BIOSCIENCE JOURNAL

EVALUATION OF POSTHARVEST AGE AND DORMANCY-BREAKING METHODS ON *Echinochloa crus-galli* SEED GERMINATION

Alexandre PISONI[1], Giliardi DALAZEN[2], Mateus GALLON[3], Catarine MARKUS[4], Aldo MEROTTO JR[4]

[1]SLC Agrícola S.A., Porto Alegre, Rio Grande do Sul, Brazil.
[2]Department of Plant Science and Plant Health, Universidade Estadual de Ponta Grossa, Ponta Grossa, Paraná, Brazil.
[3]Klabin S.A., Telêmaco Borba, Paraná, Brazil.
[4]Department of Crop Science, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

Corresponding author:
Giliardi Dalazen
giliardidalazen@gmail.com

How to cite: PISONI, A., et al. Evaluation of postharvest age and dormancy-breaking methods on *Echinochloa crus-galli* seed germination. *Bioscience Journal*. 2024, **40**, e40047. https://doi.org/10.14393/BJ-v40n0a2024-62804

Abstract

Barnyardgrass (*Echinochloa crus-galli*) is one of the most troublesome weeds in irrigated rice cultivation and has increasingly impacted rainfed crops due to the emergence of herbicide-resistant populations. Understanding its germination dynamics is crucial for developing and implementing effective management strategies. Additionally, since barnyardgrass research relies on growing plants from seeds, its dormancy characteristics are of particular interest. The present study aimed to evaluate the influence of postharvest age on barnyardgrass seed germination and the effectiveness of different dormancy-breaking methods in susceptible and herbicide-resistant populations. Germination rate (G), germination speed index (GSI), and seed viability, assessed using the topographic tetrazolium test, were measured in seed lots with four different postharvest ages: two years, one year, two months, and one day postharvest. The seeds were subjected to 15 dormancy-breaking methods, including temperature variation and the use of solutions containing H_2SO_4, KNO_3, and GA_3. Seeds that were one or two years old showed germination rates exceeding 90%, regardless of the method used. In contrast, seeds aged two months or one day postharvest only germinated when exposed to 40°C for seven days, with G values of 25.2% and 5.9%, respectively. Both herbicide-susceptible and resistant barnyardgrass populations exhibited similar dormancy levels and responses to dormancy-breaking methods. The results indicate that newly harvested seeds have high dormancy levels, and specific methods are only partially effective in overcoming barnyardgrass seed dormancy.

Keywords: Barnyardgrass. Chemical scarification. Seed bank. Seed dormancy. Thermal scarification.

1. Introduction

Barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) is one of the most problematic weeds in irrigated rice (*Oryza sativa* L.) cultivation in Brazil and other countries (Tian et al. 2020; Ulguim et al. 2021). It has also become a significant concern in soybean and corn crops (Marchesi and Saldain 2019), particularly with crop rotation systems. It is particularly troublesome due to its substantial adaptability, wide distribution, high germination capacity under water deficit, and high plasticity regarding temperature and CO_2 concentration (Bajwa et al. 2015; Dalazen et al. 2020).

Barnyardgrass has a C_4 photosynthetic metabolism (Elmore and Paul 1983) and exhibits a high

competitive capacity (Agostinetto et al. 2021). Depending on factors such as weed density, rice cultivar, and irrigation management, yield losses can be up to 90% (Pinto et al. 2008; Bajwa et al. 2015). Instances of herbicide resistance have also been documented: in Brazil, *E. crus-galli* has developed isolated resistance to several herbicide groups, including auxin mimics (group O, 4), acetolactate synthase (ALS) inhibitors (group B, 2), acetyl-CoA carboxylase (ACCase) inhibitors (group A, 1), cellulose synthesis inhibitors (group L, 29), and 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) inhibitors (group G, 9) (Matzenbacher et al. 2015; Heap 2024). Globally, 52 cases of resistance have been reported in 24 countries, ranking barnyardgrass among the top 15 most problematic weed species regarding herbicide resistance (Yang et al. 2017; Heap 2024).

Barnyardgrass reproduces by seeds, producing 2,000 to 4,000 seeds per plant, depending on environmental conditions (Gibson et al. 2002). Despite the high seed production, dormancy reduces the likelihood of plant emergence soon after seed dispersal. Dormancy contributes to the soil seed bank, allowing seeds to germinate over an extended period, leading to prolonged weed outbreaks (Clay et al. 2005; Longo et al. 2021). The viability of barnyardgrass seeds has been observed even after 12 years when buried at a depth of 20 cm (Burnside et al. 1996). Therefore, seed dormancy hampers the control of this species in agricultural areas.

Furthermore, information on the physiology, molecular regulation, and dormancy-breaking methods of barnyardgrass seeds is scarce, especially when compared to other common weeds in rice cultivation, such as weedy rice (*Oryza sativa*). In recent years, important advances have been made in understanding the molecular mechanisms of seed dormancy in weedy rice (Fogliatto et al. 2020). Over 12 quantitative trait loci (QTLs) have been identified as regulators of dormancy in weedy rice seeds (Zhang et al. 2020). Additionally, the interaction between genotype and environment plays a crucial role in dormancy in weedy rice (Fogliatto et al. 2012). Many plants show variable germination rates due to dormancy, with the intensity and duration of dormancy influenced by biotype and environmental conditions (Delatorre 1999; Guillemin et al. 2013). Efforts to control barnyardgrass are complicated by seed dormancy, as seeds tend to emerge at low or inconsistent rates due to prolonged dormancy periods. Moreover, studies on herbicide resistance in barnyardgrass require seeds to be evaluated soon after harvest to ensure rapid assessments and provide timely information to producers (Matzenbacher et al. 2013). In some cases, the limited availability of seeds for research necessitates high germination rates.

Several methods for overcoming weed seed dormancy are known. However, their effectiveness depends on several factors, such as species, biotype, seed age, and the conditions and chemical treatments applied (Nawrot-Chorabik et al. 2021; Abrantes et al. 2021). These methods include the use of concentrated sulfuric acid (H_2SO_4), potassium nitrate (KNO_3), gibberellic acid (GA_3), hot water, temperature variation, thermal shock, mechanical scarification, and fire (Azania et al. 2003).

In addition to dormancy-breaking methods, the postharvest period significantly impacts seed dormancy levels. For *E. crus-galli*, germination rates are known to increase over time (Martinkova et al. 2006). Furthermore, combining dormancy-breaking methods may be more effective when isolated methods are insufficient. For example, immersing seeds for three days in 0.25M ethanol in the dark at 35–37°C, followed by germination for 14 days at 20–30°C with a 16/8 hour (day/night) photoperiod, resulted in germination rates higher than 80% in some *E. crus-galli* populations, although rates were considerably lower in others (Kovach et al. 2010).

A comprehensive evaluation of various dormancy-breaking methods is crucial for providing consolidated information on barnyardgrass. In this regard, the present study aimed to evaluate the impact of the postharvest period and different dormancy-breaking methods on the germination and viability of barnyardgrass seeds.

2. Material and Methods

The experiment was conducted at the Molecular Biology Laboratory of the Faculty of Agronomy at the Federal University of Rio Grande do Sul (*Universidade Federal do Rio Grande do Sul* - UFRGS), Porto Alegre, Rio Grande do Sul (RS), Brazil. The experimental design was completely randomized and organized in a factorial scheme, with four replicates of 100 *E. crus-galli* seeds placed in Petri dishes containing three

layers of previously sterilized filter paper. Four barnyardgrass populations were evaluated in separate experiments: two susceptible populations (SUSSP01 from Engenheiro Coelho, São Paulo (SP); MOSTS01 from Mostardas, RS) and two populations resistant to imidazolinone herbicides (ARRG01 from Arroio Grande, RS; PALMS01 from Palmares do Sul, RS) (Dalazen et al. 2018). Seeds from the second generation (generation 2) of each population were used.

Factor A consisted of four postharvest ages of barnyardgrass seeds: two years, one year, two months, and one day postharvest. Except for newly harvested seeds (one day postharvest), the other seeds were stored in a dry place at 25°C until the experiment began. Factor B included 15 dormancy-breaking methods obtained from a literature review of methods previously applied to *Echinochloa* spp. with some methodological adaptations, as follows:

T1 – Control: Seeds were soaked in distilled water.

T2 – Gibberellic acid (GA_3): Four levels of GA_3 were evaluated, defined as $T2_a$, $T2_b$, $T2_c$, and $T2_d$. In $T2_a$ and $T2_b$, the seeds were submerged in a GA_3 solution at 0.05% and 0.10%, respectively, for 24 hours. In $T2_c$ and $T2_d$, the seeds were submerged in a GA_3 solution at 0.05% and 0.10%, respectively, until the end of the evaluations.

T3 – Potassium nitrate (KNO_3) + low temperature: The seeds were kept in a 0.2% KNO_3 solution for 24 hours, washed with distilled water, and stored at 5°C for seven days.

T4 – Hot water: The seeds were submerged in water at 40°C for 24 hours.

T5 – Sulfuric acid (H_2SO_4): The seeds were submerged in 96% H_2SO_4 (36N) for three minutes ($T5_a$) or five minutes *($T5_b$)*, then washed with distilled water.

T6 – High temperature: The seeds were kept in a forced air oven at 40°C for seven days.

T7 – Low temperature: The seeds were stored at 5°C for seven days.

T8 – Submersion in water: The seeds were submerged in water at room temperature for 24 hours.

T9 – Thermal shock: The seeds were stored at 5°C for seven days, then transferred to a forced air oven at 40°C for 48 hours.

T10 – Submersion + KNO_3: The seeds were submerged in a 0.2% ($T10_a$) or a 0.4% ($T10_b$) KNO_3 solution until the end of the evaluations.

In treatments $T2_c$, $T2_d$, $T10_a$, and $T10_b$, the seeds were submerged in the respective solutions for the entire evaluation period. In the other treatments, after the initial exposure, the seeds were transferred to Petri dishes containing three layers of sterilized filter paper, with 7 mL of distilled water added. All seeds were kept in a controlled environment at 25°C with constant light throughout the observation period.

The evaluation involved daily counting of germinated seeds over 45 days to determine the germination speed index (GSI), following the methodology described by Maguire (1962). The germination rate (G) was determined at the end of the counting period. Non-germinated seeds were assessed using the topographic tetrazolium test (Brasil 2009) to determine the viability of potentially dormant seeds.

Data were submitted to normality and homogeneity tests, transformed to $\sqrt{X}$, and subsequently submitted to an analysis of variance ($p<0.05$). Means were compared using Tukey's test ($p<0.05$).

3. Results

No significant variations were found between the populations evaluated. Therefore, the averages of the four populations are presented. Significant differences in the G values were observed across dormancy-breaking treatments and different postharvest ages (Table 1). Seeds with longer postharvest periods (two years and one year) exhibited higher G values than newly harvested seeds (two months and one day). Seeds stored for two years had a G value of 96.8%, while those stored for one year had a G value of 90.6%. In contrast, seeds stored for two months or one day exhibited a G value of 0%.

The storage period also influenced the effectiveness of dormancy-breaking methods (Table 1). For seeds stored for two years, no significant differences were found between methods regarding G values, which averaged 96.0%. Similarly, seeds stored for one year did not show a significant increase in G in response to dormancy-breaking treatments. Conversely, a 10.5% reduction in germination was observed when seeds were submerged in water for 24 hours (T8) (Table 1). For seeds with a postharvest age of two months or one day, the only method of overcoming dormancy that significantly increased G was exposure

to high temperature (40°C) for seven days (T6) (Table 1). This treatment increased germination by 25.2% for seeds stored for two months and 5.9% for seeds stored for one day compared to the control.

Therefore, the present study demonstrates that seeds stored for more than one year exhibited high G values, rendering dormancy-breaking treatments unnecessary in these cases. In contrast, recently harvested seeds showed a modest but significant increase in G when subjected to high-temperature treatment (Table 1).

Table 1. Seed germination rate of *Echinochloa crus-galli* as a function of postharvest age and methods of overcoming dormancy.

Treatment	Postharvest period			
	two years	one year	two months	one day
T1 - Control	96.8^{aA}	90.6^{aA}	0.0^{bB}	0.0^{bB}
$T2_a$ - 0.05% GA_3 for 24h	93.1^{aA}	97.3^{aA}	0.0^{bB}	0.7^{bB}
$T2_b$ - 0.10% GA_3 for 24h	97.0^{aA}	98.0^{aA}	0.7^{bB}	0.2^{bB}
$T2_c$ - Final 0.05% GA_3	99.3^{aA}	99.4^{aA}	0.0^{bB}	0.0^{bB}
$T2_d$ - Final 0.10% GA_3	98.3^{aA}	97.2^{aA}	0.0^{bB}	0.0^{bB}
T3 - KNO_3 + low temperature	98.9^{aA}	98.1^{aA}	0.0^{bB}	0.0^{bB}
T4 - Hot water	98.7^{aA}	93.4^{aA}	0.0^{bB}	0.5^{bB}
$T5_a$ - H_2SO_4 3 min	92.1^{aA}	95.0^{aA}	0.0^{bB}	0.0^{bB}
$T5_b$ - H_2SO_4 5 min	98.9^{aA}	96.2^{aA}	2.0^{bB}	0.0^{bB}
T6 - High temperature (40 °C)	98.3^{aA}	97.0^{aA}	25.2^{aB}	5.9^{aC}
T7 - Low Temperature (5 °C)	90.3^{aA}	92.6^{aA}	0.0^{bB}	0.0^{bB}
T8 - Water submersion	96.1^{aA}	80.0^{bB}	0.0^{bC}	0.0^{bC}
T9 - Thermal shock	95.1^{aA}	93.7^{aA}	0.0^{bB}	0.0^{bB}
$T10_a$ - Submersion + 0.2% KNO_3	95.8^{aA}	95.5^{aA}	0.3^{bB}	0.0^{bB}
$T10_b$ - Submersion + 0.4% KNO_3	91.1^{aA}	92.6^{aA}	0.0^{bB}	0.0^{bB}
Average	96.0	94.4	1.9	0.5
CV (%)	9.5			

Means preceded by the same lowercase letters in a column or followed by the same uppercase letters in a row do not differ by Tukey's test at 5%.

GSI evaluations revealed that, across all treatments, seeds stored for longer periods (two years and one year) had higher GSI values (9.5 and 9.4, respectively; Table 2) than those with postharvest ages of two months or one day (<0.1). The only dormancy-breaking method that significantly affected GSI was submersion in water for 24 hours (T8), which reduced the GSI to 8.0 for seeds with a postharvest age of one year (Table 2).

Non-germinated seeds were evaluated using the topographic tetrazolium test. The results show that over 95% of the seeds were classified as viable in all treatments, regardless of postharvest age. Additionally, newly harvested seeds (two months and one day) exhibited a high dormancy level (Table 3).

4. Discussion

Barnyardgrass seeds with one or two years of postharvest age had higher G values than seeds harvested two months or just one day prior (Table 1). These results align with previous studies on barnyardgrass, which found that newly harvested seeds of *E. crus-galli* exhibit primary dormancy that can be overcome by an extended storage period. For example, Martinkova et al. (2006) observed that barnyardgrass seeds stored for eight years had higher G values (52.2%) compared to those stored for one year (2.4%). Similarly, Van Acker (2009) reported higher G values in *E. crus-galli* seeds stored for eight months (50%) compared to freshly harvested seeds (1.4%). Peralta Ogorek et al. (2019) also observed higher G values eight months after harvesting barnyardgrass seeds.

In newly harvested seeds, physical and physiological barriers can affect the immediate germination process. Physical barriers, such as glumellae, pericarps, and seed coats, protect the embryo from adverse

environmental conditions and microorganisms (Baskin and Baskin 2000). In barnyardgrass, dormancy is particularly associated with physical barriers present in the integuments and, more specifically, in the caryopses (Miyahara 1974). These barriers also prevent the entry of water and oxygen into the seed tissues, delaying the initiation of the germination process. However, when seeds are stored in dry conditions for a certain period, O_2 permeates to the seed interior, aiding in overcoming dormancy and promoting seed germination (Olatoye and Hall 1973; Silveira et al. 2014). This is reflected in the increased germination observed in seeds stored for over a year (Table 1). Besides, these physical barriers, an evolutionary characteristic of the species, may account for the lack of significant differences in germination between the biotypes evaluated.

Table 2. Seed germination speed index (GSI) in *Echinochloa crus-galli* as a function of seeds' postharvest age and methods of overcoming dormancy.

Treatment	Postharvest period			
	two years	one year	two months	one day
T1 - Control	9.6^{aA}	9.0^{aA}	0.0^{aB}	0.0^{aB}
$T2_a$ - 0.05% GA_3 for 24h	9.3^{aA}	9.7^{aA}	0.0^{aB}	0.1^{aB}
$T2_b$ - 0.10% GA_3 for 24h	9.7^{aA}	9.8^{aA}	0.1^{aB}	0.0^{aB}
$T2_c$ - Final 0.05% GA_3	9.9^{aA}	9.9^{aA}	0.0^{aB}	0.0^{aB}
$T2_d$ - Final 0.10% GA_3	9.8^{aA}	9.7^{aA}	0.0^{aB}	0.0^{aB}
T3 - KNO_3 + low temperature	9.8^{aA}	9.8^{aA}	0.0^{aB}	0.0^{aB}
T4 - Hot water	9.8^{aA}	9.3^{aA}	0.0^{aB}	0.0^{aB}
$T5_a$ - H_2SO_4 3 min	9.2^{aA}	9.5^{aA}	0.0^{aB}	0.0^{aB}
$T5_b$ - H_2SO_4 5 min	9.5^{aA}	9.6^{aA}	0.2^{aB}	0.0^{aB}
T6 - High temperature (40 °C)	9.8^{aA}	9.7^{aA}	2.5^{aB}	0.6^{aB}
T7 - Low Temperature (5 °C)	9.1^{aA}	9.2^{aA}	0.0^{aB}	0.0^{aB}
T8 - Water submersion	9.6^{aA}	8.0^{bB}	0.0^{aC}	0.0^{aC}
T9 - Thermal shock	9.6^{aA}	9.3^{aA}	0.0^{aB}	0.0^{aB}
$T10_a$ - Submersion + 0.2% KNO_3	9.5^{aA}	9.5^{aA}	0.0^{aB}	0.0^{aB}
$T10_b$ - Submersion + 0.4% KNO_3	9.1^{aA}	9.2^{aA}	0.0^{aB}	0.0^{aB}
Average	9.5	9.4	0.2	0.0
CV (%)	7.6			

Means preceded by the same lowercase letters in a column or followed by the same uppercase letters in a row do not differ by Tukey's test at 5%.

Chemical scarification methods using H_2SO_4 for three ($T5_a$) and five ($T5_b$) minutes significantly increased the G values of seeds with a postharvest age of two months or one day (Table 1). This lack of effect may be attributed to the short exposure time to H_2SO_4, which may not have been sufficient to reach the caryopsis region of the seed. Sadeghloo et al. (2013) observed that 95% of barnyardgrass seeds germinated after 15 minutes of exposure to concentrated H_2SO_4 (98%). Moreover, Sung et al. (1987) observed that 40 minutes of exposure of barnyardgrass seeds to H_2SO_4 resulted in 100% germination, compared to only 28% when the treatment lasted 10 minutes.

GA_3 application, regardless of concentration and exposure time, did not increase G (Table 1). This growth regulator acts as an antagonist to dormancy induced by abscisic acid (ABA) and also regulates the hydrolysis of seed reserves. However, the balance between these hormones depends on various endogenous factors, such as gene regulation, and exogenous factors, including temperature, light, and humidity (Tuan et al. 2018). In rice seeds, reducing ABA-induced reactive oxygen species (ROS) can nullify the effect of GA_3, thereby reducing G (Ye et al. 2012).

Similarly, treatments containing KNO_3 did not increase the G values of barnyardgrass seeds (Table 1). The hypothesis regarding nitrate action involves altering the seed's osmotic potential, thereby increasing the water absorption capacity of the caryopsis (McIntyre 1997). Studies have shown that the presence of KNO_3 in seeds reduces the need for GA_3 for germination and that its effect depends on the interaction with temperature and hormones like ABA and GA_3 (Alboresi et al. 2005). KNO_3 is a nitrate

compound commonly found in nature, particularly in drained environments, and is nearly absent in flooded soils. Thus, in these non-flooded environments, its accumulation signals the presence of O_2, which, in turn, stimulates seed germination (Mollard and Insausti 2009).

Table 3. Topographic test of tetrazolium seeds from *Echinochloa crus-galli* as a function of postharvest age and methods of overcoming dormancy.

Treatment	Viability (%)			
	Postharvest period			
	two years	one year	two months	one day
T1 – control	A 96.0 [a*]	A 95.6[a]	A 96.8[a]	A 97.6[a]
$T2_a$ – 0.05% GA_3 for 24h	A 98.1[a]	A 97.3[a]	A 96.2[a]	A 97.3[a]
$T2_b$ – 0.10% GA_3 for 24h	A 97.5[a]	A 98.0[a]	A 97.0[a]	A 98.0[a]
$T2_c$ – final 0.05% GA_3	A 99.0[a]	A 99.4[a]	A 99.4[a]	A 99.4[a]
$T2_d$ – final 0.10% GA_3	A 98.0[a]	A 97.3[a]	A 98.3[a]	A 97.2[a]
T3 – KNO_3 + low temperature	A 98.0[a]	A 98.1[a]	A 98.9[a]	A 98.1[a]
T4 – hot water	A 97.7[a]	A 96.9[a]	A 98.7[a]	A 97.4[a]
$T5_a$ – H_2SO_4 3 min	A 96.1[a]	A 98.0[a]	A 97.6[a]	A 97.0[a]
$T5_b$ – H_2SO_4 5 min	A 98.9[a]	A 96.2[a]	A 95.9[a]	A 96.2[a]
T6 – High temperature	A 98.3[a]	A 97.0[a]	A 98.3[a]	A 97.0[a]
T7 – Low Temperature	A 96.3[a]	A 95.9[a]	A 97.3[a]	A 96.6[a]
T8 – Water submersion	A 96.2[a]	A 97.1[a]	A 96.1[a]	A 97.5[a]
T9 – Thermal shock	A 95.1[a]	A 97.6[a]	A 95.1[a]	A 98.7[a]
$T10_a$ – Submersion + 0.2% KNO_3	A 95.8[a]	A 96.5[a]	A 95.8[a]	A 95.8[a]
$T10_b$ - Submersion + 0.4% KNO_3	A 97.1[a]	A 98.6[a]	A 97.1[a]	A 96.6[a]
Average	97.2	97.3	97.2	97.4
CV (%)	5.5			

* Means preceded by the same uppercase letter in a row or followed by lowercase letters in a column do not differ by Tukey's test at 5%.

The present results show that high-temperature treatment (T6 - 40°C for seven days) significantly increased the G values of barnyardgrass seeds with postharvest ages of two months or one day (Table 1). This treatment was the most effective method for overcoming dormancy in barnyardgrass, aligning with findings of Brasil (2009). Taylorson and Di Nola (1989) achieved similar results by exposing barnyardgrass seeds to 38°C for eight days, resulting in a G value of 82%. However, increasing the temperature to 42°C led to a decrease in G to 28%. The authors also reported an interaction between temperature and light intensity, where higher G values were associated with increased light exposure following heat treatment. Previous research by Kovach et al. (2010) also supports these findings. They noted that at a low temperature (10 °C), germination was absent in the presence of light and 25% in the dark, indicating a negative photoblastic response. At 20°C, G values were 94% with light and 25% without light, showing a positive photoblastic response. However, at 30°C, the G value was high (85-95%), regardless of the presence of light.

In weedy rice, different environmental factors can affect seed dormancy; however, temperature is the key factor controlling dormancy breaking (Liu et al. 2013). Temperature acts in conjunction with light on the signaling and hormonal balance of ABA and GA (Gubler et al. 2008; Izydorczyk et al. 2017). Consequently, it also plays a role in the enzymatic degradation of substances involved in the germination process and the protein composition of seed cell membranes (Di Nola and Taylorson 1989; Tuan et al. 2018). As previously discussed by Kovach et al. (2010), the dormancy process in barnyardgrass seeds is highly complex. The present study further underscores the critical role of temperature in breaking dormancy and regulating germination, as the high-temperature treatment yielded the highest G values for barnyardgrass seeds (Table 1).

The low GSI values (Table 2) combined with the seed viability observed in the tetrazolium test (Table 3) for seeds with postharvest ages of two months or one day indicates that dormancy is a crucial factor in maintaining a viable barnyardgrass seed bank in the soil. This highlights the need for integrated

management strategies to control this species, including efforts to prevent seed production and dispersal, especially given recent cases of herbicide resistance, including resistance to glyphosate (Heap 2021). For experimental purposes, these results suggest that dry storage for one year is the most effective method for obtaining non-dormant seeds. However, exposing seeds to temperatures of 40°C for seven days significantly increased G, particularly for seeds with a postharvest age of two months. Thus, employing techniques that lower soil temperatures, such as using straw in no-tillage systems, can reduce barnyardgrass germination.

5. Conclusions

Herbicide-susceptible and resistant barnyardgrass populations exhibited similar dormancy levels and responses to seed dormancy-breaking methods. The postharvest age of barnyardgrass seeds significantly affects the dormancy level and, consequently, germination rate and germination speed index values.

Seeds assessed one day and two months after harvest had low germination rates and germination speed index values due to dormancy. This dormancy is naturally overcome once seeds are stored for one or two years.

Exposure to temperatures of 40°C for seven days significantly increases the germination rates of seeds harvested two months or one day after harvest, with increases of 25.2% and 5.9%, respectively.

Authors' Contributions: PISONI, A.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; DALAZEN, G.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, critical review of important intellectual content; GALLON, M.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; MARKUS, C.: analysis and interpretation of data, drafting the article, critical review of important intellectual content; MERROTO JR, A.: conception and design, analysis and interpretation of data, drafting the article, critical review of important intellectual content.

Conflicts of Interest: There are no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: The authors would like to thank the funding provided by CAPES Foundation National Council for Scientific and Technological Development (CNPq) for scholarship and fellowship to the authors.

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Received: 13 August 2021 | **Accepted:** 19 June 2024 | **Published:** 30 October 2024