Impact of Seed Size and of Ultrasounds on Seed Germination in *Lotus corniculatus* L.

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Abstract
In this paper the authors point out the impact of ultrasounds on seed germination in bird's-foot trefoil (*Lotus corniculatus* L.). The bird's-foot trefoil seeds, grouped into 4 size groups, were exposed to ultrasounds with variable intensities and exposure times ranging between 0.11 and 2.72 W/cm^2 and between 10 and 160 seconds. Research shows that both germinating energy and germinating ability measured at 10 and 21 days, respectively, were positively impacted by ultrasounds. The highest values were when we treated with ultrasounds medium- and large-size bird's-foot trefoil seeds.

Keywords: bird's-foot trefoil seeds, germination, *Lotus corniculatus* L., *ultrasounds*

1. Introduction

Applying ultrasound treatments in agriculture has been a major goal in research: it aims at enhancing bird's-foot trefoil seed germination ability and also plant growth and development during the vegetation period [1-5].
In this paper, we present the effect of ultrasound treatments on bird's-foot trefoil seed germination ability depending on bird's-foot trefoil seed size.

2. Materials and methods

Research was carried out in laboratory conditions on Livada bird's-foot trefoil cultivar seeds. grouped into 4 size groups, depending on bird's-foot trefoil seed diameter, as follows: no group bird's-foot trefoil seeds (a_1) (control), small-size bird's-foot trefoil seeds Ø = 1.0-1.4 mm (a_2), medium-size bird's-foot trefoil seeds Ø = 1.4-1.5 mm (a_3), and large-size bird's-foot trefoil seeds Ø = 1.5-1.6 mm (a_4).
Before setting bird's-foot trefoil seeds in Petri vases, they were treated with ultrasounds of different intensities and exposure durations. as follows: b_1= not treated (control), b_1 (I = 0.11 W/cm^2, t = 60 s); b_2 (I = 0.44 W/cm^2, t = 60 s); b_3 (I = 0.98 W/cm^2, t = 60 s); b_4 (I = 1.74 W/cm^2, t = 60 s); b_5 (I = 2.72W/cm^2, t = 60 s); b_6 (I = 1.74 W/cm^2, t = 10 s); b_7 (I = 1.74 W/cm^2, t = 20 s); b_8 (I = 1.74 W/cm^2, t = 40 s); b_9 (I = 1.74 W/cm^2, t = 60 s); b_{10} (I = 1.74 W/cm^2, t = 80 s); b_{11} (I = 1.74 W/cm^2, t = 106 s).
While treating the bird's-foot trefoil seeds for 21 days, we noted daily the number of germinating bird's-foot trefoil seeds; on the 10th day we measured germinating energy and on the 21st day we measured germinating ability.
The ultrasound device was developed at the Polytechnic University in Timişoara: it is made up of a generator with continuous power and frequency regulator and of a transductor.
Germination data were statistically processed through variance analysis.

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3. Results and discussion

Bird's-foot trefoil seed quality is also the result of size uniformity, which has an impact on germination ability and on sprouting plants share after bird's-foot trefoil seeding.

Research shows that, compared to the control variant (bird's-foot trefoil seeds not treated), germinating energy in bird's-foot trefoil is very much impacted by bird's-foot trefoil seed size class (Figure 1).

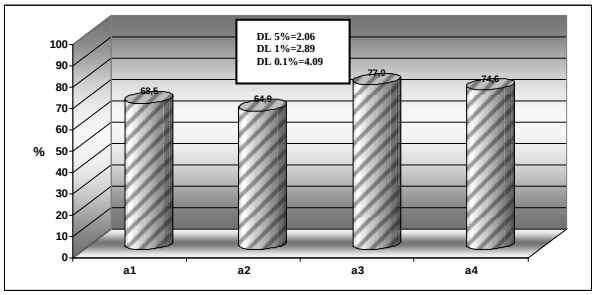

Figure 1. Impact of bird's-foot trefoil seed size on germinating energy in bird's-foot trefoil

Thus, medium- and large-size bird's-foot trefoil seeds resulted in very significant increases in yield, i.e. 12.3% and 8.1%, respectively, more. Small-size bird's-foot trefoil seed group (a_2) resulted in a decrease of the germinating energy of 5.3% compared to the control variant.

The impact of bird's-foot trefoil seed size on germination ability was also obvious on the 21st day of the treatment period. In this case the germinating ability was higher in medium-size bird's-foot trefoil seeds (80.2%) and in large-size bird's-foot trefoil seeds (77.0%) compared to the control variant (76.2%). In the small-size bird's-foot trefoil seed variant, there was a very significant decrease of the germinating ability (6.8%) as in the germinating energy we measured on the 10th day of the trial (Figure 2).

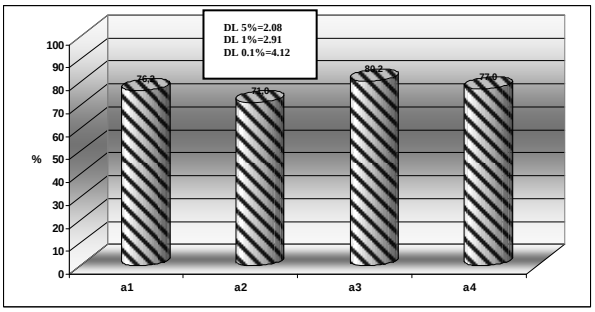

Figure 2. Impact of bird's-foot trefoil seed size on germinating ability in bird's-foot trefoil

Ultrasound treatment impacted bird's-foot trefoil seed germination on both the 10th and the 21st day of treatment, through wave intensity and exposure time. Thus, in germinating energy measured on the 10th day, the following variants of ultrasound treatment resulted in the highest increases of germination level (Table 1).

Table 1. Impact of ultrasounds on germinating energy in bird's-foot trefoil

Variant	EG (%)	Valori relative (%)	Dif. (%)	Significance
b_1	69.0	100	Mt	
b_2	72.4	104.8	3.4	*
b_3	64.7	93.7	-4.3	0
b_4	74.1	107.3	5.1	**
b_5	73.7	106.8	4.7	**
b_6	70.9	102.6	1.9	
b_7	74.6	108.1	5.6	**
b_8	67.0	97.1	-2.0	
b_9	69.3	100.4	0.3	
b_{10}	68.6	99.4	-0.4	
b_{11}	71.1	103.1	2.1	

DL 5%= 3.34; DL 1%= 4.41; DL 0.1%=5.67

- The variant W/cm^2 = 1.74 and t = 60 s had a generating energy of 74.1%, i.e. 7.3% more than the control variant (an increase in yield statistically ensured);
- The variant W/cm^2 = 1.074 and t = 20 s resulted in a germinating energy of 74.6%, i.e. 8.1% more than the control variant (an increase in yield statistically ensured).

Results concerning germinating energy show that the impact of ultrasounds is proportional not only to intensity, but also to bird's-foot trefoil seed exposure time.

Germinating ability in bird's-foot trefoil seeds determined on the 21st day was positively impacted in all the variants. The highest values (78.0% and 77.9%) were in the variants with the highest intensities (1.74 and 2.72 W/cm^2), for the same exposure time (60 s), increases in growth in these variants are distinctly significant. The increase of the exposure time (from 10 to 160 s) at the same intensity level (1.74 W/cm^2) resulted in no statistically ensured increases compared to the control variant (Table 2).

Table 2. Impact of ultrasounds on germinating ability in bird's-foot trefoil

Variant	FG %	Valori relative	Dif. (%)	Significance
b_1	72.3	100	Mt	
b_2	74.1	102.3	1.8	
b_3	76.2	105.4	3.9	*
b_4	77.9	107.7	5.6	**
b_5	78.0	107.9	5.7	**
b_6	74.8	103.4	2.5	
b_7	75.6	104.5	3.3	
b_8	75.5	104.4	3.2	
b_9	74.4	102.9	2.1	
b_{10}	75.5	104.4	3.2	
b_{11}	75.0	104.4	2.7	

DL 5%= 3.47; DL 1%= 4.59; DL 0.1%= 5.9

The most interesting outputs of the ultrasound treatment were the result of the interaction between ultrasound treatment and bird's-foot trefoil seed size groups. The strongest impact, both in germinating energy and in germinating ability, of ultrasound treatments was in medium-size bird's-foot trefoil seeds where, in all variants, there were significant growth increases: between 3.5 and 10.7% in germinating energy and between 5.4 and 11.1% in germinating ability (Tables 3 and 4).

In the no group bird's-foot trefoil seed group (control), ultrasound treatment I = 1.74 W/cm^2 and t = 60 s had a positive effect on both germinating energy and germinating ability.

In the small- and very small-size bird's-foot trefoil seed group, ultrasound treatments are efficient when I = 2.72 W/cm^2 and t = 60 s.

Table 3. Impact of the interaction between bird's-foot trefoil seed size and ultrasound treatments on germinating energy

Factor A	Factor B	EG (%)	Valori relative	Dif. (%)	Significance
a_1	b_1	68.5	100	Mt	
a_1	b_2	70.5	102.9	2.0	
a_1	b_3	52.2	76.2	-16.3	000
a_1	b_4	74.5	108.7	6.0	***
a_1	b_5	71.7	107.6	3.2	**
a_1	b_6	68.0	99.3	-0.5	000
a_1	b_7	76.1	111.1	7.6	***
a_1	b_8	64.7	94.4	-3.8	00
a_1	b_9	64.2	93.7	-4.3	000
a_1	b_{10}	65.2	95.2	-3.3	00
a_1	b_{11}	71.5	104.4	3.0	00
a_2	b_1	63.2	100	Mt	
a_2	b_2	68.1	107.7	4.9	***
a_2	b_3	52.7	83.4	-10.5	000
a_2	b_4	66.3	104.9	3.1	00
a_2	b_5	69.1	109.3	5.9	***
a_2	b_6	63.5	100.5	0.3	
a_2	b_7	67.6	106.9	4.4	***
a_2	b_8	60.8	96.2	-2.4	0
a_2	b_9	67.0	106.0	3.8	**
a_2	b_{10}	61.0	96.5	-2.2	
a_2	b_{11}	67.8	107.3	4.6	***
a_3	b_1	70.9	100	Mt	
a_3	b_2	76.2	107.4	5.3	***
a_3	b_3	78.5	110.7	7.6	***
a_3	b_4	78.2	110.2	7.3	***
a_3	b_5	77.6	109.4	6.7	***
a_3	b_6	75.7	106.8	4.8	***
a_3	b_7	77.6	109.4	6.7	***
a_3	b_8	73.4	103.5	2.5	*
a_3	b_9	74.2	104.6	3.3	**
a_3	b_{10}	76.7	108.2	5.8	***
a_3	b_{11}	77.5	109.3	6.6	***
a_4	b_1	73.7	100	Mt	
a_4	b_2	74.9	101.6	1.2	
a_4	b_3	75.7	102.7	2.0	
a_4	b_4	77.6	105.3	3.9	***
a_4	b_5	76.6	103.9	2.9	*
a_4	b_6	76.4	103.4	2.7	*
a_4	b_7	77.4	105.0	3.7	**
a_4	b_8	69.4	94.2	-4.3	000
a_4	b_9	72.0	97.6	-1.7	
a_4	b_{10}	71.7	97.3	-2.0	
a_4	b_{11}	67.8	92.0	-5.9	000

DL 5%= 2.25; DL 1%= 2.97 ; DL 0.1%= 3.82

Table 4. Impact of the interaction between bird's-foot trefoil seed size and ultrasound treatments on germinating ability

Variant	Seeds germinate (%)	Dif. (%)	%	Significance
$a_1 b_1$	71.6	Mt	100	
$a_1 b_2$	72.0	0.4	100.6	
$a_1 b_3$	75.4	3.8	105.3	*
$a_1 b_4$	79.0	7.4	110.3	***
$a_1 b_5$	81.3	9.7	113.5	***
$a_1 b_6$	72.5	0.9	101.2	**
$a_1 b_7$	77.1	5.5	107.7	
$a_1 b_8$	74.3	2.7	103.8	
$a_1 b_9$	73.2	1.6	102.2	
$a_1 b_{10}$	73.3	1.7	102.4	
$a_1 b_{11}$	79.4	7.8	110.9	***
$a_2 b_1$	67.6	Mt	100	
$a_2 b_2$	69.2	1.6	102.3	
$a_2 b_3$	70.5	2.9	104.3	
$a_2 b_4$	71.6	4.0	105.9	*
$a_2 b_5$	73.4	5.8	108.5	**
$a_2 b_6$	68.9	1.3	101.9	
$a_2 b_7$	68.6	1.0	101.4	
$a_2 b_8$	68.2	0.6	100.9	
$a_2 b_9$	73.3	5.7	108.4	**
$a_2 b_{10}$	69.2	1.6	102.3	
$a_2 b_{11}$	72.6	5.0	107.4	*
$a_3 b_1$	74.3	Mt	100	
$a_3 b_2$	79.2	4.9	106.5	*
$a_3 b_3$	80.8	6.5	108.7	***
$a_3 b_4$	81.5	7.2	109.6	***
$a_3 b_5$	79.8	5.5	107.4	**
$a_3 b_6$	78.8	4.5	106.0	*
$a_3 b_7$	78.7	4.4	105.9	*
$a_3 b_8$	79.6	5.3	107.1	**
$a_3 b_9$	78.3	4.0	105.4	*
$a_3 b_{10}$	82.6	8.3	111.1	***
$a_3 b_{11}$	79.2	4.9	106.5	**
$a_4 b_1$	75.8	Mt	100	
$a_4 b_2$	75.6	-0.2	99.7	
$a_4 b_3$	77.8	2.0	102.6	
$a_4 b_4$	79.1	3.3	104.3	
$a_4 b_5$	77.5	1.7	102.2	
$a_4 b_6$	78.5	2.7	103.5	
$a_4 b_7$	77.9	2.1	102.7	
$a_4 b_8$	79.6	3.8	105.0	
$a_4 b_9$	72.6	-3.2	95.7	
$a_4 b_{10}$	77.0	1.2	101.6	
$a_4 b_{11}$	68.8	-7.0	90.7	000

DL 5%= 3.41; DL 1%= 4.52; DL 0.1%= 5.86

4. Conclusions

Ultrasound treatment has a positive impact on both germinating energy and on germinating ability in bird's-foot trefoil depending on bird's-foot trefoil seed size.

The highest values of bird's-foot trefoil seed germination were when we treated with ultrasounds medium- and large-size bird's-foot trefoil seeds.

The effect of ultrasounds does not directly correlate with the increase of the two parameters – wave intensity and exposure time.

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