conf. 3.91%, 99% Conf. 5.17%, n = 100).

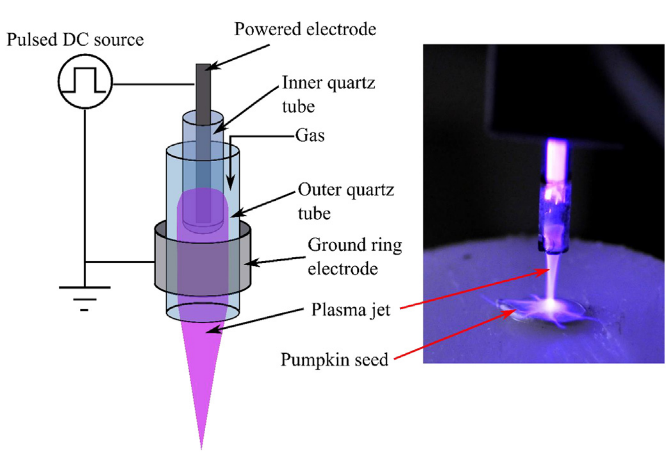


Fig. 1. Schematic diagram of CAPP treatment of seeds. (Left) Cold plasma jet and (Right) photo of a pumpkin seed in a contact with the plasma jet.

2.2. Chemicals

Hydrogen peroxide solution was purchased from *Sigma-Aldrich* (USA). Ozone test strips were purchased from Macherey-Nagel Company (Germany). These were used to determine the ozone concentration in air near the surface of a plasma ball and under the plasma jet.

2.3. Cold plasma

The CAPP source consisted of a nested pair of quartz tubes with a central tungsten rod and an outer steel ring. The rod and ring served as the powered and grounded electrodes, respectively. The outer tube (4 mm ID, 6 mm OD) was open on both ends. The inner tube (1 mm ID, 2 mm OD) had a closed end and was inserted into the outer tube. The 1 mm diameter tungsten rod was then placed inside the inner tube to rest against the sealed end. The tubes were held in place by a plastic Tee compression fitting (Swagelok NY-400-3). The grounded ring electrode was placed near the end of the outer tube. Bottled helium or argon were metered with MKS digital mass flow controllers. Both gases were flown at a rate 2 L/min in the annulus between the two tubes. The gas was ionized by the strong electric fields between the electrodes, and formed a plasma jet approximately 4 cm long measured from the exit of the outer tube (Fig. 1). The two gases were operated separately but at the same electrical conditions. The fact that both electrodes are electrically isolated from the plasma prevents arcing to the treated sample. Treatment by the jet was either via direct contact as shown in Fig. 1, or indirect with a gap. The latter kept the visible tip of the jet 1 cm from the surface of the seed. The indirect treatment prevented the conductive plasma (e.g. ions and electrons) from contacting the seed but still allowed any RONS species to affect the surface.

The plasma was powered with a high voltage pulsed DC system consisting of a Matsusada AU-10P60 10 kV DC power supply, a IXYS

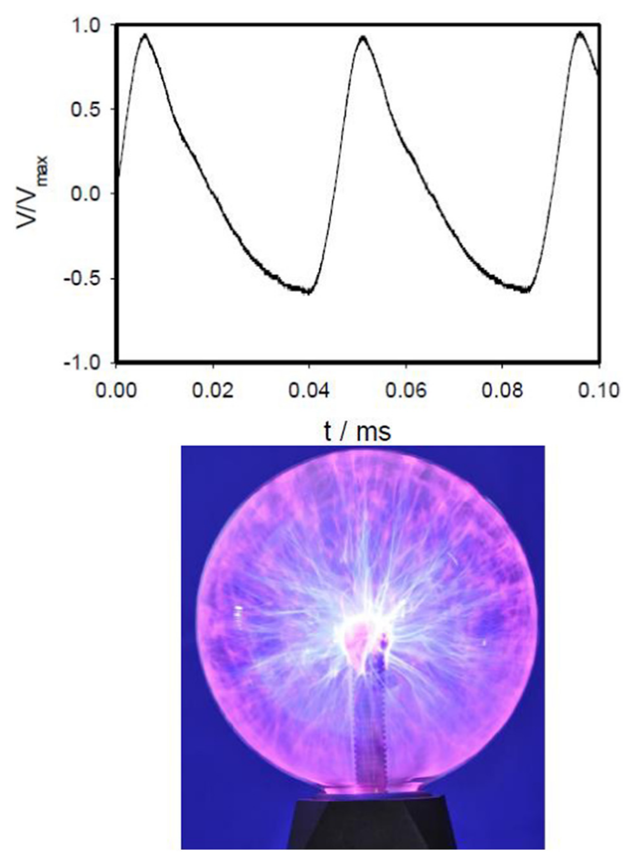


Fig. 2. The plasma ball generates electrical signals with a frequency of 21.86 kHz. The amplitude of the electrical signal 1 cm from the plasma ball was 628 V.

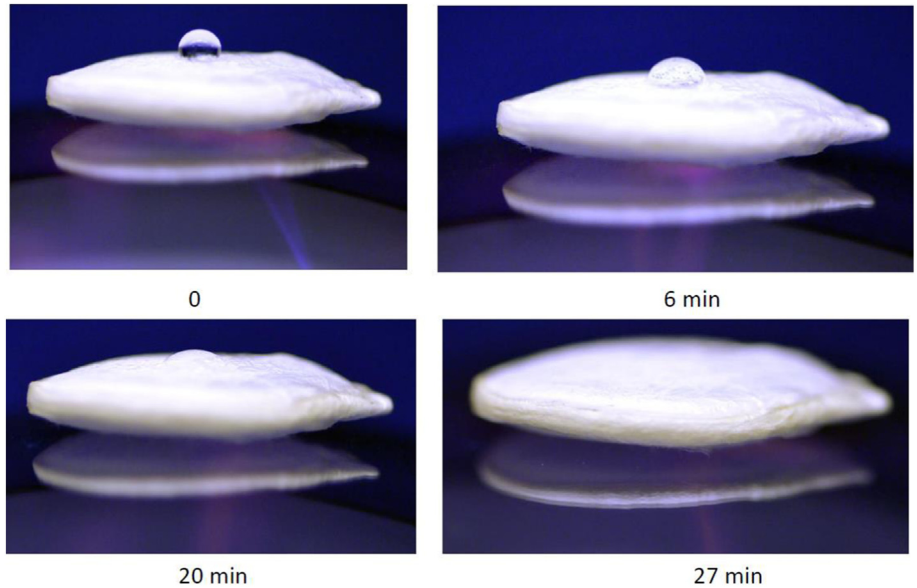


Fig. 3. The dormant pumpkin (*Cucurbita pepo* L., cv. Cinderella) seed located at the top of a plasma ball. The length of the pumpkin seed was 2 cm (mean 2.00 cm, median 2.00 cm, std. dev. 0.04 cm, std. err. 0.006 cm, n = 50). A drop of 10 μL of 3% H_2O_2 was placed in the middle of a seed. The time span until water droplet is absorbed is 27 min (Mean 26.88 min, Median 27.00 min, Std. Dev. 1.20 min, Std. Err. 0.30 min, 95% Conf. 0.65 min, 99% Conf. 0.89 min, n = 16).

PVX-4110 pulse generator, and a SRS DG-645 digital delay generator. The system was operated with an 8 kV pulse amplitude, 6 kHz pulse frequency, 1 μs pulse width, and ~70 ns pulse rise and fall time. The pulse voltage and current were measured from the pulse generator and had a square pulse shape with a slight sloping during the rising edge. The current trace had two distinct current pulses at the rising and falling fronts of the voltage with damping oscillations after the main current peaks due to both displacement and conduction currents.

A commercial plasma ball (also called globe, lamp, and sphere) was also used for electrostimulation of the seeds (Fig. 2). Plasma balls were developed by Tesla [38] and their physical properties were investigated recently [39,40]. The effects of electrical fields on vegetation have been the subject of research since the nineteenth century [41–43]. The plasma ball is an 8-in. diameter clear glass sphere filled with low-pressure noble gases such as neon or argon. The plasma is generated inside the ball by a high voltage AC signal from the central bulb. The electrical signal generated by the plasma ball is not confined by the glass sphere and propagates into the ambient air as electromagnetic interference (EMI). The EMI was measured with a PR-55 high voltage probe connected to a Tektronix MDO3024 oscilloscope. If a pumpkin seed is placed on the outer surface of the ball or near the ball, capacitive coupling can energize a high voltage load up to a few kV with a frequency of 21.86 kHz (Fig. 2). The plasma inside and the seed outside the ball act as plates of a capacitor.

2.4. Atomic force microscope

The surface of the pumpkin seed was analyzed using the Pico-Plus Atomic Force Microscope (AFM) from Molecular Imaging (now Agilent Technologies). The AFM creates a three dimensional representation of

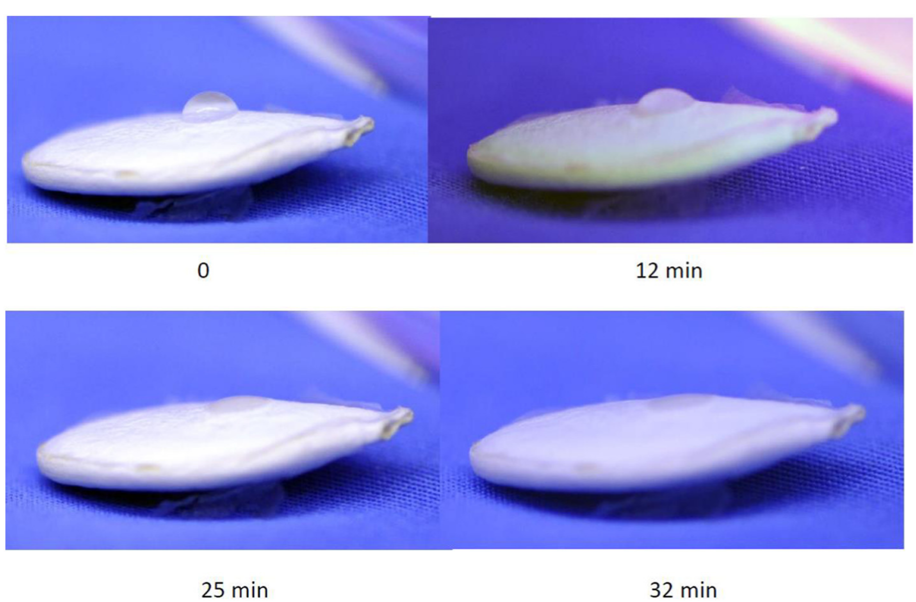


Fig. 4. The dormant pumpkin (*Cucurbita pepo* L., cv. Cinderella) seed located at 4 cm from left side of a plasma ball. The length of the pumpkin seed was 2 cm (mean 2.00 cm, median 2.00 cm, std. dev. 0.04 cm, std. err. 0.006 cm, n = 50). A drop of 10 μL of 3% H_2O_2 was placed in the middle of a seed surface. The time span until water droplet is absorbed is 31 min (Mean 31.00 min, Median 32.00 min, Std. Dev. 3.52 min, Std. Err. 0.88 min, 95% Conf. 1.88 min, 99% Conf. 2.59 min, n = 16).

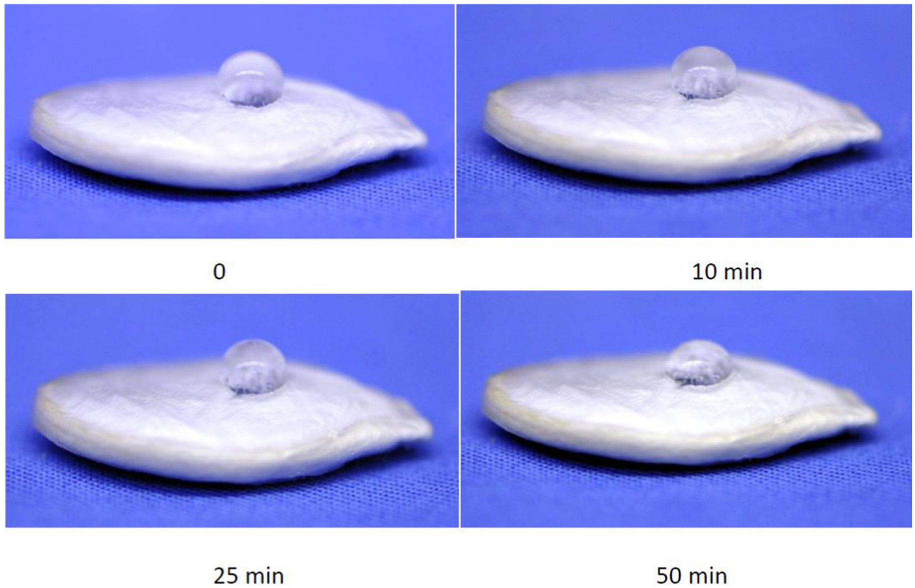


Fig. 5. A drop of 10 μL of 3% H_2O_2 was placed in the middle of a dormant pumpkin (*Cucurbita pepo* L., cv. Cinderella) seed without any treatment by a plasma ball. The length of the pumpkin seed was 2 cm (mean 2.00 cm, median 2.00 cm, std. dev. 0.04 cm, std. err. 0.006 cm, n = 50).

the sample surface by monitoring the force of interaction between the sample and a cantilever probe. AFM can obtain topographical images of pumpkin seeds in their natural state without surface damage. The pumpkin 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 topographically images of the pumpkin

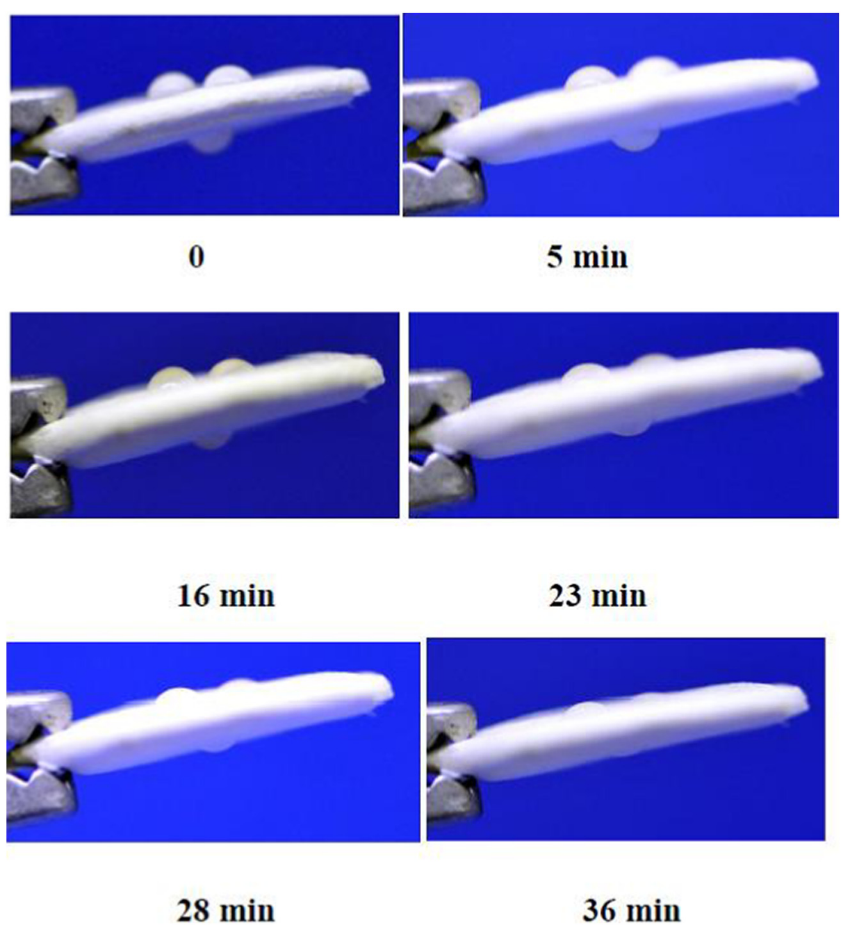


Fig. 6. Kinetics of 10 μL water drops penetration to a dormant pumpkin (*Cucurbita pepo* L., cv. Cinderella) seed coat from the top and from the bottom of the seed which was treated by He plasma jet for 1 min of each side. The length of the pumpkin seed was 2 cm (mean 2.00 cm, median 2.00 cm, std. dev. 0.04 cm, std. err. 0.006 cm, n = 50). The time span until water droplets are absorbed is 37 min (Mean 37.19 min, Median 37.00 min, Std. Dev. 1.17 min, Std. Err. 0.29 min, 95% Conf. 0.62 min, 99% Conf. 0.86 min, n = 16).

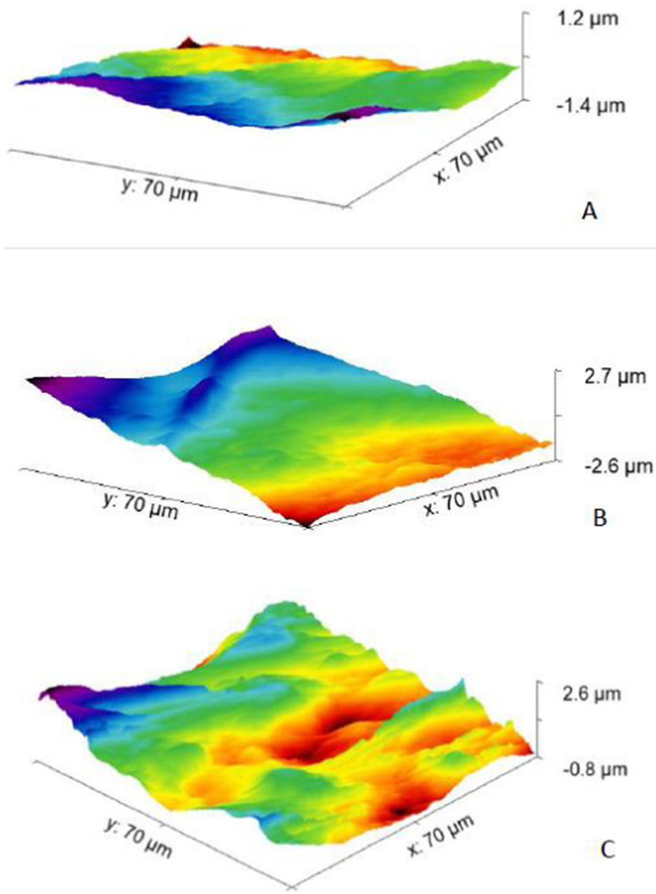


Fig. 7. AFM topographic imaging of a surface of a dormant pumpkin (*Cucurbita pepo* L., cv. Cinderella) seed covered by a coat (A), after treatment by plasma ball during 10 min (B). Panel C shows the topographic imaging of a surface of a dormant pumpkin seed without a coat treated by He plasma jet for 1 min. The surface heights are shown on the z-axis scale and the color plots in each image correspond to their respective height scales.

seeds were then analyzed. The software Gwyddion-2.51 (http://gwyddion.net/) was used as a tool for AFM data visualization and analysis.

2.5. Magnetic resonance imaging

The seeds were imaged using a 9.4 T system (BioSpec, Bruker BioSpin GmbH) equipped with a surface coil as 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 = 2500 ms, TE = 36 ms, rare facto $r = 8$, 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).

2.6. Photos

A photo camera Nikon D3x with AF-S Micro Nikkor 105 mm 1:2.8 G ED VR lens was used for the photography of seeds. All images in Figs. 3–6 were taken at the same light and distance. Photos of pumpkin seeds with water drops were taken every minute and some of them are shown in Figs. 3–6.

2.7. Statistics

The software SigmaPlot 12 (*Systat Software, Inc.*) was used for statistical analysis of experimental data. All experimental results were reproduced at least 16 times.

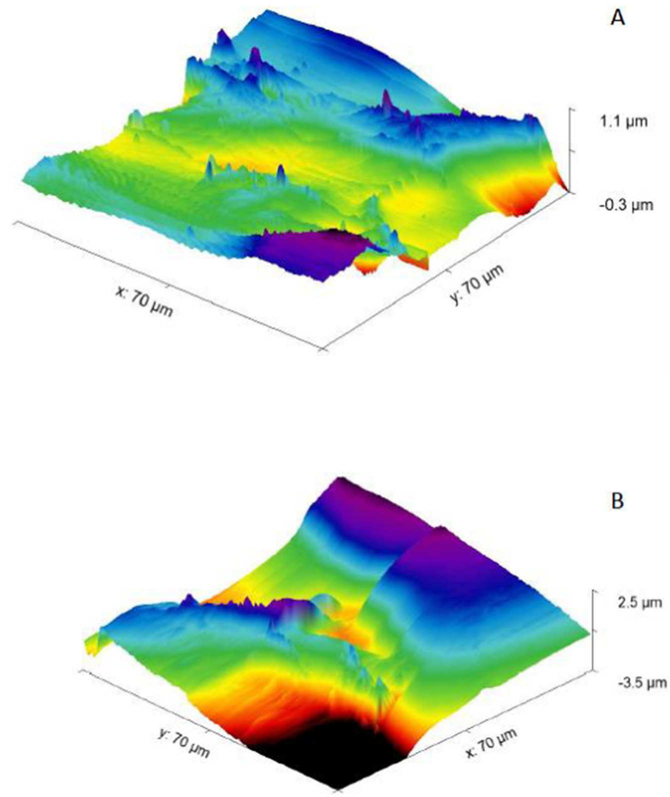


Fig. 8. AFM topographic imaging of a surface of a dormant pumpkin (*Cucurbita pepo* L., cv. Cinderella) seed covered by its coat after treatment by He (A) or Ar (B) cold plasma jet. The surface heights are shown on the z-axis scale and the color plots in each image correspond to their respective height scales.

3. Results

3.1. CAPP jet and plasma ball treatments of pumpkin seeds

The majority of RONS produced by a plasma jet are unstable and has a very short life time. The most common and relatively stable products are HNO_2, HNO_3, H_2O_2, O_3 and NO_x compounds. Because the plasma ball does not produce RONS such as OH as the cold plasma jet would, we added a small drop of 10 μL of 3% aqueous solution of H_2O_2 to the pumpkin seed as a RONS stand-in. The pumpkin seed was located on the top of the glass dome of the plasma ball (Fig. 3). The plasma ball was then turned on which led to a fast decrease in the droplet volume, indicating the liquid penetrated inside the seed. The same phenomenon exists if the pumpkin seed is located at short distance of 2–4 cm from the plasma ball (ig. 4). This effect can be caused by formation of hydrophilic pores and defects induced by electromagnetic field generated by the plasma ball. It is well known that non-thermal electromagnetic fields can induce electroporation and sterilization in bio-tissue [35]. The time span until water droplet absorption was about 4 s longer for dormant pumpkin seeds located at 4 cm away from a plasma ball compared to at the top of a plasma ball (Figs. 3 and 4).

Fig. 5 demonstrates a control experiment when a small drop of 10 μL of 3% aqueous solution of H_2O_2 was placed on a surface of a dormant pumpkin seed coat without any treatment by a plasma jet or ball. There are no changes in the shape or volume of this aqueous droplet during first 25 min. Very small changes occur after 50 min (Fig. 5). Decrease of the droplet shape and volume in Figs. 3 and 4 is not caused by evaporation of liquid phases. If a small drop of the same solution was placed on a glass Petri dish, the shape and volume of droplet stays the same over an hour without any visible changes.

To determine the types of products from the plasma which may affect the pumpkin seeds, commercial ozone test strips and UV detector

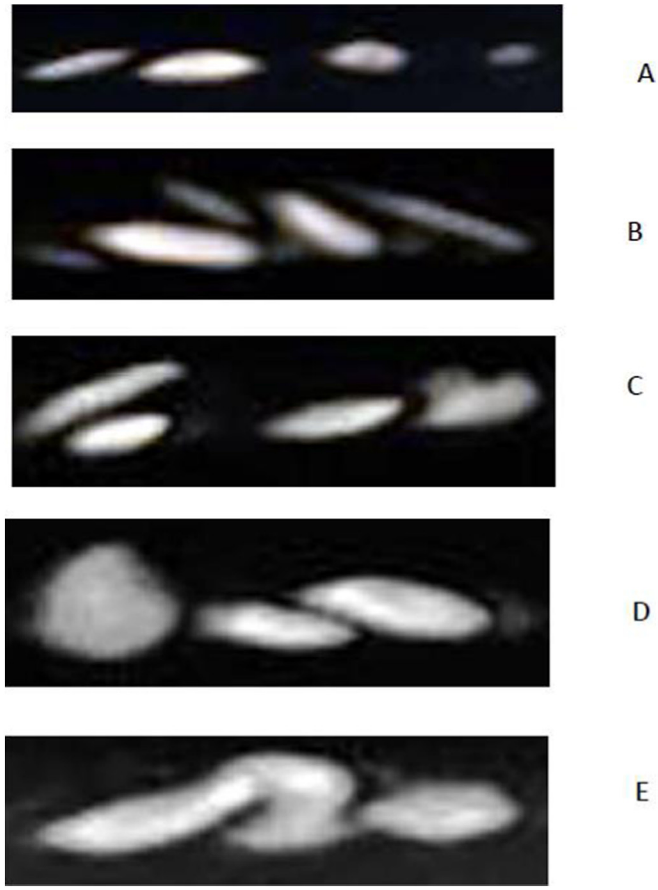


Fig. 9. MRI of pumpkin (*Cucurbita pepo* L., cv. Cinderella) dormant (A) and imbibed for 5 h (B - E) seeds. (B) Seeds untreated by plasma; (C) Seeds treated for 20 min by a plasma ball; (D) Seeds with each side treated for 1 min by Ar (D) or He (E) plasma jet. The length of the pumpkin seed was 2 cm (mean 2.00 cm, median 2.00 cm, std. dev. 0.04 cm, std. err. 0.006 cm, n = 50). The same amount of seeds (5) in a Petri dish was always used for each treatment at the same fixed measurement distance. The water in the seeds appears as the white regions seen in the figures. Thus, the overall seed sizes in (A) and (E) are the same, but (A) has much less internal water.

are used. The ozone test strips, when placed in the cold plasma jet at the same distance as the seeds for 10 min, show production of ozone by the He plasma jet in concentrations between 90 and 150 μg/m^3. While the plasma inside a plasma ball is surrounded by a glass wall and does not produce RONS outside the ball, the high frequency electromagnetic radiation does propagate outside (Fig. 2). Detection of ozone at the surface of our plasma balls by commercial ozone test strips did not show production of ozone in the air. A small amount of UV radiation penetrates through glass surface of the plasma ball. Plasma balls induce strong electromagnetic radiation (Fig. 2) which may induce electroporation of seeds (Figs. 3, 4, 6–8).

Treatment of pumpkin seeds by the helium cold plasma jet can also induce poration and corrugation of the seed coat. Both sides of the pumpkin seed were treated by the cold plasma jet for 1 min per side at a distance of 3 cm from the jet exit in direct contact mode. Formation of hydrophilic pores in a dormant pumpkin seed coat was detected with similar water drop experiments as shown in Fig. 6. Drops of pure water on the top and bottom surfaces of a plasma treated seed slowly penetrate inside the seed over 36 min. A water drop on the bottom of a seed does not fall down under the gravitational force because of balance of gravitational and capillary forces. Penetration of aqueous drops through the seed coat of a dormant pumpkin seed is induced by the difference of water potentials outside and inside the dormant seed. Formation of hydrophilic pores and surface defects in coats of pumpkin seeds, induced by cold plasma, can facilitate water transport during imbibition and germination processes.

3.2. Atomic force microscope imaging: corrugation of pumpkin seeds' surfaces by plasma

Atomic force microscopy is used to characterize the pumpkin seeds' surfaces before and after their treatment by the plasma ball electric field and direct exposure by the cold plasma jet. Others have previously studied the surfaces of cold plasma treated seeds with scanning electron microscopy (SEM) [4]. The benefit of AFM over SEM is the former readily produces a 3D topographical map and does not damage the living tissue, thus allows for potential planting of the same sample. AFM topographic images of one surface of a dormant pumpkin seed covered by a coat before and after plasma treatment are shown in Fig. 7. Dormant pumpkin seeds have a flat surface with some roughness of the seed cover (Fig. 7A). If the same seeds are placed on the surface of a plasma ball, corrugation of the pumpkin seed coat becomes visible (Fig. 7B). The plasma ball field can also lead to the formation of pores in a seed coat. Treatment with a helium cold plasma jet of the pumpkin seeds without their coats creates even further corrugation of the surface with clear spots of pore formation indicated by the dark red/black spots in the AFM map. (Fig. 7C).

Both He and Ar cold plasma jets produce very significant changes in the topography of dormant pumpkin seed (*Cucurbita pepo* L., cv. Cinderella) (Fig. 8). There is a strong corrugation of coats, formation of surface defects and possible poration of pumpkin seeds coats. The same structural effects were obtained for pumpkin *Cucurbita maxima* L., cv. Jarrahdale and *Cucurbita maxima* L. cv. Warty Goblin seeds. Though the argon plasma jet in Fig. 8B appears to have more significant surface corrugation, it is not clear whether this is always the case, or just due to random variations in the initial seed surface.

AFM is a powerful tool which can measure the topography of very small surfaces with a great degree of accuracy. The surface topography of a pumpkin seeds obtained by AFM is commonly displayed as a pseudo color plot representing the different relative heights of the surface. However, as the colors are auto scaled for each given image, they are not wholly useful for cross comparison. Thus, Figs. 7 and 8 also show the exact scales on the z-axis in μm.

3.3. Magnetic resonance imaging of pumpkin seeds

The noninvasive magnetic resonance imaging (MRI) technique allows us to obtain a spatial representation of the water distribution in seeds. MRI results of the pumpkin seeds' water content are displayed in Fig. 9. The images show the presence of water in both dormant and imbibed seeds. According to literature, dormant seeds contain 4–5% moisture [23,37]. Fig. 9A shows that dormant seeds have some inherent water content. The seeds in Fig. 9B were not treated by cold plasma, but seeds shown in other panels were treated by either the plasma ball (C), Ar plasma jet (D), or He plasma jet (E). Seeds shown in the Fig. 9B–E were placed in Petri dishes for incubation with water for 5 h after their respective treatment, or no treatment in the case of Fig. 9B. After water incubation, the surfaces of imbibed seed coats were gently dried with soft tissues and placed in dry Petri dishes before placed in the MRI. Treatment of pumpkin seeds by cold plasma before imbibing water clearly increases the aqueous content inside the coats (Fig. 9D and E). This is likely due to plasma poration and corrugation of seeds coats that enhances the water penetration into the seeds. MRI studies show enhancement of water uptake in pumpkin seeds exposed to a cold plasma jet.

3.4. Germination of pumpkin seeds

The treatment of pumpkin seeds by CAPP accelerates their germination and radicle development (Fig. 10). The efficiency of pumpkin seed germination in increasing order: not treated by plasma < plasma ball

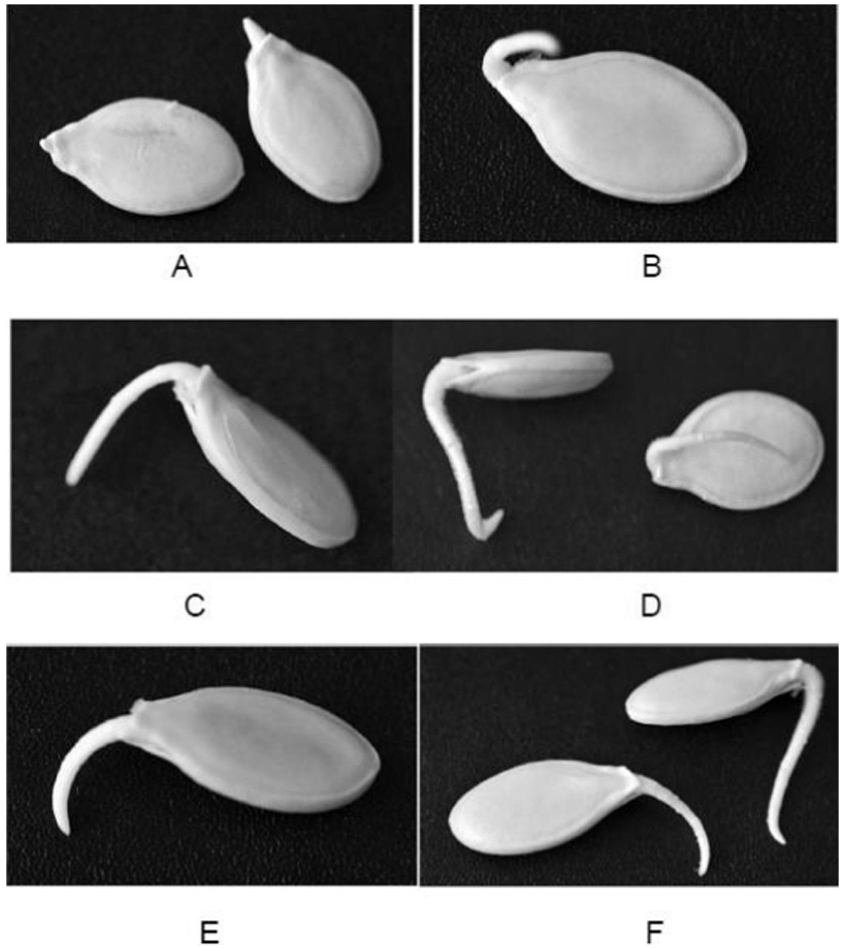


Fig. 10. Germination of imbibed pumpkin (*Cucurbita pepo* L., cv. Cinderella) seeds 3 days after incubation in water for 10 h. (A) Control; (B) Pumpkin seed was located on the plasma ball surface for 25 min; Argon (C) or He (D) plasma jet was in direct contact for one minute per side of seeds; Argon (E) or He (F) jet was in indirect contact with 1 cm gap between the plasma jet tip and the seed also for one minute. The length of the radicle was (A) 2 mm, (B) 6 mm, (C) 15 mm, (D) 20 mm 6, (E) 13 mm, (F) 19 mm. The length of the pumpkin seed was 2 cm (mean 2.00 cm, median 2.00 cm, std. dev. 0.04 cm, std. err. 0.006 cm, n = 50).

< Ar or He plasma jets. The germination of pumpkin seeds is accelerated either direct plasma jet contact or indirect contact with 1 cm gap between the plasma jet tip and seed (Fig. 10, panels C, D, E and F). (Tables 1 and 2).

3.5. Germination of pumpkin seeds by plasma jets: poration by RONS, electroporation by high frequency electromagnetic field, or UV effects?

The previous results show that CAPP jets can improve water imbibing and germination of seeds (Figs. 6, 9, 10). The AFM results show the plasma jets can produce pores in seed coats. However, the question remains if the pores are due to high frequency electromagnetic field electroporation or redox interactions with reactive oxygen and nitrogen species. Photons produced by the plasma also can improve germination of pumpkin seeds [26,27]. To try and separate these factors, a set of control experiments were done and the germination results are shown in Fig. 11. The effect of electromagnetic field can be decreased or removed if the plasma jet is contained within an electrically grounded galvanized steel mesh cage akin to a Faraday cage. The cage still allows RONS of photons to reach the seed (Fig. 11A). The UV light and RONS can be blocked by placing the pumpkin seeds inside a Petri glass dish (Fig. 11B). By placing the seeds inside a quartz cuvette, the UV light is now allowed to reach the seed as well (Fig. 11C). In both glass dish or quartz cuvette cases, the electromagnetic field still reaches the seed. Fig. 11 shows that electroporation by high frequency electromagnetic field, redox poration by RONS, and activation by UV light all accelerate germination of pumpkin seeds independently. When combined in direct contact by the plasma jet, the components combined synergistically for even faster germination of seeds (Fig. 11D).

Comparison of the water permeation through the seed coats was also done for the different control cases as shown in Fig. 12. All CAPP

Table 1
Mean, median, standard deviation, standard error, and confidence intervals for the results shown in Fig. 10.

Treatment Number	Radicle length, mm (n = 16 for all)				
	Mean	Median	Std. Dev.	Std. Err.	95%/99% Conf. Int.
A	1.63	2.00	1.02	0.26	0.55/0.75
B	6.31	6.00	1.14	0.28	0.61/0.84
C	15.35	15.00	1.32	0.32	0.68/0.94
D	20.25	20.00	1.61	0.40	0.86/1.19
E	13.13	13.00	1.06	0.27	0.59/0.81
F	18.19	19.00	2.48	0.62	1.32/1.83

Table 2
Mean, median, standard deviation, standard error, and confidence intervals for the results shown in Fig. 11.

Treatment Number	Radicle length, mm (n = 16 for all)				
	Mean	Median	Std. Dev.	Std. Err.	95%/99% Conf. Int.
A	6.65	7.00	0.89	0.22	0.48/0.66
B	3.88	4.00	0.81	0.20	0.43/0.59
C	9.63	10.00	1.09	0.27	0.58/0.80
D	28.36	29.00	3.38	0.85	1.80/2.49

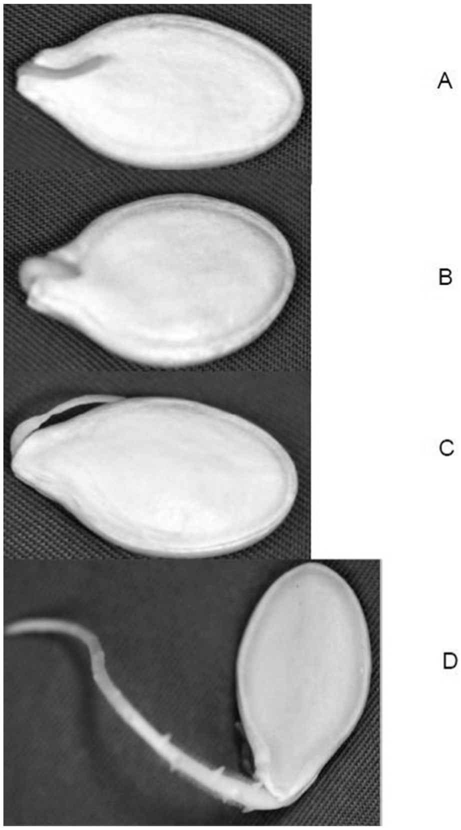


Fig. 11. Germination of imbibed pumpkin (*Cucurbita pepo* L., cv. Cinderella) seeds 3 days after incubation in water for 12 h. He plasma jet was in contact for one minute per side of seeds (A - D). The plasma jet was shielded with a grounded galvanized steel mesh (A), located inside a Petri glass dish (B) or in closed quartz cuvette (C). He the plasma jet was in direct contact for one minute per side of seeds, thus all factors combined (D). The length of the radicle was (A) 7 mm, (B) 4 mm, (C) 10 mm, (D) 29 mm. The length of the pumpkin seed was 2 cm (mean 2.00 cm, median 2.00 cm, std. dev. 0.04 cm, std. err. 0.006 cm, n = 50).

components (electromagnetic field, RONS, light) increase the rate of water permeation. A grounded galvanized steel mesh (Fig. 12B) can decrease the electrical field, but still allow RONS and UV light from the He plasma jet to reach the surface of seeds. A closed quartz cuvette with a seed inside (Fig. 12B) is permeable to light and UV and electrical field, but not permeable for RONS. A closed Petri glass dish with a seed inside (Fig. 12F) blocks UV radiation and protects seeds from RONS.

The AFM measurement showed that CAPPs induce poration and strong corrugation of pumpkin seeds coats (Figs. 7, 8). Control experiments in Fig. 13 show that RONS and photons (A), electromagnetic fields and photons (B) and electromagnetic fields without UV light and RONS can induce deformation of seed coat surfaces. Corrugation and poration by electromagnetic fields with RONS and UV photons (Fig. 13A, B) is more efficient then (Fig. 13C) electroporation by electromagnetic field only. All three components of plasma work synergistically.

4. Discussion

Cold plasma jets can induce different effects in seeds by generation of RONS, high frequency electromagnetic radiation, and UV light (Fig. 10). Cold plasma can inactivate bacteria, fungi, spores, and viruses [45,46]. Plasmas can also modify surfaces of seeds, produce pores, surface defects, accelerate water imbibing of the seeds, and accelerate germination. Plasma can also generate side effects such as modification of DNA and proteins [45], oxidation and peroxidation of bio-tissue (Scheme 1).

Redox reactions between plasma and air produce different RONS, which play different roles in biochemical processes in seeds. Nitrates and nitrites can break seed dormancy often in combination with light [27,47]. Nitric oxide NO promotes seed germination, de-etiolation, and inhibits hypocotyl and internode elongation, processes mediated by light [27,48,49].

Due to the significant differences between the plasma ball and plasma jet power sources and operating conditions, it wasn't possible to replicate the field of the plasma ball with the jet. The plasma jet is also design specifically to shield out the EMI except where the plasma actually exits. Separating the electromagnetic component from the

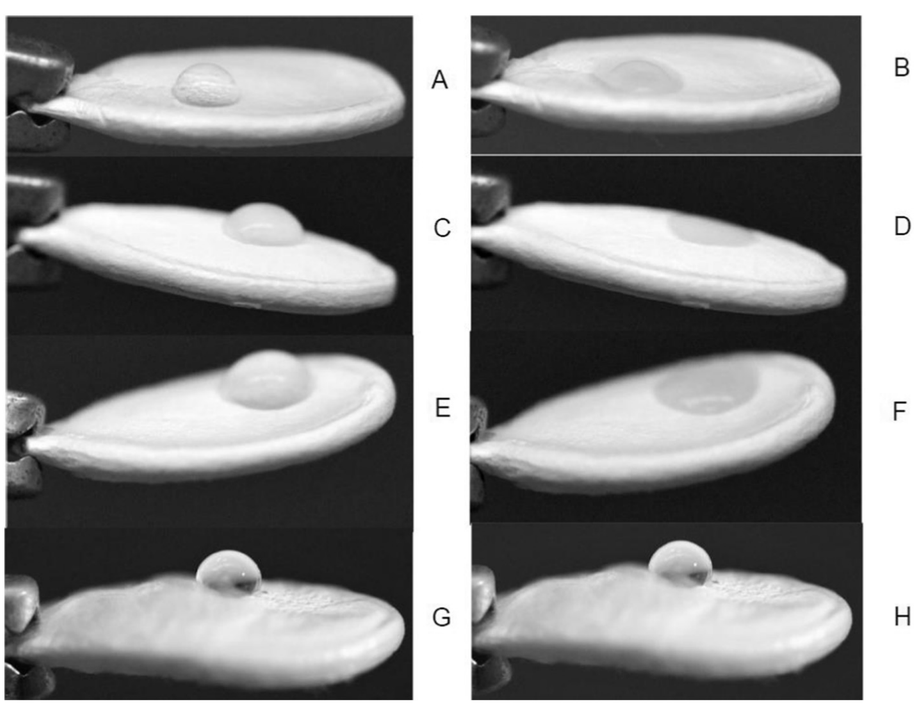


Fig. 12. Kinetics of 10 μL water drops penetration to a dormant pumpkin (*Cucurbita pepo* L., cv. Cinderella) seed coat, which was treated by He plasma jet for 1 min of each side. Photos A, C, E and G correspond to moment of drops deposition and photos B, D, F and H were taken 25 min later. Pumpkin seed was covered by a grounded galvanized steel mesh (A, B), located inside or closed quartz cuvette (C, D) or a Petri glass dish (E, F). Control experiments without plasma treatment of seeds are shown in photos (G) and (H). The length of the pumpkin seed was 2 cm (mean 2.00 cm, median 2.00 cm, std. dev. 0.04 cm, std. err. 0.006 cm, n = 50).

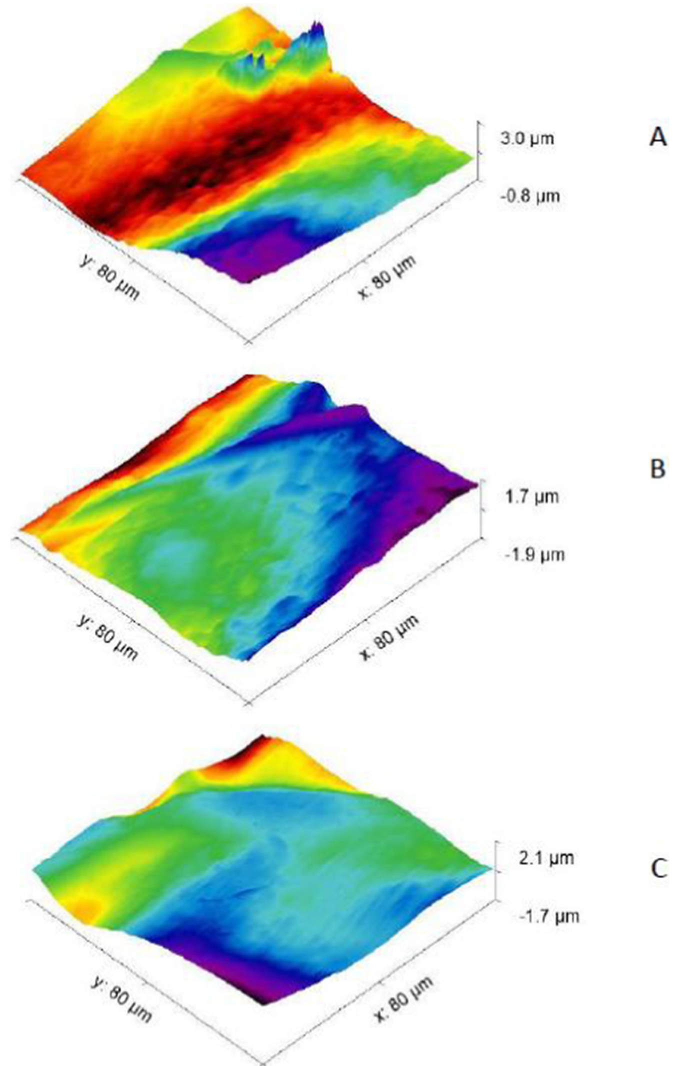
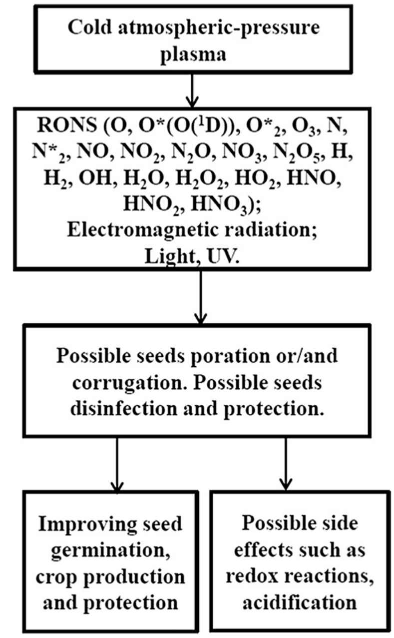


Fig. 13. AFM topographic imaging of a surface of a dormant pumpkin (*Cucurbita pepo* L., cv. Cinderella) seed covered by its coat after treatment by He plasma jet. Pumpkin seed was covered by a grounded galvanized steel mesh (A), located inside closed quartz cuvette (C) or a Petri glass dish (C). The plasma jet was in contact for one minute per side of seeds. The length of the pumpkin seed was 2 cm (mean 2.00 cm, median 2.00 cm, std. dev. 0.04 cm, std. err. 0.006 cm, n = 50). The surface heights are shown on the z-axis scale and the color plots in each image correspond to their respective height scales.



Scheme 1. Diagram of effects of cold atmospheric pressure plasma on seeds.

plasma jet would be difficult and cause interferences with electronics. Thus, the use of the plasma ball seemed a reasonable test to determine if the field alone could have an effect. The helium and argon plasma produce RONS while the plasma ball induces effects of the electric field only. Comparison of MRI and AFM for plasma and plasma ball shows that RONS from plasma jet are much more efficient in seeds poration and corrugation than the electrical field from the plasma ball.

The speed of germination and yield can be increased by CAPP treatment of pumpkin seeds. CAPP treatment of pumpkin seeds can increase wetting properties of seeds coats by decreasing the apparent contact angle between an aqueous drop and a coat surface (Figs. 3, 4). CAPP treatment of pumpkin seeds increase the roughness (Figs. 7, 8), hydrophilic properties (Figs. 3, 4, Fig. 6) and induce poration of the seed coats (Figs. 3–6). It should be noted that there is inherent variation in the speed of germination and yield percentage of seeds of the same variety. This is due to the changes in post-harvest processes, drying, storage, and age. Variability can also occur due to growing conditions and methods. Because of these variables, we can only observe trends in germination speed and yield caused by CAPP treatment and cannot derive exact improvement rates with laboratory scale tests. It should also be noted that the helium and argon plasmas appeared to have no significant difference in terms of water penetration or seed germination.

The initial absorption of water by seeds is primarily a physical process of hydration and osmosis. Water permeation across coats can occur by the partition mechanism or through pores and surface defects [50–53]. Partition transport through a thick coat to the seed can be extremely slow, thus the observed fast water transport across thick coats occurs through hydrophilic pores and surface defects. Chemical redox reactions between plasma induced RONS and seed coats can produce hydrophilic seed surfaces and generate hydrophilic pores (Figs. 5–8).

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. 6, can be done from a phase equilibrium energy analysis. In the case where three phases meet, a solid seed, aqueous water, and gaseous air, 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 the values of surface energies at all three interfaces: seed/water, seed/air, and water/air (Figs. 3–6). If the energy of interaction between the molecules of water with the seed is greater than between water with air, the aqueous phase will tend to occupy a greater area on the surface of a seed, thereby replacing the molecules of air in the same volume. The equilibrium state, corresponding to a minimum of total surface energy of the system, will be achieved when the energy gain due to replacement of air by water is compensated by expenditure of energy due to increasing of the surface area of liquid [44].

Due to the difference in water potentials inside and outside of a seed's coat, water can penetrate through a coat to a pumpkin seed. Formation of surface defects and hydrophilic pores in coats will accelerate water transport, imbibition, and germination of pumpkin seeds. Energization of ions in CAPP sheaths and boundaries can also lead to the activation of surface modifying processes of seed coats.

The imbibed seeds in their coats of *Cucurbita pepo* L., cv. Cinderella, *Cucurbita maxima* L., cv. Jarrahdale, and *Cucurbita maxima* L. cv. Warty Goblin show the same properties induced by cold plasma treatment. The plasma can also disinfect the seeds by RONS and UV radiation which can increase the percentage of germinated seeds.

Understanding mechanisms of CAPP interactions with seeds and plants could promote plasma-based technology for plant developmental control, increasing yield, growing rates and for plant protection from pathogens. Our work offers new insight into mechanisms which trigger water transport and absorbance, seed germination, and activation of metabolism by cold plasmas.

5. Conclusions

Treatment of seeds by electrically generated low-temperature plasma can accelerate hydration (Figs. 3–6), imbibition (Fig. 9), and germination (Figs. 10–12) of pumpkin seeds. Plasma-generated reactive oxygen and nitrogen species and high frequency electromagnetic field can penetrate into seed coats and modify their surface properties (Figs. 7, 8). AFM data shows that He or Ar cold plasmas produce strong corrugation of pumpkin seed coats, surface defects, and hydrophilic pores in a pumpkin seed coat. These structural deformations can enhance water uptake by seeds during the imbibing process, accelerate seed germination, and increase the growth of the seeds. Cold atmospheric pressure plasma treatment of pumpkin seeds can decrease the apparent contact angle between a water drop and a seed surface and increase wetting properties of seed surfaces. MRI studies show the acceleration of water uptake in pumpkin seeds treated by cold plasma. RONS, high frequency electromagnetic fields and photons emitted by plasma jets accelerate germination of pumpkin seeds both independently and synergistically. Our results can be used in plasma agriculture for acceleration of seed germination, increasing growth of plant seedlings, poration, and corrugation of the bio-tissue surfaces.

Disclosure of potential conflicts of interest

The authors declare no competing financial interests.

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