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POSITIVE EFFECT OF NON-THERMAL PLASMA TREATMENT ON RADISH SEEDS*

A.L. MIHAI[1], D. DOBRIN[2], M. MĂGUREANU[2*], M. E. POPA[1]

[1] University of Agronomic Science and Veterinary Medicine, 59 Marasti Blvd, Bucharest 011464, Romania,
E-mail: mihai_laura21@yahoo.com; pandry2002@yahoo.com
[2] National Institute for Lasers, Plasma and Radiation Physics, Str. Atomistilor, Nr. 409, Magurele, Bucharest, Romania,
E-mail: monimag@infim.ro; daniela.piroi@infim.ro

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Abstract. The effect of cold atmospheric plasma on radish seeds has been investigated. It has been shown that plasma treatment had little effect on the germination rate, but influenced the early growth of seeds. Sprouts and roots of plasma treated seeds were longer and heavier than those of control seeds. The best results were obtained for 20 minutes treatment time, where an increase of the length of roots and sprouts with 10-11% and a 30% enhancement of roots weight were determined.

Key words: non-thermal plasma, radish seeds, surface discharge, seed germination, *Raphanus Sativus*.

1. INTRODUCTION

Recently, non-thermal plasma has been investigated as an alternative to traditional pre-sowing seed treatment in agriculture, such as physical scratching (scarification), heat treatment, chemical treatment with various acids, etc. [1]. Promising results have been reported with respect to increasing seed germination rate and activity and reduction in germination time [1-3], increasing wettability and, implicitly, water absorption [4], and for decontamination of grains and vegetables seeds [5, 6].

Most studies were carried out using plasma produced at low pressure, mainly radio frequency [1, 4, 7, 8, 10] and microwave discharges [2, 11, 12], however, some investigations with atmospheric pressure plasma, such as corona [3] and dielectric barrier discharge [9] have been reported as well.

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Several authors obtained increased germination rate of the plasma treated seeds as compared to control (untreated) seeds, for certain optimized plasma exposure periods [1, 7, 11]. Dhayal *et al.* [1] reported 50% enhancement of germination rate of safflower (*Carthamus tinctorium L.*) seeds exposed to low pressure RF argon plasma for a very long treatment time (130 min). In contrast, shorter treatment (30 min) under the same experimental conditions slightly inhibited seeds germination, but further reducing the pressure improved the results considerably. A more spectacular result was reported by Sera *et al.* [11]: they achieved three times higher germination rate of Lamb's Quarters (*Chenopodium album*) seeds after stimulation in a microwave discharge. However, in other studies no effect of plasma treatment on the germination rate of oat exposed to microwave afterglow [2] and radish seeds treated by RF oxygen discharge [8] was noticed. This wide variation of results among different research groups may be due to diverse plasma treatment conditions, but may also be an indication of the different response of various seed types to plasma exposure.

It was found that the early growth of plants may also be affected by plasma treatment of seeds [1, 8, 9, 11]. A considerable effect of the treatment duration was observed in some studies [7, 9, 11]: too short time may be ineffective, while too long plasma exposure may even inhibit plant growth [7, 9]. In contrast, other authors did not detect any significant influence of this parameter on plant length [8]. Kitazaki *et al.* reported 30-60% increase in plant length for radish seeds exposed to oxygen RF plasma as compared to untreated seeds, but a much smaller difference in weight [8]. Even better results were obtained in [1], where the roots of safflower seeds treated by argon RF plasma were two times longer than the control ones.

The objective of this paper is to evaluate the behavior of radish seeds treated with non-thermal plasma generated in a surface discharge. The influence of non-thermal plasma on the germination rate and early growth of radish seeds was investigated.

2. MATERIALS AND METHODS

2.1. PLASMA REACTOR AND ELECTRICAL CIRCUIT

The seeds were exposed to non-thermal plasma generated in a surface discharge (Fig. 1) at atmospheric pressure and room temperature.

The high voltage electrode consists in an array of 13 copper wires, with wire diameter of 100 μm, length of 4.4 cm and distance between adjacent wires of 6 mm. The ground electrode was an aluminum tape. The electrodes were placed on both sides of a glass plate of 1.5 mm. The seeds to be treated were distributed uniformly on the wires. The plasma reactor was enclosed in a rectangular case under air flow (flow rate 1 L/min).

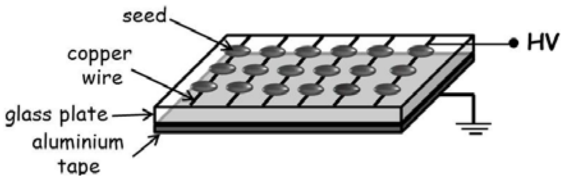

Fig. 1 – Discharge reactor.

The discharge was generated in a.c. mode using a high voltage transformer which provides sinusoidal voltage at 50 Hz frequency. The present experiments were carried out at constant amplitude of the voltage of 15 kV. The discharge voltage was measured by a high voltage probe (Tektronix P6015A, 1000×, R_p = 100 MΩ). The current was measured with a shunt resistor connected in series with the ground electrode. The total charge dissipated in the discharge was measured with a non-inductive capacitor (C = 1.5 nF), placed instead of the shunt resistor. The discharge characteristics were monitored by a digital oscilloscope (Tektronix DPO2024). The average electrical power dissipated in the discharge was calculated by the Lissajous method [13]. The area inside the Lissajous figure (charge-voltage plot) is equal to the energy deposited in the discharge in one voltage cycle, therefore the average power dissipated in the discharge was calculated by multiplying this area with the frequency of the applied voltage.

2.2. SEED MATERIAL

Radish seeds (*Raphanus sativus*, variety Icicle) were tested in the present study. These seeds are suited for open field crops. Seeds without visible defects were selected. Four seed lots were prepared, each containing 400 seeds. From these seeds 3 lots were exposed to atmospheric pressure plasma, and the seed samples without treatment served as the control. The seeds were distributed uniformly on the wires and exposed to plasma for durations of 5, 10 and 20 minutes.

Seeds were transferred to Petri dishes containing one layer of filter-paper impregnated with 4 ml distilled water. Each Petri dish contained 10 seeds. Seeds germination was carried out in an incubator at 22 ^{o}C in the dark. The seeds were monitored for duration of 12 days. In the 12th day the germination rate, the length and mass of roots and sprouts were measured. Subsequently, roots and sprouts were dried until a constant mass was reached, for weight determination.

The following parameters were measured: germination rate, average length of roots and sprouts, total dry weight of roots and sprouts and root-to-shoot mass ratio. The germination rate was defined as the number of germinated seeds divided by the total number of seeds. The average length of roots / sprouts was defined as the sum of lengths of all roots / sprouts (measured by a sling caliper, precision 0.1 mm) divided by the number of germinated seeds. The weight of roots and sprouts was measured by an analytical balance, with precision of 0.1 mg. The root-to-shoot mass ratio was defined as the ratio between the dry weight of roots and the dry weight of sprouts.

3. RESULTS AND DISCUSSIONS

3.1. ELECTRICAL CHARACTERISTICS OF THE DISCHARGE

The discharge is filamentary, consisting in numerous plasma filaments which start from the wires and spread on the glass surface. Voltage and current waveforms are illustrated in Fig. 2.

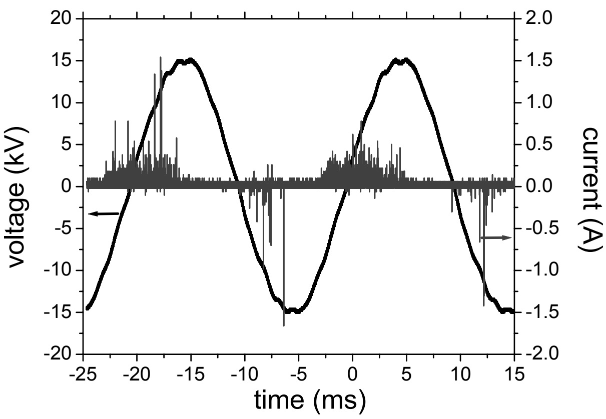

Fig. 2 – Typical waveforms of the discharge voltage and discharge current.

The current and voltage waveforms are typical for a filamentary a.c. discharge: numerous current peaks with amplitudes of tens to hundreds milliamperes, even higher, appear on the voltage rise, on both alternances of the voltage waveform.

The average power dissipated in the discharge, calculated from the Lissajous figure, was 2.7 W for the applied voltage used in the present experiments (15 kV amplitude).

3.2. EVALUATION OF THE PLASMA EFFECT ON SEED GERMINATION AND EARLY GROWTH

Under the experimental conditions used in this study, plasma treatment did not influence the germination rate of radish seeds, which was 77–78% both for the control seeds and for those exposed to the discharge. No effect of plasma on the germination dynamics was observed either.

It was found that the roots and sprouts of plasma treated seeds were longer than those of control seeds, for all treatment durations used in this work. The most substantial increase in length was obtained for the seeds treated for 20 min, therefore the following figures will illustrate the comparison between these seeds and the control ones.

Figure 3 shows the seeds distribution as a function of root length for control seeds and for 20 minutes plasma treated radish seeds. The data presented in the graph were fitted with a Gaussian distribution.

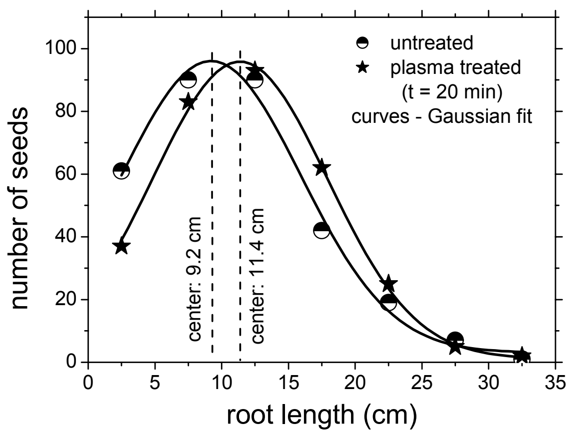

Fig. 3 – Distribution of seeds as a function of root length for untreated and 20 minutes plasma treated radish seeds.

For the plasma treated seeds the distribution is shifted towards longer roots as compared to the control sample. The distribution of untreated seeds was centered at 9.2 cm, while the treated seeds profile was centered at 11.4 cm. The standard deviation for these data was approximately 6.5 cm.

Figure 4 illustrates the seed distribution as a function of sprout length for untreated and for 20 minutes plasma treated radish seeds. Again the distribution is slightly shifted toward longer sprouts for plasma treated seeds as compared to the untreated ones. The center of the distribution of control seeds was 1.30 cm, while the plasma treated seeds profile was centered at 1.49 cm. For the sprout length the standard deviation was approximately 0.43 cm.

The main parameters characterizing the early growth of seeds (mean length of roots and sprouts, weight of roots and sprout and root-to-shoot mass ratio), measured in the twelfth day after plasma treatment, are summarized in Table 1.

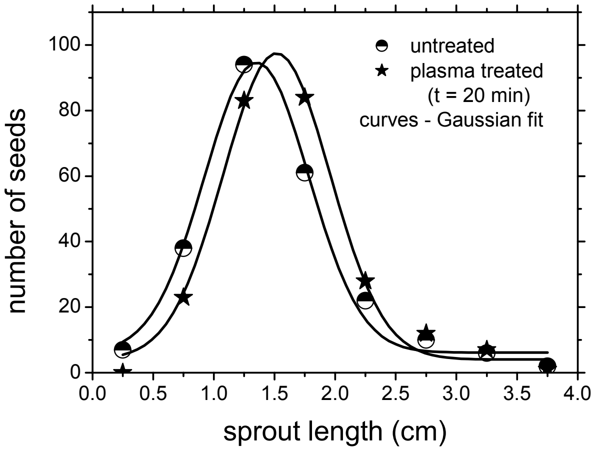

Fig. 4 – Distribution of seeds as a function of sprout length for untreated and 20 minutes plasma treated radish seeds.

Table 1

Growth parameters of radish seeds (untreated and plasma treated) in the twelfth day after plasma treatment

Parameter	Control seeds	Plasma treated seeds (20 min)
mean root length (cm)	10.66	11.83
mean sprout length (cm)	1.45	1.60
roots weight – dry (mg)	330	430
sprouts weight – dry (mg)	960	1100
root-to-shoot mass ratio	0.34	0.39

Plasma treatment of radish seeds led to an increase of root and sprout length with 11% and 10%, respectively. A more important increase of root weight (30%) was observed for the seeds exposed to plasma as compared to the untreated ones, while the sprout weight was enhanced with almost 15%. The root-to-shoot mass ratio was also 15% higher for the treated seeds.

The mechanism responsible for plasma stimulation of seed germination and plant early growth has not been entirely clarified up to now. Plasma may affect the seeds in various ways: by modification of seed coat, by reactions with electrons, ions and radicals generated in the discharge, by UV radiation emitted from the plasma etc. Different treatment parameters, such as plasma properties, power levels, working gas, treatment time, as well as the type of seeds, obviously influence the response of seeds to plasma treatment. This can explain the wide variation of results obtained by different research groups.

The antifungal action of plasma may be to a certain extent related to the increase in germination rate of plasma treated seeds. Indeed, several authors found that plasma treatment was effective for the decontamination of seeds infected with various fungi, such as *Aspergillus* [5, 6], *Penicillum* [5], *Fusarium*, *Ascochyta* [7].

Several authors observed modifications of the seed coat following exposure to plasma. Seeds of *Chenopodium album* treated by microwave plasma presented an eroded surface [11]. Dhayal *et al.* [1] also detected changes of the seed coat of *Carthamus tinctorium* seeds: both the outer surface of the seed and the Hilum (the part from where the primary root appears) are altered under the action of a RF discharge. It was suggested that plasma-induced modification of seed surface may increase transmission of oxygen and water through the seed coat [1, 11]. Indeed, Bormashenko *et al.* [4] found that wettability of plasma treated seeds was considerably higher than for untreated seeds. Enhanced hydrophilicity resulted in increased water uptake for plasma treated seeds, which was evidenced for lentils, beans and wheat treated by RF discharge in air. However, the authors did not relate this behavior to seed coat erosion (which was not observed for the short treatment times used in this work), but suggested a relationship between the improved wettability of treated seeds and the oxidation of seed surface, based on detection of oxygen-containing groups at the surface [4]. In contrast, Kitazaki *et al.* [8] did not observe any oxide formation on the surface of radish seeds exposed to RF discharge in oxygen. They also excluded etching of seed coat as the cause for improved growth, since no surface modification was noticed as a result of plasma treatment. By screening the seeds from the action of ions and radiation emitted from the plasma the authors obtained similar results as for the direct treatment. Therefore, they proposed a growth enhancement mechanism of plasma treated seeds based only on oxygen radicals generated in the discharge [8]. A similar idea, of penetration of reactive oxidant species formed in the plasma through the seed coat into the seed, is suggested also by Sera *et al.* [2]. They examined extracts from treated and control seeds and evidenced different content of some phenolic compounds, so they concluded that metabolic processes of the plants grown from plasma treated seeds were changed [2]. Obviously, further research is needed to elucidate the mechanism of plasma interaction with seed cells.

4. SUMMARY

The present investigation shown that although plasma treatment did not influence the germination rate and dynamics of radish seeds, it had a noticeable effect on plants early growth. It was found that the roots and sprouts of plasma treated seeds were longer and heavier than those of control seeds, for all treatment durations used in this work. Better results were observed for longer treatment time (20 min). Under these conditions, a 10–11% increase of the length of roots and sprouts and a 30% enhancement of roots weight were determined.

Therefore, this work confirms that non-thermal plasma treatment of seeds may have a positive effect on plant early growth. However, more research is needed in order to understand the mechanism of plasma interaction with the cells of the seed.

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