RESEARCH ARTICLE Legume Research- An International Journal, Volume 47 Issue 2: 234-241 (Fabruary 2024)

Seed Dormancy and Germination in Alfalfa (*Medicago falcata* L.)

Shushuan Wang[1], Fengling Shi[1], Ruru Shi[1], Yutong Zhang[1]

10.18805/LRF-758

ABSTRACT

Background: *Medicago falcata* L. (*M. falcata* L.) is widely distributed in Xinjiang and Inner Mongolia, which is a significant source of resistance genes. Its seeds have a high level of physical dormancy (PD). The current study aimed to study the dormancy characteristics, water absorption characteristics and methods for releasing hard seeds of *M. falcata* L., to reveal the the relationship between seed coat structure and water absorption and to understand the dormancy characteristics, which can provide theoretical basis for seed dormancy and seed biology research.

Methods: Seed germination rate (GR), absorption, germination inhibitor activity and initial site of water entry into seeds were measured to investigate the causes of seed dormancy. Treatments to break dormancy included: (1) sandpaper sanding, (2) ultrasonic treatment for 30 min after sandpaper sanding, (3) 98% sulfuric acid immersion (5,10, 20, 40, 60 and 120 min) and (4) hot water immersion at different temperatures (50, 60, 70, 80 and 90°C) for 10 min, 20 min and 30 min.

Result: The results showed that the hard rate of untreated seeds was 99.33% and the hard rate after the seed coat was scratched was 1.33%. Based on the inhibitory activity test of *M. falcata* L. seeds, high concentration seed extract has an inhibitory effect on the growth of other seeds. The toludine staining test showed that the hilum and microscope are the initial and main parts for seed water absorption. The germination rate of seeds after soaking in 98% sulfuric acid for 40 minutes and ultrasonic treatment with sandpaper for 30 minutes is higher than 94%, which is an effective method to relieve the PY of *M. falcata* L. seeds.

Key words: Absorption, Alfalfa, Breaking hardness, Dormancy, Germination rate.

INTRODUCTION

M. falcata L. is an important wild legume forage, commonly distributed in Xinjiang and Inner Mongolia, it is rich in protein and is a high-quality fodder crop. *M. falcata* L. can be planted widely in arid and cold areas (Shi *et al*., 2019; Zhou *et al*., 2021). Due to its rich genetic background and stress resistance, it has become an important gene source for breeding new varieties of improved alfalfa (Singer *et al*., 2018; Kumar, 2011).

Seed dormancy is a characteristic of plants that adapt to their environment and continue to survive, the factors affecting seed dormancy are complex and varied, such as collection time, maturity, storage conditions and the maternal environment (Gama-Arachchige *et al*., 2011). Dormancy is generally divided into five types, including morphological dormancy (MD), morphological physiological dormancy (MPD), physiological dormancy (PD), physical dormancy (PY) and combined dormancy (PY+PD) (Baskin and Baskin, 2004). PY also called hard or hardness, it is exists widely in legumes, especially wild varieties, It usually caused by seed coat impermeability (Wang *et al*., 2023). Physical methods such as mechanical treatment or temperature treatment can effectively reduce the permeability of the seed coat (Baskin and Baskin 2000; de Souza *et al*., 2001; Long *et al*., 2014). Different varieties have different types and strength of dormancy, so there are also differences in the methods for releasing dormancy. Janská *et al*. (2018) indicated that pea seeds of different dormancy types differ in imbibition capacity and seed coat permeability, dormancy release is detected after temperature oscillation and lipid removal. Besides, chemical reagent, ultrasound and radiation treatments are also effective in releasing dormancy, all of these treatments are commonly used to break seed dormancy in legumes (Patanè *et al*., 2006; Babaei *et al*., 2020).

Some scholars have already conducted research on alfalfa dormancy. Liu *et al*. (2019) found that the expression level of amino acid-associated genes in fall dormant alfalfa is higher than non-dormant alfalfa under cold induction. Scippa *et al*. (2011) inferred that in addition to the hard seed coat, another complex mechanisms are also involved in the dormancy of *Medicago marina* (L.) seeds. In addition, researchers have used multispectral imaging analysis technology to identify alfalfa hard seeds, achieving rapid and non-destructive identification of hard seeds (Hu *et al*., 2020; Wang *et al*., 2023). As an excellent source of

[1]Key Laboratory of Grassland Resources, Ministry of Education, P.R. of China, College of Grassland, Resources and Environment, Inner Mongolia Agricultural University, Hohhot 010019, China.

Corresponding Author: Fengling Shi; Yutong Zhang, Key Laboratory of Grassland Resources, Ministry of Education, P.R. of China, College of Grassland, Resources and Environment, Inner Mongolia Agricultural University, Hohhot 010019, China.
Email: nmczysfl@126.com; zyt7567@126.com

How to cite this article: Wang, S., Shi, F., Shi, R. and Zhang, Y. (2024). Seed Dormancy and Germination in Alfalfa (*Medicago falcata* L.). Legume Research. 47(2): 234-241. doi: 10.18805/LRF-758.

Submitted: 04-07-2023 **Accepted:** 07-11-2023 **Online:** 20-12-2023

resistance genes, the study of dormancy characteristics in *M. falcata* L. has important practical significance for the conservation and utilization of germplasm resources, but there are few reports on the dormancy characteristics of *M. falcata* L., especially the dormancy types and water absorption characteristics of it. This study aims to investigate the dormancy characteristics of *M. falcata* L. by using toludine staining to determine the water absorption point and water movement path of seeds. At the same time, find effective ways to relieve seed dormancy. The study of seed dormancy mechanism provides important theoretical basis for the preservation of germplasm resources, as well as the storage and breeding practices of agricultural and forestry production seeds.

MATERIALS AND METHODS

Seed material

All materials were provided by the Forage Seed Laboratory of Inner Mongolia Agricultural University (Table 1) and stored at 4°C. M1 was the main subject of the experiment, while M2, M3 and B1 were all required for the inhibitor experimental assay.

Seed germination determination

The seeds were divided into two treatment groups: (A) hard seeds without treatment and (B) scarified seed coats. Fifty seeds per group with five replications were placed in plastic boxes containing two layers of moistened filter paper and stored at an incubation temperature of 25°C and 75% relative humidity. Final germination and hardness were measured on the seventh day of incubation. Distilled water was replenished in the boxes daily.

Water absorption determination

Fifty seeds of each group (A and B) were randomly selected and weighed separately with five repeats. Then, the seeds were poured into beakers containing sufficient water and seeds were removed at 2, 4, 8, 12, 24, 36 and 48 h, after which the water on the surface of the seeds was absorbed by filter paper and the seeds were weighed. The rate of water absorption was equal to the ratio of the difference between the weight of the seeds before and after water absorption to the weight of the seeds before water absorption (Li *et al.*, 2022).

Inhibitor into seeds

An extract of 1 g seeds was diluted to 20%, 40%, 60%, 80% and 100% with distilled water. Then, 50 seeds of M2, M3 and B1 were taken and placed in Petri dishes (diameter, 9 cm) containing two layers of moistened filter paper in five replicates. Using distilled water as a control, 5 ml of the extract was added to the medium. Germination tests were performed at 25°C and the number of germinated seeds, fresh weight of seedlings and embryonic root length were counted on the fifth day.

Table 1: Details of different materials used in the experiment.

Code	Materia name	Source
M1	*M. falcata* L.	Xinjiang
M2	*M. falcata* L.	Chifeng, Inner Mongolia
M3	*M. sativa* L. 'Xinjiang Daye'	Inner Mongolia
B1	Cabbage	Inner Mongolia

Initial site of water entry into seeds

The seeds were treated with 98% sulfuric acid for 30 min to break PD and non-treated seeds were immersed in 1% toluidine blue solution. Subsequently, five seeds were randomly removed every two hours, cut longitudinally with a razor blade and observed under a stereomicroscope to determine the initial site and path of movement of water into the seeds. Staining continued for 48 h.

Breaking dormancy

Seeds of *M. falcata* L. were divided into four groups as follows: (1) Lightly sanded seeds sanded with sandpaper until the seed surface lost its luster; (2) Ultrasonic (SB-5200DTD, 45 KHz, 35°C, 120W) treatment for 30 min after sanding with sandpaper; (3) 98% sulfuric acid treatment of seeds at 5, 10, 20, 40, 60 and 120 min; and (4) seeds were immersed in hot water at 50, 60, 70, 80 and 90°C for 10, 20 and 30 min. All seeds were germinated at 25°C in the dark, with fifty seeds per box with five repetitions of each treatment.

Calcultation methods

Germination rate (GR), Germination potential (GP), Germination index (GI) and Vigor index (VI) were calculated on the seventh day.

$$GR(\%)=\frac{\text{Number of final germinated seeds}}{\text{Number of seeds tested}}\times 100\%$$

$$GP(\%)=\frac{\text{Number of seeds germinated at 3d}}{\text{Number of seeds offered for testing}}\times 100\%$$

$$GI(\%)=\Sigma(Gt/Dt)$$

Where

Gt = Number of germinated seeds on day t.

Dt = Number of days corresponding to germination.

$$VI= GI\times S$$

Seedling growth potential (S) = Total fresh weight of young shoots and young roots.

Statistical analysis

Excel 2010 and GraphPad Prism 9 were used for organizing data and graph production, SPSS 26.0 was used for statistical analysis.

RESULTS AND DISCUSSION

Seed germination percentage

Dormancy is a mechanism for seed self-protection, which may give annual plants the possibility of longevity in harsh environments was reported by Al-Namazi *et al.* (2020). Seed dormancy is influenced by a variety of factors such as mother genetics, growth environment and maturation

(Gresta *et al.*, 2007). Jaganathan (2016) has found that seeds matured under dry, warm conditions are more likely to form impermeable seed coats. Chen *et al.* (2019) have been proven fresh seed testa form impermeability after two or three years of dry storage. Hudson *et al.* (2015) found that 25% of the seeds of plants are physically dormant because of their impermeable seed coat.

The study clearly measured the germination percentage of seeds from groups A and B. The hardness rate of hard seeds was as high as 99.33%, but when the seed coat was scratched the hardness rate decreased to 1.33% (Table 2). The final germination percentage of the seeds that had been scarified was much higher than that of the non-treated seeds. These results are in accordance with most other studies (Al-Namazi *et al.*, 2020; Baskin and Baskin 2004; Naser *et al.*, 2013).

Determination of water absorption

Hudson *et al.* (2015) showed that innate dormancy and impermeable seed coat are important factors affecting delayed seed germination. After seed maturation and drying, the seed coat provides coverage and protection for the embryo, forms a barrier between the embryo and its immediate environment and may trigger seed dormancy by controlling water uptake (Qutob *et al.*, 2008).

In the germination trials (Fig 1), the seeds treated with H_2SO_4 had a significantly ($p<0.05$) higher germination rates than the control group, showing that seed coat impermeability was an important reason preventing the seeds from absorbing moisture. Most research has indicated that a hard and dense seed coat structure causes a mechanical barrier to water absorption and the germination of seeds by Zhu *et al.* (2005). In this study, the water uptake curves over 48 h varied significantly among treatments (Fig 1a). The initial weight of seeds in group A was 0.042 g and the final seed weight was 0.050 g. The weight of seeds in group B was 0.121 g at 48 h, which was 2.42 times higher than that of group A (Fig 1b). This indicates that the water absorption of seeds increased significantly after the seed coat had been scarified.

Inhibitory effects on *Medicago falcata* L. seed

Germination inhibitors have been demonstrated to delay or inhibit germination of seeds of different plants. Inhibitors are not only present in seeds and fruits, but are present in individual plant parts depending on the species (Evenari, 1949). Some secondary metabolites present in plant seeds may prevent germination. For example, 58 endogenous compounds were identified in the endocarp of *Cinnamomum migao* seeds, including a phenolic compound, aldehydes, ethers and lipids, among others (Chen *et al.*, 2022).

In this study, the seed growth of the three materials showed an overall decreasing trend with increasing concentration of the extract, indicating that a high concentration of the extract had a certain inhibition effect on seed germination and growth (Fig 2). When treated with 80% and 100% extract concentration, the GR of B1 was reduced by 10.07% and 9.48%, respectively, compared to the control, but there was no significant ($p>0.05$) difference in seedling GP, seedling growth potential or seedling length at 80% and 100% concentrations compared to the control treatment (Fig 2 B1). This indicates that the high concentration of seed inhibitor hinders the germination of alfalfa seeds, the finding that is in agreement with Zhao *et al.* (2019) and Chen *et al.* (2022). GR of M2 seeds after 20%, 40% and 60% concentration treatments were unchanged compared to the control and all treatments had a GR of approximately 24%, while GR after 80% and 100% treatments were 7.02% and 7.86% lower than the control, respectively. In addition, there was no significant ($p>0.05$)

Table 2: Germination of *M. falcata* L. seeds by different treatments.

Group	Treatment	Germination percentage (%)	Hardness rate (%)
A	Hard seeds without treatment	0.67	99.33
B	Scarified seed coat	98.67	1.33

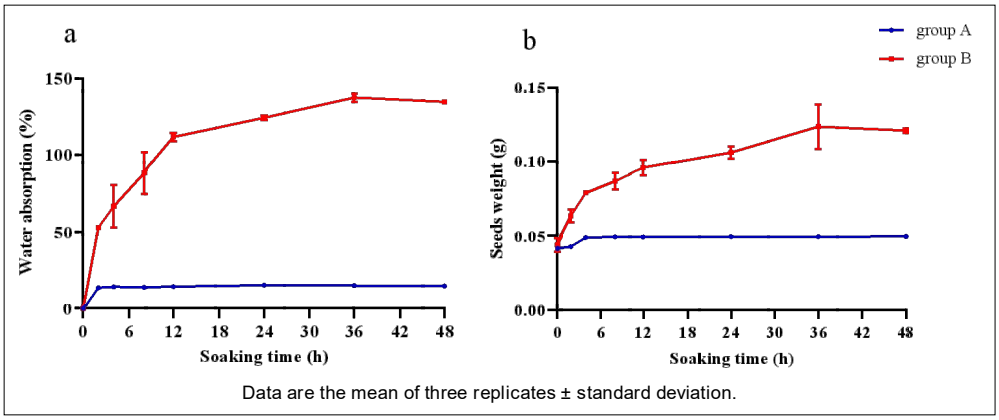
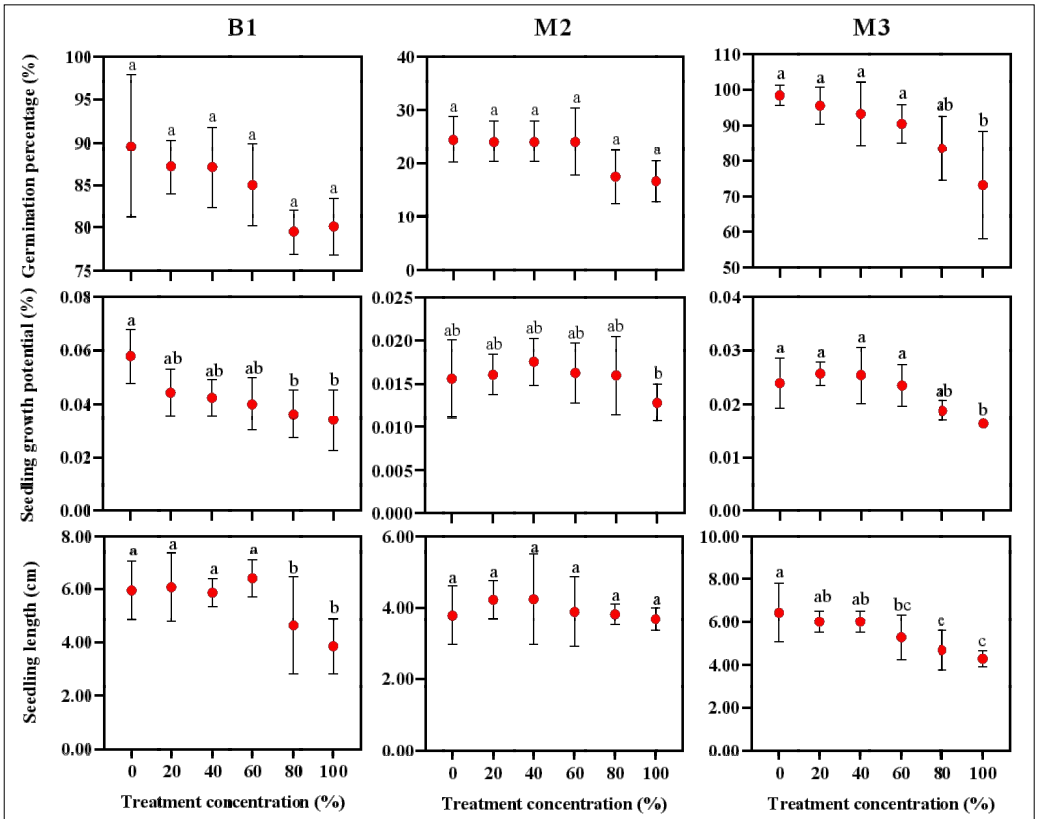


Fig 1: Time course analyses of water absorption rates (a) and seeds weight (b) of control seeds and scarified seeds during imbibition.



(B1) Effect of different concentrations of extracts on the germination of cabbage seeds; (M2) Effect of different concentrations of extracts on germination of *M. falcata* L. seeds from Chifeng; (M3) Effect of different concentrations of extracts on the germination of *M. sativa* L. 'Xinjiang Daye'. Different letters indicate significant differences (P<0.05).

Fig 2: Effect of different concentrations of extract solutions on seed germination and seedling growth.

difference in seedling growth potential and seedling length following each treatment (Fig 2 M2). The GR under the 100% treatment was significantly (p<0.05) lower than the 20%, 40%, 60% and control treatments and the GR of M3 decreased with increasing inhibitor concentration. This indicates that the dormancy of *M. falcata* L. is not only due to the impermeability of the seed coat, but may also be due to other reasons. The results of this study are similar with Bolingue *et al*. (2010), which found that the model legume *Medicago truncatula* exhibits both PY and PD. But further confirmation is needed regarding the existence of other types of *M. falcata* L. dormancy.

Initial point of water entry into seeds

The hilum, lens and micropyle are all considered to be the initial points where water enters the seed. The hilum is the mark of the seed and funiculus when the seed is shedding, with a depression in the middle of the hilum. It is symmetrical on both sides and separated by an obvious slit that points to a set of tracheid bars inside the hilum and extends to the micropyle in one section of the hilum. In legumes, seeds that are released from dormancy absorb water mainly through the micropyle and lens and these structures are associated with the swelling of seeds after breaking dormancy (Baskin, 2003; Gama *et al*., 2013; Baskin *et al*., 2008; de Paula *et al*., 2012). Lersten (1982) showed that seeds with a thicker seed coat require a shorter swelling time for water to diffuse through the micropyle to the hilum and transport water directly to the radicle via the tracheid bar.

In our study, the untreated seeds did not swell within 48 h. Fig 3 shows the staining of the seeds after the release of physical dormancy (Fig 3). At 2 h, some of the seeds absorbed the staining solution and began to swell and the hilum and the micropyle were the first to turn blue and the color deepened at 4 h, indicating that the hilum and the micropyle were the initial and main water absorption sites of the seeds. This also indicated that water uptake gradually increased with a longer seed soaking time. At 6 hours, the coloring solution permeated from the micropyle to the radicle and embryo root, followed by its diffusion to the upper end of the seed coat at 12 hours, ultimately resulting in complete coverage of the entire seed coat. After 24 hours, the rupture of the inner epidermis of the seed could be observed under a stereomicroscope. At this point, the dye solution was absorbed by the inner part of the seed and water uptake began in the embryo, with movement patterns similar to those of the seed coat. Similar results were found Erika *et al*. (2016).

The initial water uptake stage of seeds occurs at other parts of the seed ridge, but this site may vary depending on the species. There are also differences in the initial water uptake sites of legume seeds after release of dormancy and it is believed that the lens, hilum and micropyle are all closely related to the water uptake of seeds under test Smýkal *et al.* (2014) and Harrison *et al.* (2021). In this experiment, sulfuric acid was used to treat alfalfa seeds and the initial water absorption point was determined to be the hilum without destroying the seed coat structure, but the initial water absorption site of seeds treated with scarification was the seed coat break, so this experiment only analyzed the water absorption of seeds after concentrated sulfuric acid treatment.

Break dormancy

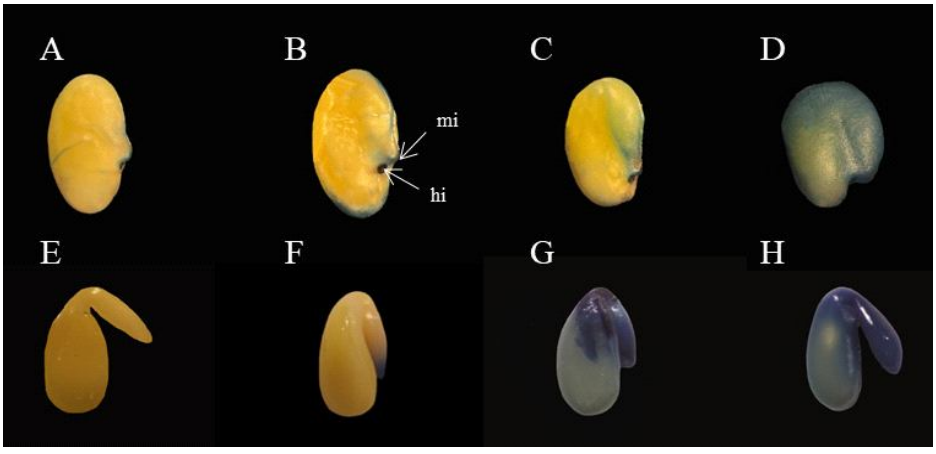
Reports on the breaking of PY in legume seeds indicate that the main principle of breaking dormancy using physical or chemical methods is to weaken the water absorption barrier of the seed coat and enhance the water absorption capacity of seeds. In this study, different treatments were used to release alfalfa seeds from hardness and the effects of different methods to reduce seed dormancy were dissimilar. There were highly significant differences in GR and GP after sandpaper + ultrasonic treatment (Fig 4) compared with the control (P<0.01), with increases of 95.33% and 90.95%, respectively. The difference in germination rate between the two treatment groups was also significant (P<0.05), indicating that the combined effect of sandpaper and ultrasound to release dormancy was stronger than that of sandpaper alone. As a rapid and effective non-destructive method, ultrasound can not only improve the GR of seeds and the growth state of seedlings, but also delay the decline in seed GR during storage, improve the vitality of aging seeds and enhance the stress resistance of seeds. Similar results were reported by Babaei *et al.* (2020); Abbaspour *et al.* (2019); Ding *et al.* (2018) and Shekari *et al.* (2015) in dormancy release studies.

Acid corrosion treatment can corrode the local seed coat, break the barrier of fenestrated tissue and increase the permeability of the seed coat. In this study, 98% concentrated sulfuric acid was used to treat seeds (for 5, 10, 20, 40, 60 and 120 min) and the GI of seeds varied greatly with different treatment durations (Table 3). Germination ability showed a trend of first increasing and then decreasing with increasing soaking time. Germination capacity decreased sharply and the GR and GI were significantly lower than other treatments except with 5 min treatment (P<0.05), indicating that the inappropriate treatment duration did not achieve the expected effect or

Table 3: Comparison of sulfuric acid treatment mean effects on the germination indices of *Medicago falcata* L.

Treatment (min)	Germination percentage	Germination potential	Germination index	Vigor index
	(%)			
5	56.00±9.17^{c}	38.00±10.58^{c}	20.67±6.63^{ab}	0.12±0.04^{b}
10	74.00±5.29^{b}	56.67±13.01^{bc}	30.40±7.49^{a}	0.17±0.07^{b}
20	82.67±11.02^{ab}	53.33±3.06^{bc}	32.38±2.81^{a}	0.29±0.09^{ab}
40	94.18±5.43^{a}	85.54±17.11^{a}	34.08±11.95^{a}	0.56±0.11^{a}
60	86.67±2.89^{ab}	78.89±8.39^{ab}	27.03±6.41^{a}	0.30±0.76^{ab}
120	40.26±21.59^{c}	38.26±24.73^{c}	10.63±2.43^{b}	0.10±0.03^{b}

Note: Different lowercase letters in the same column indicate significant differences at the 0.05 level.

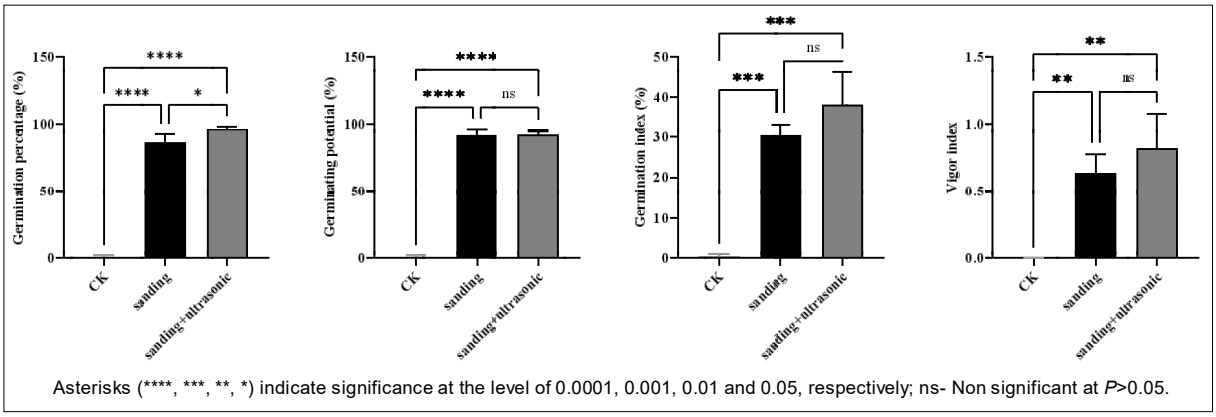


(A) Staining 2 h, (B) Staining 4 h, (C) Staining 6 h, (D) Staining 12 h, (E) Staining 24 h, (F) Staining 36 h, (G-H) Seed embryo, staining 48 h; hi- Hilum; mi- Micropyle.

Fig 3: Entry of the dye (indicated by blue color) after pretreatment of afalfa seeds by the hilum.

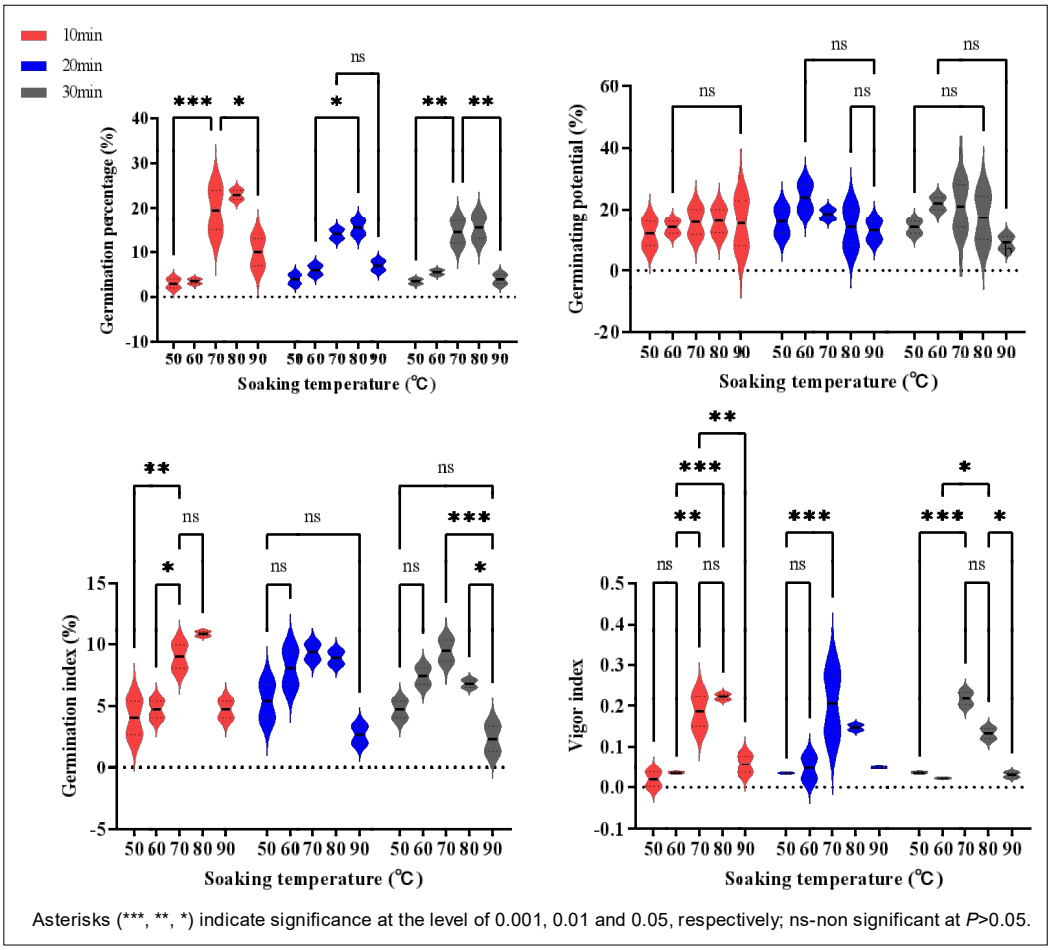
harmed the seeds themselves. In this study, 98% sulfuric acid treatment for 40 min was more effective at relieving the PY of alfalfa seeds and the operation was simple and not laborious compared with the sandpaper treatment.

Temperature treatment is also a common method to relieve seed dormancy. However, there are differences in the treatment time and temperature required for different varieties. Patane *et al.* (2006) have found that hot water treatment for 20 minutes can significantly improve the GR of *Astragalus hamosus* seeds, but there was no significant effect on the germination rate of *Medicago orbicularis* seeds. The seed GR of alfalfa was 3%~23% after hot water treatment at different temperatures (Fig 5). The GR of alfalfa treated at 70°C for 10 min was higher than that treated at 50°C (P<0.05). At the same temperature, when the treatment time was increased to 20 min or 30 min, the GR of seeds



Asterisks (****, ***, **, *) indicate significance at the level of 0.0001, 0.001, 0.01 and 0.05, respectively; ns- Non significant at P>0.05.

Fig 4: Comparison of the ultrasound and mechanical processing mean effects on the germimicropyle.ices of *Medicago falcata* L.



Asterisks (***, **, *) indicate significance at the level of 0.001, 0.01 and 0.05, respectively; ns-non significant at P>0.05.

Fig 5: Comparison of the hot water treatment mean effects on the germination indices of *Medicago falcata* L.

was significantly lower than that at 10 min, but there was no significant difference in GP among different treatments (P>0.05). The germination index of seeds treated at 90°C was lower than that treated at 70°C (P<0.05). The VI of 50°C, 60°C and 90°C treatments were significantly lower than the other two treatments (P<0.05). Therefore, the appropriate temperature could effectively improve the germination rate of seeds and enhance the vigor of seedlings. Hot water treatment also has a positive impact on the release of dormancy in other leguminous plants, such as *Ceratonia siliqua* L. and *Acacia mearnsii* seeds under treatments Cavallaro *et al.* (2021) and São José *et al.* (2019).

CONCLUSION

We concluded that PY is present in the seeds of *M. falcata* L. from Xinjiang and it has a hardness rate of 99.33%. Seed coat impermeability is the main reason for hardness formation, while the presence of seed germination inhibitors in *M. falcata* L. was also confirmed. The hilum and micropyle were identified as the initial sites of seed water uptake. Dormancy could be broken either by sulfuric acid (H_2SO_4) treatment for 40 min or by sandpaper sanding + ultrasonic treatment.

ACKNOWLEDGEMENT

F.L. Shi designed the experiment, R.R. Shi performed the experiments and wrote the manuscript. Thanks to Key Laboratory of Grassland Resources, Ministry of Education P.R. of China. This study was supported by the the National Natural Science Foundation of China (32160323), The Science and Technology Innovation 2030-Major Project (2022ZD04012). The authors would like to express their gratitude to Edit Springs for the expert linguistic services provided.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Abbaspour-Gilandeh, Y., Kaveh, M., Jahanbakhshi, A. (2019). The effect of microwave and convective dryer with ultrasound pre-treatment on drying and quality properties of walnut kernel. Journal of Food Processing and Preservation. 43(11): e14178.

Al-Namazi, A.A., Al-Ammari, B.S., Davy, A.J., Al-Turki, T.A. (2020). Seed dormancy and germination in Dodonaea viscosa (Sapindaceae) from south-western Saudi Arabia. Saudi Journal of Biological Sciences. 27(9): 2420-2424.

Babaei Ghaghelestany, A., Alebrahim, M.T., MacGregor, D.R., Khatami, S.A., Hasani Nasab Farzaneh, R. (2020). Evaluation of ultrasound technology to break seed dormancy of common lambsquarters (Chenopodium album). Food Science and Nutrition. 8(6): 2662-2669.

Baskin, C.C. (2003). Breaking physical dormancy in seeds-focussing on the lens. New Phytologist. 158: 229-232.

Baskin, C.C., Baskin, J.M. (2000). Seeds: Ecology, biogeography and evolution dormancy and germination. Crop Science. 40: 564-565.

Baskin, J.M., Baskin, C.C. (2000). Evolutionary considerations of claims for physical dormancy-break by microbial action and abrasion by soil particles. Seed Science Research. 10: 409-413.

Baskin, J.M., Baskin, C.C. A classification system for seed dormancy. (2004). Seed Science Research. 14: 1-16.

Baskin, J.M., Davis, B.H., Baskin, C.C., Gleason, S.M., Cordell, S. (2008). Physical dormancy in seeds of *Dodonaea viscosa* (Sapindales, Sapindaceae) from Hawaii. Seed Science Research. 14: 81-90.

Bolingue, W., Ly Vu, B., Leprince, O., Buitink, J. (2010). Characterization of dormancy behaviour in seeds of the model legume *Medicago truncatula*. Seed Science Research. 20(2): 97-107.

Cavallaro, V., Maucieri, C., Patanè, C., Fascella, G., Pellegrino, A. Barbera, A.C. (2021). Polyphenols leaching and seed dormancy in carob (*Ceratonia siliqua* L.) in relation to hot water treatment. Acta Physiologiae Plantarum. 43: 141-150.

Chen, D.L., Zhang, R., Baskin, C.C., Hu, X.W. (2019). Water permeability/impermeability in seeds of 15 species of *Caragana* (Fabaceae). PeerJ. 7: e6870.

Chen, J.Z., Huang, X.L., Xiao, X.F., Liu, J.M., Liao, X.F., Sun, Q.W., Peng, L., Zhang, L. (2022). Seed dormancy release and germination requirements of *Cinnamomum migao*, an endangered and rare woody plant in Southwest China. Frontiers in Plant Science. 13: 770940-770940.

de Paula, A., Delgado, C., Paulilo, M., Santos, M. (2012). Breaking physical dormancy of *Cassia leptophylla* and *Senna macranthera* (Fabaceae: Caesalpinioideae) seeds: Water absorption and alternating temperatures. Seed Science Research. 22: 259-267.

de Souza, FHD., Marcos-Filho, J. (2001). The seed coat as a modulator of seed environment relationship in Fabaceae. Brazilian Journal of Botany. 24: 365-375.

Ding, J., Hou, G.G., Dong, M., Xiong, S., Zhao, S., Feng, H. (2018). Physicochemical properties of germinated dehulled rice flour and energy requirement in germination as affected by ultrasound treatment. Ultrasonics Sonochemistry. 41: 484-491.

Erika, R.D., Joel, F., Laura, Y.E. (2016). Paths of water entry and structures involved in the breaking of seed dormancy of *Lupinus*. Journal of Plant Physiology. 192: 75-80.

Evenari, M. (1949). Germination inhibitors. The Botanical Review. 15(3): 153-194.

Gama-Arachchige, N.S., Baskin, J.M., Geneve, R.L., Baskin, C.C. (2011). Acquisition of physical dormancy and ontogeny of the micropyle-water-gap complex in developing seeds of *Geranium carolinianum* (Geraniaceae). Annals of Botany. 108: 51-64.

Gama-Arachchige, N.S., Baskin, J.M., Geneve, R.L., Baskin, C.C. (2013). Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes. Annals of Botany. 112: 69-84.

Gresta, F., Avola, G., Abbate, V. (2007). Germination ecology of *Scorpiurus subvillosus* L. seeds: The role of temperature and storage time. Plant Ecology. 190: 123-130.

Harrison, R.J., Edwards, Tom, J., Steel, E,Yates, R.J., Nutt, B.J., Howieson, J.G. (2021). Breaking hard seed dormancy in the perennial legume *Lebeckia ambigua* E. Mey. to enhance sustainable agricultural production. Agronomy for Sustainable Development. 41: 45-57.

Hu, X., Yang, L., Zhang, Z. (2020). Non-destructive identification of single hard seed via multispectral imaging analysis in six legume species. Plant Methods. 16: 116.

Hudson, A.R., Ayre, D.J., Ooi, M.K., (2015). Physical dormancy in a changing climate. Seed Science Research. 25(2): 66-81.

Jaganathan, G.K. (2016). Influence ofmaternal environment in developing different levels of physical dormancy and its ecological significance. Plant Ecology. 217: 71-79.

Janská, A., Pecková, E., Sczepaniak, B., Smýkal, P., Soukup, A. (2018). The role of the testa during the establishment of physical dormancy in the pea seed. Annals of Botany. 123: 815-29.

Kumar, S. (2011). Biotechnological advancements in alfalfa improvement. Journal of Applied Genetics. 52: 111-124.

Lersten, N.R. (1982). Tracheid bar and vestured pits in legume seeds (Leguminosae: Papilionoideae). American Journal of Botany. 69: 98-107.

Li, Z.A., Zhang, T.Y., Zhu, M.Y., Li, C.Y., Li, B., Lu, X.J., Wang, J.H., Jia, L.Q., Qi, H.N., Wang, X.M., Zhao, G.W. (2022). High-vigor seeds associated with seed hardness and water absorption rate in rice (*Oryza sativa* L.). Agriculture. 12(5): 712.

Liu, Z.Y., Baoyin, T., Li, X.L., Wang, Z.L. (2019). How fall dormancy benefits alfalfa winter-survival? Physiologic and transcriptomic analyses of dormancy process. BMC Plant Biology. 19(1): 205-217.

Long, R.L., Gorecki, M.J., Renton, M., Scott, J.K., Colville, L., Goggin, D.E., Commander, L.E., Westcott, D.A., Cherry, H., Finch-Savage, W.E. (2014). The ecophysiology of seed persistence: A mechanistic view of the journey to germination or demise. Biological Reviews. 90(1): 31-59.

Naser, S.M., Savadkoohi, Ahmadi, E., (2013). Effect of different seed treatments on dormancy breaking and germination in three species in arid and semi-arid lands. Forest Science Pract. 15(2): 130-136.

Patanè, C., Gresta, F. (2006). Germination of *Astragalus hamosus* and *Medicago orbicularis* as affected by seed-coat dormancy breaking techniques. Journal of Arid Environments. 67(1): 165-173.

Qutob, D., Ma, F., Peterson, C.A., Bernards, M.A., Gijzen, M. (2008). Structural and permeability properties of the soybean seed coat. Botany. 86(3): 219-227.

São José, J.F.B., Volpiano, C.G., Vargas, L.K., Hernandes, M.A.S., Lisboa, B.B., Schlindwein, G., Beneduzi, A., Longoni, L.S., Sampaio, J.A.T. (2019). Influence of hot water on breaking dormancy, incubation temperature and rhizobial inoculation on germination of *Acacia mearnsii* seeds. Australian Forestry. 1-5.

Scippa, G.S., Petrollini, E., Trupiano, D., Rocco, M., Falco, G., Di Michele, M., Chiatante, D. (2011). Dormancy of *Medicago marina* (L.) seed. Environmental and Experimental Botany. 72(2): 320-329.

Shekari, F., Mustafavi, S.H., Abbasi, A. (2015). Sonication of seeds increase germination performance of sesame under low temperature stress. Acta Agriculturae Slovenica. 105(2): 203-212.

Shi, H.F., He, S.J., He, X.Y., Lu, S.Y., Guo, Z. F. (2019). An eukaryotic elongation factor 2 from *Medicago falcata* (MfEF2) confers cold tolerance. BMC Plant Biol. 19: 218-230.

Singer, S.D., Hannoufa, A., Acharya, S. (2018). Molecular improvement of alfalfa for enhanced productivity and adaptability in a changing environment. Plant Cell Environ. 41: 1955-1971.

Smýkal, P., Vernoud, V., Blair, M.W., Soukup, A., Thompson, R.D. (2014). The role of the testa during development and in establishment of dormancy of the legume seed. Frontiers in Plant Science. 5: 351.

Wang, X.M., Zhang, H., Song, R., Sun, M., Liu, P., Tian, P.X., Mao, P.S., Jia, S.G. (2023). Multiple omics datasets reveal significant physical and physiological dormancy in alfalfa hard seeds identified by multispectral imaging analysis. The Crop Journal. 1458-1468.

Zhao, T.T., Qian, C.M., Gao, Y.P., Chen, L., Zhu, M.W., Pan, Y.T., Li, S.X. (2019). Germination inhibitors detected in *Sapium sebiferum* seeds. Journal of Forestry Research. 30(6): 2305-2312.

Zhou, X.Y., Han, H.J., Liu, Y.F., Qiu, R., Zhao, Y. (2021). Research progress of *Medicago falcata* L. Grassland Pratacul. 33: 1-5.

Zhu, H.N., Lu, Q.X., Abdollahi, K. (2005). Seed coat structure of *Pinus koraiensis*. Microscopy and Microanalysis. 11(S02): 1158-1159.