



Effect of pre-soaking substrate and light availability on seed germination and seedling establishment of *Dracaena draco* (L.) L., a threatened species

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Abstract

The Dragon tree, *Dracaena draco* (L.) L., is an extremely threatened species. Its natural regeneration is disturbed and population appears to decrease rapidly. To design appropriate germination and seedling establishment protocols for a species conservation and restoration program, we studied the effects of pre-soaking, substrate and light availability treatment on seed germination for 15-day period. The condition for light availability corresponded to the sowing depth (at surface or at 2 cm depth). Germination occurred better in the dark when seeds were sown at 2 cm depth. Our results showed that *D. draco* seeds sown in the sand under dark condition and after 24 hours pre-soaking in warm water germinated better and recorded 82.5% of final germination percentage (FGP). However, seedlings grew better in potting soil and recorded 54 mm of length. The maximum FGP of 51.3% was recorded under light condition. These results indicate that sowing depth and light availability could be a limiting factor for *D. draco* seed germination in natural condition. Furthermore, the establishment of *D. draco* seedlings (shoot and root length) during an 8-week period in pots was also assessed and illustrated. More research on seed ecophysiology is required to understand the mechanisms controlling seed germination of *D. draco* in arid and semi-arid conditions *viz.* salinity and drought.

Keywords

Agroforestry; Asparagaceae; Conservation; Dragon tree; Reforestation

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1 Introduction

Species of the genus *Dracaena* are found in subtropical and tropical regions of the world, but are mainly native to Africa (Lu and Morden, 2014). The regular growth form has a single trunk and an umbrella-like crown bearing the leaves on the tips of the branches (Krawczynszyn and Krawczynszyn 2014). The dragon tree, *Dracaena draco* (L.) L., is a wonderful specimen tree that is hugely popular because of its naturally strong architectural features. It is a monocot from Asparagaceae, subfamily Nolinoideae (Klimko et al. 2018). Being evergreen, drought tolerant and able to live for hundreds of years, it is a good choice of feature plant in homes and gardens (Gideon 2013; Jupa et al. 2017; Lengálová et al. 2020). Some specimens are believed to be up to 650 years old, the oldest is growing at Icod de los Vinos in Northwest Tenerife (Krawczynszyn and Krawczynszyn 2016). *D. draco* is a sub-tropical plant native to the Canary Islands, Cape Verde, Madeira and Western Morocco (Marzol et al. 2011). It is so valued in Tenerife that it has been chosen as the natural symbol of the island (Krawczynszyn and Krawczynszyn 2016).

Natural regeneration of *D. draco* is destroyed by overgrazing for decades which causes gradual population decline (Maděra et al. 2019). When grown in perfect conditions, the Dragon tree can grow to around 10-12 meters in height with a spread of up to 4 meters (Anonymous 2020). Although widespread in cultivation, the species is classified in the IUCN Red List of Threatened Species in 1998 as 'Vulnerable' in the wild where populations have been in decline for a long time (Bañares 1998). At least one reason for the population decline is the over-exploitation of the tree as a source of a medicinal resin (Nair 2000; del Arco Aguilar and Delgado 2018). Dragon's blood, a red resin exuding from the stem of *D. draco* has been used frequently as a "herbal" remedy in traditional medicine (Bruck 1999). Several studies demonstrated that the different morphological parts of *D. draco* species are rich sources of cytostatic and/or cytotoxic steroidal saponins and antioxidant (Silva et al. 2011; Valente et al. 2012). Wang et al. (2011) suggested that planting dragon trees is the only way to solve the resource shortage problem of dragon's blood.

In view of the radical reduction in the number of individuals and the consequent loss of Dragon tree genetic diversity caused by absence of natural regeneration, seed propagation offers many advantages because it potentially can facilitate large-scale production of valuable specimens and allow plant reintroduction in its natural ecosystem. Furthermore, recognizing the criteria of seed germination and seedling establishment for threatened species used in reintroduction projects is crucial to ensuring artificial seedling propagation (Ren et al. 2014). It has been shown that imbibition is an important trigger for seed germination and several species require light for high germination rates (Hegarty 1978; Bell 1999; Saux et al. 2020). Soil type is also an important factor influencing seed germination and growth (Iralu et al. 2019). This study indicated that substrate, light and pre-soaking can be important determinants of seed germination and seedling emergence of *D. draco*. Their potential interactive effects necessitate a combined assessment. In the present study, we investigated the effects of these three important environmental variables on seed germination of *D. draco*.

2 Materials and methods

2.1 Collection and seed characteristics

The seeds of *Dracaena draco* used in the present experiments were collected in November 2019 from freshly mature and ripe fruits harvested from the only three dragon trees growing in the Professional Training Centre in Agriculture (PTCA) of Misserghine (West of Oran, Algeria), located at (Latitude: 35°37'0.00" N; Longitude: 0°43'50.69" W) and 118 m of altitude. These trees are over 50 years old and around 2.5-3 m height. The fruit sample was obtained by mixing the fruits of the three trees. After harvesting, the seeds were extracted by opening the ripe fruits and removing the pulp using running water. The seeds were then air dried for one month and then stored in a glass bottle at 4 °C for one-month period to break possible dormancy, and then used for the germination experiments in January 2020 (Figure 1).



Figure 1. *Dracaena draco* (L.) L. tree, leaves, fruits and seeds.

A total of 50 fruits and 50 seeds (fresh and stored) were used for biometric determinations using a digital caliper. The length is a distance between the base and the apex and the diameter in the median region of both fruits and seeds. The fruit and seed weights were also estimated on basis of the weight of the 50-unit samples (Kheloufi et al., 2019). Fruit and seed size are shown on Table 1.

Table 1. Fruits and seeds characteristics of *Dracaena draco* (n=50).

Parameters	Ripe fruits	Fresh seeds	Seeds after storage
Weight (g)	1.83 ± 0.19	0.67 ± 0.06	0.46 ± 0.07
Length (mm)	13.6 ± 0.55	8.97 ± 0.29	8.06 ± 0.79
Diameter (mm)	14.4 ± 0.51	10.5 ± 0.32	9.39 ± 0.41
1000-sample weight (g)	1846	672	470

2.2 Experimental design

We utilized the split-plot design with pre-soaking as main plots and substrate and light availability as subplots, to study seed germination and seedling length characteristics of *D. draco*.

Substrate experiment: Two germination substrates (well washed sand and potting soil) were tested for their effects on the germination of *D. draco* seeds. The sand was collected from the dune of the desert in the region of Biskra (Algeria). The potting soil contained: 91 g.m⁻³ N, 44 g.m⁻³, P₂O₅, 28 g.m⁻³ K₂O, with a pH of 6.4 and an electrical conductivity of 49 mS.m⁻¹. The germination experiments were carried out in growth chamber with a temperature of 27 ± 2 °C. The seeds were placed on the two types of substrate in a plastic container (18 cm length × 8 cm height × 12 cm width) between two layers of sand or potting soil moistened (with distilled water) at 15%. The same seed density (50 seeds per plastic container) was used in all treatments. All treatments lasted 15 days and were replicated four times. The substrate was maintained relatively constant at 15% over the experiment.

Light availability experiment: The condition for light availability corresponded to the sowing depth. Seeds were sown either on the surface or at a depth of 2 cm of sand or potting soil (dark condition), and exposed to a 16:8 h photoperiod (light condition).

Seed pre-soaking: The control did not undergo pre-soaking. The second batch underwent 24 hours soaking in warm water at a temperature of 25 °C using a laboratory hot plate (Stuart SB162).

In summary, we had the following eight treatments (Table 2).

Table 2. List of the various treatments applied to *Dracaena draco* seeds.

Treatments	Pre-soaking	Substrate	Light availability
T1	24 h in warm water	Potting soil	Light
T2	24 h in warm water	Potting soil	Dark
T3	24 h in warm water	Sand	Light
T4	24 h in warm water	Sand	Dark
T5	0 h in warm water	Potting soil	Dark
T6	0 h in warm water	Potting soil	Light
T7	0 h in warm water	Sand	Dark
T8	0 h in warm water	Sand	Light

At the end of the experiment, the final germination percentage (FGP) and seedling total length (SL) were assessed. Seeds were counted as germinated when the radicle growth reached 2 mm.

2.3 Kinetic of seedling emergence

In order to study the kinetics of the emergence and the establishment of *D. draco* seedlings, seeds were germinated in plastic pot (12 cm top diameter; 9 cm bottom diameter; 15 cm height) containing 1.2 kg of the same potting soil described above. Pots were put under greenhouse conditions (25 ± 1 °C temperature and 35% relative humidity). Each week of the eight weeks of the study, four seedlings were randomly selected from 100 seedlings. The root length (RL), shoot length as well as the total length were measured using a digital caliper.

2.4 Data analysis

Data were analyzed by SAS Version 9.0 (Statistical Analysis System) (2002) software. For each treatment, two-way ANOVA was applied using the tested variable as a fixed factor and pre-soaking as the random factor. The mean separations were carried out using Duncan's multiple range tests and significance was determined at $p \leq 0.05$. The graphic was made with Excel 2016.

3 Results and discussion

3.1 Effect of pre-soaking, substrate and light

Substrate and light had a significant effect on seed germination of *D. draco* (Table 3). However, pre-soaking had no significant effect on seed germination. The germination percentage with pre-soaking in sand and under dark condition was significantly higher than that without pre-soaking by the respective values of 82.5% and 67.5% FGP (Figure 2). The first step in germination is the absorption of water. Water is the medium and reactant for many biochemical processes (Welbaum et al. 1998; Rifna et al. 2019). Germination takes place quickly when humidity is provided (Ali and Elozeiri 2017). Indeed, seed pre-soaking improved the germination metabolism compared with non-presoaked seeds of *D. draco* (Figure 2). Since water comes from the soil, the seed must be in direct contact with moist soil particles. However, absorption of water by seed cells is influenced by soil solution concentration of inorganic salts and/or organic substances (Woodstock 1988). If the concentration of salt is too high, then the seed cannot absorb adequate water for normal germination (Kheloufi et al. 2016). This explains partly why seeds failed to germinate in fertilizer zone or in highly saline soils (Maun 1994; Mansouri et al. 2019). Figure 2 showed that the very low FGP of 10% was recorded under T8. Indeed, seeds sown without pre-soaking, on the sand and under light condition was the extreme unfavourable condition for *D. draco*.

Table 3. Significance of substrate, light availability and pre-soaking on *Dracaena draco* seed germination.

Factor	df	SS	F-value	P
Substrate	1	344.53	8.12**	0.0089
Light	1	11819.53	278.45**	<0.0001
Pre-soaking	1	38.28	0.90 ^{ns}	0.3518
Substrate × Light	1	2907.03	68.48**	<0.0001
Substrate × Pre-soaking	1	1313.28	30.94**	<0.0001
Light × Pre-soaking	1	63.28	1.49 ^{ns}	0.2340
Substrate × Light × Pre-soaking	1	63.28	1.49 ^{ns}	0.2340

df - Degree of freedom; SS - Sum of squares; F-value - F-Snedecor; ** - Significant $p < 0.01$; ns - non significant

Germination occurred better in the dark (Figure 2). The FGP was significantly lower for non-buried seeds than for buried ones (Figure 2). Compared to 82.5% of FGP for buried seeds, seed germination percentage significantly decreased to 25% of FGP for non-buried seeds (Figure 2). The sowing depth of the germinating seed in the soil also affects the sufficient supply of oxygen and light, and consequently the emergence and establishment of seedlings (Forcella et al. 2000). The results confirmed the hypotheses that non-buried seeds have low germination, and few seedlings from

these seeds emerge from the soil. The maximum FGP of 51.3% was recorded under light condition (Figure 2). Seeds can use light to detect if they are close to the soil surface, this is especially important for small seed species since small seeds have limited resources and if they germinate too deep in the soil, these seedlings will not emerge successfully (Fenner 1991; Khurana and Singh 2001). It was reported that light response and seed mass co-evolved as an adaptation to ensure that small seed species germinate only when they are near enough to the soil surface to emerge (Limón and Peco 2016).

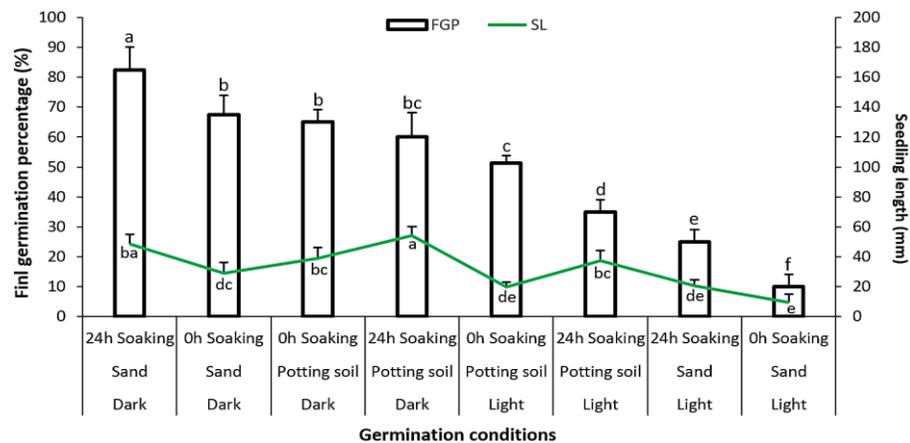


Figure 2. Effect of substrate, light and pre-soaking on *Dracaena draco* seed germination and seedling length after a 15-day period. Data represent the mean ± SD. For each variable, means with similar letters are not significantly different at the 5% probability level using Duncan's.

On the other hand, pre-soaking, substrate and light had a significant effect ($p < 0.0001$) on *D. draco* seedling length of (Figure 2). However, seedlings grew better in potting soil than in sand (Figure 2). After germination and the end of seed nutritional reserves, the potting soil contained sufficient nutrient supply for seedling establishment. Under different treatments, seedling length ranged from 9.47 mm to 54 mm.

Effective germination of a threatened species is important not only for regeneration maintenance but also for ex situ conservation of the species (Cochrane et al. 2007; Godefroid et al. 2010). This research is among the first investigations on seed germination and seedling emergence of the extremely threatened species *D. draco*. These data may help support decisions concerning the species' conservation management and restoration programs. Understanding the needs of a threatened species for germination and seedling emergence can be useful in planning programs for germplasm conservation and population restoration (Ashmore et al. 2011; Shen et al. 2015).

Seed viability and seed size are also additional factors that influence germination (Murali 1997; Kheloufi et al. 2019). Viability indicates if the embryo is alive and able to germinate under favourable conditions (Bewley and Black 2012). Seed size is an indicator of how much nutriment the seedling will receive (Gallardo et al. 2008). Larger seeds with increased nutrient supply also germinate more quickly, emerge from higher soil depths and develop a more robust seedling than smaller seeds do (Harper et al. 1970; Burmeier et al. 2010).

3.2 Kinetics of seedlings emergence

The study of plant morphology should primarily reflect variations in environmental conditions (Schneider et al. 2017). Seedling establishment follows radicle emergence (Maiti et al. 2017). The shoot length, root length and total length of *D. draco* seedlings over an 8-week period are shown in Table 4 and Figure 3. By week 1, the radicle had emerged and it continued to elongate throughout the experiment. A few roots were visible at the 3rd week and these had increased in number and length by week 8 (Figure 3).

Table 4. Shoot length (SL), root length (RL) and total length (TL) of *Dracaena draco* seedlings over an 8-week period (n=4).

Time	SL (cm)	RL (cm)	TL (cm)
Week 1	0.00 ± 0.00 ^f	1.07 ± 0.12 ^c	1.07 ± 0.12 ^f
Week 2	1.05 ± 0.11 ^e	3.99 ± 0.19 ^b	5.10 ± 0.10 ^e
Week 3	1.50 ± 0.14 ^e	4.06 ± 0.15 ^b	5.49 ± 0.07 ^e
Week 4	3.10 ± 0.36 ^d	4.14 ± 0.05 ^b	7.24 ± 0.38 ^d
Week 5	4.91 ± 0.55 ^c	4.02 ± 0.06 ^b	8.93 ± 0.60 ^c
Week 6	6.37 ± 0.90 ^b	4.07 ± 0.07 ^b	10.3 ± 0.96 ^b
Week 7	6.55 ± 0.81 ^b	4.10 ± 0.05 ^b	10.5 ± 0.80 ^b
Week 8	14.6 ± 0.72 ^a	11.3 ± 1.09 ^a	25.9 ± 1.50 ^a
F of Fisher	285.67	211.76	400.89
P	< 0.0001	< 0.0001	< 0.0001

Upon emergence, 2 to 4 weeks after sowing, the seedling develops a short 3.10 cm SL and 4.14 cm RL (Table 4). According to Table 4 and Figure 3, the root system continued to develop with secondary roots growing outward and downward from the taproot. The main root length remained relatively constant from the 2nd to the 7th week averaging nearly 4 cm.



Figure 3. *Dracaena draco* seedlings emergence over an 8-week period.

A root elongation of 175% was observed at the 8th week (Table 4). Root development is due to cell division and enlargement at the tip of the root. Shoot length increased from the 3rd week to the 6th week with an average of 1.6 cm and

remained unchanged at the 7th week with 6.55 cm. An elongation of 122% of SL was observed at the 8th week (Table 4). Root and stem growth enhance each other by adjusting their relative size in response to climatic and soil conditions, to meet the basic requirements of the entire plant (Sanders-DeMott et al. 2018; Reichardt and Timm 2020). Three weeks after emergence, the seedling develops its first true leaves (Figure 3). The plant quickly establishes a rosette with older leaves at the base increasing in size and smaller, younger leaves developing in the centre.

4 Conclusion

The present research proposes a practical way to reintroduce *D. draco* by planting artificial propagation in a specific habitat in order to carry out reintroduction programs of wild plants with extremely low populations. Thus, the species will establish a new population. We recommend that seeds should be collected from various individuals to maintain the genetic diversity of the seedling. Seed germination should be conducted at a temperature of 25-30 °C and 35% of relative humidity. Seeds should be pre-soaked and sown at 1-2 cm depth to ensure dark condition only for the two first weeks of germination. Seed should also be sown in a soil containing a sufficient supply of nutrients that will be available during seedling establishment. A further study will be carried out to assess certain abiotic and biotic factors before integrating *D. draco* into a reforestation program, particularly in the arid and semi-arid regions. This makes understanding of the conditions of its establishment essential for conservation and management.

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