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KD and FD conceived and designed the experiment; KD performed the experiment; KD and ES wrote the paper

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Competing interests

No competing interests have been declared.

Copyright notice© The Author(s) 2019. This is an Open Access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits redistribution, commercial and noncommercial, provided that the article is properly cited.**Citation**Dziurka K, Skrzypek E, Dubert F. Breaking seed dormancy of *Astragalus penduliflorus* Lam. Acta Soc Bot Pol. 2019;88(1):3617. <https://doi.org/10.5586/asbp.3617>**Digital signature**

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SHORT COMMUNICATION

Breaking seed dormancy of *Astragalus penduliflorus* Lam.

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Astragalus penduliflorus Lam. is an alpine-subalpine species. Several isolated populations occur in Europe: in the Alps, Pyrenees, Carpathians and in central Sweden. *Astragalus penduliflorus* is considered as critically endangered species in Poland, growing only at the locality in the Smytnia Valley, in the Western Tatra Mountains. The population is at risk, due to the limited reproduction caused by low rate of seed germination, periodically shortened vegetation period that prevent seed development and gnawing the aerial plant parts by deer. The aim of the study was to explain the reason for the poor germination of *A. penduliflorus* seeds. As a result of mechanical scarification, 100% of *A. penduliflorus* seeds germinated, which proved that these seeds are characterized by a water-impermeable seed coat, which classified them as hard seeds that go through physical dormancy. Results obtained in this work can be used for effective reproduction and active conservation of threatened *A. penduliflorus*.

Keywords

endangered species; seed germination; seed hardness; scarification

Introduction

Astragalus penduliflorus Lam. is a high mountain species with a disjunct distribution. It occurs in Europe in the Alps, Pyrenees, Carpathians, and in Sweden. *Astragalus penduliflorus* received the status of critically endangered species (CR) in the *Polish red data book of plants* [1]. The only relict population is located in the central part of the Smytnia Valley, the Tatra Mountains, within the limits of the strict reserve “Kominy Tylkowe”, at an altitude of 1,400–1,470 m a.s.l. *Astragalus penduliflorus* was discovered here for the first time in 1935 by Bogumił Pawłowski [2].

Astragalus penduliflorus is a perennial forming large, single tufts. Numerous stems grow from the root, up to 80 cm high, thick, empty inside, branched with dense foliage, and covered with white hairs. The leaves are imparipinnate, composed of 7–11 pairs of oblong leaflets. It reproduces generatively, forming racemes consisting of up to 14 flowers. The flowers are pendulous, intensely yellow, and the fruits are single-chamber pods – pendulous, strongly dilated and usually four-seeded. The seeds are kidney-shaped, brown and smooth. The plant blooms from June to August [2].

The population of *A. penduliflorus* in the Smytnia Valley has approximately only 35 plants [3] and is endangered due to the limited possibility of generative reproduction. This danger results from the fact that a low percentage of *A. penduliflorus* seeds germinate in natural conditions. In addition, early snowfalls and deer gnawing on the aerial plant parts prevent seed production and maturation [4].

Seeds with water-impermeable coat are common in many species of Fabaceae family. They are termed as hard seeds and undergo physical dormancy. There are several methods for breaking physical dormancy revised by Rolston [5]. Dormancy

and germination of seeds from the genus *Astragalus* has been studied in some rare and endangered species, e.g., *A. adsurgens* [6], *A. gines-lopezii* [7], *A. nitidiflorus* [8], *A. maritimus*, and *A. verrucosus* [9] due to the fact that it is crucial for their reproduction, ex situ conservation and reintroduction.

The aim of the study was to increase the rate of seed germination of *A. penduliflorus*. The experiments were carried out to investigate the effect of chemical and mechanical scarification of the seeds on germination.

Material and methods

Plant material

The seeds of *A. penduliflorus* (Fig. 1) were obtained from the collections of the Mountain Botanical Garden of the Tatra Field Station of the Institute of Nature Conservation, Polish Academy of Sciences (TFS INC PAS) in Antałówka, Zakopane. They originated from a natural locality in the Smytnia Valley. Seeds were harvested at the turn of September and October in 2004 and 2005. They were stored at 4°C. The experiments were carried out in the spring of 2005 and 2006.



Fig. 1 The seeds of *Astragalus penduliflorus* Lam.

First experiment: chemical and mechanical scarification

Chemical scarification of *A. penduliflorus* seeds was performed by treating the seeds with concentrated sulfuric acid for 2 minutes and then rinsing in distilled water twice for a few seconds and thrice for 3 minutes. Mechanical scarification was performed with sandpaper by rubbing the seeds between its two layers. Nonscarified seeds served as control. Fifty seeds were used for each treatment. The seeds were placed on a wet filter paper, on Petri dishes, 10 seeds per dish. Seed germination was carried out in an air-conditioned chamber with artificial light ($240 \mu\text{mol m}^{-2} \text{s}^{-1}$), 16-h photoperiod, and at 20°C. Germination assessment was carried out within 4 weeks.

Second experiment: mechanical scarification in sterile and nonsterile conditions

In the second experiment, half of the control and mechanically scarified seeds (using sandpaper or by puncturing the seed coat with a dissecting needle) were disinfected, treated with 96% ethanol for 1 minute followed by a 20% bleach solution (Domestos; Unilever, Poland) for 15 minutes and rinsing four times with sterile water. The seeds were placed on Petri dishes, on Murashige Skoog agar medium [10], 10 seeds per dish and five dishes per each treatment. The other half seeds were germinated as in the first experiment. Germination assessment was carried out within 4 weeks.

Statistical analysis

Data were analyzed using the statistical package STATISTICA 12 (StatSoft, Inc., USA). The Duncan test at $p \leq 0.05$ was used to determine the significance of differences between germination percentage of *A. penduliflorus* seeds under various treatments.

Results

The experiment showed a low germination capacity in nonscarified (control) amounting to 24% (Fig. 2). Of the germinating seeds, only half of them developed normal seedlings. In view of this result, a positive effect of scarification on *A. penduliflorus* seed germination has been demonstrated. After chemical scarification with sulfuric acid, twice more seeds germinated (48%), from which twice more seedlings were obtained compared to the control seeds, however, after mechanical scarification with sandpaper, seeds germinated in 100% and 5 times more seedlings were obtained compared to control. Regardless of the seed treatment method, it was found that almost half of them were infected and perhaps did not develop to the seedling stage for this reason. Therefore, the effect of scarification on seed germination was investigated both in sterile and nonsterile conditions in the second experiment. Mechanical scarification was chosen as a more efficient method, but it was complemented by point scarification of the seed coat with a dissecting needle. All scarification variants resulted in 100% seed germination (Fig. 3, Fig. 4).

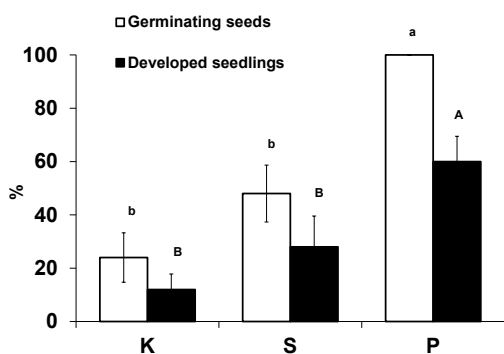


Fig. 2 *Astragalus penduliflorus* seed germination (%) and seedlings development (%), depending on the method of seed scarification. K – control – nonscarified seeds; S – seeds scarified with concentrated sulfuric acid; P – seeds scarified with sandpaper. Results are average of five biological replicates \pm SE. Different capital and small letters indicate statistically significant differences in percentage of germinating seeds and obtained seedlings ($p \leq 0.05$), respectively.

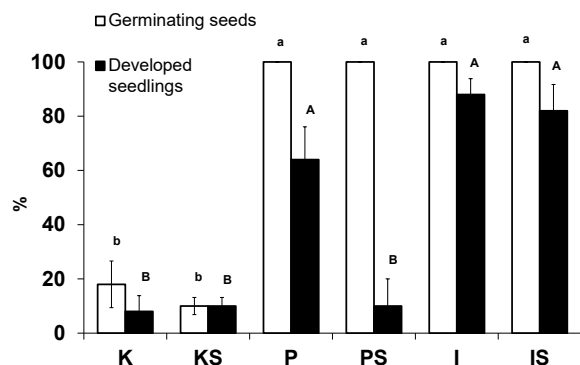


Fig. 3 *Astragalus penduliflorus* seed germination (%) and seedlings development (%), depending on the method of seed scarification. K – control – nonscarified seeds; P – seeds scarified with sandpaper; I – seeds scarified with a dissecting needle. KS, PS, IS correspond to K, P, I, but in sterile conditions. Results are average of five biological replicates \pm SE. Different capital and small letters indicate statistically significant differences in percentage of germinating seeds and obtained seedlings ($p \leq 0.05$), respectively.

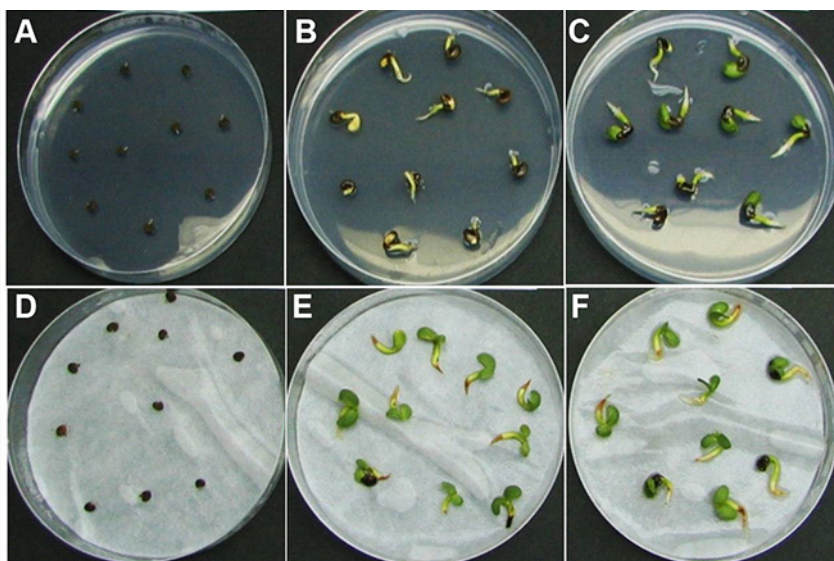


Fig. 4 The seeds and seedlings of *A. penduliflorus*, depending on the method of seed scarification. (A) Control – nonscarified seeds; (B) seeds scarified with abrasive paper; (C) seeds scarified with a dissecting needle; (D–F) correspond to (A–C), but in sterile conditions.

The new scarification method using a dissecting needle proved to be the best, because it allowed to obtain the largest number of seedlings, 88%, under nonsterile conditions and 82% under sterile conditions. Scarification with sandpaper was a less efficient method, especially in sterile conditions, because the number of seedlings was comparable to control (10%) and 8 times lower compared to the scarification with a dissecting needle. Sandpaper scarification was found to be destructive to seeds, especially when combined with sterilization, during which the seeds have been damaged.

Discussion

The seeds of *A. penduliflorus* germinated independently in a low percentage: 18–24%. This result is consistent with the information in the literature, according to which seeds stored at 4°C germinated in 18–28%, and seeds stored at room temperature in 3–12% [4]. Scarification with concentrated sulfuric acid allowed to increase the percentage of germinating seeds of *A. penduliflorus*, however, it was comparable to the control. In contrast, Kondo and Takeuchi [6] obtained high percentage of germination of *A. adsurgens* seeds treated with concentrated sulfuric acid for 20–90 min. Similarly, soaking in concentrated sulfuric acid was very effective in breaking dormancy of *A. adscendens* [11] or *A. cicer* [12]. It is possible that discrepancies in results are caused by different acid treatment time. The experiments showed a positive effect of mechanical scarification on seed germination, because this method increased the percentage of germinating seeds to 100%. It is in totally agreement with results obtained for *A. gines-lopezii* [7] and *A. siliquosus* [13] for which scarification with sandpaper significantly enhanced seed germination. Physical scarification turned out to be the best method of breaking dormancy also for *A. arpilobus* [14] and *A. cicer* [12]. Our results confirmed the assumptions that *A. penduliflorus* seeds have a water-impermeable seed coat and should be included into the group of seeds characterized by physical dormancy, according to the classification proposed by Baskin and Baskin [15].

Seed hardness is mainly hereditary, but environmental conditions during maturation and storage can affect the intensity of this feature. Dry and sunny weather favors the emergence of hard seeds, similarly as the excess of calcium in the soil [16]. Chemical analysis of soils on which *A. penduliflorus* grows in the Smytnia Valley showed that these soils are rich in calcium compounds [4], what probably favors hard seed formation in *A. penduliflorus*.

Hard seeds, due to the low water content and the proper seed coat structure, are resistant to low temperatures. This characteristic helps to safely store *A. penduliflorus* seeds in liquid nitrogen in the seed bank of the Botanical Garden, PAS in Powsin [17]. The water-impermeable seed coat also allows for effective seed disinfection for experiments in sterile conditions and minimizes seed damage during this procedure. For this purpose, sterilization should first be carried out and then the sterile material should be scarified. The reverse procedure (as in the second experiment) is destructive to seeds, because damaging the seed coat allows deep disinfectant penetration, which can cause embryo poisoning.

Thanks to the fact that dormant seeds may stay in the soil for years, without losing germination capacity, they form a natural seed bank that allows the population to recover even if plants did not produce seeds in the season for some reason [18]. On the other hand, considering the factors limiting *A. penduliflorus* seed production and the fact that the population is small, seed dormancy contributes to population aging.

In conclusion, *A. penduliflorus* seeds show physical dormancy due to the impermeable seed coat. The most efficient method of breaking *A. penduliflorus* dormancy is mechanical scarification. For the *A. penduliflorus* reproduction disinfection of seeds, mechanical scarification and germination in in vitro conditions are recommended.

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