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EFFICIENCY OF DIFFERENT METHODS IN BREAKING THE DORMANCY OF *Erythrina lysistemon* Hutch. SEEDS

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ABSTRACT. *Erythrina lysistemon* seeds imported to Libya go through a stage of being unable to germinate; we call this phase "dormancy". The present study evaluated the efficiency of the following methods in breaking the dormancy of newly collected *Erythrina lysistemon* seeds from mature pods and stored for 12 months: untreated seeds (control), mechanical scarification with sandpaper, soaking treatments (tap water for 24 h, hydrochloric acid for 60 min, hydrogen peroxide for 48 h, acetone for 72 h, Clorox cleaner for 72 h, cow dung and chicken manure for 24 h, and hot water at 100° C for 30 min), scarification with soaking (distilled water for 24 h, and cow dung and chicken manure for 12 h). All treatments showed a significant increase ($p<0.05$) in the germination rates of newly collected *E. lysistemon* seeds from mature pods, except for the treatment in which seeds were soaked in tap water for 48 h, which was ineffective. The different scarification treatments were more efficient than the other treatments, recording the highest germination percentages and lowest mean germination times, while the soaking treatments led to high seed mortality. In contrast, seeds stored for 12 months showed a significant decrease in germination percentage with a delayed mean germination time compared to newly collected seeds under all tested treatments. Soaking all treatments was ineffective in breaking the dormancy of *E. lysatetemon* seeds stored for 12 months. The results indicate that *E. lysistemon* seeds have physical dormancy that can be overcome using different scarification.

Keywords: mature seed; mechanical scarification; physical dormancy; stored seed.

INTRODUCTION

Erythrina lysistemon Huch. a member of Fabaceae family, known as South African coral, is a deciduous ornamental tree, that grows up to 10 m,

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and is widely spread in Al-Bayda City, Libya (Salih and Abdulrraziq, 2024). The *Erythrina* genus comprises roughly 120 species spread across South Africa, the tropics and subtropics, and the southern United States (Krukoff and Barneby 1974). This genus is famous for its many uses, as it is used in agriculture to restore degraded lands, for nitrogen fixation in the soil, as a source for wood production, and in handicraft production (Hardt *et al*., 2006; Alves Junior *et al*., 2016). In traditional medicine, it is utilised for the treatment of a range of conditions, including asthma, wounds, abscesses, arthritis, venereal disease, toothache, and nervous system disorders (Sarragiotto *et al*., 1981; de Lima *et al*., 2006; Patocka, 2009). Furthermore, *Erythrina* species contain the most important compounds such as erythroidine alkaloids, isoquinoline alkaloids, isoflavonoids and pterocarpans, flavonoids isoflavonoids, pterocarpanes, flavanones and chalcones (Na *et al*., 2006; Flausino *et al*., 2007; Ozawa *et al*., 2010). According to Majinda *et al*. (2005), many secondary metabolites derived from tree of the genus *Erythrina* exhibited antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. In anticipation of the decade of ecosystem restoration (2021–2030), there is a revived emphasis on enhancing wetland restoration, and climate mitigation. The first step in restoring the ecosystem is reforestation through a strategic seed-based approach (Kettenring and Tarsa, 2020).

Seeds are considered the most successful method of sexual reproduction in plants and are responsible for transmitting genetic traits across generations (Bareke, 2018). However, many seeds go through a stage of being unable to germinate, whether under suitable or unsuitable conditions, and this stage is known as dormancy (Otani *et al*., 2024). Dormancy is linked to internal factors, such as the hardness of the seed coat, immature embryos, and germination inhibitors, and external factors, such as temperature, light, oxygen, and humidity (Prudente and Paiva, 2018). Baskin and Baskin (2004), classified dormancy into five types (physical, morphological, morphophysiological, physiological, and combinational dormancy). Mature seeds of the *Erythrina* genus exhibit seed coat impermeability (physical dormancy), which must be overcome through the mechanical disruption of the impermeable seed layer to break dormancy and initiate germination (Molizane *et al*., 2018). This allows the seeds to imbibe water and exchange gases necessary for germination (Salih and Abdulrraziq, 2020). There are many techniques used to break seed dormancy in *Erythrina* species. For example, the application of mechanical scarification with sandpaper breaks the dormancy of *Erythrina falcata* Benth seeds (Artur *et al*., 2023).

Therefore, the main aim of this research was to evaluate the efficiency of different methods in breaking the dormancy of mature *E. lysistemon* mature seeds and those stored for 12 months.

MATERIALS AND METHODS

Erythrina lysistemon seeds were randomly harvested from the mature pods beginning at dehiscence, of growing trees at the University Campus and Al-Thawra Hospital- Al-Bayda City, Libya, in 2023 and 2024.

The seeds were divided into two groups: newly collected seeds from mature pods in 2024 and seeds harvested in 2023 and stored for 12 months under dry conditions at room temperature.

Seeds vitality was tested by soaking them in distilled water to remove any empty seeds, followed by soaking them in 5% sodium hypochlorite solution for 5 min and then washing them 3 times in distilled water (Luzia Delgado *et al*., 2015).

Experimental design

The laboratory experiments were conducted at the Department of Biology/Faculty of Education/Omar Al-Mukhtar University. A completely randomised design (CRD) with three replications was used. The experiment 13 treatments as follows:

1- Untreated (intact) control (C) seeds.

2- Treatment by soaking in tap water for 48 h (SW).

3- Treatment with mechanical scarification on the area opposite the hilum with the help of sandpaper (MS).

4- Treatment with mechanical scarification + soaking in distilled water for 24 h (MS+SW).

5- Treatment with mechanical scarification + soaking in cow dung for 12 h (MS+SCD).

6- Treatment of mechanical scarification + soaking in chicken manure for 12 hours (MS+SCM).

7- Treatment by soaking in hot water at 100°C for 30 min and cooling to at room temperature (SHW).

8- Treatment by soaking in concentrated Hydrochloric acid for 60 min and then washing with distilled water (HCL).

9- Treatment by soaking in hydrogen peroxide at a concentration of 6% for 48 h (H_2O_2).

10- Treatment by soaking in acetone for 72 h (C_3H_6O).

11- Treatment by soaking in Clorox cleaner (30966, 121 Oz.) for 72 h (SCC).

12- Treatment by soaking in cow dung for 24 h (SCD).

13- Treatment by soaking in chicken manure for 24 h (SCM).

The seeds were placed in 9-cm-diameter Petri plates on No. 42 filter paper with 25 seeds/plate, and incubated at room temperature; each treatment was repeated 3 times. Seeds were monitored for 10 days, and as needed, 10 mL of distilled water was added to each of the Petri plates.

Germination was calculated by recording the number of germinated seeds in all treatments starting on the second day of observation when germination frist occurred (*Equation 1*) (Salih and Abdulrraziq, 2021). The germination criterion was the appearance of the radicle outside the seed cover, and at the end of the experiment took the final results of the following qualities were obtained (*Equation 2*) (Das *et al*., 2017):

$$\textit{Germination percentage} = \textit{number of germinated seeds} / \textit{total number of seeds} \times 100 \quad (1)$$

$$\textit{Mean germination time} = \textit{total number of germinated seeds} / \textit{total number of germinated seeds at end of the experiment} \quad (2)$$

Organic Residue Preparation

A solution of cow dung and chicken manure was prepared separately, in a ratio of 500g/500mL (w/v) of organic matter and water without dilution.

Statistical analysis

The experiments followed a full randomised design (CRD). Statistical analysis including analysis of variance (ANOVA) Tukey's test at $p<0.05$, were performed using Minitab 17.

RESULTS AND DISCUSSIONS

All treatments showed a significant increase ($p<0.05$) in the germination rate of newly collected *E. lysistemon* seeds from mature pods, except for the treatment in which seeds were soaked in tap water for 48 h, which did not promote germination (ineffective) compared to non-treated healthy seeds (control) after 10 days of germination (*Figure 1*, *Figure 2* and *Figure 6*).

The scarification treatments were not significantly different from each other and recorded the highest germination percentages and lowest mean germination times. These methods increased germination percentages to 69.33, 65.33, 62.66, and 70.66% as well as reduced the mean germination time to 3.07, 3.20, 3.39, and 3.24 days with treatments of mechanical scarification, scarification + soaking in water, scarification + soaking in cow dung, and scarification+ soaking chicken manure, respectively.

The obtained results corroborate those reported by Pêgo *et al.* (2015) and Artur *et al.* (2023), who suggested that scarification treatment was an efficient method for breaking the physical dormancy of *E. verna* and *E. falcata* seeds. The scarification approach offers the highest seedling performance and germination, as shown by Pinheiro *et al.* (2021). Compared to the control group, the scarification method disrupts the water impermeability of the seed coat due to coat thickness. Manning and Staden (1985) demonstrated that *E. lysistemon* Hutch seeds had an outer layer rich in phenolic compounds that might enhance the coat thickness. Seeds soaked in hot water for 30 min showed a good germination rate compared to other treatments, as the germination percentage was 46.66% and had the lowest mean germination time (3.75 days).

This result contrasts with that of Artur *et al.* (2023), who indicated that water treatment at 100°C was most harmful to the germination of *E. falcata* Benth.

This was followed by treatments of soaking in chicken manure and cow dung for 24 h, which showed germination percentages of 34.00 and 30.66% and mean germination times of 4.00 and 4.42 days, respectively.

These results are consistent with those of Salih and Abdulrraziq (2021), who found that organic residues caused the corrosion of the seed coat by acids present in a cow dung slurry and chicken manure, which break the dormancy of *Ceratonia siliqua* L.

The treatment in which seeds were soaked in hydrochloric acid and hydrogen peroxide resulted in weak germination percentages (24.00 and 22.66%, respectively), with a clear increase in the mean germination time (4.04 and 4.86 days, respectively).

Finally, the lowest germination percentages (10.66 and 6.66%) and the highest average germination times (6.22 and 5.66 days) were obtained when seed were soaked in acetone and Clorox cleaner, respectively. The decline in germination percentages might be due to

the inability of these treatments to break the physical dormancy.

Erythrina lysistemon seeds stored for 12 months showed a significant reduction in their viability 10 days after the start of the experiment. *Figure 3* and *Figure 4* show that mechanical scarification + soaking in chicken manure for 12 h resulted in a higher germination percentage (48.00%) and a faster germination time (3.09 days). Germination percentages were lower (45.33, 44.00, and 42.66%) and the mean germination time was delayed (4.00, 4.52, and 4.35 days) for seeds pre-treated with mechanical scarification, scarification + soaking in cow dung, and scarification + soaking in water, respectively.

The treatments in which seeds were soaked in chicken manure, hot water, and cow manure resulted in the lowest germination percentages (32.00, 14.66, and 13.33%, respectively) and were associated with the longest mean germination (5.78, 4.91, and 5.36 days, respectively).

This study furthers the conclusions of Pereira *et al.* (2014), who found that the germination rates of *E. mulungu* and *E. velutina* seeds stored for one year were decreased by more than a quarter. However, de Freitas *et al.* (2020) found that newly collected *E. crista-galli* L. seeds from mature and immature pods grew naturally without dormancy breaking treatments.

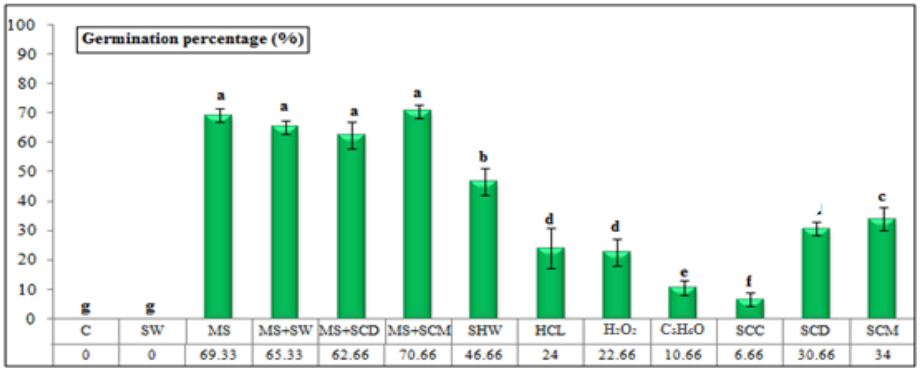
Other than these treatments, exogenous soaking in tap water for 48 h, soaking in concentrated hydrochloric acid for 60 min, soaking in hydrogen peroxide for 48 h, soaking in acetone for 72 h, and soaking in Clorox cleaner for 72 h were ineffective in breaking the dormancy of *E. lysistemon* seeds stored for 12 months.

Generally, based on the breaking dormancy experiment, seeds stored under dry conditions at laboratory temperature for 12 months showed a clear decrease in germination percentages (24.00. 22,67, 18.66, 22.66, 32.00, 17.33, and 2.00%) for seeds pre-treated with mechanical scarification, scarification + soaking in distilled water for 24 h, scarification + soaking in cow dung for 12 h, scarification + soaking in chicken manure for 12 h, soaking in hot water at 100°C for 30 min, soaking in cow dung for 24 h, and soaking in chicken manure for 24 h, respectively, compared to newly collected seeds from mature pods harvested in 2024 (*Figure 5*).

The results of our study indicate that the percentage of physical dormancy in *E. lysistemon* seeds increases with increasing storage time.

Similar findings were reported regarding the storage of *E. speciosa* seeds (Magalhae and Oliveira, 2020). This may be dormancy because of the accumulation of pectic substances and cutin within the cells of the outer palisade layer, which hinders water absorption (Magalhae and Oliveira, 2020).

Furthermore, drier storage conditions were found to cause the closure of cracks in the mucilaginous stratum for seeds according to Magalhaes *et al*. (2021). In addition, this dormancy may be caused by environmental conditions experienced by the mother plant, which affects the level of dormancy in *Erythrina* seeds during the maturation process (Molizane *et al*., 2018).



- C: control; SW: tap water for 48 h; MS: Scarification by sandpaper; MS+SW: scarification + water for 24 h; MS+SCD: Scarification + cow dung for 12 h; MS+SCM: Scarification + chicken manure for 12 h; SHW: Hot water at 100 °C for 30 min; HCL: Hydrochloric acid for 60 minutes; H_2O_2: hydrogen peroxide; C_2H_6O: Acetone; SCC: Clorox cleaner; SCD: cow dung for 24 h; SCM: chicken manure for 24 h.

Figure 1 – Germination percentages of *Erythrina lysistemon* seed (collected from mature pods) submitted to different treatments. (Mean ± Standard error). Columns with the same letters do not differ from each other according to the Tukey test (5%)

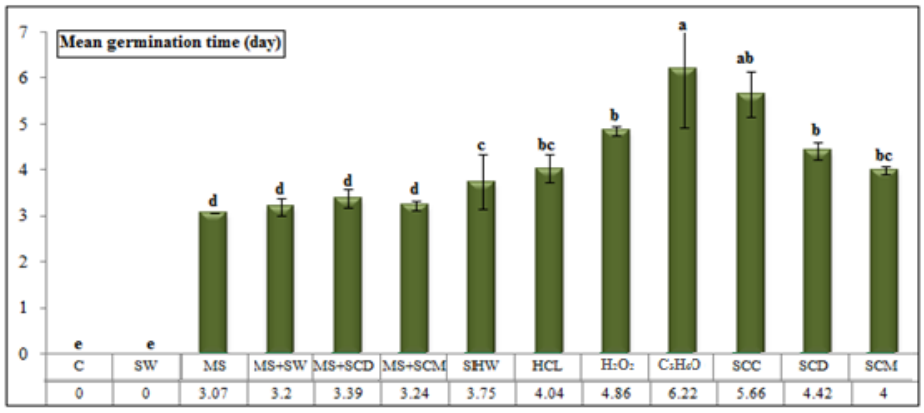


Figure 2 – Mean germination time of *Erythrina lysistemon* seed (collected from mature pods) submitted to different treatments

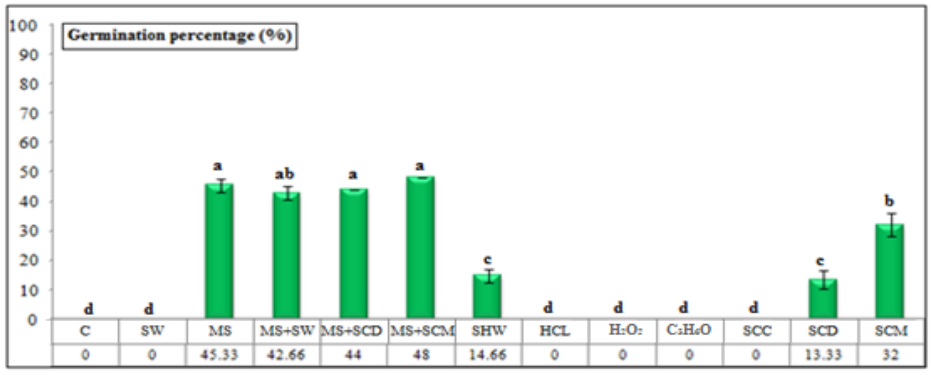


Figure 3 – Germination percentages of *Erythrina lysistemon* seed (stored for 12 months) submitted to different treatments

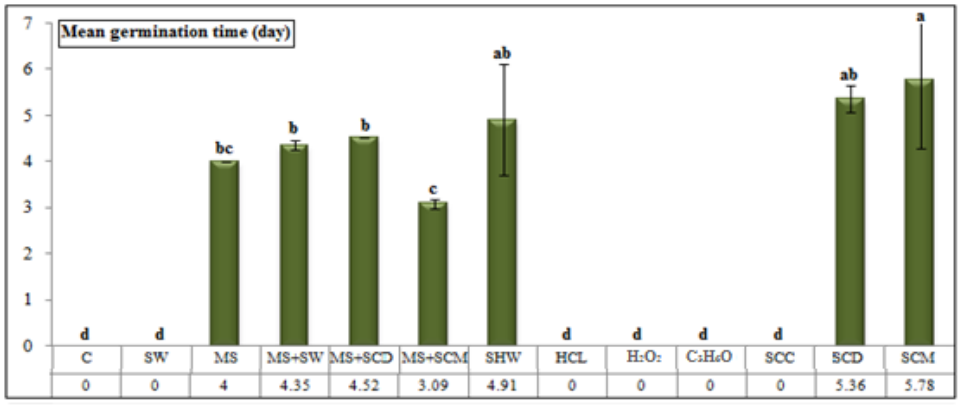


Figure 4 – Mean germination time of *Erythrina lysistemon* seed (stored for 12 months) submitted to different treatments

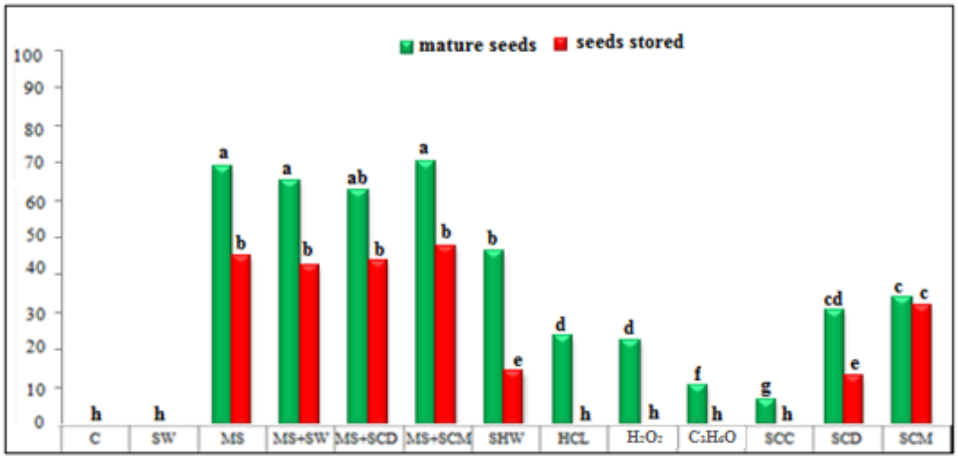


Figure 5 – Germination percentages of *Erythrina lysistemon* seed submitted to different treatments

Figure 6 – Germination after mechanical scarification (newly collected mature seeds)

CONCLUSIONS

This study discovered that various scarification techniques may be used to break the physical dormancy of both freshly harvested *E. lysistemon* Huch seeds and seeds that have been kept for a year. However, compared to freshly collected seeds under all studied treatments, seeds held for a year had a significantly lower germination percentage and a delayed mean germination time. As a result, farmers are advised to utilise scarified seeds for roadside and garden decorative trees as soon as they are harvested.

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