

Article

Effect of Different Presowing Treatments to Break Seed Dormancy and Seed Collection Methods on the Germination of *Dracaena steudneri* Schweinf. Ex Engl.

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Abstract: Research Highlights: This study is focused on the germination of *Dracaena steudneri* Schweinf. Ex Engl. seeds using different presowing treatments. Background and Objectives: The study aimed to overcome the problem of breaking seed dormancy, to facilitate artificial regeneration for conservation and development purposes. The objectives of this study were (1) to evaluate the effect of different seed treatments in breaking seed dormancy of *D. steudneri* and (2) to assess the effect of the seed collection method (seeds collected on the ground vs. from the tree) on the germination of the seed. Materials and Methods: experimental study with different seed-dormancy-breaking treatments was carried out in a greenhouse and seed laboratory. T testing and one way analysis of variance (ANOVA) were used to analyse the data. Results: The applied seed treatments (hot water, cold water, sodium hypochlorite and nicking) did not improve the germination of the species, nor the breaking of seed dormancy. One-way ANOVA results also showed no significant differences between the different seed treatments and the control on the mean germination of the species in the greenhouse ($p < 0.05$). The *t* test result also revealed no significant differences in the mean germination between fallen seeds collected from the ground and in the tree crown ($p < 0.05$). The tetrazolium test results showed that the percentage of nonviable seeds was greater than that of the viable seeds. Conclusion: The different treatments for breaking seed dormancy did not improve the germination of seeds in the greenhouse (ranging from 0%–7%) nor in the seed laboratory (0%), which might be due to the intermediate characteristics of the seeds of this species. Therefore, we recommend that more research is undertaken on the seed ecophysiology of the species, in order to understand the mechanisms controlling its seed germination.

Keywords: dragon trees; conservation; germination; seed dormancy



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1. Introduction

The genus *Dracaena* L. has more than 116 species, of which 63 occur in Africa, including Madagascar, while the remaining species are found in Asia, Australia and Central America [1–3]. In the Angiosperm Phylogeny Group (APG) IV classification system, *Dracaena* is placed in the family Asparagaceae, subfamily Nolinoideae (formerly the family Rusceae) [4,5]. Some *Dracaena* species have high importance in horticulture [6,7], while others have medicinal uses [8] or fulfill social functions by marking graves, sacred sites, and farm plots in many African societies [9]. Globally, *Dracaena* is in the top ten most important crops in floriculture [6]. The species *Dracaena steudneri* is distributed from eastern to southern Africa in moist or drier forest areas and grows mainly in moist highland forests, 1250–2100 metres above sea level (m.a.s.l.), often in gaps, along riverbanks or in gallery forests [2,10]. A review of ethnobotanical study results indicated that the species is used in

Ethiopia as a medicine to treat rabies [11] and livestock diseases [12]; in Tanzania to treat hernia, asthma, chest problems in children, fibroids, and infertility in women [13]; in Congo as a cancer treatment [14]; and against malaria in Uganda [15]. The species is also used for poles, bee forage, shade, ornamental purposes, etc. [10].

Although *D. steudneri* has a broad socioeconomic and cultural importance, the species has poor natural regeneration in its habitat in Ethiopia. Vegetation and regeneration dynamics studies in the Masha forest, Bonga Forest, Boginda Forest, Zegie Forest, and Belete forest in Ethiopia showed no records of the seedlings of the species, despite *D. steudneri* trees being available [16–19]. These results may indicate that the species has a problem with regeneration, which could be associated with seed dormancy. The major external factors that affect seed germination are temperature, water, oxygen or air, and sometimes light, while the internal factor that affects seed germination is mainly seed dormancy [20]. Seed dormancy is defined as a temporary failure of a viable seed to germinate in conditions that favour germination [21]. Dormancy is an internal condition of the seed that impedes its germination, and it can be caused by different factors, such as the presence of germination inhibitors or impermeable seed coats [22,23]. Persistency of dormancy is the period of storage (in weeks) needed by seeds from harvest until the moment that the percentage of dormant seeds reaches $\leq 5\%$ in ambient storage [24].

Breaking seed dormancy to enhance seed germination is important, and different treatments for such purposes, including plant growth regulators, chemicals, hot water, cold water, and heat treatments, have been used in different species [25]. The effectiveness of the dormancy breaking method is strongly influenced by the dormancy behaviour of the species seed [25]. Seeds from some plants can have one or more types of dormancy that must be overcome before they will germinate, and such characteristics are especially common for woody ornamental and native perennial plants [26]. Generally, evaluation of different treatments including hot water, cold water, scarification, etc., is required to overcome the seed dormancy problem of any species to enhance germination to produce more seedlings [26]. Despite the economic and environmental importance of *D. steudneri*, to date, study results on the propagation techniques and seed germination characteristics of the species have not been available. Overall, understanding the seed germination behaviour of the species by using different treatments for breaking seed dormancy is important for the sustainable management and conservation of the species. Therefore, the objectives of this study are (1) to assess the effect of seed presowing treatment on the germination of *D. steudneri*, both in a greenhouse and under laboratory conditions and (2) to evaluate the effect of the seed collection method on the germination of *D. steudneri*. At the beginning of the study, it was hypothesized that (a) in both the laboratory and greenhouse experiments, seed presowing treatment can facilitate the germination of the seeds of *D. steudneri* and (b) collection of seeds of *D. steudneri* directly from the tree can result in a higher germination percentage compared to seeds collected from the ground.

2. Materials and Methods

2.1. Seed Collection and Germination Experiment

The seeds of *D. steudneri* along with its fruit were collected in Bedele, located 500 km from Addis Abeba, the capital city of Ethiopia (Figure 1), in June 2019. Ripened seeds of *D. steudneri* were collected directly from different individual trees and from the ground under the trees. The seeds were carefully removed from the fruit and allowed to dry under shade. The dried seeds that were collected in June 2019 were sealed in a plastic jar and kept in a cold room for one month until the experiment was started. After one month, the seeds were removed from storage for the seed-dormancy-breaking tests using different treatments, and the experiment was undertaken in a greenhouse.

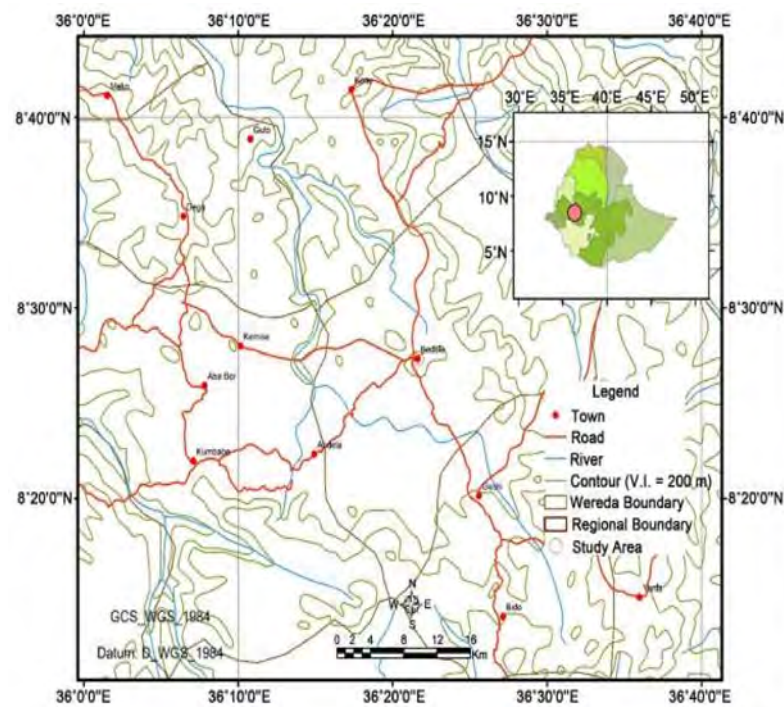


Figure 1. Location map of the seed collection area, Bedele.

Similarly, in June 2021, further batches of *D. steudneri* seeds from the same locality (Bedele) were collected from the tree. The collected seeds were depulped, dried in shade, sealed in a plastic jar and kept in a cold room for seven months until the experiment was started. The seeds collected in 2021 were used for germination testing in a seed laboratory, using different treatments for breaking seed dormancy.

For the seeds collected in 2019, the seed germination study to evaluate the effects of different presowing treatments for breaking seed dormancy was conducted in a greenhouse in the Forestry Research Center, Addis Ababa. The greenhouse had an average temperature of 32 °C. The germination experiment in the greenhouse was performed using plastic boxes filled with sand as the germination medium. The sand was continuously washed and cleaned before use. For seeds collected in 2021, the seed germination experiment using different presowing treatments was carried out in a seed laboratory at Mendel University in Brno, Czech Republic, using a Jacobson germinator. For the laboratory experiment, germination was undertaken in Petri dishes. In the Petri dish, filter paper was placed on the bottom and with pieces of cotton swab as substrate on top of this used as germination media (Figure 2). The filter paper seemed to be an insufficient substrate, as it did not provide sufficient moisture to the seeds of *D. steudneri*, and for this reason the cotton swabs were included on top of the filter paper for use as germination media (Figure 2).

Randomized complete block design (RCBD) was used for the germination test. For the greenhouse experiment, four replications were employed for each treatment, and each replication had 25 seeds. The number of seeds for each replication was limited to twenty-five due to the lack of seeds available for the experiment. The seeds in the experimental study in the greenhouse were watered daily, every morning. The seeds were sown on 7 July 2019, and the germination study in the greenhouse was conducted until 28 August 2019, for a total of 52 days.



Figure 2. Photographs showing the seeds of *D. steudneri* in a Petri dish with germination media (photo on the **left**), the seeds covered by cups inside the Jacobsen germinator (photo in the **middle**) and the partial view of the Jacobsen germinator (photo on the **right**).

For the laboratory experiment, ten replications were used for each treatment, each replication involving ten seeds. The number of replications was ten because of the large size of the seeds and the relatively small size of the Petri dishes. While attending seed germination in the laboratory, it was observed that fungus developed on the Petri dishes and seeds. To remove the fungus, the seeds were rinsed every five days in tap water, and then the cleaned seeds were transferred to a new Petri dish with new filter paper and cotton swabs. The germination experiment in the laboratory was conducted from 1–21 February 2022 for a total of 21 days.

Various methods can be used to deal with different kinds of dormancy, including scarification, stratification, chemical, biological, and irradiation methods [27]. In this study, scarification (nicking), physical (soaking in water), and chemical methods (sodium hypochlorite) were used as pre-sowing treatments. In the present study, the dormancy breaking treatments used for the greenhouse experiment were as follows: hot water (boiled water at 100 °C) for 5 min, 10 min, and 15 min; cold water (tap water with a temperature of 5 °C) for 48 h; soaking in sodium hypochlorite for 20 min, 1 h and 5 h; and the control. For the laboratory germination experiment, the presowing treatments used were: nicking; soaking in sodium hypochlorite for 20 min and 60 min; hot water treatment for 10 min and 20 min; and cold water treatment for 72 h.

2.2. Data Collection and Analysis

The number of seeds per kilogram of *D. steudneri* was determined by the following procedure: (1) the bulk of seeds of *D. steudneri* was thoroughly mixed; (2) seed samples from the different directions were taken and mixed; (3) eight plastic cups were prepared, and 100 seeds were counted and included in each cup; (4) each plastic cup containing 100 seeds was independently weighed on a sensitive balance and the weight recorded on a data collection sheet; and (5) finally, the average weight of seed per kilogram was determined.

Seed germination, both in the greenhouse and in the laboratory, was attended daily, and if a seed had germinated, it was counted and recorded on a data collection sheet. At the end of the experiment evaluating germination in the greenhouse, a viability test using tetrazolium was not performed because it was difficult to find the seeds that were buried in the sand that was used as germination media. For the seeds that were evaluated for germination using a Jacobson germinator in the laboratory, their viability was assessed using the scientific method of the triphenyl tetrazolium chloride test. After dissecting all the seeds, viable and nonviable seeds were identified visually by observing the status of the stains showing red colour in the living tissue (Figure 3). The references for determining viable and nonviable seeds of *D. steudneri* after dissecting and observing the red colour staining were set by two experts. In addition, for further confirmation, photographs of dissected seeds from each of the presowing treatment groups were taken independently. Then, the viable and nonviable seeds were identified by zooming into the pictures on

a computer. Finally, the numbers of viable and nonviable seeds were determined by averaging the visually observed result and the photographic staining results for the living tissue of the dissected seeds.

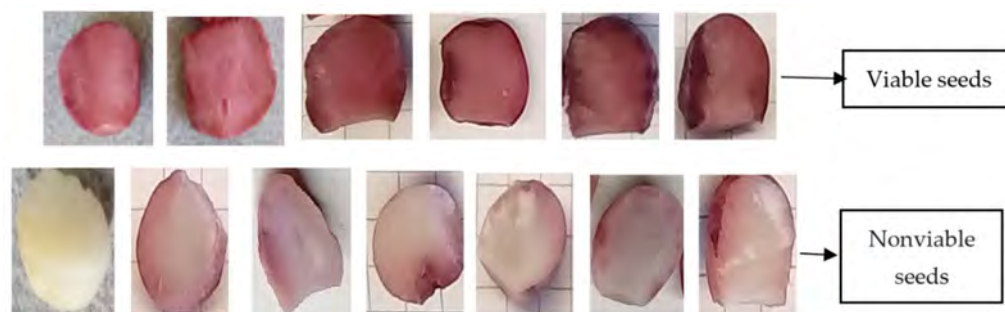


Figure 3. The different staining of the dissected seeds of *D. steudneri* after the tetrazolium test, used as a reference for the identification of viable and nonviable seeds.

Descriptive statistics such as percentages and graphs, one-way analysis of variance (ANOVA) and *t* tests were used for the data analysis. In the data analysis, a nonparametric test was used, as the data did not fulfil a normal distribution. Mean separation was performed using Fisher's least significant difference (LSD) test with a significance level of $p = 0.05$. The statistical software program SigmaPlot 13 (Systat Software, Inc., San Jose, CA, USA) was used for the data analysis.

In the one-way ANOVA, to determine significant differences in the viability of seeds from each of the different seed-dormancy-breaking treatment groups, the number of viable seeds from each of the individual petri dishes representing different treatments was considered as a replication (ten replications for each treatment \times each replication with 10 seeds). Then, after determining the number of viable seeds, results were converted into percentages for each treatment and each replication, independently. Finally, the significant differences in the relative mean percentages of viable seeds among the different seed-dormancy-breaking treatment groups were analysed.

The final germination percentage, mean germination time and germination index for the different seed-dormancy-breaking treatments applied to the species seed were analysed using the following Equations (1)–(3):

The final germination percent (GP) was calculated using Equation (1):

$$GP = \frac{TG}{TS} \times 100 \quad (1)$$

where TG = total number of seeds germinated; TS = total number of sown seeds.

The mean germination time (MGT) of seeds was calculated using Equation (2):

$$MGT = \frac{\sum_{n=1}^n T1N1 + T2N2 + \dots + TkNk}{\sum_{n=1}^n N1 + N2 + \dots + Nk} \quad (2)$$

where N = number of newly germinated seeds; T = time from the beginning of the experiment.

The germination index (GI) was calculated using Equation (3):

$$GI = \sum_{n=1}^n (T1N1 + T2N2 + \dots + TkNk) \quad (3)$$

where N = number of newly germinated seeds, T = time from the beginning of the experiment.

3. Results

3.1. Seed Germination in the Greenhouse

The analysis result of the final germination percentage of *D. steudneri* seeds collected from the ground and directly from the tree, evaluated in the greenhouse after different treatments for breaking seed dormancy, is presented in Figure 4. The results revealed that for both seed collection methods (the seeds collected from the tree crown and from the ground), seeds sown without treatment achieved the highest germination percentage relative to other seed-dormancy-breaking treatments. Soaking in sodium hypochlorite for five h resulted in no germination for the seeds collected from the ground nor those from the tree (Figure 4). Similarly, seeds collected from the tree and treated with hot water for five min resulted in no germination (Figure 4). Relative to the other seed dormancy breaking treatments, soaking in hot water for 15 min resulted in a higher germination percentage for the seeds collected directly from the tree (Figure 4). Similarly, for the seeds collected from the ground, soaking in hot water for 10 min resulted in a higher germination percentage compared with other seed-dormancy-breaking treatments. The *t* test results for the mean germination of seeds collected from the ground and from the tree and treated with different seed-dormancy-breaking treatments revealed no significant differences ($p = 0.314$). The results of the studies of the mean germination times for the different treatments of seeds collected from the ground and from the tree crown are presented in Figure 5. The results revealed that hot water treatment for 10 min for the seeds collected from the ground, and for 15 min for the seeds collected from the tree, caused seeds to take longer to germinate compared with the other treatments (Figure 5). Seeds collected from the tree and treated using sodium hypochlorite for 20 min, and seeds collected from the ground and treated with hot water for 5 min, took less time to germinate.

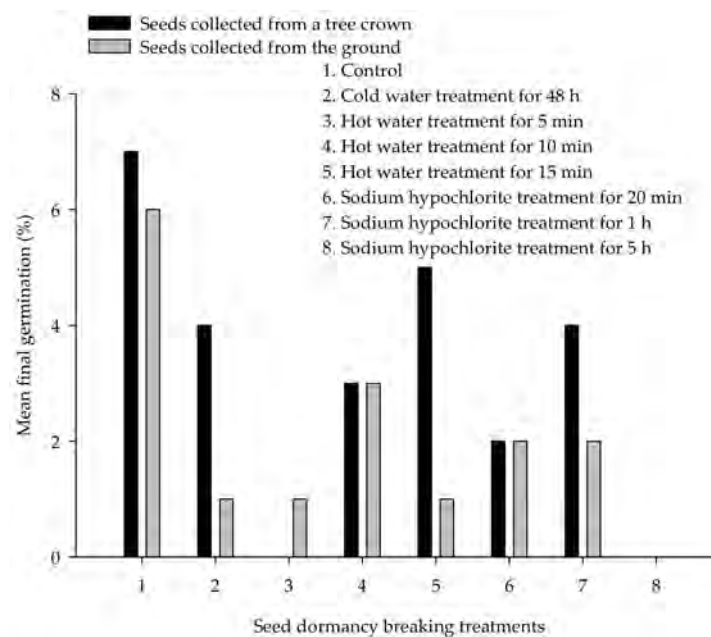


Figure 4. Final germination percentage of *D. steudneri* seeds collected from the tree and the ground with different presowing treatments in the greenhouse.

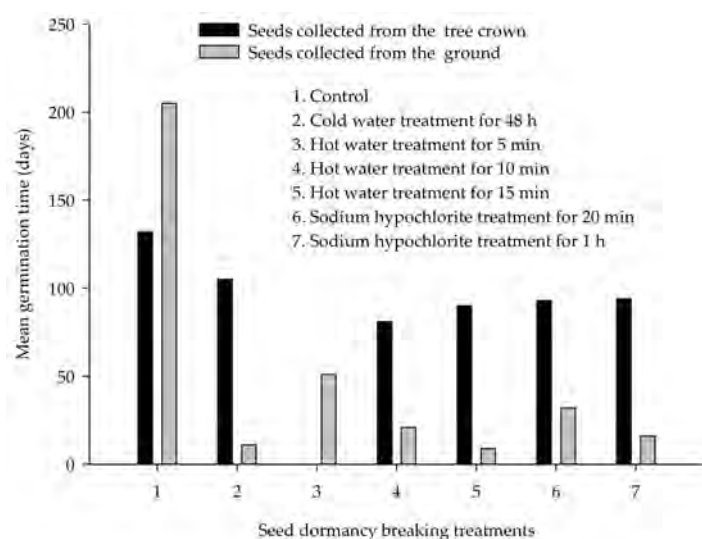


Figure 5. Mean germination time (days) of *D. steudneri* seeds collected from the tree and the ground with different presowing treatments in the greenhouse.

The results of the germination index for *D. steudneri* revealed that for the seeds collected from the ground and those collected directly from the tree, the dormancy-breaking treatments applied to the seed resulted in a lower germination index compared with the control (Figure 6). Hot water treatment for 15 min for the seeds collected from the ground resulted in a lower germination index relative to the other dormancy breaking treatments (Figure 6). The one-way ANOVA results for the seeds collected from the ground and from the tree, with different dormancy-breaking treatments, are presented in Figure 7. The results revealed no significant differences in the mean germination of the species seeds collected from the ground and treated to break seed dormancy ($p > 0.05$, Figure 7). Similarly, the results showed that there were no significant differences in the mean germination of the species seeds collected directly from the tree and treated to break seed dormancy ($p > 0.05$, Figure 7).

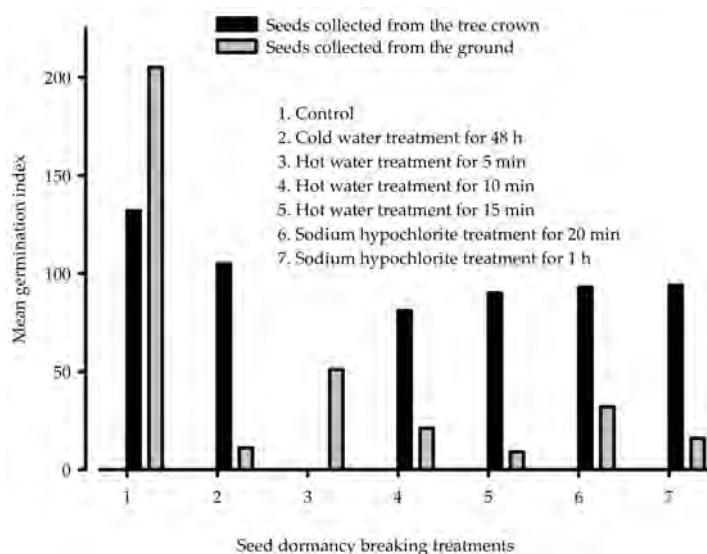


Figure 6. Mean germination index of the *D. steudneri* seeds collected from the tree and the ground with different presowing treatments in the greenhouse.

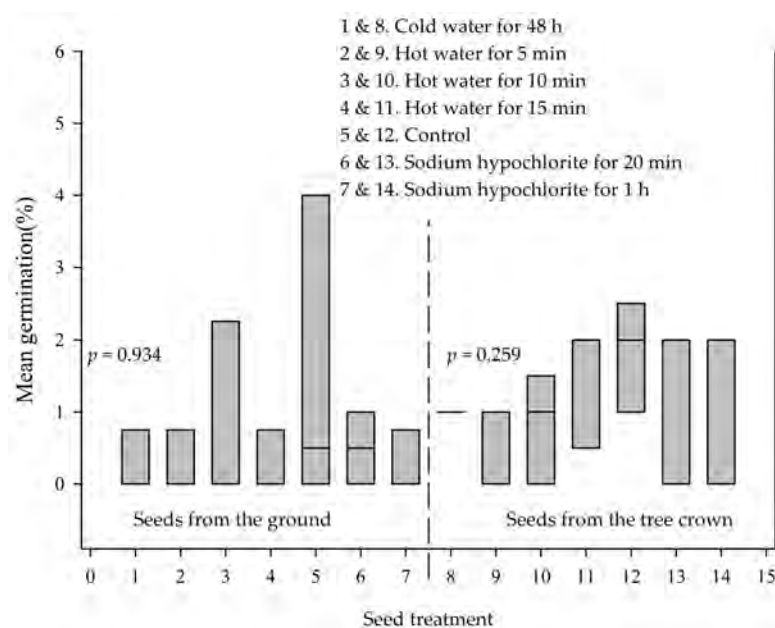


Figure 7. One-way ANOVA results of the mean germination of *D. steudneri* seeds evaluated in the greenhouse, collected from the ground (graph on the left) and directly from the tree crown (graph on the right) with different seed-dormancy-breaking treatments.

3.2. Seed Germination in the Laboratory

The analysed results showed that the number of seeds for *D. steudneri* ranged from 1888 to 1969 per kilogram. The seed germination results in the seed laboratory revealed that none of the treatments for breaking seed dormancy facilitated the germination of the seeds of *D. steudneri*. The tetrazolium test results showed the relative percentages of viable seeds at the end of the experiment in the seed laboratory, for the presowing treatments: 27% for nicking; 17% for 20 min soaking in sodium hypochlorite; 19% for 60 min soaking in sodium hypochlorite; 40% for 10 min soaking in hot water; 30% for 20 min soaking in hot water; 33% for 72 h soaking in cold water; and 46% for the control (Figure 8). The one-way ANOVA results for the mean percentage of viable seeds after the different seed-dormancy-breaking treatments used on *D. steudneri* seeds showed statistically significant differences ($p < 0.01$, Figure 9).

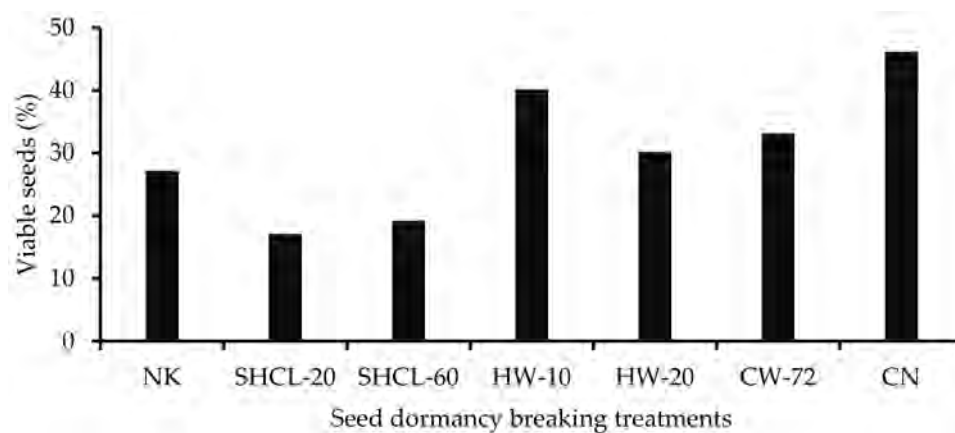


Figure 8. The relative percentage of viable seeds of *D. steudneri* after the end of the experiment in the seed laboratory. Nicking (NK), soaking in sodium hypochlorite for 20 min (SHCL-20), soaking in sodium hypochlorite for 60 min (SHCL-60), soaking in hot water for 10 min (HW-10), soaking in hot water for 20 min (HW-20), soaking in cold water for 72 h (CW-72), and the control (CN).

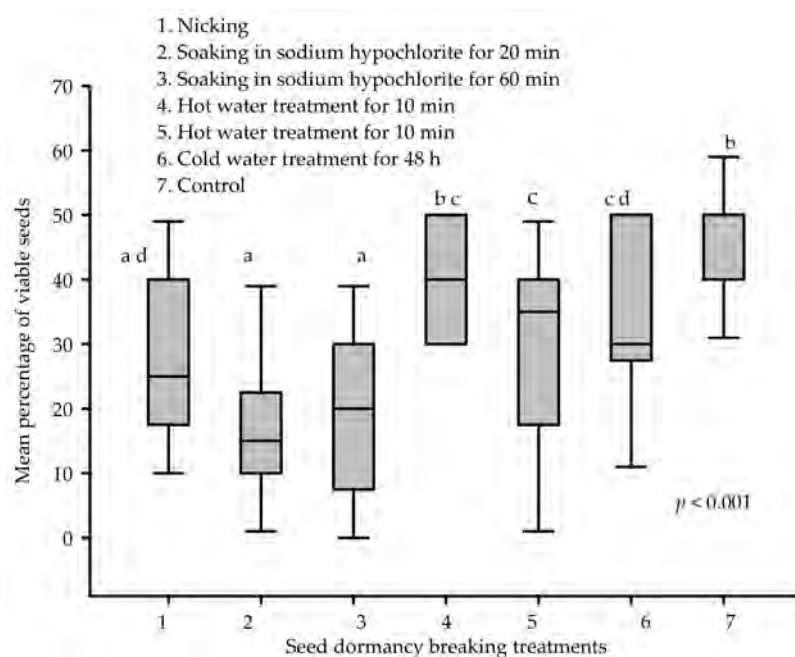


Figure 9. The one-way ANOVA results for the relative mean percentages of viable seeds among the different seed-dormancy-breaking treatments of *D. steudneri* at the end of the experiment in the seed laboratory.

4. Discussion

Seed dormancy is a suspension of germination in viable seeds, which is imposed by abscisic acid during seed development, and it may persist in some mature seeds [28]. Therefore, it is not unusual that mature seeds should fail to germinate immediately after dispersal or harvest, even under conditions favourable for germination (e.g., water, oxygen, optimal temperatures). The current study results have shown that *D. steudneri* seeds have a dormancy problem. The results indicated that the seeds collected from the tree and from the ground and sown with different treatments achieved lower germination percentages and germination indexes relative to seeds sown without treatment. These results suggest that the treatments for breaking seed dormancy did not enhance germination and help to break the seed dormancy of *D. steudneri*. This result coincides with the findings of Tang et al. [29], whose results revealed that the application of GA3 did not break the seed dormancy problem of *Sorbus alnifolia* (Siebold & Zucc.) K. Koch after two months of incubation. Ralu and Upadhaya [30] also showed that physical pregermination treatments, including surface and acid scarification, failed to overcome dormancy problems in *Elaeocarpus prunifolius*.

Soaking in sodium hypochlorite for five h resulted in no germination for the seeds collected from the ground or from the tree when tested in the greenhouse, while soaking for twenty min and soaking for one h each resulted in germination. This result could indicate that the time of soaking in sodium hypochlorite affects the germination of the seeds of *D. steudneri*. Di-Tommaso and Nurse [31] found similar results, reporting that the germination of *Amaranthus powellii* seeds increased as the soaking period increased, but declined for seeds soaking for more than 60 s. Krishnapillay and Tompsett [32] indicated that the ground collection of seeds introduces the limitation of fungal problems; seed deterioration and premature germination may be encountered, which could affect the germination of the species seed. However, the *t* test results in the present study on the mean germination of the seeds collected from the ground and from the tree showed no significant differences in the greenhouse after different seed-dormancy-breaking treatments. This may suggest that the method of seed collection (ground or tree crown) for *D. steudneri* did not affect the seed germination of the species seeds. The one-way ANOVA results showed no significant differences in the mean germination of untreated and treated seeds of *D. steudneri*, indicating that the applied seed-dormancy-breaking treatments did not facili-

tate the germination of the species seeds. Overall, in both the treated and untreated seeds, the germination of the seeds in the greenhouse was very poor. Such poor germination could be associated with the short period of the germination study time in the greenhouse, as some species' seeds require a longer time to germinate. Certain species require a longer period to germinate, for example, seeds of *Elaeocarpus prunifolius* Wall. ex Müll took six months to initiate germination after dispersal [30]. On the other hand, *Dracaena cinnabari* germination experiments showed that air temperature and air humidity during germination could play an important role; germination of fresh *D. cinnabari* seeds was very low at 20 °C, reaching only 5%, and was better at 22 °C, while germination was approximately 80% at 26 °C and 30 °C [33,34].

A nonviable seed is a seed that cannot germinate even if all the favourable conditions needed for germination are provided [35,36]. The seed germination tests in the laboratory showed that none of the presowing treatments facilitated the germination of the species seed, which might be associated with the shorter period of the experiment and the characteristics of the seeds of this species. The viability of the seeds of *D. steudneri* after different seed-dormancy-breaking treatments was less than fifty percent. This might be associated with the storage time of the seeds (seven months in this case), which affects the viability of seeds for species that have recalcitrant and intermediate seed characteristics. Intermediate seeds are between recalcitrant and orthodox seeds, and cannot be kept for more than one year [37]. We observed two large and mature *D. steudneri* trees in Hawasa city, Ethiopia, and the trees did not flower and bear fruits annually; rather, they produced flowers and fruits every two years. This may indicate that the species might have intermediate seed characteristics and cannot be stored for a longer time. Bramasto [37] stated that the main problems for intermediate seeds are their short lifetime and that, for some species, the flowering and fruiting periods do not occur every year. The lower viability results obtained from the laboratory germination study of seeds treated with sodium hypochlorite for 20 and 60 min, relative to the results for the other seed-dormancy-breaking treatments, might be associated with the length of the soaking period, which could harm the viability of *D. steudneri* seeds. Ditommaso [31] showed that the effect of sodium hypochlorite on breaking seed dormancy varies depending on the type of species, period of soaking time, and concentration.

Study results from the natural forests of Ethiopia have also shown that the *D. steudneri* species in its natural habitat exhibits very poor natural regeneration [16–19]. Such poor natural regeneration could be associated with the ecophysiology of the seed, or with external factors such as light and temperature. Studies showed that light, temperature, and sowing depth affected the seed germination of some Draceana species [34,38]. Kheloufi et al., (2020) indicated that sowing depth and light availability limited the seed germination of *D. draco* in natural conditions, and better germination for the species occurred in the dark when seeds were sown at 2 cm depth. Bauerová et al. [34] found that the germination of *D. cinnabari* in laboratory conditions was affected by temperature, and their results showed that its germination index was high at 30 °C. Chan-Chin [39] obtained the highest germination percentage for *D. concinna* at a temperature of 25 °C. For some *Dracaena* species which are difficult to germinate from seed, different propagation techniques have been developed and used [40–47].

5. Conclusions

The germination test results, both in the greenhouse and in the laboratory, showed that *D. steudneri* seeds have a problem with germination and that the applied seed-dormancy-breaking treatments did not improve it. The seed viability test results after seven months of storage demonstrated fewer viable seeds than nonviable seeds for all the seed presowing treatments, which might be associated with the intermediate characteristics of the seeds of the species. Therefore, we recommend further research on the seed ecophysiology of the species and also the investigation of the effect of light and temperature in controlling the seed germination of *D. steudneri*.

Author Contributions: S.A.M. established the experiment, harvested and analysed field data, and wrote the article. H.H. designed the experiment, analysed the data and revised the manuscript. K.H. established the laboratory experiment and analysed the data. L.K. designed the experiment and assisted in data collection. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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