Scarification of Altaic Flax Seeds With High-Power UV Radiation Generated by Plasma of Nanosecond Electric Discharges

Alexander E. Dubinov, Julia P. Kozhayeva, and Elena A. Zuimatch

***Abstract*— This paper describes an investigation of effect of high-power UV-radiation plasma, generated by electric discharges, on germination rate of Altaic flax seeds *(Linum altaicum)*. It was found that UV-radiation scarifies flax seeds by means of cracks formation in the seeds' coat. The cracks shorten moisture penetration time to a seed bud thus twice increasing the germination rate. It is found that the germination rate function has the three-stage character; the velocity-growth function has the view of three pulses, which separates the seeds into three subgroups. Division of the group into subgroups is stipulated by the probabilistic nature of the cracks formation. The number of subgroups is equal to the number of layers +1. We calculated average times of natural moisture penetration through the flax seed coat layers: $T_s = 11.8$ h through the spermoderm and $T_e = 17.7$ h through the endosperm. It is possible that scarification of the flax seed coats can shorten the germination time for these times. We also noted that some seeds could die due to the effect of high-power UV radiation. So, it is necessary to find more sparing regimes of the seeds UV irradiation.**

***Index Terms*— Crack, electric discharge, endosperm, flax seeds, germination rate, multipulse mode, plasma treatment, scarification, spermoderm, the Gompertz growth function, UV radiation.**

I. INTRODUCTION

FOR many centuries, the search of effective technologies of presowing seeds treatment, which increase the germination capacity and rate, has performed. It has been noted that partial destruction of solid waterproof seed coat is favorable for moisture access to the seed bud in the seed kernel thus increasing the germination capacity and rate. Sometimes, it is sufficient either to scratch the coat, or locally decrease its thickness. But sometimes, it is necessary to make holes or cracks in the coat. Such technologies are called scarification.

There are three types of scarification and their combinations [1]:

1) mechanical scarification [2], [3] (it is known that in ancient China and India, rice was sprinkled many times with sand, which caused abrasive action on the seeds surface);
2) temperature scarification (short-term watering of the seeds with hot and/or cold water) [4];
3) chemical scarification (seeds treatment with chemically active substances—acids, for example) [5], [6];
4) combined scarification, which uses two or three mentioned methods [7]–[11].

Recently, method of presowing seed treatment appeared—treatment of the seeds with low-temperature plasma and its radiation generated by the electric discharges. For example, Reference [12] contains the information about the results of plasma effect of dc glow discharge at low pressure on the seeds of the grain cultures—barley and oats. This paper claims that the germination rate can be significantly increased after the plasma treatment.

At the same time, Volin *et al.* [13] present the results of radish, soya, pea, beans, and maize seeds' treatment with rotating plasma of RF discharge in different gas media. Increase or decrease of the germination rate depended on the gas type.

Also, former publications about plasma presowing seeds treatment are known [14], [15]. Actually, References [12]–[15] pushed wide-scale research of plasma treatment effects on the seeds. These investigations started the new direction of the plasma research—plasma agriculture [16]–[18].

In many papers, the positive effect of treating seeds with plasma is explained by one of the three following biophysical mechanisms:

1) The fungicide and bactericide effects of plasma [12], [21]–[23].
2) The modification of biochemical processes inside the kernel [24]–[27].
3) Treatment of the seed coat, in the result of which the water inflow and ions access through the seed coat to the seed kernel increases (plasma scarification) [23], [28]–[31].

Recently, we used two types of nanosecond discharges [30] to study the effects of mustard seed plasma scarification.

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Fig. 1. General view and sizes of Altaic flax seeds.

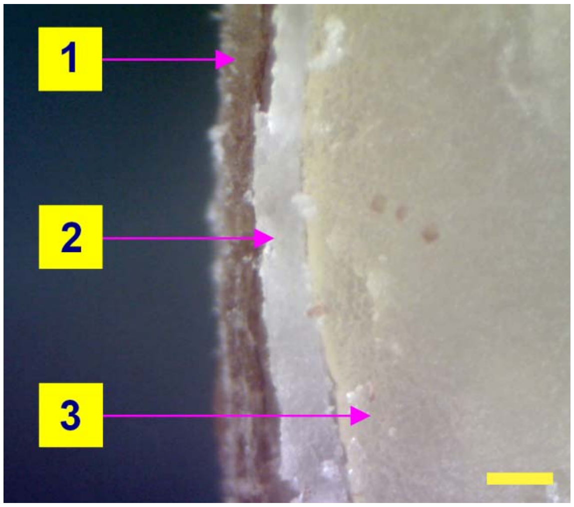

Fig. 2. Cross section of the flax seed. 1—Spermoderm. 2—Endosperm. 3—Cotyledon. (Scale bar is 100 μm.)

We detected stimulating influence of plasma on the seeds in the cases, when the cracks appeared in the seeds' coats under the effect of high-power UV radiation of the discharges.

Also, we recorded an unexpected phenomenon of the two-stage germination rate function, which corresponded to the two-pulse velocity mode. We did not explain that phenomenon at that time.

It is necessary to note that there are papers presenting the seed growth functions after other types of scarification. Some of them could be recognized as two-stage and three-stage germination rate functions [9], [30], [31]. However, there were no discussions.

The goal of this paper was to perform the treatment of other seeds with the method used in [29]. Understanding the reasons of multistage germination rate functions was one of the partial tasks of this paper.

II. MATERIALS AND EQUIPMENT

A. Seeds

In this paper, we took the seeds of Altaic flax (*Linum altaicum*). This plant is used in textile and food industries, and in medicine. Selecting the flax for scarification, we were guided by the following properties of its seeds.

1) The flax seed is rather smooth; its surface irregularities are not more than 20 μm; this allows easily detecting and recording the microcracks on its surface.
2) The flax seed has multilayer coat; this appeared to be important for realization of the multistage growth functions.

The seeds have the almond shape of ~5 mm long and ~2.5 mm width (Fig. 1). The seeds were bought at the local market. All the seeds were from the same trade batch. To make the calculation easier, the seeds were divided into groups by 100 seeds in each.

Thickness and structure of the seed coat are important for scarification. Fig. 2 shows the cross section of the flax seed. One can see that the coat has two layers. The external layer is a peel—spermoderm, and the internal white layer is endosperm. Total thickness of these layers is from 100 to 300 μm at different places of the seed surface. Cotyledons are located under the endosperm. The seed bud of the future plant is located inside the cotyledons. The main task of the scarification is to provide fast moisture access to the seed bud through all layers.

Fig. 3. General view of "Game" device, the supply unit and applicator on it.

B. Generator of Plasma and UV-Radiation Pulses "Game"

The "Game" device, which was designed to study effects of plasma and its radiation on biological objects, was used as the generator of plasma and UV-radiation pulses in our experiments. The device was successfully used to disinfect different materials; it is described in detail in [32]. It consists of two main parts—the supply unit and the plasma applicator; both are connected with a cable (Fig. 3).

The supply unit charges and discharges a capacitor battery and supplies continuous sequence of high-voltage pulses with the amplitude of 25 kV, duration of 200 ns, and controllable frequency from 0.1 to 6 Hz to the applicator. The maximum consumed power from the electric network is 100 W.

The plasma applicator consists of a polyamide head with a cylinder-to-cone adaptor. The 10-channel surface discharge

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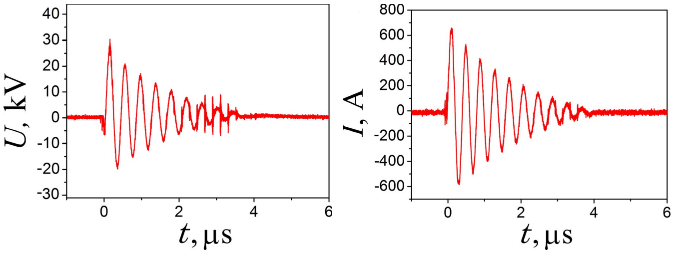

(a) (b)

Fig. 4. Oscillograms of (a) voltage and (b) total current pulse in the discharge.

Fig. 5. Photograph of integral lighting for one pulse of a multigap electric discharge on the obverse side of the applicator.

appears in the applicator, when the voltage pulse is supplied to the applicator electrodes. The discharge channels are connected in series. Fig. 4(a) and (b) shows the oscillograms of the voltage and the total discharge current. The photograph of the discharge in the applicator in the air at atmospheric pressure is shown in Fig. 5.

The larger diameter of the applicator cone is 80 mm, so that the applicator fits a standard Petri dish (Fig. 6). The discharge plasma is 25 mm from the bottom of the Petri dish. (Its diameter is 80 mm.) It influences on the objects in the Petri dish with UV radiation and charged particles flows. In the group of 100 mustard seeds (placed in one layer in the Petri dish), each seed is almost uniformly irradiated with the plasma particles and the photons of UV radiation.

Let us describe the characteristics of a multigap electric discharge measured earlier: electric energy of the pulse is 0.35 J, flow of the generated UV radiation is 0.5×10^{21} photon/s, and maximum intensity of the radiation is 6.3 kW with the average wavelength of 250 nm.

III. UV-Irradiation Regime, Method of Seeds Germination, and Method of Mathematical Analysis of Scarification

A. Seeds UV-Irradiation Regime

Fig. 6. Photograph of the applicator with the Petri dish.

The following regime of the seed treatment was realized with the "game" generator. All the seeds of the treated group were placed into the Petri dish and were subject to the influence of UV radiation and plasma. The working regime of the generator was 15 min with pulse frequency of 1 Hz. There were several control groups—they were not treated.

Just before the germination, the seeds were kept 5 min at a temperature of 13 °C.

B. Method of Seed Germination

To study the germination time, a well-known method of the seed germination in filtering paper rolls was selected [24], [33], [34]. The seeds of each group were put in line between the sheets of the filtering paper, which were rolled up in small loose rolls. The rolls were put in a tank containing some water (up to 1 cm); the seeds were incubated keeping constant conditions (18 °C).

The germinated seeds were calculated each 4 h during 10 days excluding night time. It is necessary to note that correct time interval for calculation of the germinated seeds is important. In [35]–[38], the calculation was carried out each 24 h or even more. Such a large interval does not allow plotting the growth curves with precision necessary for registration of the multistage germination rate functions.

The flax sprouts of these experiments are shown in Fig. 7.

C. Method of Mathematical Analysis of Scarification

The values of W (the amount of germinated seeds in different groups, in which the seeds were treated in the same way) obtained at the same time moment t were averaged, and the mean deviations were found. The obtained points were plotted as $W(t)$. The experimental points were approximated using a model function—the Gompertz growth function [39]

$$W = A \exp[-b \exp(-kt)] \tag{1}$$

where the parameter A determines the number of the germinated seeds, parameter k determines the growth curve slope,

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Fig. 7. Flax sprouts.

and the parameter b is not important from biological point of view—it determines the position of time reference point relatively to zero. The parameters must be calculated.

It is specified in [29] that the function (1) is the most preferable one for the approximation and the analysis of the seed germination dynamics. Roy *et al.* [37] and Šerá *et al.* [40] use another function—the Richard function. The Richard function has four independent parameters, whereas the Gompertz function has only three parameters. That is why, the Gompertz function is easier to use. Effort gain is noted at numerical approximation of multistage growth functions.

To find the values A, b, and k in this paper, we used approximation of the experimental values in the points (based on the least-squares method). Knowing A, b, and k, one can calculate time characteristics of the germination.

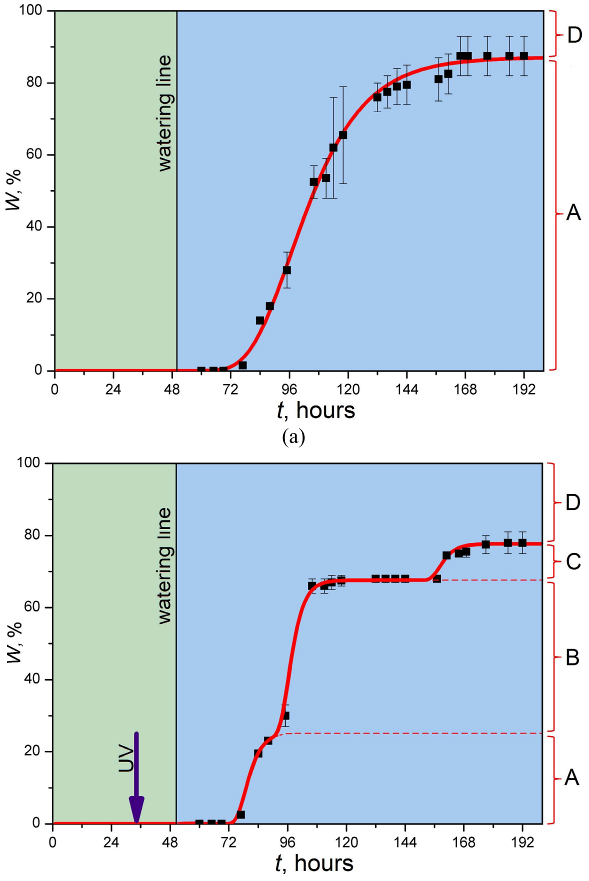
In the experimental physics, it is appropriate to determine the beginning of the pulsed transient process with the amplitude A on the level $\alpha_1 = 0.1$, and the end of the process—on the level $\alpha_2 = 0.9$ [41]. This approach was used here for calculation of the beginning T_1 and end T_2 times of the germination process using the following formula:

$$T_{1,2} = -\frac{1}{k}\ln\left(-\frac{\ln\alpha_{1,2}}{b}\right). \qquad (2)$$

IV. EXPERIMENTAL RESULTS

Fig. 8(a) and (b) shows experimental data of germination of the control seeds and seeds after treatment with plasma UV radiation. In this case, the error of each point was determined by averaging and dispersion on all control groups of the seeds and on all groups of treated seeds. After mathematical analysis, we found that germination rate of the flax control seeds is approximated by one Gompertz function (1); growth of treated seeds—by the sum of three Gompertz functions in a way that the summarized growth function has the view of three-step function

$$W = A_A\exp[-b_A\exp(-k_At)] + A_B\exp[-b_B\exp(-k_Bt)] + A_C\exp[-b_C\exp(-k_Ct)] \qquad (3)$$



(a)

(b)

Fig. 8. Diagrams of flax seed germination rate functions. (a) Control seeds. (b) Seeds treated with UV radiation (A, B, C, and D—subgroups and arrow UV—time moment of the seeds irradiation).

TABLE I
RESULTS OF THE GROWTH FUNCTION APPROXIMATION

Regime		A, %	b	k, h^{-1}	T_1, h	T_2, h
Control	A	87.1	324.4	0.060	82.5	133.9
	D	12.9	–	–	–	–
UV	A	25.1	$1.7\cdot10^9$	0.270	75.6	87.1
	B	42.5	$5.1\cdot10^{11}$	0.280	93.3	103.3
	C	10.1	$0.9\cdot10^{19}$	0.276	155.1	166.3
	D	22.3	–	–	–	–

where A, B, and C—the indices 9. (Meaning of the indices is presented below). The obtained values $A_{A,B,C}$, $b_{A,B,C}$, $k_{A,B,C}$, $T_{A,B,C}$, and $T_{A,B,C}$ are presented in Table I. One can easily see that influence of the plasma UV radiation causes accelerating effect on germination of the majority seeds. However, some parts of the seeds die after irradiation.

Differentiation of the growth functions with respect to time $V = (dW/dt)$ shows that the velocity-growth function of some control seeds represents a monopulse curve [Fig. 9(a)], whereas the velocity-growth function of the irradiated seeds takes place in three-pulse mode [Fig. 9(b)]. In this case, the amplitude of the velocity rate of the treated seeds is twice higher than that of the control seeds.

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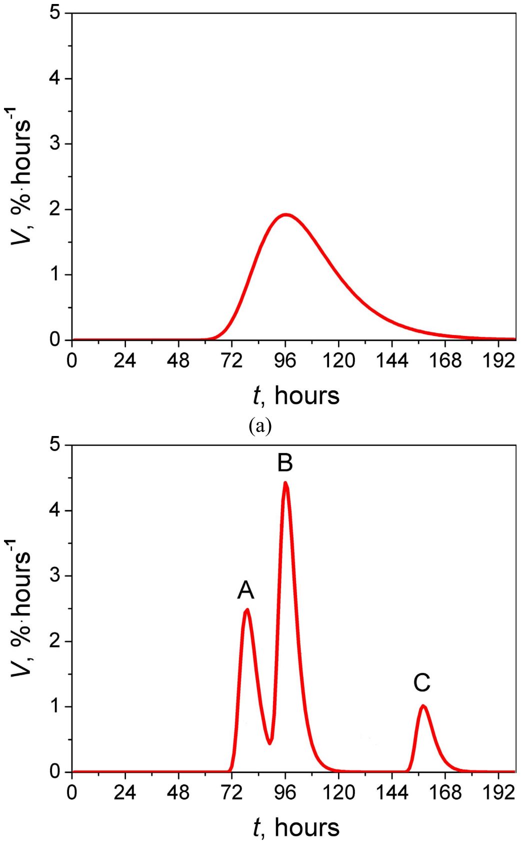

(a)

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Fig. 9. Diagrams of the flax seeds velocity-growth function. (a) Control seeds. (b) Seeds treated with UV radiation (A, B, and C—subgroups).

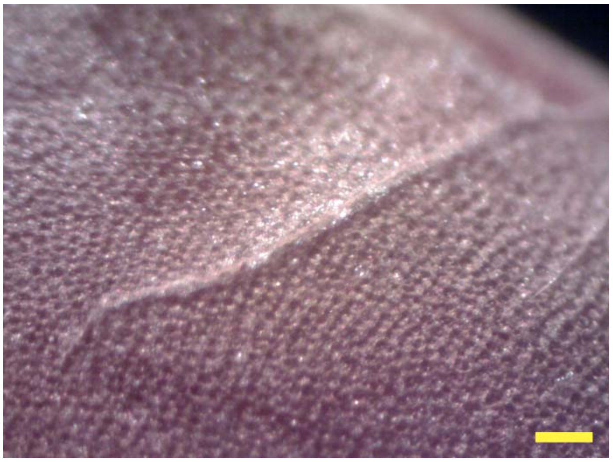
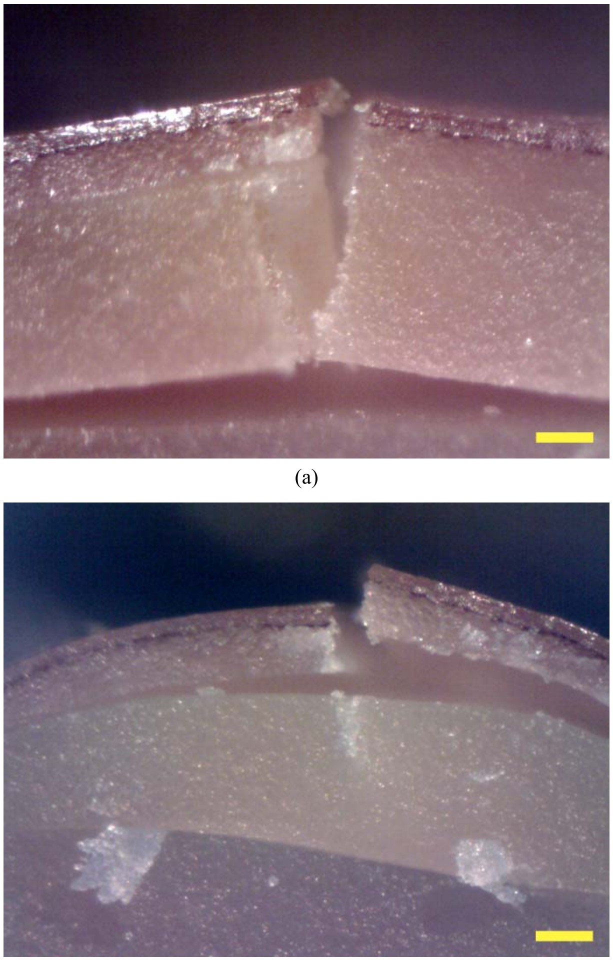


Fig. 10. Photograph of the crack on the flax seed surface after UV irradiation. (Scale bar is 100 μm.)



(a)

(b)

Fig. 11. Photographs of the cracks cross section of UV-irradiated flax seeds. (Scale bars are 100 μm.) (a) Crack in the seed of subgroup A. (b) Crack in the seed of subgroup B.

V. INTERPRETATION OF EXPERIMENTAL RESULTS

Let us interpret two-pulse velocity mode (found in [26]) and three-pulse velocity mode found in this paper.

It seems as if all seeds of one group are in similar irradiation conditions, all of them should germinate identically in the monopulse mode. However, the seeds of one group have different layer thicknesses and they are suffered with different UV-radiation dose. This causes cracks formation on the surfaces of some seeds of the group; other seeds of the same group may not have cracks. Image of typical crack (appeared under the influence of UV irradiation) on the surface of the flax seed is shown in Fig. 10. Earlier, the cracks on the surfaces of treated seeds with the plasma were observed in [27], [29], and [42].

We cut each seed across the crack and found that the following situations are possible (for the flax seeds with two-layer coat).

Subgroup A: The cracks exist; they completely pass though the spermoderm and endosperm and reach the cotyledon [Fig. 11(a)].

Subgroup B: The cracks exist; they completely pass though the spermoderm, but do not pass through the endosperm [Fig. 11(b)].

Subgroup C: There are no cracks or their depth is much smaller than the spermoderm thickness.

The specified situation splits the seed group into three subgroups—A, B, and C, respectively. Germination time of the seeds of each group is different. It is possible to separate the fourth subgroup D—dead seeds.

So, the seeds of subgroup A germinate earlier than others since moisture penetrates through the crack to the cotyledon for several minutes; the seeds of subgroup B germinate slower

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than the seeds of subgroup A since moisture passes through the crack in the spermoderm during 1–2 min and then slowly diffuses through the endosperm. Germination of the seeds from subgroup C requires more time that is necessary for moisture diffusion through the spermoderm and endosperm. In all cases, the difference $T_2 - T_1$ is the length of the germination process.

According to Table I, one can calculate average time of natural (diffusive) moisture penetration through the spermoderm $T_s = T_{1\mathrm{C}} - T_{1\mathrm{B}} - T_0$, where $T_0 = 50$ h is watering time; average time of natural moisture penetration through the endosperm is $T_e = T_{1\mathrm{B}} - T_{1\mathrm{A}} - T_0$. Calculation results are: $T_s = 11.8$ h and $T_e = 17.7$ h. In reality, if someone scarifies the seed surfaces, these times could be saved at flax seed germination. Mustard seeds have one coat; that is why only two-stage velocity mode could be realized. This was observed in [28].

VI. CONCLUSION

This paper presents investigation of effect of high-power UV-radiation plasma, generated by electric discharges, on germination rate of Altaic flax seeds (*Linum altaicum*). The results are the following.

1) UV radiation scarifies flax seeds by means of cracks formation in the seeds coat.
2) The cracks decrease time of moisture penetration to the seed bud; this leads to increase of the germination velocity rate (more than twice).
3) The growth function has three-stage character; the velocity-growth function has three pulses, which split the seed groups into three subgroups.
4) Splitting of the flax seed groups into subgroups is stipulated by existence of two-layer coat and probabilistic nature of the cracks formation.
5) Average times of natural moisture penetration through the flax seed coat are calculated: $T_s = 11.8$ h and $T_e = 17.7$ h. In reality, if someone scarifies the seed surfaces, these times could be saved at flax seed germination.
6) Loss of some seeds due to their death under the influence of high-power UV radiation is possible. So, it is necessary to search more spare regime for seeds UV irradiation.

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