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Cold plasma technology in seed science: Emerging applications and innovations

Y. Bharathi*[1], K. Rohitha[2] and P. Sai ram[3]

[1]G & PB, Agriculture Research station, Tandur, PJTAU, India
[2]Student of DSST, SRTC, PJTAU, Rajendranagar, Hyd-30, India
[3]Agriculture Student of DSST, SRTC, PJTAU, Rajendranagar, Hyd-30, India

ABSTRACT

Cold plasma technology has emerged as a promising tool in the field of plant science and agriculture. Cold plasma, also known as non-thermal plasma, is a partially ionized gas composed of ions, electrons, neutral molecules, and reactive species, generated at or near room temperature. Unlike thermal plasma, cold plasma can be applied to biological materials such as seeds and plants without causing thermal damage, making it highly suitable for agricultural applications. In the context of seed science and plant technology, cold plasma offers a range of benefits. It has been shown to enhance seed germination, improve seedling vigor, and decontaminate seeds by inactivating pathogens and degrading pesticide residues on the seed surface. The reactive oxygen and nitrogen species (ROS and RNS) generated during plasma treatment can modify the seed coat, increase water uptake, and trigger metabolic activities critical for early growth stages. Beyond seeds, cold plasma treatments are being explored for plant growth promotion, stress tolerance enhancement, and post-harvest preservation. These applications align with the goals of sustainable agriculture, as cold plasma is a chemical-free, environmentally friendly alternative to conventional treatments. Despite its growing potential, the underlying mechanisms of cold plasma interactions with plant systems are still under investigation. One of the difficulties encountered during the current investigations was the lack of defined protocols for exposure length, gas type, pressure, voltage, or the distance between the plasma and seed source coupled with underlying molecular mechanisms. Continued interdisciplinary research is essential to optimize treatment protocols, ensure safety, and fully harness this innovative technology in agriculture.

Keywords: *Cold plasma, seed enhancements, non-thermal plasma, dielectric barrier discharge and Reactive oxygen species, Seed physiology, Yield parameters, Antioxidant enzyme activity*

Introduction

Cold Plasma: Composition and Characteristics

Cold plasma, often described as the fourth state of matter, represents a partially ionized gas comprising a complex mixture of reactive and energetic species. It consists of excited atoms and molecules, positive and negative ions, electrons, free radicals, as well as reactive oxygen species (ROS) and reactive nitrogen species (RNS). These components coexist in both excited and ground states, contributing to the unique physicochemical properties of cold plasma. A defining feature of cold plasma is its non-equilibrium nature. While the electrons possess high kinetic energy, the bulk gas, gas-comprising ions and neutral molecules, remains at relatively low temperatures. This temperature disparity allows cold plasma to remain thermally benign, minimizing damage to heat-sensitive materials and biological tissues. The low overall gas temperature arises not from electron energy transfer, but rather from the efficient cooling of ions and uncharged molecules [1].

Operationally, plasma sources generating temperatures near ambient conditions, typically below 60°C, are classified as cold plasmas [2],[3]. This temperature threshold facilitates the application of cold plasma in diverse fields such as biomedicine, food processing, and materials science, where thermal sensitivity is a critical consideration.

Cold Plasma in Plant Protection and Stress Tolerance

Cold plasma generates a wide array of reactive oxygen species (ROS) and reactive nitrogen species (RNS), along with other strong oxidizing agents. These reactive components can penetrate microbial cells, leading to oxidation of the cytoplasmic membrane, disruption of cellular functions, and eventual microbial inactivation [4]. Such antimicrobial properties make cold plasma a promising tool for managing phytopathogens in agriculture.

Beyond its antimicrobial activity, cold plasma treatment has shown beneficial effects on seed physiology and early plant development. For instance, plasma exposure has been reported to significantly enhance seed germination rates and water uptake in seedlings exposed to chilling stress [5]. These improvements can be attributed to the modulation of seed surface properties and potential activation of stress-responsive pathways.

Cold plasma also plays a critical role in reducing plant disease incidence and severity, which is of great importance given that plant diseases are responsible for approximately 20-30% of global crop losses annually [6]. The antimicrobial efficacy of cold plasma has been demonstrated across various crop species [7, 8], and the application of plasma-activated water (PAW)

*Corresponding Author: ***Y. Bharathi***

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has further expanded its utility for disease control [9, 10].

Despite these promising findings, the induction of plant defense responses-, particularly in relation to cold stress tolerance- *via* cold plasma seed treatments, remains poorly understood and underexplored in agricultural research. Further, investigation is required to elucidate the underlying molecular mechanisms and optimize treatment protocols for specific crop species and environmental conditions.

Plasma, an ionized gas, can be generated under both low-pressure and atmospheric conditions. Its composition is highly dependent on operational parameters such as applied voltage, frequency, humidity, gas flow rate, and gas mixture-, factors that critically influence the effectiveness of plasma-based treatments in agricultural applications. Among the various plasma sources, dielectric barrier discharge (DBD) is the most commonly employed in agricultural contexts, followed by plasma jets, corona discharges, microwave plasmas, radiofrequency systems, and gliding arc discharges [11, 12].

Plasma treatment of seeds and seedlings may be performed either directly, by exposing them to the plasma field, or indirectly, by placing them at a distance from the discharge zone. Alternatively, plasma-activated liquids-such as plasma-activated water (PAW) and plasma-activated media (PAM)-can be used to soak seeds or water plants. Comparative analyses between gaseous plasma treatments and plasma-activated aqueous treatments have demonstrated similar outcomes in terms of macroscopic plant responses [13, 14].

Several studies have reported that the enhancement of seed germination and plant growth is significantly influenced by the type of feed gas used during plasma generation. Notably, gases such as aniline, cyclohexane, and helium have been shown to produce varying biological effects [15, 16].

Rice is particularly sensitive to low temperatures during the vegetative stage, which can significantly inhibit seed germination [17, 18] and suppress seedling development. Symptoms of cold stress include leaf curling, shoot stunting, and reduced tillering [19]. With the increasing frequency of extreme temperature events due to climate change, rice cultivation is increasingly subjected to yield penalties. Exposure to low temperatures can damage root tissues, impairing water uptake and nutrient transport to the shoot, thereby limiting overall seedling growth [20, 21].

Recent studies have shown that non-thermal plasma treatment, applied at 15.0 kV for 30 seconds, can enhance rice seed germination and seedling growth under low-temperature stress. This effect is associated with an upregulation of antioxidant enzyme activity, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) [22].

Cold plasma technology has been employed to enhance growth and yield parameters in rice [23,24,25]. In addition to agronomic benefits, cold plasma treatment has been reported to significantly improve the cooking properties of rice grains [26,27]. Treatment of rice seeds with cold plasma has resulted in notable improvements in key morphological traits, including panicle length, stem length, seedling height, panicle weight, and harvest index [25].

Furthermore, cold plasma has been effectively utilized to enhance seed coat permeability without altering the seed's moisture content. This characteristic has made plasma treatment a promising tool in agriculture for improving seed quality, enhancing seed performance, and inactivating pathogenic microorganisms [28].

Possible effect of plasma treatments at the molecular level

Several studies have demonstrated that plant development, particularly processes regulated by thiol groups, can be significantly influenced by redox reactions triggered by reactive oxygen species (ROS) generated from water vapour plasma [29].

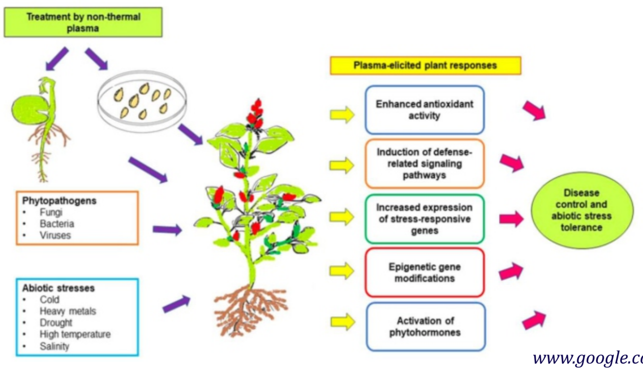
Non-thermal plasma has emerged as a promising alternative to traditional seed enhancement techniques such as scarification, stratification, and priming, with numerous reports highlighting its positive effects on plant growth and development [30]. Plasma-based treatments offer multiple advantages, including non-destructive processing, elimination of chemical pesticides, and the provision of environmentally sustainable seed treatment methods [15, 30,31]. In addition to improving seed health, plasma technology has been shown to enhance seed quality and promote robust plant growth [11, 32].

Exposure of seeds to plasma has been shown to induce changes in enzymatic activity [29] and effectively sterilize seed surfaces, reducing microbial contamination [31]. Plasma treatments can modulate seed germination dynamics, either enhancing or delaying the process depending on treatment parameters and crop type [15]. Notable advancements in plasma-based agricultural applications include the use of microwave discharges [11] and low-density radio frequency (RF) discharges [33,28] which have opened new avenues for seed treatment technologies.

Numerous studies have explored the effects of plasma on germination patterns in various crops, including wheat, maize, radish, oat, safflower, and blue lupine [11,28,30,32]. For example, safflower seeds exhibited a 50% increase in germination rate following RF plasma treatment with argon for 130 minutes [30]. Similarly, soybean seeds treated with cold plasma at varying power levels (0-120 W) for 15 seconds showed significant improvements in both germination and seedling vigour [34].

The impact of various plasma treatments on different crop species is summarized in Table 1,2 and Table 3.

Cold plasma effects on abiotic stress tolerance



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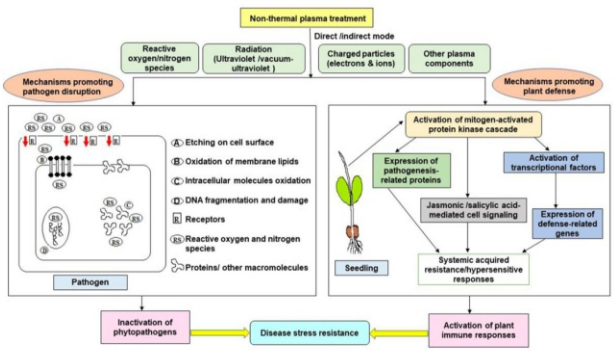

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Table 1. Common Plasma Sources and Their Applications in Agriculture

Plasma Source	Operating Conditions	Gas Types Used	Mode of Application	Agricultural Applications	References
Dielectric Barrier Discharge (DBD)	Atmospheric or low pressure High voltage AC	Air, O_2, N_2, Ar	Direct and indirect treatment PAW & PAM	Seed germination, microbial inactivation, stress tolerance	[11,12]
Plasma Jet	Atmospheric pressure Low voltage RF or microwave	He, Ar, air	Direct treatment	Germination, root growth stimulation, sterilization	[16]
Corona Discharge	Atmospheric pressure High voltage DC	Air, N_2	Indirect treatment	Disinfection, seed surface modification	[12]
Microwave Plasma	Low or atmospheric pressure	Ar, N_2	Direct and indirect	Germination enhancement, sterilization	[35]
Radiofrequency (RF) Plasma	Low pressure	O_2, N_2, He	Direct treatment	Seed coat etching, microbial inactivation	[15]
Gliding Arc	Atmospheric pressure AC power	Air, N_2, CO_2	Direct and PAW generation	Growth promotion, stress tolerance	[36]

Abbreviations:
- **PAW** – Plasma-Activated Water
- **PAM** – Plasma-Activated Media
- **RF** – Radiofrequency
- **DC** – Direct Current
- **AC** – Alternating Current

Table 2: Impact of plasma treatments on field and horticultural crops

S. No	Crop	Type of plasma	Impact summary	Reference
1	Oil seed rape	Radio frequency capacitively coupled plasma generated using helium gas	The activities of superoxide dismutase and catalase were increased by 17.71%, 16.25% and 13.0%, 13.2% in drought-sensitive and drought-tolerant cultivars, respectively. Further, increase in germination rate, soluble sugars and proteins and reduction in malonaldehyde content was noticed under water deficit conditions.	[37]
2	Tomato	Plasma-activated water	Up regulated the synthesis of pathogenesis related genes, induced hormone-mediated signaling, epigenetic modifications and proline accumulation promoting both disease resistance and drought stress tolerance	[38]
3	Wheat	Arc discharge plasma	Induced the activity of peroxidase, α-amylase and soluble proteins enhancing drought tolerance. However, the degree of tolerance varied between the cultivars	[39]
4	Wheat	Low pressure dielectric barrier discharge using (air/argon, argon/oxygen)	Reduction in pH of seeds and decreased bioavailability of cadmium. Increase in the activity of catalase and super oxide dismutase, total soluble protein content and down-regulation of cadmium transporter genes was noticed in treated seedlings	[40]
5	Water spinach	Atmospheric Pressure plasma jet	Significant reduction in the bio concentration factor of cadmium from 0.864 to 0.54. However, the concentration of lead remained un affected through plasma treatment	[41]
6	Wheat	Dielectric barrier discharge plasma	Enhanced the expression of heat shock transcription factor-4A, peroxidase activity by 25% and phenylalanine ammonia lyase activity by 21%. Increased tolerance to salt stresses besides, increase in shoots dry mass (18%), leaf area (18%) and chlorophyll-content (37.5%).	[42]
8	Rice	Atmospheric pressure cold plasma	Provoked epigenetic changes through methylation and demethylation of promoters involved in α-amylase synthesis (OsAmy1A, OsAmy1C, OsAmy3B and OsAmy3E) and abscisic acid synthesis genes (OsNCED2 and OsNCED5) promoting germination in seeds exposed to heat stress.	[43]
9	Maize	Atmospheric pressure cold plasma	Soluble sugar content, proline and peroxidase activity were increased by of 5.5%, 24.7% and 33.3% respectively. Electrolyte leakage decreased by 20.9% resulting in cold tolerance	[44]
10	Cotton	cold plasma generated using air/ argon	Significant impact in enhancing water absorption, whereas, treatment for 21 min using air could enhance both water absorption and germination followed by 81 min of argon plasma treatment increasing tolerance to cold.	[5]
11	Sunflower	cold plasma	pre-treatment of seeds with cold plasma for 30 seconds increased oil and seed yield of sunflower by 68.8 and 58.5 per cent respectively in the weed infest treatment than control	[45]

Table 3: Impact of plasma treatments targeted for pathogens in field and horticultural crops

S. No	Crop	Type of plasma	Pathogens targeted	Impact summary	Reference
1	Wheat seeds	Low-temperature plasma	*Alternaria* sp., *Fusarium* sp., *Gibberella*sp., *Pencillium* sp., *Rhizopus stolonif era*, *Trichoderma* sp. and non-spore forming fungi	Reduction in number of fungal colonies within 10 s of exposure.	[46]
2	Soybean seeds	Atmospheric pressure dielectric barrier discharge plasma using nitrogen/ oxygen	*Diaporthe/ Phomopsis* complex	Reversal of oxidative damage caused by fungal complex in the treated seeds.	[47]
3	Barley and corn seeds	Glow discharge plasma	*Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp.	Fungal load on the seeds of both crop species decreased significantly with an increase in treatment duration	[48]
4	Rice seeds	Atmospheric pressure dielectric barrier discharge plasma	*Gibberella fujikuroi*	Inhibition in the growth of fungus and 92% reduction in the number of fungal colonies on the plasma treated seeds	[49]
5	Tomato seeds	Micro dielectric barrier discharge plasma using air/argon	*Fusarium oxysporum f. sp. lycopersici*	Significant reduction in spore germination per cent after 10 min of treatment	[50]
6	Barley and corn seeds	Glow discharge plasma	*Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp.	Fungal load on the seeds of both crop species decreased significantly with an increase in treatment duration	[48]
7	Rice seeds	Atmospheric pressure plasma jet	*Burkholderia plantarii*	Disease severity index reduced to 38.6% in the plasma treated seeds.	[51]
8	Tomato seeds	Inductive Helium plasma	*Ralstonia solanacearum*	Significant increase in resistance against bacteria and improvement in antioxidants and hydrogen peroxide concentration	[52]
9	Cabbage seeds	Low pressure plasma using Argon	*Xanthomonas campestris*	Significant reduction in inoculum load after 5 min of treatment, and complete inactivation of bacteria after 40 min of treatment	[53]
10	Stored grains	Dielectric barrier discharge	*Tribolium castaneum*	The mortality of 95.0 % –100% for pre adult stages can be achieved within seconds of treatment, but longer plasma exposure (5 min) is required to kill adult insects. Cold plasma treatment reduces both the respiration rate and the weight of insects and affects the levels of oxidative stress markers in adult populations.	[54]
11	Chickpea	Cold plasma at different power 40, 50, and 60 W each for 10, 15, 20 min.	*Callosobruchus chinensis L.*	Plasma treated and untreated chickpeas were stored in an airtight ziplock pouch and found effective in controlling the pulse beetle infestation of treated chickpea samples	[55]

One of the key advantages of plasma treatment is its ability to enhance seed viability and performance without leaving behind toxic chemical residues. This is largely due to the fact that plasma is composed of naturally occurring reactive species-, such as ions, electrons, and reactive oxygen and nitrogen species-, that rapidly recombine into non-reactive forms shortly after treatment. Furthermore, plasma components typically exhibit a shallow penetration depth of approximately 10 nanometers, resulting in effects that are largely limited to surface functionalization. This makes plasma particularly effective for modifying the seed coat without compromising internal structures. Additionally, plasma technologies are generally characterized by low maintenance requirements and minimal energy consumption, making them an economically and environmentally sustainable option for agricultural applications [56].

Future scope of the study

It is necessary to investigate how cold plasma affects yield-contributing pathways and how plasma affects the genes that produce yield-contributing traits.

Compared to the phenotypic impacts, the underlying mechanisms of plasma effects are comparatively unexplored. To optimise plasma systems and their uses, additional knowledge about the modes of plasma action on plant production and sustainability is required.

Conflict of interest

The corresponding author assures on behalf of all listed authors that the subject materials described in this paper do not present any financial or non-financial conflicts of interest.

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