Review

Improving Seed Germination by Cold Atmospheric Plasma

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Abstract: Cold atmospheric plasma (CAP) is a tunable source of reactive species and other physical factors. It exerts luxuriant biochemical effects on diverse cells, including bacterial cells, mammalian cells, and plant cells. Over the past decade, CAP has shown promising application in modern agriculture. Here, we focused on the state of the art of plasma agriculture, particularly the improvement of seed germination rates. Typical plasma sources, underlying physical principles, and the chemical and cellular mechanism of plasma’s effect on plants seeds have been discussed in depth.

Keywords: cold atmospheric plasma; plasma agriculture; seed germination

1. Cold Atmospheric Plasma (CAP): Sources and Physics

As a phase of matter, plasma can easily be found in many places, from household objects to deep space, such as fluorescent lamps, lightning, nuclear fusion, the ionosphere, the sun, and all other stars [1]. The first definition of plasma was introduced by Irving Langmuir, who described plasma as an ionized gas containing ions and electrons with a roughly equal charge as a whole. In other words, plasma is an ionized gas with a quasi-neutrality [2]. Ionizations can be achieved by particle collisions with energies higher than their ionization thresholds.

Heating and applying an electric field are two common ways of energizing collisions. The former strengthens the random motion of particles, while the latter accelerates naturally existed “seed” electrons to knock out more electrons from atoms. However, the electrons with lower mass are usually accelerated prior to ions at the beginning of applying an external electric field. The higher drift motion of these electrons causes a higher random motion (thermal motion, also called “Brownian motion”) during collisions, resulting in a different temperature distribution from other species [3]. Such plasma is called thermal nonequilibrium plasma. In contrast, the accelerated electrons in thermal equilibrium plasma deliver their kinetic energy to other particles during collisions until all of them share the same temperature profile [4].

Cold atmospheric plasma (CAP) is a thermal nonequilibrium plasma with all heavy particles at near room temperature (average temperature) because the collision frequency of electrons with other particles or species is not high enough to make the plasma reach thermal equilibrium before those free electrons are lost during the diffusion, the recombination, or the attachment with atoms [5].

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CAP has been studied for many years, and multiple generators have been invented. The most popular hardware includes the Cold Atmospheric Plasma Jet (CAPJ), Dielectric Barrier Discharge (DBD) reactor, radiofrequency (RF) reactor, and surface DBD source [6]. To avoid arc discharge, either the anode or cathode will be covered by a layer of dielectric materials such as glass, quartz, ceramic, or others [7]. Noble gases such as helium (He) and argon (Ar) are used to trigger the stable and nonthermal glow-like discharge in CAP.
sources [8]. For the CAP jet, the samples do not involve the discharge process (Figure 1). Thus, the CAP jet is an indirect discharge source [9]. In contrast, the DBD source is a direct discharge source, in which samples such as seeds are involved in the discharge process [10]. Compared with the CAP jet, DBD plasma has a short length but can affect a much larger area simultaneously. However, the nature of the CAP jet makes it so that it has a high aspect ratio and can even reach 11 mm of length [11]. Thus, the DBD reactor is more suitable for a large area treatment compared to the CAP jet. Similarly, surface DBD is a unique DBD configuration, in which two electrodes have been integrated in one surface [12]. Such a design overcomes the sample areas’ three-dimensional space limitation. Even a large sample can also be treated by a surface DBD source. The discharge in a radiofrequency (RF) plasma reactor is based on the RF power input between two electrodes, rather than AC or DC power [10]. Typically, RF plasma generates in a chamber, where samples such as seeds are set in the gaps between electrodes.

![Figure 1. Some CAP sources used in plasma agriculture. (a) A CAP jet and its discharge photo [9]. (b) A DBD reactor and structure [10]. (c) A radiofrequency (RF) reactor and structure [10]. (d) Two surface DBD sources with honeycomb electrodes and strip electrodes [12].](image)

Here, we shortly explained the physical mechanism of CAP formation using the CAP jet as an example. Specifically, taking the CAP jet as an example, a single electrode or a pair of high voltage (several kilovolts) electrodes are separated from a noble gas flow by dielectric material such as a glass tube. The electric field is thus applied on the noble gas, and results in an ionization wave (IW), which is defined as the propagation of an ionization rate peak [13]. The wave can thus propagate to the areas that the noble gas flow covers. The photon emission during the IW propagation luminates the ionization region called the ‘plasma bullet’ [14]. The bullets are usually observed in an Intensified Charge-Coupled Device (ICCD).

2. Reactive Species in CAP

The electron impact ionizations and excitations are not the only result of electron collisions during discharge. An abundance of reactive species are also formed in CAP [15]. Due to the emission, many excited species can be identified from spectra, such as an Optical Emission Spectrum (OES). A typical OES of plasma in DBD was shown in Figure 2a, in which N$_2$ or N$_2^+$ usually have the top peak. It is generally understood that the reactive species in CAP build the foundation for the interaction between CAP and cells, including
bacterial, fungal, mammalian, and plant cells [16,17]. The electrons can be captured by the reactive species with strong negativity such as oxygen. The electrons can also recombine with ions, such as N$_2^+$, O$_2^+$, NO$^+$, and He$_2^+$. Besides the electron collisions, other reactive species also collide with each other to form new species. For example, the penning ionization of metastable helium (He(2$^3$S)) and nitrogen collide to form N$_2^+$ and He. Some reactions will form important chemicals such as NO, N$_2$O, NO$_2$, as well as OH. These chemicals may play a key role during the interaction between CAP and cells. Some typical reactions formulae are shown as the examples. O + O$_2^-$ => O$_3$ + e, N + O$_2$ => NO + O, N$_2$(A$^3$Σ$^+u$) + O$_2$ => N$_2$O + O, O$_2$ + N$_2$O$^+$ => NO$^+$ + NO$_2$, N$_2$(A$^3$Σ$^+u$) + H$_2$O => H + OH + N$_2$, H$_2$O$^+$ + O$_2^-$ => O$_2$ + OH + H, N$^+$ + H$_2$O => NO$^+$ + H$_2$, etc. Note that the hydrogen element comes from the humidity components in air [18]. The reaction rate coefficients of these reactions are significantly affected by the electric field, electron temperature, gas bulk temperature, as well as the densities of reactants. The initial concentration of these species is also important for these reactions. The initial mole fraction of He, N$_2$, and O$_2$ can be controlled by the flow rate of a carrying gas such as He and the geometry of the nozzle [19]. In addition, the humidity of air is another key but hardly controllable factor for the formation of reactive species in plasma [18].

**Figure 2.** Reactive species in gas phase and liquid phase. (a) An emission spectrum of plasma in the gas phase of DBD in the range from 200–400 nm [17]. (b) General picture of CAP-aqueous solution interactions [20].

Reactive species in the gas phase will have complex CAP-aqueous solution interactions when plasma touches solution (Figure 2b). The main reactive species in CAP can be divided into two categories: short-lived and long-lived reactive species. Short-lived reactive species,
such as OH and $^{1}O_{2}$, may only affect the cells near cold plasma over a short-time scale [21].
In contrast, long-lived reactive species, such as $H_{2}O_{2}$, $O_{3}$, NO, and $NO_{2}^{-}$, may be capable of affecting cells from the site even far from plasma through the diffusion in aqueous environments over a long-time scale [22]. In addition, many aqueous components, such as amino acids in medium, are modified during CAP treatment [23]. The pH in the CAP-treated solutions may also be changed; however, such a change is negligible because of the existence of buffering chemicals in solutions, such as phosphate monobasic ($H_{2}PO_{4}^{-}$), phosphate dibasic ($HPO_{4}^{2-}$), and bicarbonate ($HCO_{3}^{-}$) [24,25]. All these chemical changes may finally contribute to the biological impact of CAP on plant cells.

3. Plasma Agriculture, an Overview

The chemical effect of CAP may build the foundation for plasma agriculture [26,27]. The pathogen-based disease therapy, the resistance to abiotic stress, food sterilization during storage, as well as the improvement of germination rates are challenges in modern agriculture. CAP may help humans to overcome all these challenges through abundant and controllable reactive species generation [28–30].

Pathogen-based plant diseases are profoundly affecting crops worldwide [31]. Naturally, $H_{2}O_{2}$ production in plants can kill pathogens directly or induce defense genes to limit the infection [32–34]. CAP is a powerful source of ROS including $H_{2}O_{2}$ and others. A CAP jet was capable of curing the fungus-infected plant leaves and inhibiting the spread of infection (Figure 3) [35]. The leaves with the small (<2 mm) black spots infected with fungal cells could be completely recovered from the infection [35]. A CAP jet with a relatively high plasma density could completely kill the tomato pathogen $C. fulvum$ and decrease the rotting rate of the infected tomato seeds within a short treatment time [31]. Aspergillus niger is a dangerous pathogen of date-palm fruits due to the production of fumonisin B2 (FB2) and ochratoxin A (OTA) [36]. A double atmospheric pressure argon cold plasma (DAPACP) jet system showed promising results in the inhibition of mycotoxin release by Aspergillus niger [36]. The pathogenic microorganisms of diverse sprout seeds play a crucial role in the pathogenesis of sprout-related outbreaks [37]. Three minutes of corona discharge plasma jet (CDPJ) treatment showed strong killing capability on aerobic bacteria, models and yeast, $Bacillus cereus$, $Escherichia coli$, $Salmonella$ spp. in rapeseeds [37].

 Furthermore, CAP is a safe tool for food decontamination. Diverse pathogens are large threats to the modern food industry. The abundant reactive species components in CAP make it a flexible and low-cost food processing technology for vegetables, fruits, poultry, as well as meats [38–41]. For example, a nitrogen CAP inactivated $S. typhimurium$ on potato, strawberry, and lettuce [42]. CAP treatment is also desirable for in-package
decontamination. CAP significantly eliminated the postprocess contamination while retaining the essential quality characteristics of strawberries [43]. The foods’ cross-contamination from persistent pathogen reservoirs is a risk in processing environments. CAP rapidly decontaminated the food surface with *salmonella* biofilms [44]. CAP treatment increased the tomato’s resistance to *R. solanacearum* with an efficacy of 25%, which might be due to the regulation of peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase in tomato [45]. CAP also effectively inactivated the spoilage bacteria *P. fluorescens* and *M. caseolyticus* in packages [46]. Moreover, a short-pulsed CAP has shown its strong effect of eliminating foodborne pathogens *salmonella* and *E. coli* O157:H7 on almonds [47]. Compared with the dry air, nitrogen, as a carrying gas, resulted in a worse antimicrobial efficacy [47].

Additionally, CAP treatment can trigger the tolerance of plants to various abiotic and biotic stress. CAP was used to help oilseed rape seedlings resist the damage caused by the drought stress tomato seeds [45,48,49]. Under drought stress, the direct CAP treatment significantly improved seedling growth characteristics, including shoot and root dry weights, shoot and root lengths, and lateral root number in a drought-sensitive oilseed [48]. ROS and RNS in CAP may mainly contribute to the tolerance of plants to diverse stresses. Naturally, the \( \text{H}_2\text{O}_2 \) production in plants also induces the resistance to various stresses, including ultraviolet radiation (UV), salt stress, drought stress, light stress, metal stress, and high or low temperature [32]. \( \text{H}_2\text{O}_2 \) is involved in the abscisic acid (ABA)-induced stomatal opening and closing [32,34,50]. NO has been identified as an important signaling molecule in plant immune response [51]. NO is also involved in the ABA-induced stomatal closure in epidermal peels [52]. NO can also strongly counteract many ROS-mediated cytotoxic processes in plants [53]. Furthermore, NO possesses antioxidant properties and might act as a signal in activating the ROS-scavenging enzyme activities under abiotic stress, such as salt, drought, temperature, UV, and heavy metal stress [54–56]. Several NO donors, including sodium nitroprusside, S-nitroso-N-acetylpenicillamine, and a mixed solution of ascorbic acid and NaNO\(_2\), can prevent chlorophyll loss [57].

In addition to the direct CAP treatment on plants or seeds, aqueous solutions (particularly, water) have been used as the carriers for the CAP-originated reactive species through CAP treatment on these solutions [39,58–69]. CAP-stimulated solution (PSS) can be widely used in many circumstances independent of CAP devices (Figure 4). PSS was also named as CAP-activated solution (PAS) or water (PAW) in some references [46,70–72]. PSS contains an abundance of reactive species and has promising applications in agriculture. PSS has been shown to have a strong killing effect on bacteria [46,70–72] and viruses [41,73,74].
ABA, modifies redox status, and induces protein carbonylation [33,77,80–82]. Several RNS, promote the release of seed dormancy and seed germination via affecting metabolism, and a mixed solution of ascorbic acid and NaNO2, can prevent chlorophyll loss [57]. Several NO donors, including sodium nitroprusside, S-nitroso-acetylpenicillamine, and other downstream events in seeds to further trigger germination [33,77]. Specifically, OH• facilitates the loosening process of the cell wall, along with the protrusion and the elongation of radicles, which are the necessary steps for germination [33,78]. H2O2 stimulates the seed germination of cereal plants such as pea, apple, barley, wheat, and rice by facilitating the removal of ethanol-soluble compounds from the seeds with pericarp or by the oxidation or decomposition of the germination inhibitors in pericarp [33,79,80]. More importantly, H2O2 directly interferes with ABA and GA signaling pathways, breaks down ABA, modifies redox status, and induces protein carbonylation [33,77,80–82]. Several RNS, such as NO, NO2•, NO3•, NO2, or NO donor, such as sodium nitroprusside (SNP) also promote the release of seed dormancy and seed germination via affecting metabolism, oxidation state, as well as other signaling pathways, such as ABA/GAs-based pathways in seeds [55,77,81–88].

4.2. CAP’s Capability

As a controllable ROS/RNS source, CAP treatment has significantly improved the seed germination rate, speed, water uptake, seed vigor, as well as several key characteristics of seedling growth, including the shoot length, shoot dry weight, root length, and root dry weight of several seeds [89]. These effects were observed in chickpea seeds [75], cotton seeds [90], soybean seeds [91], brown rice seed [92], mung bean seeds [93], rapeseed [37], erthyrida velutina seeds [94], sweet basil seeds [95], wheat seed [96,97], spinach seed [98], 

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<th>Making PSS</th>
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![Diagram](Image)

**Figure 4.** The application of CAP-stimulated solution (PSS) in agriculture. Here, PSS was made by the vertical treatment on solutions. Subsequently, the PSS containing 3-nitro-L-tyrosine can be stably stored in the container at 2 °C to 8 °C for at least a week. PSS can be used to kill the pathogens grown on the plant. In addition, the soak of seeds in PSS will improve the seed germination rate.

4. Seeds Germination

4.1. Germination Mechanism

Improving the seed germination rate is a main challenge in agriculture [75]. In agriculture, most crop seeds are dispersed in a dry and mature state during storage. When environmental conditions are favorable, seeds will initiate a complex process of germination [76,77]. Seed germination is a complex physiological process involving strictly regulated signaling pathways affected by a combination of environmental and endogenous factors [33,77]. The early germination phase culminates in the testa rupture, which is followed by the late germination phase and endosperm rupture [76]. Abscisic acid (ABA) and gibberellins acids (GAs), two widely investigated plant hormones, regulate the germination process [76]. ABA maintains dormancy, while GA promotes germination [77]. ROS and RNS may exert significant impacts on seed germination by interfering with ABA-related signaling, and stimulate GA-related signaling, which will modify redox balance and other downstream events in seeds to further trigger germination [33,77]. Specifically, OH• facilitates the loosening process of the cell wall, along with the protrusion and the elongation of radicles, which are the necessary steps for germination [33,78]. H2O2 stimulates the seed germination of cereal plants such as pea, apple, barley, wheat, and rice by facilitating the removal of ethanol-soluble compounds from the seeds with pericarp or by the oxidation or decomposition of the germination inhibitors in pericarp [33,79,80]. More importantly, H2O2 directly interferes with ABA and GA signaling pathways, breaks down ABA, modifies redox status, and induces protein carbonylation [33,77,80–82]. Several RNS, such as NO, NO2•, NO3•, NO2, or NO donor, such as sodium nitroprusside (SNP) also promote the release of seed dormancy and seed germination via affecting metabolism, oxidation state, as well as other signaling pathways, such as ABA/GAs-based pathways in seeds [55,77,81–88].
hemp cultivars [99], wheat seeds [100], tomato seeds [45,48,49], watermelon seeds [60], as well as cultivar seeds [48]. In short, CAP treatment is an alternative to conventional pre-germination treatments.

The improved germination rate and the increased growth of the hypocotyl and the radicle of mung beans were strongly dependent on the feed gases used to generate CAP and CAP dose (Figure 5) [93]. The CAP using air as feed gases showed a stronger effect on the seed germination rate than the CAP using O₂, N₂, and He did [93]. In addition, a moderate-intensity DBD treatment increased the seedling growth by 24.0%, 28.0%, and 35.5% after a 4 min of air plasma, N₂ plasma, and Ar plasma treatment, respectively [96]. The O₂ plasma treatment did not cause the observable enhancement on germination [96].

Though the key role of ROS/RNS in the seed germination process has been demonstrated in plant biology, the dominating factors for the improved germination rate and seedling growth have not been strictly demonstrated yet. The acidification and formation of H₂O₂ or nitrogen compounds may mainly contribute to these performances by promoting the leathering of seed chaps and the activation of catalase expression [93]. However, the enhanced germination rate may not be simply due to the chemical factors such as ROS or RNS. It was also found that a radiofrequency (5.28 MHz) electromagnetic radiation increased the germination rate of freshly harvested R. smirnowii seeds about 70% [101]. The electromagnetic effect in CAP may also exert an impact on seeds [102,103].

Figure 5. Air plasma drastically improved the germination rate of mung bean seeds. (a) 0 h, (b) 9 h, (c) 12 h, (d) 24 h, and (e) the germination percentage of mung bean seeds treated with He, N₂, air, or O₂ plasma as a function of incubation time [93].

So far, the limited studies demonstrated that CAP treatment indeed triggered an observable cellular and molecular response in seeds [102–104]. An argon plasma promoted soybean seed germination and sprout growth by regulating the demethylation of adenosine triphosphate (ATP), target of rapamycin (TOR), and growth-regulating factor (GRF) [105]. The level of GA₃ hormone, mRNA expression of amylolytic enzyme-related gene, chlorophyll, as well as the total polyphenols in spinach seedlings have been increased by a CAP treatment [98]. It was also found that the superoxide dismutase and catalase activities have been increased by 17.71% and 16.25% in CAP-treated oilseed, respectively [48]. The expression of heat shock proteins HSP101 and HSP70 in maize grains has been increased by a CAP treatment [106]. Thus, CAP treatment may improve oilseed rape drought tolerance by improving antioxidant enzyme activities, increasing osmotic-adjustment products, and reducing lipid peroxidation [48].

Seed germination is a complicated physiological process that begins with the seeds’ water absorption (imbibition) and ends with the radicle emerging [28,62,107]. It is known that the seeds’ kinetics and quantity water uptake efficacy are affected by their surface
properties (e.g., morphology, composition, and structure) and surrounding environment, such as moisture content and temperature. The modification of CAP on the seed’s surface properties have been explored over the past decade. Some CAP-treated seeds, either experiencing a direct treatment or a soak of CAP-treated solution, showed better hydrophilicity and better water uptake efficacy [108]. For example, the seeds of a common bean (Phaseolus vulgaris L.) were treated by low-pressure oxygen plasma triggered by an inductively coupled radiofrequency (RF) discharge [109]. As shown in Figure 6, the CAP treatment drastically increased the wettability of bean seeds via changing their surface properties such as surface chemistry, surface roughness, and surface morphology. Promisingly, the improved wettability significantly increased the water uptake of these bean seeds. More attractively, these improvements were achieved by just a short treatment length (a few seconds). In short, CAP is scalable to large systems in the real agricultural applications.

PSS also improves the germination rate of seeds. The CAP-stimulated tap water, demineralized water, and liquid fertilizer have shown promising effects on increasing the germination rate and the stem elongation rate of lentils [39,59–69,110]. Reactive species in the aqueous solutions mainly contribute to the observed increased germination rate and stem growth (Figure 7) [59]. The endogenous production of NO radicals in seeds may be activated and facilitate the release of seeds from dormancy [59]. A cylindrical double DBD reactor was used for water activation and a plate-to-plate double DBD reactor was employed for the treatment of the seeds of radish, tomato, and sweet pepper [66]. The activated water has been acidified (pH ≈ 3) due to the absence of a buffer [66]. PSS showed a significant impact on germination rate as well as plant growth for all three types of seeds [66]. The stem length increased about 60% when the PSS treatment and the direct CAP treatment were combined [66].

Figure 6. CAP treatment improved the water uptake of bean seeds. (a) Visual bean seed radicle length after 4 days of incubation. (b) Water droplets on untreated and treated bean seed. SEM images of seeds’ surface were shown below. (c) The effect of plasma treatment on water contact angle on seed’s surfaces. (d) The effect of plasma treatment on water uptake of seeds [109].
will plasma agriculture be competitive compared to existing breeding technologies such as fertilizers? Many challenges are waiting to be solved before the final realization of plasma agriculture.

Over the past decade, CAP has shown its attractive potential as a novel and safe modality in agriculture. The complex reactive chemical nature of CAP builds the foundation of plasma agriculture, including the pathogen-based plant disease therapy, the resistance to abiotic stress, food sterilization, and the improvement of seed germination rates. Reactive species either kill microorganisms on seeds’ surface or trigger cellular and molecular pathways in seeds to facilitate the germination process and further development such as seedling. CAP also shows a strong potential to modify the surface properties of seeds, causing an improvement in the water uptake and later development. The CAP-stimulated (treated, activated) solutions recently attracted widespread attention due to their convenience during storage and the potential for massive use in farms. Moreover, a CAP source can consume electricity and use air to generate reactive species via careful design. Thus, plasma agriculture is an environmentally friendly, green technology.

To date, the demonstration of these practical perspectives is mainly limited to the lab level. It is far from an extensive application of CAP technology in modern agriculture. Though we know many positive outcomes of plasma agriculture, such as improved seed germination, we do not know whether CAP treatment could affect the quality of these important crops, such as rice production, and potential side effects. Furthermore, it is entirely unknown whether plasma agriculture could contribute to some significant challenges in agriculture, such as the growth of crops in saline-alkali land. Will the CAP-treated seeds give the final crop a solid resistance to diseases? In terms of efficacy and economy, will plasma agriculture be competitive compared to existing breeding technologies such as fertilizers? Many challenges are waiting to be solved before the final realization of plasma agriculture.
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