DOI: 10.1515/plass-2015-0002

Meisam Nazari¹, Amin Sharififar^{1*}, Hamid Reza Asghari¹

Faculty of Agriculture, Sharood University, Sharood, 36199-95161 Iran;
*ilam_amin@yahoo.com

MEDICAGO SCUTELLATA SEED DORMANCY BREAKING BY ULTRASONIC WAVES

ABSTRACT

In this study dormancy breaking of a hard-coated plant seed, *Medicago scutellata*, was investigated. The ultrasonic waves effect on the seed germination percentage, germination rate, radicle length and stalk length growth was assessed. Six treatments of waves exposure periods including 0, 1, 3, 5, 7, and 9 minutes were tested under laboratorial conditions. Statistical analyses were done at probability level of 0.01. Results revealed that the ultrasonic waves have a significantly positive effect on the seed dormancy breaking, but there was no linear correlation between the increasing times of exposure with any of the growth features. The best treatment for germination percentage and germination rate was the 7-minute one and the 3-minute one was the best for radicle length growth. Treatments of 3, 5 and 7 minutes had the same effect on stalk length growth and were better than all other treatments. The 9-minute treatment had a negative effect, even lessening the growth of all of the assessed features in comparison with the control treatment.

Key words: germination stimulation, *Medicago scutellata*, radicle length, rangeland plants, seed dormancy breaking, stalk length, ultrasonic waves

INTRODUCTION

Dormancy breaking and germination stimulation is important for proliferation and early production of important plants, especially rangeland plants. Because favorable environmental conditions are not always provided for growth of plant seed in the nature. This is particularly important for vigorously germinating seeds such as *Medicago scutellata* seed. Seed germination is the essential period of plant establishment in rangelands. Some different mechanisms (called dormancy) are used by plants to postpone germi-

nation and protect the seeds until the favorable conditions for seedling are provided. Seed dormancy is defined as ways of hindering germination for an intact viable seed. Anyway, when a rangeland seed is planted to grow in man-manipulated rangelands or pastures, the seed dormancy should be broken. This is particularly important when plant proliferation is significantly required for soil conservation, water entrapment and pasture or rangeland rehabilitation. *Medicago scutellata* is an annual legume used for grazing, crop rotations and sometimes cut for hay. This plant has a symbiotic relationship with the bacterium *Sinorhizobium meliloti*, which is capable of nitrogen fixation and therefor can be helpful in nitrogen maintenance in crop rotations. Its germination and retrospective regeneration is sometimes low. It has been recorded in Africa, Australasia, Europe, Middle East and Pacific Ocean, but it is mainly distributed around Mediterranean basin. It grows in neutral to alkaline soils and is classified in the *Fabaceae* family. Detailed information about this plant can be found in the databases: www.ildis.org and www.pasturepicker.com.au.

Many of seeds, which are produced in natural conditions, such as rangelands, show different levels of seed dormancy. Different methods have been applied to overcome the seed dormancy such as salinity, temperature, humidity (Bradbeer, 1998), light, seed scarification (Jun and Tao, 2004), seed stratification (Lindig and Lara-Cabrera, 2004), regulatory hormones (Sozi and Chiesa, 1995) and chemical compounds. Beside technology, development of new techniques are suggested for breaking the seed dormancy such as, Nano-compounds (Azimi *et al.* 2013), ultrasonic waves (Yaldagard, *et al.* 2008) and magnetic water (Fateh *et al.* 2012). Gordon (1971) discussed the usefulness of ultrasound application on plants and stressed the significant benefit of ultrasonic waves on plant material. There is a major research gap in the area of seed germination stimulation and dormancy breaking for rangeland plants, especially through ultrasonic application technique.

Ultrasonic waving technique seems to be easier to apply, safer and healthier than other chemical and physical methods. In the literature, study on dormancy breaking by using ultrasonic waves for rangeland plants seeds are rare.

The objective of this study is to assess the effect of ultrasonic waves periods on seed germination of *Medicago scutellata*.

MATERIALS AND METHODS

Source of seeds

Medicago scutellata seeds were collected from rangelands of Shahrood region (northeast of Iran) with mean altitude of 1500 meters above sea level

and approximate eastern longitude of 54° to 55° and northern latitude of 36° 26' to 36° 45'. The mean annual rainfall of the region is 154 mm and its annual mean temperature is 14.6 °C (according to the 55-year data of Shahrood meteorological station). The seeds showed dormancy and very low germination rates in the field.

Seed preparation

After seed collection, seeds of the same size were selected (not immature and damaged seeds). They were then soaked for 10 minutes in a solution of 1 part sodium hypochlorite (NaOCl 12.5% w/v) and 2 parts water, and subsequently rinsed by distilled water for three times.

Analysis

Experiments were performed in three replicates in sterilized petri dishes under laboratorial conditions. Seeds of each replicate were exposed to ultrasonic waves for different durations independently. The treatments were periods of ultrasonic exposure of 0 min (control), 1, 3, 5, 7 and 9 min. The frequency of waves was 42 kHz. For ultrasonic waves exposing, seeds were soaked in a plastic dish containing distilled water and placed in the ultrasonic apparatus. After waves exposure, seeds were placed on Wathman No.1 filter paper moistened with 10 ml of distilled water in sterilized petri dishes. Three germination experiments were carried out in completely randomized designs in room temperature (25 °C) and darkness. The experiments were performed in March – April 2013. The statistical analyses were done using MSTATC software. The analyses were based on probability level of $P < 0.01$ in the Duncan test.

The evaluated features for assessing the effect of ultrasonic waves on the seed germination and seedling growth were germination percentage, mean germination rate, radicle length and stalk length. The germination percentage was calculated 7 days after sowing and radicles length were measured 14 days after sowing. Stalks length were measured 21 days after sowing.

A seed was considered germinated when the tip of the radicle had grown free of the seed coat (Wiese and Binning, 1987; Auld *et al.*, 1988). The germination percentage (GP) was calculated according to the following formula:

$$\%Germination = \frac{G}{N} \times 100$$

where; G is the number of germinated seeds and N is the number of total seeds.

Mean germination rate was investigated for all of the treatments during a week with 24-hour intervals. The germinated seeds were counted each 24

hour and the cumulative germination was evaluated in correlation with time (for one week).

RESULTS AND DISCUSSION

Germination percentage

The statistical analyses results of the germination percentage are presented in Table 1 and 2. The best treatment was 7-minute exposure period and longer exposure time (9 minutes) had a negative effect on germination percentage. The results showed significant differences between treatments and control. The positive efficiency of ultrasonic waves on germination percentage was realizable. However, increasing exposure time from 1 minute to 5 and 7 minutes positively increases germination percentage, but exposure time of 9 minutes had a negative influence that decreased the germination percentage compared with the control treatment. The mechanism and reasons why this happens is not yet clear, although some researchers have mentioned possible reasons for positive effect of ultrasonic waves on seed germination, such as enlarging the pores size of seed shell that induces better hydration and cell wall fluidity in ultrasonic waves exposed seeds (Yaldagard *et al.*, 2008). Fig. 1 shows the dishes of seeds with different germination percentages with an obvious outlook.

Table 1

Means comparison of germination percentage

Ultrasonic waves treatment ¹ [min]	Germination percentage ² [%]
0	33.33-C
1	33.33-C
3	33.33-C
5	86.66-B
7	96.66-A
9	25.00-C

¹wave exposure times (minute), ²mean values of three replications, means with the same letter are not significantly different

Table 2

Analysis of variance of germination percentage results

Source	DF	Sum of squares	Mean square	F value
Treatment	5	596.28	119.26	153.33
Error	12	9.33	0.78	

Coefficient of variation: 8.58

Fig. 1. Comparison of seeds germination in 7-minute exposure treatment and the control

Mean germination rate

Analyses showed obvious different germination rates among treatments. Fig. 2 shows the graphs of number of germinated seeds during the period of one week for different ultrasonic waves exposure times. The highest growth rate was the related to the 7-minute treatment and the 9-minute treatment had the lowest growth rate, which was even less than the control treatment. It can be deduced that exposure times of more than 7 minutes are harmful for seeds.

Fig. 2. Germination rate comparison for different treatments (germinated seeds were counted cumulatively)

Radicle length

Table 3

Analysis of variance of the radicle length data

Source	DF	Sum of squares	Mean square	F value
Treatment	5	24.49	4.90	76.26
Error	12	0.77	0.06	

Coefficient of variation: 4.04

Statistical analyses of the radicle growth data are presented in Tables 3 and 4. The ultrasonic waves exposure were effective on the radicle length of the seeds and significant difference among treatments was found. The three-minute treatment was the most effective treatment that caused 7.71 cm radicle, which was the longest one among all. The relation between increasing exposure time and

radicle length was not linear. The nine-minute exposure time had a negative effect, which causes a radicle growth even lower than non-exposed seeds.

Table 4

Comparison of mean radicles length.

Ultrasonic treatments ¹ [min]	Mean [cm]
3	7.71 A
5	7.23 B
7	6.68 C
1	6.50 C
0	5.25 D
9	4.29 E

¹exposure time, means with the same letter are not significantly different

Stalk length

Statistical analyses of the stalk growth data are presented in Tables 5 and 6. The ultrasonic waves exposure was effective on the stalk growth of the seeds and significant difference among treatments was found. The 3, 5 and 7-minute exposure periods had the same influence on stalk growth. The 9-minute exposure period had a negative influence and decreased the stalk growth in comparison with the control treatment.

Table 5

Analysis of variance of the stalk length data

Source	DF	Sum of squares	Mean square	F value
Treatment	5	23.78	4.76	56.08
Error	12	1.02	0.08	

Coefficient of variation: 4.52

Table 6

Mean comparison of stalk length

Ultrasonic treatments ¹ [min]	Mean [cm]
5	7.56 A
3	7.55 A
7	7.21 A
1	6.45 B
0	5.30 C
9	4.56 D

¹exposure time, means with the same letter are not significantly different

The relation between increasing exposure time and growth features is not linear for any of the evaluated characteristics. The 9-minute exposure period had a negative effect on all of the growth characteristics. Significant differences were observed in treatments of all of the evaluated features. Therefore, we can conclude that the ultrasonic technique is positively effective for seed stimulation, plant growth and dormancy breaking. However, the exposure time should be considered as an important factor, which can cause negative and positive influences on seeds germination and seedling growth.

Unfortunately, there is no major researches in the literature on the seed of *Medicago scutellata* for dormancy breaking by ultrasonic waves. The goal of this research was not to investigate the mechanisms and causes of germination stimulation through such techniques. An assessment of ultrasonic waves effect on germination characteristics of fennel (*Foeniculum vulgare*) seed was done by Fateh *et al.* (2012). They reported a significant effect of ultrasonic waves exposure on fennel seed germination percentage at probability level of 0.01. They also reported that the germination percentage decreases by increasing exposure times of seeds by ultrasonic waves. A similar study was done by Yaldagard *et al.* (2008) on barley seed. They also reported significant positive effect of ultrasonic waves on seeds germination stimulation. Halstead and Vicario (1969) studied the effect of ultrasonic on the germination of wild rice (*Zizania aquatica*) and found a positive efficiency. They reported lower germination with increasing the intensity and duration of exposure. Bochu *et al.* (2004) studied the effect of sound wave on endogenous hormones in *Chrysanthemum*, reported its influence as effective, and stated that sound wave changes the contents in the plant. Rinaldelli (2002) investigated the effect of ultrasonic waves on seed germination of *Capparis spinosa* and reported effective influence of the waves on germination after seeds scarification with sulfuric acid. Khaef and Sadeghi (2011) studied the mechanical, physical and chemical scarification to break dormancy of *Medicago scutellata* and another species. They stated that dormancy was exclusively induced by seed coat (hardseededness), since they found the highest germination by hand scarification. They also reported some other techniques as effective on germination, but they did not study the effect of ultrasonic waves.

CONCLUSION

- Ultrasonic waves influence the seed germination percentage, germination rate, radicle length and stalk length of *Medicago scutellata* effectively, as statistical analyses show significant differences between the treatments.

- The most effective exposure periods were 7 minutes for germination percentage, stalk length and germination rate and the 3-minute treatment for radicle length and stalk length. The 5 and 7-minute treatments had the same effect as the 3-minute one for stalk growth.
- Exposure time of 9 minutes had a negative effect on all of the assessed features of the plant seeds.
- Treatments of less than 3 minutes did not show any significant effects.
- The mechanisms of such techniques on the seed dormancy breaking and growth of plant features need to be studied further.
- This study was done under laboratorial conditions. Therefore, further research needs to be done in the field under natural environmental conditions to check various factors affecting dormancy and germination of the seeds.

ACKNOWLEDGMENT

The authors would like to thank the management board of Faculty of Agriculture of the Shahrood University and the laboratory personnel for help in performing the research.

REFERENCES

- Auld D.L., Bettis B.L., Crock, J.E., Kephart D. 1988. Planting date and temperature effects on germination, and seed yield of Chickpea. *Agronomy Journal*. 80: 909–914.
- Azimi MS, Daneshian J, Sayfzadeh S, Zare S, 2013. Evaluation of amino acid and salicylic acid application on yield and growth of wheat under water deficit. *IJACS*. 5(8): 816- 519.
- Bochua w., Jipinga S., Biaoa L., Jiea L., Chuanrena D. 2004. Soundwave stimulation triggers the content change of the endogenous hormone of the *Chrysanthemum* mature callus. *Colloids and Surfaces B: Biointerfaces*, 37(3-4): 107-112.
- Bradbeer J.W. 1988. Seed dormancy and Germination. Chapman and Hall, New York, USA. 38-54.
- Fateh E., Noroozi H., Farbod M., Gerami F. 2012. Assessment of Fennel (*Foeniculum vulgare*) seed germination characteristics as influenced by ultrasonic waves and magnetic water. *European Journal of Experimental Biology*. 2 (3): 662-666.
- Gordona A.G. 1971. Beneficial effects of ultrasound on plants—a review. *Ultrasonics*, 9(2):81-84.
- Jun R., & Tao L. 2004. Effects of different presowing seed treatments on germination of 10 Calligonum species. *Forest Ecology and Management*. 195: 291-300.
- Khaef N., Sadeghi H. 2011. Improving Germination Rate of *Medicago Scutellata* and *Medicago Rigidula* as Affected by Seed-Coat Dormancy Breaking Techniques. *Legume Research*, 34(2): 98-104.
- Lindig C. R., & Lara-Cabrera S. 2004. Effect of scarification and growing media on seed germination of *Crotalaria pumila* (Ort.) Seed science and technology. 32(1): 231-234.
- Rinaldelli, E. 2002. Effect of ultrasonic waves on seed germination of *Capparis spinosa* L. as related to exposure time, temperature, and gibberellic acid. *Advances in Horticultural Science*, 14(4): 182-188.
- Sadeghi H., Rasouli M. 2012. Effective methods for improving seed germination of *Medicago scutellata* and *Medicago rigidula*. *Romanian Agricultural Research*, 29: 163-167.
- Sozi G., & Chiesa A.O. 1995. Improvement of Caper (*Capparis spinosa* L.) seed germination by breaking seed coat-induced dormancy. *Scientia Horticulture*. 62(4): 255-261.
- USDA, <http://plants.usda.gov>, 22 December 2013. National Plant Data Team, Greensboro, NC 27401-4901 USA.

- Wiese A.M. & Binning L.K. 1987. Calculating the threshold of temperature of development of weeds. *Weed Science*. 35: 177–179.
- Yaldagard M., Mortazavi S.A., Tabatabaie F. 2008. Influence of ultrasonic stimulation on the germination of barley seed and its alpha-amylase activity. *African Journal of Biotechnology*. 7 (14): 2465-2471.