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IW-01

Reactive oxygen and nitrogen species in plasma activated water: tuning their concentrations and functions in plant growth promotion

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Non-thermal (cold) air plasmas in interaction with water induce chemical activation and generate *plasma activated water* (PAW). PAW typically contains various reactive oxygen and nitrogen species (RONS), especially long-lived H_2O_2, NO_2^-, NO_3^-. Their concentrations and ratios depend on the plasma discharge and dissipated power (e.g. low power corona or DBD vs. high power spark, glow or gliding arc discharges), the gas flow system (e.g. open air vs. confined volume) and the way of interaction with water (e.g. bulk treatment vs. aerosol). Selection of these discharge/gas flow/water interaction parameters enables us to tune the concentrations and ratios of RONS in PAW for various applications [1]. Furthermore, the water solution characteristics, its pH, buffering capacity and storage temperature strongly influence RONS concentrations upon delayed application.

PAW has been successfully demonstrated to stimulate germination and plant growth [2-4]. These effects depend on the RONS concentrations and the way of application, e.g. seed imbibition in PAW, plant watering or foliar application [3-4]. We show that these effects also strongly depend on plant species. Using different types of PAW generated by glow discharges with bulk water or by transient spark with water aerosol, exhibited a positive effect on amylase activity of pea seedlings and did not inhibit seed germination, seedling length, total protein concentration or protease activity. PAW caused only moderate oxidative stress that was effectively alleviated by natural plant antioxidant enzymes (SOD, G-POX, CAT). In pea seedlings, we observed a faster turn-over from anaerobic metabolism (related to imbibition) to aerobic metabolism. RONS contained in PAW did not affect the DNA integrity. On the other hand, the high DNA damage in barley and the reduced root and shoot length and decreased amylase activity was attributed to the oxidative stress caused by PAW, which was exhibited by enhanced activity of G-POX or alcohol dehydrogenase related to grain suffocation [5].

Besides influencing the physiological plant responses upon exposure to PAW, significantly increased plant growth parameters and harvest production were detected in peas and maize. PAW of controlled properties represents a high application potential in farming.

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IW-02

Plasma-based water treatment in agricultural applications

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This contribution concerns recent advancements in our research on the application of plasma-based water treatment in the agrifood field. Specific issues addressed include the decontamination of water from poly- and perfluoroalkyl substances (PFAS) and from pesticides.

Contamination of groundwater with PFAS is affecting wide areas worldwide, including Veneto region in northern Italy, where the University of Padua is located and where a major international company, now closed, has operated with PFAS for over half a century. These compounds are taken up by plants and vegetables and bioaccumulate in human blood. Due to growing indications of possible damage to human health caused by PFAS [1], the use of well water for irrigation and animal watering has been restricted in contaminated areas, promoting instead the use of tap water for these purposes or the installation of filters to purify the well water before use. Filters based on granular activated carbon are indeed the most diffused means for PFAS removal from water. They are effective but have a limited lifetime, are not easily regenerated and become thus a troublesome waste to be disposed of [2]. To overcome these limits, new technologies are urgently needed to achieve not only the removal but also the degradation of PFAS in water. Atmospheric plasma is particularly promising for this application [3]. A self-pulsing discharge reactor specifically designed for PFAS degradation was recently developed in our laboratory [4] and tested successfully in the degradation of perfluorooctanoic acid (PFOA). The investigation on the reactive species and on the intermediate products formed during the treatment and analysed by liquid chromatography coupled to mass spectrometry was then completed to achieve a comprehensive mechanistic picture of the complex chemistry occurring in this system [5]. Improvement of the reactor design and use of combined approaches of plasma with catalysts are currently investigated in our laboratory to increase the energy efficiency of the process and decrease the treatment time.

Other important targets in the application of plasma-based water treatment in agriculture are pesticides, which are often persistent organic compounds. Aqueous solutions containing pollutants belonging to different classes of pesticides, such as herbicides and insecticides, were thus subjected to treatment in different plasma reactors. The effects of major experimental variables on the degradation kinetics and on the products of the process were investigated. The results allowed us to describe the chemical reactions responsible for the degradation of the various contaminants.

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IW-03

Cold atmospheric pressure plasma applications along the entire food production chain: examples and perspectives

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1. General Aspects

When the high hydrostatic pressure process was introduced for the minimal processing of food, pressure was also referred to as the "third dimension", in addition to the important process variables of temperature and time [1]. With cold atmospheric pressure plasma (CAPP), the so-called "fourth state of aggregation", a further area of application can now be opened up for food processing, in addition to the states of aggregation solid, liquid and gaseous that have been considered so far. The lecture is intended to identify the potential and limitations of various plasma technologies for the preservation and modification of food.

2. Short Abstract

The presentation will include a thematic introduction to the topic of cold atmospheric pressure plasma application and summarises the important fundamental aspects related to plasma application in food processing. The introduction of a new decontamination technology in industry requires understanding of the mechanisms of microbial inactivation and the associated interactions between process and product. Therefore, experimental studies will be presented with the following objectives: i) to investigate the underlying inactivation mechanisms in plasma treatment of microbial contaminants; ii) to clarify how the different process- and product-related parameters influence the inactivation process; and iii) to evaluate the treatment effects on relevant quality parameters of plant products (exemplary for food). The individual aspects follow a comprehensive overall approach, from the targeted inactivation of microorganisms on temperature-sensitive food systems to the customised influence of quality attributes along the entire food value chain. The multi-scale approach takes into account the influence of cold atmospheric pressure plasma on molecules, macromolecules, single cells, complex plant food systems and food processing equipments [2]. Finally, the future perspectives for the transfer of plasma applications to industrial food production will be discussed.

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IW-04

From Bench to Prototype for Fresh Produce Processing with Non-thermal Plasma

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1. General

The diversity of microbiological safety and quality challenges in addition to the persistent issue of antimicrobial resistance drives the research agenda and technological development for sustainable fresh food production and processing. Consumer demand for less processed goods provides further rationale for effective decontamination methods that can maintain produce quality characteristics and that can provide shelf-life advantage in the context of increasingly complex food chains.

Atmospheric cold plasma (ACP) has found increasing attention in the food processing sector, with successful and wide-ranging decontamination applications demonstrated. The concept of non- thermal plasma processing has reached a state of knowledge where industrial prototypes have been developed in cooperation with industry stakeholders.

This presentation outlines the key issues for the fresh produce sector, and how these have been addressed at bench scale, and how this has translated to prototype development. This talk will discuss how an iterative process between bench and prototype scales can promote scale up and successful adoption of gaseous and liquid mediated cold plasma technologies. The reported antimicrobial efficacy, the nature of the risks, as well as the importance of considering the mode of delivery and stage of implementation compatible with food processing unit operations are highlighted.

Keywords: Fresh Produce, Critical Process parameters, In Package, Plasma functionalized Liquids, Biofilm, Safety

IW-05

Dry atmospheric plasma priming: a sustainable approach to improve seeds germinative parameters

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Seed-stacked dielectric barrier devices (S^2DBD) operating at atmospheric pressure are processes well suited to agricultural issues, especially to improve the germination parameters of seeds such as vigor and germination rate but also to release their dormancy or reduce their pathogen charge. The efficiency and the sustainability of these biological effects depend on several properties including (i) reactive species generated in the plasma phase, (ii) the plasma-seed interaction and (iii) the amorphous solid states of the seeds.

First, the question of the reactive chemistry will be discussed in the case of S^2DBD supplied in helium (1slm) with/without reactive gas (O_2 or N_2). Based on MS, OES and WCA measurements, the role of the main reactive species will be highlighted as well as the mechanisms bridging gaseous chemistry with surface chemistry. Besides, we will show why the He-N_2 plasma drives to the most promising results (in comparison with those obtained using a He-O_2 gas mixture). The sustainability of the plasma-triggered biological effects will be also demonstrated through a 2-months ageing study, hence supporting the relevance of the process for technological transfer towards seed companies.

Second, the question of the plasma-seed interaction will be discussed, and its crucial role will be demonstrated through an equivalent electrical model of the S^2DBD (based on Peeter's model). Complementarily, the role of the plasma-seed contact surfaces in inducing stronger biological effects will be deciphered through a solid modelling of the seeds stack, enabling the distinction between areas of the seed-seed contact surfaces and of the seed-wall contact surfaces. We will explain how the characterization of these contact surfaces can contribute to design more appropriate plasma atmospheric processes.

Third, the question of the amorphous solid state of the seeds will be discussed in the case of an air DBD treatment. We will show how seeds dormancy can be drastically alleviated by controlling the amorphous solid state of the seeds which can be either rubbery or glassy. Two distinct methodological approaches will be compared: modulation of seeds water content and modulation of seeds temperature upon plasma exposure.

IW-06

The molecular mechanisms involved in plant response to stress induced by seed exposure to cold plasma

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The potential of seed irradiation with low temperature plasmas or cold plasmas (CP) for increasing agricultural production is under intensive investigation. Numerous effects on seed microbial contamination, germination and seedling growth have been reviewed recently [1-4], and the accumulated body of evidence indicates to the high complexity of seed response to CP treatment on the molecular level. The overview of the most recent findings on the molecular changes induced in dry or germinating seeds and in growing plants will be provided in the talk, including the results of our research group.

Earlier studies on effects of seed exposure to CP were focused mostly on microbial decontamination, changes in seed surface wettability and germination. Certain later studies have demonstrated enhanced plant growth for the entire vegetation period resulting in increased production yields, indicating to the persistence of CP effects on much longer time-scale due to involvement of complex molecular responses in growing plants. The most important novel findings on such response induced in dry seeds are changes in: 1) EPR signal; 2) DNA methylation; 3) protein carbonylation; 4) balance of phytohormones; 5) expression of genes; 6) expression of proteins; 7) enzyme activities; 8) seed microbiome. CP-induced changes in ROS production were reported in the germinating seeds. The events of seed response to the CP stress signal are further developed in the growing plant as multiple inter-related changes in: 1) gene expression due to DNA methylation; 2) protein expression; 3) enzyme activities (including enzymes of photosynthetic system and enzymes of secondary metabolism); 4) changes in secondary metabolism that followed by 5) a modified plant communication with microorganisms (both pathogens and plant growth promoting microorganisms, e.g. N-fixating rhizobacteria).

Thus, CP induces significant and complex changes in plant metabolism. Upregulated photosynthesis results in better plant growth, stimulated secondary metaboism leads to better plant establishment, fitness and stress resistance. CP effects “beyond plants” (plant communication with microorganisms) are important for improved agricultural performance. The persistence of CP effects implies that CP induces complex mechanisms of plant adaptation that can be exploited for Plasma in agriculture goals.

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Keywords: cold plasma; DNA methylation; phytohormones; proteome; seeds.

IW-07

Plasma activated water as disease resistance inducer in plants

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1. General

Current global food sustenance by intensive agriculture is mainly based on economic crop monocultures that, however, drastically reduces the biodiversity, increasing the yield losses due to the presence of biotic and abiotic stresses. A technology based on plasma activated water (PAW), characterized by the presence in liquid of reactive oxygen and nitrogen species (RONS), was tested to try to ensure yield stability also enhancing the plant resistance responses and to promote an eco-sustainable management of plant diseases. The exposure of sterile distilled water (SDW) to a cold atmospheric pressure plasma (CAP) causes a reduction of pH and the production of RONS, that induce plant defense responses. The use of PAW for the treatment of infected plants is developed and applied with the design, production, optimization and characterization of different CAP sources. The use of PAW on micropropagated shoots and plants in orchards and in greenhouse cultivation systems to evaluate its effectiveness as pathogen resistance inducer was exploited.

2. Studied cases

The effects of PAW applications were tested on tomato plants experimentally inoculated with *Xanthomonas vesicatoria* (Xv), phytoplasma infected periwinkle micropropagated shoots and plants, and grapevine plants in greenhouse and in vineyards. Quantitative (q)RT-PCR analyses allowed to determine the transcription level of genes involved in the plant defence response (phenylalanine ammonia-lyase, pal) and in the plant phytoalexin metabolism of PAW treated plant materials. The number of leaf spots caused by Xv in tomato plants and the number of symptomatic grapevine plants in in vineyards were significantly reduced by the treatments [1]. Transcriptional and post-transcriptional molecular analyses highlighted the PAW's ability to enhance the expression of genes encoding the main enzymes involved in the phytoalexin biosynthetic pathway (alkaloids and stilbenes) and to modulate some of the stress response genes through miRNAs regulation [2]. The PAW ability to enhance some of the plant defence mechanisms, also improving the health status of the treated plants, was experimentally demonstrated. The main results indicated the suitability of using PAW to reduce the disease severity, induce plant resistance both in open field and greenhouse, improving the plant healthy status and fruits yield production [3].

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IW-08

Plasma Treatment of Growing Plants – Effects on Plant Growth, Development and Stress Responses

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In the last decade, effects of cold atmospheric pressure plasma (CAPP) and plasma treated water (PTW) generated by CAPP on plant germination as well as on plant growth and developmental processes have been extensively studied for various kind of plant species ranging from crops, herbs, vegetables to ornamental plants. CAPP generated with air contain ions, electrons, neutral atoms and molecules, and a set of various reactive nitrogen (RNS) and oxygen (ROS) species. Depending on the specific chemical and physical environment, RONS have different half-life times and are converted to more stable chemical states, e.g. in water to hydrogen peroxide (H_2O_2), nitrite (NO_2^-) and/or nitrate (NO_3^-) ions. Gaseous and aqueous plasma-generated RONS can react with biomolecules of biological systems. Chemical modification of biomolecules alter its stability, activity and physiological function and can provoke cellular responses resulting in physiological changes affecting developmental processes and stress responses of the whole plant.

In two initial approaches, three kinds of plasma treatment modes were applied to blue lupin (*Lupinus angustifolius* L.) to evaluate plasma effects on biomass production, biochemical parameters and stress response. In the first approach, lupin roots (watering) or shoots (spraying) were treated with PTW that was generated by using a pin-to-liquid or a gliding arc discharge device. Interestingly, PTW treatment of shoots by spraying for five days resulted in higher biomass parameters after 4-5 week of growth. Biochemical parameters such as total chlorophyll content, soluble protein content or catalase activity were essentially unaltered. In the second approach, the upper shoot part including the shoot apex of lupin plants were exposed for 2 min to a surface dielectric barrier discharge reactor (SDBD). Shoots were harvested five hours after treatment and extracted soluble proteins were subjected to MS-based proteome analysis. 5608 proteins were identified in soluble protein leaf extracts. Upon those, 287 were down-regulated and 423 were up-regulated after plasma exposure. Several up-regulated proteins were assigned to secondary metabolism and to abiotic stress responses, while a set of down-regulated proteins were referred to primary metabolism, growth regulation, protein expression and turnover machinery.

In addition to RONS, other factors, such as the electromagnetic field generated by the SDBD source, may have led to the observed stress response in blue lupin.
Results of both approaches are discussed in terms of the current state of knowledge and possible future approaches to plasma treatment of growing plants for potential stress priming.

Keywords: cold atmospheric pressure plasma, plasma treated water, blue lupin, biomass and biochemical parameters, soluble leaf proteom

Oral presentations

OW-01

Removal of mixtures of pharmaceutical pollutants in aqueous solutions using non-thermal plasma

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Water pollution is a serious problem of the modern world due to the increasing prevalence of contaminants of emerging concern, especially pharmaceuticals. Their increased usage and resistance to conventional treatment lead to widespread environmental dissemination [1], [2]. New technologies are needed to remove these pollutants. One of the most promising approaches is non-thermal plasma treatment, capable of degrading a variety of organic pollutants [3], [4]. The objective of this paper is to investigate the plasma degradation of mixtures of pharmaceutical compounds.

A pulsed corona discharge above liquid was used for the removal of a mixture of amoxicillin (AMX), diclofenac (DCF) and ibuprofen (IBU). The pulse duration (110 ns), frequency (25 Hz), voltage (18 kV) and discharge gas (O_2, flow rate 300 mL/min) were maintained constant throughout the experiments. The plasma reactor was coupled with an ozonation reactor in order to use the excess ozone generated in the discharge [5]. A volume of 330 mL of contaminant solution (0.1 mM AMX, 0.1 mM DCF and 0.1 mM IBU) was circulated between the two reactors. The conductivity of the solution was adjusted to 300 μS/cm with different salts (Na_2SO_4 and $NaHCO_3$), and the initial pH was 7.7-8.

Complete removal of AMX was achieved after 5 min of treatment with $NaHCO_3$ and after 10 min with Na_2SO_4. DCF and IBU were almost completely degraded after 10 min and 20 min, respectively, regardless of the salt used. However, their degradation appeared to be faster when using Na_2SO_4. The highest mineralization degree achieved was 44% ($NaHCO_3$, after 60 min). For comparison, experiments with single contaminant solutions were performed. The concentrations of individual pharmaceuticals were adjusted to match the theoretical oxygen demand of the mixture. The complete removal of single contaminants was achieved slower as compared with their degradation in mixture. Overall, the plasma-ozonation system shows promising results for the simultaneous removal of multiple pharmaceuticals from solution.

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OW-02

Energy decomposition analysis of organic pollutants in water: a way for finding plasma degradation routes

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1. Methods

Energy decomposition analysis (EDA) [1] aims at determining bond energies inside molecules. If a complex molecule F1…F2 is expected to dissociate in 2 fragment F1 and F2, the bond energy (or binding energy) is written as: $\Delta E = E_{F1\ldots F2} - E_{F1} - E_{F2}$. $E_{F1\ldots F2}$ is the energy of the optimized complex molecule, while E_{F1} and E_{F2} are the energies of the optimized fragments. The bond energy ΔE can be divided in 2 terms: the strain energy which is the energy necessary to optimize geometry of fragments to their optimized geometry in the complex molecules and the interaction energies. EDA is decomposing the interaction energy in three terms: Coulomb interaction, Pauli repulsion, and attractive orbital interactions. The Coulomb interaction, which is usually attractive, is the energy between the unperturbed charge distributions of the prepared fragments. The Pauli repulsion consists in the destabilizing interactions between occupied orbitals of the fragments. The orbital interaction includes charge transfer and polarization.

The bond energy determination calculates all these energies for each component. This is done using Density Functional Theory using the appropriate exchange correlation functions and basis sets. The main limitation is that we must select the possible fragments before running calculations. So, the method has been extended in Interacting Quantum Atoms (IQA) developed in the frame of the so-called Quantum Theory of Atom in Molecule (QTAIM) [2] for obtaining all bond energies in one calculation.

2. Applications to plasma degradation of persistent organic pollutants

We have applied and compared these methods to analyzing bond energies of antibiotics such as amoxicillin and sulfamethoxazole (SMX) for comparison with plasma degradation experiments. The degradation of SMX in water by a corona discharge above liquid was studied experimentally and the plasma-treated solutions were analyzed by LC-MS to identify the degradation products. For short treatment time (2 min), the presence of compounds with higher masses than the parent molecule (m/z 270, 273, 284, 288, 296) suggests the addition of oxygen-based functional groups as a first step in the degradation pathway. Smaller degradation products (m/z 174, 204) resulting from the fragmentation of the SMX molecule are also detected. Since IQA is very powerful for halogens compounds [3], it will be used for addressing pesticide, such as chlordecon and atrazine, degradation by plasma.

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OW-03

Application of a surface DBD plasma source for flour treatment

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For decades non-thermal plasmas (NTPs) have been used in various industrial processes to modify surfaces of different materials. At first, mostly low-pressure NTP was used for these applications, thus imposing a limitation to the type of the sample. With the development of NTP sources at atmospheric pressure, the number of plasma material processing applications has increased, enabling applications in medicine, biology and recently agriculture and food industry [1]. Cold plasma treatment can change the functionality and improve rheological properties of wheat flour [2] due to the radicals and ozone propagated oxidation of amino acids. Additionally, plasma-created reactive species has demonstrated ability for mycotoxins detoxification. Therefore, in this study we investigated both effects of cold plasma treatments on flour – plasma influence on amino acids in flour as well as reduction of *Alternaria* toxins in contaminated flour.

The experimental setup used consists of a surface dielectric barrier discharge (SDBD) source which is placed on top of a box that contains a flour sample and openings for air. High-voltage sine signal is supplied to the powered electrode positioned at the bottom of a dielectric plate. Before treatments we characterized the source by recording electrical waveforms and by using optical emission spectroscopy to obtain information about excited species created in the plasma. For moisture content analysis NIR Spectroscopy analyzer was used, while for determination of *Alternaria* toxins content high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) measurements were performed [3]. Measurements of the different types of wheat flour samples showed linkage formation in the gluten-related proteins after plasma treatment, due to reaction with reactive oxygen species formed in the plasma. In flour samples contaminated with different *Alternaria* toxins we found reduction from 4% up to 74%, depending on the type of the toxin and treatment conditions. We used the experimental results for developing and fitting empirical models in the form of the second-order polynomials for prediction of toxin reduction and optimization of the process. Significant differences observed between treated and control samples in this study demonstrated great potential of the NTP DBD source for treatment of the wheat flour.

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OW-04

The potential of cold plasma for the stimulation of natural sweeteners biosynthesis in *Stevia rebaudiana* Bertoni

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Seed treatment with non-thermal or cold plasma (CP) stimulates seed germination, grown plant morphometric parameters, biomass production, and disease resistance in different plant species by inducing changes in plant biochemical phenotype. The activities of enzymes, the amounts and ratios of different secondary metabolites are markedly changed after some treatments, however, there are still not enough knowledge in molecular mechanisms to control and predict treatment effect.

Stevia rebaudiana Bertoni is an economically valuable plant due to its secondary metabolites steviol glycosides (SGs) that are responsible for the sweetness of stevia and are widely used as natural sweeteners. Stevioside (Stev) and rebaudioside A (RebA) are the most abundant SGs in stevia. RebA is preferred over Stev for better taste (lack of bitterness). We have demonstrated for the first time, that seed treatment by CP can increase Stev and RebA concentrations several times.

The aim of this study was to overview our research group results on the effect of *Stevia rebaudiana* Bertoni seed treatment (2-7 min) with different types of CP (dielectric barrier discharge (DBD) and capacitively coupled (CC) CP) on the amount and ratio of Stev and RebA in the leaves of stevia, the kinetics of stimulated biosynthesis and the possibility to transfer the CP-induced stimulating effect to the vegetatively propagated plants.

Both types of CP had strong stimulating effect on steviol glycosides (SGs) in stevia leaves 8 weeks after germination. CC CP increased the RebA concentration 1.5-fold and the concentration of Stev 7-11-fold depending on treatment duration. The optimal 2-min pre-sowing seed treatment with DBD CP increased the RebA concentration 2-fold, Stev - 14%, RebA/Stev ratio - 1.7-fold. The treatment of longer duration (5-7 min) had lesser effect than 2 min. Stimulating effect persisted 14 weeks (time of cutting for vegetative propagation) but only in 5 min-treatment group and vanished in 20th week (onset of buttonization). Vegetative propagation induced additional stress to plants what resulted in 2-fold decrease of SGs synthesis after 8 weeks. Plants recovered after 20 weeks; however, the CP-induced stimulating effect was lost in vegetatively propagated plants. The concentrations of other bioactive compounds as phenolics and flavonoids were decreased or unchanged by both types of CP treatment resulting in lower or unchanged antioxidant activity of stevia leaf extracts rich in SGs.

It can be concluded that a short time pre-sowing treatment of seeds with CP can be a powerful tool for the enhancement of biosynthesis/accumulation of SG in stevia plants at least for 14 weeks of vegetation. More studies are required to evaluate the possibility of stimulation transfer by vegetative propagation by adjusting cutting time.

Keywords: *Stevia rebaudiana* Bertoni, seed treatment, cold plasma, vegetative propagation

OW-05

Effects of Surface Dielectric Barrier Discharge Treatment of Some Sprout Species in Different Conditions

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Cold plasmas produced at atmospheric pressure are suitable and have been very much used for processing biological materials such as seeds. It was shown that such a powerful chemical reactor can stimulate the germination and growth among other positive effects. However, the outcome depends on the used source, but most important on the species of the seeds processed using plasma.

In our previous works we focused on establishing the results of direct and with intensified reactive species exposure of some species (*Raphanus sativus* L. var *longipinnatus* – Japanese radish, *Bassica oleranceae* L. var *italica* – broccoli, *Lepidium sativum* – garden cress). The sDBD device used for the experiments has a simple flexible configuration that can be either used for the direct treatment of different surfaces or can be attached to a closed package with the processed materials (in our case seeds or sprouts) placed inside.

Sprouts are becoming popular in the diet of Europeans because of the health benefits brought by bioactive molecules such antioxidants and enzymes, which are found in sprouts. However, they have a short shelf life and might carry microorganisms harmful for the human consumption. So, there are two directions we turn our attention on: stimulating the growth while studying the mechanisms of plasma-seeds interaction, and increase shelf life and consumption safety. To achieve our goals, we perform a detailed characterization of plasma and of the outcome of plasma treatment starting from seeds (surface morphology imaged with Environmental Scanning Electron Microscopy, wettability – water contact angle measurements, imbibition), germination behavior (germination potential, germination speed), sprouts physical and biochemical properties (biometric measurements, chlorophyl and carotenoid contents, etc.), shelf life of sprouts produced after seeds or sprouts plasma treatment.

Our results show significant differences between species behavior: for some the germination and sprouting is stimulated, for other is inhibited; some species exhibit strong morphological changes of the surface of seeds, with increasing the hydrophilic character, while others show only minor modifications; biochemical parameters are changing for most species, especially when the seeds are treated inside a closed package, probably due to the intensified action of the reactive species that cannot diffuse in the surrounding air but remain in the closed proximity of the samples. In all cases we found an increase of the life shelf of the sprouts.

Keywords: plasma agriculture, cold plasma, sDBD, sprouting, germination.

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Tailoring plasma sources towards plasma agriculture: at the interface with liquids and solids

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Plasma discharges are rapidly gaining importance as versatile tools for material processing, since they are easy to use, technologically simple and environmentally friendly. Applications of these plasmas are widely spread, including: plasma technologies (involved in industry: food to textile, construction to automotive), plasma medicine (e.g.: biology, pharmaceutics, oncology) and, recently, plasma agriculture; therefore, it is important to characterize and monitor plasma sources from electrical and optical point of view, in order to fulfill the applications requirements.

Our goal is to use atmospheric pressure plasma jet sources to mitigate on one hand the plasma-liquid medium interaction (treatment / production), and on the other hand to optimize the plasma-solid surface interaction in the plasma-seed direction. More precisely, in this report, by using the principle of dielectric barrier discharge, in a cylindric geometry, we powered up an atmospheric pressure plasma jet (appj) in helium, and used it two scenarios:

1. in the food industry direction (for wine making), we used the plasma source to treat liquid media as follows: treating fresh must (white grape juice) in order to improve the storage / quality of wine;
2. in the plasma seed and plasma activated medium direction, we directly treat seeds with plasma and indirect, by means of plasma activated water (paw), and studied the effects of these interactions upon the plant evolution (from cultivation to harvest).

Furthermore, we used a 2 SLM He flow through the discharge tube and applied a 16 kVpp sinusoidal voltage on the discharge electrodes, at 48 kHz, keeping a gap of 5 mm between the discharge tube and the sample (liquid or solid). The plasma source was characterized via electro-optical diagnosis: electrical methods for applied voltage, discharge current, and power, charge and energy, as well as optical emission spectroscopy.

Plasma-treated medium (must, water or seed) was investigated by means of UV-Vis absorption spectroscopy, ART-FTIR, pH, conductivity, and optical microscopy. The results, as a correlation between the plasma characteristic parameters and treated medium properties, are in the favor of using plasma treatment for activating the medium (PAW) / preserving (wine) or stimulating processes (accelerated germination) in the studied medium. Preliminary results support the plasma treated must procedure in the preservation of young wine (1-2 years old) as well as the promotion of germination of seeds (both for the direct treatment and using paw on seeds).

Further studies, implying more analysis methods should be implicated for a better and perspective view on the topic of plasma agriculture.

Keywords: plasma physics, plasma agriculture, plasma&wine, plasma-bioengineering

Regular contributions

PW-01

The effect of a cold atmospheric filamentary plasma on seeds of beans (Phaseolus vulgaris)

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Because of climate changes conditions, the soil contamination with microorganisms and chemicals, low quality of seeds, the rate of seeds germination and implicitly the crop yield continue to decrease. To overcome these economic problems, non-thermal plasma emerged nowadays as a promising technology in agriculture [1]. Recently, plasma treatment technology has been focused on practical ways to increase the germination percentage, at large quantity [2].

In this study, the effect of the cold radiofrequency (RF) plasma treatment on beans seeds (Phaseolus vulgaris) is investigated. Firstly, a preliminary research was made using a singular filamentary jet [3]. The effects of the reactive gas introduced in the Ar discharge and of the applied power were studied. It was observed that the germination was accelerated when a small amount of O_2 was introduced, at lower RF power.

Figure 1. Image of the scaled- up plasma system during operation

Secondly, in order to overcome the laboratory studies and to make the first steps towards field trials (to increase the treated seed quantity), a scaled-up cold filamentary plasma system was successfully implemented for seeds treatment, as presented in Figure 1. The importance of seed shapes was evaluated. Therefore, we treated different shapes, colors and dimensions of the seeds, under identical plasma parameters. Moreover, the importance of plasma treatment time on the germination efficiency was highlighted. These results showed that plasma treatments promote the germination rates and plant growth, which, therefore, can increase crop yields. As such, we can state that this newly developed plasma system can be successfully implemented in pre-harvest agriculture and this system will probably be transformed into an especially useful tool for future field trials.

Keywords: cold plasma, beans treatment, germination, scaled-up system
Acknowledgement. This work was performed under the COST Action CA19110. The study was financed by the Romanian Ministry of Research, Innovation and Digitalization under Nucleus Programme 4N/2019.

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PW-02

The influence of air atmospheric pressure plasma treatment on the germination of *Tagetes erecta* seeds under saline stress

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The applications of atmospheric-pressure plasmas in agriculture grow rapidly. As shown in some recent studies, several methods of treatment are used in agriculture to enhance the rate of germination and growth in plant seeds. A large experimental flexibility is envisaged, as the seeds might be exposed to the plasma active agents either directly, as well indirectly by using the plasma activated water or plasma modification of soils, leading to promising solutions of technological transfer from laboratory to farms.

In this study, we discuss the influence of atmospheric pressure plasma exposure on *Tagetes erecta* 'Petite Yellow' seeds in saline and non-saline solutions. The seeds were exposed to the pulsed plasma generated using a dielectric barrier discharge (DBD), in air at atmospheric pressure. The DBD setup consists of one circular plane electrode, covered by glass and a second mesh electrode, with a discharge gap of 5 mm. The discharge was driven by an AC power supply at 50 Hz and 13 - 16 kV peak-to-peak amplitude. Treatment time ranged from 2 to 5 minutes. In order to monitor some of the reactive oxygen and nitrogen species in the gas phase, during the *Tagetes erecta* seeds plasma exposure, we used the Fourier-transform infrared spectroscopy (FTIR) technique. The following reactive species were identified using gas phase FTIR: HNO_2 (3630-3545 cm^{-1}, 1735-1670 cm^{-1}, 890 - 760 cm^{-1}), N_2O (2265-2145 cm^{-1}), N_2O overlapped with HNO_2 (1330-1210 cm^{-1}) and O_3 (2122 cm^{-1}, 1053 cm^{-1}) [1]. The seed lots (75 seeds/lot) were placed in Petri dishes (25 seeds/plate) with distilled water, 0.1 M and 0.05 M NaCl solutions respectively, at 22 °C, in a light incubator and monitored for 6 days.

We calculated the germination percentage/day (GP), the final germination percentage (FGP) the coefficient of velocity of germination (CVG), the germination index (GI), the germination rate index (GRI), the mean germination time (MGT) [2]. The highest final germination percentage (FGP) [4] in distilled water was recorded for the seeds treated at 15.5 kV for 5 minutes and the highest FGPs in both saline solutions, were recorded for the seeds treated at 13.5 kV for 5 minutes. Our observations prove the positive influence of the atmospheric pressure plasma on the seeds germination capacity and velocity in both saline and non-saline solutions.

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PW-03

Effect of non-thermal plasma treatment on sunflower seeds

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The rapid growth of world population and consumption surpasses crop production and as a result hundreds of million people are undernourished [1]. Improving sustainability in agriculture requires new and efficient technologies to enhance productivity, while reducing the use of chemicals and their negative impact on environment. Pre-sowing seed treatment by non-thermal plasma is a promising approach, capable of improving seed germination, enhancing the early growth as well as further development of plants, decontamination of seeds, inducing resistance to environmental and biological stress etc. [2-4].

This work investigates the effect of seed treatment with a dielectric barrier discharge (DBD) on sunflower germination and early growth. The plasma was generated in a coaxial DBD reactor with the inner electrode (Φ 21.7 mm) connected at high voltage and the outer electrode (Φ 34 mm, L 230 mm) grounded. The sunflower (*Helianthus annus* L.) seeds, a semi-early hybrid P64LE99 (Pioneer®), production from 2019, were packed between the electrodes, i.e. in the discharge gap of 4.5 mm, approximately 30 g in each experiment. The discharge was generated in air, with sinusoidal voltage of 16 kV amplitude and 50 Hz frequency. The average power dissipated in the discharge was 5 W, calculated by the Lissajous method [5], and the treatment time was 10 min. The seeds were incubated at 22°C, 60% UR and light/darkness program 8/16 h for seven days at the Institute for Plant Protection Bucharest. Four replications of 25 seeds each were used for the two variants. The germination of seeds on filter paper in Petri dishes, measured for three consecutive days (3rd, 4th and 5th) was 98% both for the untreated and plasma-treated samples.

The radicles, measured on the 5th and 7th day, were shorter for the treated seeds, and the difference was statistically significant (ANOVA, $p = 0.05$). On the 7th day, the seeds were transferred in pots with organic substrate under lab conditions (25°C, 60%UR, 8/16h), where the plant growth was further observed for three weeks. The stem length (from the soil up to the cotyledons) was measured at two-day intervals, and showed significantly higher values for the treated seeds as compared to the control (up to 18-19% difference). The seedling height was also considerably larger for the plasma-treated seeds. On the other hand, the cotyledons dimensions, measured on the 10th day, did not seem to be influenced by plasma exposure. After 30 days, the plants were removed from soil, measured and weighed. The root length and weight were similar for the treated and control samples. However, the total plant weight was significantly increased as a result of plasma treatment, by almost 30% with respect to the control.

Further work is planned to detect the optimum plasma parameters specific for sunflower seeds treatment, as well as to investigate long-term effects of plasma exposure.

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PW-04

Plasma-induced morphological and biochemical changes in dwarf bearded iris (*Iris reichenbachii* Heuff.) calli

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Expansion of the plasma agriculture and plasma medicine and the demand for precise and localized *in vivo* treatments of living cells and tissues resulted in fast development of various plasma devices that operate at atmospheric pressure [1,2]. Irises can be regenerated *in vitro* by process of somatic embryogenesis and/or organogenesis by formation of shoot or root meristems on calli. During the induction of regeneration process, three types of calli could be distinguished, two friable regenerative calli: white embryogenic and green organogenic and the most abundant yellow, compact, nodular type of non-regenerative calli, designed as non embryonic [3]. Due to its lack of morphogenetic response and/or their low regeneration potential, the regeneration of non-embryogenic iris calli is one of the greatest challenges in this field of investigation. In the current study plant undifferentiated compact tissue (calli) of Balkan endemic dwarf bearded iris (*Iris reichenbachii* Heuff.) was treated using a RF plasma needle device operating with He as a working gas and changes at morphological and biochemical level were investigated. The plasma needle was positioned 3mm above the callus surface enabling direct contact between the active plasma volume and the surface. Direct plasma treatment triggered significant morphological alterations in structure of non-embryonic calli. Observed changes could be attributed to the enhanced cell division of the plant cells at the surface of the compact calli and differentiation of friable calli type stimulated by reactive species formed in the low temperature plasma. Indicated morphological changes were followed by the significant alteration in secondary metabolites in derived different calli types. Our results implicate that direct plasma treatment could serve as a significant elicitor of secondary metabolites production in dwarf bearded iris calli.

Keywords: low temperature plasma, RF plasma needle, calli, iris

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PW-05

Treatment of *Chlorella vulgaris* by gliding arc discharge plasma

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Microalgae have been identified as a promising feedstock for energy and high-value products [1,2] A major challenge for extracting valuable compounds (proteins, lipids, polysaccharides or carotenoids) from microalgae is their strong and rigid cell wall. Conventional extraction processes have different disadvantages and limitations which could be overcome by application of various plasma methods [1-2]. The aim of this work was to investigate the influence of plasma treatment parameters on the viability of algae cultivated in different medium.

The gliding arc discharge technology was used for the treatment of *C. vulgaris* cultivated in BG-11 medium and aquaculture wastewater. Compressed air (with total flow rate of ~22.8 l/min) was used as the plasma gas. The distance between the "knife-edge" type electrodes and surface of the algae suspension was 30 mm. The plasma treatment durations were 300 s or 600 s. The plasma treatment of the suspension was performed at various power supply output power values ranging from 35 W to 265 W at frequency of 270 kHz. Chlorophyll A content was measured as an indication of algae viability after gliding arc discharge plasma treatment. Pigment concentrations were determined on the 5th day after the plasma treatment using methanol extraction. Then, the absorbance of samples was measured at 470 nm, 665 nm and 720 nm wavelengths using a UV-visible spectrophotometer [3].

Obtained results showed that the chlorophyll A content in microalgae depends on the treatment duration and the discharge power. The increase in the discharge power and/or treatment duration resulted in a decrease in the pigment concentration. It was demonstrated that the concentration of chlorophyll A in *C. vulgaris* after plasma treatment was reduced up to 50 % comparing with untreated sample.

Keywords: Microalgae, Chlorella vulgaris, Air plasma, Gliding arc discharge, Cell disruption.

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PW-06

Application of plasma activated water for phenol degradation using a gliding arc discharge at atmospheric pressure

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Phenol and phenolic derivatives are common by-products use as antioxidants or synthesis intermediates in significant industrial processes such as in plastics, pharmaceutical and food industries. These toxic pollutants present in water due to the discharge of these human activities are known to induce several damage both on human and animals health acting as carcinogen products and damaging the red blood cells [1]. Then, along the extended available methods, the use of Plasma Activated Water (PAW) as chemical medium for phenol degradation remains a promising technology [2]. In this context, the present work deals with the analysis of phenol degradation in PAW using a gliding arc discharge as plasma reactor at atmospheric pressure. Based on previous works on the characterization of the key species generated in PAW by our plasma source [3], the present research will focus on application for phenol degradation. Then, distilled water solutions (V=50 ml) with initial phenol concentrations of 1000 mg.l^{-1} have been exposed to the plasma source for different time treatment (0-10 minutes) using air as feeding gas. Analysis of the water composition after plasma treatment have been carried out by spectrophotometry for semi-quantitative evaluation (cf. figure 1) and by GC/MS after liquid/liquid extraction using dichloromethane for phenol concentration measurements/degradation by-products inventory (cf. figure 2). The results highlights the effect of time experiment on the phenol degradation and by-products formation. Analysis of the GC/MS chromatograms allows to clearly identify the phenol degradation and the by-products composition according to the experimental conditions. In addition, the fall of phenol concentration after plasma treatment have been determined after a calibration step using different phenol solutions at known concentrations.

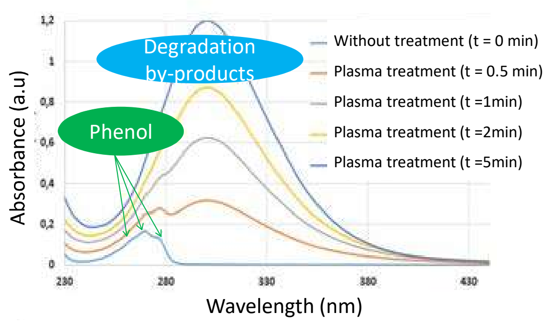

Figure 1: UV-visible spectra as a function of the time treatment.

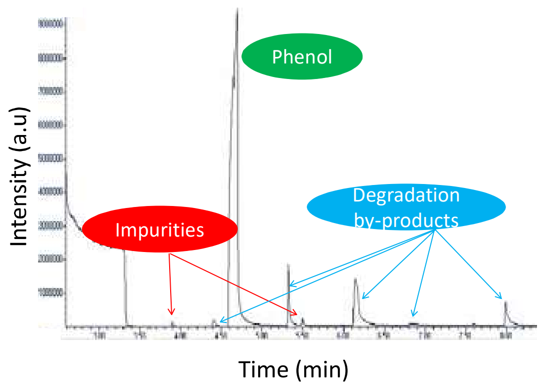

Figure 2: GC/MS chromatogram obtained after plasma exposure.

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PW-07

Hydrophilization of corn seeds by non-equilibrium gaseous plasma

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Plasma agriculture is a promising niche of interdisciplinary research where the physics, of non-equilibrium gases meets surface chemistry and biological responses. Despite numerous scientific papers, the interaction of gaseous plasma with seeds is not understood enough to make the technique useful in practical agriculture. An obstacle is an improper methodology adopted by different authors. In this paper, we show that the surface wettability does not depend on discharge parameters such as power and pressure, but rather on the fluence of oxygen atoms onto the seed surface. The proper methodology is demonstrated for the case of corn seeds. The surface activation, which enables improved water uptake or good adhesion of a coating, progresses relatively linearly up to the O-atom fluence of 3×10^{24} m^{-3} and remains constant thereafter. The minimal water contact angle achievable using oxygen plasma treatment is a few degrees.

Keywords: Corn seeds, Fungi, Gaseous plasma, Hydrophilization, Sterilization

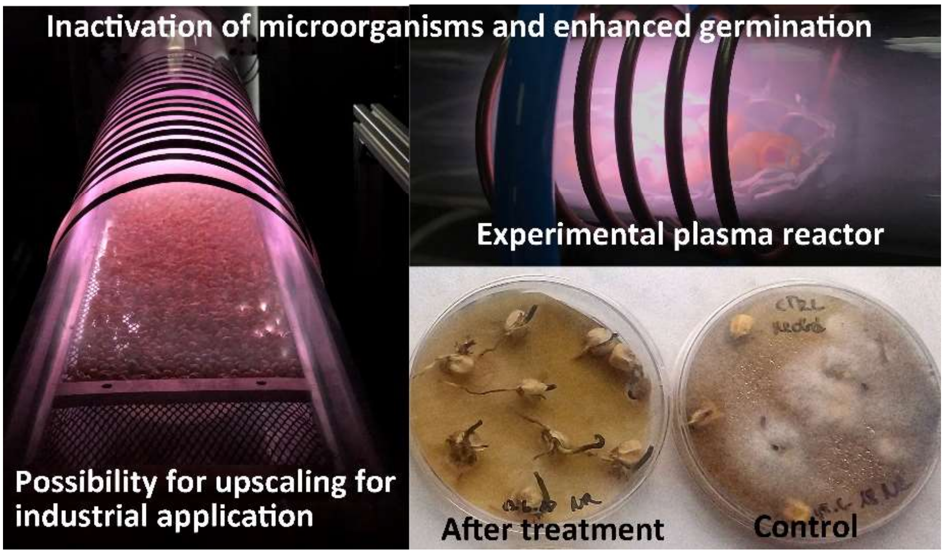

Figure 1: Graphical abstract

PW-08

Listeria monocytogenes dynamics on multispecies biofilms formed on anti-biofilm coatings applied by Non-Equilibrium Atmospheric Plasma on stainless steel

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Biofilms are considered an important source of microbial contamination on food industry. In a previously published work [1], we described the development and characterization of anti-biofilm coatings applied with an atmospheric-pressure plasma jet system on AISI 316 stainless steel, with the best results obtained against *L. monocytogenes* (*Lm*) biofilms formed at 12ºC (90% reduction of biofilm production). The present study assesses the anti-biofilm activity of this plasma-polymerized coating against multispecies biofilms developed from food industry environmental samples, together with the efficacy of conventional sanitizers. The biofilm formation levels on stainless steel with or without the plasma-polymerized anti-biofilm coating after seven days at 12ºC was evaluated for (i) a *Lm* three-strain cocktail, (ii) the indigenous microbiota of food-contact and no food-contact surfaces from processing environments of three different industries and (iii) the previous environmental samples artificially inoculated with the three *Lm* strains. In addition, the disinfection effectiveness of a 15-min treatment with sodium hypochlorite and peracetic acid at 0.5% was assessed for the three types of biofilms on both surfaces. The biofilm populations of *Lm* and the total aerobic plate count, before and after disinfection, were enumerated by selective agar plating after the recovery of the cells from the biofilms through scraping with swabs. The biofilm formation by the *Lm* cocktail was approximately 10-fold higher on the uncoated stainless steel than on the coating. However, the anti-biofilm activity of the coating against the multispecies biofilms developed from environmental samples was dependent on the industry, with the coating showing anti-biofilm activity on two of them and a slight pro-biofilm activity on the third one. The growth of *Lm* seems to be partially controlled by the microbiota present in the industrial samples as the biofilm counts are reduced from mean values of 6.0±6.2 log CFU/cm^2 in the *Lm* mono-species biofilms to 3.4±3.6 log CFU/cm^2 on the artificially inoculated industrial samples. In these samples, the differences between *Lm* counts on stainless steel with and without coating are not significant, which indicates that the coating anti-biofilm activity against *Lm* might be influenced by the microbiota present in the industrial sample. The disinfection treatments were insufficient to eliminate the biofilms but no *Lm* were detected in most of the cases (<10^2 CFU/cm^2). These results continue the characterization of this anti-biofilm coating that is successfully applied by atmospheric pressure plasma-polymerization on stainless steel. They contribute to the understanding of population dynamics of mixed-species biofilms of indigenous microbiota and *Lm* under conditions that better resemble the food processing environments where the coating would be applied.

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PW-09

Wheat seed surface changes after cold plasma treatment

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1. Abstract

The nonthermal or cold plasma technology is a relatively new technology in agriculture. The research focuses on two aspects of this technology: Firstly, it could be used as a new decontamination method of seed material and fresh food products, such as berries [1]. Secondly, cold plasma technology could be used as a tool for seed priming; improving seed germination, seedling growth and potentially even improve crop yield and alleviate abiotic stress from the environment [2–4].

The study's main purpose was to detect changes on seed coat and to evaluate effects on germination rate and malondialdehyde (MDA) content in wheat seeds after cold plasma treatment. Morphological changes on the seed surface after cold plasma treatment were studied by scanning electron microscopy (SEM). The results show that the longer exposure of seeds to cold plasma treatment, seed surface becomes "smoother" compared to untreated seeds, because of the etching effect of plasma components on seed surface. The X-ray photoelectron spectroscopy (XPS) showed altered surface chemistry of seed surface already after 10s of plasma treatment; an increase in oxygen and decrease in carbon content was detected. Slight traces of potassium and sulphur were also detected in seeds after plasma treatment.

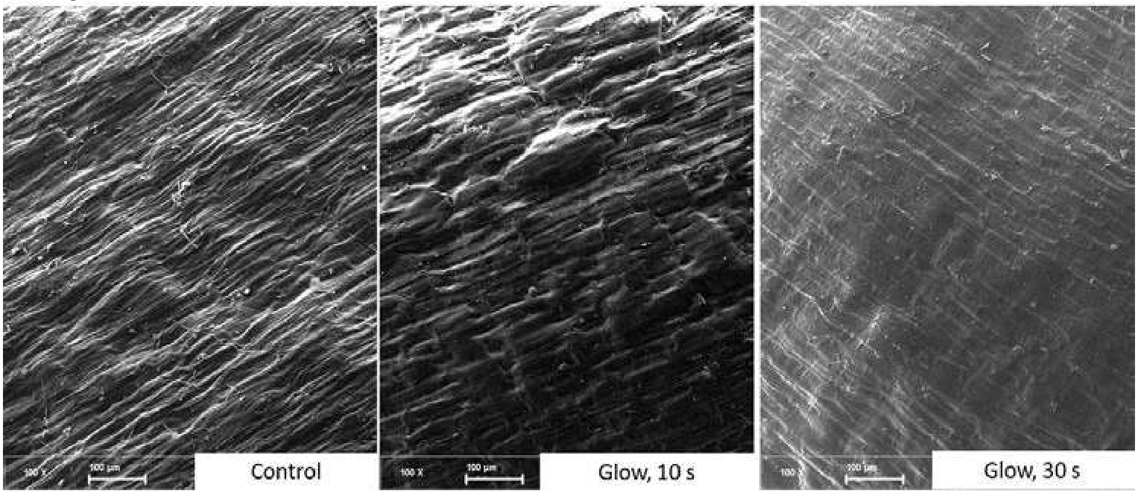

Figure 1: Seed surface of wheat seeds; from left to right: seed surface of control (untreated) seeds, seed surface after 10 s and seed surface after 30 s direct plasma treatment.

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Keywords: plasma, plant, seeds, XPS, SEM

PW-10

Effect of plasma activated water (PAW) as a fertilizer improving the outdoor plant yield: maize case

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The activation of water by cold plasma induces some chemical changes such as: formation of reactive particles derived from oxygen and nitrogen (hydrogen peroxide, nitrate/nitrite ions, peroxynitrite, etc.) [1]. Amongst these reactive particles, Hydrogen peroxide, nitrate/nitrite ions are considered as key species which provide to the PAW an interest in the agriculture field. These key species play an important role for the rapid germination of seeds and, plant growth enhancement by providing nitrogen from the PAW to the plant. Based on the PAW's properties, it could be an interesting source of nitrogen provider for the whole growth cycle (from seed to harvest) [2].

We used two types of PAWs generated by transient spark (TS) discharge with water electrospray and by glow discharge (GD) with water cathode, and Hoagland solution. After plasma treatment, each PAW was characterized by measuring long-lived RONS concentrations (H_2O_2, NO_2^-, NO_3^-) by UV/VIS absorption spectroscopy.

After the harvest, the effect of PAWs generated by the two plasma sources and Hoagland solution on maize yield were analyzed by measuring some growth parameters (plant height, product length, product weight).

As shown in Fig.1 (right), we found that the PAW could be effective for watering the plant from seed to harvest. GD 2 (PAW from glow discharge activated for 2 min) showed a similar result to Hoagland solution (HS) which is a common synthetic fertilizer (micro and macro nutriment). The results show us that the PAW effects during the plant cycle up to harvest depend on the concentration of hydrogen peroxide nitrate/nitrite ions.

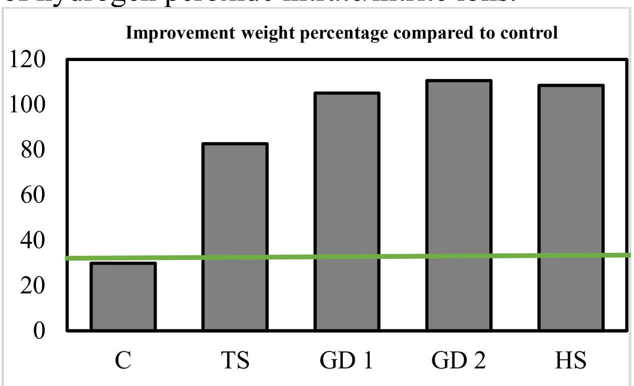

Figure 1: (left) Experiment field, and (right) improvement of the average product weight percentage compared to control (C).

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