

Review

Plasma Agriculture from Laboratory to Farm: A Review

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Received: 30 July 2020; Accepted: 14 August 2020; Published: 17 August 2020

Abstract: In recent years, non-thermal plasma (NTP) application in agriculture is rapidly increasing. Many published articles and reviews in the literature are focus on the post-harvest use of plasma in agriculture. However, the pre-harvest application of plasma still in its early stage. Therefore, in this review, we covered the effect of NTP and plasma-treated water (PTW) on seed germination and growth enhancement. Further, we will discuss the change in biochemical analysis, e.g., the variation in phytohormones, phytochemicals, and antioxidant levels of seeds after treatment with NTP and PTW. Lastly, we will address the possibility of using plasma in the actual agriculture field and prospects of this technology.

Keywords: non-thermal plasma; plasma agriculture; seed germination; growth enhancement

1. Introduction

Plants regularly undergo a multitude of stresses, e.g., scarcity of water, waterlogging, toxicity, high salinity, and extreme temperatures. These stresses result in less yield of crops. Countries such as India, Australia, China, USA, South America, Central Asia, and Africa are significant producers of food crops and are facing droughts at regular intervals. To enhance seed germination and growth under the changing environment, techniques such as chemical, physical, and biological treatments are developing [1–4]. However, in the framework of seed technology, the physical invigoration treatments in seeds can result in the change of seed morphology, gene expression, and protein level [5]. These physical changes can result in increased germination and growth enhancement. The physical methods for pre-sowing seed treatments are magnetic fields, electromagnetic waves, ionizing radiations, ultrasounds, non-thermal plasma, etc., as shown in Figure 1. In this review, we will discuss the role of non-thermal plasma (NTP) in stimulating germination and growth in plant seeds.

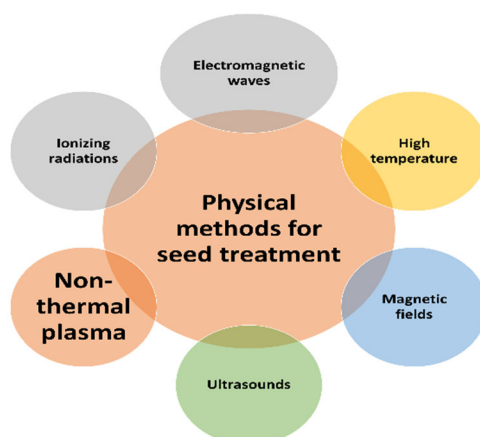


Figure 1. Schematic representation of various physical methods used for seed treatment.

NTP has received considerable attention in recent years due to its increasing applications in medicine, sterilization, agriculture, etc., as displayed in Figure 2 [6–26]. NTP discharges generate the reactive charged species such as electrons and ions, and neutral species, and emit ultraviolet radiation, and electric fields. The plasma generated reactive oxygen and nitrogen species (RONS) and change in solution properties pH, electrical conductivity, and oxidation-reduction potential. These solutions affect the rate of seed germination, enhancement in plant growth, and well as an increase in agricultural yields. NTP applications in agriculture possess advantages over conventional treatments such as short treatment time, easily accessible, and low temperature during operations. Moreover, NTP can be used to treat seeds and crops without damaging them. The use of different feeding gases can alter the plasma chemistry that leads to an increase in variation of seed coating technology in comparison to traditional methods [8].

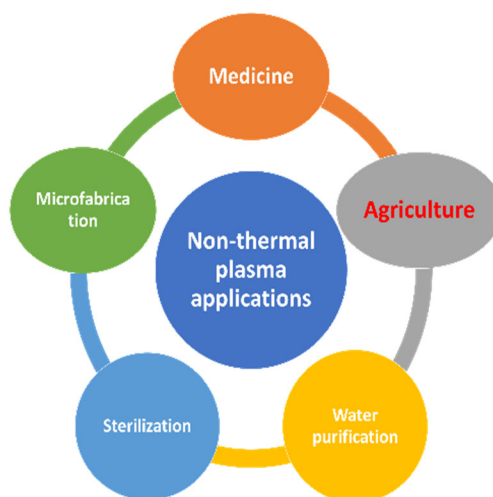


Figure 2. Schematic representation of non-thermal plasma (NTP) applications.

Generally, plasma applications in agriculture classified as preharvest and post-harvest. The use of NTP in post-harvest processes such as food preservation and food processing are discussed briefly in previously published reviews, as demonstrated in Figure 3 [27–32]. On the other hand, limited reviews are available for the NTP use in preharvest [33–36]. NTP technology is used in preharvest at different levels like sterilization of seeds, improving seed germination, reducing pathogen invasion in soil, etc. Although in this review, we will focus on the role of NTP generated at variable pressure ranges on seed germination and seedling growth. In addition, we will also discuss the possible impact of plasma-treated water (PTW) (also known as the plasma-activated water (PAW)) on seed germination and plant growth. At last, we will discuss the probable mechanism of NTP and PTW treatment in plasma agriculture and the prospects of this technology in the real scenario.

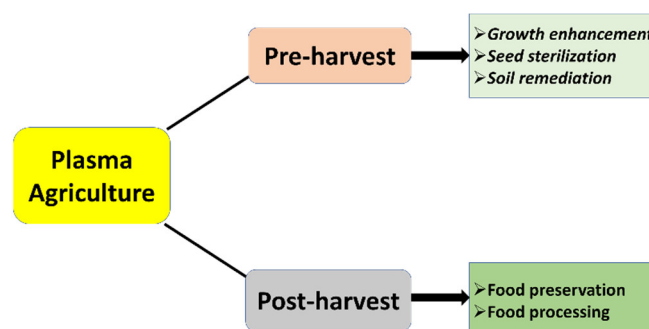


Figure 3. Role of plasma in preharvest and post-harvest processes.

This review aims to discuss the current status of plasma treatment in the pre-harvest stage and its possible mechanism. Additionally, explore the possibilities of using plasma in the actual agriculture field.

2. Effect of NTP at Low/Medium (6.7×10^{-2} to 53,328 Pa) Pressure on Seed Germination and Growth Enhancement

The NTP treatment applied at low or medium pressure, to treat various seed for the different time intervals, in the presence of different types of feeding gases as described in Table 1.

Table 1. Enhanced seed germination and growth due to low/medium/atmospheric pressure plasma and plasma-treated water treatment, as reported in the literature.

Seeds	Plasma Type	Results	Reference
Radish sprouts	Low-pressure plasma (100 Pa)	Radish sprouts growth increases with O ₂ plasma treatment, while no effect was observed for seed germination. In contrast, no effect on the average length of sprouts for N ₂ Low-pressure plasma.	[37]
Radish sprouts	Low-pressure plasma (40 Pa)	Growth enhancement.	[38]
Radish sprouts	Scalar dielectric-barrier discharge (DBD) plasma	Growth enhancement.	[39]
Radish sprouts	Scalar DBD plasma	The average seedling length was 250% longer than the control samples.	[40]
Radish sprouts	Scalar DBD plasma	Enhanced plant growth for O ₂ , Air and NO (10%) + N ₂ feeding gases plasma. While no significant growth enhancement for He, N ₂ , and Ar gases plasma.	[41]
Radish sprouts	Plasma jet	The total mass and average lengths of radish sprouts increased.	[42]
Radish sprouts	Surface discharge plasma	No effect on the germination dynamics but the length of root and sprout increased.	[43]
Radish sprout, rice, Zinnia, <i>Arabidopsis thaliana</i> and <i>Plumeri</i>	Scalable DBD plasma	Enhanced plant growth.	[44]
<i>Arabidopsis thaliana</i>	Low-pressure plasma (20 Pa)	Lengths of the leaves and stems of <i>Arabidopsis</i> increased \approx 1.5 times over the control.	[45]
<i>Arabidopsis thaliana</i>	Scalar DBD plasma	Enhanced growth, shorter harvest, and increased total weight.	[46]
Wheat	Medium pressure glow discharge plasma (1333 Pa)	Increased growth activity and dry matter accumulation.	[47]
Wheat	Low-pressure plasma	Plasma treatment increased the grain and spike yield.	[48]
Wheat	Low-pressure plasma	Enhanced seed germination rate.	[49]
Wheat	Low-pressure plasma (150 Pa)	Improved germination potential, germination rate.	[50]
Wheat	Surface discharge plasma	Little effect on the germination rate while a substantial impact on growth parameters.	[51]

Wheat	DBD plasma	Improved the germination and seedling growth.	[52]
Wheat	DBD plasma	The germination rate, germination potential, germination index, and vigor index increased after plasma treatment.	[53]
Wheat	DBD plasma	The germination rate, germination potential, root length, and shoot length of the wheat seedlings increased.	[54]
Wheat	Plasma jet	Increased dry weight after plasma treatment.	[55]
Wheat	Low-pressure plasma (140 Pa)	Rate of germination increases after plasma treatment.	[6]
Sunflower	Scalar DBD plasma	Adverse effects on germination kinetics.	[56]
Sunflower	Streamer like plasma	Growth enhancement and increased dry weight.	[57]
Sunflower	DBD plasma	The distribution of sprouts length and the dry weight increased after plasma treatment.	[58]
Soybean	Low-pressure plasma (150 Pa)	Germination and vigor indices significantly increased after plasma treatment.	[59]
Soybean	DBD plasma	Total fresh weight increased by 1.2-fold for DBD plasma	[60]
Pea	Coplanar type of dielectric surface barrier discharge (DCSBD) plasma	Increased in germination percentage and growth parameters.	[61]
Pea and Zucchini	FSG plasma (a semi-automatic device) system.	Germination of Pea and Zucchini increased after plasma treatment.	[62]
Mung bean	Microplasma array plasma.	Germination index increased for Air and O ₂ plasma, and no significant difference observed for He or N ₂ plasma compared to control.	[63]
Beans	Low-pressure plasma (6.7 ×10 ⁻² Pa)	The final germination percentage of seeds was not affected by plasma treatment. However, the rate of germination was improved for the plasma-treated samples.	[64]
Artichoke	Low-pressure plasma (1.8 Pa)	Improved the germination rate and seedling growth.	[65]
Ajwain	Low-pressure plasma (9.9 Pa)	Improved seed germination percentage and germination index.	[66]
Poppy	Plasonic AR-550-M	Enhanced seed germination	[67]
Oilseed rape	Low-pressure plasma (50 Pa)	Improved germination rate and seedling growth.	[68]
Hemp	Gliding arc and downstream microwave devices (low-pressure, 40 Pa)	Gliding arc treatment increased the length of seedlings, seedling accretion, and weight of seedling, while downstream microwave plasma treatment had an inhibiting effect.	[69]
Garlic seed bulbs	Low-pressure plasma (15–60 Pa)	Increased dried bulb mass after plasma treatment.	[70]
Sweet basil	Low-pressure plasma (40 Pa)	Increased germination and seedling vigor after plasma treatment.	[71]
Black gram	Medium pressure DBD plasma (53,328 Pa)	Enhanced seed germination rate and seedling growth.	[72]
Tomato	Coaxial DBD reactor plasma	The root-to-shoot ratio (R/S) ratio increased significantly for plasma-treated samples.	[73]
Pumpkin	Plasma jet	Plasma jets accelerated the germination of pumpkin seeds.	[74]
<i>Brassica napus</i>	DBD plasma	No significant difference in seed germination.	[75]
<i>Andrographis paniculata</i>	DBD plasma	Increased seed germination.	[76]
Fenugreek	Plasma jet.	Enhanced seed germination rate.	[77]
Mulungu	Plasma jet	Enhanced seed germination rate.	[78]
<i>Hybanthus calceolaria</i>	Plasma jet	Enhanced seed germination rate.	[79]
Nasturtium	DBD plasma	Enhanced seed germination for short plasma treatment.	[80]
Thuringian Mallow	GlidArc reactor	Enhanced seed germination.	[81]
Cucumber and Pepper	DCSBD plasma	Improved germination observed for both seeds.	[82]

Spinach	High voltage nanosecond pulsed plasma and micro DBD plasma.	Germination and dry weight of seedlings increased after both plasma treatment.	[83]
Barley	Surface DBD plasma	Accelerated the early growth of sprouts and enhance bioactive phytochemicals in the sprouts.	[84]
Barley	Low-pressure plasma (26 Pa)	No effect of plasma treatment.	[7]
Oat	Low-pressure plasma (13 Pa)	Quantity of germination seeds increased by 27% after plasma treatment than control on 5th day.	[7]
Oat	Low-pressure plasma (140 Pa)	No significant difference in rate of germination.	[6]
Chili pepper	Plasma jet	Enhanced seed germination.	[44]
Black Pine	DCSBD plasma	The germination index increased for short treatment time.	[85]
Basil	DBD plasma	Increased overall germination rate.	[86]
Gram	PTW	Increased cumulative germination and vigor index.	[87]
Radish sprout	PTW	Increased growth of sprouts.	[88]
Radish sprout	PTW	Enhanced seeds germination rate and the seedling growth.	[89]
Soybeans	PTW	Enhanced seeds germination.	[90]
Mung bean	PTW	No significant difference in growth rate.	[91]
Zinnia	PTW	Increased germinability and growth of flowers of Zinnia annual.	[92]
<i>Brassica rapa</i>	PTW	Increased dried weight of the plant.	[93]
<i>Lentils</i>	PTW	Enhanced seeds germination as compared with commercial fertilizer.	[94]
Tomato	PTW	Enhanced shoot and root length.	[95]
Rapeseed	PTW	Significant improvement in germination rate and seedling vigor.	[96]

Low/medium-pressure plasma used to treat the seeds of radish sprouts, wheat, ajwain, black gram, poppy, oilseed rape, garlic, sweet basil, and bean. Radish Sprouts (*Raphanus sativus*) seeds were treated by low-pressure radiofrequency (RF) plasma at 100 Pa pressure with O₂ and N₂ as feed gases. The discharge power and frequency were 50 W and 13.56 MHz, respectively, see Figure 4a [37].

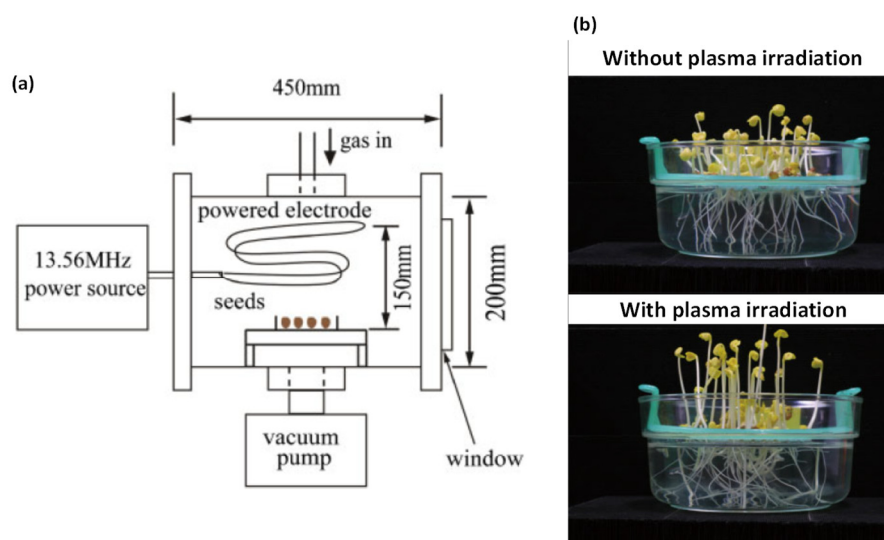


Figure 4. (a) Schematic diagram of the low-temperature plasma reactor and (b) Radish sprouts cultivated with and without O₂ plasma irradiation. Reproduced from reference [37], Copyright (2012) The Japan Society of Applied Physics.

No effect on the seed germination was reported for O₂ and N₂ low-pressure plasma treatment. However, the average length of sprouts was 60% higher for O₂ low-pressure plasma as compared to the control sample, as indicated in Figure 4b. In contrast, no change was observed for N₂ low-pressure plasma [37]. Hayashi et al. studied the effect of inductively coupled RF plasma on the radish sprouts

seeds. RF plasma worked at 13.56 MHz frequency, 40 Pa pressure in the presence of ambient air for 20 min, with an input power of 50 W. Enhancement in radish sprouts growth observed in their study [38]. In another study, *Arabidopsis thaliana* and *Raphanus sativus* seeds were treated with O₂ low-pressure plasma with RF power at power 60 W, 20–80 Pa pressure, and 13.56 MHz frequency. The length of *Raphanus sativus* varied with changes in the pressure, although at pressure 20 Pa maximum length was obtained. Whereas, plasma treatment on *Arabidopsis thaliana* seeds results in increased length of stems by 1.5 times and area of leaves by 2 times as compared with control (without plasma treatment) [45].

Wheat is an essential strategic crop; therefore, many researchers used NTP to treat the wheat seeds. The wheat seeds (*Triticum aestivum*) treated by glow discharge plasma with a mixture of air/O₂ gases at 1333 Pa pressure and 3–5 kHz frequency for 3–9 min. They observed that 6 min treatment of glow discharge plasma could result in 95–100% seed germination and a 20% increase in wheat yield [47]. In a very recent study, RF plasma reactor operated at 13.56 MHz frequency in the air for 180 s used to treat the same wheat seeds (*Triticum* spp.). They found that after plasma treatment, the grain and spike yield was enhanced to 58 and 75%, respectively, compared to control in the presence of haze stress [48]. Iqbal et al. treated the wheat (*Triticum* spp.) seeds with Ar low-pressure plasma at variable voltages (600–850 V). They observed that the germination rate was 57–60% higher with changing the voltage from 600 to 850 V as compared to control [49]. Another group used the same wheat seeds (*Triticum* spp.) and treated with He plasma for 15 s at 150 Pa pressure and 3×10^9 MHz frequency with 60–100 W variable power. The plasma treatment at 80 W showed 6 and 6.7%, improvement in seed germination potential and germination rate, respectively, as compared to control. Additionally, the plant height, root length, and fresh weight increased to 20.3, 9, and 21.8%, respectively, at the seedling stage. At the same time, the wheat yield was increased to 5.89% with respect to control [50]. Šerá et al. showed that germination rate of wheat increases when treated with Plasonic AR-550-M at power 500 W and pressure 140 Pa, whereas no significant effect observed for Oat caryopses treatment [6]. On the other hand, Dubinov et al. revealed 27% increase in the quantity of germination of Oat seeds treated with glow discharge plasma at pressure 13 Pa with respect to reference seeds [7].

Bormashenko et al. used the inductive air plasma discharge to treat beans (*Phaseolus vulgaris* L.) seeds for 2 min at 10 MHz frequency, 6.7×10^{-2} Pa pressure, and 20 W power. No significant change in germination percentage was observed between plasma-treated sample and control, although speed of germination was faster for plasma-treated samples than control [64]. In a sperate study, a commercial computer-controlled plasma device (HD-2N) treated the soybean (*Glycine max*) seeds. HD-2N plasma device works at a frequency of 13.56 MHz and pressure of 150 Pa with variable powers from 60 to 120 W. The improvement in the seed germination and seedling growth obtained at 80 W power. Shoot length, shoot dry weight, root length, and root dry weight increased by 13.77, 21.95, 21.42, and 27.51%, respectively, after plasma treatment with respect to control [59].

Hosseini et al. treated the artichoke seeds (*Cynara cardunculus* var. *scolymus*) with capacitively coupled RF plasma at a pressure of 1.8 Pa with the power of 10 W. Authors showed that length of root increased by 28.5 and 50% after 10 and 15 min plasma treatment, respectively. The dry weight of roots was increased by 13 and 53% after 10 and 15 min plasma treatment, respectively [65]. Gholami et al. treated the ajwain seeds by RF capacitively coupled plasma at a frequency of 13.56 MHz and pressure of 9.9 Pa for 2 min at variable power. Ajwain seeds treated at 50 W power plasma showed 11.1, and 1.22% increase in germination percentage and germination index, respectively. At the same condition, root length was increased by 34% as compared to control. However, root length increments were 2 and 10% at powers of 80 and 100 W, respectively. The authors concluded that the germination percentage and germination index values decreased when power was more than 50 W [66].

Poppy seeds (*Papaver somniferum*) treated with 2.45 GHz microwave power source with a magnetron input of 500 W (Plasonic AR-550-M) at different time intervals. The seed germination rate on the fifth day was a maximum of 104 and 102% for 3 and 5 min plasma treatment, respectively, as compared to reference samples [67]. Ling et al. showed that oilseed rape (*Brassica napus* L.) seeds

treated for 15 s with He-plasma at 13.56 MHz frequency, 100 W power, and 50 Pa pressure results in the improved germination rate of Zhongshuang 7 and Zhongshuang 11 by 6.25 and 4.44%, respectively [68]. Sera et al. investigated the effect of low-pressure plasma on Hemp (*Cannabis sativa* L.) seeds. They used 2.45 GHz microwave power source with a magnetron input of 500 W at a pressure of 140 Pa in the presence of O₂ and Ar gases. The authors observed the inhibitory effect of plasma treatment on all tested hemp cultivars [69].

Recently, garlic seed bulbs (*Ptujski spomladanski*) treated with O₂ low-pressure RF plasma at 15–60 Pa pressure for 60 s. The authors noticed increased dried bulb mass by 11% at 15 Pa pressure. Additionally, treatment at 30 and 45 Pa pressure exhibit little increase in dry bulb mass. Further, an increase in pressure (60 Pa) results in decreased dried bulb mass [70]. Another recent study by Singh et al. showed the increased in germination percent of sweet basil (*Ocimum basilicum* L.) seeds when treated with RF plasma at 13.56 MHz frequency, 40 Pa pressure with variable power 30–270 W in the mixture of O₂ (80%) and Ar (20%) gases. The germination percentage increased by 16.3 and 20.5% than control at power 90 and 150 W, respectively [71].

In a very recent study, DBD plasma working at a pressure of 53328 Pa with 45 W power at the applied voltage of 5 kV and frequency of 4.5 kHz used to treat black gram (*Vigna mungo*) seeds. After plasma treatment, the rate of seed germination and seedling growth was increased by 13.67 and 37.13%, respectively, as compared to control [72].

3. Effect of NTP at Atmospheric Pressure on Seed Germination and Growth Enhancement

Recently reported work reveals the increasing use of atmospheric pressure non-thermal plasma (AP-NTP) than low-temperature plasma to treat the seeds. This increase was due to the difference in the treatment cost of both devices as well as the user-friendly operation of atmospheric pressure plasma devices (easily operated). The various seeds, such as radish sprouts, wheat, sunflower, pea, bean, maize, rice, pumpkin, cucumber, pepper, barley, spinach, basil, black pine, etc., were treated by AP-NTP. It was showed that scalar dielectric-barrier discharge (DBD) treated the *Raphanus sativus*, *Oryza sativa*, *Arabidopsis Thaliana*, *Plumeria*, and *Zinnia* seeds at 9.2 kV discharge voltage and 0.2 A discharge current and 1.49 W/cm² of discharge power density. The growth enhancement was 250, 80, 60, 30, and 20% for *Raphanus sativus*, *Oryza sativa*, *Arabidopsis Thaliana*, *Plumeria*, and *Zinnia*, respectively, after scalar DBD treatment [39,97]. Similar scalar DBD used to treat *Arabidopsis thaliana* seeds see Figure 5a, which results in accelerated growth, shorter harvest time, increased total seed weight, and increased seed number, as shown in Figure 5b [46]. Further, Kitazaki et al. also used the same scalar DBD plasma to analyze the growth of radish sprouts (*Raphanus sativus* L.) using combinatorial analysis. Authors observed 250% growth enhancement when seeds were placed at x = 5 and y = 3 mm [40]. In a separate study, this scalar DBD treated the radish sprouts (*Raphanus sativus*) seeds for 3 min in the presence of different feed gases like He, Ar, N₂, Air, O₂, and NO (10%) + N₂. For He, N₂, and Ar feeding gases, plasma treatment showed a limited influence on plant growth; however, for O₂, Air and NO (10%) + N₂ gases plasma had significant on growth enhancement [41].

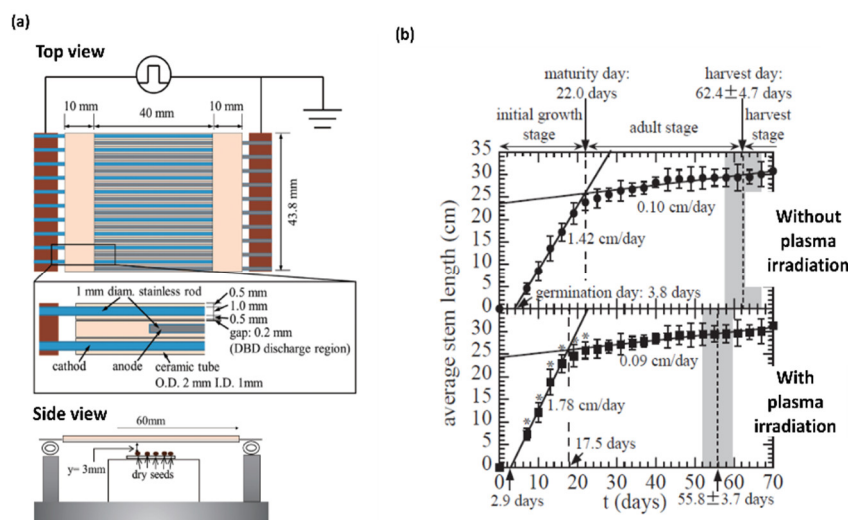


Figure 5. (a) Schematic depiction of scalable dielectric barrier discharge plasma device (b) Average stem length for plants as a function of time with and without plasma treatment. Reproduced from reference [46], Copyright (2016) The Japan Society of Applied Physics.

Hayashi et al. treated the seeds of radish sprouts (*Raphanus sativus* var. *longipinnatus*) with plasma torch for 60 min. Plasma device with O₂ and air feeding gas had a frequency of 12 kHz with a varied applied voltage of 7–10 kV. The total length (stem and root length) increased by 1.6 times for O₂-plasma and 1.2 times for air-plasma treatment than control (without plasma treatment) [98]. In another study, the radish sprouts seeds (*Raphanus sativus*) treated with an Ar-plasma jet. Plasma treatment at 140 W power results in increased total mass by 9–12% and increased average length by 3 cm in comparison with untreated seeds [42]. In a separate study, surface discharge plasma treatment of radish sprouts (*Raphanus sativus*) seeds for 20 min results in no change in the germination dynamics. The surface discharge plasma operated in AC mode with the sinusoidal voltage of 15 kV at 50 Hz frequency with an average power of 2.7 W. However, root and sprout lengths were increased by 11 and 10%, and root and sprout weight were increased to 30 and 15%, respectively, after plasma treatment [43].

Substantial the effect of plasma treatment on wheat seeds studied by both low-pressure plasma and atmospheric pressure plasma. Dobrin et al. treated the wheat seeds (*Triticum aestivum* L.) with surface discharge plasma with airflow of 1 L/min had a sinusoidal voltage of 15 kV at 50 Hz frequency with an average power of 2.7 W for 15 min. The plasma treatment had a small effect on the germination rate, but at the same time, plasma treatment showed a pivotal impact on growth parameters. The root-to-shoot (R/S) ratio was higher for treated samples than untreated samples [51]. Meng et al. treated the wheat seeds (Xiaoyan 22) with DBD plasma had discharge voltage 0–50 kV and frequency of 50 Hz with different working gases like Air, N₂, Ar, and O₂. After plasma treatment, germination potential increased to 24, 28, and 35.5% for Air, N₂, and Ar feeding gases plasma, respectively, as compared to control [52].

Li, et al. used the Air-DBD (1.50 W discharge power and 13.0 kV discharge voltage) to treat the wheat seed (Xiaoyan 22) for 7 min. The authors observed that the germination rate, germination potential, germination index, and vigor index were increased by 9.1, 26.7, 16.9, and 46.9%, respectively [53]. Further, the same group studied the adverse effects of drought stress on wheat seed (Xiaoyan 22) germination and seedling growth in the presence of the DBD as mentioned earlier. The germination potential and germination rate were increased to 27.2 and 27.6%, respectively, after plasma treatment. Additionally, root and shoot length increased after DBD treatment [54]. Recently, Lotfy et al. used the N₂-plasma jet at discharge voltage and discharge current of 2.6 kV and 38.1 mA, respectively, to treat wheat seeds (Giza 168). The N₂-plasma jet treatment for 4 min results in 54.3% higher mean dry weight than control samples [55].

Sunflower seeds and sprouts are in high demand due to its application in food industries, biofuels, cosmetics, and lubricants. Therefore, researchers are using plasma to enhance the productivity of sunflowers. Sunflower (*Helianthus annuus*) seeds treated with scalar DBD for 7 and 11 min at a discharge voltage of 9.2 kV, and the current of 0.2 A, due to plasma treatment the adverse effect on germination kinetics was observed. Although root length was increased by 44%, and the weight of seedlings and leaves were increased by 16 and 15%, respectively. Moreover, DBD plasma treatment for 11 min did not affect root length and sprout weight. However, DBD treatment results increased leaf weight and leaf number by 15 and 10%, respectively, with respect to control. In addition, no effect on the stem length of sunflower seedlings was observed [56]. Matra et al. treated the same sunflower (*Helianthus annuus* L.) seeds with NTP had average discharge voltage and current of 7.451 kV and 0.073 mA, respectively, with Ar: O₂ feeding gases. After plasma treatment, the dry weight was 1.79 times heavier, and the average shoot length was 2.69 times higher, than control [57]. A recent study showed the DBD treatment effect on sunflower (*Helianthus annuus*) seeds for 120 s at 90 W power, which results in the enhancement in sunflower seed germination [58].

Coplanar type of dielectric surface barrier discharge (DCSBD) used to treat the Pea (*Pisum sativum*) seeds with 14 kHz frequency, 10 kV sinusoidal voltage, 370 W input energy, and 2.3 W cm⁻² of average power density. After the DCSBD plasma treatment for 120 s, the total percentage of seed germination significantly increased to 95%, whereas the germination percentage was 77.5% for control. Similarly, the length of the shoot and root, as well as dry seedling weight also increased as compared to control samples after DCSBD treatment [61]. Khatami and Ahmadiania, treated pea seeds for 30 and 60 s with a plasma device (self-made FSG plasma (a semi-automatic device)) system with an applied voltage of 15 kV and air gas flow of 5 L/min. After 30 and 60-s plasma treatment, 80 and 74% of seeds, respectively, were germinated, although, in the control sample, only 40% seeds were germinated, after 14 days [62].

Zhou et al. used a micro-plasma array with an AC frequency of 9.0 kHz to treat Mung bean (*Vigna radiata* (Linn.) Wilczek.) seeds in aqueous media in the presence of different feed gases such as Air, O₂, N₂, and He. The authors observed that seed germination and growth of mung bean were dependent on the feed gases and treatment time. Air micro-plasma array treatment improved the seed germination rate and seedling growth in comparison to other feeding gases plasma (O₂, N₂, and He) treatment. Air and O₂ micro-plasma array treatments substantially improved the germination index by 58.3% and 41.7%, respectively, whereas no significant difference observed for He or N₂ plasma as compared to control [63]. Pérez-Pizá et al. treated the soybean (*Glycine max* (L.) Merrill) seeds with DBD plasma with AC power supply (0–25 kV) operating at 50 Hz with N₂ or O₂ as carrying gases. N₂ and O₂ plasma treatment for 3- and 2-min results in 1.2-fold incremented in total fresh weight than control, and the full length of soybean plant increased to 4–10% after plasma treatment [60].

Zahoranová et al. used DCSBD plasma (operated at 14 kHz frequency, 20 kV (peak-to-peak) sinusoidal high voltage, and 80 W cm⁻³ power density with an input power of 400 W) to treat maize (*Zea mays* L.; cv. Ronaldinio) seeds. After 60- and 120-s plasma treatment, no significant difference in germination was noted in comparison to untreated maize seeds. However, for 300 s of DCSBD treatment, the germination rate was decreased to 7%. However, the vigor index increased by 23% for 60-s plasma treatment compared to control [99]. Further, rice (*Oryza sativa*) seeds were treated with hybrid cold plasma (HCP) (high voltage of 14 kV_{pp}, 700 Hz frequency, and 4.8 W power (Matsushita Electronic components)), working in Ar gas. The final germination was 98% for plasma-treated rice seeds, whereas 90% for control. However, seedling length remains the same for both non-treated and treated rice seeds [100]. Amnuaysin et al. treated the rice (*Oryza sativa* L.) seeds with Air-DBD operated at 5.5 kHz frequency for 60 s. The germination rate was 92.67% after plasma treatment, while 85% for control. Additionally, the vigor index, germination rate, seedling growth, fresh weight (root and shoot), and dry weight (root and shoot) showed improvement after plasma treatment [101].

Tomato seeds (*Lycopersicon esculentum*) hybrid Belle F1 treated with a coaxial DBD reactor with air as a working gas had a flow rate of 15 L/min with 50 Hz frequency and 1.43 W discharge power. They noted a 77% germination rate for 5 min DBD treatment while 68% for the control. Although 5

min DBD exposure results in three times increase of average root length than control seeds. Additionally, the root-to-shoot ratio (R/S) for control seeds was 0.51, whereas for 5- and 30-min plasma treatment, it was 0.87 and 0.73, respectively [73]. Pumpkin (*Cucurbita pepo*) treated with plasma jet with a high voltage pulsed DC system operated with pulse amplitude 8 kV, pulse frequency 6 kHz, pulse width 1 μ s, and pulse rise and fall time \sim 70 ns. After the treatment, seed germination, and seed growth was enhanced [74]. Schnabel et al. showed that *Brassica napus* L. seeds treated with DBD had a frequency of 5.7 kHz and showed no significant change in seed germination than control. However, 10 min DBD treatment results in increased germination percentage by 3 and 13% after 24 and 48 h, respectively, compared with control seeds [75].

Andrographis paniculata seeds treated with DBD plasma at 4250 V for 10 s and 5950 V for 20-s results in enhanced seed germination [76]. Fenugreek (*Trigonella foenum-graecum*) seeds treated with Ar-plasma jet (applied voltage 16 kV and an applied frequency of 24 kHz). After plasma treatment, the seed germination rate improved by 7 and 4 times with and without an accelerating grounded electrode, respectively [77]. Mulungu (*Erythrina velutina*) seeds treated with He-DBD at 10 kV applied voltage, 750 Hz frequency, and 150 W power for different time intervals. At 60 s He-DBD treatment, the seed germination rate was 5% higher than control [78]. *Hybanthus calceolaria* seeds treated with He-plasma jet working at 8.1 kV discharge voltage, 720 Hz frequency for 1 min showed an increase in seed germination by 3.5 times than untreated seeds [79]. Molina et al. treated the Nasturtium seeds (*Tropaeolum majus*) with He-DBD plasma for 10- and 30-s results in increased germination to 68.3 and 61.7%, respectively, as compared with control seeds under drought conditions. However, more extended plasma treatment results in decreased germination efficiency [80]. Thuringian Mallow (*Lavatera thuringiaca*) seeds treated with GlidArc reactor with dry nitrogen as working gas at 50 Hz discharge frequency, 680 V applied root mean square (RMS) voltage, 33 mA RMS current and 40 W of mean power. The germination was 60% for both 2- and 5-min plasma treated seeds, while 36.25% for control seeds [81]. In another study, pre-treatment of cv. “Bialobrzieskie” and cv. “Finola” hemp with Gliding arc working at 50 Hz power frequency at a flow rate of 10 L/min of humid air, resulted in increased length of seedlings, seedling accretion, and weight of seedling [46].

Cucumber (*Cucumis sativus*) and Pepper (*Capsicum annuum*) seeds treated with DCSBD plasma in ambient air, had 15 kHz frequency, and 400 W input power. After the DCSBD treatment, the germination percentage for both cucumber and pepper increased for short plasma treatment time, while decreased for more prolonged plasma exposure [82]. Mitra et al. treated the *Cicer arietinum* L. seeds with surface micro-discharge (SMD) plasma in ambient air had a power density of 10 mW/cm². After 1 min SMD treatment, the seed germination was 89.2% improved, and the mean germination time also decreased to 2.7 days than control [102]. In another study, high voltage nanosecond pulsed plasma (NPP) and micro DBD plasma used to treat the Spinach (*Spinacia oleracea*) seeds. NPP plasma had 6 kV and 0.7 kA of discharge voltage and current, respectively, with 0.3 J per one-shot pulse discharge energy. Whereas, micro DBD plasma had 6 kV, 14 mA, and 22 kHz of discharge voltage, current, and frequency, respectively, with Air and N₂ working gases. The authors observed that seeds treated with NPP showed 75–80% increased germination with one or five shots in comparison to 60% for untreated seeds. Whereas the germination decreases for ten shots of NPP. Seedlings growth and dry weight were increased after Air DBD treatment, while no substantial changes observed after N₂ DBD treatment [83].

Very recently, barley (*Hordeum vulgare*), chili pepper (*Capsicum*), black pine (*Pinus nigra*), and basil (*Ocimum basilicum*) seeds treated with different types of plasma [44,84–86]. Song et al. treated the barley (*Hordeum vulgare* L.) seeds with surface dielectric barrier discharge (SDBD) plasma operated at 14.4 kHz driving frequency, 8 kV peak-to-peak voltage, and 51.7 W average power. The 6-min plasma exposure increased the fresh weight of whole barley seedlings by 137.5% [84]. Chili pepper seeds treated with Ar-plasma for 15 s with a high voltage DC pulse operated at different powers. Seed germination was 86.53, 100, 95.51, and 94.23% at 0.41, 0.48, 0.55, and 0.61 W power, respectively, whereas control was 24.35%. The seed germination percentage was highest at 0.48 W [44]. Black Pine (*Pinus nigra*) seeds treated with DCSBD plasma for 1–60 s with an input power of 400 W for each treatment period. The highest germination index was for 3 s and the smallest for 60 s of

plasma treatment [85]. Basil (*Ocimum basilicum*) seeds treated with DBD in humid air, resulted in the rapid germination rate for 1 and 3 min DBD treatment as compared to control [86].

4. Effect of Plasma Treated Water on Seed Germination and Growth Enhancement

In the last few decades, plasma-treated water (PTW) showed the potential application in the agriculture and food industry [103–106]. The plasma exposor to water results in the production of different reactive oxygen and nitrogen species (RONS); therefore, PTW has a mixture of different RONS, mainly those that have a long lifetime, e.g., H_2O_2 , NO_2^- , NO_3^- , etc. In this review, we will discuss the influence of PTW on the germination and growth of plants.

Gram (*Vigna mungo*) seeds treated with H_2O - O_2 discharge plasma generated PTW. H_2O - O_2 discharge plasma worked with high voltage (3–6 kV, 3–10 kHz) power supply. The PTW produced from H_2O - O_2 discharge plasma for 3, 6, 9, 12, and 15 min, revealed the significant improvement in seedling growth of black gram seeds was observed after PTW treatment as compared to control. PTW generated by 6 min plasma treatment showed the foremost cumulative germination, while 3 min plasma treatment produced PTW showed the higher vigor index. Seeds treated with PTW created after 3-, 6- and 9-min plasma treatment showed longer shoot and root as compared to untreated samples. Additionally, PTW produced after plasma treatment for 12 min displayed the highest dry weights of shoots among other PTW treated samples [87].

Sarinont et al. showed that PTW generated by scalable DBD device using various gases like Air, O_2 , N_2 , He, and Ar used to treat the Radish sprouts (*Raphanus sativus* L.) seeds with discharge frequency of 14 kHz. The power was 5.85, 4.51, 3.54, 8.95, and 9.64 W for air, O_2 , N_2 , He, and Ar working gases, respectively. The PTW kept for 1 h and 1 day after scalable DBD treatment at room temperature to minimize the effect of short-lived reactive species. The PTW kept for 1 h produced from Air, O_2 , He, N_2 , and Ar plasmas showed 1.62-, 1.38-, 1.13-, 1.12-, and 1.04-times increased in seedling length of sprouts as compared to control. Although one day kept PTW produced from Air, O_2 , He, N_2 , and Ar plasmas showed 1.52, 1.28, 1.13, 1.10, and 1.08 times increased length of sprouts, respectively, more than control [88]. In another study, Sivachandiran and Khacef treated the Radish sprouts (*Raphanus sativus* L.) seeds with PTW created from double dielectric barrier discharge reactors (DBD) for 15- and 30-min treatment. The PTW generated from the double DBD discharge plasma (high voltage pulsed power supply (40 kV, 1 kHz)) showed enhance seeds germination rate and seedling growth. The PTW produced from 15- to 30-min plasma treatment had 60 and 100% germination rate as compared to control (40%). They also noticed that PTW produce from 15- to 30-min plasma treatment after three days had an average seedling length of 15 mm and 5 mm, respectively [89].

Soybeans (*Glycine max*) seeds were treated with PTW obtained from NTP in Air at voltage 80 kV and a frequency of 50 Hz in AC. The soybean seed germination was enhanced by PTW treatment in comparison with untreated seeds [90]. Very recently, Mung bean seeds treated with PTW produced at atmospheric pressure in specially designed two electrochemical cells using HV-DC power supply. The plasma discharged with circulating water for 3 h. No significant difference was observed on the growth rate of mung bean sprouts for control and cathodic electrochemical cell water. However, the growth rate was significantly lower for anodic electrochemical cell water as compared to control [91].

Zinnia annual (*Zinnia elegance*) seeds treated with PTW produced from underwater electric front type discharged for 5 min, operated at a voltage not more than 1.5 kV, and discharge current was 50–70 mA. Germinability rate increased by 50%, and plant roots rose to 1.5- to 2-fold as compared to the control after PTW treatment [92]. *Brassica rapa* var. *perviridis* seeds treated with PTW generated by underwater discharge with a repetition rate of 250 pps had a peak voltage of 30 kV using pulsed power generator for 10- and 20-min. The authors noticed that the dry weight of the plant increased to 3.9 and 6.6 times, additionally, leaf length increased to 2.1 and 2.5 times after PTW treatment generated by plasma for 10- and 20-min, respectively, as compared to control [93]. Zhang et al. treated the *Lentils* seeds with PTW created with He-APPJ, showed 80% germination rates, and for control was 30%. Additionally, they also noticed the higher stem elongation rates and final stem lengths in PTW treated samples than commercial fertilizer (control) [94].

Recently, Adhikari et al. treated the Tomato (*Solanum lycopersicum* L.) seeds with PTW generated from the plasma jet. The frequency, current, and applied voltage of the plasma jet discharge were 83.5 kHz, 70.39 mA, and 0.66 kV, respectively. PTW generated from plasma jet for 15- and 30-min treatment showed better morphological growth compared to control seedlings. More PTW produced at 15- and 30-min plasma jet treatment showed higher shoot and root length, but there is no significant change observed for the PTW generated after 60 min plasma treatment [95]. In another recent study on Rapeseed (*Brassica napus*) seeds showed a significant improvement in germination rate and seedling vigor as compared with control when treated with PTW produced with Ar and O₂ feed gases [96].

5. Patents Related to Seed Germination and Seed Growth Using Low-pressure/Medium-Pressure/Atmospheric-Pressure Plasma

A few dozen patents using discharge plasma for plant seed to induce seed-activity have been taken out according to PATENTSCOPE by WIPO [107]. Typically, one of those is direct irradiation. Masaru et al. unveil direct radiation of the atmospheric-pressure plasma jet operated at 50,662–202,650 Pa pressure on rice (*Oryza sativa* L.) seeds, which results in growth improvement [108]. The working gas was Ar or inert gas; the plasma density was 1×10^{14} – 1×10^{17} /cm³, and the plasma temperature was 1000–2500 K. According to the Masaru group, the preferable value of multiplication of plasma density with irradiation time was 6×10^{16} – 2×10^{17} s/cm³ for a positive effect. Additionally, Hayashi et al., showed the improved method for the growth of radish (*Raphanus Sativus* L.) by plasma irradiation to dry seeds [109]. The DBD type plasma generated in stainless chamber $\varnothing 200$ mm \times 450 mm at 80 Pa. The feeding gas was O₂, the frequency and power of the plasma source were 13.56 MHz and 50 W, respectively, and the exposure time was 60 min. The whole stem and root length, and cotyledon width of seedling with the plasma irradiation were 167 mm, 70 mm, 97 mm, and 10.6 mm, and those of control was 120 mm, 41 mm, 79 mm, and 9.5 mm, respectively. They revealed that not only the stem and root length but also the amount of endogenous thiol increased with plasma-irradiation time by changing water vapor pressure from 30 to 270 Pa. Konstantinovich et al. combined the exposure of low-pressure plasma and activated water containing anolyte and catholyte. Low-pressure plasma worked at 0.01–0.1 W/cm³ and 2–150 Pa under inert gas, oxygen, nitrogen, or a mixture of oxygen and nitrogen environment at 20–40 °C for 10–45 s. The authors reduced the seed germination period of radish, pea, beet, cabbage, tomato, barley, lentils, pumpkin, corn, wheat, etc., by 2–4 days [110]. Another group used plasma at 184–188 W for 8–10 s, rested for 4–5 s, 103–107 W for 13–15 s, rested for 2–3 s, and 264–270 W for 11–13 s for black bean (*Phaseolus vulgaris*) seeds; they observed the acceleration in the seed germination after plasma treatment [111]. Shi et al. used plasma seed at 51–53 W for 150–170 s, 141–145 W for 90–110 s, and 77–79 W for 190–210 s twice for dry pumpkin (*Cucurbita* L.) seeds, and at this treatment condition, the seed germination process was promoted [112]. Guo et al. used an atmospheric-pressure DBD plasma generated with a frequency of 3–4 kHz and a voltage of 4–7 kV to treat dry peony seed for 40–60 s [113]. Another research group irradiated the wheat (*Triticum*) seeds with low-pressure plasma with He as the working gas at a power of 1–500 W for 15–20 s. The highest germination rate of 12.72% observed at 80 W plasma power and irradiated for 18 s [114]. Chengdong et al. found that irradiation of an atmospheric-pressure DBD plasma generated at a frequency of 2.5–3.5 kHz and a voltage of 3.5–4.5 kV for 65–85 s to Chinese rosewood (*Dalbergia odorifera* T. Chen) seeds preceding soaked in the distillate of wood for several h, resulted in improved seed germination and subsequent growth [115]. Later, a group of researchers treated the peony seed by atmospheric-pressure DBD plasma with a frequency of 3–5 kHz and a voltage of 5–7 kV for 50–60 s, showed the improvement in germination [116]. On the other hand, Li et al. observed the increased seed viability and the crop yield by 6.5% after maturation compared to the control after plasma treatment working at 220 ± 22 V with a frequency of 50 Hz and a power of 55 W or less [117]. The reviewed patents emphasize the positive effects of plasma treatment on the improved seed germination.

6. Probable Mechanism and Future Perspectives

Overall research outputs by several research groups showed that the germination rate and growth of seedling had increased with direct plasma treatment (low/medium/atmospheric pressure plasma) or through PTW treatment. In this review, we discussed the probable mechanism of enhanced germination rate and growth of the seedling as well (as shown in Figure 6) as the possibility of genetic mutation in seeds observed by various research groups around the world. Hayashi et al. revealed active oxygen species produced from O₂-low pressure plasma-activated the genes in *Arabidopsis thaliana* seeds that were responsible for the cell elongation proteins. Moreover, in the second generation, these genes are not activated; this concluded that there was no genetic mutation in the seeds [45]. Further, the enhanced germination rate and growth of seedling mechanism were discussed below and in Figure 6.

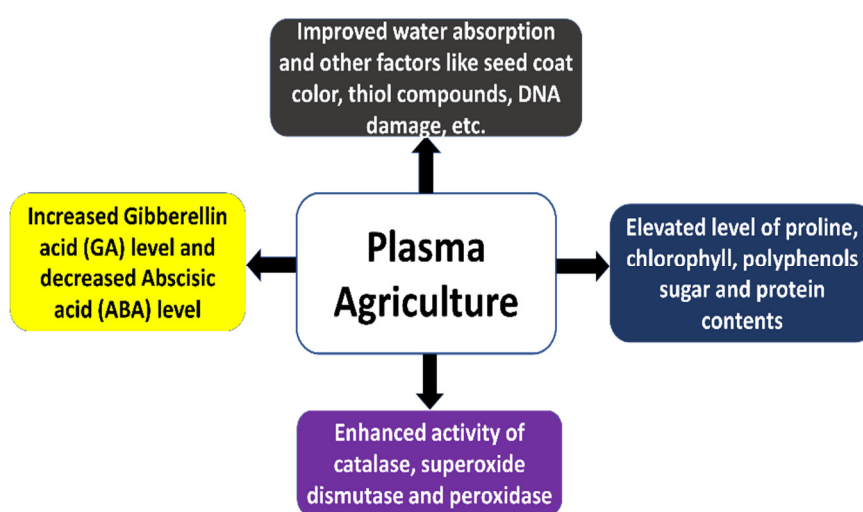


Figure 6. Possible mechanism in plasma agriculture.

Volin et al. showed that plasma treatment in different feeding gases could alter germination time [8]. Treatment of seeds in the presence of carbon tetrafluoride delays the germination of two pea cultivars (*Pisum sativum* cv. Little Marvel, *P. sativum* cv. Alaska) and radish (*Raphanus sativus*). At the same time, plasma treatment in the presence of cyclohexane can significantly accelerate the germination percentage of soybean [8]. Hence, the different feeding gas alters the plasma chemistry that provides alternatives for seed coating [8].

Sarinont, et al. revealed an increase in chlorophyll and carotenoid concentration in Radish sprouts seeds after scalar DBD treatment [118]. Saberi et al. showed improvement in the photosynthesis rate, stomatal conductance, and chlorophyll content in low-pressure plasma-treated wheat seeds [48]. Ji et al. showed an increased level of chlorophyll and total polyphenols contents in spinach seedlings after air DBD treatment [83]. In a very recent report, Sajib et al. showed the increased chlorophyll content in gram seeds after PTW treatment [87].

Phytohormones played an essential role in plant growth. Zukiene et al. showed increased/decreased gibberellin (GA)/abscisic acid (ABA) ratio that results in positive or negative effects on germination and/or seedling growth of sunflower seeds after treatment with scalar DBD [56]. Whereas Stolarik, et al. observed the changes in catabolites, conjugates and endogenous hormones (auxins and cytokinins) of pea seedlings after treatment with DCSBD plasma [61]. In another study, Degutye-Fomins et al. showed a decrease and increase in ABA and GA contents in radish sprouts, respectively, after DBD treatment [119]. Ji et al. observed an elevated level of GA3 hormone in spinach seedlings after NPP treatment [83].

On the other hand, Li et al. showed an elevated level of proline, soluble sugar contents, and osmotic-adjustment products after DBD treatment on wheat seeds [53]. Another study showed that low-pressure plasma treatment on the soybean seeds results in increased protein and soluble sugar

content [41]. Whereas, Ling et al. observed the elevated level of insoluble sugar and protein contents, whereas reduction in malondialdehyde content after low-pressure plasma treatment on oilseed rape seeds [68]. Similarly, Guo et al. observed improvement in proline and soluble sugar contents and decreased the malondialdehyde content in wheat seeds treated with DBD [54].

Hosseini et al. showed improved seed's water uptake in artichoke seeds after low-pressure N₂ plasma treatment [65]. Meng et al. showed that DBD treatment on wheat seeds results in an improvement in the capacity for water absorption and activation of several physiological reactions [52]. Bormashenko et al. showed that low-pressure plasma treatment noticeably increased the water imbibition in bean seeds through the testa, it is independent of the micropyle effect [64]. Alves Junior et al. observed the change in hydrophilicity and water absorption of Mulungu seeds after plasma jet treatment [78]. Volkov et al. showed that pumpkin seeds treated with plasma jet showed structural deformations of seeds such as surface defects, and hydrophilic pores that results in enhanced water uptake [74]. Fadhlalmawla et al. reported that Fenugreek seeds treatment with cold atmospheric pressure plasma jet showed increased water imbibition and absorption that was due to etching on seed coat surfaces [77]. Additionally, Li et al. showed that improvement in the water absorption capacity of wheat seed after DBD treatment is due to the etching effect [53]. Saberi et al. demonstrated the improved tolerance of wheat plants against the haze and increased relative water content after low-pressure treatment [48].

Further, Amnuaysin et al. showed that the production of reduction-type thiol compound changed in radish sprout seeds after treated with plasma torch; these thiol compounds were responsible for growth regulation mechanisms in plants [101]. Hayashi et al. mention that thiol content in seeds with plasma irradiation may be associated with plant growth. Moreover, the redox properties of plasma irradiation as a function of water vapor pressure results in the variation of cysteine peak values at 2587 cm⁻¹ (thiol group; -S-S-) and 520 cm⁻¹ (disulfide bond; -SH) using Fourier transform infrared spectroscopy (FTIR). The increased amount of thiol is due to the reduction of cystine by active hydrogen. The increased cysteic acid was due to oxidative modification of cysteine during plasma irradiation [109]. Very recently, Song, et al. demonstrated the enhanced contents of the primary metabolites, especially the free amino acids and soluble sugars, as well as secondary metabolites like phytochemicals, e.g., saponarin, GABA, and policosanols in barley seeds after treated with SDBD plasma [84].

Other observations in plasma treated seeds by different authors such as Kyzek et al. noticed the DNA damage in Pea seeds due to DCSBD plasma treatment [120]. Wang et al. observed the etching in cotton seeds after DBD treatment [121]. Whereas Adhikari et al. found the elevated expression of pathogenesis-related (PR) gene and defense hormones in tomato seeds treated with PTW [95]. Tong et al. and Hosseini et al. observed the increase in catalase activity after DBD and low-pressure treatment [65,76]. Additionally, Li et al. showed increased activities of superoxide dismutase and peroxidase [53]. In a very recent study, Koga et al. noticed that different seed coat color of radish sprouts responds differently to the scalar DBD treatment. Hence, pigments in the seed coat play a vital role in plasma treatment [122].

All the above studies showed that plasma treatment and PTW treatment had a positive effect on seeds; it improves the germination percentage, seedling growth, and yield. Although these results are laboratory-based; hence, the real field study will be more helpful, which can open new prospects in farming. Ahn et al. conducted the actual field experiments [123]. Authors treated the yellow dent corn hybrid seeds with different types of plasma-like medium-pressure RF plasma, microwave-driven plasma (atmospheric pressure), and DBD plasma (atmospheric pressure). Corn seeds treated by medium-pressure RF (13.56 MHz) plasma at 13332 Pa with N₂ as process gas. Separately, corn seeds treated with a microwave atmospheric plasma jet with 500 W microwave power. DBD plasma with He as feed gas treated the corn seeds with a high voltage of 15 kV at 35 kHz. The yield obtained for medium-pressure RF was relatively higher than other plasma systems but not as high as the control. The authors concluded that there was no statistically significant difference in the yield found between the control and plasma treatment [123].

7. Conclusions

In this review, we concluded that the direct plasma treatments working at low/medium/atmospheric pressure and PTW treatments could change the physical and biochemical properties of seeds. These changes result in the enhancement of seed germination and seedling growth. However, it was imperative to do the real field experiments with the plasma-treated seeds to make it useful to society. Otherwise, plasma agriculture will be limited to the research articles and will not be of value to society.

Author Contributions: P.A. designed and wrote the review. K.I. and M.S. checked the contents, K.K. provided the figures and T.O. compile the patent data for the study. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by JSPS-KAKENHI grant number 20K14454. Additionally, partly supported by JSPS KAKENHI Grant Number JP16H03895, JP19H05462, JP19K14700, JP20H01893, JP20K14454, Plasma Bio Consortium, and Center for Low-temperature Plasma Sciences, Nagoya University.

Conflicts of Interest: The authors declare no conflict of interest.

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