

Gravitational and Space Research

Reviewing Plasma Seed Treatments for Advancing Agriculture Applications on Earth and Into the Final Frontier

Annie Meier¹, Deborah Essumang¹, Mary Hummerick², Christina Johnson³, Mirielle Kruger¹, Gioia Massa¹, Kenneth Engeling¹

¹NASA Exploration Research and Technology Office, Kennedy Space Center, FL 32899; ²AECOM Management Services, Inc., Kennedy Space Center, FL 32899; ³NASA Postdoctoral Program, Universities Space Research Association, Kennedy Space Center, FL, United States;

Abstract

With benefits such as environmentally safe treatment methods to stimulate growth, to increase plant yield, and improve disinfection efficiency, literature on the field of plasma treatment of seeds is growing. Generalized variables and success criteria have not been well correlated between studies, so this review paper serves to connect plasma and agriculture technologies to coordinate future efforts in this growing area of research. The authors have particular interest due to space agriculture, where seeds are sanitized before being sent into space for crop production. In order to supply a spectrum of nutritional needs, it is necessary to provide a variety of crops and ensure biological decontamination before the seeds are being sent into space. Traditional seed sanitization methods are not viable for all seed types, so exploration of other options is needed to expand the astronaut diet on long-duration space missions. This review paper brings together the current state-of-the-art reported literature to aide in understanding plasma seed application apparatus, seed or crop performance pertaining to germination, growth, water interactions, inactivation of bacteria, and surface sanitization results. These recent works include evolving research themes for potential seed treatment sanitization processes for various seed types to ensure the viability of plants for future growth in microgravity crop production systems.

Keywords

Plasma for agriculture • Plasma treatment of seeds • Space agriculture • Space crop sanitization • Seed sanitization • Seed vitality

INTRODUCTION

When plants are grown during a spaceflight to supplement the astronaut's diet, plants that are safe to eat are preferred for production. Pathogens are a concern in this process, and we go to great lengths to remove potential pathogens from the plant growth hardware, equipment, and seeds themselves prior to packing for a launch. Seed sanitization is an important process in the agriculture industry to prevent the spread of plant disease and reduce possible contamination on crops. Traditional spaceflight seed sanitization treatment methods include alcohol soaking and chemical gas fumigation, which can have harmful effects on the environment and human health. Earth-based sanitation methods also include the use of chlorine (Filatova et al., 2013; Jo et al., 2014). For the same reasons that these methods are not optimal for crop production in Earth, these methods are also not practical for growing plants during spaceflight. Since some of these traditional methods are not viable for plant seeds and are ecologically unsafe, it is important to explore other alternatives for sanitizing. A new promising technological approach for seed sanitization is through the application of plasma. Plasma is formed when gases are excited with a high temperature or high electric fields, whereby free electrons and ions are generated. These reactive ions can

influence bacteria inactivation on the seed surface (Ono et al., 2017; Laroussi, 2005; Šerá and Šerý, 2018; Šimončicová et al., 2019; Kim et al., 2017; Nishioka et al., 2016; Butscher et al., 2016; Mitra et al., 2014; Selcuk et al., 2008; Feizollahi et al., 2020; Ito et al., 2018; Bafail et al., 2018; Randeniya and Groot, 2015; Anna Zahoranová et al., 2018; Butscher et al., 2016; Adhikari et al., 2020; Pérez-Pizá et al., 2019). Plasma has been historically used for decontamination of surfaces in many arenas, including harvested crops such as grains and legumes. Decades of research has evolved the use of plasma as more than just an agent for decontamination of surface crops. It has been demonstrated that certain types of plasma do not produce harmful effects to the environment and human health, and plasma treatment can also change seed properties such as water absorption capacity, germination rate, growth rate, and increased seed metabolism due to increased free radicals on the seed surface. With such promising additional benefits besides sanitization, plasma applications to seeds can improve crop vitality and productivity, which makes it a promising avenue of research for an application to aide in the global agriculture food crisis (Kitazaki et al., 2014; Puač et al., 2018). With benefits such as environmentally safe treatment methods to stimulate growth, increased plant yield, and improved disinfection of seeds,

[†]Corresponding author: Kenneth Engeling
E-mail: kenneth.engeling@nasa.gov

literature on the field of plasma treatment of seeds is growing rapidly, and understanding these variables and success criteria has not been well correlated. The research ranges far and wide from pathogenic microorganism inactivation on seeds (which saves time as compared to the traditional methods which may have toxic reagents), hydrophilization of organic surfaces (which has advantages for wettability), and adhesion without impacting bulk properties (Shapira et al., 2017). This paper reviews the use of plasma technologies to sanitize seeds and surveys the literature to coordinate future efficient efforts in this hopeful research field.

Space agriculture investigations are underway on the International Space Station (ISS), and seeds are sanitized before being sent into space for crop production (Massa et al., 2020). Since traditional methods are not viable for all seed types, it is important to explore other options in order to expand the astronaut diet on long-duration space missions. The traditional methods may be time consuming, and seeds can only be treated in small batches. For long-duration space missions, the ability to grow crops reduces the storage and launch costs of astronaut food requirements, but in order to supply a wide spectrum of nutritional needs, it is necessary to bring a variety of crops and ensure biological decontamination before the seeds are being sent to the ISS and beyond. Further studies are needed to be performed in order to obtain proper experimental procedures for the various seed types and ensure the viability of plants for future growth in microgravity crop production systems. Plasma seed applications have included crops such as corn (Ahn et al., 2019; Anna Zahoranová et al., 2018), pepper (Shapira et al., 2017; Shapira et al., 2018; Mošovská et al., 2018), mimosa (da Silva et al., 2017), soybean (Sinegovskaya et al., 2019; Ling et al., 2015; A. Zahoranová et al., 2016; Švubová et al., 2021), cabbage (Ono et al., 2017), wheat (Guo et al., 2018; Dobrin et al., 2015; Kordas et al., 2015; Zahoranová et al., 2016; Filatova et al., 2009; Selcuk et al., 2008; Feizollahi et al., 2020), lentil (Shapira et al., 2018), rice (Khamsen et al., n.d.; Jo et al., 2014), spinach (Ji et al., 2016), broccoli (Nishioka et al., 2016; Kim et al., 2017), quinoa (Gómez-Ramírez et al., 2017), radish (Matra, 2016; Puligundla et al., 2017), peanut (Li et al., 2016), tomato (Măgureanu et al., 2018), pea (Tomeková et al., 2020; Stolárik et al., 2015; Švubová et al., 2020), chickpea (Mitra et al., 2014), sprouts (Butscher et al., 2016; Waskow et al., 2018), and cotton (de Groot et al., 2018; Wang et al., 2017) to name a few. Different types of plasma sources include radiofrequency (RF) discharges including inductive or capacitive types, alternating current (AC) or direct current (DC) plasma jets, dielectric barrier discharge (DBD), packed bed dielectric barrier discharge (PBDBD), glow discharge plasma, and diffuse coplanar surface barrier discharge (DCSBD).

Several reviews have been performed on plasma applications which investigated non-thermal plasmas for food production

and crop yield applications (Bourke et al., 2018; Randeniya and Groot, 2015; Ranieri et al., 2021; Attri et al., 2020); fertilization (Ranieri et al., 2021); seeds, dry fruits, and grains (Šerá and Šerý, 2018); fungi treatment, food safety, plasma medicine, and animal or bacteria breeding (Šimončicová et al., 2019); crop stress (Song et al., 2020); biomedical (standalone bacteria) applications (Laroussi, 2005); plant disease (Adhikari et al., 2020); and biochemical and molecular factors (Waskow et al., 2021; Attri et al., 2020). Several dozen patents have also emerged in the topic of using discharge plasma on plant seeds to induce seed activity (Attri et al., 2020). The early stages of the successful research investigations are laying down a foundation in order to optimize the process and also focus toward scaled up or commercial applications. Despite the groundbreaking concepts and positive results, there is still work to be done. In this review, literature was surveyed for plasma seed sanitization methods and other treatment effects to understand the consistencies and variables in the current state-of-the-art research. The interesting synergy between botany and plasma physics is somewhat convoluted—in this research area, agriculture and crop production scientists tend to focus on posttreatment results of seed treatment, often lacking the depth of plasma physics, whereas plasma physicists focus on agricultural advantages, which sometimes minimizes the thoroughness of botany. It is intriguing to find this application join the two fields together, and this paper suggests ways in which to improve the relationship of the fields in further studies for coordination in effective and thorough results. This review paper is set up in such a way to first discuss the experimental apparatus and setup of the research or technology according to the plasma application (when available). Next, the performance results of germination and growth are reviewed, followed by water interactions, analytical chemistry, and emission analysis of the plasma seed treatment (i.e., scanning electron microscopy (SEM)/x-ray photoelectron spectroscopy (XPS)/ Fourier-transform infrared spectroscopy (FTIR)/optical and emission spectroscopy (OES), and finally, microorganism/bacteria inactivation and sanitization results are presented. This literature review was used as a basis for understanding the current state of the art in the field of plasma seed applications, as well as optimizing the seed/plasma interface with the three plasma treatments being used at NASA's Kennedy Space Center (KSC): AC DBD, DC atmospheric plasma jet, and RF subatmospheric plasma.

EXPERIMENTAL SETUPS

The general process of treatment and analysis with plasma and seed applications is displayed in Figure 1. Performance benefits and sanitization benefits are two major foci in the results within the literature. The general approach is to perform

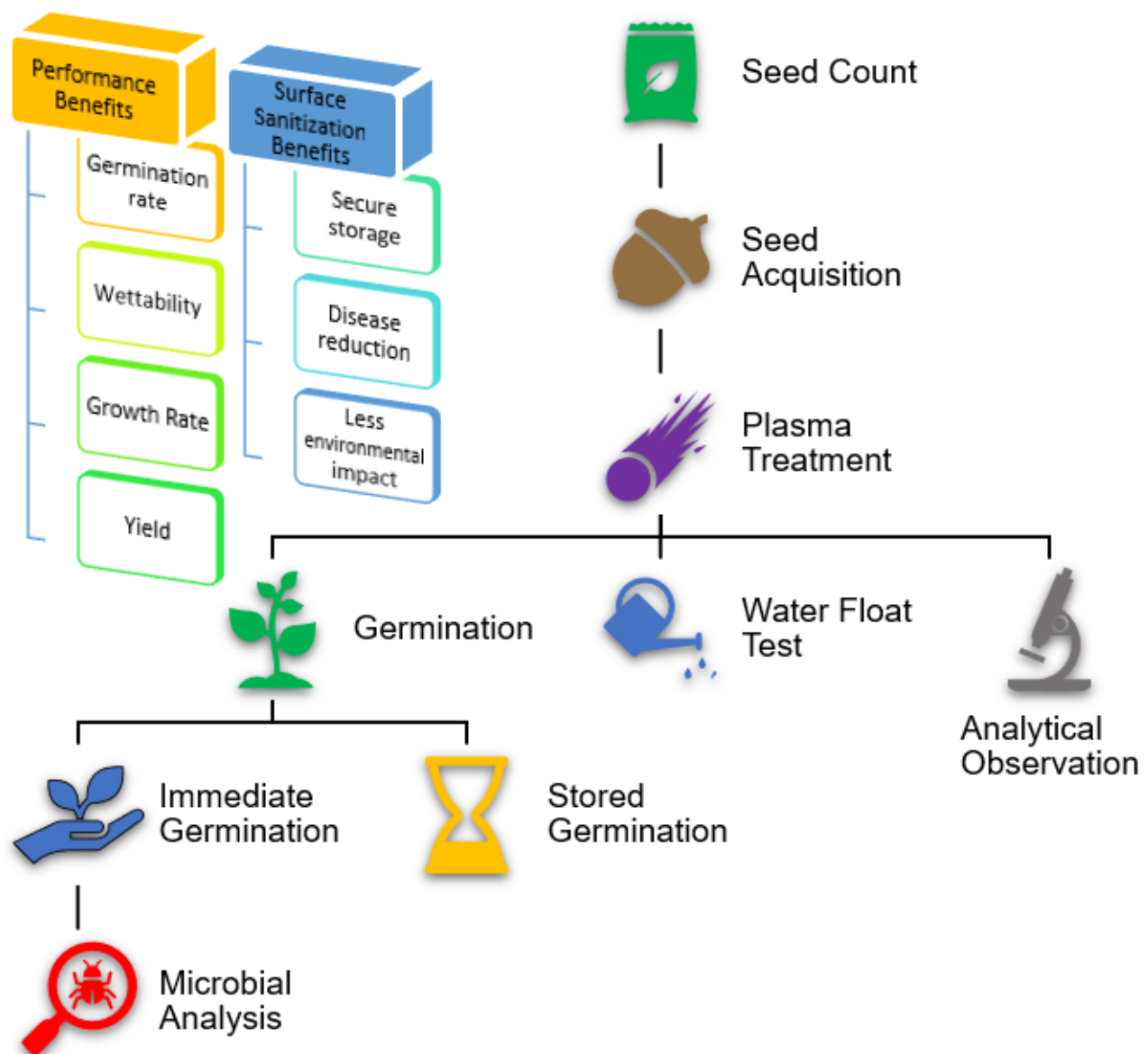


Figure 1. Process involved in plasma seed treatment, along with performance and surface sanitization benefits that are implied by data from literature review.

acquisition of seeds and baseline a general control analysis prior to the plasma treatment of the seeds. Each control seed and plasma-treated seed should undergo identical analysis for consideration of the plasma effects. If plasma causes a temperature increase in microbial reduction studies, then the control should also show the same temperature profile. In the literature surveyed, plasma sources varied greatly. RF plasma is diffusive and has lower power, which provides a non-thermal, uniform treatment of the seed via diffusion processes of the plasma. RF plasma is typically generated

within a vacuum system at partial pressures with controlled environments. Plasma jets involve producing a plasma via a power source and then transporting the plasma to a target area via a gas flow. Standard AC or DC discharges include high voltage electrodes and may use a continuous gas supply to control reaction mechanisms or cooling. Common plasma carrier gases include air (nitrogen (N_2) and oxygen (O_2)), argon (Ar), helium (He), and carbon dioxide (CO_2). Air plasma is the most economical one compared with other isolated gas supply options. Electrode configuration within

chambers is important for activation of large volume ions for sanitization. AC or DC plasma discharges typically produce thermal energy which could be detrimental to the viability of the seed. In order to mitigate this, dielectric barriers (insulating materials) are placed between electrodes to limit current in the plasma and distribute it among the dielectric material. This leads to the “non-thermal” or “cold plasma” which has a more uniform treatment that is desired for many of the aforementioned applications. AC/DC sources may be pulsed for durations on the microsecond or nanosecond timescales, limiting the amount of power delivered per second and therefore mitigating thermal effects. Pulsing does change the relative densities of plasma species, which allows for further plasma manipulation during treatments.

After plasma treatment, seeds were typically tested for germination and water uptake (a water float test or contact angle test), and analytical observations were taken. Analytical tools such as SEM with energy-dispersive X-ray spectroscopy (EDS), XPS, FTIR, and OES were used to examine the physical and chemical changes of seed surfaces, plasma emission properties, seed coat properties, and chemical reactions that impacted further growth performance. FTIR and OES have been used to observe in situ reactions and formations, especially C–H (methyl) and (–OH) hydroxyl radicals generated between plasmas during and immediately following plasma treatments. The investigations from this literature survey have typically targeted pathogenic bacteria and fungi such as *Salmonella* or *E. coli* (Butscher et al., 2016), *Xanthomonas campestris* pv. *Campestris* (Nishioka et al., 2016), *Gibberella fujikuroi* (Jo et al., 2014), *S. aureus* and *L. monocytogenes* (Kim et al., 2017), and *Bacillus*, *Mucor*, *Fusarium*, and *Alternaria*, which cause plant disease (Takemura et al., 2014). Wettability was typically studied in parallel with germination because germination first begins with rapid initial water uptake on the surface. Germination performance before and after storage is an important feature but not always studied. For spaceflight seed supply, germination of seeds after storage which underwent plasma treatment will be important as the seeds may be sitting dormant undergoing launch preparation and spacecraft operation logistics for many days/weeks/months and even years prior to use. Plants on the ISS have been grown from seeds that have been stored for up to 2.5 years, and for a Mars mission, seeds may be sent in advance of astronauts, which would also be on the order of years. This will be an important factor that is considered in the work for NASA sanitization. Microbial analysis is important to perform on seeds, especially those that undergo successful germination. Even if the plasma treatment damages the seed and prevents germination, data collected in this field are important to understand phenomena or mechanisms that may be hindering germination induced by the plasma treatment.

The artificial plasma generated in these laboratory environments

was produced by using a variety of creative mechanisms and styles and is discussed in detail in this section. The key design parameters noted in the majority of literature include two main categories: the seed and the plasma system. For seeds, the seed type, volume, and apparatus setup/support material are key parameters; for plasma, the plasma discharge or formation configuration, plasma treatment time, available gas species for plasma ionization, and power are key parameters. The design of the plasma treatment setup generally includes a housing or platform for the seed, a plasma chamber, and a mechanism for gas and seed exposure to maximize the exposure of seed surface. The seed holder/platform material varies widely. In general, the seeds need to rest on a chemically inert or high-temperature integrity material that will not switch off the gas supply or impact the chemical interaction during plasma treatment. Therefore, glass or ceramic is the common and preferred seed holder material. For some plasma systems, heat and pressure are important design variables, as heat can damage a seed coat and reduce viability. Additional experimental variables to consider include the following: plasma power source, system pressure, thermal vs. non-thermal plasma, electrode configuration, and degree of ionization. Plasma treatment times are typically on the order of pulsed nanoseconds up to many hours. Since this is a fairly new area of research, it is understandable that the plasma treatment time has such a high range in order to understand the impacts. Volume DBD and DCSBD (surface) plasma treatments were exclusively reported in atmospheric pressure and almost all in air. Pulsed DBD-style discharges and variants of them are classified to be low-temperature (nonequilibrium) plasma, which make them ideal for direct seed treatments. Low-temperature plasma means that energy is mostly transferred to electrons as opposed to ions. The electrons therefore have high energy so they can generate plasma chemical reactions, reactive components of a plasma, and radiation with lower gas temperatures that are closer to room temperature. There are additional efforts to create uniform discharges using nanosecond pulses and certain gas mixtures (Brandenburg, 2018; Ollegott et al., 2020). Typically, DBDs are filamentary and localized in formation but are seen to be more uniform in time-averaged treatments. RF plasma treatments were exclusively reported at a rough vacuum pressure with various carrier gases, and a common exposure was observed at a frequency of 13.56 MHz. This frequency is common in plasma applications as it is a selection of the radio spectrum reserved internationally for industrial, scientific, and medical purposes, limiting any interference with telecommunication systems. Once the plasma treatment was performed, many authors had various agendas or focus areas to report results on: germination, growth of the seedling, sterilization, and inhibition/inactivation of microbes on the seed surface. Table 1 displays the experimental parameters

Table 1. Experimental parameters of plasma agriculture experiments.

Reference	Seed		Carrier gas		Experimental parameter			Plasma		Primary focus of investigation
	Type	Amount	Type	Flow rate	Pressure	Seed holder material type	Treatment time	Type	Power	Indication of success (\pm /0)
(Filatova et al., 2013)	Spring wheat (<i>Triticum aestivum</i> L.), blue lupine (<i>Lupinus angustifolius</i>), and maize (<i>Zea mays</i> L.)	(1) 50 (2) 60	Air	N/A	(1) 0.5 Torr (2) 0.3–0.5 Torr	Petri dish	(1) 2.5 min, 5 min, 8 min, 10 min (2) 1 min, 5 min, 7 min, 10 min, 20 min	RF	(1) 5.28 MHz; 0.2–0.6 W/cm ² (2) 13.56 MHz; 50 W, 100 W, and 200 W	Germination (+); Seedling health (\pm) treatment dependent; microorganisms (\pm) treatment dependent
(Shapira et al., 2018)	Lentil (<i>Lens culinaris</i>), pepper (<i>Capsicum annuum</i>)	2 (1 pair)	Air	N/A	0.5 Torr	Silk wires inside a capacitor	60 s	RF	Frequency: 13.56 MHz; RF discharge: 18 W; voltage: 6 kV	Surface charge density and kinetics (+); wettability: (+)
(Filatova et al., 2009)	Grain crops (rye, wheat, barley), legumes (peas and narrow-leaved lupin), and aster	100	Air	N/A	0.3–0.7 Torr	Two parallel round Cu electrodes	7 min, 15 min, and 30 min	RF	5.28 MHz; RF discharge: 5.28 MHz; power: 0.9 W/cm ³	Germination (+); pathogenic microbes (+) (<i>Escherichia coli</i> ATCC 8739, <i>Staphylococcus aureus</i> ATCC 6538, <i>Bacillus subtilis</i> ATCC 6633)
(Ling et al., 2015)	Soybean (<i>Glycine max</i> (L.) Merr)	N/A	He	N/A	1.1 Torr	Polar plates	15 s	RF	0 W, 60 W, 80 W, 100 W, and 120 W	Germination (+); growth (+); water uptake (+); soluble sugar and protein content (+)
(Li et al., 2016)	Peanut	N/A	He	N/A	1.1 Torr	Conveyer belt	15 s	RF	60–140 W; 13.56 MHz	Germination (+); yield (+); growth (+)
(Ono et al., 2017)	Cabbage	1,000	(1) Air, (2) O ₂	N/A	0.45 Torr	Plastic pot	0–3 h	RF	13.56 MHz, 100 W	Inactivation of bacteria on plant seed (+)
(Shapira et al., 2017)	Pepper, lentil	N/A	N/A	N/A	0.5 Torr	N/A	60 s	RF	13.56 MHz, 18 W	Seed surface charging (0)
(Selcuk et al., 2008)	Wheat, barley, oats, lentil	1–8 g	Air and SF ₆	N/A	500 mTorr	Quartz tube	30 s to 30 min	Low pressure cold plasma	1 kHz, 300 W, 20 kV	Microbial reduction (+); germination (+)
(Ahn et al., 019)	Yellow dent corn hybrid	(1) 1,500 (2) 120; 1,512 total (3) N/A (4) 1,500	(1) N ₂ (2) He + N ₂ (3) He (4) He + air	(1) N/A (2) 15 + 3 LPM (3) 10 LPM (4) 10 + 5 LPM	(1) 100 mTorr, 10 ⁻⁶ Torr (2) Atm. (3) Atm. (4) Atm.	(1) Mesh plate (2) Al mesh plate (3) Plate (4) Al mesh plate	(1) 2 min., 10 min (2) 3 s (3) 10 s (4) 10 min	(1) RF (2) MW (3) DBD (4) MW	(1) 800 W, 13.56 MHz (2) 500 W (3) 15 kV; 35 kHz (4) 800 W	Seed growth (0); germination (0); yield (0)
(Sinegovskaya et al., 2019)	Soybean	N/A	(1) Air (2) Ar + air	N/A	Atm.	N/A	N/A	(1) DBD (2) MW jet	(1) 28–32 kHz, 6–12 kV, AC (2) 2.45 GHz	Germination (\pm) treatment dependent

Table 1. Experimental parameters of plasma agriculture experiments.

Reference	Seed		Carrier gas		Experimental parameter			Plasma		Primary focus of investigation
	Type	Amount	Type	Flow rate	Pressure	Seed holder material type	Treatment time	Type	Power	Indication of success ($\pm/0$)
(Ji et al., 2016)	Spinach (<i>Spinacia oleracea</i> (L.))	(1) 50 (2) 25	(1) Air + N ₂ (2) N/A	(1) 1.5 LPM (2) N/A	Atm.	(1) Petri dish (2) Wire gauze	30 s, 1 min, 3 min	(1) DBD (2) Pulsed	N/A	Germination (\pm); growth (\pm); treatment dependent
(Gómez-Ramírez et al., 2017)	Quinoa	N/A	Air	(1) N/A (2) 0.005 LPM	(1) 375 Torr (2) 0.08 Torr	(1) Quartz plate (2) N/A	10 s, 30 s, 60 s, 180 s, 900 s	(1) DBD (2) RF	(1) 6.4 W (2) 15 W, 13.56 MHz	Germination (\pm); treatment dependent; surface chemistry (+)
(de Groot et al., 2018)	Cotton	350	(1) Air (2) Ar	1 LPM	Atm.	Borosilicate glass cylinder	(1) 0 min, 3 min, 27 min (2) 81 min	DBD	N/A	Germination (+); water uptake (+)
(Guo et al., 2018)	Wheat	50	Air	1.5 LPM	Atm.	Wire netting in a plexiglass cylinder	4 min	DBD	Discharge voltage: 0.0 kV, 9 kV, 11 kV, 13 kV, 15 kV, and 17 kV, 50 Hz	Vitality (+); growth (+); water uptake (+)
(Kitazaki et al., 2014)	Radish sprouts	10 per line	Air	N/A	Atm.	Glass plate	180 s	DBD	10 kHz AC. P2P discharge voltage and current 9.2 kV/0.2 A. Discharge power: 1.49 W/cm ²	Growth (+); NO _x and O ₃ emissions (0)
(Wang et al., 2017)	Cotton	120 g	Air N ₂	1 SLPM	Atm.	Needle-plate structure	3 min, 9 min, 27 min	DBD	19 kV, 1 kHz AC	Seed spectral characteristics (0); water uptake (+)
(Khamseen et al., n.d.)	Rice	25	Air, air + Ar	N/A	Atm.	Glass plate	15 s, 30 s, 45 s, 1 min, 2 min	DBD	N/A	Sterilization(+); germination (+); water uptake (+)
(da Silva et al., 2017)	Mimosa caesalpiniaefolia Benth	100	N/A	N/A	Atm.	Glass tubes, metal mesh screen	3 min, 9 min, 15 min	DBD	17.5 kV, 990 Hz	Seed wettability (+); imbibition (+); germination (+)
(Hayashi et al., 2014)	Rice	N/A	Air	N/A	Atm.	Dish (unknown material)	20 min	DBD	10 kHz	Surface sterilization of seed and fruit surfaces (+)
(Dobrin et al., 2015)	Wheat	105	N/A	N/A	Atm.	Glass plate	5 min, 15 min, 30 min	DBD	2.7 W, 15 kV, 50 Hz	Germination (+); water absorption (+); root length (+)
(Pérez-Pizá et al., 2019)	Soybean (<i>Glycine max</i> (L.) Merrill)	500	(1) O ₂ (2) N ₂	6 NL min ⁻¹	Atm.	N/A	60–180 s	DBD	50 Hz, 0–25 kV, AC	Pathogenic reduction (+); plant growth (+)
(Butscher et al., 2016)	Sprout seeds: onion (<i>Allium cepa</i>), radish (<i>Raphanus sativus</i>), cress (<i>Lepidium sativum</i>), alfalfa (<i>Medicago sativa</i>)	10 g	Ar	5.6 NL min ⁻¹	Atm.	PC	500 ns	DBD	2.5–10 kHz, 6–10 kV	Germination (+); microbial inactivation (+) (<i>Salmonella</i> and <i>E. coli</i>)

Continued **Table 1.** Experimental parameters of plasma agriculture experiments.

Reference	Seed		Carrier gas		Experimental parameter			Plasma		Primary focus of investigation
	Type	Amount	Type	Flow rate	Pressure	Seed holder material type	Treatment time	Type	Power	Indication of success (\pm /0)
(Nishioka et al., 2016)	Brassicaceous (<i>Brassica campestris</i> var. <i>amplexicaulis</i>)	20	Air	0.5–1 LPM	10.7–16.0 kPa	Mesh sheet, unknown material	0 min, 5 min, 10 min, 20 min, 40 min	DBD	AC, 10 kHz, 2.5–5.5 kV	Disinfection and DNA of pathogen (+) (<i>Xanthomonas campestris</i> pv. <i>Campestris</i>)
(Jo et al., 2014)	Rice (<i>Oryza sativa</i> L.)	3–5	Air	N/A	Atm.	Aluminum holder	0 min, 0.5 min, 1 min, 2 min, 3 min	DBD	30 kV, 22 kHz, 3 W dishcharge	Fungal pathogen reduction (+) (<i>Gibberella fujikuroi</i> and <i>cnoidia</i>)
(Mitra et al., 2014)	Chickpea (<i>Cicer arietinum</i>)	NA	Air	N/A	Atm.	Polyoxy-methylene-copolymer	0.5–5 min	DBD	17 kV _{pp} , 5 kV _{pp} , 0 kV _{pp}	Germination (+); microbial reduction (+)
(Feizollahi et al., 2020)	Barley grain	11–12	Air	N/A	Atm.	Plastic cup	0 min, 2 min, 4 min, 6 min, 8 min, 10 min	DBD	0–34 kV; 3,500 Hz; duty cycle 70%; 1 Amp; 300 W	Microbial reduction of DON (+); germination (+)
(Kordas et al., 2015)	Winter wheat grain	200	Air	N/A	Atm.	Packed bed	3 s, 10 s, 30 s	PBDBD	100 Hz, 83 kHz, 8 kV (AC)	Fungus colonization reduction (+)
(Anna Zahoranová et al., 2018)	Maize (<i>Zea mays</i> L; cv. <i>Ronaldinio</i>)	200–250	Air	N/A	Atm.	Ceramic plate	30–300 s	DCSBD	14 kHz, up to 20 kV, 400 W, AC	Inhibition of surface microorganisms (+); seedling growth (+/0) treatment dependent
(A. Zahoranová et al., 2016)	Wheat (<i>Triticum aestivum</i> L. cv. <i>Eva</i>)	100–300	Air	N/A	Atm.	Ceramic plate	30–300 s	DCSBD	14 kHz, up to 20 kV, 400 W, AC	Inhibition of surface microorganisms (+); germination (+); water uptake (+)
(Štěpánová et al., 2018)	Cucumber (<i>Cucumis sativus</i> L.), Pepper (<i>Capsicum annuum</i> L.)	100	Air	N/A	Atm.	Ceramic	20–50 s 4–12 s	DCSBD	400 W, 20 kV, 15 kHz AC	Germination (+); Pathogenic microbes (+) (<i>Didymella lycopersici</i> spores)
(Waskow et al., 2018)	Lentil	1 g	Air	N/A	Atm.	Alumina ceramic plate	0–10 min	DCSBD	400 W	Germination (+); microbial inactivation (+) (several strains of bacteria and fungi)
(Mošovská et al., 2018)	Black peppercorn	5 g	Air	N/A	Atm.	Ceramic plate	60 s, 120 s, 180 s, 240 s, and 300 s	DCSBD	400 W DC, 18 kHz, 10 kV	Pathogenic bacteria inhibition (+)
(Puligundla et al., 2017)	Radish	3 g	Air	2.5 m/s	Atm.	Petri dish	0–3 min	Plasma jet	20 kV DC, 1.5 A, 58 kHz	Germination (\pm); treatment dependent; decontamination (+)

Continued **Table 1.** Experimental parameters of plasma agriculture experiments.

Reference	Seed		Carrier gas		Experimental parameter			Plasma		Primary focus of investigation
	Type	Amount	Type	Flow rate	Pressure	Seed holder material type	Treatment time	Type	Power	Indication of success ($\pm/0$)
(Matra, 2016)	Radish	10	Ar	4 LPM	Atm.	Acrylic box	2 min, 4 min, 6 min	Plasma jet	(1) 90 W (21.2 kV) (2) 140 W (30 kV)	Germination (+)
(Takemura et al., 2014)	Black pepper	1 g	(1) Ar (2) Ar + CO ₂ (3) Air (4) Ar + H ₂ O	(1) 20 LPM (2) 0.5 LPM, 20 LPM (3) 20 LPM (4) 20 LPM	Atm.	Petri dish	5 min	Plasma jet	Pulse – 280 V, 8 A, 16–20 kHz	Sterilization (\pm) treatment dependent
(Kim et al., 2017)	Broccoli (<i>Brassica oleracea</i> var. <i>kialica plen.</i>)	1 g	N/A	N/A	N/A	Petri plate	0–3 min	Plasma jet	220 V AC, 20 kV DC, 58 kHz	Microbial reduction (+); germination (+)
(Bafoil et al., 2018)	<i>Arabidopsis Thaliana</i>	150–300 seeds	(1) Air (2) He (2b) He	(1) N/A (2) 3 L/min	(1) Atm	(1) Glass plate (2) Eppendorf tube (2b) Glass beaker	(2) 15 min	(1) DBD (2) Plasma jet (2b) Plasma jet/DBD	(1) HV (2) 10 kV, 9.7 kHz,	Germination (+); growth (+)

(+), plasma had positive impact on primary focus of investigation; (–), plasma a negative impact on primary focus of investigation; 0, plasma had a neutral or no impact on primary focus of investigation; AC, alternating current; Ar, argon; Atm., atmospheric; Al, aluminum; DON, deoxynivalenol; DBD, dielectric barrier discharge; DCSBD, diffuse coplanar surface barrier discharge; DC, direct current; g, gram; hr, hour(s); He, helium; SLPM, standard liters per minute; min, minutes; N₂, nitrogen; O₂, oxygen; PBDBD, packed bed dielectric barrier discharge; PC, polycarbonate; RF, radiofrequency; s, seconds.

of the reviewed plasma agriculture works and includes information on the seed, carrier gas, experimental apparatus, plasma type, and research focus.

RF and Cold Plasma

RF plasma is usually formed when a RF field is applied with sufficient voltage to cause plasma formation. Typically, RF systems are operated within a low pressure environment and with a gas flow. The gas is typically selected based on desired chemical interactions, and the chamber is usually designed to process seeds in batches. Non-thermal or cold plasma is a property of all RF systems and that is why RF plasma was common throughout this review. Cold plasma treatments are used widely for activation or decontamination of surfaces. In this literature survey, seed treatment, germination, seed growth, and inactivation of microbes on seed surface and surface charging were investigated.

Filatova et al. (Filatova et al., 2009) developed an RF discharge operation at 5.28 MHz plasma to investigate the effects of electromagnetic fields on plant seeds to observe the shoot length of seedlings, rate of microorganism survival on seed surface, and the emission spectrum of the air plasma during seed irradiation. The crop seeds investigated were

grain crops (rye, wheat, barley), legumes (peas, narrow-leaved lupin), and aster. Each batch contained 100 seeds, and treatment times were 7 min, 15 min, and 30 min. The air plasma pressure was between 0.3 Torr and 0.7 Torr. Two parallel round copper electrodes with a diameter of 120 mm were separated at a distance of 20 mm, see Figure 2A. The RF discharge operated between the two electrodes. The seeds were placed on a grounded electrode (91.44 cm long) in a petri dish. A vacuum gauge, Rogowski coil (8), and a voltage meter (6) were the diagnostic tools used for operation. The RF was delivered at a power of 0.9 W/cm³.

Filatova et al. (Filatova et al., 2013) also developed two RF plasma systems with air carrier gas to investigate spring wheat (*Triticum aestivum* L.), blue lupine (*Lupinus angustifolius*), and corn (*Zea mays* L.) seeds. The first plasma frequency setting was set to 5.28 MHz and consisted of an electrode made of two identical water cooled disks with an operating power ranging between 0.2 W/cm² and 0.6 W/cm². The plasma treatment pressure was 0.5 Torr. Each petri dish with 50 seeds was exposed to RF plasma treatments for 2.5 min, 5 min, 8 min, or 10 min, and the treatment was repeated four times for each petri dish. The controlled batch of seeds was subjected for 15 min at the 0.5 Torr gas pressure. The temperature in the

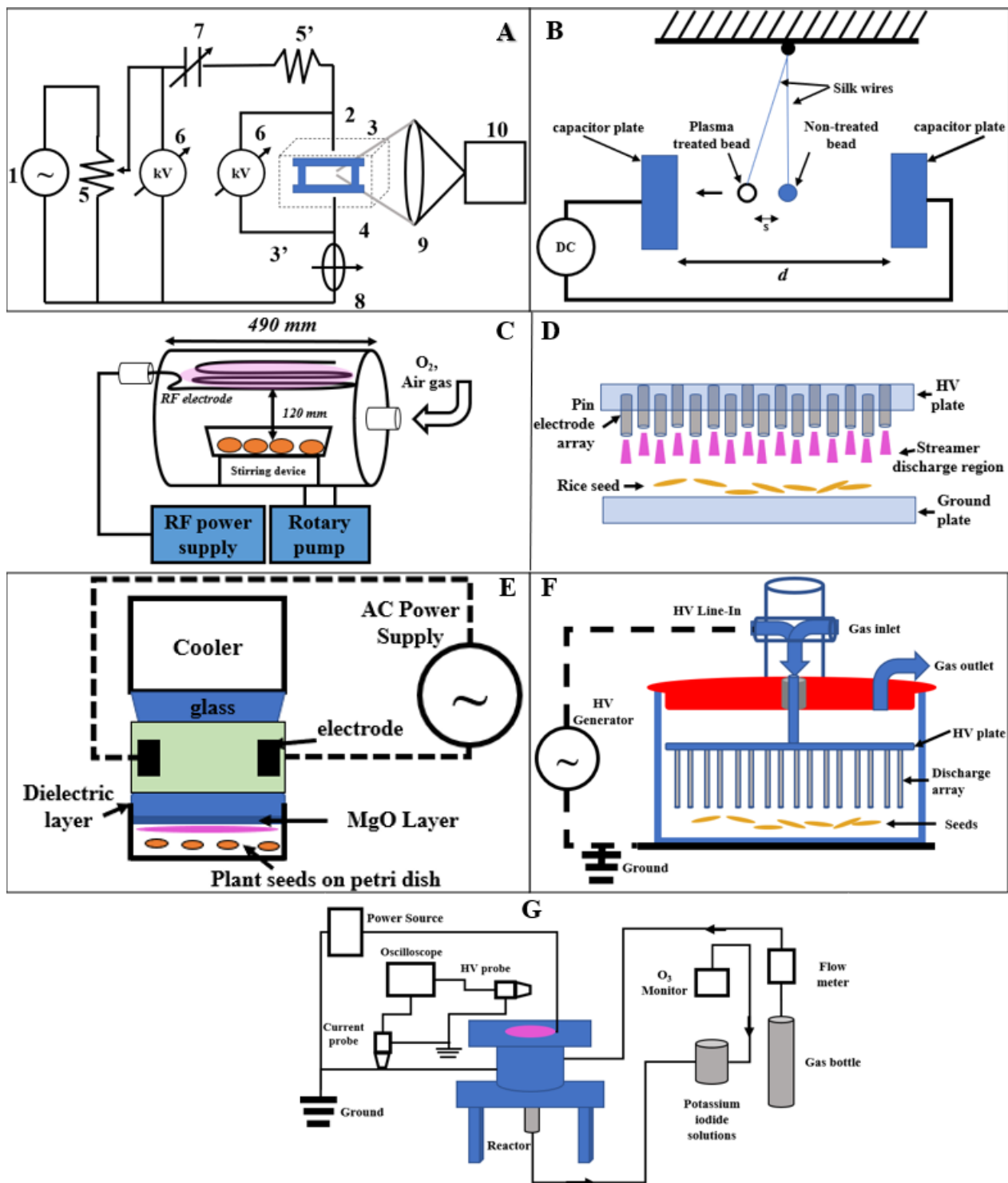


Figure 2. A. RF plasma experimental set-up: Alternator (1), vacuum chamber (2), electrodes (3, 3'), quartz window of discharge chamber (4), inductor (5,5'), voltage meter (6), tuning capacitor (7), Rogowski coil (8), lens (9), monochromator (10). (Filatova et al., n.d.); B. RF plasma system for treating lentil and pepper seeds. (Shapira, Chaniel, and Bormashenko 2018); C. Low plasma and vibrating stirring device with seeds. (Ono et al. 2017); D. Non-thermal plasma to sterilize rice seeds. (Khamsen et al., n.d.); E. Micro DBD plasma for sanitizing spinach seeds. (Ji et al. 2016); F. Plasma system cross section view and equipment on shaker. (de Groot et al. 2018); G. Schematic diagram of discharge plasma reaction system. (Guo et al. 2018).

planar geometry reactor did not exceed 310°C. The second plasma source was a cylindrical capacitively coupled plasma (CCP) reactor run at 13.56 MHz, powered by an aluminum electrode. In this second system, three petri dishes were used, and each dish contained 20 seeds, except corn. Ten seeds of corn were placed in each petri dish for the treatment. Wheat seeds were treated at 50 W and 100 W at 500 mTorr, while corn seeds were treated at 200 W at 300 mTorr. The plasma treatment times were 1 min, 5 min, 7 min, 10 min, and 20 min (Filatova et al., 2013).

Shapira et al. (Shapira et al., 2018) exposed lentil (*Lens culinaris*) and pepper (*Capsicum annuum*) seeds to inductive RF plasma discharge. The plasma frequency, power, pressure, and radiation time were 13.56 MHz, 18 W, 0.5 Torr, and 60 s, respectively. Polyethylene (PE), polycarbonate (PC), polystyrene (PS), and poly(methyl methacrylate) (PMMA) particles of a similar seed diameter (3–6 mm) were treated as control particles. The seed or particle was located below the silk wire between capacitor plates (see Figure 2B for the experimental setup). This study investigated surface charge density as induced by the inductive RF cold plasma discharge as well as kinetics of surface charge leakage according to exposure time (between 0 min and ~180 min).

Ono et al. (Ono et al., 2017) investigated inactivating bacteria on seeds by using a low pressure RF plasma. A plasma device with an inner diameter of 210 mm and a width 490 mm had an internal volume of 17 L, as seen in Figure 2C. The RF electrode was formed in a wave shape to provide spatially uniform plasma species on cabbage seeds. The seeds were stirred for 2–3 s by the vibrating device once every 30 min. Using a light emission spectrometer, the location of the seeds was observed; the active species generated were identified. The cabbage seeds were exposed to plasma for 0–3 h. The seed holder was a plastic pot placed on a vibrating, stirring device below the electrode. The seeds were investigated for germination, light emission intensity, SEM analysis, and inactivation of *Xanthomonas campestris* pv. *campestris* (Xcc).

Gómez-Ramírez et al. (Gómez-Ramírez et al., 2017) used RF plasma to sanitize quinoa (*Chenopodium quinoa*, Willd) seeds. The RF-treated seeds were placed on a sample holder and treated for 10 s, 30 s, 60 s, 180 s, and 900 s at 0.1 mbar at 15 W. Germination and water uptake tests were performed, as well as XPS and SEM analysis.

Ling et al. (Ling et al., 2015) investigated the effects of cold plasma treatment on seed germination and seedling growth of soybeans (*Glycine max* (L.) Merr). The experimental apparatus consisted of a low pressure (1.1 Torr), helium device, cold plasma generator, transmission device, and inlet/outlet hopper. The device was composed of two parallel plates and has a metal suspension shell. The area between the plates and the metal shell was filled with insulating

materials. Seeds received the plasma treatment when they were in the mesh cell that was insulated between the two polar plates. Each plasma treatment was carried out for 15 s with a power range of 0–120 W.

Kordas et al. (Kordas et al., 2015) used an AC, PBDBD plasma system to determine the potential effect of plasma on fungi colonizing winter wheat grain and the seed quality. Approximately, twelve fungal colonies were isolated from winter wheat grain, and those of the genus *Fusarium* were called out to be of particular importance. A packed-bed reactor of wheat grain containing gaps was used to form the discharge. The gas supply was air, and the grain was treated for 3 s, 10 s, and 30 s at atmospheric pressure. The plasma discharge for sterilizing the grain was created by the increase of electric field strength in the gas gaps due to the higher permittivity of the grain than air. Dielectric material locally polarizes and therefore causes regions of enhanced electric field. The localized increase in field strength results in less voltage requirements for plasma breakdown and streamer propagation.

Sinegovskaya et al. (Sinegovskaya et al., 2019) used a microwave (RF) generator at atmospheric pressure to treat soybean seeds for 60 and 120 s. Using 2.45 GHz frequency, the treatment was 2 cm away from the plasma torch edge. The characteristics of the plasma plume were determined with optical emission, electrical probes, and chemical methods. The plasma plume emission spectra ranges presented were from 300–400 nm to 700–800 nm. An argon-air mixture was employed as the gas supply. The flux density of the 2.45 GHz power frequency did not exceed 1 W/cm². The plasma components were kept 45 mm away from the plume surface.

Volume DBD

DBDs form with a variety of power sources but typically involve an AC or pulsed DC discharge. To create a DBD plasma, a dielectric material is placed between the electrodes of the plasma system. In doing so, the charge is spread over the surface of the dielectric until fully charged. This leads to a termination of the discharge before arc formation which limits the current transferred and therefore limits thermal effects of the plasma. The spreading of the charge also leads to a more uniform plasma discharge that is distributed evenly in the gap between the electrodes in time averaged imaging, nanosecond pulsed systems, or certain gas mixtures. A uniform, non-thermal application is ideal for plasma treatment of surfaces, resulting in an increased interest in this technology for plasma interactions with seeds. DBD is the most prevalent plasma selection for seed treatment that was found during this review. For more information on DBDs, the reader is referenced to R. Brandenburgs extensive review on the topic and Ollegott's review of applications for plasma catalytic reactions in DBDs (Brandenburg, 2018; Ollegott et al., 2020). The DBDs

described in this section are considered volume DBDs as plasma interacts within the interstitial regions between the electrodes and dielectric material; thus, a volumetric plasma is observed. In a later section, surface DBDs will be discussed in which plasma is limited to surface interactions and does not propagate into the volume.

Gómez-Ramírez et al. (Gómez-Ramírez et al., 2017) developed a DBD plasma for treating quinoa seeds to improve germination and increase water uptake of the etched seed coat. The plasma durations were 10 s, 30 s, 60 s, 180 s, and 900 s. Seeds were placed between two parallel quartz plates that acted as dielectric barriers. Seeds rested on the bottom quartz plate, which laid on the grounded electrode and treated at 6.4 W. The plasma worked at a sub-atmospheric pressure of 500 mbar.

Khamsen et al. (Khamsen et al., n.d.) used the non-thermal DBD plasma to observe the sterilization and germination rates of rice (*Oryza sativa* L.) seeds. The plasma and seed interaction is displayed in Figure 2D. The seeds were treated in air at atmospheric pressure in batches of 25 seeds and operated at 25°C. Another study on rice seeds for fungal pathogen reduction was performed by Jo et al. (Jo et al., 2014) that utilized an aluminum plate holder for small volume seed treatment (three to five seeds).

Ji et al. (Ji et al., 2016) used a micro DBD plasma to investigate seed germination, growth, and physiological activities of treated spinach (*Spinacia oleracea* L.) seeds. This plasma used two electrodes and a dielectric layer made of SiO₂ and layers of additional coatings to prevent hydration. The electrodes were 5 µm thick and 200 µm wide, and the gap was 200 µm apart. Air and N₂ carrier gasses were supplied at 1.5 liters per minute (LPM). The discharge voltage, current, and frequency were 6 kV, 14 mA, and 22 kHz respectively. Fifty spinach seeds were placed on a petri dish with a dried filtered paper 1 mm away from the DBD plasma. The plasma treatment times were 30 s, 1 min, 3 min, and 5 min. Control samples were defined as gas-treated seeds with no plasma treatment. After each treatment, 3 mL of distilled water was added to the filter paper to moisturize the seeds. The experimental setup is displayed in Figure 2E.

Sinegovskaya et al. (Sinegovskaya et al., 2019) applied a DBD plasma jet on soybean seeds to observe seedling development. The plasma was formed in air at a temperature of 30°C and at atmospheric pressure, operating at a voltage and frequency range of 6–12 kV and 28–32 kHz, respectively. The dielectric was made of a 3-mm-thick polymer which was inserted between copper electrodes. The seeds were 2 cm away from the edge of the plasma jet, but full details of the structure or apparatus were not revealed.

de Groot et al. (de Groot et al., 2018) applied a DBD plasma on cotton (Sicot 74BRF) seeds for investigation of germination under the optimal (warm) and sub-optimal (cold) conditions. A

compressed inlet of air or Ar gas was connected to an array of electrodes to form plasma, while 350 seeds were placed in a 5 L borosilicate glass beaker, as seen in Figure 2F. The systems operated at 45°C with a gas flow rate of 1 LPM. The chamber was placed on a 120-rpm shaker to aid in uniform seed treatment. Hundred “needles of plasma” were formed, and the spatial distance was 13 mm. Seeds were treated in air at 3 min and 27 min, while Ar gas treatment was performed at 81 min.

Guo et al. (Guo et al., 2018) developed a DBD system using air at atmospheric pressure to treat wheat seeds. The DBD plasma setup is displayed in Figure 2G and had an AC high-voltage power supply. The surface of wheat seed was uniformly irradiated with atmospheric plasma as displayed in Figure 3A. The voltages of plasma treatments were 0 kV, 9 kV, 11 kV, 13 kV, 15 kV, and 17 kV.

Wang et al. (Wang et al., 2017) utilized the DBD plasma with air or N₂ gas at a flow rate of 1 SLPM, with plasma generated in a glass container at a needle plate gap of approximately 15 mm. Then, 120 g of cotton seeds was present in each group and spread evenly on the bottom of the glass container. Seeds were treated at 3 mins, 9 mins, and 27 mins. Seed surfaces were investigated with FTIR using a universal attenuated total reflection accessory with a pressure arm, as seen in Figure 3B. Moisture uptake was determined by immersing the seeds in 25 mL of H₂O for 20 h, and then, seeds were dried in air, and the mass was collected as soon as surface moisture was removed.

Hayashi (Hayashi et al., 2014) used a plasma technology to surface sterilize fruit (lemon) and rice seeds by exposing them to O₂ (from air) and ultraviolet (UV) light at ambient pressure (Figure 3C). To reduce the emission of NO_x products, silicone was spread on the discharging electrode. A chemical indicator was placed in the sample chamber to confirm the active oxygen species formations.

Kitazaki et al. (Kitazaki et al., 2014) used air DBD to observe growth enhancement of radish seeds (*Raphanus sativus* L.). The treatment device had 20 stainless steel rod electrodes that were 1 mm in diameter and 60 mm in length, covered with ceramic tubes. The spacing distance between the tubes was 0.2 mm, and the temperature range was 24–26°C. Seed treatment time was 180 s. Seeds rested on a glass plate at varying distances of 10 seeds per line. The experimental setup is displayed in Figure 3D. The system used a gas detector to monitor NO, NO₂, and O₃ emissions.

Dobrin et al. (Dobrin et al., 2015) investigated what they called a surface discharge of air plasma to investigate germination and growth of treated wheat seeds. The plasma reactor had two electrodes placed near both sides of the glass plate (top and bottom) (Figure 3I) with an air flow of 1 LPM. Thirteen copper wires were used to produce a high-voltage electrode, and seeds were distributed uniformly on each wire. The

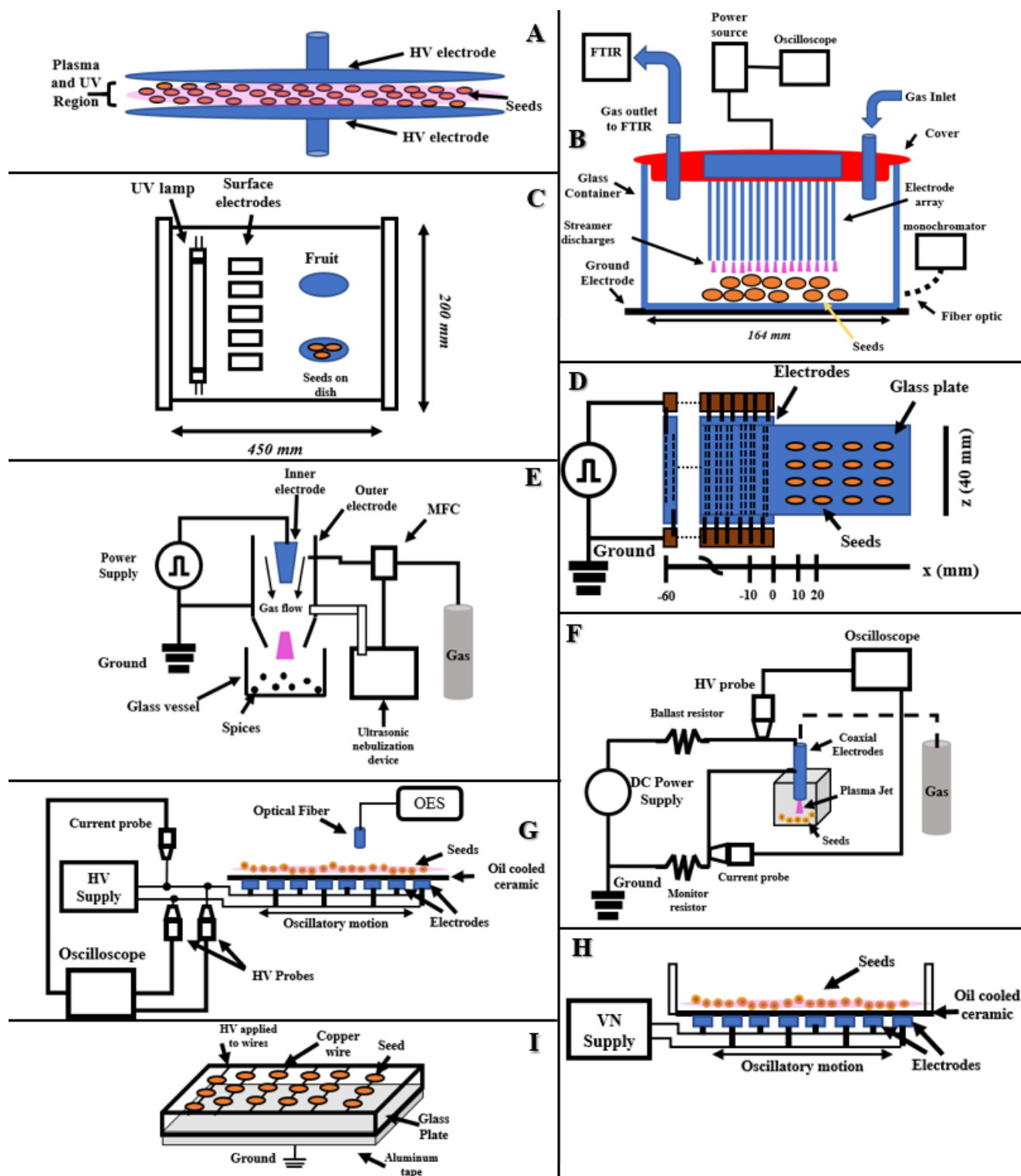


Figure 3. A. Picture of wheat seeds between two electrodes showing UV irradiation. (Guo et al. 2018); B. Needle-plate set-up on cotton seeds (Wang et al. 2017); C. Plasma and UV light for sterilizing fruits and seeds (Hayashi et al. 2014); D. Plasma irradiation on radish seeds via DBD plasma (Kitazaki et al. 2014); E. Germicidal treatment system (Takemura et al. 2014); F. Plasma experimental setup (Matra 2016); G. Schematic diagram for DCSBD plasma treatment of maize (Zahoranová et al. 2018); H. DCSBD plasma tool sterilizing black peppercorn sample. (Mošovská et al. 2018); I. Surface discharge plasma made of 13 copper wire (Dobrin et al. 2015).

discharge in this work was similar to a traditional DBD with the seeds in the vicinity of the electrodes for enabling plasma species interaction with the seed surface.

Pérez-Pizá *et al.* (Pérez-Pizá *et al.*, 2019) investigated soybean (*Glycine max* (L.) Merrill) seeds using DBD. The plasma discharge consisted of needle array electrodes with the ground electrode plate covered by polymer dielectric barriers, with the full setup described in an earlier work (Pérez-Pizá *et al.*, 2018). The sine AC power supply operated at 50 Hz with O₂ and N₂ carrier gases with a discharge input power of 65 W or 85 W depended on the polymer barrier. A total of 500 seeds were treated at a time, with mechanical movement for uniform treatment, ranging from 60 to 180 s of plasma exposure time.

Plasma jet

Plasma jets, also known as atmospheric pressure plasma jets (APPJ), are unique in that the plasma is generated within a flow tube with plasma exiting the tube and interacting with a surface external to the discharge. These jets are typically in a concentric DBD arrangement but may also be concentrated RF waves. The feedstock gas provided to the flow tube allows for control of the plasma produced species, jet depth external from the tube, and operating parameters. Research interest in plasma jets has been recently increasing in the plasma agriculture and plasma medicine communities as they allow fine control of the surface being treated with plasma.

Takemura *et al.* (Takemura *et al.*, 2014) developed a germicidal treatment system utilizing a 5-mm plasma jet nozzle with multiple carrier gases. The surface of black pepper seeds was uniformly irradiated with atmospheric plasma as displayed in Figure 3E. The model contaminant was *B. subtilis* strain ATCC6633 prepared on petri dishes and then spread over 25-mm diameter glass fiber filters that were exposed to plasma for varying durations.

Matra (Matra, 2016) designed a plasma jet model which used Ar gas. The plastic tube was used to supply gas to the copper cathode tube. While gas was fed through the tube, the high-voltage source generated a plasma jet at the tip of the copper tube, discharging into the acrylic box, as seen in Figure 3F. Ten radish seeds were placed in the acrylic box per each experiment. Then, 90 W of power created a self-pulsing discharge waveform mode during the experiment. The plasma treatment times were 2 min, 4 min, and 6 min for each power value using Ar gas at 4 LPM. Control and treated seeds were cultivated in a dark environment.

Puligundla *et al.* (Puligundla *et al.*, 2017) developed a corona discharge plasma jet (CDPJ) to treat radish seeds. The plasma operated at 1.5 A and 58 kHz. Air was used as the jet emitting 2.5 m/s velocity of gas at the electrode tip with an interelectrode gap of 5 mm. The seeds were placed in a petri dish and treated for a duration of 0–3 mins. The samples were 2.5 cm away from the electrode.

DCSBD (Surface DBDs)

DCSBDs are similar in concept to that of a DBD with a main difference being the electrode layout that restricts plasma processes to surface-based interactions. A DCSBD will have a row of electrodes of alternating high voltage applied with plasma and are typically embedded into dielectric material. The alternating row of electrodes embedded within the material lead to a more controlled uniform discharge than the traditional DBD. Typically, DCSBDs are used in higher power density applications where increasing the power of the system would lead to arc discharge formation in a DBD. An increase in power density of a DCSBD allows for a higher plasma density and a change in plasma kinetics that would otherwise be absent.

Zahoranová *et al.* (Anna Zahoranová *et al.*, 2018) investigated the use of DCSBD cold atmospheric plasma on surface microorganism inactivation. DCSBD is a planar type of discharge generating plasma in a thin layer (~0.3 mm) on a ceramic plate with a highly active plasma area (20 cm × 8 cm). Maize (*Zea mays* L.; cv. *Ronaldinio*) seeds were placed in an orbital shaker for uniform treatment around seeds during treatment period (Figure 3G). A similar study by Zahoranová *et al.* (Zahoranová *et al.*, 2016) used the similar DCSBD setup to treat wheat seeds (*Triticum aestivum* L. cv. *Eva*). This method was impactful to treating a larger seed surface area because the seeds were in a continuous rotational motion which also helped maintain a constant temperature range between 50 and 55°C. A detailed description of the DCSBD source is reported in the 2009 work by this team (Černák *et al.*, 2009). Mošovská *et al.* (Mošovská *et al.*, 2018) developed a DCSBD plasma to sterilize black peppercorn seeds inoculated with bacteria. In that study, 5 g of the seeds was placed on a ceramic plate and exposed to air plasma at atmospheric pressure. The seeds were treated at different plasma times including 60 s, 120 s, 180 s, 240 s, and 300 s. The seeds were shaken at 270 rotations per minute during treatment to ensure all sides of the seeds are exposed to the plasma, as seen in Figure 3H. Two systems of parallel silver electrodes were used.

Štěpánová *et al.* (Štěpánová *et al.*, 2018) created a prototype device for the DCSBD treatment of cucumber seeds (*Cucumis sativus* L.) type spined pickling, and variety Regina F1 and pepper seeds (*Capsicum annuum* L.) type Hungarian sweet wax, variety Amy – which had a vibrating dispenser, four discharge units, and a collecting vessel for plasma treated seeds. The approach enabled continuous treatment of the seeds. The study of Štěpánová *et al.* also ensured that the DCSBD ceramic temperature did not exceed 50°C for any treatment times.

High-voltage nanosecond pulsed plasma

The high-voltage nanosecond pulsed plasma used by Ji *et al.* (Ji *et al.*, 2016) charges capacitors in parallel and discharges

them in series. This system consisted of 15 stages, and each capacitor had a capacitance of 8 nF, connected with two 20 Ω resistors in parallel. Each spark was located between every two stages of the capacitor. A point-to-point electrode was made of stainless steel with a 3-mm diameter and a gap electrode distance of 10 mm. The peak voltage was 27 kV, and the current was 2.3 kA. The discharged voltage and current measured were 6 kV and 0.7 kA, respectively, with the discharged energy as 0.3 J per one shot pulse. A charged coupled device (CCD) with a spectral range from 200 nm to 1,000 nm was used to measure the reactive oxygen and nitrogen species. The 25 spinach seeds were wrapped in a wire gauze, and 1, 5, or 10 shots of high-voltage nanosecond pulses were applied to the seeds indirectly, as they were wrapped in the filter paper resting on a petri dish.

SEED PERFORMANCE RESULTS

Germination and Growth

Germination is the process of initiating growth of a dormant embryo. This is an important stage of the life cycle where environmental factors such as temperature, light, pH, and water are key factors for successful growth (Ji et al., 2016). The plasma results seem to, on more occasions than not, positively enhance the germination rate. This section is organized to reflect plant family and the performance results after plasma treatment when compared with the control samples.

Poaceae

Poaceae/Gramineae is the grass family. It includes wheat, barley, rice, maize, and other similar grains. When investigating wheat, Guo et al. (Guo et al., 2018) showed significant improvements in germination rate potential with 11 kV and 13 kV DBD plasma when compared with the control. These two voltages were also best compared with the 9 kV, 15 kV, and 17 kV plasmas. The 11 kV and 13 kV plasma treatments also had the best performance in fresh weight gain, dry weight gain, shoot length, and root length. When germination rates were measured in the Dobrin et al. (Dobrin et al., 2015) study with wheat seeds and surface discharge plasma, on day four, the performance rate of 15 seeds of both control and plasma-treated seeds was 95% and 98%, respectively.

The germination results of Kordas et al. (Kordas et al., 2015) for winter wheat grain were improved by the cold plasma treatment when observations were collected on day 4 and 8 of germination. This work used a petri dish as the germination test medium and a germination temperature of 21°C with two independent series of ten repetitions for each exposed duration.

When conducting RF treatment for 5–7 days at 20–21°C, (Filatova et al., 2009) saw no difference in germination for plasma-treated barley seeds compared with the control (untreated). Filatova et al. (Filatova et al., 2013) also investigated the impact of planar discharged RF plasma on spring wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). They found that treated seedlings grew faster, measuring 2.1 cm longer than the controls. There were also increased germination factors (germination time and growth rate of seedling) with the CCP discharge for spring wheat with 100 W at a shorter plasma treatment time. The highest germination rate was at 5 min plasma exposure. The germination percentage decreased from almost 95% to 73% with an increase in time from 7 min to 20 min. The 50 W plasma had the highest germination percentage at 7 min exposure. There was 95% germination for 7 min and 85% for 20 min.

Zahoranová et al. (Anna Zahoranová et al., 2018) found that the DCSBD plasma treatment of maize (*Zea mays* L.; cv. *Ronaldino*) seeds at 180–300 s led to a significant reduction of germination. On the other hand, the 60 s treatment showed significantly improved root length, shoot length, and overall vigor, compared with the control. In the wheat seed (*Triticum aestivum* L. cv. *Eva*) study (Zahoranová et al., 2016), the plasma treatment increased the germination rate by 21% at 30 s but negatively impacted all growth parameters (germination decreased by 14% and 25%) at the longest plasma treatment times of 70 s and 80 s. There was indeed an optimal plasma treatment time observed for growth studies between 20 s and 50 s.

Plasma-treated dent corn (*Zea mays* cv. *indentata*) seeds in Ahn et al.'s. (Ahn et al., 2019) study were planted in outdoor fields in 6 test plots in Illinois, USA. Some of the seeds were treated with a biological aid to protect the seeds after planting (Poncho/VOTIVO with Acceleron). Overall, no significant difference was found between the plasma treatments and the engineered commercial hybrid corn seeds planted in the USA, which already have a nearly 100% germination rate. Their earlier work within the laboratory had shown improvement in desired traits with plasma-treated seeds and water, which was not seen in the field. They concluded that the external environmental factors such as weather conditions, soil nutrient levels, fertilizers, etc. played a more important role than the plasma treatment in plant development. It was recommended to apply plasma treatment to crops where such genetic and seed engineering improvements were not already achieved through other means.

Rice and legume seeds were treated with plasma for pathogenic fungi decontamination in Selcuk et al.'s (Selcuk et al., 2008) study. The use of air and SF₆ plasma was investigated due to its bio-compatible nature that avoids damage to the living seeds while still providing surface disinfection. No significant germination difference was observed at 5 min, 10 min, and

15 min of air and SF₆ plasma gas, with air having slightly more of a stimulant effect than SF₆. The markers were not that much improved, however, when compared with the control tests.

Pathogenic fungi, *Gibberella fujikuroi*, and oomycetes were also treated on rice via DBD plasma in Jo et al.'s study (Jo et al., 2014). Increased exposure to the seeds with plasma dramatically reduced the colony-forming units (CFUs) of *G. fujikuroi* from the surface. At 180 s exposure, >99% of the pathogen was reduced. The study made note that low-power DBD was more effective for sterilization than the gliding arc discharge.

Barley grain was treated with DBD in a study by Feizollahi (Feizollahi et al., 2020) in which deoxynivalenol (DON), a trichothecene mycotoxin, was reduced by the treatment at 48% in 6 min of treatment time.

Brassicaceae

Brassicaceae/Cruciferae is commonly known as the mustard family. This family contains many important vegetable crops including broccoli, cabbage, and radish. The CDPJ treatment in the study by Puligundla et al. (Puligundla et al., 2017) on radish (*Raphanus sativus*) seeds had the highest germination rates at 1 min and 2 min with a germination rate of 81% and 78%, respectively, compared with the control seeds which was 76%. The longer 3 min treatment reduced germination rates to 50%.

In Matra's (Matra, 2016) plasma jet work with Ar, the growth rate was determined by measuring a 7 day radish root length and the mass of dehydrated radish sprouts. The root length and stem length of the plasma-treated seeds were longer than those of the control. The average length of the plasma-treated seed roots was 7.5 cm as compared with 4.5 cm of the controlled cases. A power of 140 W produced a slightly higher total mass as compared with 90 W. The 140 W generated 9–15% higher total mass than controlled cases. Increase of the plasma time for 90 W enhanced the germination rate, while 140 W decreased the germination rate. The decreased germination rate may have been due to the higher power causing more heat, therefore damaging parts of the seeds.

Kitazaki et al. (Kitazaki et al., 2014) reported that plasma-induced cracks and holes affected plant growth in radish. Oxidative stress of the radicals was stronger within 7 days of plasma treatment that may have caused secretion of hormones such as auxin and gibberellin, which stimulate plant growth. After 7 days, the seed metabolism slowed down hormone production, which may have slowed growth. Plasma-treated seeds were 250% longer than the non-irradiated seeds (Kitazaki et al., 2014). The low-pressure RF plasma germination results from Ono et al. (Ono et al., 2017) on cabbage (*Brassica oleracea*) seeds concluded no difference between the plasma-treated and control seeds.

Brassicaceous (*Brassica campestris* var. *amplexicaulis*) (Nishioka et al., 2016) was also studied under the works of

Nishioka et al. (Nishioka et al., 2016), Kim et al. (Kim et al., 2017), and Ito et al. (Ito et al., 2018) using DBD, CDPJ, and DBD, respectfully with specific focus on microbial reduction.

Cucurbitaceae

Cucurbitaceae/Cucurbits is the gourd family of flowering plants. Common members of this family include gourds, melons, cucumbers, squash, and pumpkins. In the study of cucumber (*Cucumis sativus* L.) and pepper (*Capsicum annuum* L.) seeds of Štěpánová et al. (Štěpánová et al., 2018), all germination tests were performed according to the International Seed Testing Association standards. Germination power was calculated using a ratio of germinated seeds after a certain number of sowing days to the total number of planted seeds × 100. Germination was calculated similarly but after longer sowing times; cucumbers germinated in 4 days, and a second batch germinated in 8 days; peppers germinated in a 7- and 14-day batch. An optimum seed treatment time of 20 s for cucumber seeds and 4 s for pepper seeds was correlated with maximum germination. Longer plasma treatment times confirmed seed mortality increase, but longer plasma treatment times also eliminated pathogens which improved seed viability. This study emphasized that germination is influenced by the seed shape, size, weight, and surface. In the Štěpánová et al. study, cucumber germination rate increased to 96% with plasma treatment of 20 s and 89% for pepper at only 4 s. This study did include optimization parameters and how plasma treatment time has an optimal threshold on the seeds; in this study, cucumber seeds required five times longer treatment time than the pepper seeds.

Fabaceae

Fabaceae/Leguminosae is the legume family. It includes beans, peanuts, soybean, clover, alfalfa, and legumes. These crops form a symbiotic relationship with a group bacterium called rhizobium, which assists the plant with nitrogen fixation from the atmosphere, adding nutrients to the root zone. There may be benefits to investigating the impact of plasma treatment on the whole symbiotic relationship for this plant family. Sinogovskaya et al. (Sinogovskaya et al., 2019) observed a 21% increase in the laboratory germination rate of soybean under DBD and microwave plasma treatment compared with untreated control seeds. Filatova et al. (Filatova et al., 2013) noted that the optimum times for planar discharged RF plasma treatment were 2.5 min and 5 min for blue lupine (*Lupinus angustifolus*). In Sinogovskaya's work with soybean, there was an increase in germination and preservation of plants for the DBD plasma treatment (Sinogovskaya et al., 2019). Higher concentrations of free radicals were noted in metabolically active parts of the soybean seeds (Sinogovskaya et al., 2019). After plasma treatment, stability in phosphorous, calcium, and magnesium contents was also observed in the seeds for 5 months

(Sinegovskaya et al., 2019). The percentage of potassium (K) stored decreased by 0.13 after treatment due to an increase in permeability (Sinegovskaya et al., 2019). Ling et al. (Ling et al., 2015) found no treatment with significant effect of cold RF sub-atmospheric plasma on soybean germination potential or rate. The vigor index increased compared with the control, with 80 W plasma treatment performing best. For all seedling growth assessment measures, the mid-range treatments (80 W and 100 W) showed significant improvement, while the other treatments (60 W and 120 W) did not show significant improvement.

In the Sinegovskaya et al. (Sinegovskaya et al., 2019) study with soybeans exposed to an Ar microwave plasma, an increase of free radicals was found on the metabolic active part of the seeds, as well as activation of protein enzyme systems and a 1.3–1.5% decrease in the amount of protein. The number of seedlings after 48 h was 73% for the 60 s treatment (higher than the control at 30%) but declined to 37% after the 120 s treatment. For the 60 s and 120 s treatments, field germination rates were 83% and 79%, respectively, and 82% for control.

The soybean investigation of Pérez-Pizá et al. (Pérez-Pizá et al., 2019) focused on healthy seed quality aspects, including germination, vigor, and health; this included vigor index I (VI I) and vigor index II (VI II) and electrical conductivities of seed leachates. The study concluded that the DBD plasma treatments positively influence germination rates when compared with controls, with an increase of 5–7%. VI I incremented 1.4–2.25 times compared with the control; VI II had no difference compared with the control, and electrical conductivities treated with plasma were 18–27% lower than the controls. Plant growth improvements in this work correlate with those of other plasma-treated soybean seeds (Ling et al., 2015).

Asteraceae

Asteraceae/Compositae is the plant family that includes lettuce, sunflowers, zinnia, and daisies. It includes agriculturally relevant crops. Lettuces and zinnia have been grown successfully on the ISS. Filatova et al. (Filatova et al., 2009) did a germination test in a petri dish with a moist filter paper for both the capacitively coupled RF-treated and untreated (control) aster (*Aster* sp.) seeds. The test was performed for 5–7 days at 20–21°C. The seedlings with a shoot length of 0.5 cm were considered germinated. The germination performance was determined on the third, seventh, and tenth day. The germination “energy”, germination test and growth rate of seedling, increased as germination “ability” decreased with increased plasma treatment time. All were at rates higher than the control. In contrast, Filatova (Filatova et al., 2009) saw no impact of this treatment on germination of barley seeds.

Amaranthaceae

Amaranthaceae is the amaranth family. Amaranth (Inca wheat) itself is an important staple crop, but this plant family also includes beet, chard, sugar beet, spinach, and quinoa. It also includes many salt-tolerant species within the clade commonly called chenopods. The RF results of the study by Gómez-Ramírez et al. (Gómez-Ramírez et al., 2017) on quinoa (*Chenopodium quinoa*) seeds indicated that, on day 12, 10 s, 30 s, 60 s, and 180 s plasma treatment times had 100%, 80%, 85%, and 60% germination rates, respectively. The DBD results indicated that, on day 12, 10 s, 30 s, and 900 s treatment times had 10%, 80%, and 100% germination rates, respectively.

No drastic change was observed in the DBD plasma treatment of spinach (*Spinacia oleracea*) after a 7 day germination test when compared with the control (Ji et al., 2016). It was noted that the air plasma enhanced seed germination more than the N₂-generated plasma. Ji et al. (Ji et al., 2016) noted that the germination rate was 75–80% with 1 or 5 shots of high-voltage nanosecond pulse treatment, while the control was 60%. Germination with 10 shots was lower than the control, as well as the 1 and 5 shot treatments. The air plasma treatment showed a higher germination rate as compared with the control.

Malvaceae

Malvaceae is also known as the mallow family. It is commonly known for its flowers, but this family includes cotton, okra, and durian. Wang et al. (Wang et al., 2017) tested germination experiments on pre-treated and control cotton (*Gossypium hirsutum* var. Sicot 74BRF) seeds. The germination test results with Ar gas had the lowest germination rate. Germination improved for both 3 min and 27 min of air treatment. The highest overall germination was obtained for the 27 min air treatment. For the air germination test, there was a higher germination rate as compared with the control at 4 days of germination. They observed that the 4 days air germination more than doubled with the 3 min air and 81 min Ar. At 7 days and 10 days, only the Ar-treated seeds showed increased germination rates while the air-treated seeds showed decreased germination rates.

SURFACE ANALYSIS, ANALYTICAL CHEMISTRY, AND OPTICAL EMISSION

Water Interactions

Water contact angle was investigated in some studies. A decline in the water contact angle implied an increase in hydrophilicity of the seed coat. Excessive moisture on a seed can decrease O₂ absorption which affects the germination process, so it is an important parameter to investigate when

possible. A water contact angle decline was observed in the study by Zahoranová *et al.* (Anna Zahoranová *et al.*, 2018) using maize (*Zea mays* L.; cv. *Ronaldinio*) seeds under DCSBD plasma in air, and Dobrin *et al.* (Dobrin *et al.*, 2015) observed the same effect on wheat seeds in a surface discharge plasma. Longer plasma treatment time in experiments had lower contact angle trends. Water uptake increased in some studies of soybean seed and quinoa seeds after DBD plasma and RF plasma treatment (Sinegovskaya *et al.*, 2019; Gómez-Ramírez *et al.*, 2017).

Plasma treatment time did appear to be an important factor since, in one study, the first few hours appeared to have the larger difference and increase in water uptake (Anna Zahoranová *et al.*, 2018), while in another study, an ideal performance was found in a mid-range DBD plasma power application on wheat (Guo *et al.*, 2018). The water uptake in wheat seeds (Zahoranová *et al.*, 2016) was the highest during the 2-h range compared with the 8-h imbibition.

Other works with soybean (*Glycine max* (L.) Merr) found no significant increase in water uptake but had an impact on water contact angle and increased soluble sugar and protein contents due to the plasma treatment (Ling *et al.*, 2015). In the DBD plasma study of Khamsen *et al.* (Khamsen *et al.*, n.d.) with rice seeds, a 2 μ l water droplet experiment test was performed right after the treatment of each different treatment time of seeds. It was observed that the shorter treatment time had higher water imbibition time. In the same study, the highest imbibition time was of the untreated seeds and the lowest was air-Ar mix plasma.

SEM

SEM analysis was typically used to observe morphology changes of the seed coat (Ahn *et al.*, 2019; Ji *et al.*, 2016). Ono *et al.* (Ono *et al.*, 2017) observed damage to cabbage seeds after treatment with low-pressure O₂ and air plasma in the form of crack-like damage. These cracks may have been caused by oxidation, collision of active species generated by the plasma, or thermal expansion via plasma treatment. Guo *et al.* (Guo *et al.*, 2018) compared the wheat seed coat before and after DBD plasma treatment. The seeds before treatment had a clear boundary layer, and after treatment, the seed had a softer structure and a hard to identify boundary layer with crack formations. The cross section of the wheat seeds displayed a reticular or netlike formation in the endosperm of the seed, with dissociative starch grains before plasma treatment. After plasma treatment, there was a clear release of the starch grain from the reticular formation, increasing the amount of free starch grain available. In the study by Gómez-Ramírez *et al.* (Gómez-Ramírez *et al.*, 2017), the quinoa seed coat was reported to have etching on the surface after plasma treatments (Gómez-Ramírez *et al.*, 2017). In the study by Bafail *et al.* (Bafail *et al.*, 2018), SEM images displayed significant

alteration of the *Arabidopsis thaliana* seed coat morphology after plasma exposure. The study reported that this may correlate with water uptake and potentially a direct impact to germination rates, but this required more investigation.

Other studies did not report any major or significant degradation of the seed coat when utilizing SEM for analysis, including those of maize (Anna Zahoranová *et al.*, 2018) (even after longer plasma exposure times (300 s)), fruits (Hayashi *et al.*, 2014), black peppercorns (Mošovská *et al.*, 2018), and cucumber and pepper seeds (Štěpánová *et al.*, 2018). A “softer” structure with wrinkles was present in some of the study observations, when compared with the control seeds, but not all. Shapira *et al.* (Shapira *et al.*, 2018) reported wrinkling, associated with irreversibility of wetting properties after the RF plasma treatment, which likely had some impact on the morphology change observed.

One item often missing for consideration from the morphology studies using SEM is the clarification of effect on the system pressure on the seed coat. For example, it is recommended to run control tests with and without vacuum, if the plasma treatment involves vacuum, to see if that is also the cause of seed surface crack formation. Plasma is also known to have an etching effect, as it is used for layer removal and surface chemistry (i.e., XPS). High-voltage “shots” of micro DBD plasma were observed to destroy the surface of spinach seeds in the work of Ji *et al.* (Ji *et al.*, 2016) with spinach seeds, especially as the “shots” increased. It was believed that >10 shots was so damaging that the germination was impacted and declined; however, “moderate” shots (<5) of the DBD plasma may have accelerated germination. There was no correlation between the surface changes and seed germination though to report in the study, and changing the plasma gases (N₂ and air) did not create a difference in the surface coat disruption.

XPS

XPS can be a powerful tool to understand the surface chemistry, and it is recommended that XPS should be used to observe seed surface chemical properties before and after plasma treatment whenever possible to examine the carbon and oxygen bonding behavior at the surface. Elemental analysis at the surface level can also be collected with XPS to determine if elements related to nutrition or surface protection are being deteriorated due to plasma treatments. XPS can also be used to etch beneath the surface of the seed coat and reveal alterations or changes in the atomic levels of surface bonds. Since XPS is in essence a plasma treatment on the seed surface, it should be considered that this may impact the surface properties of the seed. Not enough information or detail is available to correlate a conclusion on these concerns, but the authors felt it relevant to address and will be seeking this out during XPS treatment during the NASA KSC study

that will be reported in a future manuscript. It would be most beneficial if XPS was accompanied by *in situ* OES or FTIR during experimental application of plasma to the seeds to examine real-time chemistry interactions from the plasma field. Ramirez et al. (Gómez-Ramírez et al., 2017) used XPS for the elemental analysis of the different treatment times of quinoa seeds and compared them with the control. The longest treatment time caused a significant increase in O₂, N₂, and K on the seed surface coat. In general, XPS was an underutilized tool when surveying the literature. Štěpánová et al. (Štěpánová et al., 2018) used XPS for surface composition and chemistry changes, detecting primarily C, O, and N with surface impurities of Mg, K, S, and P. An increase in hydroxyl- and carbonyl carbon-containing functional groups was present during the DCSBD plasma treatment, as well as increased oxygen-containing functional groups, even up to nine days after plasma treatment.

FTIR/OES

FTIR and OES data can be complimentary in that they are observing spectra generated by the plasma during seed treatment. The FTIR and OES can observe ionized molecules as well as transient compounds formed during plasma emission and chemical reactions on the surface of the seed coat and plasma-generated ions. OES was commonly used to characterize the baseline plasma emission spectra. FTIR was used to observe surface chemistry between the seed and plasma-ionized field or the surface of the seed coat using a specialized accessory called attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), which takes a reading directly on the solid surface. This data collection method has been the limiting factor that is recommended to be performed on all future plasma agriculture applications when available. The correlations that can come of these data may be key reference indicators to unlocking the mechanisms and behaviors coupled with botany and plasma performance. To date, the studies discussed in this section highlight the variability in the FTIR/OES use and objectives. In some cases, basic observation of spectra is collected and reported, while other cases seek to understand the mechanism of microbial inactivation or correlations with growth and vitality comparing water uptake, growth, and SEM observations. We selected these works to discuss as they are all specific to various objectives of the paper and should be called out for availability to those wishing to pursue future work in this field and see what benefit OES and FTIR might bring to enhancing the results and correlations. If this research is to continue its potential, it must be documented clearly for understanding in the biology, chemistry, and physics to move the technology and applications forward.

In the study by Zahoranová et al. (Anna Zahoranová et al., 2018), the ATR-FTIR data were used to characterize

the presence of specific chemical groups on the surface of maize seeds before and after plasma treatment. The plasma treatment caused surface activation on seeds, and the spectra confirmed the presence of carbohydrates, lipids, and proteins. Lipids were removed, as noticed by the increase in FTIR activity of species containing O₂ and N₂ released from the seed surface (polar groups), which correlated with the contact angle results, supporting the removal of the hydrophobic lipids.

In the study by Takemura et al. (Takemura et al., 2014), OES data were collected in the UV and visible range from 200 nm to 1,000 nm. Since the experimental conditions were for Ar, Ar + H₂O, and Ar + CO₂, most emission lines from the radical generations included those with strong Ar peaks. In the study for bacterial interactions, it was believed that the radical reaction species made available impacted the bacterial performance and are shown in Table 2, Eqs. (1–4). (Takemura et al., 2014). The OH available species suggested a potential mechanism on the microbial inactivation due to the oxidative stresses present in the active oxygen radicals believed to be caused by both the gas and ambient air, which included any present water.

In the work of Kitazaki et al. (Kitazaki et al., 2014), the radical density was studied to show the importance of distance and spatial profiles of concentration of ions such as NO_x, and O concentrations, and specifically NO, NO₂ and O₃. The concentration of O₃ species decreased as the y-value (distance in mm) increased in distance from the seed surface. In air discharge, the most commonly observed species for NO_x compounds were NO and NO₂. This study revealed that the concentration of NO decreased as the distance from the seed increased, but NO₂ remained fairly stable when the distance varied. From the theoretical analysis and reference data collected, the major reactions in the discharge region are estimated, as shown in Table 2, Eqs. (1–10) (Kitazaki et al., 2014). NO₂ requires three reactions to reach a stable form, while NO requires only two reactions. It was difficult to find correlation between radical concentration emissions from this work, so the reaction rate constant, k (as displayed in Table 2 for some reactions), likely plays an important role in gas concentration production.

The species identified from the treated grain crops of the study by Filatova (Filatova et al., 2009), using RF plasma, were neutral molecular nitrogen, N₂, and ionized molecular nitrogen, N₂⁺. There was an observed increase in atomic lines O-I, N-I, and H-I. The CO and O₂⁺ molecules seen in the spectra confirmed that the plasma chemical etching of the seed surface also influenced the seed germination. The plasma treatment was effective for surface decontamination due to the oxygen atoms and active molecules generated in the air plasma. The low-pressure air plasma of the 5.28 MHz RF discharge treatment was used to stimulate plant seeds

Table 2. Potential reactions in the plasma discharge region.

Eq. #	Equation	Reference
(1)	$e^- + H_2O \rightarrow e^- + OH^\bullet + H$	(Takemura et al., 2014)
(2)	$e^- + CO_2 \rightarrow CO + O + e^-$	
(3)	$e^- + O_2 \rightarrow O(^1D) + O(^3P) + e^-$	
(4)	$O(^1D) + H_2O \rightarrow OH^\bullet + OH^\bullet$	
(1)	$e + O_2 \rightarrow 2O + e$	(Kitazaki et al., 2014)
(2)	$O + O_2 + M \rightarrow O_3 + M, k = 3.4 \times 10^{-34} \text{ cm}^6/\text{s}$	
(3)	$e + N_2 \rightarrow e + 2N$	
(4)	$N + O_2 \rightarrow NO + O, k = 7.7 \times 10^{-17} \text{ cm}^3/\text{s}$	
(5)	$N + O_3 \rightarrow NO + O_2, k = 3.7 \times 10^{-13} \text{ cm}^3/\text{s}$	
(6)	$O + NO_2 \rightarrow NO + O_2, k = 9.7 \times 10^{-12} \text{ cm}^3/\text{s}$	
(7)	$NO + O_3 \rightarrow NO_2 + O_2, k = 2.1 \times 10^{-14} \text{ cm}^3/\text{s}$	
(8)	$H_2O + e \rightarrow OH + H + e$	
(9)	$H + O_2 + M \rightarrow HO_2 + M, k = 1.8 \times 10^{-32} \text{ cm}^3/\text{s}$	
(10)	$NO + HO_2 \rightarrow NO_2 + OH, k = 7.8 \times 10^{-12} \text{ cm}^3/\text{s}$	

and positively impact their strength and branching of sprouts and roots.

In the study by Wang et al. (Wang et al., 2017), FTIR and OES data were used to observe interactions between the DBD air and N_2 plasma and cotton seeds for 3 min, 9 min, and 27 min. ATR-FTIR was applied to the seed surface, in addition to analysis of the exhaust discharge during plasma applications on the seed. The seed surface analysis revealed that the plasma treatment duration affected the intensity of the FTIR spectra bands but not the position or type of species generated. The intensity declines after plasma treatment for both air and N_2 . The reactive species that interacted with seeds were more easily generated when using N_2 flow discharge rather than air flow and were confirmed by the FTIR and OES data in the gas phase observation of the study. This observation was likely due to the electronegative nature of air due to O_2 ($O_2 + e^- \rightarrow O_2^-$) or ($O_2 + e^- \rightarrow O + O^-$) which potentially consumes extra electrons that would participate in further plasma ion activity. FTIR confirmed plasma-induced chemical etching on the surface, which likely caused hydrophilic behavior and therefore imbibition of water by seeds. The etching-enabled surface wetting and increased water crossing the hard coating of seeds resulted in the partial degradation of seed surface. NO , N_2O , NO_2 , and O were observed in FTIR. If the seed coat partially broke down and these chemical species entered or penetrated the seed, it may have stimulated biochemical processes for seed germination, in addition to deactivation and removal of pathogenic microorganisms. CO_2 , CO , and $-C(CH_3)_3^-$ groups may have been responsible for the chemical etching. The N_2 plasma may generate higher levels of oxygen-containing species and therefore could be a more efficient species to use in plasma gas (i.e., use air or N_2 , rather than

CO_2). Cellulose and wax were present on the cotton seeds. The hydrophobic (wax) and hydrophilic (cellulose) properties affect the behavior of the seed coat, with main impacts of plasma being on the seed epidermis cell wall. Wax is a protective barrier for prevention of water loss during exposure to elevated temperatures. When plasma degrades the wax, the water permeation is impacted, which is a main trigger for germination (Wang et al., 2017). It was theorized in this study that the decline in FTIR bands after plasma treatment on the seed surface was due to plasma chemical etching. Other studies researched in this review paper include that plasma may also impact metabolic rates or mRNA protein production activity, which could actually be the main trigger or surface etching, although increasing permeation may be the most easily observed reason (Panngom et al., 2014; Ling et al., 2015). This could also be very seed-type dependent, and more research would be required to verify these theories.

Along the topic of genetic studies, DNA damage was investigated by Švubová et al. (Švubová et al., 2021) on soybean seeds (*Glycine max* L. cv. Nížina). Švubová et al. concluded that the DCSBD plasma damaged DNA with increasing treatment times in each type of working gas, and so, DNA damage was considered negligible for this case. In the work of Tomeková et al. (Tomeková et al., 2020), DNA damage of DCSBD plasma treated pea seeds (*Pisum sativum* L.) concluded that using a working gas of air had minimal to no DNA damage, while increasing the Nitrogen working gas as well as that working gas plasma treatment time presented some DNA damage.

Khamsen et al. (Khamsen et al., n.d.) detected the excited species in pure air with OES during cold plasma treatment of rice. OES was performed for 20 s under air during plasma operation. N_2 species peaks were observed between 300 nm and 400 nm, and same was observed with air + Ar. These peaks confirmed reactive nitrogen and reactive oxygen species (RNS/ROS) generation under ambient air. N_2 - and Ar-excited species were important for surface modification and imbibition enhancement since results showed that, after plasma treatment, water imbibition was dramatically reduced and achieved worthy surface activation.

Ji et al. (Ji et al., 2016) used OES to observe emission lines of N_2 and air plasma during the treatment of spinach seeds. The reactive species of nitrogen was of most interest. In air plasma, both O_2 and atomic oxygen were observed along with high amounts of N^+ and N^{2+} . N_2 and NO were observed in both spectra, and authors theorized that these excited N_2 species are involved in oxidation of other molecules via removing electrons from them which had influence on biological processes and reactive states of the biological molecules.

In the plasma treatment of soybean seeds, Sinogovskaya et al. (Sinogovskaya et al., 2019) used OES to observe the air DBD and Ar/air microwave plasma emission spectra.

Corresponding peaks of Ar, NO, OH, N₂, NO₂, and O₃ were observed in the DBD system, while Ar, OH, N₂, NO₂, and O₃ were observed in the microwave plasma system. It was stated that, due to the plasma treatment, an increase in activation occurred for seeds to exit dormancy and cause an increase in germination and growing power, as observed by the increase in sprouts. The plasma activation was used to modify the seed cover and the internal structure. The internal structure was thought to initiate biological protection that increased resistance of the soybean to adverse factors.

The DBD plasma treatment of wheat seeds in the work of Guo et al. (Guo et al., 2018) utilized OES to detect and confirm the presence of N-radicals and O-radicals. Encouraging results were reported in the germination findings, and it was reported that the oxidation of the wheat seed coat occurred and a softening of the seed coat contributed to the improved water uptake and permeability. The active species detected with OES also likely penetrated into the seed to activate physiological reactivity that correlated to the study results of soluble protein and α -amylase activity enhancement.

Mošovská et al. (Mošovská et al., 2018) employed OES during the treatment of black peppercorn seeds to observe the spectra emission of the cold air plasma generated which emits in a UV region. The reported spectrum was fairly typical with molecular nitrogen radicals, and results reported that these species along with the UV radiation contribute to the decontamination effect on the seed. ATR-FTIR was also used to observe the seed coat surface before and up to 14 days after the plasma treatment, and minimal differences were observed as time increased, which concluded that the plasma treatment did not significantly affect sensory properties of the seed.

Microorganism Results

General observations from studies that investigated bacterial growth or inactivation typically resulted in positive outcomes to those treated with plasma when compared with the control. Bacteria appear to be more resistant microorganisms than filamentous fungi as well. A study of black peppercorn seeds confirmed a decreased amount of inoculated bacteria after DCSBD plasma treatment, as compared with the control (Mošovská et al., 2018). In the study by Takemura et al. (Takemura et al., 2014), *Bacillus subtilis* bacterial spores had the lowest bacteria survival rate in 5 min with the Ar + CO₂ plasma jet, followed by Ar + H₂O and Ar (Takemura et al., 2014). One reason for this may be due to the amount of UV light emitted from the Ar + CO₂ plasma versus Ar + H₂O or Ar, although this is not alluded to in the work. The manuscript discusses the potential impact of the OH radical produced in the Ar water vapor plasma and emphasizes that it should be taken into consideration as well. The DBD plasma study on rice seeds by Khamsen et al. (Khamsen et al., n.d.) showed

no pathogenic fungi observations on the treated seeds as compared with the untreated ones.

Zahoranová et al. (Anna Zahoranová et al., 2018) found a significant reduction of microorganisms on corn seeds after DCSBD plasma treatment, both naturally occurring and artificially inoculated on the seeds. As the plasma treatment time increased, the amount of microbiota and filamentous fungi decreased, which shows that there is a better inhibition effect on bacteria than fungi. The filamentous micromycetes on the seeds decreased in the order > *F. nivale* > *F. culmorum* > *T. roseum* > *A. flavus* > *A. clavatus* with varying treatment times.

The study carried out by Zahoranová et al. (Zahoranová et al., 2016) on wheat seeds artificially infected the seeds with cultures of filamentous fungi (*Fusarium nivale*, *F. culmorum*, *Trichothecium roseum*, *Aspergillus flavus*, and *A. clavatus*) to demonstrate the efficacy of the DCSBD plasma treatment.

Although the low plasma and vibrating-stirring device created by Ono et al. to irradiate species inactivated bacteria on the seeds, they expected a decrease in the germination rate, the damage caused to the seed surface, and occurrence of abnormal cotyledons, rot, and mold (Ono et al., 2017). Other works such as the study by Mitra et al. (Mitra et al., 2014) recommended shaking seeds for uniform exposure during plasma treatment, as used in their design, which would help influence the exposure area on the seed.

Mošovská et al. (Mošovská et al., 2018) demonstrated the efficacy of a low temperature plasma treatment generated by DCSBD in the reduction of vegetative bacterial cells and endospores inoculated onto black peppercorns. Whole black peppercorn seeds were sterilized by autoclaving at 121 °C for 20 min before inoculation with 3,500 μ L of 16-hour cultures (approximately 10⁸ cells per mL) of *Escherichia coli*, *Salmonella enteritidis*, or *B. subtilis*. In addition to the inoculation with vegetative cells of *B. subtilis*, endospore suspensions (1,750 μ L of approximately 10⁷/mL) prepared of the bacterium were prepared and inoculated onto the peppercorn seeds. The inoculated seeds were shaken for 10 min to obtain a homogenous coating and incubated to promote adherence to the seed surface. The viable bacterial load on the surface of the treated and untreated control peppercorn seeds was determined by surface extraction of the bacteria, followed by serial dilution and plating to determine CFU per gram. The reduction in viable cell load was calculated for the treatment as well as the inactivation kinetics. Devitalization of the vegetative cells and endospores increased with plasma treatment time; however, this varied by individual genera. The time to achieve a 1 log reduction or the decimal reduction time (D-value) for *B. subtilis* was the shortest exposure time at 43 s, followed by *E. coli* at 47 s and *S. enteritidis* at 58 s. Significant reductions were seen with increased plasma exposure times. After 300 s of treatment,

viable *B. subtilis* cells were reduced by $5.06 \log_{10}$, and *E. coli* and *S. enteritidis* cells were below the detection limit, a $>6 \log_{10}$ reduction. *B. subtilis* spores, as expected, were more resistant to the lethal effects of the plasma treatment. The D-value for the spore population was 142 s, and after 300 s of exposure, there was only a log reduction of 2.03 CFU/g.

In the study by Nishioka et al. (Nishioka et al., 2016), plasma treatment of brassicaceous seeds, *Xanthomonas campestris* pv. *Campestris* (the pathogenic black rot of cabbage and other cruciferous crops), observed a favorable reduction. The highest voltage studied was the most effective for inactivation of the *X. campestris* with approximately 5 log reduction in the viable cell number. It was also realized that varying the argon gas flow rate from 0.5 LPM to 1 LPM did not impact inactivation of the pathogen. The control seed was treated in 70% ethanol for surface disinfection, followed by rinsing with sterile distilled water. The researchers also pointed out that their experiments in treating Brassicaceous used higher pressures than those of any other previous studies and noted that the remaining air in the experimental chamber was potentially reacting. They also confirmed that ozone presence or increase in temperature as dry heat did not impact the inactivation of the pathogenic reduction based on control behavior. Nishioka et al. also did an intriguing investigation on the pathogen DNA and noted that plasma treatment inhibited DNA amplification. Specifically, from their QPCR (quantitative polymerase chain reaction) data, the target DNA of *hrpF* gene on the *X. campestris* chromosome inside the bacterial cells was damaged but still under debate if this was due to the plasma ionization or UV radiation from the plasma.

Puligundla et al. (Puligundla et al., 2017) investigated the inactivation of naturally occurring bacteria on radish seeds using CDPJ with the aim of utilizing the technology to increase the food safety of sprouted seeds. In this study, using selective plating techniques, several potentially pathogenic contaminants were detected on radish seeds, namely *B. cereus*, *E. coli*, and *Salmonella* spp., as well as yeasts and mold. Seeds were placed in petri dishes and subjected to the plasma treatment with a gap of about 2.5 cm between the electrode tip and sample at time points up to 3 min. The viable bacterial load was then determined utilizing selective plating, and a “D-like” value (D') was calculated similar to the decimal reduction value for the plasma treatment. Total aerobic bacteria, *E. coli*, and *B. cereus* decreased by 2.2, 2.0, and 1.2 log CFU/g, respectively. The *Salmonella* spp. and mold and yeast decreased by 1.7 log CFU/g. The 3 min plasma exposure showed significant reduction of microbes by 90–99% compared with the untreated seeds. The D value, or the time required to reduce bacterial viability by 90%, varied with specific organisms. *E. coli* had the lowest D value of 0.79 min, followed by mold and yeast (1.37 min) and *Salmonella* spp. (1.53 min). The D value was the highest for *B. cereus* at 2.20 min. Treated and untreated

seeds were germinated and analyzed for microbial load. The reduction of 1–2 \log_{10} CFU/g in counts for all genera, as well as total bacteria, was maintained for 4 days after germination in sprouts from plasma-treated seeds, improving the microbiological quality of sprouts.

Sprout seeds have complex surface structure which may cause challenges to plasma decontamination. In the study of Butscher et al. (Butscher et al., 2016), the increased surface area and roughness may have protected the microorganisms from the plasma-generated reactive species that aide in deactivation. This work particularly shows that the maximum germination and maximum microbial reduction were not simultaneous.

The inactivation of mold spores (*Aspergillus oryzae* and *Penicillium digitatum*) by active oxygen species generated from DBD plasma in combination with UV light was investigated in the work of Hayashi et al. (Hayashi et al., 2014). Fungal spores on the surface of lemon fruits and rice grains were microscopically examined and cultured on media after plasma/UV treatment. The mold spores were not inactivated with plasma or UV alone, but in combination, for a treatment period of 20 min, sterilization (no growth on medium) was achieved; however, microscopic examination of rice seeded with mold spores showed conidial growth indicating a viable but non-culturable state. Samples from the treated lemon fruits and rice grains were also cultured on a medium for 24 h at 58°C for the growth of endospores producing bacteria and thermophiles. The results after the incubation (no growth) indicated that the active oxygen inactivated the endospores similarly to the fungal spores. The proposed mechanism for cell death is the oxidation of cell wall components such as proteins and polysaccharides and in the case of endospores, dipicolinic acid.

Filatova et al. (Filatova et al., 2013) showed the decrease of the fungus, *Fusarium* spp., after using two plasma systems (plan-parallel and cylindrical) on spring wheat seeds. Infection of the seeds with *Fusarium* was decreased after 10 min plasma treatment; however, an increase in *Fusarium* spp. infection was observed for the planar discharge plasma for 100 W at 20 min, probably due to the damage of seed coat which may increase the seed susceptibility to the fungi. In another study by Filatova et al. (Filatova et al., 2009), *E. coli*, *S. aureus*, and *B. subtilis* were inoculated onto glass, plastic, and metal surfaces and subjected to RF discharge plasma treatment for 5 min, 10 min, 15 min, and 20 min. Results included a decrease of the microorganism survival rate to almost 0% at 10 min on the glass test surface. On the metal and plastic surfaces, there was a linear decrease in viable organisms over time of treatment with no detectable bacteria after 20 min of treatment.

The work of Kim et al. (Kim et al., 2017) resulted in aerobic bacteria having the highest counts at 8.5 log CFU/g, followed by *B. cereus*, molds and yeasts, *E. coli*, and *Salmonella* spp.

after plasma jet treatment. Pathogenic bacteria, namely *S. aureus* and *L. monocytogenes*, were not detected on the seed surfaces. The reduced microbial loads corresponded with enhanced germination rates that also did not impact physiochemical or sensory characteristics of the sprouts, including moisture content, reducing sugar content, phenolic content, DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity, and volatile basic nitrogen (VBN) content Kordas et al. (Kordas et al., 2015) tested 200 seeds from each plasma experiment and controls for mycological testing. The fungal colonies were counted and identified both macroscopically and microscopically with the morphology of hyphae, conidia, and sporangia. The fungal counts were compared with the disinfected seeds, and results showed that there was a reduction in the colonies of fungi which made the cold plasma treatment successful (Kordas et al., 2015). The number and frequency of fungal colonies isolated from non-disinfected grains for winter wheat grain were observed for 0 s, 3 s, 10 s, and 30 s of plasma exposure and included the following genera: *Alternaria alternata* (Fr.) Keissl, *Alternaria botrytis* (Preuss) Woudenberg and Crous, *Aspergillus brasiliensis* Varga. Frisvad and Samson, *Fusarium culmorum* (W.G. Sm.) Sacc, *Fusarium oxysporum* Schltdl., *Gibberella avenacea* R.J. Cook, *Gibberella intricans* Wollenw, *Gibberella zeae* (Schwein.) Petch, *Penicillium* spp., *Rhizopus stolonifer* (Ehrenb.) Vuill., *Trichoderma* spp., and non-spore-forming fungi. In most cases, as the duration of plasma exposure increased, overall fungal colonies decreased, but this varied widely between genera.

The investigation of disease reduction on seeds in the study of Štěpánová et al. (Štěpánová et al., 2018) concluded that the DCSBD plasma treatment can reduce the disease at the seed surface, shell, or endosperm but not in the core of cucumber (*Cucumis sativus* L.) and pepper (*Capsicum annuum* L.) seeds. No presence of *Cladosporium cucumerinum* was observed on the cucumber seeds in the study of Štěpánová et al. (Štěpánová et al., 2018) after DCSBD plasma treated seeds, and 60–80% reduction of *Didymella bryoniae* was observed. There was no effect on the presence of CMV (cucumber mosaic virus), ZYMV (zucchini yellow mosaic virus), and WMV (watermelon mosaic virus) diseases on the cucumber seeds. For the pepper seeds, a 50–80% reduction of *Didymella lycopersici* spores was observed as compared with the untreated seeds; and no impact on CMV when the pepper seeds were treated with the plasma was observed.

Pathogenic fungi, *Aspergillus* spp. and *Penicillium* spp., were artificially contaminated on rice and legume seed surfaces in the study by Sulcuk et al. (Sulcuk et al., 2008). Results after the air and SF₆ plasma treatments were successful on immediate and 10 day, 4°C stored plasma treated samples. Seed infection reduced to <1% without lowering the seed quality below any type of commercial threshold.

DISCUSSION

The literature surveyed in this review is vast, with each study having independent seed selections for various applications. Each individual study appeared to focus on a downstream application or incentivized marketing scale-up potential, rather than correlating differing plasma phenomena based on the seed shape, size, or traditional germination time. Therefore, the number of variables from each is too overwhelming to allow for careful correlation between various investigation focus topics or even plasma types. It is obvious that some standardization among tools and nomenclature is required for future work to adhere to consistency from study to study, including plasma system description (gas, flow rate, pressure, spatial distance of seed to plasma plume, power, seed amount), as well as performance results (specifically defined definition of “germination” and detailed description of microbial studies), and control parameters that consider system pressure effects on seed, rather than solely “untreated” seeds.

The general design of a plasma seed treatment system appeared to be most effective when the seeds have the closest possible proximity to the plasma field to receive effects of radical species for interaction onto the seed surface or permeation to the undercoat. The distance from plasma to seed location is an important feature to note, especially when considering the spatial distance of plasma radical formations, but is not always reported in literature. Hence, it is reported that the area of highest radical activity is where the seed is closer to the plume. Moving, shaking, or rotating the seeds is important for avoiding rise in temperature as plasma exposure is occurring, while also expanding the area of plasma treatment.

Analytical data collected from multiple instruments and observations are important to bring together and form an overall explanation of results, rather than just one specific instrument or measurement. For some studies, germination performance seems to have a threshold, a type of “optimal zone” (i.e., not enough, too much, or just right [optimal]) of plasma exposure. This zone could be understood best in combination with observations collected from water uptake, OES/FTIR, microorganism growth study, and pre/post plasma treatment SEM imaging to determine what may be happening to the seed during the plasma reaction phase, morphological changes of the seed coat, as well as microorganism viability and interactions. It can be summarized that initial treatment generating H₂O₂ and NO will trigger the breakdown of ABA (abscisic acid) and promote GA (gibberellic acid) biosynthesis to activate germination, while the prolonged plasma treatment time correlates with negative impacts on germination due to interactions with the seed coat performance (Ranieri et al., 2021). Excessive moisture on a seed can potentially decrease oxygen absorption, which impacts the germination process, so it is an important parameter to investigate when possible.

N₂ plasma may also be more efficient to use (in conjunction with air), rather than CO₂, in order to generate more oxygen-containing species available for interaction on the seed coat. Multiple studies using N₂ in the plasma system observed that the nitrogen species and radicals were indeed involved in the support of oxidation of other molecules that either allowed more penetration into the seed coat or electron removal of the seed coat, which had an influence on the biological or nutrient molecules and subsequent processes. Disrupting the seed coat as plasma degrades permeation layers may also lead to additional impact on metabolic rates or RNA. Correlations are not observed at this time and might be very seed-type dependent. In general, the seed coat hardness and thickness of the endosperm greatly impact the plasma effects. Nearly, every study that investigated the microbial reduction on the seed coat had some type of disinfecting or reducing effect on microbial survival after treatment. This has been ascribed to the active oxygen radicals generated at the surface between the plasma-generated species and impacts of the surface chemistry of the seed (Ranieri *et al.*, 2021). The short-lived radicals from plasma have a great impact for effective decontamination, but more so with flat surfaces, applications such as those with DBDs at room temperature and large volume (with low plasma density) take a longer time to process, compared with others. This is why the design of the experiment as well as analysis is so important.

These impacts of the plasma gas and generated ions are important to follow-up with water uptake tests to investigate the imbibition process and follow on germination or sterilization performance. Water imbibition (absorption) is the most important event to supply nutrients and activate seed growth, and so, water uptake is likely an important correlation to mark with germination. It is unclear if OES can detect any interaction from the seed holder if a non-inert material is used, but seed holder impacts were not greatly investigated in the literature. OES and FTIR are model tools that the authors strongly recommend being used in every plasma seed treatment study, based on the correlation of results that can be found on control versus plasma-treated seeds. XPS appears to be an underutilized tool when surveying the literature, and it is unknown if etching effects are a detriment to its use in this work since XPS is an inherent etching process but can reveal surface chemistry shifts based on treatments.

CONCLUSION

This review surveyed plasma applications to seeds to observe and understand the consistencies or inconsistencies in the area of this emerging research field and bring together the current state of the art in one complete report. This work involved two different communities of practice coming together:

plasma physics and agricultural sciences. The two often do not commonly overlap in research, and so, this very unique and niche area of growing interest does require some coordination on results and reporting. The general literature overview was of specific interest to the authors: the space agriculture team of NASA, as crop production is underway, and seeds are sanitized before being sent into space. Traditional methods such as gas fumigation are time consuming and involve noxious chemicals and are not viable for all seed types.

The analysis of the plasma type and system orientation, as well as performance results of seed germination, plant growth, and microbial analysis, is considerable. Crops investigated included corn, pepper, soybean, cabbage, wheat, legumes, grains, radish, cotton, and others. The most common plasma sources included RF, plasma jet, and DBD. The most common elements of analysis reported in results included germination and growth, water uptake, gas analysis using OES and FTIR, and seed analysis using SEM, XPS, and FTIR. The most consistent plasma apparatus was that of stationary seeds in as close proximity as possible to the plasma for uniform irradiation.

Of all literature surveyed, general results of primary focuses of investigation (germination, seedling health, microorganism treatments, wettability, sterilization) were favorable or had areas of improvement due to the application of plasma compared with controls. Some positive results were treatment dependent. There were no direct correlations between plasma treatment times, types, and germination rates in a broad sense; however, there seems to potentially exist an optimal threshold for plasma treatment time based on performance. It appeared that each seed type responds differently to plasma exposure and experimental parameters, and while plasma treatment time varied between each study, a magnitude of <10 min of exposure was typical. Plasma applications drastically reduced the number of pathogenic bacteria and fungi after treatment of the seeds. Results from multiple studies support the hypothesis that oxygen and nitrogen radicals permeate through seed coats to promote deactivation of bacteria and induce positive growth and performance. Some studies show that storage time (although short) did not impact properties of the seed. Room for improvements in reporting includes inclusion of key parameters such as seed type, seed amount, seed distance to plasma, plasma power, plasma system pressure, plasma OES/FTIR chemistry without and with seed interaction, plasma gas and flow rate, and control parameters that consider the plasma system pressure effects on the seed.

ACKNOWLEDGMENTS

Funding for this work was provided by the NASA KSC Independent Research and Technology Development (IR&TD)

Program. The authors would like to thank inputs from the project team at Kennedy Space Center (KSC), including Dr. Ray Wheeler, intern Aniya Norvell, and Dr. Ryan Gott.

DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

- Adhikari B, Pangomm K, Veerana M, Mitra S, Park G (2020) Plant disease control by non-thermal atmospheric-pressure plasma. *Frontiers in Plant Science* **11**(February), 77. doi: 10.3389/fpls.2020.00077
- Ahn C, Gill J, Ruzic DN (2019) Growth of plasma-treated corn seeds under realistic conditions. *Scientific Reports* **9**(1). doi: 10.1038/s41598-019-40700-9
- Attri P, Ishikawa K, Okumura T, Koga K, Shiratani M (2020) Plasma agriculture from laboratory to farm: a review. *Processes* **8**(8), 1002. doi: 10.3390/pr8081002
- Bafoil M, Jemmat A, Martinez Y, Merbahi N, Eichwald O, Dunand C, Yousfi M (2018) Effects of low temperature plasmas and plasma activated waters on arabidopsis thaliana germination and growth. *PLoS ONE* **13**(4), 1–16. @doi: 10.1371/journal.pone.0195512
- Bourke P, Ziuzina D, Boehm D, Cullen PJ, Keener K (2018) The potential of cold plasma for safe and sustainable food production. *Trends in Biotechnology* **36**(6), 615–626. doi: 10.1016/j.tibtech.2017.11.001
- Brandenburg R (2018) Corrigendum: dielectric barrier discharges: progress on plasma sources and on the understanding of regimes and single filaments (2017 *Plasma Sources Sci. Technol.* 26 053001). *Plasma Sources Science and Technology* **27**(7), 079501. doi: 10.1088/1361-6595/aaced9
- Butscher D, Loon HV, Waskow A, von Rohr PR, Schuppler M (2016) Plasma inactivation of microorganisms on sprout seeds in a dielectric barrier discharge. *International Journal of Food Microbiology* **238**(December), 222–232. doi: 10.1016/j.ijfoodmicro.2016.09.006
- Černák M, Černáková L', Hudec I, Kováčik D, Zahoranová A (2009) Diffuse coplanar surface barrier discharge and its applications for in-line processing of low-added-value materials. *The European Physical Journal Applied Physics* **47**(2), 22806. doi: 10.1051/epjap/2009131
- da Silva ARM, Farias ML, da Silva DLS, Vitoriano JO, de Sousa RC, Alves-Junior C (2017) Using atmospheric plasma to increase wettability, imbibition and germination of physically dormant seeds of mimosa caesalpiniaefolia. *Colloids and Surfaces B: Biointerfaces* **157**(September), 280–285. doi: 10.1016/j.colsurfb.2017.05.063
- de Groot GJJ, Hundt A, Murphy AB, Bange MP, Mai-Prochnow A (2018) Cold plasma treatment for cotton seed germination improvement. *Scientific Reports* **8**(1), 14372. doi: 10.1038/s41598-018-32692-9
- Dobrin D, Magureanu M, Mandache NB, Ionita M-D (2015) The effect of non-thermal plasma treatment on wheat germination and early growth. *Innovative Food Science & Emerging Technologies* **29**(May), 255–260. doi: 10.1016/j.ifset.2015.02.006
- Feizollahi E, Iqdiar B, Vasanthan T, Thilakarathna MS, Roopesh MS (2020) Effects of atmospheric-pressure cold plasma treatment on deoxynivalenol degradation, quality parameters, and germination of barley grains. *Applied Sciences* **10**(10), 3530. doi: 10.3390/app10103530
- Filatova I, Azharonok V, Gorodetskaya E, Mel'nikova L, Shedikova O, Shik A (2009) *Plasma-Radiowave Stimulation of Plant Seeds Germination and Inactivation of Pathogenic Microorganisms*. 4. Bochum, Germany.
- Filatova I, Azharonok V, Lushkevich V, Zhukovsky A, Gadzhieva G, Spasi K, Živkovi S (2013) Plasma seeds treatment as a promising technique for seed germination improvement. 4.
- Gómez-Ramírez A, López-Santos C, Cantos M, García JL, Molina R, Cotrino J, Espinós JP, González-Elipse AR (2017) Surface chemistry and germination improvement of quinoa seeds subjected to plasma activation. *Scientific Reports* **7**(1), 5924. doi: 10.1038/s41598-017-06164-5
- Guo Q, Meng Y, Qu G, Wang T, Yang F, Liang D, Hu S (2018) Improvement of wheat seed vitality by dielectric barrier discharge plasma treatment: seed treatment by discharge plasma. *Bioelectromagnetics* **39**(2), 120–131. doi: 10.1002/bem.22088
- Hayashi N, Yagyu Y, Yonesu A, Shiratani M (2014) Sterilization characteristics of the surfaces of agricultural products using active oxygen species generated by atmospheric plasma and UV light. *Japanese Journal of Applied Physics* **53**(5S1), 05FR03. doi: 10.7567/JJAP.53.05FR03
- Ito M, Oh J-S, Ohta T, Shiratani M, Hori M (2018) Current status and future prospects of agricultural applications using atmospheric-pressure plasma technologies. *Plasma Processes and Polymers* **15**(2), 1700073. doi: 10.1002/ppap.201700073
- Ji S-H, Choi K-H, Pengkit A, Im JS, Kim JS, Kim YH, Park Y, et al. (2016) Effects of high voltage nanosecond pulsed plasma and micro DBD plasma on seed germination, growth development and physiological activities in spinach. *Archives of Biochemistry and Biophysics* **605**(September), 117–128. doi: 10.1016/j.abb.2016.02.028
- Jo Y-K, Cho J, Tsai T-C, Staack D, Kang M-H, Roh J-H, Shin D-B, Cromwell W, Gross D (2014) A non-thermal plasma seed treatment method for management of a seedborne fungal pathogen on rice seed. *Crop Science* **54**(2), 796–803. doi: 10.2135/cropsci2013.05.0331
- Khamen N, Onwimol D, Teerakawanich N, Dechanupaprittha S, Kanokbannakorn W, Hongesombut K, Srisophon S (n.d.) Rice (*Oryza sativa* L.) seed sterilization and germination enhancement via atmospheric hybrid non-thermal discharge plasma. 9.

- Kim J-W, Puligundla P, Mok C (2017) Effect of corona discharge plasma jet on surface-borne microorganisms and sprouting of broccoli seeds: effect of corona discharge plasma jet on broccoli sprouting. *Journal of the Science of Food and Agriculture* **97**(1), 128–134. doi: 10.1002/jsfa.7698
- Kitazaki S, Sarinont T, Koga K, Hayashi N, Shiratani M (2014) Plasma induced long-term growth enhancement of *Raphanus sativus* L. using combinatorial atmospheric air dielectric barrier discharge plasmas. *Current Applied Physics* **14**(July), S149–S153. doi: 10.1016/j.cap.2013.11.056
- Kordas L, Pusz W, Czapska T, Kacprzyk R (2015) The effect of low-temperature plasma on fungus colonization of winter wheat grain and seed quality. *Polish Journal of Environmental Studies* **24**(1), 433–438.
- Laroussi M (2005) Low temperature plasma-based sterilization: overview and state-of-the-art. *Plasma Processes and Polymers* **2**(5), 391–400. doi: 10.1002/ppap.200400078
- Li L, Li J, Shen M, Hou J, Shao H, Dong Y, Jiang J (2016) Improving seed germination and peanut yields by cold plasma treatment. *Plasma Science and Technology* **18**(10), 1027–1033. doi: 10.1088/1009-0630/18/10/10
- Ling L, Jiafeng J, Jiangang L, Minchong S, Xin H, Hanliang S, Yuanhua D (2015) Effects of cold plasma treatment on seed germination and seedling growth of soybean. *Scientific Reports* **4**(1). doi: 10.1038/srep05859
- Măgureanu M, Sirbu R, Dobrin D, Gidea M (2018) Stimulation of the germination and early growth of tomato seeds by non-thermal plasma | SpringerLink. *Plasma Chemistry and Plasma Processing* **38**, 989–1001. doi: 10.1007/s11090-018-9916-0
- Massa GD, Newsham G, Hummerick ME, Morrow RC, Wheeler RM (2020) Plant pillow preparation for the veggie plant growth system on the international space station. *Gravitational and Space Research* **5**(1), 24–34. doi: 10.2478/gsr-2017-0002
- Matra K (2016) Non-thermal plasma for germination enhancement of radish seeds. *Procedia Computer Science* **86**, 132–135. doi: 10.1016/j.procs.2016.05.033
- Mitra A, Li Y-F, Klämpfl TG, Shimizu T, Jeon J, Morfill GE, Zimmermann JL (2014) Inactivation of surface-borne microorganisms and increased germination of seed specimen by cold atmospheric plasma. *Food and Bioprocess Technology* **7**(3), 645–653. doi: 10.1007/s11947-013-1126-4
- Mošovská S, Medvecká V, Halászová N, Ďurina P, Valík L, Mikulajová A, Zahoranová A (2018) Cold atmospheric pressure ambient air plasma inhibition of pathogenic bacteria on the surface of black pepper. *Food Research International* **106**(April), 862–869. doi: 10.1016/j.foodres.2018.01.066
- Nishioka T, Takai Y, Mishima T, Kawaradani M, Tanimoto H, Okada K, Misawa T, Kusakari S (2016) Low-pressure plasma application for the inactivation of the seed-borne pathogen *Xanthomonas campestris*. *Biocontrol Science* **21**(1), 37–43. doi: 10.4265/bio.21.37
- Ollegott K, Wirth P, Oberste-Beulmann C, Awakowicz P, Muhler M (2020) Fundamental properties and applications of dielectric barrier discharges in plasma-catalytic processes at atmospheric pressure. *Chemie Ingenieur Technik* **92**(10), 1542–1558. doi: 10.1002/cite.202000075
- Ono R, Uchida S, Hayashi N, Kosaka R, Soeda Y (2017) Inactivation of bacteria on plant seed surface by low-pressure rf plasma using a vibrating stirring device. *Vacuum* **136**(February), 214–220. doi: 10.1016/j.vacuum.2016.07.017
- Pannongom K, Lee SH, Park DH, Sim GB, Kim YH, Uhm HS, Park G, Choi EH (2014) Non-thermal plasma treatment diminishes fungal viability and up-regulates resistance genes in a plant host. Edited by Yong-Sun Bahn. *PLoS ONE* **9**(6), e99300. doi: 10.1371/journal.pone.0099300
- Pérez-Pizá MC, Prevosto L, Grijalba PE, Zilli CG, Cejas E, Mancinelli B, Balestrasse KB (2019) Improvement of growth and yield of soybean plants through the application of non-thermal plasmas to seeds with different health status. *Heliyon* **5**(4), e01495. doi: 10.1016/j.heliyon.2019.e01495
- Pérez-Pizá MC, Prevosto L, Zilli C, Cejas E, Kelly H, Balestrasse K (2018) Effects of non-thermal plasmas on seed-borne diaporthe/ phomopsis complex and germination parameters of soybean seeds. *Innovative Food Science & Emerging Technologies* **49**(October), 82–91. doi: 10.1016/j.ifset.2018.07.009
- Puač N, Gherardi M, Shiratani M (2018) Plasma agriculture: a rapidly emerging field. *Plasma Processes and Polymers* **15**(2), 1700174. doi: 10.1002/ppap.201700174
- Puligundla P, Kim J-W, Mok C (2017) Effects of nonthermal plasma treatment on decontamination and sprouting of radish (*Raphanus sativus* L.) seeds. *Food and Bioprocess Technology* **10**(6), 1093–1102. doi: 10.1007/s11947-017-1886-3
- Randeniya LK, de Groot GJJ (2015) Non-thermal plasma treatment of agricultural seeds for stimulation of germination, removal of surface contamination and other benefits: a review. *Plasma Processes and Polymers* **12**(7), 608–623. doi: 10.1002/ppap.201500042
- Ranieri P, Sponsel N, Kizer J, Rojas-Pierce M, Hernández R, Gatiboni L, Grunden A, Stapelmann K (2021) Plasma agriculture: review from the perspective of the plant and its ecosystem. *Plasma Processes and Polymers* **18**(1), 2000162. doi: 10.1002/ppap.202000162
- Selcuk M, Oksuz L, Basaran P (2008) Decontamination of grains and legumes infected with aspergillus spp. and penicillium spp. by cold plasma treatment. *Bioresource Technology* **99**(11), 5104–5109. doi: 10.1016/j.biortech.2007.09.076
- Šerá B, Šerý M (2018) Non-thermal plasma treatment as a new biotechnology in relation to seeds, dry fruits, and grains. *Plasma Science and Technology* **20**(4), 044012. doi: 10.1088/2058-6272/aaacc6
- Shapira Y, Chaniel G, Bormashenko E (2018) Surface charging by the cold plasma discharge of lentil and pepper seeds in comparison with polymers. *Colloids and Surfaces B: Biointerfaces* **172**(December), 541–544. doi: 10.1016/j.colsurfb.2018.09.004
- Shapira Y, Multanen V, Whyman G, Bormashenko Y, Chaniel G, Barkay Z, Bormashenko E (2017) Plasma treatment switches

- the regime of wetting and floating of pepper seeds. *Colloids and Surfaces B: Biointerfaces* **157**(September), 417–423. doi: 10.1016/j.colsurfb.2017.06.006
- Šimončicová J, Kryštofová S, Medvecká V, Ďurišová K, Kaliňáková B (2019) Technical applications of plasma treatments: current state and perspectives. *Applied Microbiology and Biotechnology* **103**, 5117–5129
- Sinegovskaya VT, Kamanina LA, Vasil'ev MM, Petrov OF (2019) Effect of plasma treatment of soybean seeds on their quality and development of seedlings. *Russian Agricultural Sciences* **45**(1), 26–29. doi: 10.3103/S1068367419010142
- Song J-S, Kim SB, Ryu S, Oh J, Kim D-S (2020) Emerging plasma technology that alleviates crop stress during the early growth stages of plants: a review. *Frontiers in Plant Science* **11**(July), 988. doi: 10.3389/fpls.2020.00988
- Štěpánová V, Slaviček P, Kellar J, Prášil J, Smékal M, Stupavská M, Jurmanová J, Černák M (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 Processes and Polymers* **15**(2), 1700076. doi: 10.1002/ppap.201700076
- Stolárik T, Henselová M, Martinka M, Novák O, Zahoranová A, Černák M (2015) Effect of low-temperature plasma on the structure of seeds, growth and metabolism of endogenous phytohormones in pea (*Pisum sativum* L.). *Plasma Chemistry and Plasma Processing* **35**(4), 659–676. doi: 10.1007/s11090-015-9627-8
- Švubová R, Slováková L, Holubová L, Rovňanová D, Gálová E, Tomeková J (2021) Evaluation of the impact of cold atmospheric pressure plasma on soybean seed germination. *Plants* **10**(1), 177. doi: 10.3390/plants10010177
- Švubová R, Kyzek S, Medvecká V, Slováková L, Gálová E, Zahoranová A (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 Chemistry and Plasma Processing* **40**(5), 1221–1240. doi: 10.1007/s11090-020-10089-9
- Takemura Y, Umeji S, Ito K, Furuya S, Furuta M (2014) Inactivation treatment of bacterial spores contaminated spices by atmospheric plasma jet. *Plasma Medicine* **4**(1–4), 89–100. doi: 10.1615/PlasmaMed.2014011969
- Tomeková J, Kyzek S, Medvecká V, Gálová E, Zahoranová A (2020) Influence of cold atmospheric pressure plasma on pea seeds: dna damage of seedlings and optical diagnostics of plasma. *Plasma Chemistry and Plasma Processing* **40**(6), 1571–1584. doi: 10.1007/s11090-020-10109-8
- Wang X-Q, Zhou R-W, de Groot G, Bazaka K, Murphy AB, Ostrikov K (2017) Spectral characteristics of cotton seeds treated by a dielectric barrier discharge plasma. *Scientific Reports* **7**(1). doi: 10.1038/s41598-017-04963-4
- Waskow A, Howling A, Furno I (2021) Mechanisms of plasma-seed treatments as a potential seed processing technology. *Frontiers in Physics* **9**(April). doi: 10.3389/fphy.2021.617345
- Waskow A, Betschart J, Butscher D, Oberbossel G, Klöti D, Büttner-Mainik A, Adamcik J, von Rohr PR, Schuppler M (2018) Characterization of efficiency and mechanisms of cold atmospheric pressure plasma decontamination of seeds for sprout production. *Frontiers in Microbiology* **9**(December). doi: 10.3389/fmicb.2018.03164
- Zahoranová A, Henselová M, Hudecová D, Kaliňáková B, Kováčik D, Medvecká V, Černák M (2016) Effect of cold atmospheric pressure plasma on the wheat seedlings vigor and on the inactivation of microorganisms on the seeds surface. *Plasma Chemistry and Plasma Processing* **36**(2), 397–414. doi: 10.1007/s11090-015-9684-z.
- Zahoranová A, Hoppanová L, Šimončicová J, Tučeková Z, Medvecká V, Hudecová D, Kaliňáková B, Kováčik D, Černák M (2018) Effect of cold atmospheric pressure plasma on maize seeds: enhancement of seedlings growth and surface microorganisms inactivation. *Plasma Chemistry and Plasma Processing* **38**(5), 969–988. doi: 10.1007/s11090-018-9913-3