Germination of Chenopodium Album in Response to Microwave Plasma Treatment*

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Abstract The seeds of Lamb's Quarters (Chenopodium album agg.) were stimulated by low-pressure discharge. The tested seeds were exposed to plasma discharge for different time durations (from 6 minutes to 48 minutes). Germination tests were performed under specified laboratory conditions during seven days in five identical and completely independent experiments. Significant differences between the control and plasma-treated seeds were observed. The treated seeds showed structural changes on the surface of the seat coat. They germinated faster and their sprout accretion on the first day of seed germination was longer. Germination rate for the untreated seeds was 15% while it increased approximately three times (max 55%) for seeds treated by plasma from 12 minutes to 48 minutes.

Keywords: early growth, germination, Lamb's Quarters, seed enhancement, stimulation, surfatron discharge

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1 Introduction

Plasma treating is a well known technology in solid material applications. Recently, it has been applied in biology and biomedicine as well. Preliminary experiments on using plasma exposure to seeds of agricultural plants and to some non-commercial species have been described elsewhere [1~6].

DUBINOV [1] treated oat and barley seeds by the air glow discharge with the aim to increase their germination activity. The seeds were treated for several minutes in both a continual and pulsed regime. The continual regime was more effective than the pulsed regime for seed germination. The long-time stored seeds of Carthamnus tinctorum L. were tested using low-pressure Ar discharge by DHAYAL [6]. This experiment showed not only an increase in seed germination activity but a reduction in germination time as well. Similarly, YIN [3] used magnetized plasma on tomato seeds and investigated its effect on growth under laboratory and field conditions. The optimum pre-treatment by magnetized plasma was determined in a range of 1.0 A to 2.0 A and the tomato yield increased by about 21%.

The pre-treatment with low temperature air plasma stimulating light-induced germination of Paulownia tomentosa Steud. seeds was described by ŽIVKOVIČ and her team [5]. The maximum effect was observed after $4\sim6$ minutes of treatment, with a power of 100 W and a pressure of 200 mTorr. The stimulation effect of plasma pre-treatment was not a direct photoreceptor-mediated phenomenon. The authors considered that the seed stimulation with plasma pre-treatment could have been explained on the basis of three different physical mechanisms: etching, surface functionalization, and deposition of small bioactive molecules.

The last mentioned mechanism was investigated in more detail by VOLIN [2]. His team used plasma chemistry for protection during storage and increase of water affinity to accelerate germination in several important agricultural species. In the case of delayed germination, the seed surfaces were affected via plasma-deposition of hydrophobic materials that would decrease water absorption. In contrast, hydrophilic materials were deposited (or etching processes were initiated) for a promotion of water uptake. CARVALHO [4] studied the applicability of TEOS-plasma deposition by the treated seeds of beans. Seeds with a silicon film at the surface did not show any biological degradation after being exposed to a saturated water vapour environment. However, their germination was enhanced if the seeds were continuously moistened.

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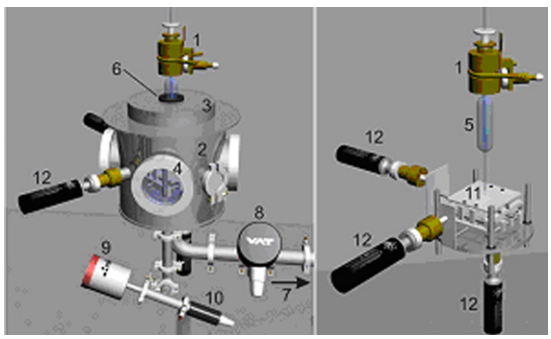
Our short preliminary experiment on germination of Lamb's Quarters (Chenopodium album agg.) seeds after low-pressure plasma treatment manifested a positive simulation [7]. The aim of this study was to find the dependency between low-pressure plasma treatment and the germination of Lamb's Quarters.

2 Material and methods

2.1 Experimental apparatus

The configuration of the experimental apparatus is shown in Fig. 1. A commercial (Sairem) surfatron and microwave generator (Sairem GMP03 KE/D) were used for plasma generation. Experimental arrangement comprise of a stainless steel vacuum vessel and an upper flange made of plastic in order to enable the propagation of the surface wave. Four windows facilitated observation of the discharge and served as ports for handling the samples into and out of the chamber.



1 Surfatron, 2 Vacuum vessel, 3 Upper flange, 4 Window, 5 Quartz tube, 6 Mixing of working gases, 7 Exhausting, 8 Butterfly VAT valve, 9 MKS Baratron 626A, 10 Pirani vacuum gauge, 11 Moving table, 12 Stepping-motors

Fig.1 A general view of experimental apparatus consisted of surfatron and facility inside of the vacuum chamber

A quartz tube with an inner diameter of 6 mm was inserted into the surfatron cavity. The end of the tube protruded into the vacuum vessel. The working gas flowed through the tube. The microwave power was coupled to the surfatron resonator cavity and evoked ionisation of the flowing gas. Plasma generated in this way was sustained further downstream by a surface wave and exited out of the open tube end [8]. The required gas composition used was prepared by mixing the working gases (technical Ar, N_2 and O_2 of the purity grade 4) and using MKS mass flow-controllers.

The volume of the vessel was continuously exhausted with a large rotary vacuum pump Lavat RV100/1 down to an ultimate pressure to the order of one pascal. The stepping-motor-controlled butterfly VAT valve was placed between the vessel and the vacuum pump. Pressure in the vessel was monitored with MKS Baratron 626A. More accurate measurement at lower pressures ($0.1 \sim 100$ Pa) was achieved by a Pirani vacuum gauge. The table movable in three dimensions was inserted in the vessel. The movement in perpendicular directions x and y was realized via linear vacuum feed-through Huntington L21 equipped with stepping-motors. In the vertical direction z the table was moved via feed-through Huntington L20 (hand-operated).

2.2 Seed material

Seeds of Lamb's Quarters (Chenopodium album agg.) were collected from a healthy population with at least 20 plants in the České Budějovice region (Czech Republic) in October 2005. The seed lot was manually purified from vegetative residues and visually screened. Only ripe intact seeds without visible defects were selected. One part of the seed lot was used for the investigation of dormancy [9] and the second part for this study. The details in screening the seed viability are as described by MURDOCH [9].

The seeds for this study contained a very small number of brown seeds that could not be used for our experiments. Only the black ones were chosen for use. The seeds were stored in unfavourable conditions (six months at the temperature of about 22°C). About 17% germinated in laboratory conditions (light, 20°C) at the start of this experiment. The weight of one thousand seeds was 0.720 g.

2.3 Plasma treatment

The samples of treated seeds were placed into glass Petri dishes (40 mm in diameter). The plasma discharge in a fog-like form (Ar/O_2, Ar/N_2 generated at a mixture pressure 590/10 in sccm, $p = 40$ mbar, $P = 100$ W) was spread over the whole diameter of the dish. In this way the seed was treated homogeneously. The distance between the nozzle outlet and the bottom of Petri dish was 2 cm. The temperature of discharge, measured simply by thermistor, was about 53°C under the described experimental conditions. The first tests proved that a reduced pressure during plasma treatment did not affect the treated seeds' quality. The exposure time varied from 6 minutes up to 48 minutes (eight times of plasma treatments, each step with a duration of 6 minutes). The seed treatment was performed on 15 April 2006.

2.4 Electron microscope scanning

Digital photos of seed coats were taken using a scanning electron microscope of JEOL 6300 with a TESCAN 1101 system for image analysis. A standard preparation for this scanning was used on dried-up seeds of Lamb's Quarters. We prepared two sets of seeds: the control seeds and the seeds being treated by plasma for 18 minutes.

2.5 Seed germination test

Seeds were imbibed in 9 cm glass Petri dishes containing two layers of FILPAP filter paper (KA O, filtration rate 6 s, percentage of ash max 0.4%) and 4 mL of

deionised water. The seeds from each plasma treatment and the control were tested on five Petri dishes with 30 seeds per dish. Thus, five replicates and 150 seeds per plasma treatment were tested.

All tested and control seeds were incubated under light-dark conditions at a temperature of about 20°C for six days. On the seventh day many dishes were contaminated with mould and many sprouts began to decay. The germination tests were done from the 18$^{\text{th}}$ to 24$^{\text{th}}$ of April, 2006.

The number of germinated seeds and the length of sprouts were monitored every day during incubation (monitoring period was 24 hours). The radicle protrusions or seed coat disruptions were recorded as the criterion for germination. The length of sprouts was measured without cotyledon.

List of all investigated characteristics (for control/treatment):

Number of germinated seeds

$$N_g[j],$$

Percentage of germinated seeds

$$\frac{N_g(j)}{N} \cdot 100\%,$$

Length of sprout (mean per Petri dish);

$$\frac{\sum\limits_k L_{gk\mathrm{Pi}}(j)}{N_{\mathrm{gPi}}(j)},$$

Total length of sprout (sum of sprouts per Petri dish)

$$\sum_k L_{gk\mathrm{Pi}}(j),$$

Sprout accretion (per growing seed)

$$l_{gk\mathrm{i}}(j) - l_{gk\mathrm{Pi}}(j-1),$$

Mean sprout accretion (mean per Petri dish)

$$\frac{\sum\limits_k [l_{gk\mathrm{Pi}}(j) - l_{gk\mathrm{Pi}}(j-1)]}{N_{\mathrm{gPi}}(j)}.$$

i is the index of Petri dish, j is the day of cultivation, k is the index of germinated seed, N is the number of seeds (150 seeds), $N_{gPi}(j)$ is the number of germinated seeds per i-Petri dish, $l_{gk}(j)$ is the length of sprout (k-germinated seed).

The data obtained were analyzed using the one-way ANOVA method in STATISTICA'99 program. The differences among the number of germinated seeds and length of sprout were calculated through post hoc comparison of Tukey HSD test for equal numbers. To obtain their normal distribution, percentage and proportional values were transformed into their first arcsin ($y = \mathrm{arcsinSQRT}(x/n)$).

3 Results

The seeds under plasma irradiation of 12∼48 minutes started to germinate before the first data monitoring. These seeds started to germinate during the first 24 hours of the germination test. The seeds with 6 minutes of plasma irradiation and the seeds in the control conditions started to germinate before the second data monitoring (between 24 hours and 48 hours of the test).

On the fourth day of the germination test we observed a significant difference between the plasma treated seeds and the control ones (Tukey HSD test, $p < 0.05$) (Fig. 2). The major differences between the control and plasma treated seeds were found also on the sixth day. Only 15% of the control seeds germinated while at least 55% of the plasma treated, for 30 minutes and 48 minutes, ones germinated, i.e., three times more. Germination of the seeds also depended on the duration of plasma exposure. During all days of cultivation, no differences were found between the seeds that underwent plasma irradiation for 6 minutes and the control seeds.

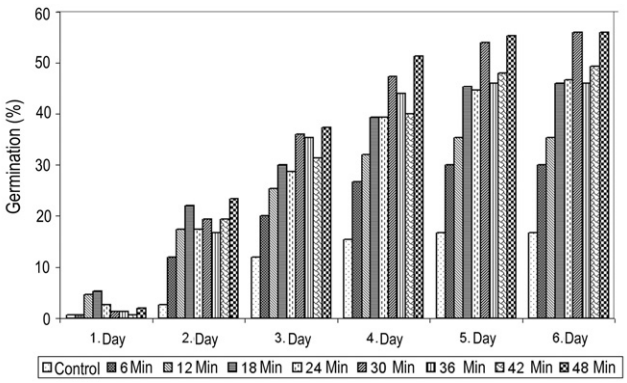


Fig.2 Percentage of the germinated seeds of Chenopodium album treated with plasma for different durations

Divergences were obvious in all four characteristics of the sprout lengths (Table 1). The differences in both lengths (length of sprouts, grand total of length of sprout) and both sprout accretions (sprout accretion, mean sprout accretion) were greater in the treated seeds than those in the control. Significant differences in the length of sprouts between control and the seeds exposed to plasma irradiation for 48 minutes (on the first day), and the control and the seeds treated for 30∼48 minutes (on the fifth day) were observed (Tukey HSD test, $p < 0.05$). The length of sprouts from the 48-minute plasma treated seeds was 51.6 mm on the fifth day compared to 39.2 mm in the control seeds. The grand total length of sprouts from the control seeds differed from the plasma treated seeds exposed for 48 minutes as well as from those exposed for 18∼48 minutes from the second to fifth day cultivation (Tukey HSD test, $p < 0.02$). The greatest difference was observed between the 48-minute plasma treated seeds (883 mm) and the control ones (259 mm) on the fifth day. Almost all sprouts arrested growth and began to rot on the sixth day of the cultivation.

Table 1. The length of sprout (mm) and B total length of sprout (mm) of growing seeds of Chenopodium album treated with plasma for different time of exposition. The types of plasma treatments followed with the same letters were not significantly different in multiple range analysis (arcs in transformation, Tukey HSD test, $P < 0.05$, see columns HSD)

	Plasma		1.day			2.day			3.day	
	treatment	Mean	SD	HSD	Mean	SD	HSD	Mean	SD	HSD
A	control	5.2	3.0	a	12.3	2.1	a	20.0	4.8	a
	6 min	6.5	3.1	a	13.4	4.6	a	20.8	0.7	a
	12 min	3.8	1.2	a	14.7	2.4	a	19.8	4.7	a
	18 min	4.6	2.3	a	14.8	2.0	a	20.3	2.3	a
	24 min	5.2	2.8	a	12.9	2.9	a	19.5	3.7	a
	30 min	4.7	2.2	a	14.1	1.9	a	21.3	2.4	a
	36 min	4.7	1.4	a	13.4	3.0	a	19.1	2.1	a
	42 min	6.4	2.3	a	15.8	3.4	a	22.4	1.8	a
	48 min	3.1	5.4	b	11.7	9.3	a	19.9	8.4	a
B	control	6.2	10.8	a	41.4	33.8	a	87.6	39.2	a
	6 min	14.2	7.3	a	73.2	15.3	ab	153.0	39.2	bc
	12 min	24.2	7.7	ab	96.8	33.7	ab	199.2	28.7	abc
	18 min	20.6	17.1	ab	130.0	41.5	bc	233.6	64.9	bd
	24 min	20.4	9.0	ab	129.0	43.3	bc	242.8	71.7	bd
	30 min	21.6	9.4	ab	139.4	41.4	bc	275.4	52.2	cd
	36 min	22.4	12.9	ab	145.8	34.5	bc	281.4	51.3	cd
	42 min	27.2	15.8	ab	128.0	42.5	bc	232.0	59.7	bd
	48 min	41.0	14.4	b	174.8	39.6	dc	346.4	76.1	d
	Plasma		4.day			5.day			6.day	
	treatment	Mean	SD	HSD	Mean	SD	HSD	Mean	SD	HSD
A	control	29.7	3.8	a	39.2	4.2	a	51.9	7.9	ab
	6 min	27.8	5.1	a	41.3	5.4	ac	52.1	6.1	ab
	12 min	29.2	3.9	a	40.5	2.9	ac	48.5	5.9	ab
	18 min	28.5	2.6	a	40.0	3.0	ac	54.2	5.5	ab
	24 min	30.4	1.6	a	46.0	3.8	a	56.7	5.0	ab
	30 min	35.7	3.1	a	50.8	3.1	b	60.5	2.9	b
	36 min	29.0	3.9	a	47.2	4.5	bc	57.5	4.1	ab
	42 min	35.5	2.5	a	51.8	1.4	b	59.1	2.9	ab
	48 min	34.5	7.9	a	51.6	4.3	b	61.5	3.2	b
B	control	163.0	45.8	a	259.0	100.9	a	305.0	108.2	ab
	6 min	261.2	75.4	ab	339.4	118.0	ab	471.0	197.6	ab
	12 min	294.4	84.8	ab	441.0	129.7	abc	547.0	110.1	abc
	18 min	395.4	80.8	bc	562.2	112.2	bcf	703.0	172.2	bd
	24 min	392.6	105.3	bc	563.4	106.6	bcg	760.6	144.5	bd
	30 min	497.6	23.8	cd	770.4	74.9	cd	947.0	63.2	cd
	36 min	478.0	67.8	cd	703.0	126.0	cd	858.0	139.7	cd
	42 min	420.4	87.0	bd	690.6	146.1	defg	883.4	197.0	d
	48 min	592.4	134.5	d	883.0	173.0	d	997.0	211.8	d

The major differences in the sprout accretion were found only on the first day of seed germination (Fig. 3). Significant differences between the control and the seeds with plasma irradiation for 6 minutes to 48 minutes were observed (Tukey HSD test, $p < 0.05$, treatment for 18 minutes with $p < 0.07$). The greatest difference was found between the seeds treated for 48 minutes (6.4 mm) and the controls (3.1 mm). The mean sprout accretion was the most obvious from all sprout characteristics. We found the most important trends in seed germination after various durations of plasma treatment. The mean sprout accretion significantly differed between the control and the seeds treated by plasma for $12 \sim 48$ minutes (on the first day) and for $18 \sim 48$ minutes (from the second to the fourth day cultivation) (Tukey HSD test, $p < 0.04$). The highest difference was observed between the control and the 48-minute plasma treated seeds (13.9 mm, while the control was 4.6 mm) on the fifth day. The sprout growth process is shown in Fig. 4.

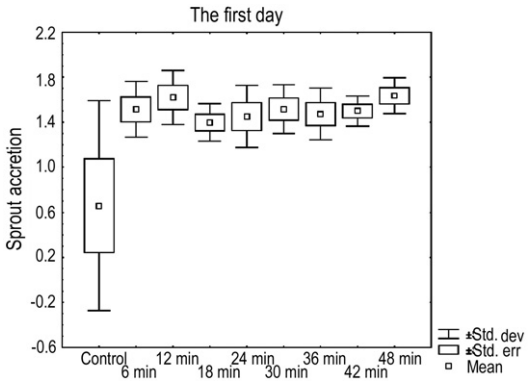


Fig.3 Sprout accretion of growing seeds of Chenopodium album treated with plasma on the first day of cultivation. Standard deviation and standard error of the mean are shown. Significant differences between the control and treated seeds were registered (Tukey HSD test; for details, see the text). The arcsin transformation is used before the figure creation

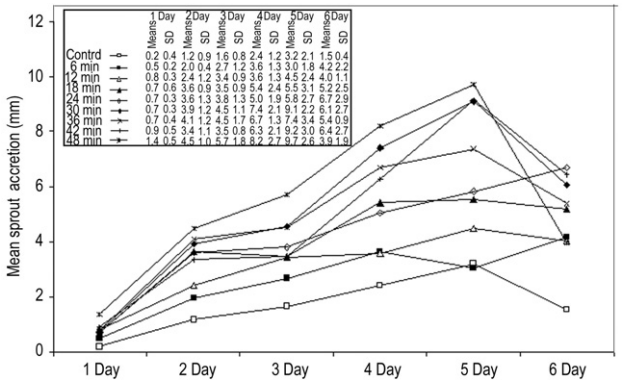


Fig.4 Mean sprout accretion of growing seeds of Chenopodium album treated with plasma for different times of exposure. Standard deviation and mean are shown

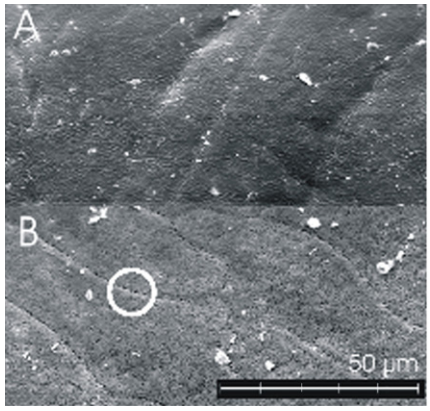


Fig.5 Seed coat surface of Chenopodium album from scanning electron microscope. Non-treated seed (A) and seed treated with plasma for 18 minutes (B) are shown

4 Discussion

Lamb's Quarters is one of the most widely restrained summer annual weeds in the world. Their seeds show considerable polymorphisms [10]. There are two different seed forms on one plant: brown seed and black seed. The brown seed has a thin seed coat and can germinate after the harvest, while the black seed has a thick seed coat and is dormant. Brown seeds are larger than black seeds. The surface of the black seeds is either smooth or reticulate. In our experiments only the black seeds with a reticulate seed coat are used. These seeds became non-dormant with nitrate treatment but not with cold stratification [11]. We expected low seed germination after six months of dry storage at about 22°C, because C. album is a species with increased seed germination after cold storage (5°C) [12,13]. The germinating part of the tested seed lot was about 15%.

We tested the seed germination that was triggered by microwave plasma treatment under light-night conditions at laboratory temperature. Our results confirmed the results of other research groups: the number of germinating seeds after plasma treatment increased, germination time was reduced, and the growth rate of seedlings increased. All results of our experiments confirmed that plasma treatment is a useful process for overcoming dormancy of C. album. This physical treatment may be functional for other plant species with analogous physiological seed dormancy.

ŽIVKOVIČ [5] proposed and discussed three possible plasma treatment effects on seed outside: etching, surface functionalization, and deposition of small bioactive molecules. We focused on the mechanical outside changes in the seed coat. We confirmed that the plasma treated seeds of C. album had a changed surface. The pictures of seed coats (control and treated seed) in Fig. 5 show that the plasma treated seeds have an etched/eroded surface. Analogous changes were observed on the surface of treated seed of Carthamnus tinctorium [6]. It was suggested that the seeds with an etched/eroded seed coat would germinate faster than healthy ones (or than those with a thick seed coat) [14]. Changed seed surface in plasma treated seeds could probably enhance and accelerate water uptake. The seeds can also change their dormancy and germination processes [15]. The process of etching has an analogous effect to the seed scarification (e.g., in the digestive tract of the bird, the seeds with a thick seed coat) [12].

Generally, three responsible agents may be assumed in the process of germination: **a.** changes in the seed coat, **b.** internal physiological changes connected with seed hormonal activities and all the other hormonally regulated processes, and **c.** all the changes in seeds evoked by outer and inner factors. There is no direct evidence for understanding the influence of plasma treatment on seeds overcoming dormancy, germination, and early growth. However, it is necessary to perform more experiments to obtain more evidence for the determination of these principles. New experiments may be based on using various plasma discharge settings and on seeds from various plant species. These plant species may be chosen according to their different germination characteristics so that all characteristics will be represented. Our future study will further clarify the a-c agent effect on the seed germination.

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