# Plasma applications for smart and sustainable agriculture-possibilities and challenges

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## OUTLINE

- Plasma agriculture: A rapidly emerging field
- Some examples:
  - 1. Treatment of medicinal plant seeds
  - 2. Treatment of synseeds
- Possibilities and Challenges of Plasma Agriculture





## Plasma agriculture: A rapidly emerging field



N. Puač, M. Gherardi and M. Shiratani, Plasma agriculture: A rapidly emerging field, PPP, 2017, e1700174

## Why should we bother with this?



Food and Agriculture Organization of UN - Major headlines

- Working together for Zero Hunger Building the #ZeroHunger Generation
- Path to Zero Hunger by 2030
- Time is running out for the world's forests
- Antimicrobial Resistance: their overuse and misuse is causing antimicrobial resistance







## Why should we bother with this?



Source: Food and Agriculture Organization of UN (FAO)



Brandenburg et al, PPP, 2018;e1700238.



Action Chair: Dr Nevena Puač (Serbia) Vice Chair: Prof. František Krčma (Czech Republic) (October 6<sup>th</sup>, 2020-October 5<sup>th</sup>, 2024)

www.plagri.eu



#### The main aims:

- to investigate the potential of non-thermal plasmas as a green alternative in agriculture to improve yields, increase size and robustness of plants and to reduce (or eliminate) the need for pesticides
- to use plasmas for treatments of final products treatments of food and its packaging

## PlAgri Action structure and WGs



WG1. Dissemination and communication (Tasks 1.1-1.6)

WG2. Low temperature plasma treatment of SEEDS

WG3 Low temperature plasma treatment of PLANTS

WG4 Plasma treatment of agricultural wastewater, growth media, manure and production of PAW

WG5 Applications of plasma processes and technologies in food industry

- to advance the current understanding of the physical and biochemical changes induced by plasma treatments of seeds
- to define standard protocols and procedures for usage of plasma and/or PAW aiming to speed up and increase plant growth and development resulting in better yields
- developing procedures for efficient decontamination of wastewater of agricultural origin and for treatment of growth media
- identification of the applications in the food production chain transferable to the industrial processing environment.

All contacts can be found at the PlAgri Action Website (www.plagri.eu) and on social platforms.

## Plasmas in agriculture and food processing

#### Main goals:

- (1) to advance the current understanding of the physical and biochemical changes induced by plasma treatments;
- (2) to develop guidelines and protocols that can be adopted by the rapidly growing community working on plasma treatment of seeds, plants and soils;
- (3) identifying applications in the food production chain where plasma technology could have a potential application translatable to the industrial processing environment.
- (4) development of plasma sources and technologies with attention to legislative, energy consumption, food safety and quality aspects





## Plasmas in agriculture and food processing

#### Questions:

- What are the fundamental mechanisms responsible for the positive effects of low temperature plasmas on seeds and plant or plant tissue ?
- How can plasmas be used to protect seeds from fungus, virus and bacteria without harming the seed (or in concert with stimulation of seeds)?
- Are there unique properties of varieties of seeds that may produce different responses to the same set of plasma parameters?
- Can these similarities and differences in response to plasma parameters be used to define standard procedures and protocols that can be broadly implemented in agriculture?
- Can these methods be extended to seedlings to further stimulate growth, and to protect from bacteria and fungus?
- What about the quality and safety characteristics of plasma treated foods?





## Non-Thermal Plasmas (NTP) and SEEDS

- NTPs have been used in treatment of seeds since the beginning of the century
- The seeds in direct contact with plasma or with plasma afterglow.
- Plasma treatments induced positive results, such as increasing the germination percentage of the treated seeds, independently from the type of the seed. Apart from the increase in germination percentage, NTP treatment of seeds lead to significant changes in enzyme activity, enzyme content and endogenous hormones content, higher seed activity including earlier germination, higher water uptake, higher germination rate and faster growth of developed plants.
- Another very important conclusion which was drawn from those first experiments is that for each type of seed the optimal plasma treatment parameters for obtaining maximum germination are different.
- The capabilities of NTPs as a bactericide and fungicide just add to the overall usefulness and efficiency of plasma treatments.
- NTPs can be used in decontamination of large quantity of grains that will go into cattle food reducing the possibilities, for example, of outbreaks of large quantities of aflatoxins in the milk.



#### List of most valuable crops and livestock products



From Wikipedia, the free encyclopedia

Сгор	Global gross production value in billion USD	Global production in metric tons	Country with highest gross production value in billion USD	
Rice, paddy	\$332	751,885,117	\$117 (Mainland China)	
Pig, meat	\$280	118,956,327	\$167 (Mainland China)	
Cattle, meat	\$269	64,568,004	\$52.8 (United States)	
Cow's milk, whole fresh	\$238	665,596,536	\$34.7 (United States)	
Chicken, meat	\$192	106,638,508	\$27.4 (United States)	
Maize (Corn)	\$191	1,126,990,585	\$61.2 (Mainland China)	
Wheat	\$168	748,392,150	\$50.7 (Mainland China)	
Soybeans	\$107	335,613,801	\$40.7 (United States)	
Eggs, hen, in shell	\$93.6	74,180,272	\$26.1 (Mainland China)	
Potatoes	\$92.7	356,952,488	\$32.2 (Mainland China)	
Vegetables, not elsewhere specified	\$89.1	292,920,885	\$53.6 (Mainland China)	
Tomatoes	\$87.9	178,158,747	\$28.9 (Mainland China)	
Sugarcane	\$87.3	1,874,611,396	\$40.9 (Brazil)	
Grapes	\$67.8	74,089,693	\$14.4 (France)	
Seed Cotton	\$56.7	67,622,193	\$21.4 (Mainland China)	
Buffalo milk, whole fresh	\$56.4	115,204,379	\$43.1 (India)	
Cotton lint	\$50.5	23,274,797	\$18.6 (Mainland China)	
Apples	\$45.9	84,743,988	\$20.8 (Mainland China)	
Onions, dry	\$42.1	94,838,690	\$23.9 (Mainland China)	
Sheep, meat	\$40.4	9,567,978	\$13.8 (Mainland China)	
Cucumbers and Gherkins	\$40.2	79,844,838	\$29.6 (Mainland China)	
Garlic	\$39.3	26,821,718	\$31.5 (Mainland China)	
Bananas	\$38.5	112,627,980	\$8.13 (India)	

- Improved germination and plant growth
- Plasma treatment for microbial decontamination
- Plasma treatment of seeds for inducing stress resistance by activating the plant defense system



## NTPs and PLANT/SOILS/WASTEWATER

- The most successful application of plasma to agricultural products is growth enhancement of mushroom using pulsed high voltage discharge plasmas. NTPs applied to various kinds of mushrooms in mushroom farmers and mushroom factories, and the products have been sold in market in Asian countries over 10 years.
- Plasma irradiation to seedlings of strawberry induces 25% increase in anthocyanin content in strawberry. This is an example of enhancement of functions of agricultural products using plasma, because anthocyanin pigments and associated flavonoids have demonstrated ability to protect against a myriad of human diseases.
- Recent research demonstrates that PAW can reduce the minimum amount of water needed for healthy growth of plants. This may be due to efficient uptake of PAW compared with untreated water, together with the plasma fertilizer effect.
- In recent years, plasma has been studied as an ecological alternative to chemicals for soil decontamination and soil modification, because plasma provides a high flux of radicals of a lifetime less than hours, being more reactive and much less residual than conventional chemicals. Plasma modification of tobacco-soil and bed-soil in farmland has been successfully demonstrated to protect from injury by continuous cropping.

#### There are nearly **400,000 plant species** ! Only about **31,000 of these species have at least one documented use**.



- Improved plant growth and inducing plant's stress resistance, fighting diseases
- Creation of PAW for watering/hydroponics
- Plasma decontamination and enrichment of water for agriculture
- Plasma treatment for enrichment of manure (plant based, animal based)



## NTPs and FOOD

- NTPs a non-thermal process capable of disinfecting different foods (and also food processing equipments) from various microorganisms, with limited impact on food characteristics. Additional positive features are that plasma is a dry technology and that the produced antimicrobial agents, mainly reactive species and UV radiation, are created locally and are not associated to harmful residues.
- Plasma disinfection potential was shown against several food-borne pathogens (e.g. Aspergillus niger, Salmonella typhimurium, Listeria monocytogenes) and for a variety of different foods, such as rice, egg shells, spices, fruits and fresh produce.
- NTPs are successfully used in production and treatments of food packaging. a first demonstration of CAP food disinfection at industrial scale was recently provided in the frame of the European project SAFE-BAG.
- NTP modification of starch, a biopolymer extensively used in the food sector and which typically undergoes chemical and physical treatments to improve its functional properties; PAW as a nitrite source for meat curing; as an accelerated oxidation technology for the analysis of food oils and oilcontaining food products.



## NTPs and FOOD



Key food applications:

- 1. Disinfection
- 2. Functional modification of food matrices
- 3. Functional modification of packaging material



## Treatment of rare & medicinal plant seeds

- Same family different outcomes
- Model plant Empress tree (Paulownia tomentosa)
- Oxidation of the surface has relevant role







## Protected, rare and medicinal plants

#### Family Paulowniaceae

#### Family Gentianaceae



Paulownia tomentosa Model plant



Gentiana asclepiadea



Gentiana lutea





## Protected, rare and medicinal plants

#### Family Gentianaceae

- Remedies and tonics
- Active substances for drugs
- Antidotes for some poisons



Gentiana asclepiadea



Gentiana lutea





#### Treatment of *Paulownia tomentosa* seeds



- Vacuum has almost no effect on seed germination.
- For longer treatment times or higher particle fluxes, inhibition occurred.
- When Argon is used as working gas there is almost no effect



#### Treatment of Gentiana asclepiadea seeds



100 seeds were placed in each, 6 cm in diameter, 3 Petri dish and then on a flat platform inside chamber



- Seed similar in morphology to Empress tree seed.
- Treatment of protected and medicinal plants with low germination percentage and high dormancy potential.





#### Treatment of Gentiana lutea seeds



• Even for same family different seeds demand different treatment parameters





Treatment of rare & medicinal plant seeds

- Plasma treatment improved germination though in case of the argon plasma the improvement was very small.
- For longer treatment times or higher particle fluxes, inhibition occurred.
- For GA, GL and PT samples: increase of O/C ratio with time => Surface oxidation of seeds
- For GA and GL samples: increase of relative concentration of oxygen based molecules: COOH, C-O-C, C-OH, O-C-O, C=O
- For PT sample: small increase of relative concentration of oxygen based molecules: COOH, C-O-C, C-OH, O-C-O, C=O since initial PT surface had already a lot of oxygen based groups on sourface
- New elements on surfaces after plasma treatment:
  - K, N, S
  - F, Si, Al (from plasma system)





## Treatment of synthetic seeds

- What are Synseeds?
- Air DBD plasma treatments
- Plant development potential



#### What are synthetic seeds?

#### WHAT IS A SYNTHETIC SEED?

It is defined as encapsulated plant tissues, such as somatic embryos, shoot buds, axillary buds, shoot tips, cell aggregates, and any other micro propagules that have the potential to grow like a plant, under in-vivo or in-vitro conditions when sown as seeds.

ADVANTAGES OF SYNSEEDS PRODUCTION The advantages of synseeds production are given below:

- Easy handling.
- Short- and long-term storage capacity.
- Genetic uniformity.
- Low-cost quality plant materials are required.



#### APPLICATION

- The production of synseeds facilitates the growth of several plants that have low seed viability, seedless fruit, and poor germination rates, and that depend on mycorrhizal-fungal symbiosis for germination.
- It is very useful when it comes to genotypes selection, germplasm preservation, and in vitro propagation of endangered, rare, and commercially important plants.
- It also allows the conservation of plant species through short- and medium-term preservation.







#### Air DBD plasma source





N Škoro, S Živković, S Jevremović and N Puač, Treatment of Chrysanthemum synthetic seeds by Air SDBD plasma, Plants 11 (7), 907 (2022)

#### Plasma treatment - synseed

Chrysanthemums (*Chrysanthemum morifolium* Ramat.) are the second, besides roses, economically the most important ornamental crop in the world.

Problems:

- seed propagation, i.e. plants that produce non-viable seeds or seedless plants, such as chrysanthemum cultivars
- need for new cultivars
- contamination

#### Chrysanthemum synseed germination under *in vitro* conditions:

Agar medium

Vermiculite+liquid medium

Chrysanthemum synseed germination under *ex vitro* conditions:











#### Plasma effects on development of plants









Effect on different cultivars

Plasma	Cultivars				
treatment [min]	BSC	Q	P.Cl	P.Cn	
0	$6\pm1^{a}$	$17\pm2^{a}$	$28\pm1^{a}$	$14\pm1^{a}$	
10	$22\pm6^{\text{b}}$	$40\pm3^{b}$	$44\pm2^{\text{b}}$	$49\pm3^{\text{b}}$	
Plantlets increase [%]	~370	~240	~160	~350	

N Škoro, S Živković, S Jevremović and N Puač, Plants 11 (7), 907 (2022)

## Treatment of synthetic seeds

- Synseed technology may be useful technique as a propagation system in terms of fast reproduction of seedless plants, preservation of genetic uniformity of plants and straight delivery to field
- The difficulties of sowing artificial seeds directly in soil or on commercial substrates under non-sterile conditions are considered to be one of the main limitations for the practical use of this technique
- Plasma treated chrysanthemum synseeds has better survival rate and whole plantlet growth under greenhouse conditions







## Conclusions

#### POSIBILITIES

- Improved germination and plant growth
- Plasma treatment for microbial decontamination
- Induced plant's stress resistance, activating the plant defense system, fighting diseases
- Creation of PAW for watering/hydroponics
- Plasma decontamination and enrichment of water for agriculture
- Plasma treatment for enrichment of manure (plant based, animal based)
- Key food applications: disinfection, functional modification of food matrices and packaging material

#### CHALLENGES

- Complexity of plant system requires significant research needs in manpower
- Scalability, benchmarking efficiency and cost, toxicity & by-products
- Definition of protocols, standardization of procedures for plasma treatments in order to be comparable
- Creating standards (especially in food):
  - ✓ to evaluate the characteristics of CAP or PAW
  - ✓ to evaluate the treatment efficacy
  - ✓ to evaluate the safety and quality of plasma treated products
- Addressing legislation in different countries

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