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Does Cold Plasma Affect Breaking Dormancy and Seed Germination? A Study on Seeds of Lamb’s Quarters (Chenopodium album agg.)*

Božena ŠERÁ1, Michal ŠERY2, Vítězslav STRAŇÁK2, Petr ŠPATENKA2,3, Milan TICHY4

1Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Na Sádách 7, CZ-370 05 České Budějovice, Czech Republic
2University of South Bohemia in České Budějovice, Faculty of Education, Department of Physics, Jeronýmovna 10, CZ-371 15 České Budějovice, Czech Republic
3Technical University of Liberec, Department of Material Sciences, Hálková 6, CZ-461 17 Liberec 1, Czech Republic
4Charles University in Prague, Faculty of Mathematics and Physics, V Holešovičkách 2, CZ-180 00 Praha 8, Czech Republic

Abstract Low-pressure discharge is applied for stimulation of germination of two seed lots of Lamb’s Quarters (Chenopodium album agg.) with different starting germinations (17%, 8%) and in different stages of dormancy. Different exposition durations with cold plasma treatment were applied. The variable of the ratio cumulative germination was calculated. The Richards’ equation was used for curve-fitting and simulation of the growth curves. Population parameters, namely Vi - viability, Me - time, Qu - dispersion, and Sk - skewness, counted from the curves described the germination rate well. Significant differences among Qu confirmed the erratic dormancy and gradual germination of Lamb’s Quarters. No difference in the Me parameter was found between two tested seed lots, and no interspecies characteristics were changed using low-pressure discharge. The results suggested that plasma treatment changed seed germination in Lamb’s Quarters seeds.

Keywords: chenopodium album, fitting, seed germination, low-pressure discharge, plasma treatment, parameter.

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1 Introduction

Lamb’s Quarters (Chenopodium album agg.) is a general summer annual plant widely distributed all over the world. Large number of produced seeds[1], erratic dormancy[2–4], and repeated germination during the vegetative season[5] are the causal characteristics of this plodding weed. Seed germination conditions were elaborated in detail by KARSSEN[2,6,7]. Lamb’s Quarters seeds need sufficient light for optimal germination. Plasma effect on the modification of seed germination was investigated in the past few years. An increase in the number of seedlings and the rate of germinated seeds was found[8–16].

Our team tested selected seed and seedling characteristics of Lamb’s Quarters after microwave plasma exposition. Plasma treatment eroded the seed surface, enhanced and accelerated the seed germination[15]. In this experiment we compared data of two seed lots of Lamb’s Quarters. These seed lots had the same origin and time of ripening. We wittingly decreased the portion of germination in both seed lots, so that the start of seed germination was different in each seed lot. Then we applied the cold plasma treatment to both seed lots while keeping an untreated sample in each lot. The aim of our experiment was to demonstrate that the number of germinated seeds and Richards’ equations are comparable in both seed lots. That would mean that the response to plasma treatment is not accidental.

2 Material and methods

2.1 Experimental apparatus

The setup of the experimental apparatus was based on the surfatron principle. A commercial (Sairem) surfatron and microwave generator (Sairem GMP03 KE/D) were used in our experiments. A quartz tube was inserted into the surfatron cavity with working gas flowed through it. The microwave power was supplied to the surfatron resonator cavity and caused ionization of the working gas. Plasma generated in this way was sustained further downstream by a surface wave exited out of the open tube[17]. This after-glow discharge (not further supported by the surface wave) was directly applied onto the seed samples. The gas composition was prepared by mixing working gases (technical argon, nitrogen and oxygen of a purity grade 4.6). The setup of the experimental apparatus is described in detail in ŠERÁ[15].

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2.2 Seed material

Seeds of Lamb’s Quarters (Chenopodium album agg.) were collected in October 2005 in the České Budějovice region, the Czech Republic. Seeds were collected from a fit, vital population of at least 20 plants. Vegetative residues were manually eliminated from the collected seeds which were visually screened. Only the ripe (black) undamaged seeds without visible defects were chosen. Among the seeds there was a small number of brown ones that were used for our experiments. The weight of one thousand seeds was 0.720 g.

From the seeds selected using the above described procedure, two seed lots were prepared, each containing 1350 seeds. Both seed lots were stored in darkness and at a temperature of about 10ºC. The first seed lot was stored for six months, and its germination was about 17%, in laboratory conditions of light-dark and 20ºC. The plasma treatment and the germination test of the first seed lot was undertaken in April 2006. The primary sub-data were particularly published [15]. The second seed lot was stored for seven months, and its germination was about 8%, also in laboratory conditions of light-dark and 20ºC. Plasma treatment and germination test of the second seed lot was undertaken in May 2006.

2.3 Plasma treatment

Each seed lot was divided into nine samples each of which is with 150 seeds. Eight of them were plasma treated and one was left untreated as a control sample. Each treated sample was put on a glass Petri dish, of 40 mm in diameter, then the dish was put on a movable desk in the vacuum vessel where a plasma source was installed. The distance between the nozzle outlet and the bottom of the Petri dish was 2 cm. The plasma discharge generated in fog-like form, in argon/oxygen, argon/nitrogen discharges described above, was applied over the whole diameter of the dish. In this way the seeds were treated homogeneously. The temperature of the discharge, measured by a thermistor, was about 53ºC. Detailed experimental conditions are described in ŠERÁ [15]. Eight samples for each seed lot were treated with different plasma treating durations, from 6 min to 48 min. The plasma treatment of the first seed lot was undertaken on 15 April 2006 and the second one on 13 May 2006.

2.4 Seed germination test

Each treated sample was further subdivided onto five glass Petri dishes of 90 mm in diameter with 30 seeds each time. Seeds were germinated on the top of filter paper. The Petri dishes contained two layers of FILPAP filter paper (KA O, a filtration rate of 6 s, a percentage of ash of max 0.4%). Filter paper was wetted with 4 mL of deionised water at the onset of the experiment. We tested 150 seeds in each plasma treatment (and for the control), 1350 seeds per seed lot, and 2700 seeds were tested per experiment as presented.

The germination test with the first seed-lot was performed from 18 to 25 April 2006 and the second seed lot from 16 to 23 May 2006. Seeds were germinated in laboratory conditions at a temperature of about 20ºC under a light-dark regime for 8 hours to 16 hours per day for six days.

We registered the number of germinated seeds in each Petri dish. Germination was recorded once a day and a seed was regarded as germinated when the radicle had protruded at least 1 mm.

2.5 Data processing

Data of the germinated seeds per Petri dish were treated in relative. We calculated the variable of the ratio cumulative germination:

\[
C_{gi}(j) = \frac{N_{gi}(j)}{\max\{N_{gi}(k)\}_{k=0}}, \quad (1)
\]

where, \( j \) and \( k \) are the day of cultivation, and \( N_{gi} \) is the number of germinated seeds per i-Petri dish.

Then a higher order model was adopted. To derive the biological information from the germination experiment, Richards’ curves were fitted to a number of the germinated seeds [18–22]. The Richards’ equation is a double asymptotic sigmoid function with a variable point of inflection. The function (\( Y_t \)) was calculated according to

\[
Y_t = \frac{a}{[1 + b \cdot d \cdot \exp(-c \cdot t)]^{\frac{1}{d}}}, \quad (2)
\]

where \( t \) represents time and \( a, b, c, \) and \( d \) are parameters. The functional parameters were calculated by a least squares scheme using a commercial code MS EXCEL. For replication of each of the plasma treatments, an individual germination curve was fitted. Totally ninety curves from the germination tests on both seed lots were obtained. The population parameters, namely \( Vi \) - viability, percentage at infinity; \( Me \) - time, median of time; \( Qu \) - dispersion, quartile deviation of time; and \( Sk \) - skewness, were calculated from the functional parameters as in HARA [23]:

\[
Vi = a, \quad (3)
\]

\[
Me = \frac{1}{c} \ln \frac{b \cdot d}{2d - 1}, \quad (4)
\]

\[
Qu = \frac{1}{2c} \ln \frac{4d - 1}{(4/3)^d - 1}, \quad (5)
\]

\[
Sk = 2 \cdot \left\{ \frac{\ln \frac{d}{(4/3)^d}}{\ln \frac{4d - 1}{(4/3)^d - 1}} \right\}, \quad (6)
\]
Since the number of germinating seeds in both germination experiments did not match, we converted the Richards’ curve to a relative ratio $V_i^{[23]}$. To examine the variability in the population parameters ($M_e$, $Q_u$, and $S_k$) among various plasma treatments, the estimates from the curve-fitting procedure were used in the variance analysis (ANOVA). Pair-wise comparisons of the first and second germination tests were performed by t-test. The ANOVA and t-test were performed with ANOVA/MANOVA and basic statistics packages of STATISTICA’99 code.

3 Results

The application of fitted Richards’ curves was quite successful in describing the temporal evolution of the germination process. We recorded the number of germinated seeds in one-day periods, so that the curves were fitted to the data obtained in rather coarse time intervals. Nevertheless visual inspection of the curves plotted in Fig. 1 shows the ability of the applied Richards’ curves to follow the experimentally observed seed germination rather well (data from the first repetition of the first germination test are given).

Table 1 shows the average and standard deviation of the population parameters for each plasma treatment and control in both germination tests. Both experiments were analysed separately. No differences were found among $M_e$, and $S_k$ parameter values in various plasma treatments for both germination tests.

While testing dissimilarities between the population parameters of the first germination experiment and the second experiment listed in Table 2, significant differences in the $Q_u$ parameters, in samples plasma treated for 18 min, 30 min, 36 min and 42 min, were found. No other differences were found.

![Fig.1 Richards’ curve fitted to the experimentally estimated number of germinated seeds of Lamb’s Quarters. Each subfigure shows the result per Petri dish for different durations of plasma exposition](image-url)
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Table 1. Mean and standard deviation of the \( V_i \), \( Me \), \( Qu \), and \( Sk \) population parameters for germination tests in plasma treated seeds of Chenopodium and the associated F-statistic probability (\( P \)) from the variance analysis. \( V_i \) viability, \( Me \) time, \( Qu \) dispersion, \( Sk \) skewness, details see in text

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( V_i )</th>
<th>( Me )</th>
<th>( Qu )</th>
<th>( Sk )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Test 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>1.001</td>
<td>0.015</td>
<td>2.467</td>
<td>0.707</td>
</tr>
<tr>
<td>6 min</td>
<td>0.988</td>
<td>0.046</td>
<td>2.311</td>
<td>0.616</td>
</tr>
<tr>
<td>12 min</td>
<td>1.013</td>
<td>0.027</td>
<td>2.162</td>
<td>0.367</td>
</tr>
<tr>
<td>18 min</td>
<td>0.981</td>
<td>0.029</td>
<td>2.301</td>
<td>0.664</td>
</tr>
<tr>
<td>24 min</td>
<td>1.029</td>
<td>0.035</td>
<td>2.570</td>
<td>0.408</td>
</tr>
<tr>
<td>30 min</td>
<td>1.015</td>
<td>0.026</td>
<td>2.519</td>
<td>0.273</td>
</tr>
<tr>
<td>36 min</td>
<td>0.989</td>
<td>0.038</td>
<td>2.307</td>
<td>0.246</td>
</tr>
<tr>
<td>42 min</td>
<td>1.014</td>
<td>0.018</td>
<td>2.275</td>
<td>0.220</td>
</tr>
<tr>
<td>48 min</td>
<td>1.019</td>
<td>0.013</td>
<td>2.421</td>
<td>0.336</td>
</tr>
<tr>
<td>( P )</td>
<td>0.924</td>
<td>&lt; 0.001*</td>
<td>0.440</td>
<td></td>
</tr>
<tr>
<td>Test 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.876</td>
<td>0.182</td>
<td>2.416</td>
<td>0.683</td>
</tr>
<tr>
<td>6 min</td>
<td>0.993</td>
<td>0.070</td>
<td>2.146</td>
<td>0.711</td>
</tr>
<tr>
<td>12 min</td>
<td>1.054</td>
<td>0.047</td>
<td>2.368</td>
<td>0.426</td>
</tr>
<tr>
<td>18 min</td>
<td>0.997</td>
<td>0.005</td>
<td>1.881</td>
<td>0.373</td>
</tr>
<tr>
<td>24 min</td>
<td>0.978</td>
<td>0.070</td>
<td>2.512</td>
<td>0.533</td>
</tr>
<tr>
<td>30 min</td>
<td>0.996</td>
<td>0.042</td>
<td>2.690</td>
<td>0.567</td>
</tr>
<tr>
<td>36 min</td>
<td>1.008</td>
<td>0.015</td>
<td>1.729</td>
<td>0.589</td>
</tr>
<tr>
<td>42 min</td>
<td>0.997</td>
<td>0.016</td>
<td>1.755</td>
<td>0.391</td>
</tr>
<tr>
<td>48 min</td>
<td>0.984</td>
<td>0.027</td>
<td>2.084</td>
<td>0.413</td>
</tr>
<tr>
<td>( P )</td>
<td>0.064</td>
<td>0.025*</td>
<td>0.082</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference

Table 2. Results of the probability associated a Student’s t-test between population parameters (\( V_i \) viability, \( Me \) time, \( Qu \) dispersion, \( Sk \) skewness) of the first and second seed lots for different plasma exposition time

<table>
<thead>
<tr>
<th>Control</th>
<th>6 min</th>
<th>12 min</th>
<th>18 min</th>
<th>24 min</th>
<th>30 min</th>
<th>36 min</th>
<th>42 min</th>
<th>48 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_i )</td>
<td>0.220</td>
<td>0.912</td>
<td>0.062</td>
<td>0.354</td>
<td>0.178</td>
<td>0.456</td>
<td>0.300</td>
<td>0.288</td>
</tr>
<tr>
<td>( Me )</td>
<td>0.991</td>
<td>0.631</td>
<td>0.325</td>
<td>0.257</td>
<td>0.749</td>
<td>0.597</td>
<td>0.099</td>
<td>0.097</td>
</tr>
<tr>
<td>( Qu )</td>
<td>0.652</td>
<td>0.157</td>
<td>0.725</td>
<td>0.002*</td>
<td>0.093</td>
<td>0.012*</td>
<td>0.007*</td>
<td>0.005*</td>
</tr>
<tr>
<td>( Sk )</td>
<td>0.736</td>
<td>0.192</td>
<td>0.182</td>
<td>0.847</td>
<td>0.105</td>
<td>0.116</td>
<td>0.190</td>
<td>0.242</td>
</tr>
</tbody>
</table>

*Significant difference

4 Discussion

Erratic dormancy and random gradual germination of Lamb’s Quarters were eliminated by the variable of the ratio cumulative germination. In this manner, the population parameters of the Richards’ curve became comparable for each Petri dish [23]. The equation parameters preserve all the information in the fitted germination curve and comprehensively describe the germination properties of a seed-lot [23∼26]. Parameter \( Qu \) shows the effect of the seed coat soaking on a uniformity of emergency during early seed growth [23,24]. Shape changes in the Richards’ curve related to changes in parameter \( Qu \) are shown in Fig. 2. Differences in parameter \( Qu \) (Tables 1, 2) confirm the erratic dormancy and random gradual germination of Lamb’s Quarters. Uniformity in parameter \( Qu \) among various seed lots is characteristic in cultural crops (e.g., varieties of cereals). Plasma influence was manifested by the increase in the parameter \( Qu \). It means the increase in variability at the beginning of germination among different seeds.

Fig. 2 Illustrative figure of \( Qu \) parameter over Richards’ curve shape (model example by Hara [23]). \( Qu \) reflects the uniformity of growth. \( Qu \) shows the effect of the duration of seed soaking on the uniformity of emergence, indicating that it would be useful for the quantification of seedling growth population dynamics. Arrow shows the \( Qu \) growth

Parameter \( Me \) is most important for the description of the germination process and early growth of seeds,
because it distinguishes a species/variety’s plant behaviour during the germination [23]. No difference in parameter $M_e$ is a satisfactory result (Tables 1,2). This result suggests that plasma treatment evokes changes in the germination characteristics of Lamb’s Quarters [15], but does not evoke the biological character of this plant species. No interspecies characteristics of Lamb’s Quarters were changed using the low-pressure discharge.

Seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate. Seed dormancy is one of the important ecological plant properties that enable the species to reproduce generatively to survive [27]. The most frequent source of the innate (primary) dormancy [28] is a high content of inhibitory compounds in the seed [29−32] and seed coat impermeability [33−35].

Cold plasma, a physical way of breaking down dormancy, is known as a recent stimulation treatment [8−16]. Several agents that can play a role in dormancy breaking (e.g., UV radiation, radicals, chemical reactions) are generated by plasma treatment. If the plasma is directly applied to a material, the etching and erosion of the surface are probably the most dominant processes [36]. The seed coat is then modified by cold plasma treatment [10,13,15]. We do not know in detail how this process is achieved, probably the seed surface loses its water-impermeability character, consequently, the dormancy is more likely to break down under the influence of moisture.

This study enriches the information to the knowledge of seed stimulation and seed dormancy-breaking by a physical process. A deeper understanding of these phenomena can contribute above all to saving rare and disappearing plant species used in conservative ecology. To our knowledge a study on the effect of the plasma treatment on seed surface at a microscopic level has not been performed yet. Thus this study contributed in a systematic investigation of the influence of plasma treatment on seed germination, alteration of the seed surface properties, and the impact of these changes on the breakdown of dormancy.

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E-mail address of Božena ŠERÁ: sera@usbe.cas.cz