

EXPERIMENTAL PAPER

Influence of storage and pre-sowing treatment of southern sweet-grass seeds on their germination and initial growth of seedlings

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Summary

Introduction: Southern sweet-grass (*Hierochloë australis* /Schrad./ Roem. et Schult., *Poaceae*) is a perennial tuft-grass occurring in North-Eastern part of Europe. Its leaves are collected from wild growing plants as an aromatic raw material used in alcohol industry. Due to overharvest, attempts to introduce the plant into cultivation have been undertaken. **Objective:** This work aims to assess the influence of southern sweet-grass seeds (spikelets) storage and pre-sowing treatment on their germination and the initial growth of seedlings. **Methods:** The seeds were assessed directly after harvest and after 6, 18 and 30 months of storage. The investigated parameters, i.e. 1000 seed weight, moisture content, germinability and viability of seeds (tetrazoline test) were determined according to ISTA. **Results:** After 30 months of storage, the germinability of seeds decreased from 42.6 (after harvest) to 4.6% but their viability remained high (70.9%). Stratification, short rinse of stratified seed in H_2SO_4 and application of KNO_3 or GA_3 had increased the seeds germination (over 70%). **Conclusion:** Southern sweet-grass seeds become dormant shortly after maturing. The improvement of germination requires the application of combined treatments, i.e. stratification, scarification and growth-promoting substances.

Key words: *Hierochloë australis*, spikelets, stratification, scarification, growth-promoting substances

INTRODUCTION

Southern sweet-grass (*Hierochloë australis* /Schrad./ Roem. et Schult., *Poaceae*), popularly named bison grass, is a perennial tuft-grass occurring in coniferous and mixed forest, in North-Eastern part of Europe [1, 2]. In Poland, it has been protected since 1983 [3], however, its leaves are still collected from the wild growing plants. The raw material is used for aromatization of alcoholic beverages and tobacco products since it is rich in coumarin, responsible for specific sweet, cherry-like smell [4, 5]. In the past, it was also used as perfume, for storing with clothes, and in medicine – to impart strength and virility [5].

Due to high demand for these raw material, nowadays the species is seriously endangered [1, 6, 7]. The most effective way to protect the plant *in situ* is to produce the valuable raw material in cultivation system. Such actions, aiming to achieve a balance between consumption, conservation and cultivation, are recommended by a number of world agencies (WHO, IUCN, WWF) working on wild growing plants protection [8]. In Poland, the first trials to bring southern sweet-grass into cultivation were undertaken in the 1970' [9-11]. Due to drastic overharvest, the problem was undertaken again a few years ago in Department of Vegetable and Medicinal Plants, WULS-SGGW in Warsaw. Up to now, some scientific papers on southern sweet-grass clonal reproductive ability and cultivation demands have been published. The results indicate that the species is very diversified in the morphological traits and the accumulation of secondary metabolites [6, 7, 12, 13]. The plant has also specific light requirements affecting its development, e.g. period of flowering, number of flowering shoots and seed quality [14].

The aim of our study was to assess the influence of southern sweet-grass seeds storage period and pre-sowing treatment on its germination and the initial growth of seedlings.

MATERIAL AND METHODS

Plant material

The southern sweet-grass spikelets filled with mature caryopsis (in the further part of the article called seeds, fig. 1) were obtained from 2-years-old plants cultivated at the Experimental Station of Department of Vegetable and Medicinal Plants WULS-SGGW. The maternal plants were described in the previous work as a clone 14 [7]. Their seeds are deposited in National Centre for Plant Genetic Resources, Polish Genebank.

Seed storage experiment

The evaluation of seeds was performed directly after harvest, and successively after six, twelve and thirty months of storage. The seeds were stored in 21°C, in

darkness. They were sealed in polyethylene bags until used for experiments. The investigated parameters, i.e. 1000 seed weight, moisture content, germinability and viability (tetrazoline test) were determined according to The International Seed Testing Association ISTA [15].

1000 seed weight

1000 seed weight was performed in 8 replicates of 100 seeds in each repetition. The result was calculated as the mean weight of 100 seeds, multiplied by 10.

Moisture content

The water content in seeds was determined in 2 replicates, by the direct method. Water was removed from seed by heating in oven at 130°C for 2 hours. Moisture content (%) was calculated as follows: (weight of fresh sample – weight of dry sample) x 100/weight of fresh sample.

Germinability

Germination was determined using sterile 8 cm Petri dishes filled with filter paper. The seeds were spread out evenly in the dishes. Four replication were used, 100 seeds each. The experiment was performed in a climatic chambers MLR 350 Sanyo, in a controlled conditions (irradiation 150 $\mu\text{E}/\text{m}^2 \times \text{s}$; temp. 20°C for 24 h). The germination status was recorded after 14 and 21 days. The seeds were defined as germinated when 2 mm of radicle had emerged. After germination, the seeds were removed.

Viability test

The seeds not germinating in the germinability test were used to carry out the viability tests. The test was carried out on fruits (caryopsis) purified out of spikelets (fig. 2). Fruits were soaked in distilled water at a room temperature for 24 h and then for a further 18 h in a 1% solution of 2,3,5-triphenyltetrazolium chlorine, pH 7.0. After this time, the fruits were rinsed with water, cut along and evaluated. The viable fruits were defined as those stained completely or partially. The coloration must have covered those parts of the fruit, which ensure the normal development of seedlings (fig. 3). Non-viable fruits were not stained or they were stained very weakly (fig. 4).

Pre-sowing treatment experiment

The seeds stored for 18 months were subjected to pre-sowing treatments:

- stratification: untreated control and cold stratification in 2°C for 3 weeks;
- hydration: rinsing with water, soaking with water (at 30°C for 24 h) directly prior to tests;
- scarification: rinsing with concentrated sulfuric acid (H_2SO_4), soaking with concentrated sulfuric acid (2 and 5 min.);
- the application of growth-promoting substances into the filter paper used in germination tests: water (H_2O as a control), potassium nitrate (KNO_3) – 0.2%, gibberellic acid (GA_3) – 500 ppm.

The treatments were applied in 15 combinations as follows:

- stratification, rinsing with water;
- stratification, rinsing with water, the application of growth-promoting substances (KNO_3 or GA_3);
- stratification, soaking with water;
- stratification, soaking with water, the application of growth-promoting substances (KNO_3 or GA_3);
- stratification, rinsing with H_2SO_4 ;
- stratification, rinsing with H_2SO_4 , the application of growth-promoting substances (KNO_3 or GA_3);
- stratification, soaking with H_2SO_4 for 2 min.;
- stratification, soaking with H_2SO_4 for 2 min., the application of growth-promoting substances (KNO_3 or GA_3);
- stratification, soaking with H_2SO_4 for 5 min.;
- stratification, soaking with H_2SO_4 for 5 min., the application of growth-promoting substances (KNO_3 or GA_3).

Germination tests were conducted in the same way as in the seed storage experiment explained above.

The initial growth of seedlings

The germinating seeds from the pre-sowing treatment experiment (the combination: stratification, rinsing with H_2SO_4 and application of KNO_3), were used to observe an initial growth of seedlings. The seeds were placed in sterile 8 cm Petri dishes filled with filter paper (germinating bed). The paper was wetted systematically with distilled water. On each Petri dishes 5 seeds were placed (8 replications were applied). The experiment was performed in a climatic chambers MLR 350 Sanyo, in a controlled conditions (irradiation $150 \mu E/m^2 \times s$; temp. 20°C for 24 h). The length of the radicle, number of lateral roots, length of coleoptile and number of leaves were measured and recorded each 6–8 days. The observations were conducted until the 44th day of experiment (fully developed seedlings).

Statistical analysis

The obtained data were subjected to statistical analysis using Statistica® software. The mean values were compared by using the one way analysis of variance (ANOVA) followed by Tukey's multiple range test. The differences between means were deemed to be significant at $p < 0.05$.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

Southern sweet-grass is a perennial from a group of tuft grasses [16]. The plant begins its growth very early. Fully developed panicles are already present in the leaf sheaths in previous autumn (unpublished data). Thus, flowering shoots appeared before the leaves, in April. Inflorescence consist of spikelets arranged in panicles. Each spikelet, with two glumes, consist of three small florets. However, only the middle one is fertile and the two marginal ones are unisexual (male). Southern sweet-grass seeds (fig. 1) called spikelets (caryopsis tightly covered with lemma, palea and male flowers residues), alike the elements of inflorescence from which the seeds develop, are mature and ready to spread in May. The generative development is followed by the vegetative growth, occurring from the end of May [1, 17-19]. Some data on the factors influencing the generative development of *H. australis* are available. The number of flowering shoots and the mass of seeds are affected by the light conditions [14]. According to Bączek *et al.* [12], the number of southern sweet-grass inflorescences produced by 2-years-old plants vary from 27.4 to 40.7 per plant, their length from 57.0 to 61.8 cm and the mass of seeds obtained from those plants from 2.41 to 4.62 g per plant. However, no dormancy/germination research on this material have been done yet. In order to understand the biology of seed germination, many factors have to be taken into consideration. The process is affected by the growth potential of embryo, the restraints imposed by surrounding tissues as well as external factors to which the seeds are subjected.

In present study, the influence of storage time on the sowing parameters of *H. australis* seeds was investigated. The 1000 seeds weight as well as the seed moisture did not depend on the factor. The mass varied from 0.910 to 0.981 g and the moisture from 8.03 to 8.31% (tab. 1). However, the time of storage had the clear effects on germination. A relatively low germination of seeds evaluated directly after harvest decreased significantly during the period of their storage. On the other hand, their viability was relatively high. After 30 months of storage it had decreased only up to 70.9% (tab. 1). The low germination rate after storage combined with relatively high viability may be related to a deep seed dormancy.

Table 1.

The influence of storage period on seed germination parameters of *H. australis*

Period of storage	1000 seed weight [g]	Seed moisture content [%]	Germinability [%]	Seed viability [%]
Non-stored	0.981a	8.31a	42.6a	86.24a
6 months	0.969a	8.17a	35.1a	83.97a
18 months	0.922a	8.03a	20.5b	79.07a
30 months	0.910a	8.00a	4.2c	70.90b

Values in columns marked with different letters differ at $p < 0.05$, Tukey's test

On natural sites southern sweet-grass seeds are spread over long distances by the wind only occasionally. After maturing they shed very quickly and easily in the vicinity of the maternal plant, and remain there. Like in many other wild species, phenotypic polymorphism of seeds expressed by uneven germination allows to "bypass" the adverse effects of habitat. It is manifested mainly through the "escape" of diaspores in time, that is, slowing germination and creating a gene bank in the soil. This happens most often for the plants whose seeds lack wild dispersal and are not moved a distance. The germination of such seeds is usually reduced, while their viability relatively high [20-22]. Such phenomena of a great importance in an evolutionary point of view was described previously for many species, including grasses [23, 24].

To break the dormancy, many pre-sowing treatments are applied. Temperature, water and a wide range of growth regulators are the most important factors used to release the seed dormancy. However, in a number of species the seeds respond to more than one factor. In such situations, dormancy breaking requires the application of two or more factors, including special sequences of their usage [25]. In our experiment stratification, hydration, scarification and application of growth-promoting substances were used to break the dormancy of southern sweet-grass seeds.

Short rinse of stratified seed in sulfuric acid and application of potassium nitrate or the gibberellin resulted in the highest percentage of germinating seeds (tab. 2). The application of only one factor gave little effects. However, stratification significantly stimulated the process irrespective of other treatments. In turn, the application of potassium nitrate and gibberellin, substances that stimulate the activity of amylases, resulted in much higher percentage of germinated seeds only after scarification treatment. The increase in germination percentage caused by the removal of glumes in some grass species was reported previously [23]. Probably, brief exposure to sulfuric acid caused micro injuries in the cover of seeds which accelerated better penetration of water and growth-promoting substances. The improvement of seeds germination in such conditions may be associated with an increase in starch hydrolase activity (α - and β -amylase) and as a consequence with increased metabolism of starch and soluble proteins [26]. The obtained results suggest that southern sweet-grass has developed different mechanisms in seeds to prevent seed germination in unfavourable environment.

The seeds display both physiological and physical dormancy. According to Baskin and Baskin [27], such a type of dormancy is called combinational dormancy. The seeds need to go through scarification and stratification to imbibe water and then – specific physiological treatment to germinate.

In the present study, the initial southern sweet-grass seedling growth was also described. The emergence of the radicle was preceded by a clear root sheath thickening, which interrupted the seed coat. Radicle has appeared on the eighth day of the experiment. At that time it had the length of about 1 mm. At the end of the observation (after 44 days) the length of the main root was 12.90 mm, on average. At its base, lateral roots appeared. Their number in different periods of the observation is shown in table 3. At the time when the seed coat was interrupted by the radicle, it was also pierced by the leaf sheath (coleoptile). Cotyledon was strongly reduced and difficult to recognize. Leaf was released after eighteen day of the observation (fig. 5). According to Moore *et al.* [28], this particular moment, i.e. the emergence of first leaf is crucial for perennial grasses. It is the end of germination stage and the beginning of vegetative phase.

Table 2.

The influence of seed pre-sowing treatment on the germinability of *H. australis* (%)

Hydration/scarification		Growth-promoting substances	Stratification	
			non-stratified seeds	Stratified seeds
Hydration	rinsing with H ₂ O	control	0h	22g
		KNO ₃	22e	33e
		GA ₃	21e	35de
	soaking with H ₂ O for 24h	control	0h	21g
		KNO ₃	28cd	30e
		GA ₃	26d	31e
Scarification	rinsing with H ₂ SO ₄	control	16f	54b
		KNO ₃	39a	71a
		GA ₃	40a	72a
	soaking with H ₂ SO ₄ for 2 min.	control	5g	24fg
		KNO ₃	36b	48c
		GA ₃	40a	49c
	soaking with H ₂ SO ₄ for 5 min.	control	3g	26f
		KNO ₃	29cd	39d
		GA ₃	32c	52bc
mean			22	40*

Values in columns marked with different letters differ at $p < 0.05$, Tukey's test

* $p < 0.05$ (concerning the mean values for stratification – in a row)



Figure 1.
Southern sweet-grass seeds (photo: A. Pawełczak)



Figure 2.
Southern sweet-grass fruits (photo: A. Pawełczak)

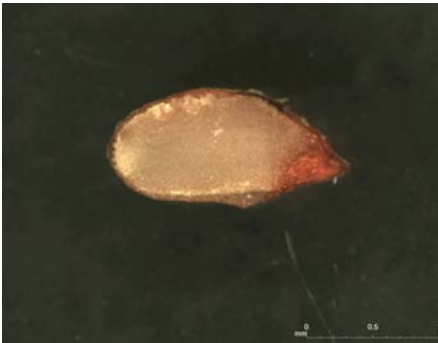


Figure 3.
Viable fruit – longitudinal section (photo: E. Pióro-Jabrucka)



Figure 4.
Non-viable fruit – longitudinal section (photo: E. Pióro-Jabrucka)



Figure 5.
Stages of the initial growth of southern sweet-grass seedlings (photo: E. Pióro-Jabrucka)

Table 3.

The initial seedling development of *H. australis*

Days after seed setting on germinating bed	Length of the main root [mm]	Number of lateral roots per seedling	Length of coleoptile [mm]	Number of leaves per plant
8	1.0	0.4	0.5	-
14	1.9	1.1	1.5	-
18	3.8	2.2	2.0	1.0
26	6.2	3.5	3.0	1.0
32	9.0	4.0	3.0	1.0
38	9.9	4.7	3.5	1.0
44	12.9	5.4	3.5	1.0

CONCLUSIONS

- Southern sweet-grass seeds become dormant directly after their harvest.
- The seeds lose their germinability gradually. After 30 months of storage, their ability to germinate becomes close to zero, but viability remains at a high level.
- The seeds dormancy can be overcome through sequential application of following factors: stratification, scarification, application of growth-promoting substances.
- The seeds germinate after eight days. In next ten days, the first leaf appears and the stage of vegetative development begins.

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WPŁYW PRZECHOWYWANIA NASION TURÓWKI LEŚNEJ I ZABIEGÓW PRZEDSIĘWNYCH NA ICH KIEŁKOWANIE I POCZĄTKOWY ROZWÓJ SIEWEK

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Streszczenie

Wstęp: Turówka leśna (*Hierochloë australis* /Schrad./ Roem. et Schult., *Poaceae*) jest wieloletnią trawą kępkową występującą w północno-wschodniej Europie. Jej liście zbierane są z roślin dziko rosnących jako surowiec aromatyczny wykorzystywany w przemyśle alkoholowym. W związku z nadmiernym zbiorem tej rośliny podjęto próby wprowadzenia jej do uprawy. **Cel:** Celem pracy było określenie wpływu przechowywania nasion (kłosek) turówki leśnej oraz zabiegów przedsiewnych na ich kiełkowanie i początkowy rozwój siewek. **Metody:** Nasiona zostały ocenione bezpośrednio po ich zbiorze oraz po 6, 18 i 30 miesiącach przechowywania. Masa 1000 nasion, wilgotność nasion, zdolność kiełkowania i żywotność nasion (test tetrazolinowy) zostały określone wg przepisów ISTA. Stratyfikacja, skaryfikacja, uwodnienie oraz substancje wzrostowe (KNO_3 and GA_3) zostały zastosowane w celu poprawy kiełkowania nasion. **Wyniki:** Po 30 miesiącach przechowywania zdolność kiełkowania nasion zmalała z 42,6 (po zbiorze) do 4,6%, z tym, że ich żywotność pozostała na wysokim poziomie (70,9%). Stratyfikacja, krótkie płukanie nasion w roztworze H_2SO_4 oraz zastosowanie KNO_3 lub GA_3 zwiększało istotnie zdolność kiełkowania nasion (do ponad 70%). **Wnioski:** Nasiona turówki leśnej po dojrzeniu szybko wchodzą w okres spoczynku. Poprawa ich kiełkowania wymaga zastosowania zabiegów łączonych tj. stratyfikacji, skaryfikacji oraz substancji wzrostowych.

Słowa kluczowe: *Hierochloë australis*, kłoski, stratyfikacja, skaryfikacja, substancje wzrostowe