

Original Article

Environmental drivers of *Euphorbia resinifera* seed germination and seedling establishment for conservation purpose

Fatores ambientais da germinação de sementes de *Euphorbia resinifera* e estabelecimento de mudas para fins de conservação

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Abstract

Euphorbia resinifera O. Berg is a prickly, leafless and succulent, Moroccan endemic shrub. Field data indicate that the plant faces many challenges related to its natural regeneration and its gradual decline that can lead to a probability of extinction, at least in some areas. Successful seed germination and survival of *E. resinifera* seedlings during the dry period is one of the main obstacles encountered in establishing natural seedlings. With this in mind, 3080 seeds of two morphotypes of *E. resinifera* (M1 and M2) were harvested in the Atlas of Beni Mellal to study their germinative potential and determine suitable conditions for growth and development of the seedlings. In the laboratory, five temperatures (10 °C, 15 °C, 18 °C, 25 °C, and 35 °C) and two photoperiods (12 h light/12 h dark and 24 h dark) were tested. Whereas in field research, two factors were considered: the availability of water and the type of substrate (clay, peat, and limestone). Results show a maximum germination rate of around 52% for M2 at 15 °C and 48% for M1 at 18 °C. The Monitoring of plant seedling establishment and growth revealed a high vulnerability to prolonged periods of drought. However, consolidated soil is more conducive to seedling establishment. For this species, it is therefore essential to conserve the habitat within the karst geosystem. Furthermore, the variability of this species' morphotypes and their growth form architecture shows a tendency to favor the dwarf, cushion-shaped morphotype, which is the most widespread in the study area.

Keywords: *Euphorbia resinifera*, germination, seedlings growth, prolonged drought.

Resumo

A *Euphorbia resinifera* O. Berg é um arbusto espinhoso, sem folhas e suculento, endêmico do Marrocos. Os dados de campo indicam que a planta enfrenta muitos desafios relacionados à sua regeneração natural e ao seu declínio gradual, que pode levar à probabilidade de extinção, pelo menos em algumas áreas. A germinação bem-sucedida das sementes e a sobrevivência das mudas de *E. resinifera* durante o período seco é um dos principais obstáculos encontrados no estabelecimento de mudas naturais. Com isso em mente, 3.080 sementes de dois morfotipos de *E. resinifera* (M1 e M2) foram colhidas no Atlas de Beni Mellal para estudar seu potencial germinativo e determinar as condições adequadas para o crescimento e o desenvolvimento das mudas. No laboratório, foram testadas cinco temperaturas (10 °C, 15 °C, 18 °C, 25 °C e 35 °C) e dois fotoperíodos (12 h claro/12 h escuro e 24 h escuro). Já na pesquisa de campo, dois fatores foram considerados: a disponibilidade de água e o tipo de substrato (argila, turfa e calcário). Os resultados mostram uma taxa de germinação máxima de cerca de 52% para M2 a 15 °C e 48% para M1 a 18 °C. O monitoramento do estabelecimento e do crescimento das mudas de plantas revelou uma alta vulnerabilidade a períodos prolongados de seca. Entretanto, o solo consolidado é mais propício para o estabelecimento das mudas. Portanto, para essa espécie, é essencial conservar o hábitat dentro do geossistema cárstico. Além disso, a variabilidade dos morfotipos dessa espécie e sua arquitetura de forma de crescimento mostram uma tendência a favorecer o morfotipo anão, em forma de almofada, que é o mais difundido na área de estudo.

Palavras-chave: *Euphorbia resinifera*, germinação, crescimento de mudas, seca prolongada.

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1. Introduction

The germination of seeds and the establishment of seedlings are two crucial stages in the life cycle of a plant (Talská et al., 2020). In fact, these two successive stages are the most vulnerable to climatic variations, especially in Mediterranean regions, with alternating wet, mild periods and hot, dry ones (Cristaudo et al., 2019). It ensures the natural regeneration of species, preserving their continuity and maintaining the ecosystem's biodiversity (Klupczyńska and Pawłowski, 2021). Also, the main factors linked to seed germination and growth in arid environments are temperature, soil type and water availability (Vicente-Serrano et al., 2020).

Euphorbia resinifera O. Berg. (Euphorbiaceae), is a "columnar cacti-like" shrub endemic to Morocco. As a species of section *Euphorbia* (Dorsey et al., 2013), it is distinguished by its succulent, leafless, polygonal stems, usually with four ribs (rarely 3, 5 or 6). The plant generally nests along the central High Atlas mountains, on rocky, limestone and dolomitic substrates, at altitudes between 640 and 1,900 m (Ettaqy et al., 2020). Although the species has been well studied for its phytochemical, medicinal and pharmacological qualities, no studies have been published on its biology features or regeneration. Currently, most arid and semi-arid cactiforms are in danger of extinction (Taha et al., 2023).

In Morocco, although the species was widespread over a vast area of five regions covering the High Atlas Mountains (Lawant and Winthagen, 2001), both area and density have declined sharply. Of the five regions where the plant was present, two have survived: The Central High Atlas in central

Morocco and Tizi N'Taraktin in the south. What's more, natural seedlings are generally no longer to be found on sites where the plant once thrived, except in very rare humid localities.

Conservation and management of rare, threatened and declining species requires knowledge of biology and ecology, as well as an understanding of the environmental factors that contribute to the genetic diversity of species (Negrón-Ortiz, 2018). Such genetic diversity is particularly important, as it affects a population's ability to adapt to a changing environment (Chen et al., 2020). At the same time, the plant's architectural structure plays an essential role in reducing water loss, through which the plant adapts to the transition between humid, arid and temperate tropical climates (Anest et al., 2021).

The aim of the present study is to evaluate the germination efficiency of seeds of two distinct morphotypes in the High Atlas population of *E. resinifera* at Beni-Mellal, as well as their growth under natural and controlled conditions. This will enable us to complete the missing data on the biology of this species in Morocco, with a view to improving its conservation.

2. Material and Methods

2.1. Morphotypic traits

E. resinifera in Morocco's Central High Atlas (Beni Mellal region), is characterized by great morphological and genetic diversity (Abd-dada et al. 2023). Two very distinct morphotypes, named M1 and M2, have been selected. Morphotypic traits presented in Table 1 and Figure 1 are

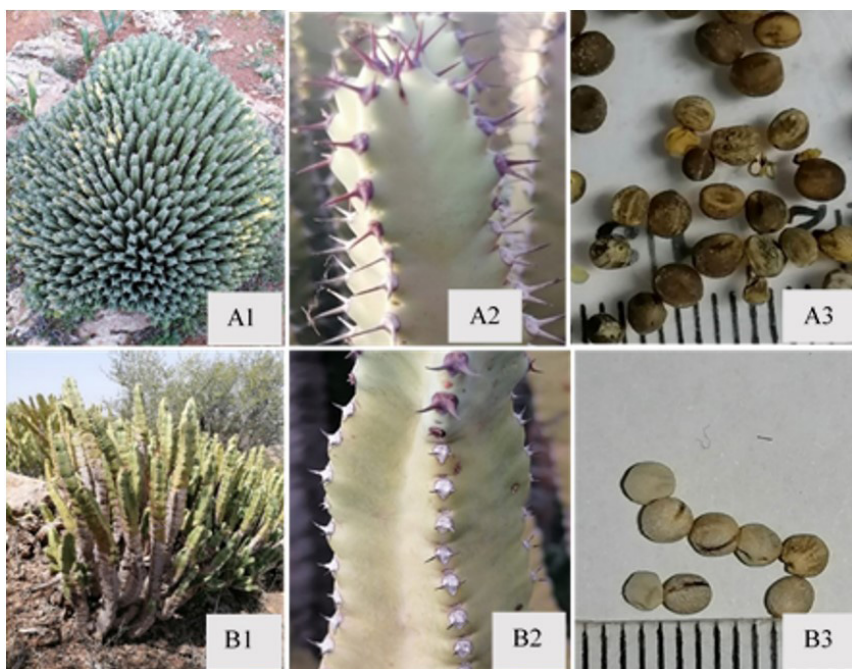


Figure 1. Photos of two *E. resinifera* morphotypes (M1 & M2) with the main phenotypic features: A1, half-spherical bush shape like an "echinus"; A2, square stems with straight spines; A3, oval and brown seeds; B1, bush with long stem; B2, rectangular stems and curved spines like an ox's head; B3, globular and gray seeds.

Table 1. Seed and stem traits measured for the two *E. resinifera* morphotypes M1 & M2 from the Atlas Mountains of Beni Mellal.

Morpho-type	Seed features				Stem features				
	1000-Seed Weight (g)	Seed length (mm)	Seed width (mm)	Seed shape	Seed colour	Stem width (mm)	Stem length (mm)	Stem height (cm)	Stem cross-section
M1	4.81 ± 0.05	2.95 ± 0.12	2.37 ± 0.08	Oval	Brown	25.72 ± 2.34	26.37 ± 2.07	73.6 ± 10.59	Square
M2	4.17 ± 0.03	2.76 ± 0.06	2.56 ± 0.07	globular	Grey	29.44 ± 3.68	39.16 ± 3.75	113.6 ± 11.10	Rectangular

based on plant architecture, including stem shape and size, as well as seed characteristics and thousand-seed weight (Park et al., 2023).

2.2. Seed materials

Mature fruits of the two different morphotypes of *E. resinifera* (M1 and M2) were collected during the period of dehiscence and seed dispersal, in July and August 2022, in Foum El-Anceur area located at the north of Beni Mellal town (latitude: 32°22'42"N, longitude 6°14'58"W). The capsules were air-dried for one month in aerated linen bags, until the seeds separated naturally, after being dispersed by opening the capsules. The seeds were stored in airtight bottles at 4 °C under dry conditions, until they were used to examine seed germination (experiment 1) and seedling establishment (experiment 2) (Park et al., 2023). For each germination experiment, harvested seeds were surface disinfected by shaking in a (0.5%) sodium hypochlorite (NaClO) solution for 30 s (second) and washed three times with distilled water (Danakumara et al., 2021).

2.3. Experimental 1

A thousand seeds of each morphotype of *E. resinifera* were tested for germination under light and dark conditions (two light treatments: 12 h light/12 h dark per 24 h (hours) cycle, and 0/24 h dark), and at five temperatures (10; 15; 18; 25, and 35 °C). The experiments were carried out with triple factorial treatments; 2 x 5 x 2 (two photoperiods, five temperatures and two morphotypes of *E. resinifera*). One hundred seeds for each treatment were divided into four replicates in 90 mm Petri dishes, each containing 25 seeds. All the Petri dishes contain filter paper moistened with 6 ml distilled water and sealed with parafilm. Germination tests were carried out from November to December 2022.

2.4. Experimental 2

The second experimentation was conducted in nursery under natural conditions in November 2022. The experimental design adopted was a randomized complete block (criss-cross sowings), with five replicates in 6 block (each bloc content 60 pots). Five hundred forty (540) seeds of each seed provenances were distributed over 180 pots. Each pot was filled with 500 g (grams) of the soil type collected in the study area: clay, peat and limestone. Three seeds were sown in each pot, and subjected to two water regimes: irrigation and non-irrigation (natural rainfall). A total of 1,080 seeds were used throughout the experiment (3 seeds x 3 soil types x 2 morphotypes x 2 irrigation modalities x 6 blocks x 5 replications = 1080 seeds).

2.5. Seed germination variables and statistical analysis

For the daily seedling count, the standard test value is that the radicle must reach a length of at least 2 mm (Khajeh-Hosseini et al., 2019). The number of germinated seeds was counted daily until day 14. To assess seedling growth and development, seedling height, root length and hypocotyl were measured after two weeks from the start

of the germination test (Danakumara et al., 2021). The seed germination parameters we used are: GermPercent (Equation 1), time taken for seeds to reach half germination percentage or t50 (Equation 2), GermIndex (Equation 3) and GermSpeed (Equation 4), according to (Aravind et al., 2023):

- GP: Germination percentage is computed as follows the Equation 1:

$$GP = \frac{Ng}{Nt} \times 100 \quad (1)$$

Where, Ng is the number of germinated seeds and Nt is the total number of seeds.

- t50: Median germination time was obtained according to the Equation 2:

$$t50 = Ti + \frac{\left(\frac{N}{2} - Ni\right)(Tj - Ti)}{Nj - Ni} \quad (2)$$

Where, t50 is the median germination time, N is the final number of germinated seeds, and Ni and Nj are the total number of seeds germinated in adjacent counts at time Ti and Tj Similarly

- GS: Germination speed is calculated using the Equation 3:

$$GS = \sum_{i=1}^k \frac{Ni}{Ti} \quad (3)$$

Where, Ti is the time from the start of the experiment to the ith interval, Ni is the number of seeds germinated in the ith time interval, and k is the total number of time intervals.

- GI: Germination index was obtained according to the Equation 4:

$$GI = \sum_{i=1}^k \frac{(Tk - Ti)Ni}{Nt} \quad (4)$$

Where, Ti is the time from the start of the experiment to the ith interval (day for the example), Ni is the number of seeds germinated in the ith time interval, Nt is the total number of seeds used in the test, and k is the total number of time intervals.

Germination data were subjected to one-way variance analysis (ANOVA) with a significance threshold of (5%) using R (version 4.2.3) (Rstudio Team, 2023). The mathematical equations for germination variables were calculated with germination metrics through the R Package germination metrics according to Aravind et al. (2023).

Concerning the effect of natural conditions on seedling establishment according to substrate type, morphotype and water availability in experiment 2, a bidirectional ANOVA was used to assess the combined effects of substrate and morphotype on seedling growth. Also, the normality of

the distribution and the homogeneity of the variance, were previously verified.

3. Results

3.1. Effect of temperature, light and morphotypes on seed germination

The one-way ANOVA (analysis of variance) test for germination rate, root length and hypocotyl length in *E. resinifera* were all significant according to the incubation temperature. Non-significant differences were observed for the effect of light on seed germination (p -Value = 0.615). However, a significant difference was observed for the effect of the morphotype on both germination rate and root growth as shown in Table 2. Therefore, no significant effect of light on germination rate and growth of the primary root and hypocotyl of *E. resinifera* was observed.

The germination rate obtained remains low, not exceeding (52%). However, both germination index and germination speed increase with temperatures between 15 °C and 18 °C. These three variables peak at 18 °C for M1 and 15 °C for M2 (Figure 2A, B, D). In contrast, the time required to reach (50%) of final/maximum germination was particularly short at temperatures above 15 °C and up to 35 °C, whereas at 10 °C it was much longer (Figure 2C).

3.2. Effect of temperature on seedling growth

The temperature range for root elongation was between 15 °C and 25 °C, with a peak at 18 °C (Figure 3C). However, hypocotyl elongation is highly sensitive to temperature variation (Table 2), and is particularly higher around 25 °C (Figure 3A). Also, the ratio of root length to hypocotyl length is positively correlated with lower temperatures, decreasing from 10 °C to 25 °C (Figure 3D). In fact, hypocotyl growth of seedlings showed a significant dependence on temperature. In addition, seedling size over the two-week germination test was clearly dependent on hypocotyl elongation (Figure 3B).

3.3. Effect of substrate type and water on seedling establishment

In the field, as shown in Figure 4, *Euphorbia resinifera* is so abundant that it covers the dolomitic and limestone rocks. The clay substrate is less densely covered. On peat, the results obtained are poor in terms of seedling survival, even with irrigation (Figure 5).

E. resinifera seedlings grow very slowly, more so on calcareous substrates, and there is a slight, non-significant morphotypic difference in favor of M2 (Table 3, Figure 5C). This growth under irrigated conditions reached a maximum height of 6.5 (cm) in clay, and a minimum of 1.2 (cm) in limestone, measured after one year of monitoring under irrigated conditions.

3.4. *Euphorbia resinifera* diversity related to water deficit tolerance

All seedlings watered only by rainfall (not irrigated) died by dehydration in early summer (June and July) (Figure 5A, B). Moreover, no seedlings of the M2 morphotype survived after only two months of consecutive drought (June) (Figure 5B). Indeed, morphotypic diversity reveals a greater tolerance of the M1 morphotype to drought, in contrast to the M2.

Two-way ANOVA on *E. resinifera* plantlets growth by measuring stem size against substrate (limestone and clay) and morphotype was non-significantly different (p -Value = 0.294). However, there was a highly significant difference in the effect of substrate on seedling growth (Table 3). In this analysis, the effect of water availability was not considered, as all seedlings that receive water from precipitation are dry out.

4. Discussion

Temperature is an important factor in the germination process, as well as in seedling growth and development (Soltani et al., 2022). Likewise, sunlight has an influence on seed germination and the development and growth of

Table 2. Analysis of variance (ANOVA) and the effects of temperature and light on germination rate, roots and hypocotyl length of *Euphorbia resinifera*.

Source	Factor	df	Sum of squares	Mean square	f-Value	p-Value
GermPercent	Temperature	1	3582	3582	33.09	1.64 e-07***
	Photoperiod	1	39	39.2	0.255	0.615
	Morphotype	1	650	649.8	4.476	0.0376*
Root length	Temperature	1	210	209.98	2.812	0.0976
	Photoperiod	1	3.9	3.934	0.489	0.486
	Morphotype	1	32.2	32.18	4.191	0.044*
Hypocotyl length	Temperature	1	1528	1528.4	26.45	1.96 e-06***
	Photoperiod	1	1	1.12	0.017	0.896
	Morphotype	1	14	13.61	0.209	0.649

Significance codes: df: degree of freedom *****: Highly significant ($p \leq 0.001$) **: Significant ($p \leq 0.05$)

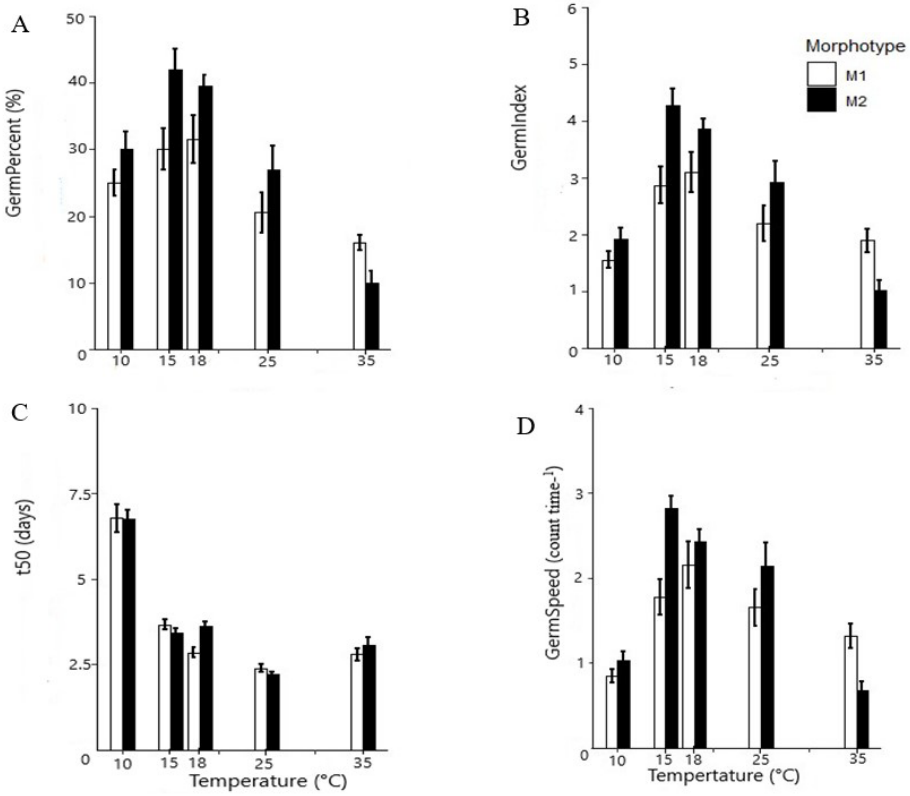


Figure 2. Germination indices for both *Euphorbia resinifera* morphotypes (M1 & M2): A, GermPercent (%); B, GermIndex (number of germinated seeds per day); C, t50 (time to reach 50% of final/maximum germination); D, GermSpeed (total number of seeds that germinate in a time interval (day)).

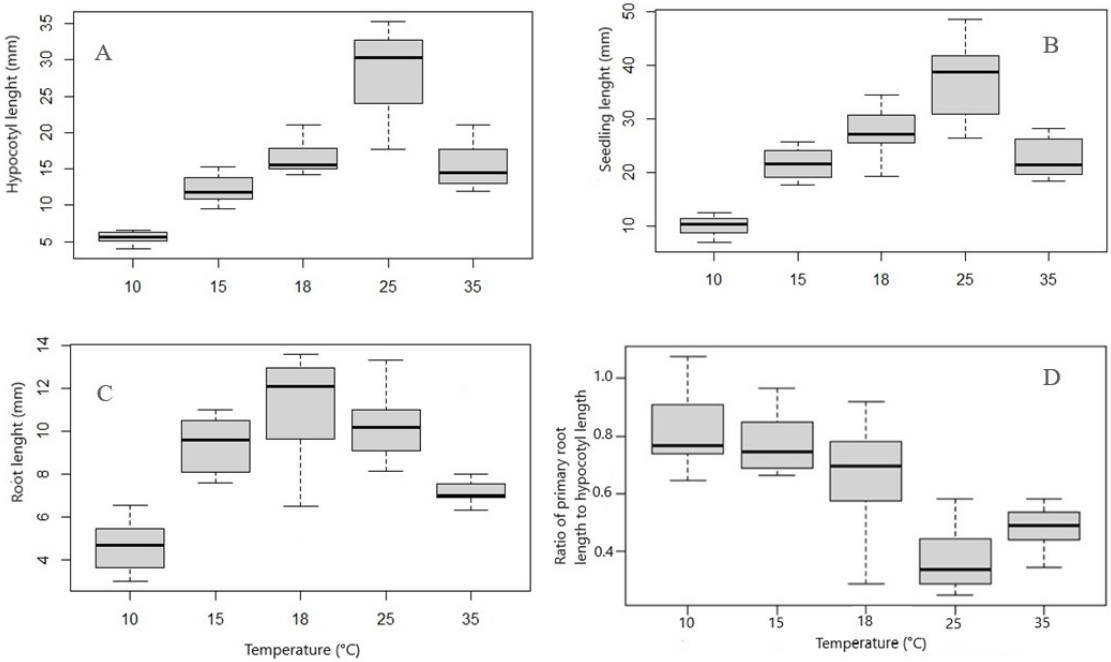


Figure 3. Effect of temperature on the growth of *Euphorbia resinifera* seedlings: A, Hypocotyl length; B, Seedling length; C, Root length; D, Ratio of primary root length to-hypocotyl length.



Figure 4. Photos of *Euphorbia resinifera* establishment on different real field substrates: A, Plant distribution according to substrate; B, Natural sowing of *E. resinifera* in clay soil; C, Seedling over a year old associated with a fern; D, Seedling more than 2 years old, well delimited by stem narrowing; E and F, Seedling trial on peat substrate; G, Seedling trial on limestone substrate; H, Seedling trial on clay substrate.

Table 3. Analysis of variance (ANOVA) and the effects of substrate and morphotype of *E. resinifera* plantlets growth.

Source	Factor	df	Sum of squares	Mean square	f-Value	p-Value
Seedling growth (stem height)	Substrate	1	25.74	25.74	7.534	1.78e-07 ***
	Morphotype	1	1.575	1.575	2.909	0.0999
	Substrate × Morphotype	1	0.475	0.475	0.874	0.3579

Significance codes: df: degree of freedom ****: Highly significant ($p \leq 0.001$)

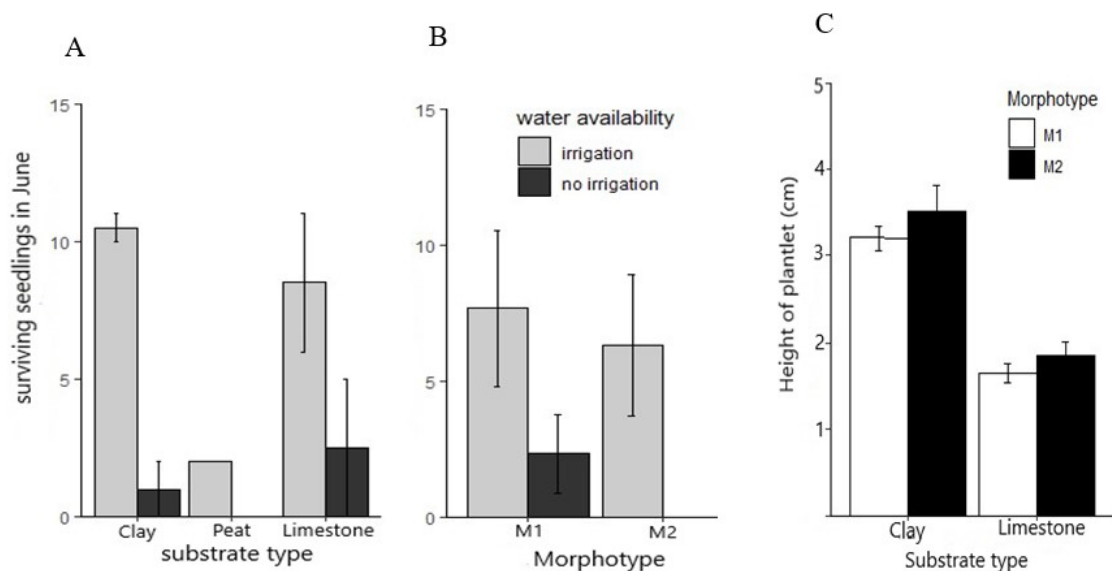


Figure 5. Effects of water availability, substrate type and morphotype on seedling establishment and growth: A, Combined effect of substrate and water availability on plantlet establishment; B, Combined effect of morphotype and water availability on plantlet establishment; C, Combined effect of substrate and morphotype on plantlet growth.

seedlings of many species (Mao et al., 2022). In fact, these factors play a key role in seedling establishment, as the seed loses its tolerance to desiccation after germination (Baskin and Baskin, 2014). In other words, the high radiation levels of Mediterranean environments are likely to adversely affect seedling performance (Castro et al., 2004). This is why seeds and seedlings react sensitively to these two factors and require well-hydrated conditions, provided that environmental situations remain favorable.

The low germination rate is linked to seed viability, which depends on seed maturity, both biotic and abiotic stress during the ripening phase, and the timing of capsule collection (Kameswara Rao et al., 2017; Pérez-López et al., 2023). In the case of *E. resinifera*, it should be noted that all these conditions were met, namely extreme drought during seed maturation and capsule collection carried out before ballistic seed dissemination. As well, *E. resinifera* seed germination was unaffected by photoperiod, the same result was found by Nunes et al. (2017) in *Jatropha curcas* L. (Euphorbiaceae), and in succulent species from Mexico (Flores et al., 2016), particularly columnar cacti within Cactaceae family and also in the Agavaceae (Jiménez-Aguilar and Flores, 2010). Therefore, the maximum germination temperature for the M2 morphotype is 15 °C and 18 °C for M1. This temperature seems to be very close to that found in a species of columnar cacti, *Oreocereus trollii*, native to the semi-arid Andes, whose optimum germination temperature is between 15 °C and 20 °C (Rojas-Aréchiga and Vázquez-Yanes, 2000). As well as the results obtained by Vicente-Serrano et al. (2020) on *Hypericum ericoides*, a succulent shrub from semi-arid Mediterranean climate. Similarly, the germination behaviour of *E. resinifera* seeds in response to temperature and light could be linked to growth form, since columnar cacti are insensitive to light,

unlike barrel-shaped cacti (Jiménez-Aguilar & Flores, 2010; Meiado et al., 2016).

Our results concerning the effect of temperature on the growth of *E. resinifera* seedlings reveal that root growth is only slightly affected by temperature, with elongation observed at temperatures ranging from (15 °C to 25 °C). However, hypocotyl elongation was significantly sensitive to temperature, with maximum elongation around 25 °C. These results are very similar to those published in *Arabidopsis*, by Fei et al. (2019) from (16 °C to 29 °C) for root growth, and by Ma et al. (2016), around 27 °C for hypocotyl elongation. Nunes et al. (2017) obtained the same result in *J. curcas* for seedling length, indicating that seedlings with the longest length and thickness were obtained at the same temperature of 25 °C. In addition, t50, high root/hypocotyl ratios and GI reflect both optimal germination and vigorous seedlings (Novak et al., 2015). The above variables were recorded at temperatures of 15 °C for M2 and 18 °C for M1. Thus, a high ratio between root length and hypocotyl length would be very important for good seedling establishment (Njimona and Baluška, 2022).

Field observations throughout the range of *E. resinifera* show that the adult plant is generally distributed on calcareous and dolomitic substrates. This predominant distribution of the plant is due to its affinity for this substrate, which is the most favorable for the establishment of seedlings. This dominance of the plant on the sunnier, drier south-facing slopes means that it has an affinity with the rocky substrate. However, the low water retention capacity of this type of substrate in a humid environment favors the establishment of stem succulents (Evans et al., 2014). Thus, this distribution of adult plant on the bedrock of the southern slopes could be a good indicator that this was a wetland environment. Therefore, the absence of

seedlings on these slopes is due to aridity. These results are in agreement with Sun et al. (2016) who suggest that Morocco's climate has changed significantly, confirming that *Euphorbia* ecosystems already existed before the emergence of the Sahara in the south of the country.

Water availability is an essential factor for the germination and establishment of species in arid ecosystems subject to rainfall limitations (Duncan et al., 2019). Thus, the tolerance of *E. resinifera* seedlings to mitigate the impact of late rains means that their stems do not have sufficient water reserves, particularly as drought intensity increases. The adult plant, with its large succulent stems, enables it to persist in rocky, arid habitats. Some leafless cactus species can persist in a rocky habitat with low, late rainfall of 150 to 400 (mm) in a semi-arid environment (Pérez-López et al., 2023). In the Beni Mellal study area, rainfall varies from 490 to 550 (mm) per year. Our results show that seedlings of both *E. resinifera* morphotypes are vulnerable to lack of rainfall, making them dependent on irrigation. Consequently, current conditions characterised by low and late rainfall combined with a prolonged period of drought have tended to limit the plant's regeneration, which could explain its rapid decline throughout its range over the last few decades. The small, cushion-shaped M1 morphotype is better adapted to drought conditions (late rains). In contrast, the higher M2 morphotype is the most affected by water deficit. This result justifies the predominance of the M1 morphotype and the rarity of the M2 morphotype in the field. This can be explained by the evolution of the architecture of cactiforms *Euphorbia* through its passage from tree to shrub to dwarf following the change in climate from humid to arid (Anest et al., 2021; Taha et al., 2023).

In general, *E. resinifera* is found in karst geosystems, mainly in limestone and dolomite. Most of the world's karst environments are highly fragile and vulnerable (Hu et al., 2022) as they have been subjected to intense anthropic pressures since the industrial revolution (Qiu et al., 2022). The loss of vegetation cover in these ecosystems increases the degenerative process of soil productivity, leading to karst desertification and making their restoration more difficult than ever (Wang et al., 2018). Indeed, overexploitation of the limestone surface layer could destroy the environment in which rock plants are found, complicating ecological restoration (Liu et al., 2015), notably as is the case for this species in Morocco.

Germination tests carried out under natural conditions show that *Euphorbia resinifera* has difficulty germinating and establishing itself. This is linked to both a consolidated substrate and a humid environment. Therefore, restoration of the plant could be carried out under controlled conditions of temperature and humidity until the seedlings have acquired the ability to mitigate the effects of drought and sunlight. In conclusion, the over-exploitation and destruction of limestone substrate for industrial purposes, coupled with the impact of global warming, explain why the plant's natural regeneration and colonization of its range are so difficult. These trends can predict the future of the species, which could become threatened and vulnerable in the short and medium term. So, protecting the plant also means preserving its habitat. Given these results, it is

imperative to preserve this endemic species for multiple uses in Morocco and it should be designed according to an ecosystem vision that considers its phenotypic diversity, seed bank creation, and regeneration through nursery production.

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