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Impact of Conditions of Water Supply on the Germination of Tomato and Pepper Seeds

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Abstract

The influence of the cold radiofrequency air plasma treatment on the imbibition and germination of tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) seeds, exerted to conditions appearing in natural seedbeds, was investigated. Various conditions of water supply (from nonrestricted water supply to water drought stress) to the germinating seeds were studied. Plasma treatment markedly increased the water imbibition in the case of tomato seeds under limited water availability conditions. Cold radiofrequency plasma treatment had no noticeable impact on the germination of tomato and pepper seeds under the conditions of nonrestricted water supply. In the case of drought stress for both studied cultivars, the cold plasma treatment essentially influenced the germination rate and the kinetics of germination (the median of the Richards' curve was changed essentially under conditions of water drought).

Keywords: plasma treatment, tomato and pepper seeds, drought stress, water imbibition, germination

1. Introduction

Interaction of cold plasmas with plant seeds has drawn the evident attention of scientists in the last decade [1–8]. The investigators mainly concentrated on the possibility to control the germination by exposure of seeds to various types of plasma, including atmospheric and low-pressure plasma discharges. It should be stressed that experimental data related to the influence of plasma treatment (carried out with capacitive and inductive discharges) on the germination time and percentage are contradictory. The first profound investigation of the impact of plasma discharges on seed germination was reported by Volin et al. [1]. These authors exposed seeds

of radishes (*Raphanus sativus*) and two pea cultivars (*Pisum sativum* cv. "Little Marvel" and *P. sativum* cv. "Alaska") to CF₄ and octadecafluorodecalin plasma and observed an essential delay in germination [1]. Since then, investigators focused on the following main fields: (1) decontamination of seeds by plasma, (2) breaking of dormancy with plasma, (3) the impact of plasma treatment (PT) on the rate and kinetics of germination, and (4) impact of PT on the root generation (sprouting).

Decontamination and inactivation of pathogenic microorganisms of seeds by PT have been communicated recently by various groups [2, 3, 5, 6]. Nishioka et al. reported the effectiveness of low-pressure plasma treatment in the inactivation of the seed-borne plant pathogenic bacteria [6]. Researchers reported the impact of PT on germination, sprouting, and dormancy breaking of seeds. Sera et al. investigated the influence of PT on wheat and oat germination. The authors reported that PT did not affect germination of oat seeds, but they did note accelerated root generation in plants grown from plasma-treated seeds [7]. The same group also communicated that PT did change seed germination in Lamb's Quarters seeds [8]. Similar results were reported for wheat seeds (*Triticum aestivum*) by Dobrin et al. [9].

In contrast, Ji et al. communicated the significant improvement of the germination rate of *Coriander sativum* under nonthermal atmospheric pressure treatment [10]. Dhayal et al. showed about 50% increase in the germination rate, and the activity was increased twice after plasma treatment of safflower [11]. The essential augmentation of the germination rate by low-temperature plasma treatment was registered by Stolárik et al. for pea (*Pisum sativum* L. var. Prophet). Stolárik et al. related the observed effect to the chemical modification of the pea surface by plasma [12]. These results support the observations of the modification of the physical structure of seed coat by the low-pressure argon gas discharge reported by Dhayal et al. [11].

A stimulating effect of cold plasma on both the germination and sprouting of tomato seeds (*Lycopersicon esculentum* L. Mill. cv. "Zhongshu No. 6") has been reported [13]. Similar results were reported for *Pauwlonia tomentosa* seeds [14]. Kitazaki et al. studied growth enhancement of radish sprouts (*Raphanus sativus* L.) induced by low-pressure O₂ radio-frequency plasma irradiation [15]. Dobrin et al. reported that the roots and sprouts of plasma-treated wheat seeds (*T. aestivum*) were longer and heavier than those of the non-treated seeds [9]. The improvement of the germination rate of the seeds of legumes and grain crops (*Lupinus angustifolius* (blue lupine), *Galega virginiana* (catgut), and *Melilotus albus* (honey clover and soy)) by low-pressure (5.28 MHz) plasma was reported by Filatova et al. [16].

The experimental results revealed that oxygen-related radicals strongly enhance growth, whereas ions and photons do not [15]. The positive effect of cold helium plasma treatment on seed germination, growth, and yield was reported recently for wheat [17]. Treatment of spinach seeds by magnetized arc plasma increased the germination rate by 137% [18]. It has been demonstrated that cold atmospheric plasma treatment had little effect on the final germination percentage of radish seeds, but it influenced their early growth [19]. The contradictory data related to the impact of plasmas on the seed germination were summarized in the recent review

by Randeniya and de Groot [20]. Ji et al. suggested that plasma can enhance seed germination by triggering biochemical processes in seeds [4].

Basically, the investigators admit that cold plasma treatment is an efficient and “green,” non-waste method to improve seed germination and crop yield. It plays essential roles in a broad spectrum of biological processes in plants, including reducing the bacterial bearing rate of seeds, modification of the seed coat chemical composition, hydrophilization of seed coats, homogenization of the kinetics of germination, and influence on the seedling growth [16, 21].

Our group recently reported the impact of cold air plasma on the surface properties of lentils (*Lens culinaris*) and beans (*Phaseolus vulgaris*) [22, 23]. We established that cold plasma treatment leads to essential hydrophilization of the cotyledon and tissues constituting the testa when they were separately exposed to the plasma discharge. Contrastingly, when the entire bean is exposed to plasma treatment, only the external surface of the bean has been hydrophilized by the cold plasma [23]. The pronounced hydrophilization of seeds by plasmas was reported also by other groups [9, 21]. Actually, the effect of hydrophilization of natural organic surfaces by plasma treatment has been already extensively studied [24, 25].

The cold plasma treatment, which inspired change in wettability, is followed by a consequent change in the water imbibition of the seeds [9, 22, 23]. At the same time, the relation between the change in the wettability, induced by the plasma treatment of seeds, and the parameters of germination (time and rate) remains obscure. In our paper we address the problem of the impact of the cold radiofrequency air plasma treatment, performed with the inductive plasma discharge [24], on the germination of seeds exerted to various conditions of water availability. The main goal of the research is the establishment of the influence of cold plasma treatment on the germination rate and kinetics under the conditions of limited water supply conditions.

2. Methods and procedures

2.1. Plasma device

Plasma treatment (PT) was carried out with the plasma unit EQ-PDC-326 manufactured by MTI Co., USA. The scheme of the experimental unit used for plasma treatment of the seeds is depicted in **Figure 1**. The unit generates the inductive plasma discharge [26].

2.2. Seed materials and plasma gases

Seeds of tomato (Efrat-70, *Solanum lycopersicum*) and pepper (Roni-272, *Capsicum annuum*) were supplied by Hazera Co. (Israel).

Dry air (99.999%) was supplied by Mifalei Hamzan Co. (Israel).

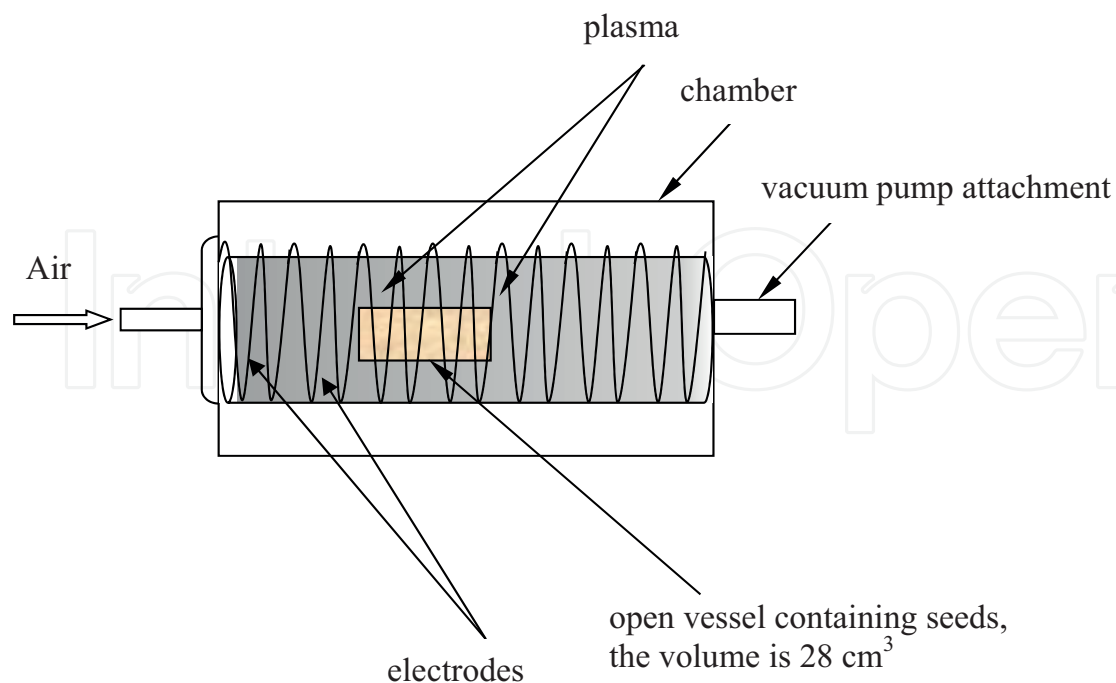


Figure 1. Plasma unit used for treatment of seeds.

2.3. Treatment conditions

Healthy uniform seeds without visible damages were selected and exposed to the inductive plasma discharge under the following parameters: the plasma frequency was 13.56 MHz (the RF matching is automatic), the pressure was 0.5 Torr, the supplied power of plasma discharge was 18 W, and the volume of the discharge chamber was 45 cm³.

The parameters of plasma for the used pressure of 0.5 Torr were temperature of electrons (11.76 ± 0.51 eV) and concentration of ions $4.97 \pm 1.16 \times 10^{15} \text{m}^{-3}$ (as established with the double Langmuir probe (AIP150, Impedans Ltd. Plasma Measurement, Ireland). The time span of irradiation was varied from 30 to 60 s. During the plasma treatment, the temperature in the discharge chamber was ambient. The seedling measurements and content analyses mentioned in detail below were carried out immediately after the PT of seeds.

2.4. Modeling of water availability conditions

Water supply to seeds was controlled as follows: the seeds were germinated in 90-mm-diameter Petri dishes. Seedbed was composed in various series of experiments of three filter paper layers (well-watered (WW)), which means nonrestricted water supply); four filter paper layers (medium drought stress (MDS), below in the text); or five filter paper layers (harsh drought stress (HDS), below in the text) of the filter paper (500-H, the thickness was 112 μm , and the maximal pore size was 48 μm).

The stacks were moistened with 4 ml of distilled water (dH₂O) dripped on the stacks by a syringe (see **Figure 2**). The specific conductivity of distilled water was 18M Ω /cm.

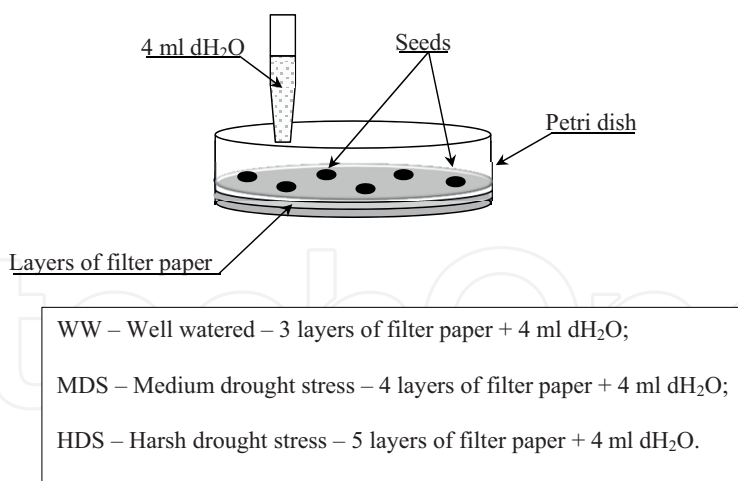


Figure 2. Experimental setup for treatment of seeds.

2.5. Study of the influence of water availability on imbibition

For the study of the time dependence of seed water absorption (imbibition) by irradiated and nonirradiated tomato and pepper seeds, 100 seeds were placed on humid filter paper stacks in different water conditions (for details see Section 2.4). Seeds were weighed at 1, 3, 6, 9, and 24 hours after plasma treatment with a MRC ASB-220-C2 analytical balance. The relative water imbibition (absorption) was defined as $\frac{\Delta m(t)}{m_0} 100\% = \frac{m(t) - m_0}{m_0} 100\%$, where m_0 is the total initial mass of seeds and $m(t)$ is the running total mass of seeds [23]. The comparative study of imbibition was carried out for non-treated pepper/tomato seeds and seeds treated by plasma during 30 and 60s. The experiment was planned with three replications for each treatment.

2.6. Study of influence of water drought and temperature on germination

For the study of influence of water availability and germination chamber temperature on germination by irradiated and nonirradiated tomato and pepper seeds, 15 pepper/tomato seeds were placed on the upper layer of the filter paper stack and germinated in different water conditions (for details see Section 2.4). The Petri dishes were covered with lids and sealed using a strip of Parafilm in order to prevent water evaporation. Seeds were placed in an incubator at 21 or at 27°C. The incubation cycle included 12 hours of darkness and 12 hours of light per day. The seeds were considered to be germinated when the radicals were half the seed length. The constant conditions of germination were provided by growth chamber model PGI-500H (Illumination 40 W × 5 tubes) (MRC, Israel). The germination percentage was recorded every 24 hours for 12 days in a case of pepper seeds and every 24 hours for 10 days in a case of tomato seeds. The experiment was planned as a completely randomized design with five replications.

The comparative study of germination was carried out for non-treated pepper/tomato seeds and seeds treated by plasma during 30 and 60s.

Germination rate (%) was defined as the number of seeds germinated in 10 or 12 days related to the total number of seeds.

3. Results

3.1. Seed water imbibition

The previous findings suggest acceleration in the water uptake of PT tomato seeds, under MDS and HDS water conditions compared to non-treated seeds [22, 23]. It is distinctly seen that the fastest water imbibition was observed for 30s plasma-treated seeds (see **Figure 3**). In the case of pepper seeds, the rate of water imbibition is almost the same in plasma-treated and non-treated samples (see **Figure 4**). Under free water supply conditions (WW), the imbibition of PT seeds and non-treated seeds is almost the same for both cultivars.

In addition, it is distinctly seen that the fastest water imbibition was observed under free water supply conditions (WW), and the slowest water absorption was registered under HDS conditions for both cultivars in non-treated and plasma-treated seeds (see **Figures 3 and 4**). The water availability is an important factor influencing the rate of water imbibition.

3.2. Seed germination

Positive influence of the cold plasma treatment on the germination rate and on the kinetics of germination was recorded for the pepper and tomato seeds under the conditions of medium and harsh drought stress (see **Figures 5 and 6; Table 1**) at temperature of 21°C. The change in the germination rate (denoted by V_i (viability) in **Table 1**) was much more pronounced for tomato seeds when compared to the pepper ones. Under MDS and HDS water conditions, the germination rate of non-treated seeds was significantly reduced by 8 and 11%, respectively, for tomato seeds and by 1 and 13% for pepper seeds, compared to the well-watered

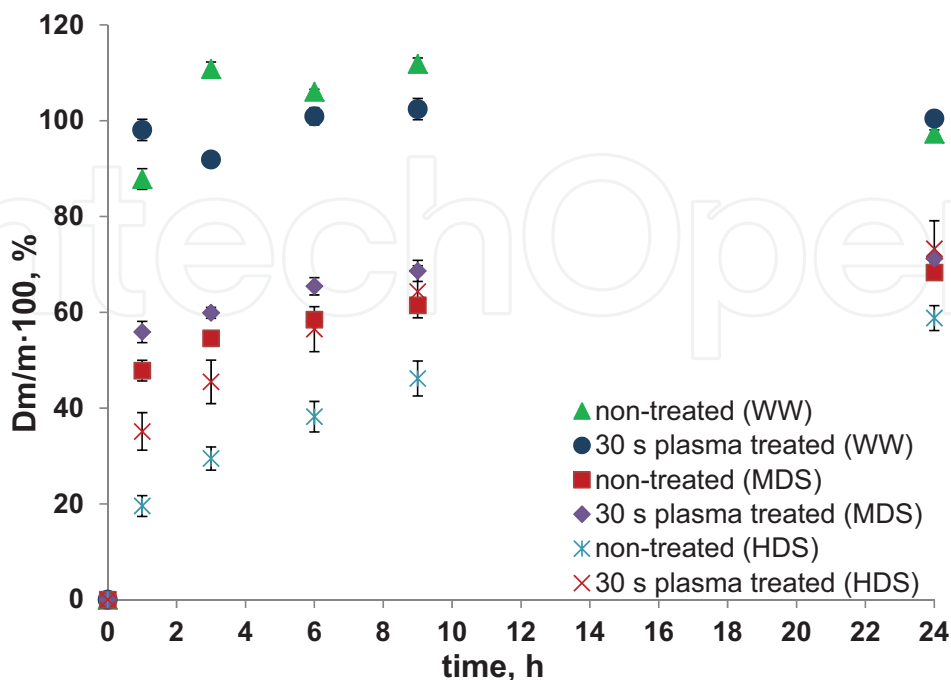


Figure 3. Comparative study of water imbibition by non-treated and 30s plasma-treated tomato seeds under WW, MDS, and HDS conditions.

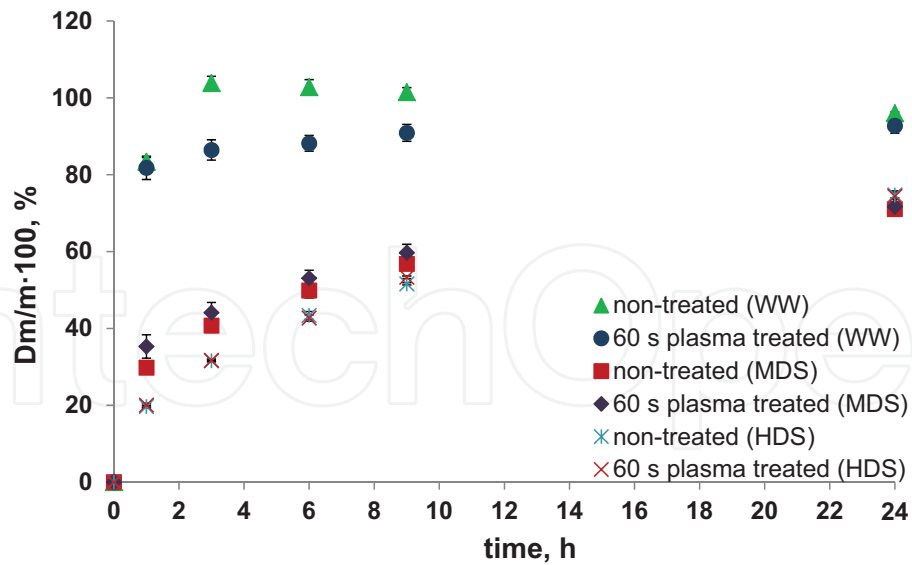


Figure 4. Comparative study of water imbibition by non-treated and 60s plasma-treated pepper seeds under WW, MDS, and HDS conditions.

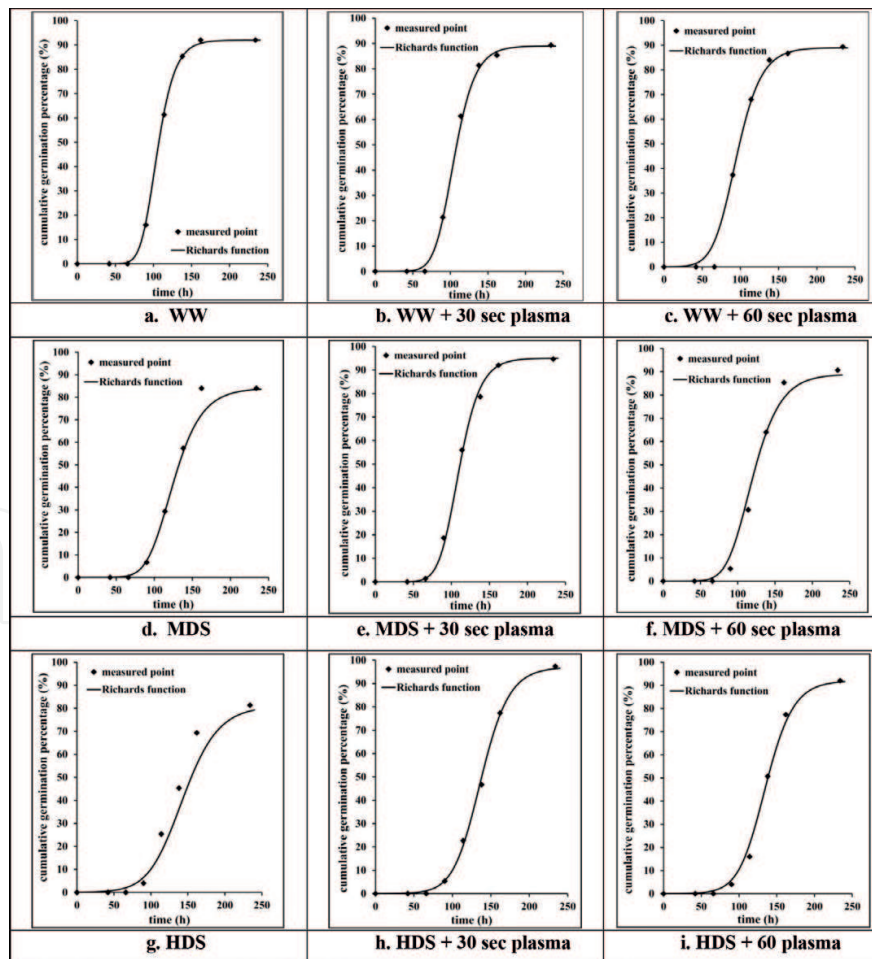


Figure 5. Germination curves calculated using Richards' fitting function [25] for tomato seeds (at 21°C). (a) WW, (b) WW + 30 s plasma, (c) WW + 60 s plasma, (d) MDS, (e) MDS + 30 s plasma, (f) MDS + 60 s plasma, (g) HDS, (h) HDS + 30 s plasma, and (i) HDS + 60 s plasma.

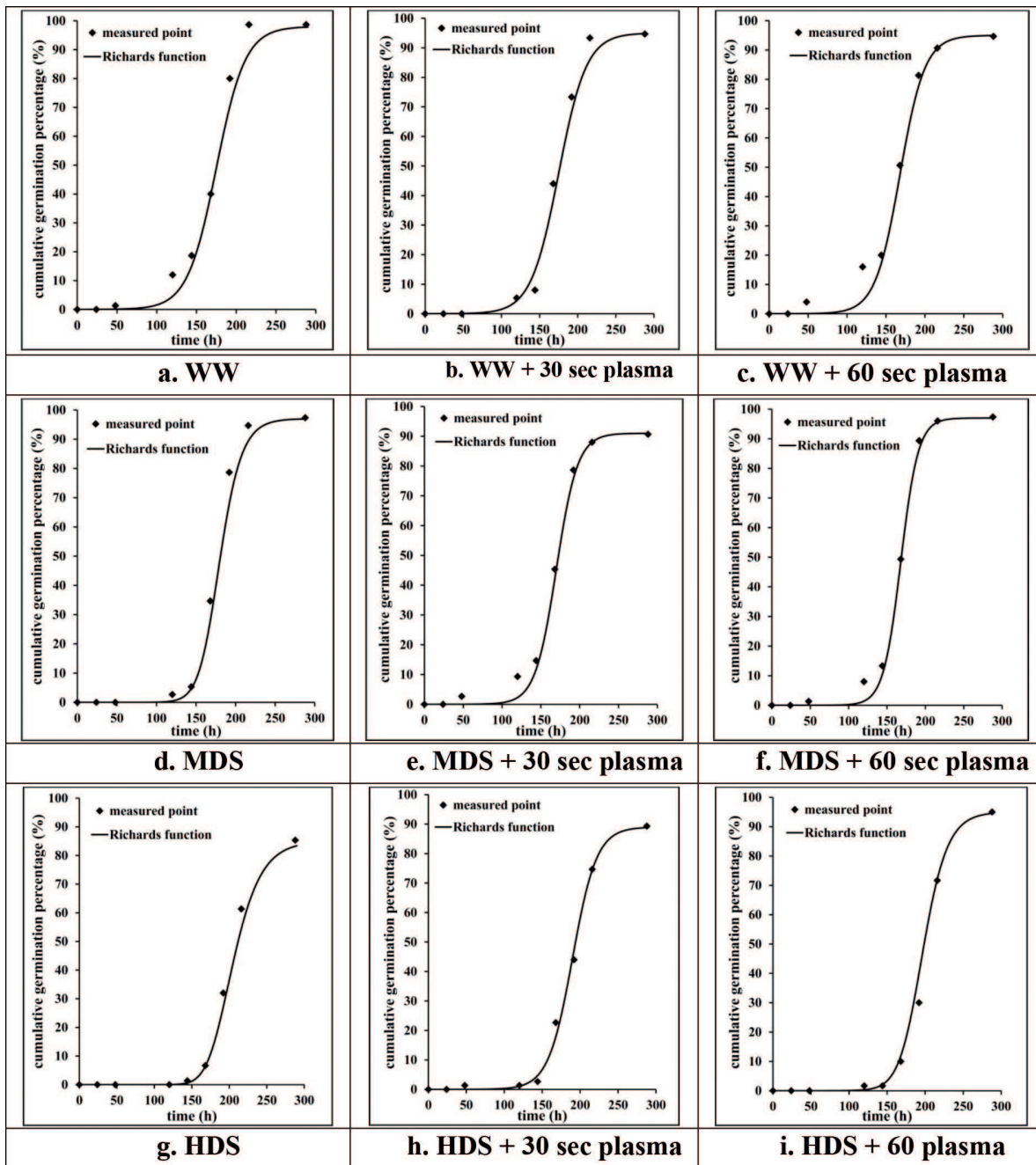


Figure 6. Germination curves calculated using Richards' fitting function [25] for pepper seeds (at 21°C). (a) WW, (b) WW + 30 s plasma, (c) WW + 60 s plasma, (d) MDS, (e) MDS + 30 s plasma, (f) MDS + 60 s plasma, (g) HDS, (h) HDS + 30 s plasma, and (i) HDS + 60 s plasma.

seeds (see **Table 1**). PT significantly increased the germination rate (V_i) of tomato seeds. It increased by 11% for 30 s PT and by 7% for 60 s compared to the medium drought-stressed non-treated seeds, and it increased by 16% for 30 s PT and 11% for 60 s PT compared to the harsh drought-stressed non-treated seeds.

For pepper seeds in HDS, the cold plasma treatment increased the germination rate by 4% for 30 s PT and 10% for 60 s PT seeds when compared to harsh drought-stressed non-treated seeds. However, in the case of medium drought stress, the final percentage of germination rate was almost the same in 60 s plasma-treated and non-treated samples (see **Table 1**).

Cultivar	Treatment	<i>Vi</i> (%)	<i>Me</i> (h)	<i>Qu</i> (h)	<i>Sk</i> (%)
Tomato	WW	92±3a	105±5	12.5	0.3
	WW + 30 s PT	89±3a	105±2	15	0.23
	WW + 60 s PT	89±3a	100±2	17.5	0.2
	MDS	84±4b	125±6	20	0.3
	MDS + 30 s PT	95±2	110±4	14.5	0.23
	MDS + 60 s PT	91±2ab	115±5	20	0.3
	HDS	81±3b	145±5	26	0.13
	HDS + 30 s PT	97±2a	140±5	20	0.17
	HDS + 60 s PT	92±1a	135±5	20	0.11
Pepper	WW	98±1a	175±5	20	0
	WW + 30 s PT	95±2a	175±5	20	0
	WW + 60 s PT	95±2a	168±4	17.5	0
	MDS	97±2a	180±5	15	0.15
	MDS + 30 s PT	91±2a	170±5	15	0
	MDS + 60 s PT	97±2a	168±4	12.5	0
	HDS	85±3b	205±5	20	0.3
	HDS + 30 s PT	89±2ab	190±5	17.5	0
	HDS + 60 s PT	95±2a	197±10	17.5	0.12

Note: WW, well-watered; WW + plasma, well-watered + plasma; MDS, medium drought stress; MDS + plasma, medium drought stress + plasma; HDS, harsh drought stress; HDS + plasma, harsh drought stress + plasma

Vi is the viability, *Me* is the time, *Qu* is the dispersion, and *Sk* is the skewness, details in text.

The values of each experiment (bold line in the table) were significantly marked by different statistical letters at $P \leq 0.05$ according to Student's t-test.

Table 1. Effect of water availability conditions, temperature (21°C), and cold plasma treatment on seed germination in tomato and pepper seeds.

Under free water supply conditions (WW), the germination percentage of PT seeds is slightly and insignificantly decreased in both cultivars.

The positive influence of plasma treatment on germination of tomato seeds at 27°C temperature was also shown (see **Table 2**). The cold plasma treatment significantly increased germination rate (*Vi*) under MDS conditions by 8% for 30 s PT and by 12% for 60 s PT compared to the MDS non-treated seeds. In HDS conditions, germination increased by 6% for 30 s PT and by 11% for 60 s PT when compared to the HDS non-treated seeds. In the case of pepper seeds, the 27°C temperature significantly decreased the germination percentage in all water regimes (see **Table 2**). Interestingly, at 27°C, while in WW conditions, germination rates of non-PT pepper seeds were much higher than the rates of treated seeds, when grown under MDS and HDS, the rates of germination of PT seeds were higher than those of WW-grown pepper seeds.

In order to elucidate the data describing the kinetics of germination, Richards' curves were fitted to a number of experiments [23, 27, 28]. Fitting experimental data by Richards' curves is shown in **Figures 5** and **6**. The solution of Richards' differential equation worked out for the growth of modeling results in Richards' curve, which is an extension of the logistic or sigmoid

functions, which are the S-shaped curves describing the kinetics of germination. The Richards' function Y_t demonstrating a variable inflection point was calculated according to Eq. (1):

$$Y_t = \frac{a}{[1 + b \cdot d \cdot \exp(-c \cdot t)]^{\frac{1}{d}}} \quad (1)$$

where Y_t is the germination percentage; a , b , c and d are the fitting parameters; and t is the time.

Fitting of experimental data by Eq. (1) supplied the best values of the fitting parameters summarized in **Table 1**, in which Me (median) denotes the time of 50% germination and characterizes the rate of this process. The quartile deviation of germination time Qu describes the deviation range of Richards' curve relative to Me , and Sk (skewness) represents the asymmetry of Richards' curve relative to the inflection point (mode) (see **Figure 7**). For calculation of these quantities, the useful formulae developed by Hara were implemented [28].

Cultivar	Treatment	Vi (%) at 27°C
Tomato	WW	84±3b
	WW + 30 s PT	83±5b
	WW + 60 s PT	97±2a
	MDS	81±2b
	MDS + 30 s PT	89±3ab
	MDS + 60 s PT	93±4a
	HDS	85±3b
	HDS + 30 s PT	91±2ab
	HDS + 60 s PT	96±2a
Pepper	WW	64±7a
	WW + 30 s PT	20±4b
	WW + 60 s PT	28±6b
	MDS	53±19a
	MDS + 30 s PT	59±11a
	MDS + 60 s PT	61±11a
	HDS	31±12a
	HDS + 30 s PT	44±11a
	HDS + 60 s PT	45±9a

Note: WW, well-watered; WW + plasma, well-watered + plasma; MDS, medium drought stress; MDS + plasma, medium drought stress+ plasma; HDS, harsh drought stress; HDS + plasma, harsh drought stress + plasma.

The values of each experiment (bold line in the table) were significantly marked by different statistical letters at $P \leq 0.05$ according to Student's t-test.

Table 2. Effect of water availability conditions, temperature (27°C), and cold plasma treatment on seed germination on tomato and pepper seeds.

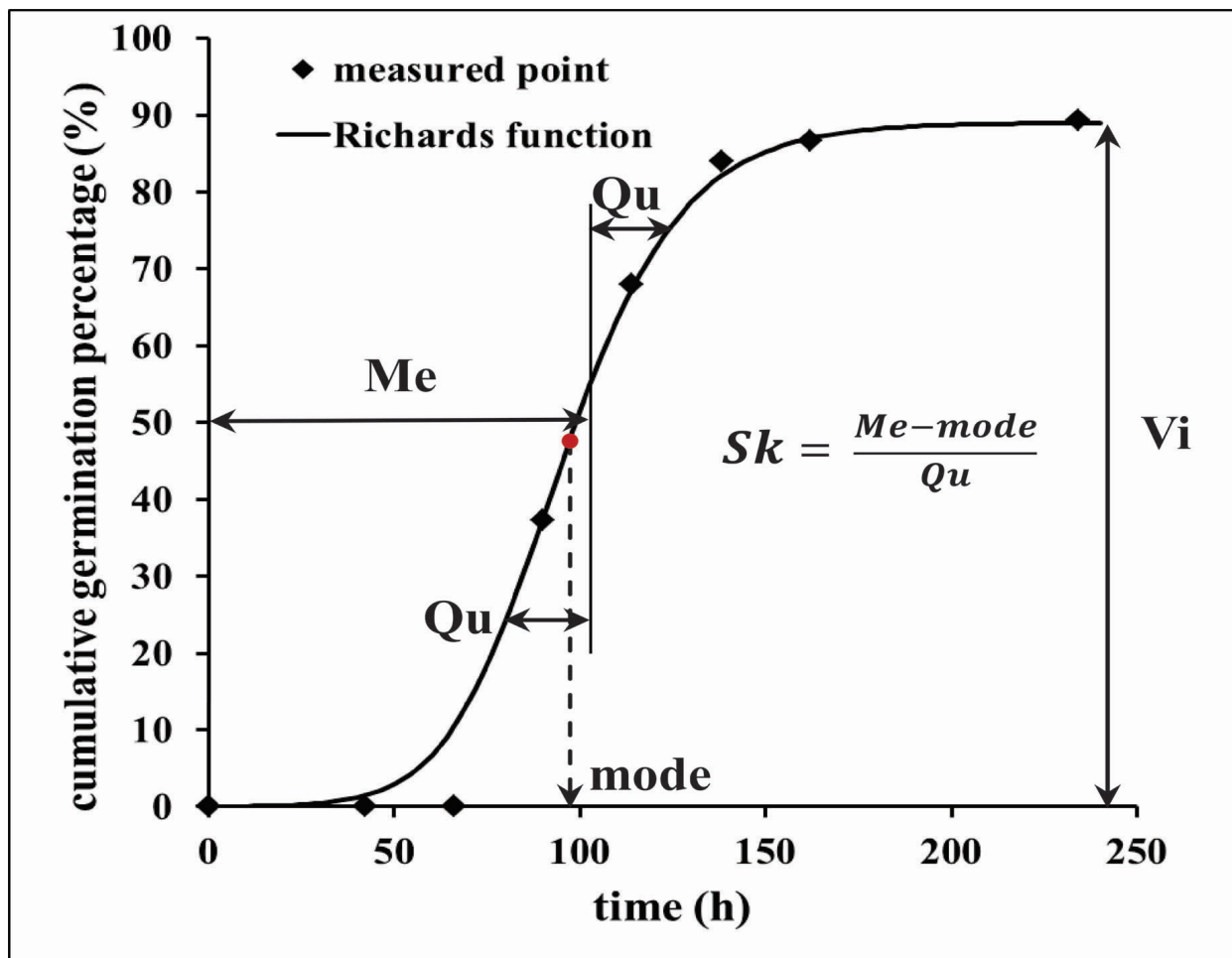


Figure 7. Richards' germination curve (see Eq. (1)). The quantitative parameters of germination are shown.

As it is recognized from the data supplied in **Table 1** for tomato seeds, the value of *Me* is distinctly lower (by *ca.* 5–15 and 5–10 hours) after 30 and 60 s of plasma treatment in the cases of MDS and HRS, respectively. Consider that germination is accelerated as *Me* decreases [28].

As it is seen from **Table 1**, for pepper seeds the value of *Me* is distinctly lower (by *ca.* 10–15 and 8–12 hours) after 30 and 60 s of PT in the cases of MDS and HRS, respectively. Parameters *Qu* and *Sk* were not affected markedly by the cold plasma treatment, as is seen from **Table 1**.

4. Discussion

In the present study, we demonstrated that the cold radiofrequency air plasma treatment has a crucial impact on the water imbibition and seed germination of pepper and tomato seeds. This effect is reasonably attributed to the hydrophilization of the surface of seeds by the plasma treatment [22, 23, 29]. The imbibition and germination in turn were adversely affected by water availability conditions of the seedbed. This effect was well expected, and it was already addressed by investigators [30–32]. However, at the conditions of the lower water availability, both the germination rate and the kinetics of germination were essentially

positively affected by the cold radiofrequency air plasma treatment. The MDS conditions used in our work represent the naturally occurring conditions typical for seedbeds, where no free water is usually present, but water is given abundantly and then drained. HDS conditions represent lower water availability, causing water stress during germination. It should be emphasized that seed germination and early seedling growth are critical stages for plant establishment, and plants are more sensitive to drought stress during these stages [33]. Our results support the findings reported recently by investigators, which studied the influence of the cold plasma treatment on the oilseed rape (*Brassica napus* L.) seed germination under drought stress [31]. Ling et al. reported that, under drought stress, cold plasma treatment significantly improved the germination rate by 4.4–6.25% for various species of the oilseed rape [34]. Seedling growth characteristics, including shoot and root dry weights, shoot and root lengths, and lateral root number, were significantly improved after the cold plasma treatment. It is noteworthy that in our experiments the similar improvement of the germination rate was obtained and confined in the range of 1–12% for pepper and tomato seeds at 21°C, under various conditions of restricted water availability. Indeed, for tomato seeds it was shown that there is a direct relationship between uptake of water and germination rate (this conclusion is supported by recent results reported by other groups [35]), while for pepper seeds, no such correlation was found. This means that other possible effects of PT are responsible for the improvements in germination rates of pepper under plasma treatment (such as the regulation of energy metabolism [36]).

In addition, it was also shown that temperature plays a key role in the effect of plasma on seed germination of pepper. Our experiment demonstrates that at a high temperature of 27°C, in WW conditions, germination rates of non-PT pepper seeds were much higher than the rates of treated seeds, grown under MDS and HDS. The rates of germination of PT seeds were higher than those of WW-grown pepper seeds but considerably lower than those at 21°C. The reason for the drastic decrease in germination rates of PT seeds under WW conditions at higher temperatures remains unclear and would be further investigated. For tomato seeds the differences between germination rates at 27°C and those at 21°C were not so dramatic. These preliminary results demonstrate the importance of determining the right combination of germination temperature and PT duration for each cultivar tested. They may explain some of contradictory effects in investigations of the impact of plasma treatment on germination rates and germination speeds found reported by other researchers.

5. Conclusions

The influence of the cold radiofrequency air plasma treatment on the imbibition and germination of tomato and pepper seeds exerted to conditions appearing in natural seedbeds has been investigated. Plasma treatment markedly hydrophilized surface of seeds and increased the water imbibition (absorption). Various conditions of water supply to germinated seeds were studied. Drought stress essentially affected both germination rates and the kinetics of seed germination.

The intensity of harmful effects due to the water stress markedly depended on the species. In our study, a drought stress had a negative effect on the seed imbibition, germination rate, and the speed of germination of *S. lycopersicum* and of *C. annuum* seeds. This observation clarified the importance of plasma treatment, improving the water absorption by seeds. It was established that plasma treatment essentially enhanced the germination rate and the speed of germination in the case of drought stress for both studied cultivars. It was also shown that there is a direct relationship between uptake of water and germination rate of tomato seeds. Under conditions of nonrestricted water supply, the plasma treatment did not influence the germination rate.

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Ethics

Does not apply.

Data accessibility

All the data are contained within the manuscript.

Competing interests

We have no competing interests.

Author contributions

E.B. and E.D. conceived the research; Y.S. carried out plasma treatment of seeds, measured the water imbibition and germination rate, and analyzed the data; G.W. analyzed the data; and B.C.L. studied wetting properties of seeds. All authors reviewed the manuscript.

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