# 2 Exploitation of cold plasma technology in agriculture

## 3 ABSTRACT

Cold plasma is an emerging nonthermal technology primarily used for microbial disinfection and 4 surface modification. Nowadays, the principle of plasma surface modification is exploited in 5 food and agriculture. The aim of the present review is to give some insights on cold plasma 6 7 technology exploitation for enhancement of seed germination. The seed germination rate can be increased on application of cold plasma by both direct and indirect treatments. Recently, the 8 9 indirect treatment through the application of plasma activated water (PAW) has drawn some attention. The formation of reactive oxygen species and reactive nitrogen species in the plasma 10 11 are mainly responsible for increase in seed germination rate. Of all those reactive species formed in the PAW, the nitrate ions serves as the fertilizer and NO radical breakdown dormancy which 12 13 enhanced the seed germination rate. The synergistic effect of cold plasma can replace the traditional seed disinfection solutions and chemical seed germination enhancers. 14

15 Key words: Seed; germination; cold plasma; plasma activated water; reactive species

# 16 **1. INTRODUCTION**

17 The present human population statistic says that the population of the earth reached to about 7.6 billion. It is insufficient for the total cultivable land to meet the demand of increasing population 18 consumption. In order to meet this demand, the only way is to increase the crop yields. 19 Enhancing the seed germination rate and a draught resistant seed is the best way to improve crop 20 production. The most commonly used methods to enhance the seed germination are the chemical 21 methods (agrochemicals, fungicides, insecticides and hormones). But these types of treatments 22 leave chemical residues which are harmful to the human health and environment. Therefore, it is 23 24 necessary to develop new alternative technologies to enhance seed germination to keep up with pace of growing population. Filatova et al [1] reported that the nonthermal and electromagnetic 25 26 technologies have been successfully applied to enhance the seed germination. Cold plasma is one of such technologies recently drawn interest in food and agriculture sector. 27

Plasma is known as the fourth state of matter, charged gas with strong electrostatic 28 interactions. Plasma consists of neutral and excited atoms, free radicals, negative and positive 29 30 ions, UV photons with net electric charge zero. Bogaerts et al [2] reported that the plasma is 31 divided into high temperature (local thermal equilibrium) and low temperature (non-local thermal equilibrium). The cold plasma contains low temperature particles like neutral molecules, 32 atomic species and relatively high temperature electrons because of this the plasma is cold and 33 34 does not affect the sensitive materials which comes into contact [3]. Ling et al [4] stated that cold plasma treatment is thought to be a fast, economic and pollution-free method to improve seed 35 performance, plant growth and ultimately plant yield. 36

Till date there are many reviews on the antimicrobial effects of cold plasma treatment but only few reviews on the seed germination enhancement. The aim of the present review is to give some insights on the seed germination enhancement by the cold plasma treatment.

#### 40 2. EFFECT OF COLD PLASMA ON SEED GERMINATION

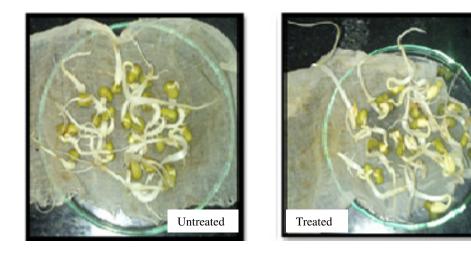
Sirova et al [5] reported that the seedling growth during germination involves two key steps 1) 41 42 primary cell elongation of the axial part of the embryo, and 2) simultaneous or delayed cell division in the radicle meristem. Ji et al [6] reported that the seed germination is initiation of 43 44 embryo breaking the dormant stage and always start with imbibition of water. Seed germination activity involves several physiological and biochemical changes such as protein synthesis, 45 46 enzyme activation and starch metabolisms [7]. The cold plasma can be applied by two different ways 1) direct treatment of seeds, 2) indirectly treating the seeds with plasma activated water 47 (PAW) or plasma acid. 48

#### 49 **2.1 Direct treatment**

50 In direct treatment method the seeds are directly placed in between the electrodes or placed under the plasma regime like in plasma jets. Direct exposure of chickpea seeds to atmospheric 51 cold air plasma for 5 min by [8] observed an overall increase in seed germination by 89.2%. The 52 authors have reported that the increase in the seed conductivity and seed roughness after the 53 plasma treatment is the main reason for enhancement. The increase in seed roughness or etching 54 caused by bombardment of reactive species may be the reason for increase in hydrophilicity of 55 seeds. Thirumdas et al [9] reported that the radio frequency low pressure plasma treatment of 56 brown rice increased the hydrophilicity. An investigation carried out by Bormashenko et al [10] 57 on the wettability of seeds using cold air plasma decreased the contact angle was from 115° to 58

 $0^{\circ}$ . The plasma treatment resulted in complete hydrophilicity of seeds. The water imbibition of plasma treated seeds was increased by 30% after the 12 h of germination.

Dorbin et al [11] exposed barely seeds to both continuous and pulsed glow discharge plasmas, with a pulse repetition rate of 0.5 Hz and pulse duration of 150–200 ms in air under 0.1-0.2 Pa. The authors reported that the number of germinated seeds after the exposure was increased more than 27% after the 5<sup>th</sup> day of germination. Low pressure plasma treatment of mung beans with a radio frequency (13.56 MHz) significantly enhanced the germination rate by 36.2%, radicle root length by 20% (Figure 1) and conductivity of seeds by 102% when compared to the untreated samples [12].



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Figure 1 Seedling growth of radio frequency cold plasma treated mung beans after 24 h ofgermination [12]

71 A detailed study conducted by Ji et al [7] on germination of coriander seeds using nitric oxide (NO) gas produced from microwave plasma torch and explained all the possible ways for 72 increase in seed germination rate. The authors have observed 91-97% germination of seeds after 73 treatment whereas only 60% in the case of control/untreated seeds. The NO formed in the plasma 74 75 serve as a signaling pathway, triggers activation of several biological processes and a crucial regulator for cellular activation. The authors finally concluded their argument that the NO 76 77 formed in the plasma is responsible for seed germination enhancement. A similar explanation was given by Zhang et al [13] on the synergistic effect of formation of endogenous NO radicals 78 79 and breakdown of seed dormancy resulted in increase in higher germination rates.

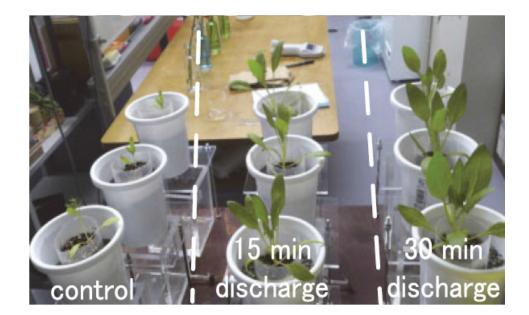
80 Violleau et al [14] reported that the oxygen plasma treatment of corn seeds increased the germination rate and higher yields. Another study conducted by Puligundla et al [15] on effect of 81 82 corona discharge plasma jet on sprouting of rapeseeds. Exposure of rapeseeds for 1 min increased the germination rate by 7.7% compared to untreated seeds. Sera et al [16] studied the 83 effect of cold plasma on seed germination of oat corns and wheat at 500 W at different time 84 durations. The authors have observed that plasma treatment did not affect the germination of oats 85 but there is slight increase in germination rate in wheat. Similar increase in the germination rate 86 of rice was observed after the treatment with static magnetic fields [17]. Table 1 report on the 87 different seeds germination enhancement caused by cold plasma. 88

### 89 **2.2 Indirect treatment**

In this type of treatments the seeds are treated with plasma activated water (PAW). The PAW is 90 91 generated by application of cold plasma on the water surface or underneath water using different plasma sources. Shen et al [18] reported that based on the working gas, discharge type and 92 93 chemical composition of plasma several reactions are initiated in the water resulting in primary 94 and secondary reactive species changing the physico-chemical properties of water. The important 95 reactive species formed in the PAW are reactive oxygen species (ROS) like atomic oxygen, singlet oxygen, superoxide, hydroxyl radicals and reactive nitrogen species (RNS) like 96 97 peroxynitrite, nitric oxide, nitrates and nitrite ions. The formation of these reactive species attributes some important change in properties of water are pH, oxidation-reduction potential, 98 99 conductivity,  $H_2O_2$  concentration, nitrites and nitrates concentration.

Naumova et al [19] conducted a study on underwater electric front type discharge to 100 101 generate PAW and treatment of PAW on rye seeds. The rye seeds treatment with PAW for 5 min increased the germinability by 50% and the number of seeds germinated. Along with the 102 103 hormones, the ROS and NO radicals participate in several signaling pathways involved in the 104 seed germination [20]. The studies related to germination suggested that the ROS including superoxide, hydroxyl radicals, hydrogen peroxide and atomic oxygen are responsible for seed 105 germination [21]. Sirova et al [5] reported that the reactive nitrogen species (RNS) particularly 106 107 NO can break the seed dormancy and enhance the seed germination.

The effect of PAW (generated using cylindrical double DBD (Cyl-DBD) reactor and tap water on seed germination was studied [22]. The authors have observed 60% and 100% germination rate when seeds treated with PAW-15 and PAW-30 respectively along with improved seedling growth whereas only 40% with tap water. Takaki et al [23] reported that the nitrate species formed in PAW served as a fertilizer which resulted in enhanced growth. The activation of tap water with the atmospheric plasma jet increased the germination rate of lentil seeds to 80% compared to 30% of untreated seeds (Figure 2).



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Figure 2 Photographs of Spinacia oleracea cultivated for 0 (upper) and 28 days (lower) under
control conditions, PAW generated for 15 and 30 min (Adapted from[23])

# 118 **3. CONCLUSION**

It can be concluded from the reports of several researchers that the seed germination rate enhancement is achieved from cold plasma technology. The plasma etching or scratching effect on the seed coat increased the hydrophilicity of seeds. Few authors have reported that few reactive species formed in the plasma has the ability to breakdown the seed dormancy and increased the seed germination rate. Cold plasma can be considered as the alternative technology for enhancement of seed germination rate.

# 125 **COMPETING INTERESTS**

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- 127 Authors have declared that no competing interests exist.
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# 129 **REFERENCE**

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220 Table 1 Key findings of different studies conducted on the seed germination rate using cold

221 plasma technology

Seeds	Plasma source	Feed gas	Key findings	References
Rapeseed	DBD plasma	Argon	No change in the germination rate. Increase in seed viability was observed	[24]
Pea	Diffuse coplanar surface barrier discharge	Air	Increase in germination percentage, root length, shoot length, seed vigor	[25]
Herbaceous plant	DBD plasma	Air	Permeability of the seeds was improved significantly, acceleration of seed germination and seedling emergence	[26]
Radish sprout	atmospheric pressure plasma torch	Oxygen	Enhancement of the germination and lengths of the stem and root of plants are observed after seeding	[27]
Tomato	Arc discharge Plasma	Air	In pot experiments the yield is increased by 20.7%. Sprouting rate after the treatment is 32.75%, whereas the untreated was only 4.75%	[28]
Wheat	Atmospheric pressure surface discharge	Air	Plasma had little effect on germination rate but the distribution of roots was shifted towards higher lengths as compared to untreated samples	[11]
Rapeseed	Radio frequency discharge	Helium	Significant improvement in germination rate by 6.25% in drought sensitive variety, and 4.44% in drought tolerant variety	[29]
Soybean	Radio frequency discharge	Helium	Germination and vigor indices significantly increased by 14.66% and 63.33%, respectively. Water uptake improved by 14.03%	[4]
Mung beans	Radio frequency capacitively coupled plasma	Air	Increase in following parameters- germination rate by 36.2%, radicle root length by 20% and conductivity of seeds by 102%	[12]
Coriander seeds	DBD	Nitrogen	After 7 days of germination there is 90% of seed germination compared to 40-60% for control seeds	[6]