Research Scientific Report

2017-2018

concerning the implementation of the project
PN-III-P4-ID-PCE-2016-0277, no. 15/12.07.2017

Increasing the Agricultural Production in Greenhouses using Non-Thermal Plasma Activated Water Technology for Irrigation
(AWAG)

The following is a synthesis of the scientific activity for the years 2017 and 2018. The report assesses the degree of implementation of the project in correlation with the scientific dissemination in scholarly journals, as well as the increase of the project's visibility on a national and international scale through participations at scientific conferences.

The objectives have had an experimental and research character, doubled by the intermediary dissemination of data obtained by the project team.

We have built the power supply and mini reactors and we analysed of power per reactor. We reconfigured the power supply to operate at lower power around 2 W and at frequencies, in the range 50 Hz-200 Hz.

To assess the characteristics and the efficiency of the reactor, we studied it effects on soluble organic compounds removal; therefore, a soluble organic dye (Reactive Blue 19 dye) solution has been treated in an NTP mini-reactor. The efficiency of the NTP treatment was enhanced by spraying the solution into the plasma zone through a two-way nozzle.

The organic dye removal from the solution, the hydrogen peroxide and nitrates formed in water sprayed in the plasma has been measured using colorimetric methods. In order to optimize (from electrical and physical-chemical parameters point of view) the NTP treatment of the organic compounds dissolved in water, we calculated the energy efficiency (g/kWh) of the dye destruction.

With the developed NTP Multi-Reactor Device we studied the non-thermal plasma activated water effect on the plants growing. To emphasize the changes that occur due to PAW treatment on plant development stages specific horticultural tests and biochemical analysis in the leaves were performed supplemented by numerous biometric phenological and production determinations.

Using Plasma Activated Water we studied the effect of nitrites on *Lactuca sativa* (lettuce) growth. The plasma was generated by a pulse high voltage electrical discharge that takes place between two electrodes, one attached to the nozzle, the other one being the outlet tube of the reactor.

The voltage and the current have been measured through a voltage divider and a shunt resistor, using a digital oscilloscope. The NO_3 and H_2O_2 concentration in PAW after treatment have been measured based on colorimetric method using an UV spectrophotometer.

We measured the chemical parameters of the PAW using a pH-meter and we performed colorimetric tests for NO_3 and H_2O_2 concentrations: the H_2O_2 concentration was determined based on $TiOSO_4$ method, by measuring the absorbance of the formed yellow complex and the NO_3 concentration was determined by using the Viscolor ECO (Macherey-Nagel), by measuring the absorbance.

The PAW was applied in controlled conditions on seeds/seedlings/plants subsequently to measurement of PAW parameters.

The activated water acts on the plants in two ways: first, the H_2O_2 formed due to the plasma chemical reactions induced in water acts as a disinfection agent on the plants leaves (inactivates the pathogen micro-organisms); second the nitrates/nitrites formed in water (with the nitrogen from air),through the leaves absorption process, go into the plants acting as a fertilizer (organic – no chemical containing).

We performed cytogenetic investigations of the Lactuca sativa L. genotype. For cytogenetic investigations, the PAW treated and controlled embryonic roots were collected, fixed with Carnoy solution, hydrolyzed with HCl, stained with carbol-fuchs in solution (Carr reagent).

In the experiment, we analyzed the following parameters: mitotic index (MI) calculated as the number of dividing cells as a fraction of the total observed cells; chromosome aberrations (CA) expressed in percentage of number of aberrations: bridges, fragments, bridges and fragments, retarded chromosomes, multipolar anatelophases, C-metaphases, micronuclei.

We used the mitotic index (MI) to estimate the ratio of the number of undergoing mitosis cells to the number of not undergoing mitosis cells.

The chromosome aberrations assay is a useful and sensitive test for detection of genotoxins. To assess the chromosome aberrations induced by PAW we also analyzed two parameters:

(a) the number/percentage of cells in aberrant phases;

(b) the type and the frequency of chromosomal aberrations.

In Figure 1 may be observed the chromosome aberrations (CA) induced by PAW in *Lactuca sativa* root types.

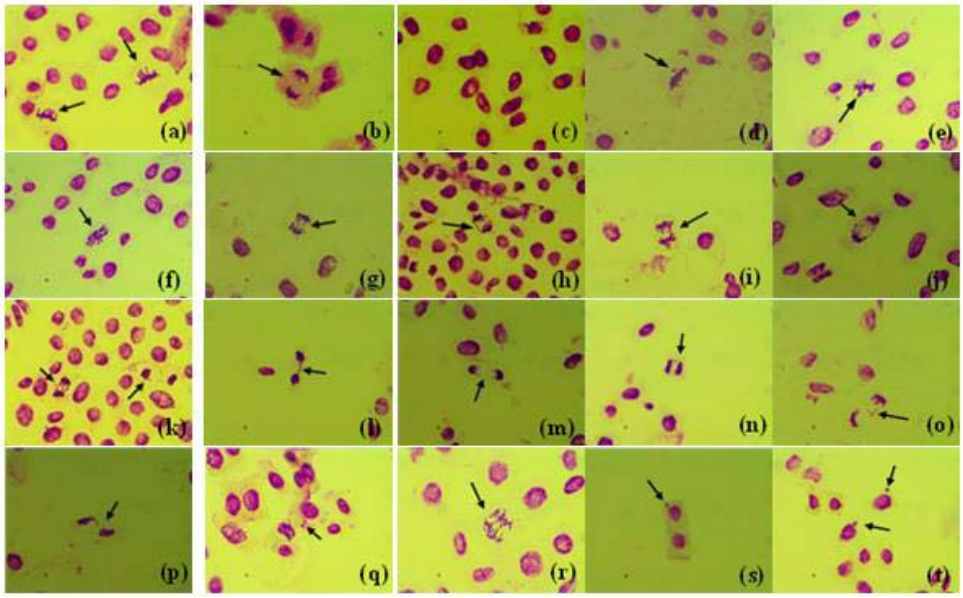

Figure 1 CA induced by PAW in *Lactuca sativa* root types (arrows): (a)-normal metaphases, (b)-normal anaphase, (c)-normal interphases, (d)-metaphase fragment, (e)-retarded chromosome in metaphase, (f)-C-metaphase, (g), (h) and (i)-anaphase whole bridges, (j)- anaphase torn bridges, (k)-anaphase whole bridges and telophase torn bridge, (l)-telophase thick bridge, (m)-telophase torn bridges, (n)-telophase fragment, (o) and (p)-anaphase fragments, (q)-early anaphase with retarded annular chromosome, (r)-multipolar anaphase (s) and (t)-micronuclei in interphase cell (1000 X).

From the cytogenetic investigations of the Lactuca sativa L. genotype it was found that NO_3 concentrations have affected to a different degree the mitotic index of the plantlets and have produced chromosomal mutations in their genome. The most common chromosomal mutations were: chromosomal bridges and micronuclei.

It was concluded that the chromosomal aberrations affect genetic information with repercussions on synthesis of biomolecules. The length growth of plantlets of lettuce show positive differences from control, statistically assured.

As the plasma activated water, has a notable effect on the living organism plants and micro-organisms (bio-disinfection), we performed a study focused on the effect of plasma activated water on the plant's germination and growing process. In this purpose, the seeds germination of lettuce was treated with plasma activated water. PAW leads to a shorter germination period increasing the physical development of plants and in the same time acting as a disinfection agent.

For the experimental bioassay, seeds of Lactuca sativa L. var. Kagraner were used as material. The experimental set-up used seeds placed in a Sanyo-MLR growth chamber. The seeds were irrigated with PAW for experimental variants and with distilled water for control tray. The germination rate has been determined, calculated as the percentage of

germinated seeds 48 h after initial exposure. The germinated seeds in each treatment were counted and reported percentage.

Two concentrations of PAW 34 ppm and 54 ppm NO_3 were applied in controlled conditions on seeds/seedlings/plants subsequently.

The results demonstrate that the length of the embryonic root, stem and leaf of the two variants (PAW 34 mg/l and PAW 54 mg/l) are statistically assured.

It was proved that the germination of lettuce seeds is not affected by PAW, on the contrary, PAW [NO_3] stimulates to some extent the germination capacity. The two concentrations of PAW [NO_3] have a genotoxic effect, demonstrated by induction of chromosomal mutations.

The growth dynamics of lettuce plantlets is not affected by PAW [NO_3], except for the stem, in which case PAW [NO_3] 54 mg/L has a slightly different atypical rhythm compared to control. From the morphological indices of mature plants treated with PAW, we have found that the cold plasma contributes to increasing plant yields in greenhouses.

Another research was performed to establish the influence of the application of non-thermal plasma treatments to a lettuce, on root area, to Shangore cultivar.

The experiment was carried out in a greenhouse, from University of Agricultural Sciences and Veterinary Medicine Iasi.

For the study, a comparative crop was organized, using the variety of the approved lettuce, respectively Shangore F1®.

Seed germination was achieved in a Sanyo MLR germinator that offered controlled conditions while the germination factors have been adjusted in optimal parameters.

The lettuce average root length plantlets treated with PAW 34 mg/l increased by 15%, while the lettuce root length treated with PAW 54 mg/l decreased with 10%. The content of chlorophyll pigments in the lettuce leaves was determined by means of a portable apparatus CCM 200, OPTI Sciences Inc. The method is non-destructive and allowed us to determine the optical absorbance of clorofolieni pigments, the results being expressed in units BCC (chlorophyll content index). Biometrics on the length, width and the foliar area were carried out using the ADC AM 350.

For the lettuce root treatment were used three concentration (NO_3) of PAW in the range (3 ÷14) mg/l resulting three samples, V1, V2 and V3.

Figure 2 presents the number of leaves on the cold plasma treated lettuce plant. The minimum value was recorded in V2, which has the average number of leaves on the plant equal to 27.4, the maximum value being recorded in V3 being of 33.8 leaves per plant compared to the witness to which a value of 29.2 leaves was registered on the plant.

The content of chlorophyll pigments is presented in the Figure 3 for lettuce treated with non-thermal plasma. The minimum value was recorded at V2, which has an average content of chlorophyll pigments of 5.73 CCI

units per leaf, the maximum value being recorded at V3, having a content of chlorophyll pigments of 7.2 CCI units, compared with the control to which the obtained a value of 6.3 CCI units. Regarding the results achieved, significant differences between versions 2 and 3 are observed.

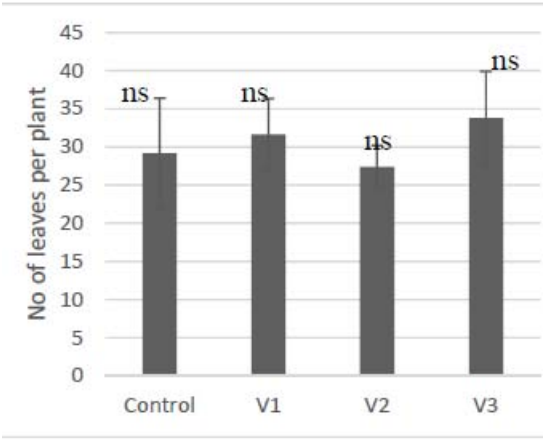

Figure 2 The number of leaves on the plant

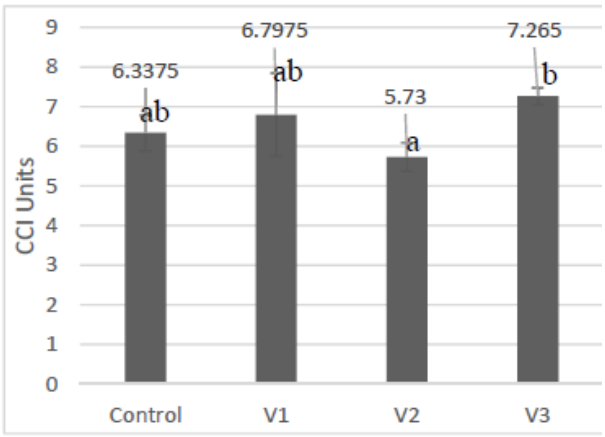

Figure 3 The content of chlorophyll pigments

The evaluation of the content of chlorophyll pigments in leaves (Figure 4) is extremely important from a physiological point of view; because the chlorophyll pigments (A and B) found in the chloroplasts of the leaves have the role of converting the energy light into energy through the photosynthesis process.

Moreover, the amount of luminous energy taken by the leaf is determined by the content of chlorophyll pigments, which directly affect both the process of photosynthesis and primary production. Determination of chlorophyll pigments content indirectly provides information about mineral nutrition because chlorophyll stores a significant amount of nitrogen. The control and V1 are close to the value, ranging from V2 to V3.

The minimum foliar surface value was obtained in control, which has an average total area of 54.8 cm^2, the maximum value falling at V1, with an average total area of 60.2 cm^2. Regarding the results achieved, no significant differences are observed between the three variations treated with cold plasma and the untreated version.

Figure 4 Determination of the lettuce content of chlorophyll pigments

Increasing the international visibility

The project's website was built and continuously updated. All the members of the project have participated to five major scientific events (three abroad and two in Romania). Two members of the project ware elected chairman of a session at EPE International Conference (Prof.PhD.eng. Radu Burlică and Prof.PhD.eng. Maricel Adam).