THE EFFECTS OF ULTRASOUND EXPOSURE ON THE GERMINATION CAPACITY OF BIRDSFOOT TREFOIL (*LOTUS CORNICULATUS* L.) SEEDS

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Abstract. The present study investigates the influence of ultrasound exposure on the germination capacity of the seeds of the plant *Lotus corniculatus* L. The seeds were grouped in four size categories according to their diameter: mixed sizes, small seeds, medium seeds and large seeds. Ultrasounds were applied to aliquots of about 100 seeds. For ultrasound treatment, the seeds were transferred into a sample holder, placed in contact with the magnetostrictive transducer of an ultrasound generator of fixed frequency, $f = 20$ kHz, and adjustable power. Each group was exposed to various ultrasound intensities, ranging from 0.11 W/cm^2 to 2.72 W/cm^2, and various exposure times, ranging from 10 seconds to 160 seconds. Following ultrasound treatment, the germination capacity of the seeds was evaluated after 21 days, 31 days and 137 days. We have also performed an extensive statistical evaluation of the obtained data. Multiple quadratic correlations have been established between the physical parameters of the applied ultrasounds and the germination capacity of the seeds. Based on these correlations, the optimal exposure conditions were identified for each size category. According to the analyis, for medium-sized seeds the largest germination capacity (86.11%) is obtained for an ultrasound intensity of 1.46 W/cm^2 applied for 72.3 seconds. For large seeds the best germination capacity (81.85%) is obtained for an ultrasound intensity of 1.79 W/cm^2 applied for 62.3 seconds.

Key words: ultrasounds, germination, quadratic correlations, *Lotus corniculatus* L.

INTRODUCTION

In the agro-environmental farming system, the controlled use of some physical factors on the biological development of crops is a modern method for achieving high yields [1, 7]. For safety reasons, the use of physical factors is preferred instead of chemical treatments, which may contaminate the plant of interest.

Ultrasounds are mechanical waves of a frequency $f \geq 20\ \text{kHz}$, which is too high for the human ear to detect. Ultrasounds have a vast range of applications in materials science, medicine, biotechnology and industry [7,10,13].

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Research on the benefits of ultrasounds in agrobiology dates back to more than half a century [3], but it is still highly attractive [4, 7]. Ultrasounds were found to improve the morphophysiological and biochemical parameters of plants [2, 11, 14]. Treatment with ultrasound at $f = 28$ kHz of a suspension culture of rice callus (*Oryza sativa* L., 'Nipponbare') cells stimulated the growth and proliferation of the cells, as evaluated by colorimetric methods and by fresh weight measurements. Optimal stimulation was found when ultrasounds were applied for 5 seconds; a more prolonged ultrasonic agitation inhibited cell growth and proliferation [9]. Low-intensity ultrasound treatment stimulated the germination of seeds of several plant species: barley [15,16], carrot [2], chickpea [4], corn [6], fodder beans [11], lentils [1], Norway spruce [5], pepper [4], radish [12], watermelon [4], and wheat [1,4]. Comparison of these results, however, is difficult because they were obtained at different frequencies, different ultrasound intensities and different durations of the treatment.

Research has shown that stimulation of seed germination by ultrasound is due to increased activity of enzymes, such as alpha-amylases, participating in the metabolism of maltose and maltodextrines [4, 8, 16, 17]. Ultrasonic stimulation was also associated with enhanced fluidity of the cell wall and cell membrane [9]. Adverse effects were ascribed to impaired enzymatic activity caused by the disruption of structural elements of the cells, such as membranes and the cytoskeleton [9].

Birdsfoot trefoil (*Lotus corniculatus* L.) is a plant of importance for the nutrition of animals, widespread in the natural vegetation of Romanian grasslands, but its seeds have low germination capacity. This paper presents the influence of low frequency, low intensity ultrasound treatment on the germination capacity of seeds of Bird's-foot trefoil (*Lotus corniculatus* L).

MATERIALS AND METHODS

Before ultrasound treatment, bird's-foot trefoil seeds were classified into four size groups: ungrouped seeds (group **a1**); small seeds, from 1.0 mm to 1.4 mm in diameter (group **a2**); medium seeds, from 1.4 mm to 1.5 mm in diameter (group **a3**); and large seeds, from 1.5 mm to 1.6 mm in diameter (group **a4**). To separate the seeds of the three size groups (**a2**, **a3**, and **a4**), a set of four sieves was used.

Before being placed in Petri dishes to study their germination, the seeds were treated with ultrasound under ten different conditions depending on the intensity of the beam of ultrasounds and on the duration of the treatment. For a given exposure time of 60 s, five values of the ultrasound intensity have been used (0.11 W/cm², 0.44 W/cm², 0.98 W/cm², 1.74 W/cm², and 2.72 W/cm²). Then, for an intermediate value of the ultrasound intensity, of 1.74 W/cm², five different exposure times were considered (10 s, 20 s, 40 s, 80 s, and 160 s).

The experimental device that generated the ultrasounds (Fig. 1A) was built at the "Politehnica" University of Timisoara, Romania. The instrument consists in a generator with continuously adjustable power (ranging from 0 W to 60 W) and frequency (18 kHz to 25 kHz), equipped with a transducer.

The ultrasound transducer (Fig. 1B) is based on magnetostriction, the deformation of ferromagnetic materials as a result of their magnetization. When the magnetic flux generated by the current passing through the winding (4 on Fig. 1B) penetrates the ferrite cores (3 on Fig. 1B) and capsule (2 on Fig. 1B), their length changes. When the current is oscillatory, so is the magnetic flux and the corresponding change in length. Thus, mechanical vibrations emerge, whose frequency is equal to the frequency of the electric current oscillations. In the present study, the frequency of the generator was set to 20kHz, which is the lower limit of the ultrasound domain. The resulting ultrasounds penetrate the aluminum sample holder (1 on Fig. 1B), in which the seeds are placed.

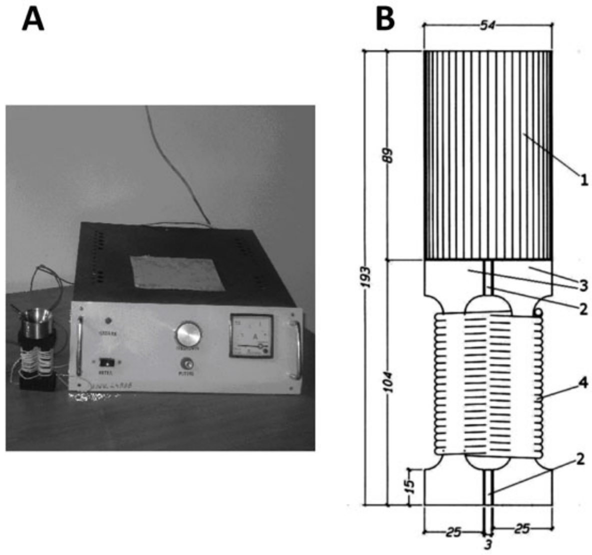

Fig. 1. The picture of the experimental device (**A**) and the scheme of the magnetostrictive ultrasound transducer (**B**). In panel **B**, the aluminum sample holder (1) is in contact with the ferrite capsule (2) placed between the I-profile ferrite cores (3) of the winding (4) that generates the magnetic flux when current flows through it.

To assure an unbiased, random choice of the seeds to be treated, for each size group, we have built plastic cups that accommodate about 100 seeds. Thus, for each treatment, a cup of seeds has been placed in the sample holder.

Five distinct experiments were done for each of the ten exposure protocols, and also for the control (untreated) seeds. Such control experiments were done for each size group and were included in the statistical analysis.

After ultrasound treatment, the seeds were placed in plastic petri dishes, on humidified paper towels, and were observed for five months, under laboratory conditions, at temperatures between 22 ºC and 25 ºC, at constant humidity, and natural light.

RESULTS AND DISCUSSION

At all time intervals chosen for the determination of germination (21 days, 31 days, and 137 days), the effect of ultrasound treatment on *Lotus corniculatus* L. seed germination manifested mostly on medium-sized seeds (group **a3**) and on large seeds (group **a4**).

The results of our quadratic multiple correlation analysis are shown in Fig. 2, panels A, C, and E for medium-size seeds, whereas panels B, D and F refer to large seeds.

The quadratic multiple correlation study indicates that for medium sized seeds (group **a3**) the interaction between the ultrasound field intensity and the duration of their action has led to statistically significant correlations, as revealed by the multiple correlation coefficients: R = 0.77 after 21 days, R = 0.86 after 31 days and R = 0.89 after 137 days. (Correlations are considered statistically significant if the multiple correlation coefficient exceeds the value 0.7.)

In order to optimize the exposure time and intensity of ultrasounds, the technical optimum of these parameters was calculated for the two groups of seeds.

Table 1

Technical optima for medium sized seeds (1.4 mm to 1.5 mm in diameter, group **a3**)

Equation	t [s] optimum	I [W/cm^2] optimum	*Eg/sg* calc.
$Eg = 80.86 - 0.0527x - 2.6459y - 0.0001x^2 + 0.0502xy - 0.4103y^2$ $R = 0.74$	74.73	1.347	77.11
$sg_{21\ \text{days}} = 83.54 - 0.0425x - 1.4823y - 0.0004x^2 + 0.0716xy - 1.1319y^2$ $R = 0.77$	61.03	1.275	83.89
$sg_{31\ \text{days}} = 83.72 - 0.0308x - 1.0485y - 0.0006x^2 + 0.0814xy - 1.5598y^2$ $R = 0.86$	62.92	1.306	82.06
$sg_{137\ \text{days}} = 87.66 - 0.0432x + 0.019y - 0.0006x^2 + 0.0887xy - 2.1953y^2$ $R = 0.89$	72.33	1.465	86.11

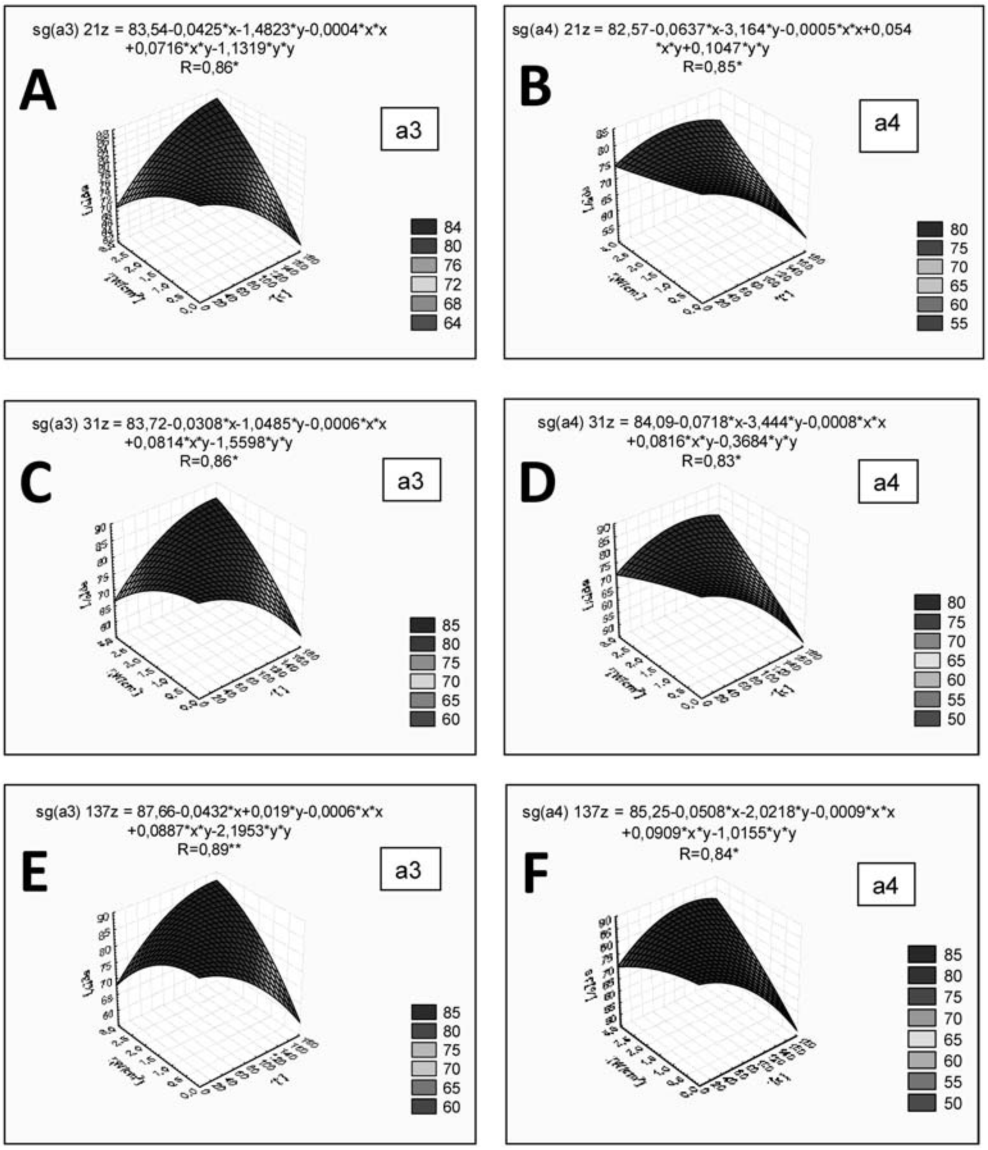

Fig. 2. Quadratic multiple correlations between the duration of ultrasound exposure (*t*[s], on the *Ox* axis), the ultrasound intensity (*I* [W/cm^2], on the *Oy* axis) and the germination capacity (*sg* [%], on the *Oz* axis). Shown are the results obtained after 21 days (A, B), 31 days (C, D) and 137 days (E, F). Panels on the left (A, C, E) refer to medium seeds (1.4 mm – 1.5 mm in diameter, group **a3**), whereas panels on the right (B, D, F) refer to large seeds (1.5 mm – 1.6 mm in diameter, group **a4**).

For medium-sized seeds (group **a3**) the maximum extent of germination is obtained after 137 days (86.11%) when the ultrasonic treatment is applied at an intensity of 1.465 W/cm² for 72.33 seconds. If the germination period is 21 days, the germination calculated value is 83.89%, while the ultrasound intensity is 1.2754 W/cm², and the exposure time is 61.03 seconds. If we consider the power or the germination energy, read at 10 days after placing seeds for germination, we get a germination of 77.11%, when applying ultrasound with an intensity of 1.347 W/cm² for 74.73 seconds (Table 1).

Also in the case of large seeds (group **a4**), we have found a close relationship between the physical parameters (ultrasound intensity and duration of exposure) and seed germination, as quantified by the multiple correlation coefficients: $R = 0.85$ after 21 days, $R = 0.83$ after 31 days and $R = 0.84$ after 137 days (see Fig. 2, panels B, D, F, and Table 2).

For the large seed group (**a4**) the technical optimum of the parameters characterizing the ultrasonic field differs from the optimal parameters obtained for medium-sized seeds. Indeed, for large seeds, the best germination capacity 81.85% is obtained if an ultrasound beam of 1.79 W/cm^2 in intensity is applied to the seeds for 62.3 seconds (Table 2).

Table 2

Technical optima for large seeds (1.5mm to 1.6 mm in diameter, group **a4**)

Equation	*t* [s] optimum	*I* [W/cm^2] optimum	*Eg/sg* calc.
$Eg = 80.65 - 0.04x - 5.89y - 0.0005x^2 + 0.0512xy + 0.9286y^2$ $R = 0.76$	50.70	1.770	74.40
$sg_{21\ \text{days}} = 82.57 - 0.0637x - 3.164y - 0.0005x^2 + 0.054xy + 0.1047y^2$ $R = 0.85$	50.39	2.113	77.62
$sg_{31\ \text{days}} = 84.09 - 0.0718x - 3.444y - 0.0008x^2 + 0.0816xy - 0.3685y^2$ $R = 0.83$	60.95	2.075	78.33
$sg_{137\ \text{days}} = 85.25 - 0.0508x - 2.0218y - 0.0009x^2 + 0.0909xy - 1.0155y^2$ $R = 0.84$	62.29	1.792	81.85

CONCLUSION

We have investigated the germination of Bird's-foot trefoil (*Lotus corniculatus* L.) seeds after various protocols of ultrasound treatment.

Seeds were grouped according to their size (mixed sizes, small seeds, medium seeds, and large seeds), and ultrasounds with a frequency of 20 kHz were applied to aliquots of about 100 seeds. After treatment, the seeds were cultured under laboratory conditions, and germination was monitored for five months. The data were studied by quadratic multiple correlation analysis. Significant correlations have been found between the physical parameters of the applied ultrasounds (intensity and duration of exposure) and the germination capacity of seeds. As a result of the analysis, the optimal exposure conditions were identified for medium and large seeds.

In agreement with a vast amount of published data regarding different plant species, our study confirms that ultrasound applied in agriculture may contribute to the improvement of agrobiological indicators, including seed germination.

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