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Germination response of *Erigeron sumatrensis* (Retz.) seeds to drying and freezing

Condón, F. [1]; Kaspary, T. [2]; González, S. [1]

[1] *Instituto Nacional de Investigación Agropecuaria (INIA), Unidad de Semillas y Recursos Fitogenéticos, Colonia, Uruguay*

[2] *Instituto Nacional de Investigación Agropecuaria (INIA), Departamento de Protección Vegetal, Colonia, Uruguay*

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Nicolás Glison
Universidad de la República, Montevideo, Uruguay

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Correspondence
Federico Condón
fcondon@inia.org.uy

Abstract

Erigeron species form a complex of weed species that have evolved herbicide resistance in Uruguay. Genetic studies using seeds from different biotypes are frequently conducted to understand how this evolution occurred. However, these seeds are extremely small, and there is limited information available on their long-term conservation in germplasm banks. In this context, the aim of this study was to characterize drying strategies for long-term seed storage, comparing prolonged low-temperature drying (20 days at 20 °C, 15% RH) with shorter, higher-temperature drying (10 days at 40 °C or 7 days at 60 °C), and to assess seed germination responses to subsequent freezing (–20 °C for 30 days). The results showed that germination was not affected by any of the drying treatments. Moreover, freezing the seed batches increased germination, particularly in the more recently harvested seed lots (2020 and 2021), and especially when combined with drying at 60 °C. These findings indicate that *E. sumatrensis* seeds can be classified as orthodox and that long-term conservation in germplasm banks using standard drying and freezing protocols is feasible.

Keywords: orthodox seeds, long-term seed storage, genebank conservation, seed dormancy, weed biology

Respuesta de germinación de las semillas de *Erigeron sumatrensis* (Retz.) al secado y congelamiento

Resumen

Las especies de *Erigeron* forman un complejo de malezas que han desarrollado resistencia a herbicidas en Uruguay. Con frecuencia se llevan a cabo estudios genéticos con semillas de diferentes biotipos para comprender cómo ocurrió esta evolución. Sin embargo, estas semillas son extremadamente pequeñas y existe información limitada sobre su conservación a largo plazo en bancos de germoplasma. En este contexto, el objetivo de este estudio fue caracterizar estrategias de secado para el almacenamiento prolongado de semillas, comparando un secado prolongado a baja temperatura (20 días a 20 °C, 15% HR) con secados más cortos a temperaturas más elevadas (10 días a 40 °C o 7 días a 60 °C), y evaluar las respuestas de germinación tras la congelación subsiguiente (-20 °C durante 30 días). Los resultados mostraron que la germinación no se vio afectada por ninguno de los tratamientos de secado. Además, la congelación de los lotes de semillas aumentó la germinación, especialmente en los lotes cosechados más recientemente (2020 y 2021), y sobre todo cuando se combinó con el secado a 60 °C. Estos hallazgos indican que las semillas de *E. sumatrensis* pueden clasificarse como ortodoxas, y que la conservación a largo plazo en bancos de germoplasma, utilizando protocolos estándar de secado y congelación, es factible.

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Palabras clave: semillas ortodoxas, almacenamiento a largo plazo de semillas, conservación en bancos de germoplasma, dormancia de semillas, biología de malezas

Resposta de germinação das sementes de *Erigeron sumatrensis* (Retz.) à secagem e congelamento

Resumo

Espécies de *Erigeron* formam um complexo de plantas daninhas que desenvolveram resistência a herbicidas no Uruguai. Estudos genéticos com sementes de diferentes biótipos são freqüentemente realizados para entender como essa evolução ocorreu. No entanto, essas sementes são extremamente pequenas, e há informações limitadas sobre sua conservação de longo prazo em bancos de germoplasma. Nesse contexto, o objetivo deste estudo foi caracterizar estratégias de secagem para armazenamento de sementes a longo prazo, comparando uma secagem prolongada em baixa temperatura (20 dias a 20 °C, 15% UR) com secagens mais curtas em temperaturas mais altas (10 dias a 40 °C ou 7 dias a 60 °C) e avaliar as respostas de germinação após congelamento subseqüente (-20 °C por 30 dias). Os resultados mostraram que a germinação não foi afetada por nenhum dos tratamentos de secagem. Além disso, o congelamento dos lotes de sementes aumentou a germinação, principalmente nos lotes colhidos mais recentemente (2020 e 2021), e especialmente quando combinado com a secagem a 60 °C. Esses achados indicam que as sementes de *E. sumatrensis* podem ser classificadas como ortodoxas, e que a conservação de longo prazo em bancos de germoplasma, utilizando protocolos padrão de secagem e congelamento, é viável.

Palavras-chave: sementes ortodoxas, armazenamento de sementes a longo prazo, conservação em bancos de germoplasma, dormência de sementes, biologia de plantas daninhas

1. Introduction

The genus *Erigeron* (Asteraceae) includes herbaceous plants of ecological, medicinal, horticultural, and agronomic interest. Some species have been employed in traditional medicine for their anti-inflammatory and diuretic properties, while others, such as *Erigeron breviscapus*, contain bioactive compounds with antioxidant and potential neuroprotective effects[1][2]. Conservation of these genetic resources in germplasm banks can support future pharmacological research, horticultural improvements, and ecological restoration efforts.

Several *Erigeron* species are also considered weedy, including hairy fleabane (*E. bonariensis*), sumatran fleabane (*E. sumatrensis*), and horseweed (*E. canadensis*). These weeds have shown remarkable adaptability, high seed output, widespread dispersal, and resistance to different herbicides[3][4]. In Uruguay, *E. bonariensis* populations resistant to multiple herbicides (e.g., glyphosate and ALS inhibitors) have been reported[5][6]. Likewise, *E. sumatrensis* populations resistant to multiple herbicide groups, including EPSPS-, ALS-, and PSI-inhibiting herbicides, as well as to 2,4-D, have been documented in Uruguay and South America[7][8][9]. The evolution of herbicide resistance may reduce genetic diversity in natural populations, potentially leading to the loss of susceptible biotypes[10]. Germplasm banks can serve as reservoirs of genetic material from different biotypes, preserving diversity for research on resistance mechanisms, potential reintroduction, or restoration projects.

For orthodox seeds (those that tolerate drying and freezing), long-term storage is typically achieved by reducing seed moisture to about 7-6% and then storing at –20 °C in hermetically sealed containers[11][12]. Standard seed storage theory indicates that each 1% decrease in seed moisture content or 5 °C reduction in temperature approximately doubles seed longevity[12]. Online databases indicate that all tested *Erigeron* species are likely orthodox[13], but direct evidence for *E. sumatrensis* is lacking. Dormancy is also an important factor. Variable dormancy has been documented in *Conyza* species (synonymous with certain *Erigeron* species), influ-

enced by temperature and light conditions[14][15][16]. However, the exact nature of dormancy and the methods to overcome it remain unclear.

This study tested the hypothesis that short-duration drying at higher temperatures (e.g., 40 °C or 60 °C) could be as effective as the standard drying at 20 °C without affecting germination. Additionally, we aimed to confirm the orthodox behavior of *E. sumatrensis* seeds by evaluating the effect of a short-term (–20 °C for one month) freezing period on germination. The objectives were: (1) to evaluate the effect of different drying treatments on seed germination and (2) to assess the impact of freezing on germination of seed lots from different years (2019, 2020, and 2021).

2. Materials and Methods

Seeds of *Erigeron sumatrensis* were initially collected in 2019 from an agricultural field in Soriano, Uruguay (33°23'32.58"S, 57°59'4.08"W). Additional seed lots from 2020 and 2021 were produced under greenhouse conditions from the original collection at INIA La Estanzuela (34°20'23.72"S, 57°41'39.48"W), Colonia, Uruguay, during the indicated years. After harvest, seeds were cleaned manually and stored at approximately 7 °C until treatments were applied simultaneously to all seed lots, during the months of May and June, 2023, therefore using seeds of different age.

Three drying procedures were evaluated: (a) 20 days at 20 °C and 15% RH in a seed drying chamber (routine genebank protocol); (b) 10 days at 40 °C in a forced convection oven, and (c) 7 days at 60 °C in a forced convection oven. Moisture content was determined before and after drying using the oven method (105 °C for 17 hours)[17]. Following drying, seeds were subjected to different combinations of soaking and freezing (**Table 1**), as follows: Control: No drying, no freezing, no soaking; Soaking only: 24 h soaking in distilled water at 20 °C before germination; Drying at 20 °C + Soaking; Drying at 40 °C + Soaking; Drying at 60 °C + Soaking; Soaking + Freezing (–20 °C for 30 days); Drying at 20 °C + Freezing + Soaking; Drying at 40 °C + Freezing + Soaking; and Drying at 60 °C + Freezing + Soaking.

Due to the extremely small seed size and difficult handling, the one-month freezing period was selected based on the times used to determine conservation behavior for *Erigeron* species reported in the Seed Information Database[13], which started from 17 days of freezing treatment (i.e., *Erigeron acris*, *https://ser-sid.org/species/a3102298-df3e-40b6-9afb-04a4dfc58ae9*). After treatments, four sub-samples of 50 seeds per lot were germinated in 90-mm Petri dishes on three layers of Whatman No. 1 filter paper moistened with 8 mL distilled water.

Germination took place in a chamber at 20 ± 2 °C, 90% RH, and a 12/12 h light/dark photoperiod, and germinated seeds were counted at 7 and 14 days, as found optimal in previous studies[18][19]. Seeds were soaked into water to remove dormancy and facilitate germination as described by Kaspary and others[10]. A seed was considered germinated when all essential structures were present: root, stem, and leaves.

Statistical Analysis: Germination percentage was analyzed using a mixed model with a binomial distribution (SAS Proc GLIMMIX)[20]. Treatments were considered fixed effects and seed lots random. Germination count dates (7 and 14 days) were treated as repeated measures. Significant interactions were explored through contrasts (ESTIMATE and CONTRAST statements) and multiple comparisons using Dunnett's test.

Table 1. Description of seed drying, storage time, soaking treatment, and humidity content measured in *Erigeron sumatrensis* seeds before germination

Treatment number	Seed drying procedure	Freezing	Soaking treatment	Humidity content	All seed lots	2019 seed lot	2020 seed lot	2021 seed lot
9	7 days 60 °C	30 days, –20 °C	24 h, 20 °C	7.5%	75.8% a	55.7% ab	92.8% a	82.7% a
7	20 days 20 °C	30 days, –20 °C	24 h, 20 °C	9.0%	70.6% a	65.1% ab	82.9% abc	65.8% ab
8	10 days 40 °C	30 days, –20 °C	24 h, 20 °C	8.5%	66.9% ab	49.9% ab	85.5% ab	65.0% ab
6	Absent	30 days, –20 °C	24 h, 20°C	10.9%	62.9% abcd	49.0% ab	76.0% abcd	64.6% ab
3	20 days 20 °C	None	24 h, 20 °C	9.0%	61.0% abcd	66.7% ab	71.1% bcdef	49.9% bc
4	10 days 40 °C	None	24 h, 20 °C	8.5%	59.5% abcde	69.7% a	75.2% bcde	42.2% cd
2	Absent	None	24 h, 20 °C	10.9%	52.5% bcde	45.0% ab	70.0% bcdef	44.2% cd
5	7 days 60 °C	None	24 h, 20 °C	7.5%	50.6% de	45.0% ab	66.1% def	43.0% cd
1	Absent	None	No	10.9%	45.9% e	28.4% c	64.1% def	50.2% bc

Germination least squared estimated means for the 14th day analyzed for all the combined and for each of the seed lots considering count dates as repeated measures. Values with the same letters within each column are not statistically different.

3. Results

A significant interaction between seed lot and treatment was found in germination at 14 days, leading to separate analyses by seed lot. Overall, soaking seeds for 24 h before germination increased average germination by about 6.6% across all lots. This effect was most pronounced for the older (2019) seed lot, where germination increased by 16.6%.

Drying at any of the tested conditions did not significantly reduce germination, indicating that even high-temperature drying (up to 60 °C) did not damage the seeds. Freezing at –20 °C for 30 days consistently increased germination, especially in newer (2020 and 2021) seed lots. The 2021 seed lot showed the strongest response to freezing, with germination increasing by approximately 26.2%. The combination of 60 °C drying followed by freezing yielded the highest germination rates. The older (2019) seed lot showed less improvement from these treatments, possibly due to natural aging and partial loss of viability over time.

The results showed that a 24-hour soaking treatment caused a significant increase in *Erigeron* seeds germination. The soaking treatment of seeds before sowing increased mean germination by 6.6% at the 14th day (45.9% vs. 52.5%); the significance for this contrast is presented in **Table 2**, when compared to treatment without soaking (corresponding to the comparison between Treatments 1 and 2, **Table 2**). When analyzed by seed lot, the differences were 16.6% in the 2019 seed lot, 5.9% in the 2020 seed lot, and non-significant for the 2021 seed lot.

Table 2. *p-value* for the contrast between groups of treatments

Treatment groups Contrasted	Seed Lot			
	All. random	**2019**	**2020**	2021
Soaking vs. no soaking	0.0507	**0.0202**	**0.0001**	0.4995
Frozen vs. not frozen. Not dried	0.1671	0.7680	0.2376	**0.0313**
Frozen vs. not frozen. Dried at 20 ºC	0.1485	0.8565	0.0523	**0.0367**
Frozen vs. not frozen. Dried at 40 ºC	0.2682	0.3118	0.2334	**0.0053**
Frozen vs. not frozen. Dried at 60 ºC	**0.0090**	0.2350	**0.0039**	**0.0012**
Frozen vs. not frozen. All temperatures	**0.0053**	0.9942	**0.0010**	**0.0001**
Dried vs. not dried. Not frozen	0.4124	0.2268	0.5614	0.9474
Dried vs. not dried. Frozen	0.1773	0.2702	0.0960	0.3292

The germination percentage of the 2019 seed lot (**Table 1**) was less responsive to drying and storage treatments, which could be due to the fact that it was an older seed lot, in which any dormancy had already been lost.

The results also proved that germination increased 14.1% after freezing the seeds at –20 ºC (the average of treatments 3, 4, and 5 vs. 7, 8, and 9). This effect showed a variation of responses in the different seed lots, with no response for the 2019 seed lot, and a response that increases to 16.3% and to 26.2% for the 14th-day germination for the 2020 and 2021 seed lots, respectively; with a clear response for the 2021 seed lot across all drying temperatures (**Table 1**). The germination increase due to freezing was significant in all seed lots (Frozen vs. not frozen, all temperatures, **Table 2**), and also for the case of drying at 60 ºC and then freezing, for the 2020 and 2021 seed lots. The seed drying treatments across all drying temperatures and time combinations did not change germination significantly neither before nor after storage at –20 ºC (**Table 2**).

4. Discussion

The present study confirmed that *Erigeron sumatrensis* seeds can be classified as orthodox, demonstrating the feasibility of long-term conservation through standard drying and freezing protocols. Contrary to initial expectations, the 2021 seed lot did not consistently yield the highest germination rates despite its relatively recent harvest date. A common assumption in seed biology is that newer seed lots tend to have higher initial viability and higher levels of dormancy than older seed lots. While the 2021 seeds did show a strong response to freezing (particularly following high-temperature drying), the germination of 2021 seed lot was lower than the 2020 seed lot in all treatments.

Several factors may explain this outcome. First, seed maturation conditions in the greenhouse environment vary from year to year. Slight differences in temperature, humidity, day length, and nutritional status of the mother plants can influence seed quality, dormancy depth, and the ability to tolerate drying and freezing[(1)(2)]. Although the seed production environment was controlled, it is possible that environmental changes during seed formation led to variations in seed fill, chemical composition (e.g., lipid and protein content), or initial dormancy status that did not strictly correlate with harvest year. Another possibility is that the 2021 seed lot may have had a higher degree of primary dormancy at harvest compared to the 2020 lot, requiring more pronounced dormancy-breaking treatments. Dormancy expression can differ annually depending on maternal plant stress and seed maturation conditions[(21)(22)(23)]. Thus, while the 2021 seeds responded positively to freezing and high-temperature drying, the starting point in terms of dormancy depth or viability may have prevented them from outperforming the 2020 seeds.

When viewed in the context of other studies on *Erigeron* and closely related *Conyza* species, our results align with previous reports that highlight environmental and maternal effects on seed germination behavior. Studies on *Conyza bonariensis* and *C. canadensis* —taxa closely related to *E. sumatrensis*— have demonstrated that germination is influenced not only by seed age but also by seed production environments and storage conditions[(14)(15)]. For example, Karlsson and Milberg[(15)] found that after-ripening in *C. canadensis* and *C. bonariensis* was sensitive to storage environment; and Wu and others[(14)] showed that seed persistence and emergence patterns in *C. bonariensis* depend on environmental cues. Similarly, variability in maternal effects and dormancy mechanisms has been documented in other Asteraceae species[(7)(8)(9)].

Within the Asteraceae family, species often exhibit variable dormancy mechanisms responsive to environmental signals, making direct comparisons between seed lots difficult. Research on other Asteraceae members has shown that germination can be strongly modulated by maternal environmental conditions and seed maturation timing[(21)(22)(23)]. These studies support the idea that annual fluctuations in seed quality and dormancy depth can obscure a direct correlation between seed age and germination performance under standardized test conditions.

In summary, although one might assume that the most recently harvested seeds would have the highest germination potential, the complexity of dormancy mechanisms and maternal effects in *Erigeron sumatrensis* may have contributed to the observed patterns.

5. Conclusions

The results confirmed that *Erigeron sumatrensis* seeds behave as orthodox, indicating that long-term seed conservation with normal seed drying procedures is feasible. Both soaking treatments and drying at 60 °C in recently harvested seed lots may increase germination, perhaps by reducing seed dormancy. Conserving *Erigeron* seeds in a genebank will thus maintain the genus's genetic variability for future exploration in aca-

demic research, and may also support their use in restoration, species reintroduction, or evaluation for other applications.

Transparency of data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

FC: Conceptualization; Investigation; Formal analysis, Writing-original draft

TG: Conceptualization, review & editing

SG: Conceptualization; Methodology, review & editing

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