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COMPARATIVE STUDY OF SPECIES OF THE GENUS *LUPINUS* ON THE SOWING QUALITIES OF SEEDS DURING THEIR TREATMENT WITH ULTRASOUND

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Abstract

The main purpose of the present study was to determine the effect of ultrasound treatment of seeds of different lupine species of the genus Lupinus on their sowing qualities. The experiments were carried out with seeds of two species of lupine (Lupinus polyphyllus Lindl.) and tarwi (Lupinus mutabilis Sweet.). The treatment of the seeds was performed with ultrasound, testing the following sound durations: 3, 6 and 9 minutes. Germination energy, germination, mean germination time and uniformity of germination, length of hypocotyls and embryo root, fresh and dry matter of seedlings were studied. Sonication with a duration of 6 minutes causes the highest increase of germination. As a result of treatment, the fresh weight of seedlings also increases. Polynomial regressions with high coefficients of determination about the effect of ultrasound on germination energy and germination were found. A higher effect was observed in the species Lupinus polyphyllus Lindl. It is recommended to improve the sowing qualities of lupine seeds to apply the sonication of 6 minutes.

Key words*: germination, seeds, ultrasound, dry matter, embryo root.*

INTRODUCTION

The genus *Lupinus* is one of the most widespread in natural conditions, characterized by a great diversity of species. It is found mainly in the Mediterranean, as well as in North and South America. Except for food, it is also widely used for decorative purposes (Wolko et al., 2011). According to Aniszewskiet al. (2001) did not find a significant difference in the seeds of ornamental species compared to the others. The authors emphasized that one of the widely used species in floriculture, such as *Lupinus polyphyllus* Lindl. is the result of mixing different species and botanical forms, which determines its great diversity in flowering and the shape and color of the seeds.

Other researchers report that these species are characterized by great adaptability, especially in the time of seed development, which explains the wide application of propagation by seed in ornamental horticulture (Sõber and Ramula, 2013). The great plasticity of the species of the genus *Lupinus*, as well as the diversity in their decorative manifestations, represents significant opportunities to expand the range of ornamental plants and to enrich the cultivated flora (Pantsyreva, 2019). Yves et al. (2020) observed uneven germination and sprouting of lupine seeds, especially *Lupinus polyphyllus* Lindl. and reported that their viability depends on both factors climatic conditions and the method of cultivation as well as their stage of development.

About the improvement of the germination processes of the seeds, various methods and ways of exposure with physical agents are applied, as one of the most widely used in the treatment with ultrasound (Panayotov, 2015). Sonication is an inexpensive and easy-to-apply way to treat seeds and thus the water uptake is accelerating, the seed porosity increases and the metabolic processes are improved (Hielscher Ultrasound Technology, 2015). When applying temperature treatment, as one of the physical methods, Carl et al. (2011) found a species response to improvement of the germination in different members of the genus *Lupinus*. Guo and Pan (2016) found that after exposure to ultrasound on lupine seeds, their viability, germination and vigour are improved and at the same time the soluble protein content is increased. Aguilar-Acosta et al. (2020) point out that the effect of ultrasound application depends on the lupine species. In *L. mutabilis*

L. the improvement in the seed status is significant and the increase in the protein content is higher, while in *L. mutabilis* L. there are almost no differences.
The main purpose of the present study was to determine the effect of ultrasound treatment of seeds of different lupine species of the genus *Lupinus* on their sowing qualities.

MATERIALS AND METHODS

The experiments were carried out in the Scientific laboratory of the Department of Horticulture at the Agriculture University-Plovdiv- Bulgaria. Seeds from two species of lupines (*Lupinus polyphyllus* Lindl.) and tarwi (*Lupinus mutabilis* Sweet.) were subjected to ultrasonic treatment. The different periods from 3 minutes, 6 minutes and 9 minutes of sonic were applied, as control was not treatment seeds (0 minutes). The apparatus Ultrasonic Water Bath "Nahita" model 620-1 with the capacity of 0.6 L, frequency of 50 Hz and total power (220-240 V) was used.
After treatment, the seeds were placed to analyze their sowing parameters. The germination energy (first cont) and germination (final count) were determined according to ISTA Rules (ISTA, 2013) in four replicates each from 100 seeds. The mean germination time (MGT) by the method of Battle and Whittington and uniformity of germination by the method of Strona, both described in Panayotov (2015) were calculated. The length of embryo root and length of hypocotyls on 15 seedlings of each replicate as well as fresh and dry weight of each developed seedlings was measured on the day when the germination was established. The dry weight was determined by the method described in detail by Georgiev et al. (1980).
The dispersion analysis of data (ANOVA) and regression analysis were processed (Foel and Cohen, 1992).

RESULTS AND DISCUSSIONS

Germination energy (Table 1) is one of the main signs that describe the possibility for earlier and faster germination of better seeds with the highest viability (Black et al., 2008). Strong differences in this behavior between both investigated lupine species were registered. The germination energy was higher in the seeds of *Lupinus mutabilis* Sweet., two to three times more, in comparison with other species. Application of ultrasonic wave also provokes higher effect in this species and maximum was in variant 3 minutes -35.0% after that decrease. In *Lupinus polyphyllus* Lindl. however, with an increase in the time of treatment, the values gradually increase and reached 30.0 % in variant 9 minutes. In this variant, but in other species, a considerable reduction towards the control with 8.33% was observed. There is a statistical significance of differences between the variants. Polynomial regression for the influence of studied ultrasound treatment was established (Figures 1 and 2) The determination coefficients are high $R^2 = 0.98$ and $R^2 = 0.99$ in *Lupinus polyphyllus* Lindl. and *Lupinus mutabilis* Sweet., respectively. This indicates that around 99% of the treatment of the above-mentioned tendency will be obtained.
Data for germination provide essential information on the seed status and this identifies this indicator as one of the most important elements for the qualification of the sowing material (Copeland and McDonalds, 2001). The germination in *Lupinus mutabilis* Sweet., regardless of the higher germination energy, was much lower than in the other lupine. In both species it increases in the application of the ultrasonic treatment with 6 minutes and the highest values are 78.33 and 35.00 for *Lupinus polyphyllus* Lindl. and other lupines, respectively. The differences between separate variants are small while the variations with control are higher. For the first species (*L. polyphyllus*) they are between 45% (9 minutes) to 46.66% (6 minutes). Each sonication with ultrasound helped for higher germination except for 9 minutes in *L. mutabilis*, when a very small decrease to the control was observed. In this variant for other lupines, the decrease towards the previous variant also has been registered. The recommended duration of sonication for lupine can be pointed out which is 6 minutes. Similar results about the higher effect of 6 minutes sonication s reported also Farahani et al. (2015). The positive effect of ultrasound seeds treatment established also Chilingirov et al. (2018) and Dakova et al.

(2018). The differences between variants and control in *L. polyphyllus* are with statistical significance. The effect of ultrasound treatment on germination also described by polynomial regression (Figures 3 and 4) with a high determination coefficient $R^2 = 0.94$ and $R^2 = 0.99$. This suggests that more than 90% of the application of ultrasound the effect discussed above trend is expected to be obtained.

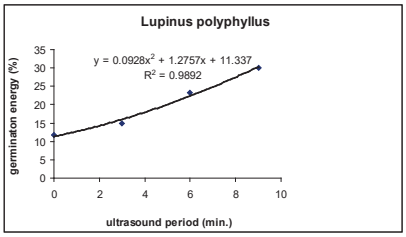

Figure 1. Regression dependence of sonication to germination energy *of L. polyphyllus* seeds

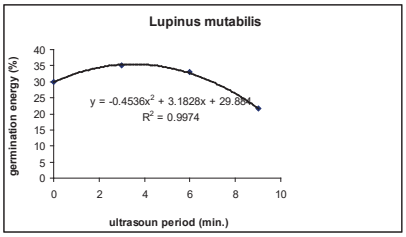

Figure 2. Regression dependence of sonication to germination energy of *L. mutabilis* seeds

Table 1. Viability characteristics of lupinus seed after treatment with ultrasound

Variants	Germination energy (%)		Germination (%)	
	Lupinus polyphyllus	*Lupinus mutabilis*	*Lupinus polyphyllus*	*Lupinus mutabilis*
Control	11.67	30.00	31.67	32.67
3 min	15.00	35.00	76.77	35.00
6 min	23.33	33.33	78.33	35.00
9 min	30.00	21.67	76.67	31.67
LSD p=5.0%	3.2	2.8	6.3	5.2

About a more complete assessment of seed potential Panayotov (2015) recommended evaluating the additional behaviors such as needed days for germination of one seed (MGT) and their uniformity. Table 2 is shown the results for these two parameters. The mean germination time in both investigated species after sonication improves, especially for variant 6 minutes with 1.17 and 0.36 days. The next increase of ultrasound wave decreased this characteristic in comparison with the previous variant, but the data are higher than the control. Better results are obtained for *L. mutabilis*.

A similar trend was observed for uniformity of germination. The highest results also were established for treatment with 6 minutes 10.78% and 14.33% for *L. polyphyllus* and *L. mutabilis*, respectively and after that, the percentage of germinated seeds per day decreased. A small decrease also indicated the seed of *L. mutabilis* in 3 minutes. Most of the differences between the variants and control are statistically significant. López-Ribera and Vicient (2017) also emphasized that the ultrasound improves the sowing parameters of the seeds.

The fresh weight of the seedlings very often use as an indicator for seed vigour index (Panayotov, 2013). The data are presented in Table 3. The higher period for sonication provokes an increase of this index and it reached in 9 minutes to 144.74 mg (*L. polyphyllus*|) and 102.18 mg (*L. mutabilis*), which is more towards the control with 56.58 mg and 34.67 mg, respectively.

The dry weights of the seedlings are characterized by small differences between species as well as investigated variants. It is in diapason 8.95% (control) to 10.92% (6 minutes) and 8.82% (9 minutes) to 9.86% (6 minutes, for first and second species, respectively. The data are mathematical proved.

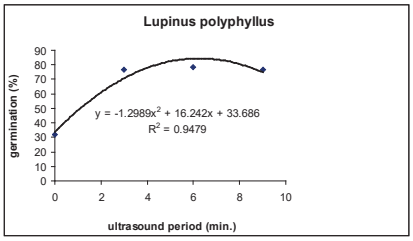

Figure 3. Regression dependence of sonication to germination of *L. polyphyllus* seeds

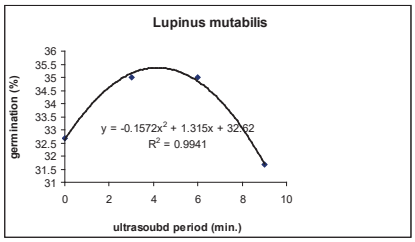

Figure 4. Regression dependence of sonication to germination of *L. mutabilis* seeds

Table 2. Sowing behaviors of lupinus seed after treatment with ultrasound

Variants	Mean germination time (day)		Uniformity of germination (%)	
	Lupinus polyphyllus	*Lupinus mutabilis*	*Lupinus polyphyllus*	*Lupinus mutabilis*
Control	6.87	3.69	5.45	12.08
3 min	8.58	3.77	10.23	10.33
6 min	8.56	4.05	9.31	14.33
9 min	7.16	5.42	10.79	9.72
LSD p=5.0%	1.4	1.9	4.0	3.6

Seedling morphology is also influenced by sonication (Table 4). Species differences are recorded about the length of the embryo root. The effect is higher in *L. polyphyllus*. In this species, the higher period of treatment provokes a decrease while the contrariwise was established for *L. mutabilis*. The highest values are measured in the first species in 3 minutes - 7.30 cm or with 86.70% above the control while for other species it is in 9 minutes - 3.46 cm that is 45.99% more than non-treated seeds.

Table 3. Behaviors of lupinus seedlings after treatment with ultrasound

Variants	Fresh weight (mg)		Dry weight (%)	
	Lupinus polyphyllus	*Lupinus mutabilis*	*Lupinus polyphyllus*	*Lupinus mutabilis*
Control	88.16	67.51	8.95	9.10
3 min	136.78	69.79	9.88	9.18
6 min	142.75	100.33	10.62	9.86
9 min	144.74	102.18	9.99	8.82
LSD p=5.0%	12.1	10.8	1.6	1.2

The lengths of hypocotyl were stimulated highly from treatment with ultrasound. The highest for *L. polyphyllus* was registered in variant 6 minutes - 3.66 cm and for other species - in 3 minutes - 1.81 cm, this is with 50% more than the control seedlings. The strong effect of sonication on the development of seedlings root also reported Machikow et al. (2013). The effect of ultrasound treatment is due to better absorption of nutrients from the endosperm, as well as increased activity of enzymes and other reactions in the seed (Miano et al., 2015).

Table 4. Morphological characteristics of lupinus seedlings after treatment with ultrasound

Variants	Length of embryo root (cm)		Length of hypocotyl (cm)	
	Lupinus polyphyllus	*Lupinus mutabilis*	*Lupinus polyphyllus*	*Lupinus mutabilis*
Control	3.91	2.37	2.34	1.29
3 min	7.30	2.59	3.66	1.81
6 min	5.57	3.42	3.95	1.44
9 min	5.91	3.46	3.48	1.53
LSD p = 5.0%	3.30	1.24	1.6	0.92

CONCLUSIONS

Ultrasound treatments highly improve the viability status and the sowing parameters of lupine seeds and the effect depends on species responses.

The germination energy and germination increase after sonication and the highest values were found in the period of treatment of 6 minutes. This duration of the ultrasound wave is recommended to apply for augmentation of sowing quality of lupine seeds. The seedling morphological behaviors are also with better characteristics.

Effect of sonication on the germination energy and germination of lupine seeds described by polynomial regression with high determination coefficients.

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