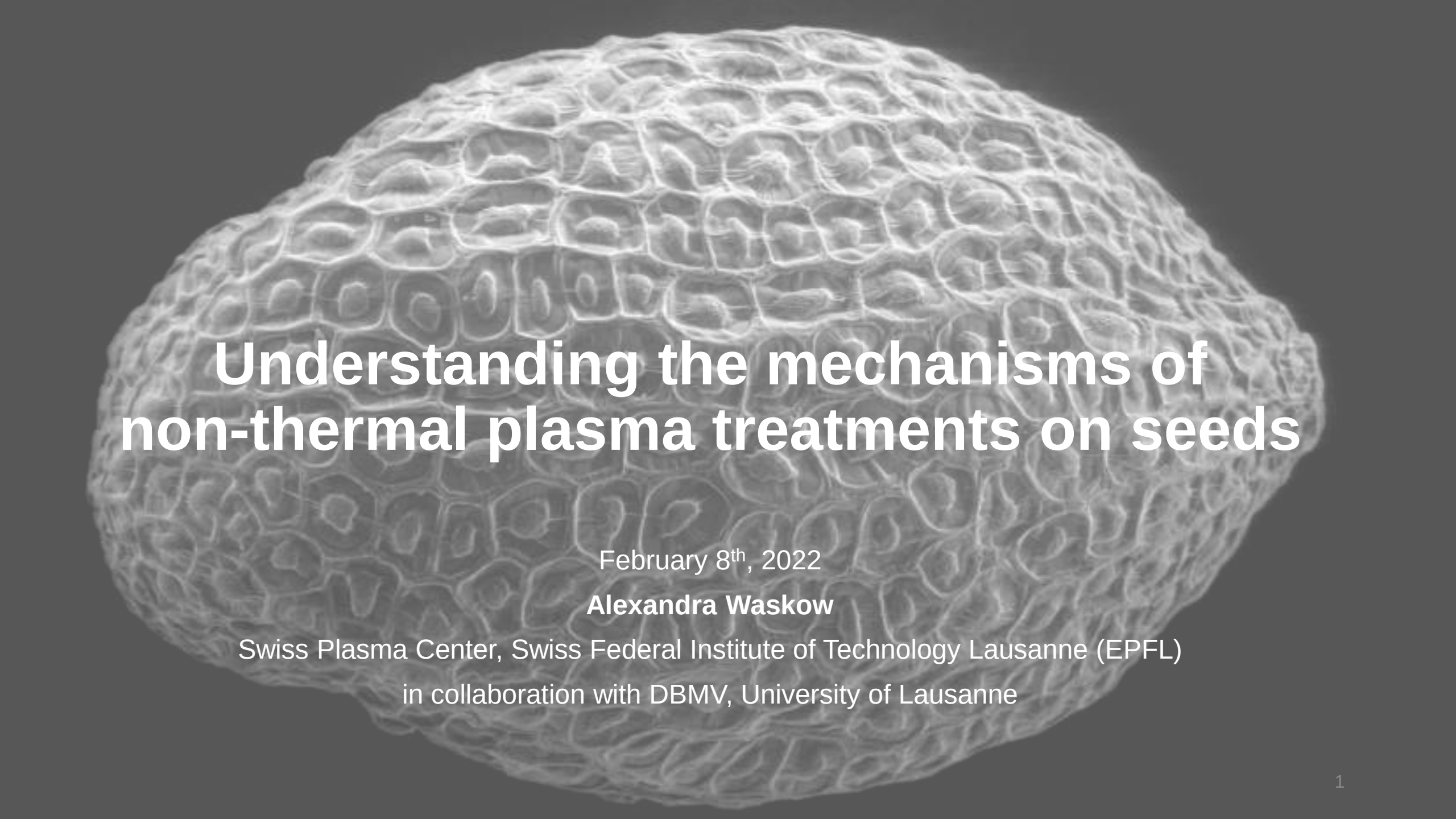


Understanding the mechanisms of non-thermal plasma treatments on seeds

February 8th, 2022

Alexandra Waskow

Swiss Plasma Center, Swiss Federal Institute of Technology Lausanne (EPFL)

in collaboration with DBMV, University of Lausanne

Presentation outline

- Introduction to plasma-seed treatments
- Importance of Surface Dielectric Barrier Discharge (SDBD) design
- Surface analysis techniques
- Germination rate of plasma-treated *Arabidopsis thaliana* seeds
- *In situ* Fourier Transform Infrared Spectroscopy (FTIR) plasma chemistry characterization
- RNA sequencing results for changes in gene expression

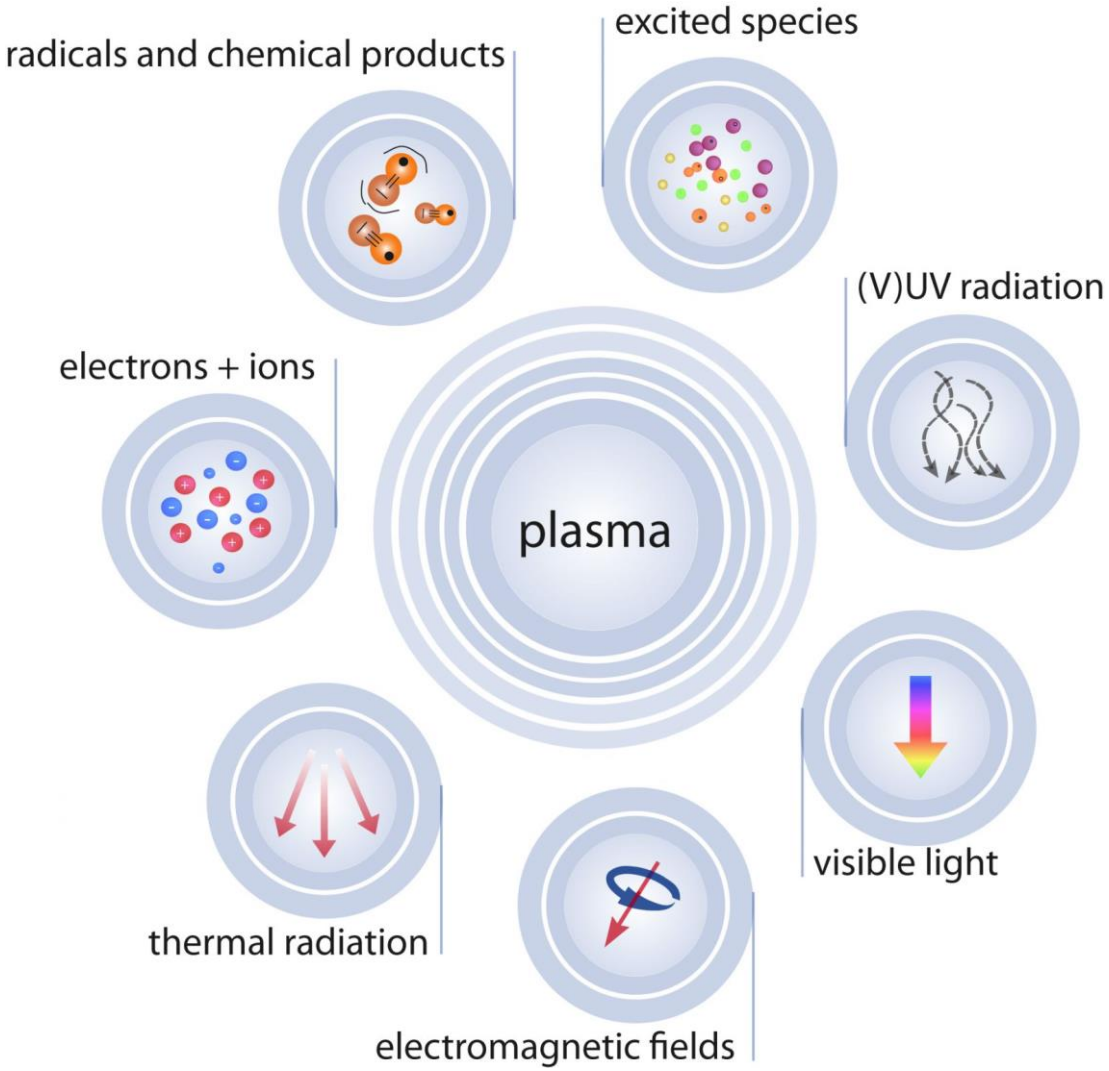
Take away message

Accelerated germination after plasma treatment is observed.

It is not yet clear which component of plasma is responsible for this effect.

However, plant stress and defense response is observed and the response is dependent on the plasma treatment time exposure.

What is plasma?

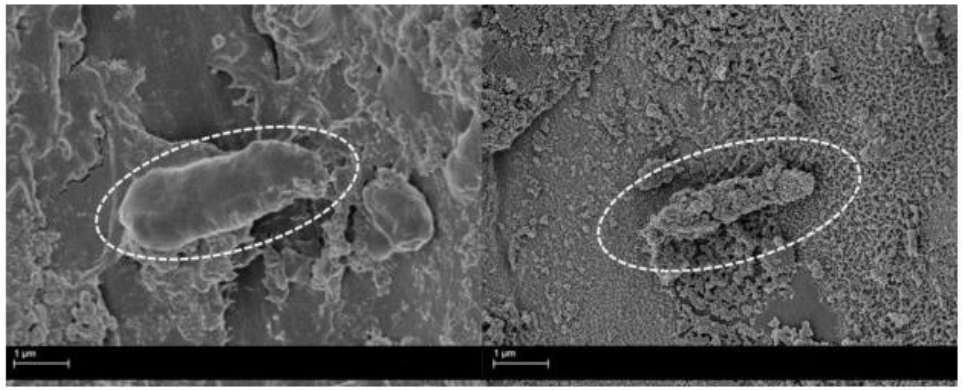


Picture: INP Greifswald

We still don’t understand how plasma affects seeds

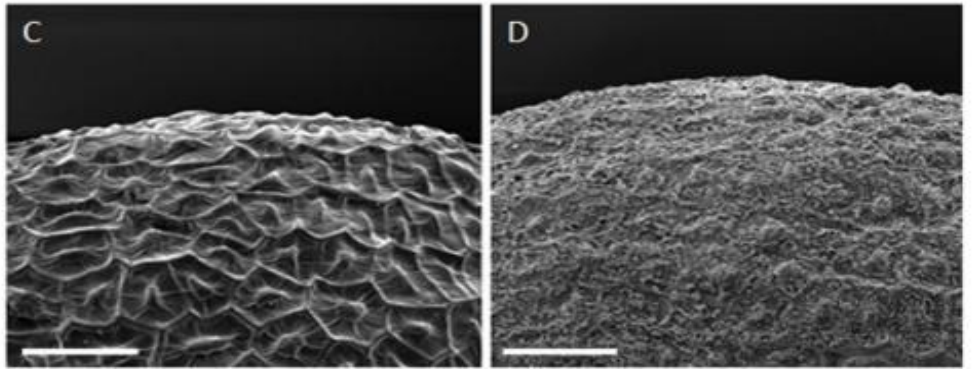
Disinfection of surface microorganisms

Butscher et al., 2015



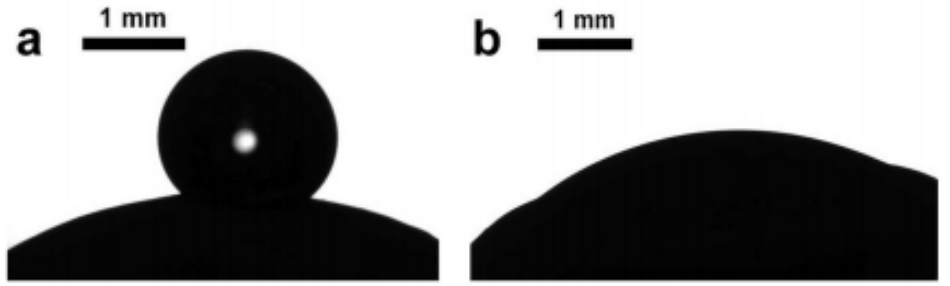
Seed coat erosion

Bafoil et al., 2017

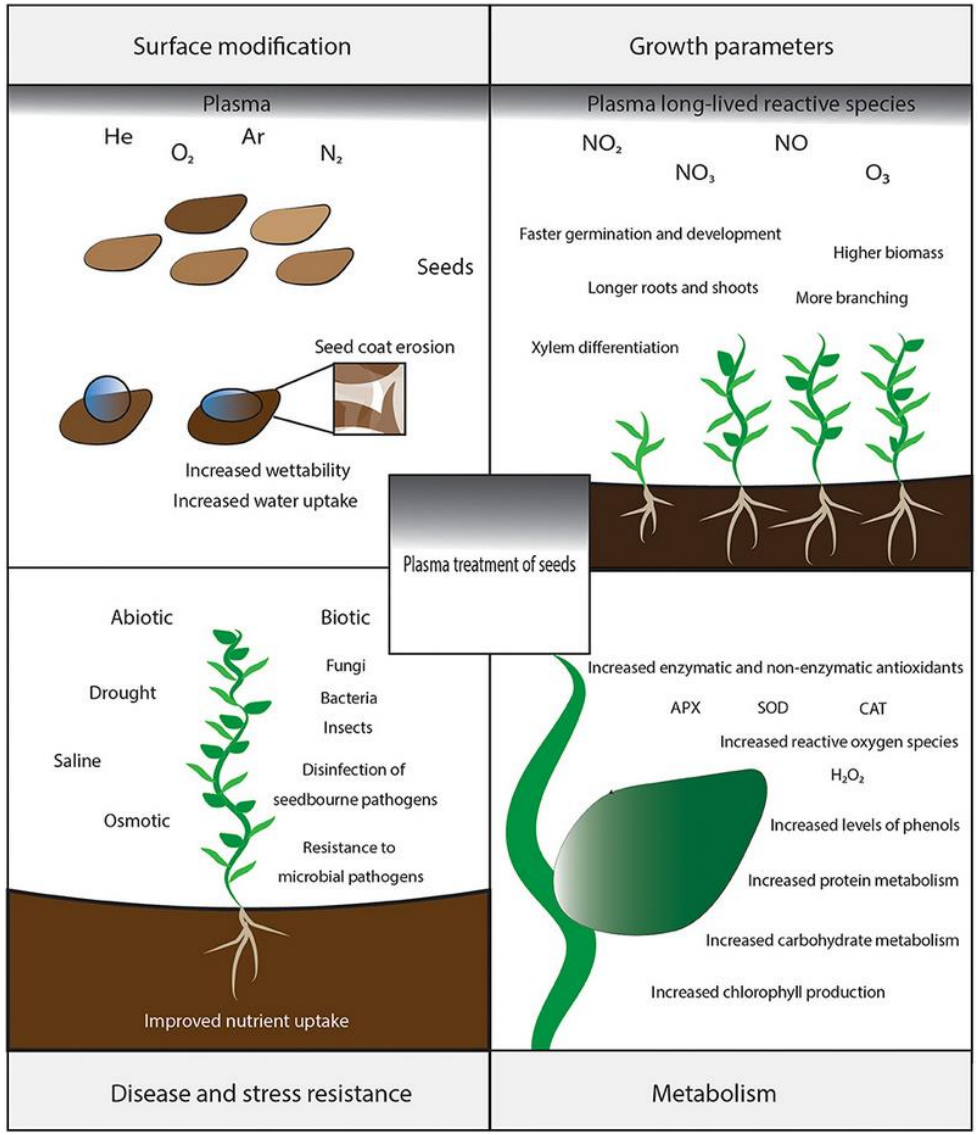


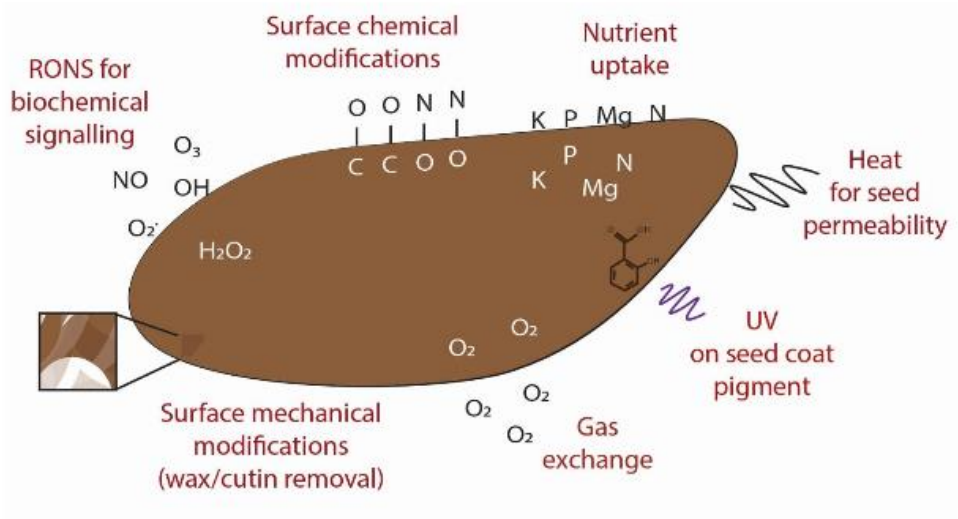
Seed surface modification

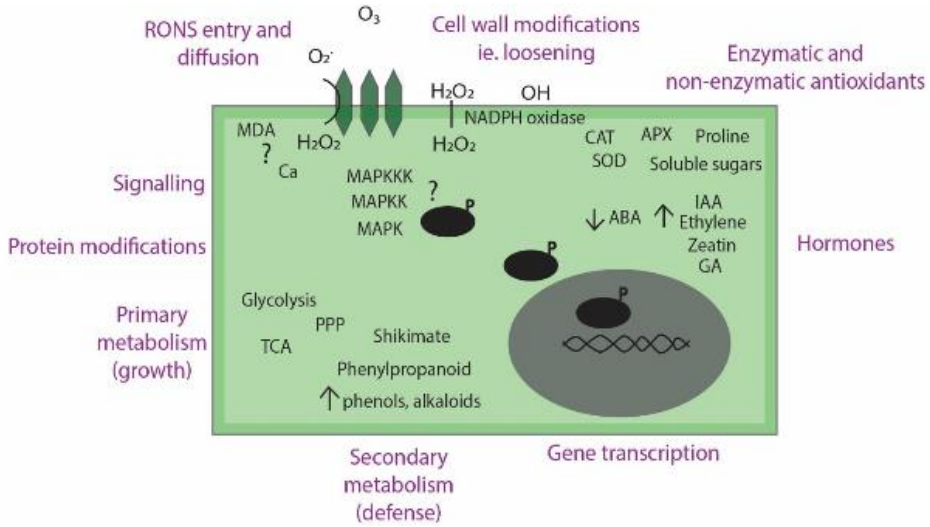
Bormashenko et al., 2012



Mechanisms of plasma-seed treatments are complex





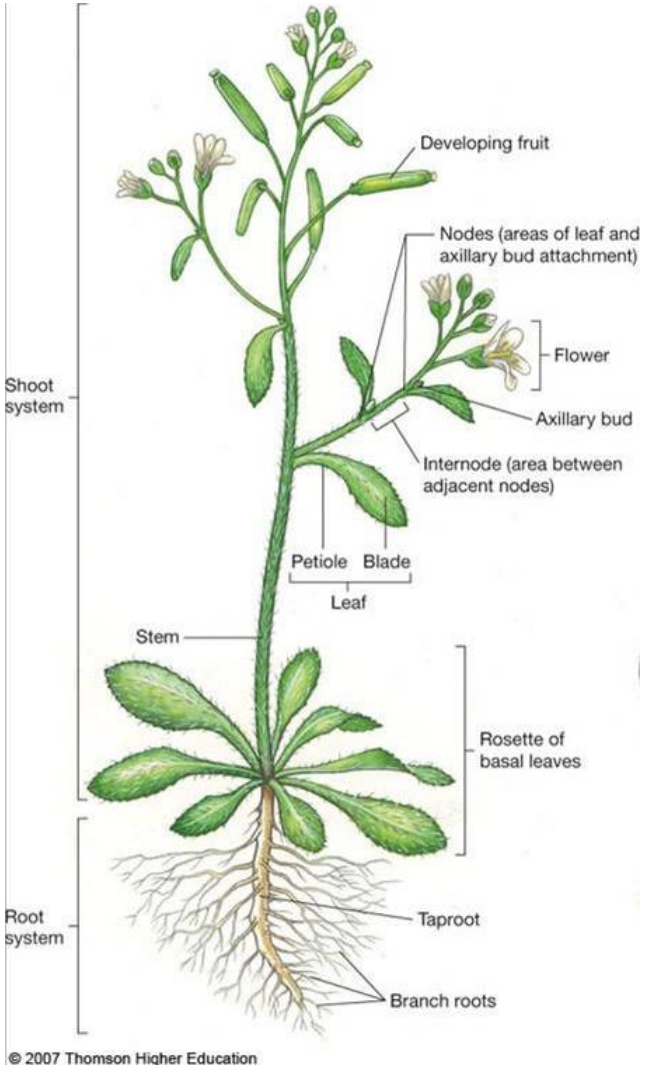


Mechanisms of plasma-seed treatments as a potential seed processing technology (Frontiers in Physics)

Bioplasmas lab of the BPPA group at the Swiss Plasma Center

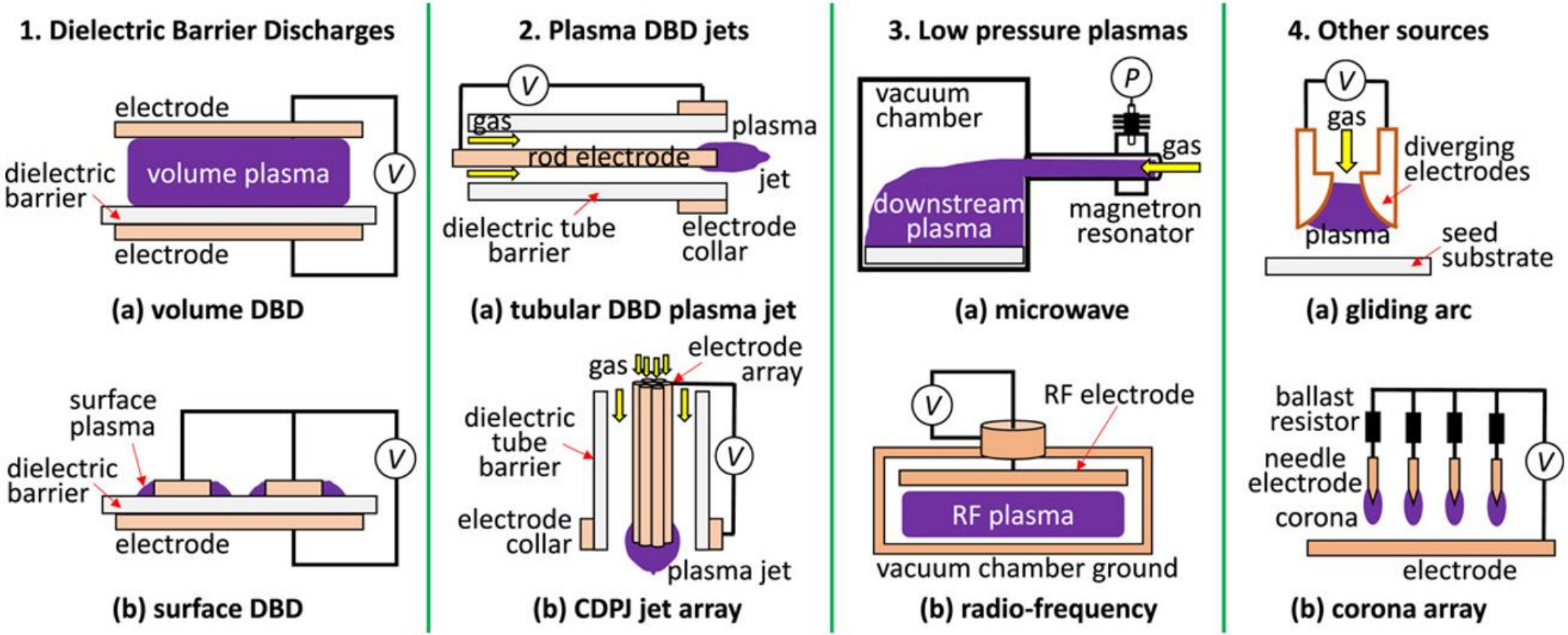
Virtual tour: https://my.matterport.com/show/?m=prTDR6c7qMh

All relevant experiments done with plant model organism, *Arabidopsis thaliana*



Brief life cycle ~3 months/generation
Well-annotated genome
Many seeds per generation
Easy to genetically manipulate

How can we make a plasma?

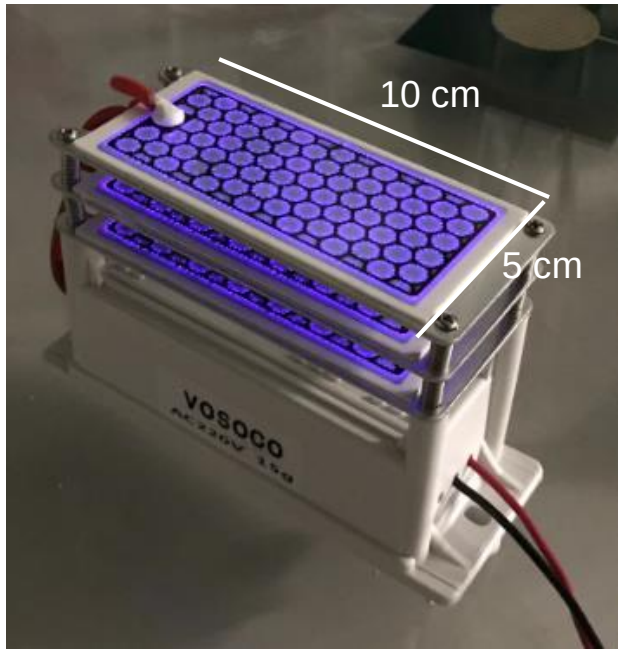


Entering the plasma agriculture field: an attempt at standarizing protocols for plasma treatment of seeds (Plasma Processes and Polymers)

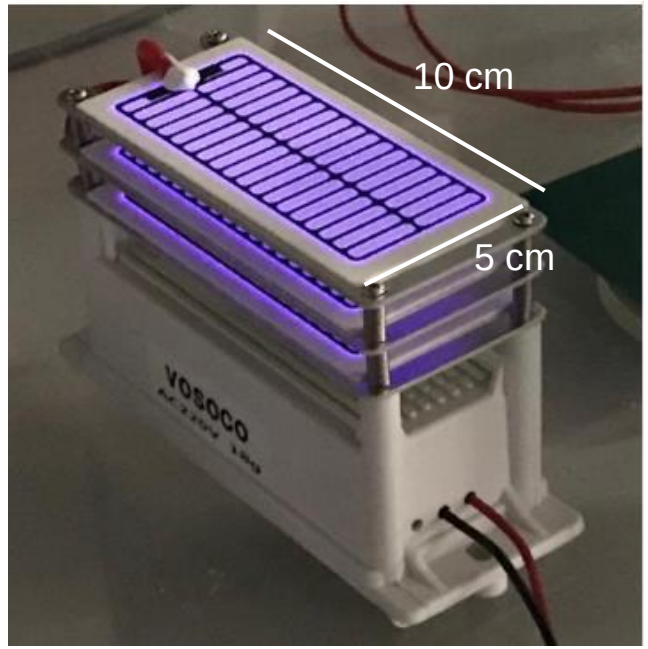
Surface Dielectric Barrier Discharges (SDBD) used to treat seeds

Flat finger configuration

Honey configuration

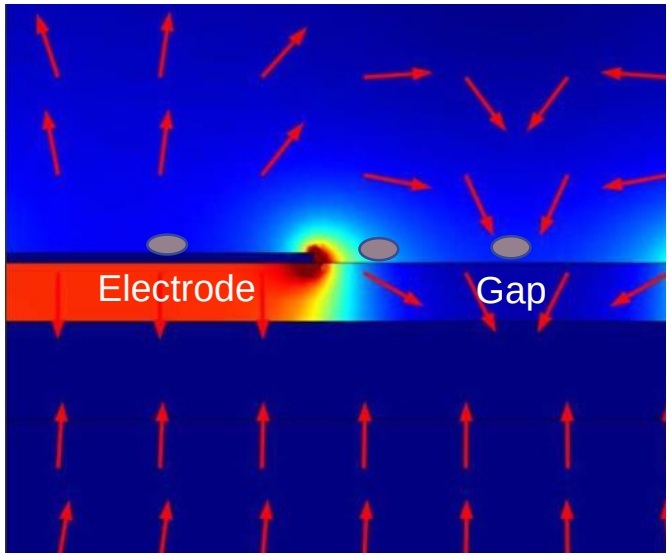


Stripes configuration



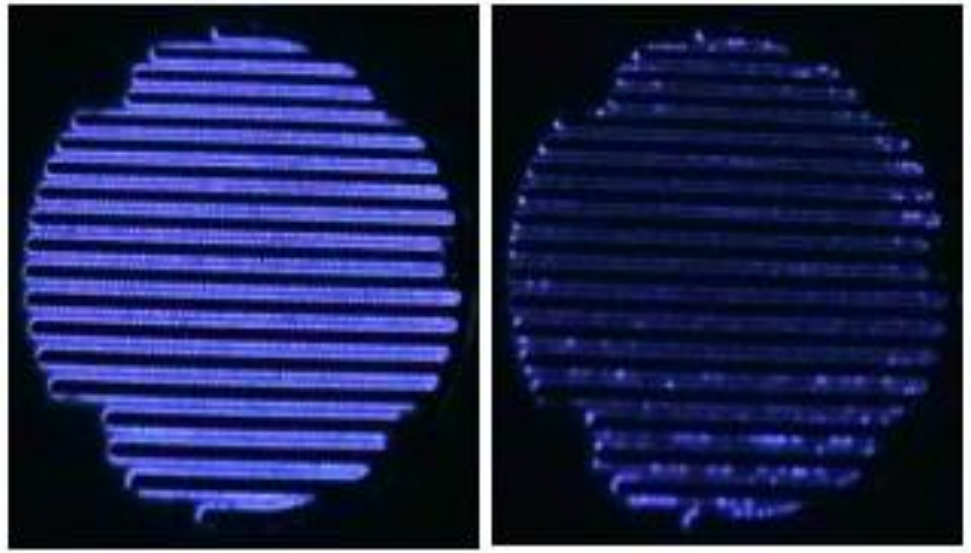
Different geometries

Complex number of variables in plasma-seed treatment design



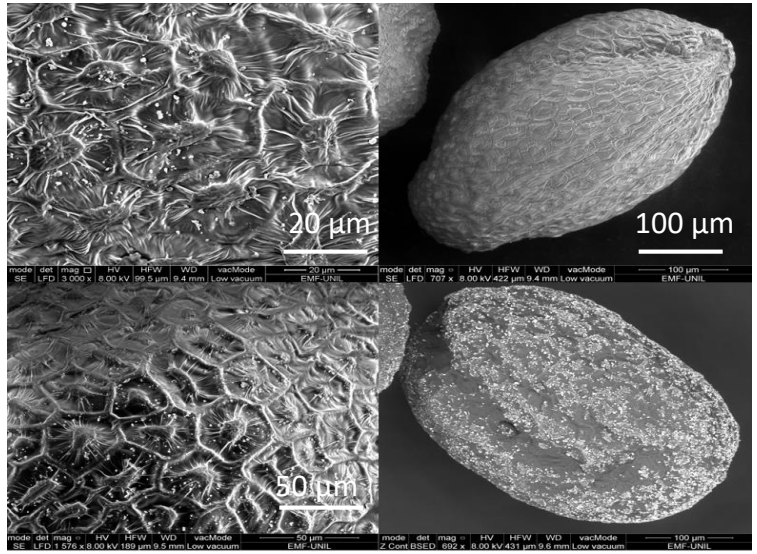
Seed positioning

Seeds might not be exposed nor uniformly to the plasma



Humidity

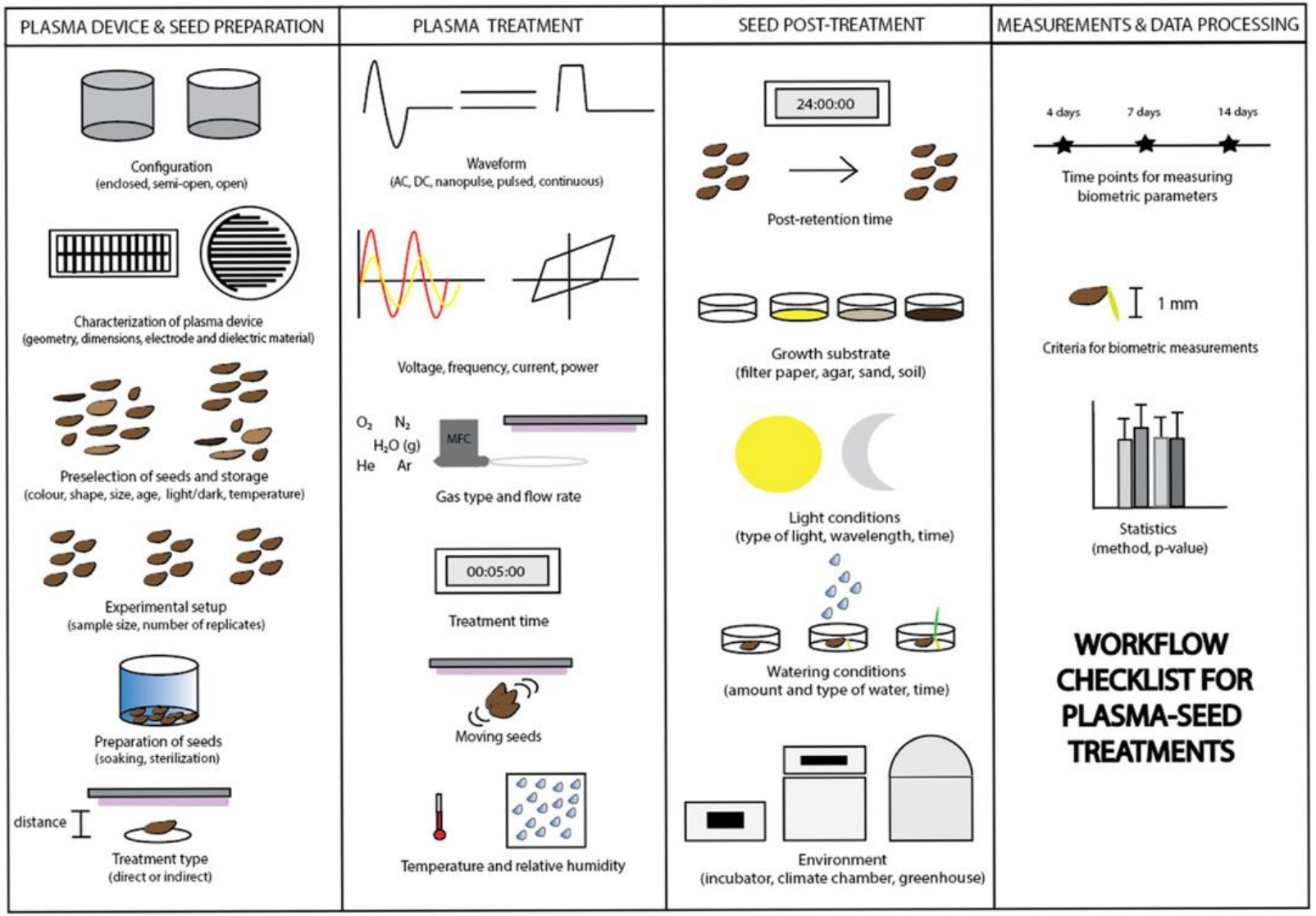
Not reproducible in terms of germination and plasma brightness



Nanoparticle deposition from DBD electrodes

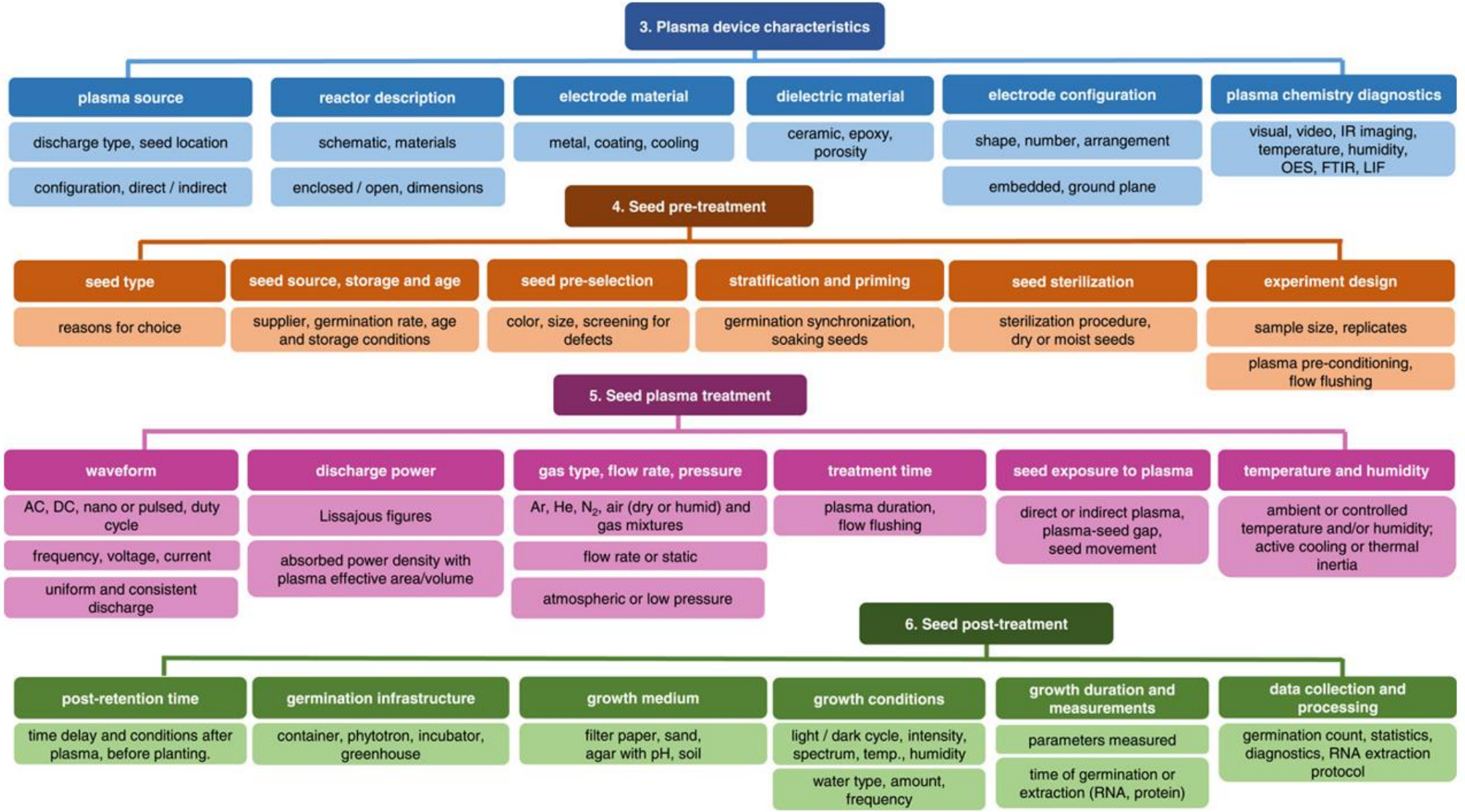
Need to determine whether this is beneficial or problematic

The importance of protocols for reproducibility



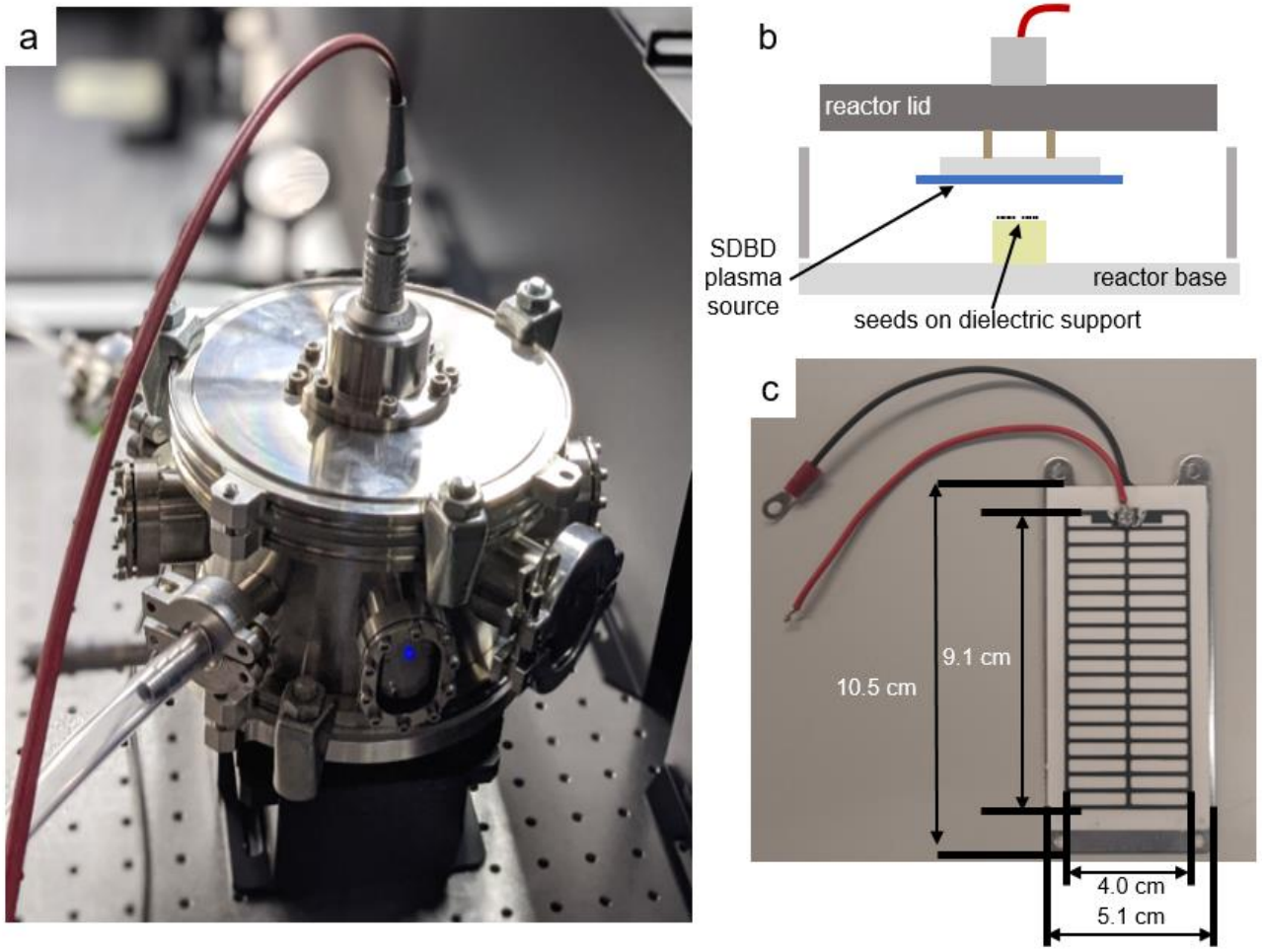
Entering the plasma agriculture field: an attempt at standarizing protocols for plasma treatment of seeds (Plasma Processes and Polymers)

Plasma device and seed characterization should be carefully recorded



Entering the plasma agriculture field: an attempt at standarizing protocols for plasma treatment of seeds (Plasma Processes and Polymers)

Plasma-seed treatment using SDBD stripes and *Arabidopsis thaliana*

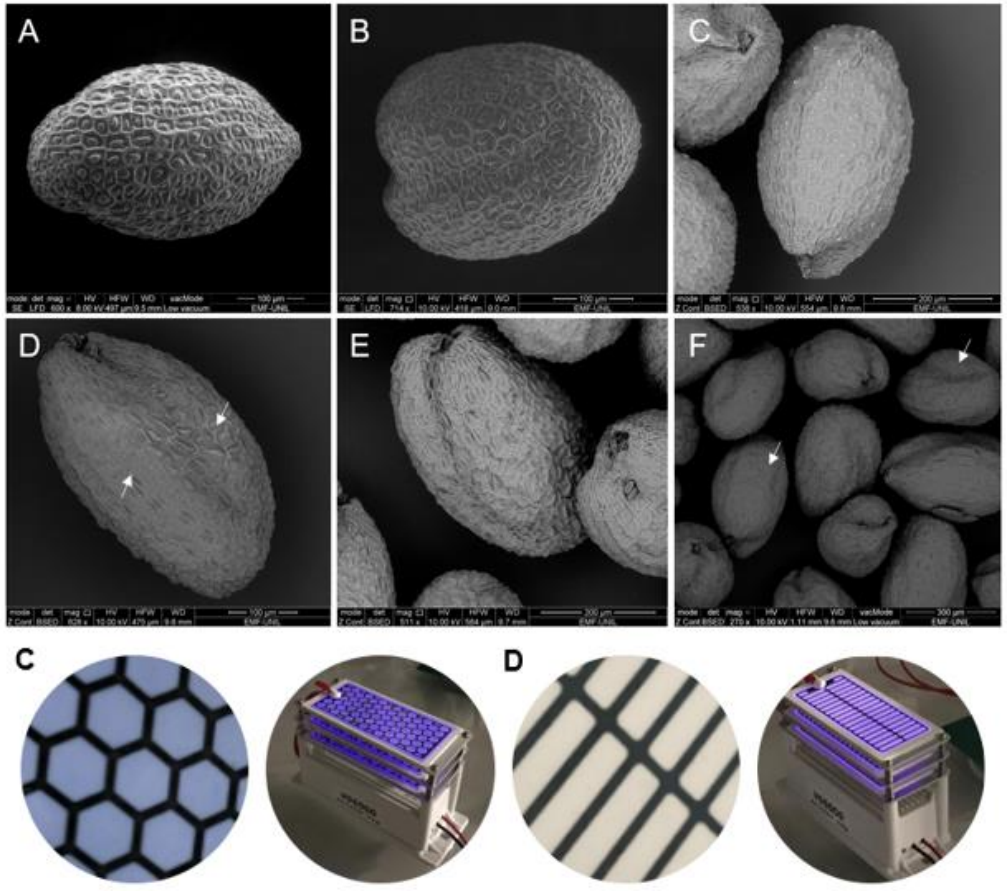


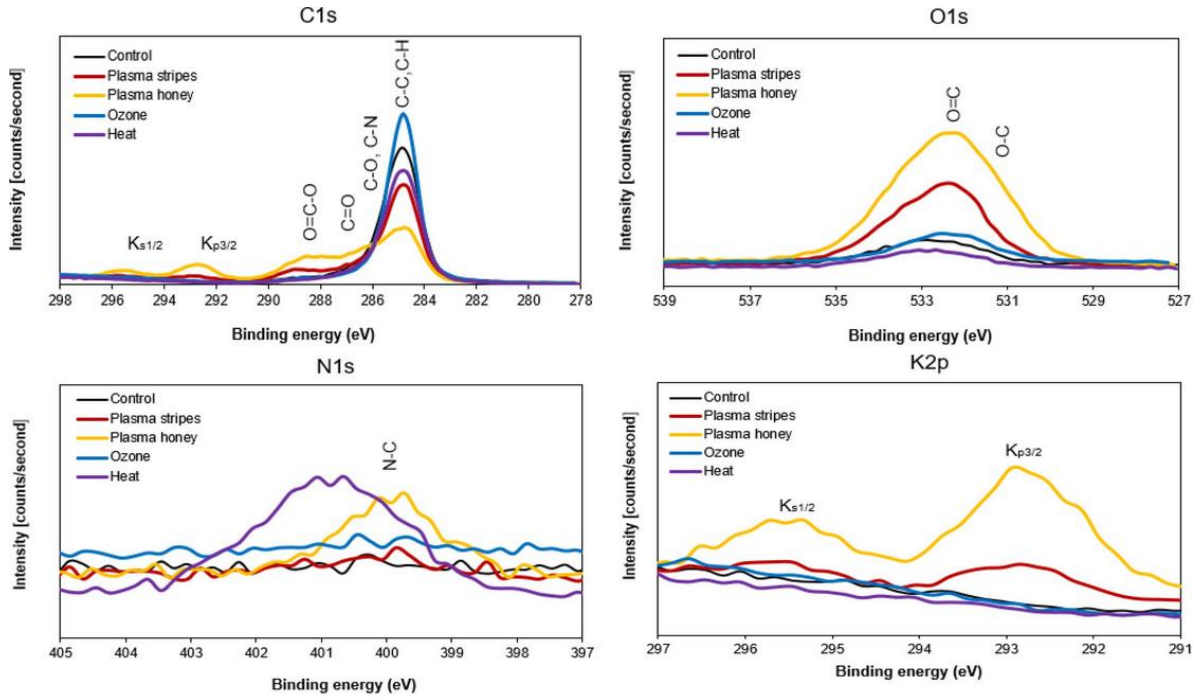
Parametric study

- Stripes vs. mesh
- AC vs. nanopulse
- Frequency:
  - 500 Hz, 1 kHz, 2 kHz (nanopulse)
  - 5, 7, 10 kHz (AC)
- Time: 20, 60, 80 s
- Voltage: 6 - 9.5 kV
- Distance: 4.33, 3.7, 3 mm
- Flow rate: 2 - 6 L/min

An in situ FTIR study of DBD plasma parameters for accelerated germination of *Arabidopsis thaliana* seeds (MDPI)

Scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) are the most useful techniques





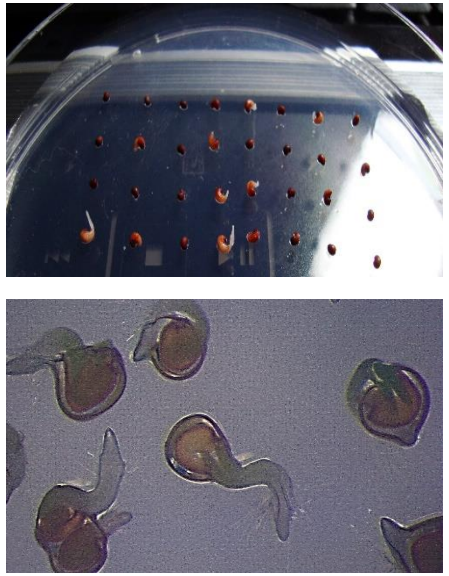
Treatment	% C1s	% N1s	% O1s	% K2p
Control	91.34	1.31	6.40	0.07
Heat 65°C 3 min	93.24	0.00	6.25	0.10
Ozone 200 ppm 3 min	90.44	0.31	7.12	0.26
Plasma stripes 3 min	75.20	0.09	21.37	0.43
Plasma honeycomb 3 min	59.04	3.14	34.76	1.86

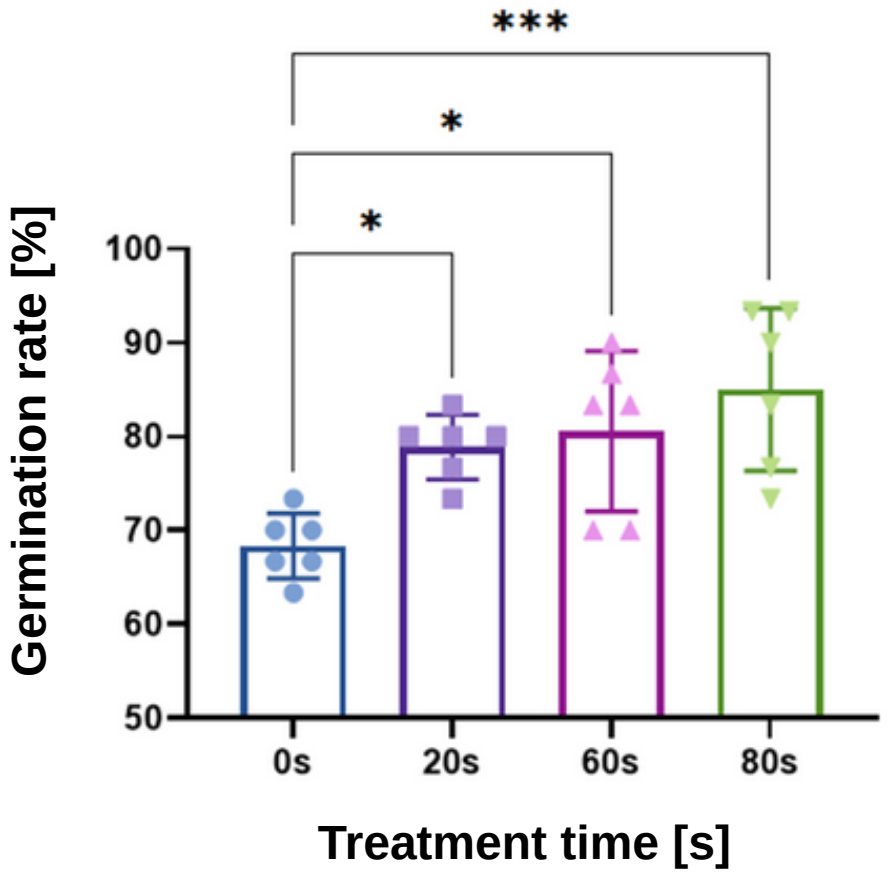
SEM only reveals information about erosion and nanoparticles

XPS reveals oxidation of carbons only with plasma

Advantages and limitations of surface analysis techniques on plasma-treated seeds (Frontiers in Materials)

Stripes SDBD powered with AC showed accelerated germination rate





Plasma conditions:

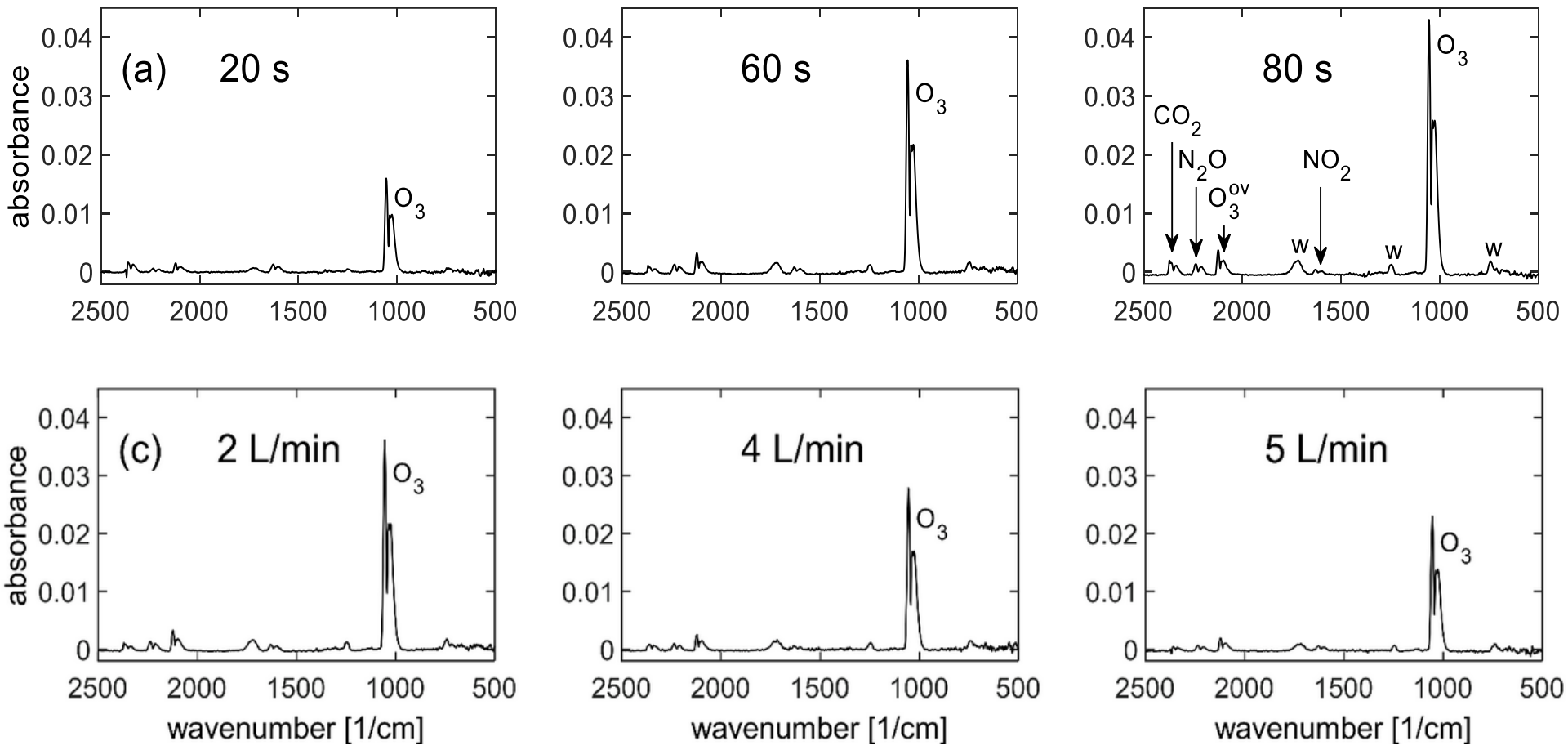
- 1 min flow flushing before treatment
- 2 L/min with dry synthetic air (80% N_2, 20% O_2)
- 2 - 3% relative humidity
- 3.7 mm
- 10 kHz; 8 kV
- 1 min treatment as standard
- modulated AC power supply, 10% duty cycle
- 1.2 – 3 W for nominal voltages for 4-9 kV_{pp}
- 0.03 – 0.08 W/cm^2 power density
- 31ºC at 80 s

Seed handling:

- 30 seeds x 3 biological replicates
- experiment repeated independently
- water agar
- measured at 48 hours
- grown in continuous light, 23ºC, 66% RH

Asterisks denote statistical significance where * signifies p <0.05; ** is p <0.01; and *** is p <0.001.

In situ FTIR revealed no obvious differences in plasma chemistry

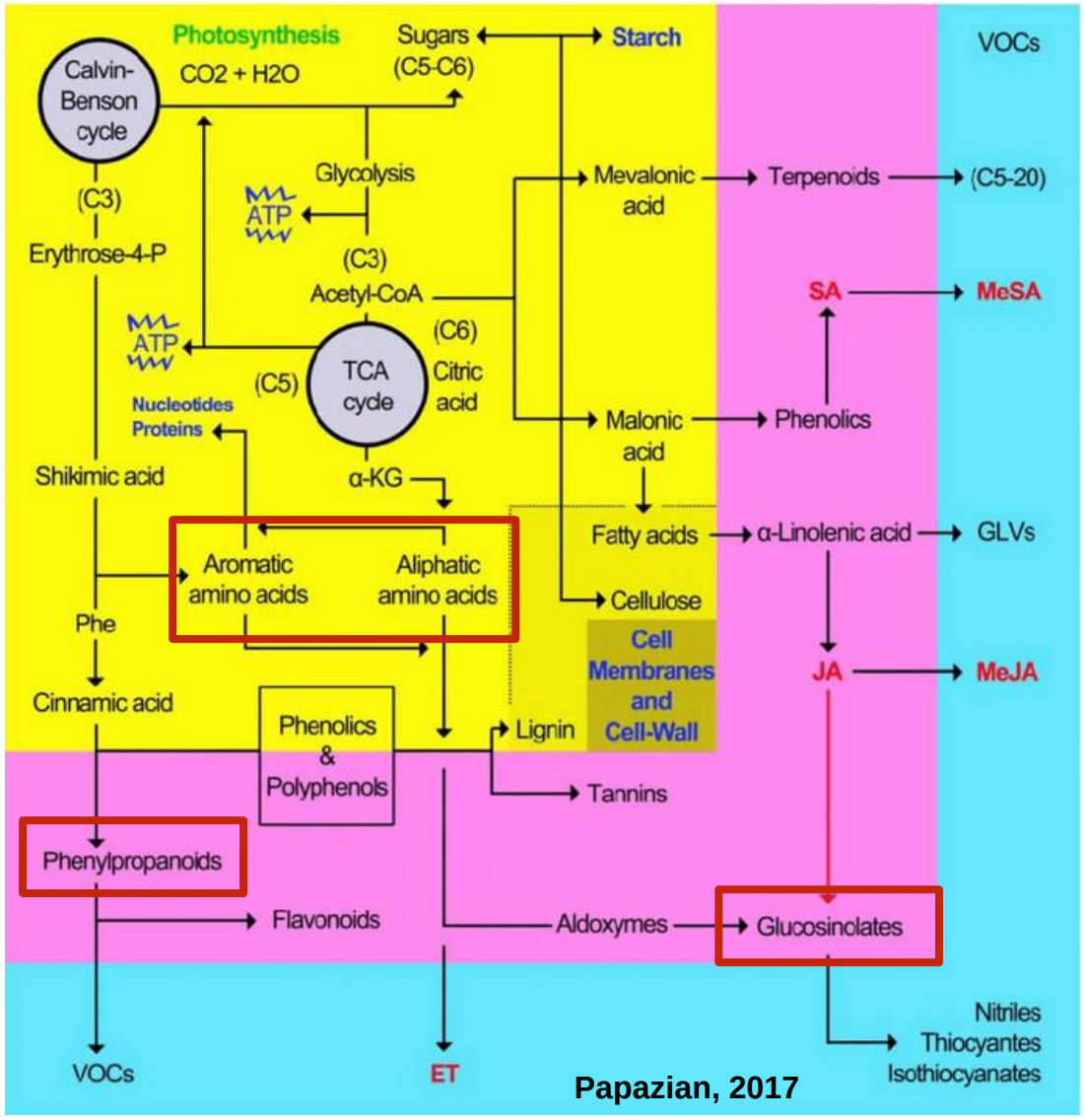


Plasma treatment time could determine the type of defense response

Primary metabolism (growth)

Secondary metabolism (defense)

Primary and secondary metabolism (interactions with other biota)



Main Trends from RNA-seq

- Most genes are downregulated after plasma-seed treatment
- Defense responses can be up- and downregulated
- Cell wall most affected

RNA sequencing of *Arabidopsis thaliana* reveals upregulation in stress and defense pathways. Waskow [1,*#], Guihur[2#] et al. (submitted)

Take away message

Accelerated germination after plasma treatment is observed.

It is not yet clear which component of plasma is responsible for this effect.

However, plant stress and defense response is observed and the response is dependent on the plasma treatment time exposure.

Acknowledgments

- BPPA group EPFL
  - Marion von Allmen
  - Max Leftley
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- Laboratory of Food Microbiology ETH
  - Dr. Schuppler

Relevant papers

- Waskow, A., Howling, A., and Furno, I. (2021) Advantages and Limitations of Surface Analysis Techniques on Plasma-Treated *Arabidopsis thaliana* Seeds. Front. Mater. 8:642099.
- Waskow, A., Howling, A., and Furno, I. (2021) Mechanisms of Plasma-Seed Treatments as a Potential Seed Processing Technology. Front. Phys. 9:617345.
- Waskow, A., Ibba, L., Leftley, M., Howling, A., Ambrico, P.F., Furno, I. (2021) An In Situ FTIR Study of DBD Plasma Parameters for Accelerated Germination of *Arabidopsis thaliana* Seeds. *Int. J. Mol. Sci. 22*, 11540.
- Waskow, A., Avino, F., Howling, A., Furno, I. (2021) Entering the plasma agriculture field: An attempt to standardize protocols for plasma treatment of seeds. *Plasma Processes and Polymers*. e2100152
- Waskow, A., Guihur, A., Howling, A., Furno, I. (2022) RNA sequencing of plasma-treated *Arabidopsis thaliana* seeds reveal upregulation in plant stress and defense pathways. Manuscript submitted.
- Waskow, A., Guihur, A., Howling, A., Furno, I. (2022) RNA sequencing reveals the effect of voltage in plasma-treated *Arabidopsis thaliana* seeds. Manuscript in preparation.

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Future outlook

Where I think the focus of research will be in the coming years…

- understanding of plasma-seed treatment design and possible standardization and evaluation criteria
- determining which plasma applications can thrive by comparing to other technologies
- more –omics approaches used to understand the effects
- more long term and generational studies
- understanding the effect on soil health and surrounding biota
- possibly used to generate mutants for fundamental research and plant breeding