# EFFECT OF CHEMICAL AND PHYSICAL FACTORS ON GERMINATION CAPACITY OF REED CANARY GRASS (*Phalaris arundinacea* L.) SEED DEPENDING ON STORAGE TIME

### Tomasz R. Sekutowski

Institute of Soil Science and Plant Cultivation, National Research Institute in Puławy Department of Weed Science and Tillage Systems, Orzechowa 61, 50-540 Wrocław, Poland e-mail: t.sekutowski@iung.wroclaw.pl

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#### Abstract

This paper presents the research results regarding the effect of temperature (-15°C) and sulfuric and gibberellic acids on germination capacity of 1-, 2- and 4-year-old seed of reed canary grass (P. arundinacea). According to the results obtained, the stimulatory and inhibitory effect of the above mentioned factors depended on seed age and the time of seed exposure. A significant increase in germination capacity under the influence of low temperature (-15°C) was recorded only for 2- and 4-year-old seed after 6-hour exposure. However, one-year-old seed showed a significant decrease in germination capacity after 48 hours of freezing. The application of sulfuric acid significantly increased seed germination capacity only in the case of 4-year-old seed, regardless of the time of exposure, while a decrease in germination capacity, also not related to the time of sulfuric acid application, was observed in 1-year-old seed. A significant increase in germination, resulting from seed treatment with gibberellic acid, was recorded for 4-year-old seed subjected to soaking for 12 h and for 2-year-old seed (soaking time 6 h) as well as for 1-year-old seed exposed to this factor for 1h. The inhibitory effect of gibberellic acid was observed in 1-year-old seed affected by this factor for 6h and 12 h as well as for hormone treatment of 2-year-old seed for 1 h and 12 h.

Key words: *Phalaris arundinacea*, seed, germination capacity, temperature, gibberellic acid, sulfuric acid, storage time

### **INTRODUCTION**

Reed canary grass (*Phalaris arundinacea* L.) is a perennial species belonging to the family Poaceae; they are common as native plants forming permanent grassland [1,2]. On purposefully established grazing lands, this species can also be a typical component of sward, often used as feed [3,4]. Another use of reed canary grass (*P. arundinacea*) involves energy purposes [5–10]. Therefore, reed canary grass (*P. arundinacea*) seed, intended for sowing, should meet defined quality requirements [11]. The common property of seed material of this species is the presence of seed featuring decreased germination capacity or abnormal germination, which especially relates to seed stored for a long time [12–14].

In such cases, seed dormancy disturbance takes place through pre-sowing stimulation using a number of stimuli. Most often, they involve physical factors, i.e. electric or magnetic field, helium-neon laser light, ultraviolet radiation, visible light (the phenomenon of positive photoblastism), or thermal factors (freezing or soaking in hot water) [15–20]. As far as chemical factors are concerned, the following compounds: sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), nitric acid (HNO<sub>3</sub>), peracetic acid (CH<sub>3</sub>COOH), gibberellic acid (GA<sub>3</sub>), or potassium hydroxide (KOH), are most often applied [21–24]. A biological factor, in the form of effective microorganisms (EM), has also gained an increasing popularity in this type of research [25,26].

The use of seed processing through the introduction of some physical, chemical or biological factors can positively contribute to the acceleration and increase in even emergence, especially in the case of seed germinating under unfavorable environmental conditions as well as seed of decreased germination capacity, e.g. old, hard or damaged seed [27–31].

Therefore, the aim of this research was to determine the effects of low temperature, sulfuric acid and gibberellic acid on germination capacity of reed canary grass (*P. arundinacea*) seed according to its storage time.

### MATERIALS AND METHODS

The material for investigation consisted of reed canary grass (*P. arundinacea*) seeds, collected from a plantation established for energy purposes. The obtained seeds were stored in a biological laboratory in a dry state (about 10.0% of water) at a temperature of 20–25°C and a relative air humidity of 40–50%, for a period of one, two, and four years. In this laboratory experiment, the following factors were applied: one physical factor – seed stratification – and two chemical factors – seed scarification and hormone treatment.

Stratification consisted in storing seeds in a closed container for 6 h, 24 h, and 48 h at a temperature of -15°C (so-called freezing). Chemical scarification involved soaking seeds in 5.0% sulfuric acid for 5, 10 and 20 min. and then rinsing them three times for 3 min. in distilled water, followed by drying on filter paper. Hormone treatment involved seed soaking in gibberellic acid (1 mmol GA<sub>3</sub>) for 1 h, 6 h and 24 h and then drying on filter paper. Seeds not treated with any of the above mentioned factors were the control treatment. Seeds prepared in that way (separately for each year and combination) were sown at an amount of 30 pieces in a Petri dish in three replications. The Petri dish was kept in the biological laboratory under controlled conditions (temperature 25°C and humidity 70%). Germination capacity was evaluated after 14 days on the basis of the quantity of germinated seed, expressed as a percentage [32].

The results obtained from observations were presented as mean values and were subjected to statistical analysis using analysis of variance and Tukey's test to determine the significance of differences between means, at a significance level  $p \le 0.05$ .

### RESULTS

The analysis of the research results regarding the evaluation of seed germination of reed canary grass (*P. arundinacea*) under laboratory conditions proved the selected physical and chemical factors (stratification, chemical scarification, and hormone treatment) to have a significant effect on seed germination capacity, depending on storage time. The highest variation in germination was recorded for seeds subjected to stratification, followed by seeds that underwent chemical scarification and seeds after hormone treatment with gibberellic acid (Table 1–3).

Stratification of reed canary grass (*P. arundina-cea*) seed using low temperature.

The stimulating or inhibitory effect of low temperature (-15°C) on germination of reed canary grass (*P. arundinacea*) seed depended on the time of seed exposure to this factor as well as on the age of stored seed.

In the group of the oldest seed (4-year-old), seeds stored at the low temperature for 6 h were characterized by the highest germination capacity. This seed showed increased germination capacity by 14.5% in comparison to control seed, which was statistically proved. Also the seed subjected to freezing for 24 h improved its germination capacity in relation to the control treatment, yet this difference (4.5%) was not statistically confirmed. In the case of the seed exposed to low temperature for 48 h, a 4.0% decrease in germination capacity was recorded as compared to the control, although this difference was not statistically proved (Table 1).

In 2-year-old seed, only the application of low temperature for 6 h resulted in a significant (by 11.5%) increase in germination capacity in comparison to the control treatment. The seed stimulated for 24 h also showed increased germination capacity by 5.0%, but this result was statistically insignificant. Only the seed exposed to low temperature for 48 h responded by decreasing germination by 5.0% in relation to control seeds, yet also in this case the difference was not statistically confirmed (Table 1).

Seed stored for the shortest period (1 year) did not show any significant increase in germination capacity under the influence of low temperature in a time unit. In seed subjected to freezing for 6 h, the germination capacity increased by 2.0% as compared to the control, but this difference was not statistically confirmed. As regards seed stored for 24 h, it was found that its germination capacity decreased by 5.0%, although this finding proved to be statistically insignificant. The only exception was found to be the seed that underwent stimulation for 48 h and was characterized by a significant decrease in germination capacity, which was statistically proved (Table 1).

 Table 1

 Influence of temperature (-15°C) and storage time on

 germination [%] of reed canary grass (*P. arundinacea*) seed

Freezing -	Storage time			
	4 years	2 years	1 year	
0 h (control)	55.5bc	58.5bc	66.5ab	
6 h	70.0a	70.0a	68.5a	
24 h	60.0b	63.5b	61.5bc	
48 h	51.5c	53.5c	58.5c	

Means in columns marked with the same letters do not differ significantly at  $p \le 0.05$ 

Chemical scarification of reed canary grass (*P. arundinacea*) seed using sulfuric acid.

Significant differences in germination could be found only in one group of seed stored for 4 years and

subjected to chemical scarification. Clearly increased germination capacity was observed in seed affected by sulfuric acid for 20 min, since it increased by 17.0% in relation to control seed and this fact was statistically confirmed. Similarly, seed soaked in sulfuric acid for 5 min and 10 min increased its germination capacity in comparison to the control treatment by 8.0% and 9.0%, respectively. In both cases, the differences were statistically significant (Table 2).

2-year-old seed treated with sulfuric acid was also found to show a stimulating effect of this factor, as expressed by a slight increase in germination capacity (1.0-1.5%) compared to the control treatment. However, regardless of the time of seed exposure to the scarifying factor (5, 10 or 20 min), the recorded differences were not statistically proved (Table 2).

As far as the youngest seed is concerned, i.e. the one stored for one year, the application of chemical scarification, in the form of sulfuric acid, resulted in a reduction in germination capacity of all examined seeds. Depending on the time of treatment with the scarification factor (5, 10 or 20 min), germination diminished by 1.5%, 3.0%, and 5.0%, respectively, in comparison to control seed, but these data were not statistically confirmed (Table 2).

 Table 2

 Influence of sulfuric acid (5%) and storage time on

 germination [%] of reed canary grass (*P. arundinacea*) seed

Sulfuric acid	Storage time		
	4 years	2 years	1 year
0 min (control)	55.5c	58.5a	66.5a
5 min	63.5b	60.0a	65.0a
10 min	64.5b	59.5a	63.5a
20 min	72.5a	60.0a	61.5a

Means in columns marked with the same letters do not differ significantly at  $p \le 0.05$ 

Hormone treatment of reed canary grass (*P. arundinacea*) seed using gibberellic acid.

In the sample of seed stored for 4 years, a significant increase in germination capacity was recorded solely for seed subjected to gibberellic acid for 12 h. The examined parameter increased by 14.0% as compared to the control treatment, which was statistically proved. Also seed that underwent hormone treatment for 1 h and 6 h increased its germination by 1.0% and 4.5%, respectively, in relation to the control, but these differences were not statistically confirmed (Table 3).

Two-year-old seed showed a significant increase in germination capacity (by 14.0%) in relation to the control treatment only when affected by gibberellic acid for 6 h. Seed subjected to gibberellic acid for 1 h and 12 h reduced its germination by 1.0% and 2.0% in comparison to the control treatment. Nevertheless, these differences were not statistically proved (Table 3).

In 1-year-old seed treated with gibberellic acid for 1 h, significantly higher values of germination capacity (by 7.0%) were observed compared to the control. Seed that underwent hormone treatment with gibberellic acid for 6 h and 12 h revealed decreased germination capacity by 1.5% and 5.0%, respectively, in relation to the control. The obtained differences could not be statistically confirmed (Table 3).

Table 3 Influence of gibberellic acid (1 mmol GA<sub>3</sub>) and storage time on germination [%] of reed canary grass (*P. arundinacea*) seed

Gibberellic _ acid	Storage time			
	4 years	2 years	1 year	
0 h (control)	55.5b	58.5b	66.5b	
1 h	56.5b	57.5b	73.5a	
6 h	60.0b	72.5a	65.0b	
12 h	69.5a	56.5b	61.5b	

Means in columns marked with the same letters do not differ significantly at  $p \le 0.05$ 

#### DISCUSSION

Intense aging of seed due to long-lasting and inappropriate storage is mainly expressed by a rapid reduction in its germination capacity. This very often leads to uneven, weak and thinned emergence of seedlings, which, in consequence, can negatively affect yield size and quality [33]. K  $u \dot{z} d o w \dot{i} c z$  [34] as well as S t e i n e r and R u c k e n b a u e r [35] believe that, in order to maintain a suitable sowing value, seed has to be stored in appropriate thermal and humidity conditions. The research results obtained by other authors indicate the fact that seed response to long-term storage can also be dependent on particular species, cultivar as well as on the size of seed stored [36–38].

The research results obtained by the author proved that the effect of reed canary grass seed stimulation with selected physical and chemical factors depended on seed age. The oldest seed, i.e. seed stored for 4 years, showed the strongest response to freezing at the temperature of  $-15^{\circ}$ C. 2-year-old seed was characterized by similar response, while 1-year-old seed showed the poorest response to low temperature. According to Y a w a l i k a r et al. [24], the germination capacity of *Pentapetes phoenicea* seed was considerably influenced not only by temperature, but also by storage time. Seed stored for 15–18 months showed the best germination capacity, while seed stored for 3 and 30 months was found to have the lowest capacity value. A similar conclusion can be drawn from the research by Steiner and Ruckenbauer [35], namely that the germination capacity of *Hordeum vulgare* and *Avena sativa* seed depended on storage time as well as on thermal and humidity conditions.

Treating reed canary grass (P. arundinacea) seed with sulfuric acid (chemical scarification) contributed to the improvement in germination capacity only in the case of 4-yeaar-old seed. The maximum increase in germination capacity of the above mentioned seed was 17.0%. A different opinion was expressed by Doliński [39] who claimed that only those seeds of Sida hermaphrodita that were stored for 1 year and then subjected to 30 min treatment with sulfuric acid showed the best germination. Older seed, 2.5 year old, was characterized by decreased germination capacity, regardless of the time of its exposure to sulfuric acid. Also Emongor et al. [21] obtained similar results regarding the effect of sulfuric acid on Corchorus tridens seed depending on its storage time. The above mentioned researchers proved that 1-year-old seed was characterized by the best germination, regardless of the time of its treatment with sulfuric acid. On the other hand, 2-year-old seed demonstrated decreased germination capacity.

In the conducted experiment, only hormone treatment with gibberellic acid significantly affected the germination capacity of reed canary grass (P. arundinacea) seed, independently of seed storage time. A significantly higher value of germination capacity was recorded for 4-year-old seed subjected to hormone treatment for 12 h, 2-year-old seed soaked in gibberellic acid for 6 h as well as for 1-year-old seed exposed to this factor for 1 h. A different opinion was held by Emongor et al. [21] who proved that C. tridens seed stored for 1 year showed the best response to pre-sowing stimulation with gibberellic acid (regardless of its concentration). In the same experiment, 2-year-old seed either did not respond at all or its response was statistically insignificant. On the other hand, Yawalikar et al. [24] reported that application of different concentrations of gibberellic acid did not significantly influence the germination capacity of P. phoenicea seed.

### **CONCLUSIONS**

- 1. The response of reed canary grass (*P. arundinacea*) seed to physical and chemical factors (stratification, chemical scarification, and hormone treatment) depended on seed age and the time of seed exposure to these factors.
- 2. A significant increase in germination capacity resulting from the effect of low temperature (stratification) was recorded only in 4- and 2-year-old seed exposed to this factor for 6h. 1-year-old seed sub-

jected to freezing for 48h showed a considerable decrease in germination capacity.

- 3. The application of sulfuric acid (chemical scarification) significantly increased the germination capacity only in 4-year-old seed that underwent treatment with this factor for 5, 10, and 20 min. An apparent, although not statistically confirmed, decrease in germination capacity was observed for 1-year-old seed, regardless of the time of its exposure to sulfuric acid.
- 4. Significantly higher values of germination capacity under the influence of gibberellic acid were proved for 4-year-old seed subjected to hormone treatment for 12h, 2-year-old seed soaked in gibberellic acid for 6h as well as for 1-year-old seed exposed to this factor for 1h. An apparent, although not statistically confirmed, inhibitory effect of gibberellic acid was recorded for 1-year-old seed treated with this factor for 6h and 12h as well as for 2-year-old seed subjected to hormone treatment for 1h and 12h.

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### Wpływ czynników fizycznych i chemicznych na zdolność kiełkowania nasion mozgi trzcinowatej (*Phalaris arundinacea* L.) w zależności od czasu przechowywania

## Streszczenie

W pracy przedstawiono wyniki badań dotyczących wpływu: temperatury (-15°C), kwasu siarkowego i kwasu giberelinowego na zdolność kiełkowania 1,

2 oraz 4 letnich nasion mozgi trzcinowatej (P. arundinacea). Z przeprowadzonych badań wynika, że stymulujące lub inhibicyjne działanie było uzależnione od wieku nasion oraz czasu działania tych czynników. Istotne zwiększenie zdolności kiełkowania w wyniku działania niskiej temperatury (-15°C), stwierdzono jedynie dla nasion 4 i 2 letnich, które były wystawione na jej działanie przez okres 6 h. Natomiast istotne obniżenie zdolność kiełkowania stwierdzono dla nasion 1 rocznych, które były przemrażane przez okres 48 h. Zastosowanie kwasu siarkowego istotnie zwiększyło zdolność kiełkowania jedynie nasion 4 letnich, niezależnie od czasu działania tego czynnika. Natomiast obniżenie zdolności kiełkowania stwierdzono dla nasion 1 rocznych, również niezależnie od czasu oddziaływania kwasu siarkowego. Istotne zwiększenie zdolności kiełkowania w wyniku działania kw. giberelinowego, stwierdzono dla nasion 4 letnich, które były poddane hormonizacji przez okres 12 h oraz dla nasion 2 letnich (czas moczenia 6 h), a także dla nasion 1 rocznych wystawionych na działanie tego czynnika przez okres 1 h. Natomiast działanie inhibicyjne kwasu giberelinowego stwierdzono dla nasion 1 rocznych wystawionych na działanie tego czynnika przez okres 6 h i 12 h oraz dla nasion 2 letnich, które były hormonizowane przez okres 1 h i 12 h.

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