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Cold Plasma: Novel Technology Enhances Seed Germination

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Introduction

The agriculture sector produces a significant amount of the world's food requirements, and seeds are critical components. New technologies of farming are playing a more and more significant part in seed quality enhancement, leading to entirely new technological innovations. The conventional way to improve seed germination is by using seed priming, scarification, pesticidal and hormonal seed treatment. In recent years, physical methods such as the magnetic fields, ultraviolet light and mechanical processes have been gaining attention. Each one of these techniques has drawbacks of being slow, complicated, and risky for the seed germination and survival.

When seeds are exposed to various physical and biological stresses during germination, water uptake plays a major role in germination as it is determined by the permeability of the seed coat. To enhance seed coat permeability during germination, various technologies have been adopted. Among them, cold plasma (CP) has emerged as a developing and environmentally sustainable physical seed treatment method.

Cold plasma technology can be applied during different stages of the crop growth period, including treatment of seeds for the promotion of germination rates and plant growth, which can increase crop yields and shorten harvest time. How effective the treatment is going to be depends on the type of seed, as well as environmental factors, such as climate, water availability, and soil conditions. Therefore, the time of plasma treatment has to be optimized for each type of seed individually (Bourke *et al.,* 2018). Several publications and patents since the 1990s have shown enhancements of seed germination, and the research is still ongoing (Ito *et al.,* 2017). Improvement of germination, longer shoots and roots of the seedlings, and higher yields of plasma-treated seeds have been reported by several authors using various seeds.

Fundamentals of Cold Plasma Technology

Plasma is commonly recognized as the fourth state of matter that exhibits a higher energy state than solid, liquid, or gas. It comprises excited atomic, molecular, ionic, and radical species, along with various constituents like electrons, positively and negatively charged ions, free radicals, gas molecules, and atoms in both ground and excited states (Feizollahi *et al.,* 2021). Additionally, electromagnetic radiation quanta, including ultraviolet photons and visible light, are integral components of plasma

(Feizollahi *et al.,* 2021). The presence of free electric charges, such as electrons and ions, renders plasma electrically conductive and highly responsive to electromagnetic fields (Cherif *et al.,* 2023).

Plasma is categorized as thermal (hot) or non-thermal (cold) depending on the thermal equilibrium between electrons and heavier particles. CP has an electron temperature, that is significantly higher than the macroscopic gas temperature resulting in a lack of local thermodynamic equilibrium (Feizollahi *et al*., 2021). This non-equilibrium state results from the asymmetrical momentum transfer during collisions influenced by an electrical potential difference (Misra *et al.,* 2018). In contrast, thermal plasma exhibits consistent average temperatures among electrons, ions, and neutrals. The temperature of any plasma is commonly defined by the mean energies of neutral and charged plasma species along with their corresponding degrees of freedom.

Historically, plasma discharges were generated under low-pressure conditions, effectively decreasing the number density of gas molecules (i.e., the number of gas molecules per unit volume) and enabling ionization at lower electric potential differences. However, with technological advancements, CP can now be initiated and sustained under ambient atmospheric conditions. This atmospheric pressure plasma generation system is simpler and more user-friendly compared to the low-pressure plasma generation system.

Working principle

Plasma properties are governed by its chemistry, involving numerous reactions and a mix of positively and negatively charged particles, depending on the initial gas mixture's composition. Chemical reactions in the air are mainly initiated by electron collisions with oxygen and nitrogen. The power input, working gas, and mode of operation significantly influence the species formed and their degree of formation in a plasma system. Various gases, including Ar, He, N_2, O_2, CO_2, specific gas mixtures, and air, have been utilized for plasma treatment. Air is the most suitable due to its abundance and low cost. Reactive oxygen and nitrogen species (RONS) are typically formed due to the collision between electrons that drift in the electric field and diffuse through the medium of neutral atoms, resulting in ions and excited atoms. Common RONS formed in plasmas include ROS, such as hydrogen peroxide (H_2O_2), ozone (O_3), superoxide anion (O_2•-), hydroperoxyl (HO_2•), alkoxyl (RO•), peroxyl (ROO•), singlet oxygen ($^1O_2$), hydroxyl radical (•OH), and carbonate anion radical (CO_3•-), and RNS, such as nitric oxide (NO•), nitrogen dioxide radical ($^{\bullet}NO_2$), peroxy nitrite (ONOO-), peroxy nitrous acid (OONOH), and alkyl peroxy nitrite (ROONO) (Arjunan *et al.,* 2015).

These constituents induce surface oxidation, erosion, corrugation (i.e., change of shape), and hydrophilization of seed coats, thereby improving seed quality (Gao *et al.,* 2019).However, the efficacy of CP treatment varies depending on seed coat properties, moisture content, and hormesis, a phenomenon where a small amount of typically harmful substances (e.g., ROS) stimulates beneficial effects on living organisms (Dauwe *et al.,* 2021).

H_2O_2 interacts with the seed surface and can cross the plasma membrane, while superoxide, carrying a charge, necessitates voltagedependent anion channels (porins) for passage through the plasma membrane (Waskow *et al.,* 2021). Despite its charge, superoxide can decompose into hydroxyl and singlet oxygen, which can more easily traverse the plasma membrane. Occasionally, RONS can bypass the plasma membrane through aquaporins, membrane proteins facilitating the diffusion of water and small molecular weight solutes, including ROS and RNS (Billah *et al.,* 2020). It is important to understand the attributes of plasma constituents, particularly reactive species, due to their extended lifespan and complex reactions. A brief overview of selected reactive species and their impacts have been presented below.

UV radiation can have negative and positive effects on the various physiological processes related to seed growth (Luuand Maurel., 2013). It can facilitate the entry of RONS into the cell membrane by causing surface-etching damage to the seed coat.

Hydrogen peroxide is a long-lived ROS with a half-life from 8 h to 20 days (Sun *et al.,* 2012). It serves as a signaling molecule and is highly interconnected with hormones, metabolism, and gene transcription. H_2O_2 diffuses through anion channels and is converted to superoxide and hydroxyl radicals (Priatama *et al.,* 2022).

Hydroxyl radical is a short-lived ROS with a half-life of 10^{-9}s. It is an extremely potent ROS and can lead to the oxidative breakdown of cell wall polysaccharides, resulting in cell wall loosening (Richards *et al.,* 2015).

A plethora of studies have been conducted to examine the impact of cold plasma on germination. These experiments have demonstrated that using cold plasma may significantly improve seed germination rates and their bioactive phytochemical contents. Furthermore, seeds can be preserved and kept for extended periods after cold plasma treatment. An approach of exposing water to plasma, i.e., "plasma-activated water" (PAW), may contain nitrates and reactive species that can supplement plant nutrition. Studies have demonstrated increased rates of germination and plant growth of soybeans through plasma treatment. PAW is enriched with a range of chemically active species RONS (Reactive Oxygen and Nitrogen Species). Nitrates generated in PAW may have fertilizing effects, contributing to plant nutrition.

Cold plasma is believed to stimulate chemical and hormonal response in the seed that breaks its dormancy by weakening the endosperm and moving the stocked-up resources of the seed to a ready-to-use location. This allows the seedling to erupt easily from the seed and to grow faster and healthier.

How exactly does cold plasma boost plant growth?

Although many studies investigated the effects of plasma on seeds, the mechanisms resulting in germination enhancement and promotion of plant growth are not entirely clear; the overall result can be a combination of various factors:

- **Changes in seed surface wettability resulting in increased water absorption**: less irrigation water would be needed for plant growth, which would be particularly important in countries in which water resources are limited.
- **Seed Germination and Vigor:** Cold plasma treatment alters seed coat structure, increasing water absorption and enhancing

enzyme activity (e.g., α-amylase) to speed up germination and improve seedling vigor.

- **Breaking the seed dormancy**: reactive species formed during plasma discharge such as nitric oxide, can break the seed dormancy and lead to faster germination.
- **Seed coat erosion:** some seeds require scratching or nicking of the hard seed coat to allow moisture to enter the seed to begin the germination process. It was observed that as a result of plasma treatment, seeds often have a slightly damaged surface.
- **Removal of microorganisms**: cold plasma treatment inactivates bacteria and fungi present on the seeds, which means that plasma-treated seeds would be less likely to pose health risks related to microbial contamination and cause economic losses.

Adhikari *et al.* (2020) emphasized that plasma discharge over seeds removes organic contaminants and induces surface modifications such as oxidation, erosion, corrugation, and hydrophilization. These alterations impact the seed coat's water contact angle, roughness, and hydrophilicity, enhancing water uptake during germination (Billah *et al.,* 2020).

Volkov *et al*. (2020) reported seed surface roughness affects wetting, and it affects the seed imbibition. Greater roughness enhances surface properties that attract or repel water. (θ?) If $\theta < 90^{\circ}$, water enters the cavities, improving wetting. If $\theta > 90^{\circ}$, water does not penetrate, reducing wetting. Further, they found that longer plasma treatment time (up to19 min) decreased the water droplet contact angle on bush bean seeds, as well as increased the hydrophilic pore sizes on the seeds. Stolarik *et al*. (2015) reported that increasing the surface barrier plasma discharge from one to ten min increased the surface-modifying electroporation of the outermost waxy layer of peas and resulted in a higher imbibition process. Billah *et al*. (2020) also observed an increased erosion of the cuticle layer and the subsequent thinning of the seed coat of black gram (*Vigna mungo* L.) following dielectric barrier discharge (DBD) plasma treatment for three min. The surface lipid erosion and an enhanced water absorption rate due to plasma etching were also reported in peas (Stolarik *et al.,* 2015), black gram (Sajib *et al*., 2020), and mung beans (*Vigna radiata* L., (Zhou *et al.,* 2019). Moreover, an increase in the water imbibition is also related to an increased interaction between RONS and micropyles located on the seed coat, thus resulting in a larger pore size.

Cold plasma against seed borne diseases and insects:

While cold plasma significantly improves the seed germination properties, it is also known to counter the seed borne fungi, bacteria and insects. It has the potential to serve as a safe and eco-friendly alternative to the traditional fungicidal and insecticidal seed treatments, considerably reducing the toxic residues. CP contains highly reactive free radicals, ions, UV photons, protons and electrons. Reactive Oxygen and Nitrogen Species (RONS) damages the microbial cell wall, proteins and DNA. The plasma physically erodes or detaches the fungal spore and biofilm present in the outer seed coat (Sutar *et al*. 2021). Studies indicate that cold plasma can control variety of pathogens and insect pests. CP is highly effective against fungi like *Fusarium*, *Aspergillus* and *Alternaria*. It can also effectively control the different stages of insect pests (egg, larva, pupa and adult) of Red Flour Beetle, Rice Weevil etc., (Martami *et al.* 2025). However, the duration of exposure, targeted developmental stages are to be explored scientifically across all major species.

Conclusion

Cold plasma technology is a promising convergence of physics and agriculture, with potential to improve seed germination, seed vigour and counteract the seed pathogens and insects. It proves itself as an innovative, efficient and ecofriendly physical method for improving seed germination and plant growth. Despite its potential, cold plasma technology faces challenges such as the high initial cost of equipment, need for treatment optimization for different crop seeds, and limited commercial-scale adoption. Further research is required before widespread use in agriculture. As research is underway to understand the mechanism by which the cold plasma improves the seed quality aspects, this technology has the potential to emerge as a key component for the future seed treatment practices complementing existing seed treatment methods as a safe, eco-friendly and chemical-free technology.

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