Effects of low temperature plasmas in ambient air on germination and plant growth of different genotypes of *Arabidopsis thaliana* seeds

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Abstract: In order to investigate the effects of low temperature plasma on germination rate and development of *Arabidopsis thaliana* seeds, we used a DBD device generating the plasma directly in ambient air. To highlight the plasma effects on seed germination stimulation, we considered the reference ecotype Col-0 of *A. thaliana* and also two further seed coat mutants *gl2* and *gpat5* to better analyse the plasma effects of the seed surface.

Keywords: Low-temperature plasma, *Arabidopsis thaliana*, Germination, *gl2* and *gpat5* mutants

1. Introduction

Germination is a crucial step in the life of the plant. Its groups together all the mechanisms involved in the absorption of water by the quiescent dry seed and ending with the lengthening of the embryonic axis. In order to pass this process, the direct environmental conditions must be favourable at a given moment.

In general, the conditions are optimal only over a short period of a few hours to a few days. In fact, the acceleration of the germination rate is an essential asset for the prosperity of a species. In order to accelerate this germination rate, seeds are often treated with phytosanitary products before sowing [1]. These chemical products can have adverse effects on both the human health and the biosphere. In an effort to reduce the use of pesticides, several alternatives are underway to find substitutes for these inputs.

The use of atmospheric pressure plasmas can be one of these alternatives. The aim of the collaboration between plasma physics and plant biology labs is to increase the germination rate while preserving the quality of the seed and trying to understand the necessary process.

Low temperature plasmas can be produced by several types of electric discharges using different background gas compositions and feed sources [2]. The resulting weakly ionized gas is composed of low densities of energetic electrons with dissociated, excited and ionized species. The average plasma temperature does not generally exceed body temperature (about 37 ° C), which allows living cells to be treated without significant thermal effects.

In fact, low temperature plasmas are good candidates for plant biology, since they have already been studied in animal biology. For several years, the antibacterial properties of low temperature plasma have been used for the sterilization of medical equipment and the decontamination of surfaces, or for the induced modifications of surface biological properties [3] Low temperature plasmas are also studied for their healing properties, or on the reduction of tumour cells [4] and can even partially permeabilize cell membranes for gene transfection applications [5].

In the field of plasma agriculture, studies have shown that the germination rate of various seeds of agronomic interest such as tomato, mustard, soybean, etc. [6] can be stimulated by low temperature plasmas. However, the mechanisms involved in this plasma stimulated germination as well as in the plant growth are very little known [7]. Further researches are therefore needed to better explain the processes involved during germination and seed development.

In this work, the model plant *Arabidopsis thaliana* was chosen more particularly because its growing conditions and short life cycle are favourable for laboratory experiments.

2. Seed germination

The treatment time using a floating-electrode DBD device generating low temperature plasma in ambient air is set at 15 minutes with the following parameters: voltage: 10 kV; frequency: 10 kHz; duration of the pulse: 1µs (see [2] for the description of the ambient air plasma device). As displayed in fig. 1, there is an increase in germination rate (testa and endosperm ruptures) after 40h with an increase of 16.6% for the endosperm and 9% for the testa rupture.

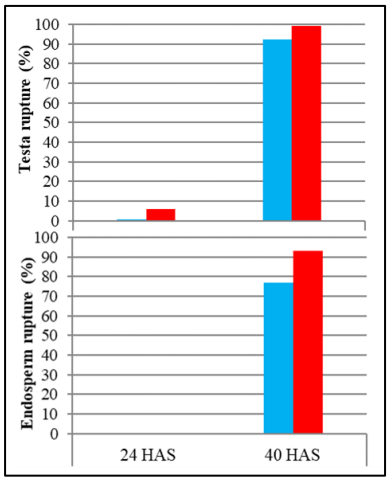

Fig. 1. Bar-chart oh the percentage of testa and endosperm ruptures after low-temperature plasma treatment. Observations are done 24 and 40 hours after sowing (HAS). Red bar: treated seeds; Blue bar: control seeds.

To check whether the effect of the plasma treatment was preserved, germination tests were performed 2, 4, 7 and 9 days after plasma treatment (Fig. 2). The effect on the endosperm rupture persists until 9 days after treatment, with more than 3% of increase. However, this positive effect is no longer significant after two days.

With those results, we showed that the treatment with low-temperature plasma induces an increase in the germination rate. At that point, the hypothesis of an effect on the seed's surface is advanced. To image this, the seeds are observed under a scanning electron microscope (Fig. 3). A change in the appearance of the seed surface is observed. Indeed, we can see that the cell structure is still present, but we can see an appearance of "melted wax" on the surface.

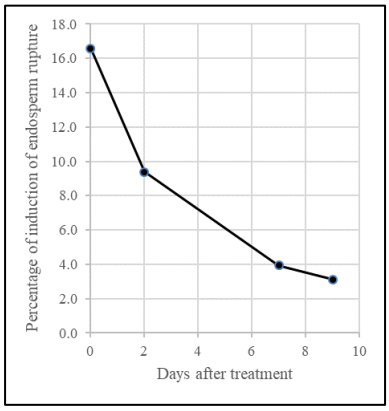

Fig. 2. Diagram of the plasma treatment persistence in time on the endosperm rupture. The seeds are sowing 0, 2, 7 or 9 days after treatment. Observations are made 40 hours after sowing.

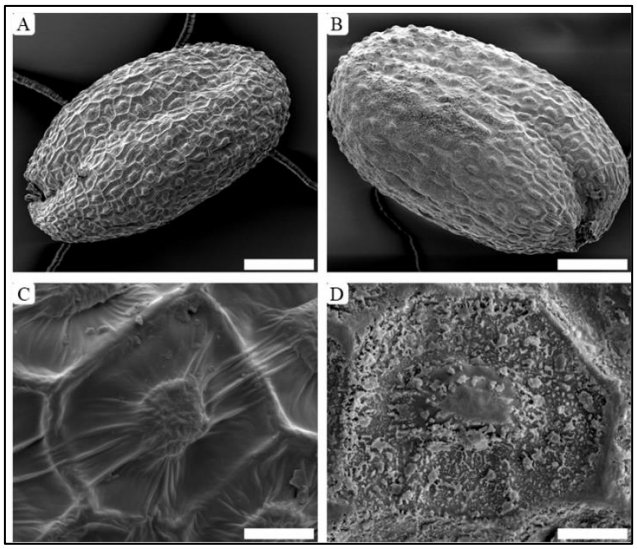

Fig. 3. Scanning electron microscopy pictures of *Arabidopsis thaliana* seeds. A and C are pictures of control seeds. B and D are pictures of plasma treated seeds. Scale bars: A and B: 100µm; C and D: 10µm.

This aspect leads us to the hypothesis of a presence of lipidic compounds. The lipids being hydrophobic, the permeability of the seeds is therefore tested.

Fig. 4 shows that the plasma treated seeds are less permeable than control seeds. This result confirms the presence of hydrophobic compounds on the seed surface due to plasma treatment.

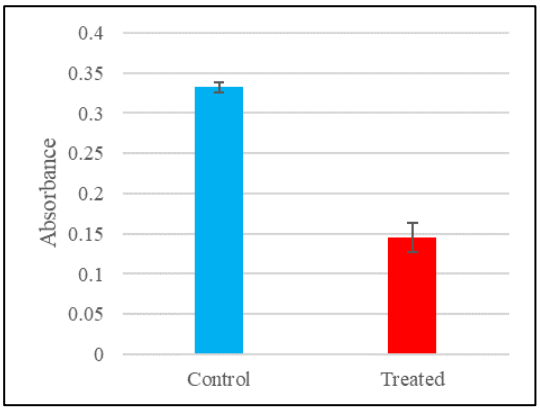

Fig. 4. Bar chart of the absorbance of tetrazolium red on seed with or without plasma treatment. The blue one is the control seeds and the red one the treated seeds.

Nevertheless, the permeability of seeds allows water to enter the seed and thus initiate the germination process. But in this case, the permeability is reduced which therefore prevents water from entering the seed and in the same time avoids the endogenic humidity to be evaporated, which could explain the germination increase. Anyway, it is possible that the observed effect is linked to the integrity of the seed coat.

For a better understanding, in addition of the treatment of the reference ecotype Col-0 of *A. thaliana* shown in figs 1 to 4, we also considered two *A. thaliana* mutants (*gl2* and *gpat5*) which are mutated respectively for the storage lipids and the structural lipids.

After observation of the germination, we see in Fig. 5 a positive effect of the plasma on the seeds of the *gl2* mutant line with an increase of 30% of the testa rupture after 64 hours. However, the treatment completely inhibits the germination of *gpat5* mutants which reach less than 10% of germination. This may indicate the importance of the protective layer of the embryo which *gpat5* mutant is lacking.

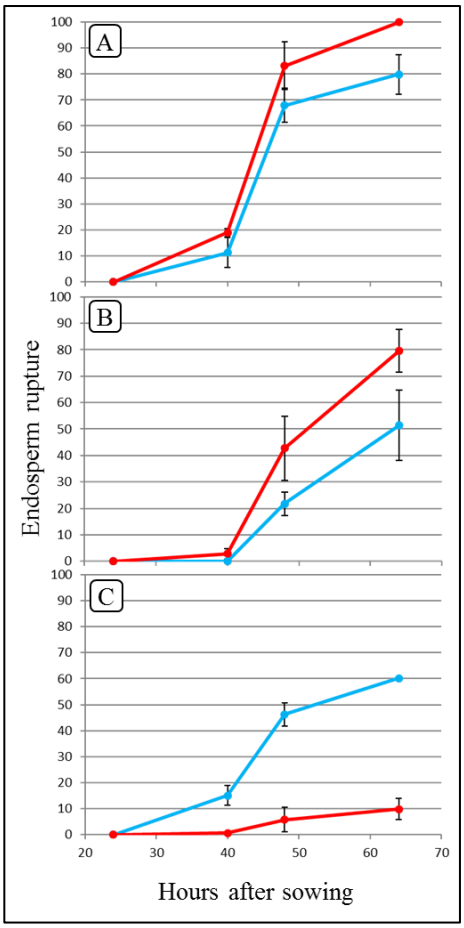

Fig. 5. Diagram of the percentage of endosperm rupture versus the time after sowing. A: germination of the Col-0 ecotype of *A. thaliana*; B: germination of the *gl2* mutant: C: germination of the *gpat5* mutant. Red curves correspond to the treated seeds and blue curves to the control seeds.

3. Plant growth

Moreover, to study the indirect effect of the plasma treatment, a helium plasma jet was used to activate tap water (PAW) which is used to watering plant. The setup used to water active is described elsewhere [8]. As displayed in Fig. 6, it appears that the plants watered by the PAW have more leaves together with bigger rosette.

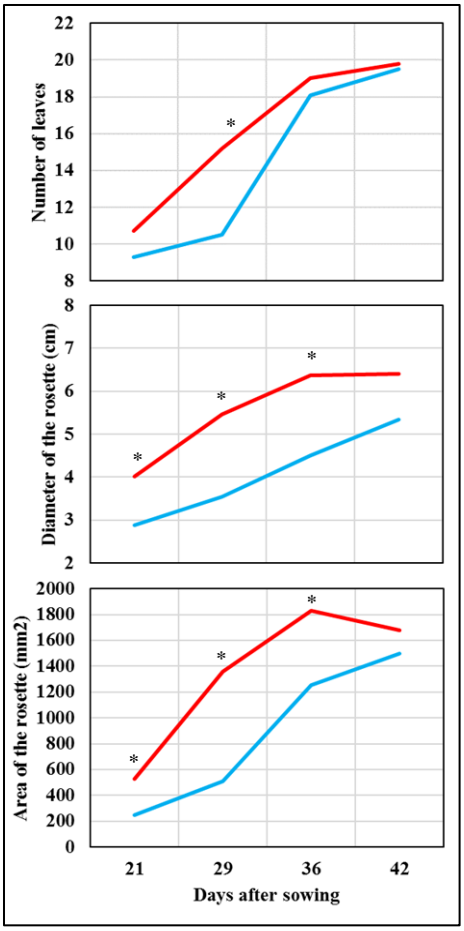

Fig. 6. Plant growth kinetic of *A. thaliana* plant. Various parameters are noted such as the number of leaves, diameter and area of the rosette. Red curves depict the plant watering with Plasma-Activated Water (PAW) and the blue curves are the plants watering the distilled water. The * is the significance of the Wilcoxon test (p-value<0.05).

4. Conclusion

The seed treatment using a low temperature plasma generated in ambient air has a positive impact on the germination of *A. thaliana* seeds in the case of the reference ecotype Col-0 *A. thaliana*. It appears that this could be due to a change of the seed surface. The SEM observations suggested that those changes due to the plasma treatment seem to be a consequence of the rearrangement of lipidic surface compounds. These latter are confirmed by the measured decrease of the

permeability of the plasma treated seeds. After these observations on the reference seed Col-0, mutants *gl2* and *gpat5* are then treated by the low temperature air plasma. The obtained results of the mutant germination allow us to support the hypothesis of the lipidic origin of those surface compound. Thus, it is possible that the surface modification comes from a protective layer of the seed coat: the cuticle. Indeed, the *gpat5* mutant that have not this protective layer, does not show any visible plasma stimulated germination.

For the developmental and growth of the plant, the watering with the plasma activated water (PAW) has a positive effect on the plant growth. In fact, this could be explained by the numerous reactive oxygen et nitrogen species (ROS and RNS) produced by the plasma treatment of the water.

5. References

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