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Enhancing germination of *Dianthus lusitanus*: A Sustainable approach to plant production and biodiversity conservation

Carlos Manuel Gaspar Reis[1,2*], Maria da Graça Diogo[1]

[1]Polytechnic University of Castelo Branco, School of Agriculture, 6001-909 Castelo Branco, Portugal
[2]Plant Biotech Centre of Beira Interior (CBPBI), Polytechnic University of Castelo Branco, School of Agriculture, 6001-909 Castelo Branco, Portugal

***Corresponding author: creis@ipcb.pt**

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Abstract: Incorporating native spontaneous flora species into urban landscapes enhances biodiversity, supports ecosystem services, and contributes to sustainable development. *Dianthus lusitanus*, an Ibero-North African endemic chamaephyte from the Caryophyllaceae family, thrives in full sun exposure and sandy, acidic soils. The aim of this study is to develop a germination protocol for *D. lusitanus* by evaluating the effects of two seed conservation methods, as well as different treatments with hydrogen peroxide (H_2O_2) on germination. Seeds were stored at either 6 °C for three months (lot A) or at room temperature (lot B). Five germination treatments were applied: T1) sterilized water (control); T2) immersion in 1% H_2O_2 for 30 minutes; T3) immersion in 3% H_2O_2 for 30 minutes; T4) immersion in 1% H_2O_2 for 15 hours (overnight); and T5) periodic watering with 50 mM H_2O_2. Ninety seeds per treatment were tested in a randomized complete block design and incubated at alternating temperatures of 25/22 °C (day/night) with a 16/8-hour photoperiod. Germination percentages were quantified after 20 days. Seeds stored at 6 °C exhibited significantly higher germination rates compared to those stored at room temperature (p = 0.007). A positive effect on seed germination percentage was observed when seeds were soaked in H_2O_2 solutions. The highest germination percentage (83.3%) was recorded in treatment T4, while the lowest values were observed in treatments T1 and T5 for seeds from Lot B (11.1% and 22.2%, respectively). This protocol has practical applications in ornamental plant production, biodiversity conservation, and the restoration of degraded areas, contributing to sustainability and the preservation of natural resources.

Keywords: biodiversity, hydrogen peroxide, rock carnation, seed dormancy, urban agroecology.

Introduction

The species *Dianthus lusitanus* Brot., commonly known as rock carnation or Portuguese pink, is an Ibero-North African endemic chamaephyte. This perennial, woody, and tufted plant is characterized by slender, erect stems that can reach heights of up to 45 cm. The leaves, measuring 10.0–30.0 × 0.5–1.0 mm, are linear to linear-filiform, somewhat fleshy, and lack evident veins. The epicalyx consists of 4 ovate-acuminate scales, each approximately one-third the length of the calyx. The calyx measures 17.0–23.0 × 2.5–3.5 mm, is cylindrical-conical in shape, and features teeth extending to about two-thirds of its length. These teeth are tapered with very narrow scarious and ciliate margins. The petal limb is 7.0–10.0 mm long, sparsely bearded or glabrous at the throat, and displays an incised-dentate margin with acute teeth. The petals are pink in color. The species has a chromosome number of 2n=30 (Franco 1971; Bernal et al. 1990). Its flowering period spans from late May to mid-July. Renowned for its resilience, *D. lusitanus* thrives in locations with full sun exposure. Historically, its numerous stems were used to make brooms. The species is well-adapted to acidic, rocky, and arid terrains, including rock crevices, cliffs, and gravelly alluvial deposits (Fig. 1).

Spontaneous flora can produce aesthetically appealing plants that can be cultivated in an ecologically sustainable manner in natural areas and public or private gardens. These plants exhibit notable agro-biological traits, such as ecological plasticity and high resilience to adverse conditions (Ilie and Cosmulescu 2023). Native plants, naturally adapted to the local climate and soil conditions, provide nectar, pollen, fruits, and seeds that serve as food sources for insects, birds, and other animals. The sustained use of native plants plays a key role in protecting biodiversity and ecosystems. Incorporating spontaneous flora species into urban landscapes is a significant goal in the framework of sustainable development. Today, there is growing awareness regarding plant selection in urban planning, emphasising that choices should align with the

local ecological context, particularly by prioritising species that belong to the region's natural vegetation potential (Machado 2023).
Due to its abundant flowering, perennial nature, hardiness, and ease of maintenance, *Dianthus lusitanus* represents a highly attractive ornamental option for urban planning, particularly in areas with sandy and acidic soils. However, its seed germination rate is low, and propagation through conventional seed-based methods remains challenging.
Several studies indicate that seed imbibition in hydrogen peroxide (H_2O_2) solutions stimulates germination (Fontaine et al. 1994; Barba-Espín et al. 2012; Ahsan et al. 2022; Barba-Espín et al. 2022), although the mechanism of action of H_2O_2 in germination is not yet fully understood (Dufková et al. 2019).
Understanding the favorable conditions for the germination of *Dianthus lusitanus* and developing a germination protocol can facilitate the production of plants for ornamental purposes. Additionally, such a protocol has practical applications in biodiversity conservation and the revegetation of degraded areas, promoting sustainability and the preservation of natural resources.
Aiming to develop a germination protocol for *D. lusitanus* seeds, we investigated the effects of two seed conservation methods and different hydrogen peroxide treatments on the germination percentage of *D. lusitanus* seeds.

Results and discussion

ANOVA and interaction effects

Significant differences were observed between the two seed storage methods (storage temperatures), F (4, 20) = 8.882, p = 0.007, partial η^2 = 0.308 (Table 1). Regarding the effect of H_2O_2 treatments, significant differences were found between treatments, F (2, 20) = 38.651, p < 0.005, partial η^2 = 0.885. The interaction effect between seed storage temperature and hydrogen peroxide treatment was not statistically significant, F (4, 20) = 1.676, p = 0.195, partial η^2 = 0.251 (Table 1).

Germination under different H_2O_2 treatments

In the set of treatments, higher germination percentages were observed in the seed lot stored at 6° C (Lot A) compared to the seeds stored at room temperature (Lot B) (Table 2). However, for treatments T3 and T4, the germination percentage values were the same in both lots (50.0% and 83.3%, respectively).
The highest germination percentage (83.3%) was recorded in treatment T4, where seeds were soaked in a 1% H_2O_2 solution for 15 hours (Table 2). The lowest germination percentage values were observed in treatments T1 (control) and T5, for seeds from Lot B (11.1% and 22.2%, respectively).
These results align with previous studies that report a positive effect on seed germination percentage when seeds are soaked in H_2O_2 solutions (Zeinalabedini et al. 2009; Barba-Espín et al. 2012; Muñoz-Salinas et al. 2021; Sorokin et al. 2021; Barba-Espín et al. 2022; Chu et al. 2022). Zeinalabedini et al. (2009) observed that a combination of stratification and pre-treatment with H_2O_2 (0.5%, 24 h) and gibberellic acid (GA_3, 1 mg. L^{-1}, 30 min) reduced germination time and increased the germination rate of stored wild almond (*Prunus* spp.) seeds. Barba-Espín et al. (2012) tested different concentrations of H_2O_2 (5, 10, and 20 mM) and imbibition periods on pea seed germination. They concluded that, compared to the water control, imbibition with 20 mM hydrogen peroxide enhanced germination and seedling growth, leading to seed invigoration. Sorokin et al. (2021) tested various concentrations of H_2O_2 solution (H_2O_2 1% H_2O_2, 3% H_2O_2, 5% H_2O_2, or 10% H_2O_2), as well as a sterile water control, to evaluate the sterilization and germination efficiency of *Cannabis sativa* L. seeds. The 1% H_2O_2 solution resulted in faster and higher germination rates compared to both higher H_2O_2 concentrations and the water control on day 1. Similar results were obtained by Ahsan et al. (2022), who tested different H_2O_2 concentrations on *Cannabis sativa* L. seed germination. Among the concentrations tested, the 1% H_2O_2 solution led to the fastest and most successful germination for all tested genotypes of *C. sativa* seeds. In another study, Muñoz-Salinas et al. (2021) soaked alfalfa (*Medicago sativa* L.) seeds for 12 hours in solutions containing 0, 98, 294, 490, 784, or 980 mM of hydrogen peroxide. The results indicated that, compared to the water control, hydrogen peroxide generally enhanced total germination except at the highest concentration (980 mM), which had an inhibitory effect. Chu et al. (2022) reported that seed germination in two sugar beet (*Beta vulgaris* subsp. *vulgaris* L.) lines improved by over 70% when incubated in a hydrogen peroxide (H_2O_2) solution compared to seeds incubated in water. Barba-Espín et al. (2022) documented that imbibition of peach seeds without an endocarp in 10 mM H_2O_2 for 24 hours, following 8 weeks of stratification, increased the germination rate and resulted in seedlings with good vegetative growth. An additional benefit of using H_2O_2 to promote germination stems from its antimicrobial properties, which protect germinating seeds from pathogens (McDonnell 2014; Szopińska 2014; Sorokin et al. 2021).
However, high concentrations of H_2O_2 can cause significant damage to germinating seeds. Duval and NeSmith (2009) stated that hydrogen peroxide concentrations exceeding 2% resulted in severe injury to germinating seeds of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Ahsan et al. (2022) reported that higher H_2O_2 concentrations (3.0%, 5.0%, and 10.0%) adversely affected the germination rate of *Cannabis sativa* seeds compared to 0.0% or 1.0% H_2O_2. In another study, Barba-Espín et al. (2012) observed that H_2O_2 concentrations higher than 20 mM induced a pronounced curvature as well as an abnormal growth of the radicle of the pea seeds (*Pisum sativum* L.). Furthermore, H_2O_2 concentrations above 100 mM reduced the germination rate of the pea seeds. Given these detrimental effects, the H_2O_2 concentration and treatment duration must be optimized for each plant species (Chu et al., 2022).
Hydrogen peroxide may act as a signalling molecule at the onset of seed germination, involving specific changes in proteomic, transcriptomic, and hormonal levels, leading to the acceleration of the germination process (Barba-Espín et al. 2012; Wojtyla et al. 2016). H_2O_2 treatment not only promotes gene activities related to cell proliferation and growth but

Table 1. ANOVA summary table

Source of variation	Sum of squares	df	Mean squares	F Value	Sig.	Partial Eta squared
Storage temperatures	0.105	1	0.105	8.882	0.007	0.308
Treatments	1.834	4	0.458	38.651	0.000	0.885
Interaction treatments x temperature	0.080	4	0.020	1.676	0.195	0.251
Error	0.237	20	0.012			
Total	19.715	30				

Figure 1. *Dianthus lusitanus*: A – general appearance of the plant; B – flower detail; C – seed morphology.

Table 2. Effect of different H_2O_2 treatments on the germination percentage of *Dianthus lusitanus* seeds from two lots stored under two different conditions. Lot A – seeds stored at 6°C; Lot B – seeds stored at room temperature; T1 – watering with sterilized water (control); T2 – soaking seeds in a 1% H_2O_2 solution for 30 minutes; T3 – soaking seeds in a 3% H_2O_2 solution for 30 minutes; T4 – soaking seeds in a 1% H_2O_2 solution for 15 hours; T5 – periodic watering with a 50 mM H_2O_2 solution.

	Germination (%)	
Treatment	Lot A	Lot B
T1 (Control)	$23.3^{c} \pm 6.7$	$11.1^{d} \pm 5.1$
T2	$60.0^{ab} \pm 5.8$	$46.7^{bc} \pm 11.6$
T3	$50.0^{bc} \pm 10.0$	$50.0^{b} \pm 8.8$
T4	$83.3^{a} \pm 12.0$	$83.3^{a} \pm 8.8$
T5	$47.8^{bc} \pm 8.4$	$22.2^{cd} \pm 8.4$

Means with different alphabetical superscripts in the same column differ significantly ($p < 0.05$) according to Tukey's post-hoc test

also accelerates the degradation of messenger RNAs stored in the seeds to maintain dormancy. (Chu et al. 2022). H_2O_2 overcomes both physical and physiological dormancy, speeding up the transition from dormancy to germination.

Different mechanisms have been suggested to explain the stimulation of seed germination by H_2O_2, including: the production of O_2 for mitochondrial metabolism and respiration as a consequence of H_2O_2 scavenging (Katzman et al. 2001); the facilitation of seed coat cracking; the oxidation of germination inhibitors, such as abscisic acid (ABA) (Ogawa et al. 2001); and the activation of redox-sensitive proteins, leading to changes at the proteome, transcriptome, and hormonal levels (Díaz-Vivancos et al. 2013; Barba-Espín et al. 2011).

Specifically, two distinct pathways have been proposed through which H_2O_2 reduces ABA levels in seeds. First, exogenous H_2O_2 can trigger a decrease in ABA content via mitogen-activated protein kinase (MAPK) signalling (Barba-Espín et al. 2011; Barba-Espín et al., 2012). Second, H_2O_2 may inhibit ABA transport from the cotyledon to the embryonic axis, either directly or indirectly, further reducing ABA levels (Wojtyla et al. 2016; Černý et al. 2018).

Beyond its role in seed germination, H_2O_2 also functions as a key signalling and regulatory component in plants, influencing various physiological processes. At nanomolar levels, it facilitates seed germination, enhances chlorophyll content, promotes stomatal opening and flowering, and delays senescence. However, at elevated levels, H_2O_2 induces oxidative damage to organic molecules, ultimately leading to cell death (Nazir et al. 2020).

Materials and Methods

Plant material and germination experiments

Dianthus lusitanus seeds were collected from wild plants in early July 2022 near the city of Castelo Branco, Portugal (39°48′57.36″ N, 7°29′32.83″ W; 405 m a.s.l.). The effects of two seed storage methods and different H_2O_2 treatments on seed germination percentage were studied. The two storage methods were: i) cold storage at 6 °C for three months (lot A), and ii) storage at room temperature for the same period (lot B). After three months of storage, the seeds were sterilized in a NaOCl solution with a few drops of Tween-20 for 10 minutes and then rinsed three times with distilled water, following Lindsey et al. (2017). Seeds from both lots (A and B) were subjected to five germination treatments: i) watering with sterilized water (T1, control); ii) immersion of seeds in 1% H_2O_2 solution for 30 minutes (T2); iii) immersion of seeds in 3% H_2O_2 solution for 30 minutes (T3); iv) immersion of seeds in 1% H_2O_2 solution for 15 hours (T4); and v) periodic watering with 50 mM H_2O_2 solution (T5). For each treatment, 90 seeds were tested in three replicates of 30 seeds in a randomized complete block design. The seeds were placed in 9-cm Petri dishes on filter paper moistened with sterilized water (except for treatment v) and incubated at alternating temperatures of 25/22 °C (day/night) with a 16/8 h photoperiod. Germination percentage was quantified after a 20-day period. Seeds were considered germinated when the radicle tip reached a length of 1–2 mm.

Data analysis

Arcsine and square root transformations of the percentage data were performed to address issues of non-normality, heterogeneity of variance, and lack of additivity (Ahrens et al. 1990).

Data were subjected to analysis of variance (ANOVA) using the General Linear Model available in IBM SPSS Statistics (version 26) after verifying that the ANOVA assumptions were met (namely, homogeneity of variance and normal distribution as verified by Levene's and the Shapiro–Wilk tests, respectively). No outliers were found, residuals were normally distributed ($p > 0.05$), and homogeneity of variances was detected. The Tukey's post-hoc test was used to detect significant differences ($p < 0.05$) among treatment means.

Conclusions

The introduction of spontaneous flora species into urban landscapes is an important goal in the context of sustainable development. Due to its abundant flowering, perennial nature, hardiness, and ease of maintenance, *Dianthus lusitanus* is a highly attractive ornamental alternative for both public and private spaces. A germination protocol for *D. lusitanus* seeds has been established. Soaking *D. lusitanus* seeds in a 1% H_2O_2 solution for 15 hours significantly increases the percentage of germinated seeds compared to the control treatment. Developing a protocol to improve seed germination rates has practical applications in biodiversity conservation, revegetation of degraded areas, and contributes to promoting sustainability and the conservation of natural resources. This optimized protocol enhances seedling production of *D. lusitanus*, facilitating its use in ornamental horticulture, ecological restoration, and conservation initiatives.

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Author contributions

Carlos Reis designed the experiments, performed the statistical analysis, and wrote the manuscript. Maria Diogo carried out the analytical measurements.

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