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Research Article

Seed Germination Characteristics of *Rhus tripartitum* (Ucria) Grande and *Ziziphus lotus* (L.): Effects of Water Stress

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Ziziphus lotus (L.) Lam. (Rhamnaceae) and *Rhus tripartitum* or Sumac (Anacardiaceae) are two indigenous species from arid and semiarid regions of Tunisia, characterized by a severe climate where dry seasons are very long. The combined action of anthropogenic factors and climate in arid regions caused a gradual threat of plant assets. In this context, an experimental study of the effects of water stress (0 to −1 MPa) on seeds has identified the water requirements germinal stage of both species. The results showed that both species were able to germinate at relatively low water potentials. However, beyond −0.6 MPa, germination was completely inhibited for *R. tripartitum*, when it reached for another 50% for *Z. lotus*. Increasing the concentration of PEG_{6000} progressively inhibited germination in both species. Only *Z. lotus* could be considered tolerant of water stress, because, to −1 MPa, seeds germinated with a rate of 17%. It resulted in that the species *Z. lotus* presented an adaptive capacity to aridity much greater than that observed for *R. tripartitum*.

1. Introduction

Drought stress is considered to be the main environmental factor limiting plant growth and yield of many agronomic and horticultural crops, especially in semi-arid areas. In Mediterranean-type ecosystems, seasonal water shortage is the main factor constraining survival and growth of plants.

Soil depth and texture are considered the most important edaphic properties that influence the moisture regime in arid environments with episodic rainfall. To date, a great deal of effort has been focused on physiological process underlying plant responses to drought stress.

The current situation of arid and desert areas of Tunisia (three quarters of the area) is in rapid decline of natural vegetation cover associated with an erosion of biodiversity [1, 2]. This decline is attributed to particular stressful environmental conditions, land clearing, and overgrazing, resulting in effects of increasingly adverse ecological (desertification) and economical.

In this area, the steppic vegetation is dominated by tall perennial grasses [3]. But, sparse trees and shrubs are among the most threatened species because of their excessive use for domestic purposes and their poor regeneration performances [4, 5]. On the other hand, under arid bioclimate, the flora has been subjected to a high, permanent increase of human pressure since at least the last century. Such a situation contributed and might have induced the phenomenon of desertification [5, 6].

In Tunisia, several attempts have been made to restore degraded rangelands [7–9]. The improvement of the methods based on seed dispersal and seed sowing would enable to know more about seed response to main environment factors especially drought stress in arid land. This factor could influence germination parameters [10, 11]. Under these conditions, seed germination monitoring in relation to water is very important to determine the colonisation capacity of species [12].

In this area, several native species are potentially interesting under aspects of dune stabilization and extension of plant cover, including some *Ziziphus* species and *Rhus tripartitum*. In addition, they improve the stability of ecosystems where

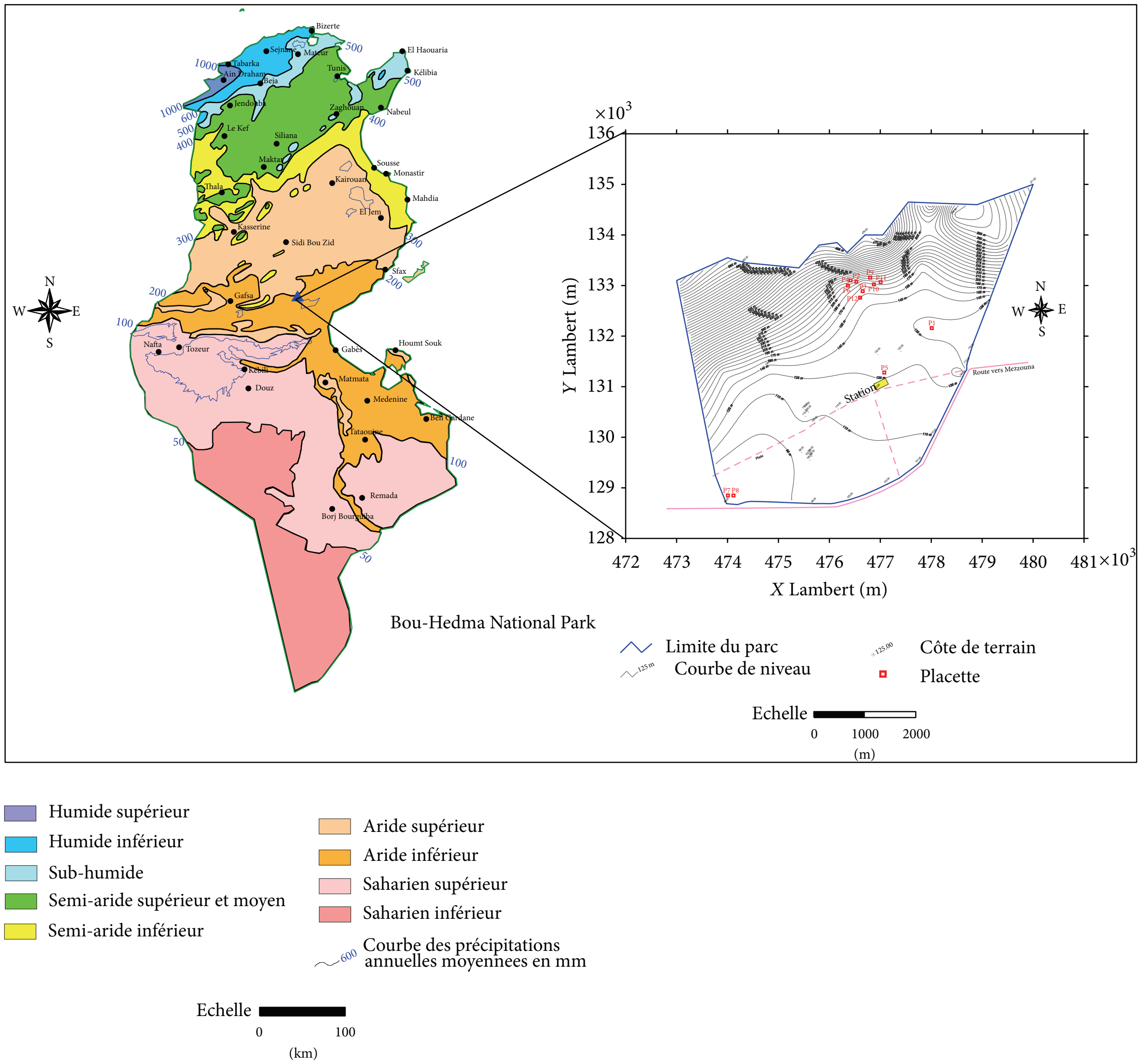


Figure 1: Location of the Bou-Hedma National Park in the bioclimatic map of Tunisia [32].

they are present, contribute to reduce the risks of desertification, and are helpful in restoring degraded ecosystems [6, 13–16].

These shrubs are also a feeding resource for livestock especially during the summer, when the alternative herbaceous species have wilted [17]. According to Barroso et al. [18], these species relieve sand movement. Currently, *Z. lotus* L. and *R. tripartitum* are rarefied in this area under overgrazing and their artificial reintroduction is necessary for the restoration of degraded ecosystems.

Accurate knowledge on the germination requirements of *Z. lotus* L. and *R. tripartitum* species is now required for successful uses of these two species in operations of artificial regeneration in Tunisia. Therefore, the effects of low water availability, simulated by PEG_{6000}, on seed germination were studied for these two tree species located in arid and semi-arid areas of Tunisia.

2. Materials and Methods

2.1. Site Collection Seed. The seeds of both species of *Ziziphus lotus* and *Rhus tripartitum* were collected from native shrubs (or low trees) in the National Park of Bou-Hedma (34°39′ N and 9°48′ E) (Figure 1) in September 2009. This park is located in the Governorate of Sidi Bouzid (central southern Tunisia). The bioclimate in this site is Mediterranean arid, with temperate winters and with large interannual and interseasonal variations of precipitation.

The mean annual average of rainfall is 180 mm in the plain and 250 mm on the crest of the mountain. Mean

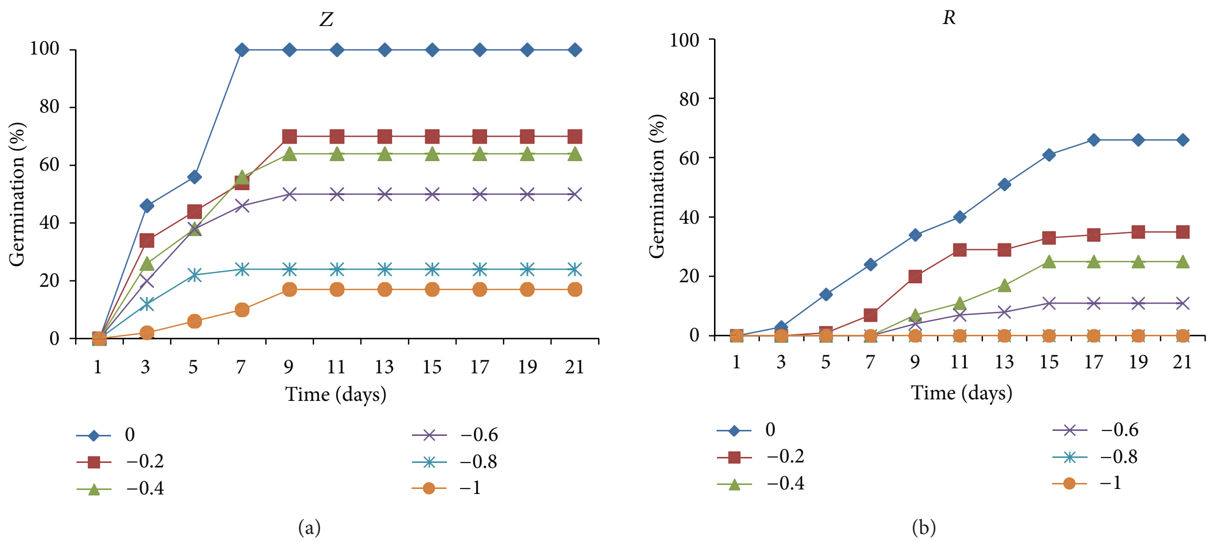


FIGURE 2: Cumulative germination percentage of *Ziziphus lotus* (*Z*) and *Rhus tripartitum* (*R*) seeds at different PEG_{6000} solutions of different osmotic potentials (0 to −1 MPa) ($n = 5$).

temperature ranges between 3.9°C throughout the coldest month (January) and 36.2°C throughout the hottest month (August).

2.2. Germination Experiments. Drought stress was induced by polyethylene glycol (PEG_{6000}) treatments. Six levels of osmotic potential ($\Psi\pi$): 0, −0.2, −0.4, −0.6, −0.8, and −1.0 MPa were tested and prepared from the formula developed by Mickel and Kaufman (1973) in [19]:

$$\Psi h = -\left(1.18\cdot 10^{-2}\right) * C - \left(1.18\cdot 10^{-4}\right) * C^2 + \left(2.67\cdot 10^{-4}\right) * CT + \left(8.39\cdot 10^{-7}\right) * C^2 T, \quad (1)$$

where C is the concentration of PEG_{6000} in $g{\cdot}L^{-1}$ of water, T is the temperature in °C, and Ψh is osmotic potential in bars. Distilled water served as a control. Seeds of the same form for both species (*Z. lotus* and *R. tripartitum)* have been selected and sterilized by aqueous solution of 75% ethanol for 5 min to prevent fungal attack and rinsed in several changes of sterile distilled water. The seeds were germinated in 9 cm sterile Petri dishes lined with two sterile Whatman number 1 filter papers with 5 mL of distilled water or the respective test solutions [20]; there were 20 seeds per Petri dish and five replicates in each treatment. Germination tests were conducted in complete darkness at 30°C. Distilled water was added into the Petri dishes each day to maintain the concentration of the test solutions. Seeds were considered to be germinated when the radicle emergenced. After seed sowing, the germinated seeds were counted and eliminated every second day for 21 days.

2.3. Methods of Germination Expression. The characteristics of seedling emergence were (FG), number determined: final germination percentage of days to first germination (delay of germination), critical limits of germination and mean time to germinate (MTG).

(i) Final germination percentage (FG) was calculated as the cumulative number of germinated seeds with normal radicles [19].

GP $= \sum n$, where n is the number of seeds that had germinated at each counting.

(ii) Mean Time of germination: MTG was estimated according to the formula:

$$\text{MTG} = \frac{\Sigma\,(ni \times di)}{N}, \quad (2)$$

where n is the number of seeds germinated at day i, d the incubation period in days, and N the total number of germinated seeds in the treatment [21].

2.4. Statistical Analysis. Statistical analysis was performed using two-way analysis of variance (ANOVA and DUNCAN) to test the effects of water stress, species, and their interactions, on germination characteristics. All statistical methods were performed using SPSS, version 16.

3. Results

3.1. Influence of Water Stress on the Kinetics of Germination. The kinetics of germination by osmotic stress conditions is presented in Figure 2. It reflects the sensitivity of the species to water stress. In control condition, the kinetic curves show three phases of germination: latency, accelerating exponentially, and finally step corresponding to a stop germination after attaining the maximum germination. The depressive effect of water stress on germination occurs during one or all of these three phases, depending on the degree of the lowering of water potential and species studied.

It results in a slower processing speed visible from −0.2 MPa, and thereafter increases. Whose, the delay of germination the most extensive (3 days) is obtained to −1 MPa for *Z. lotus* while 7 days to −0.6 and −0.8 MPa for *R. tripartitum*.

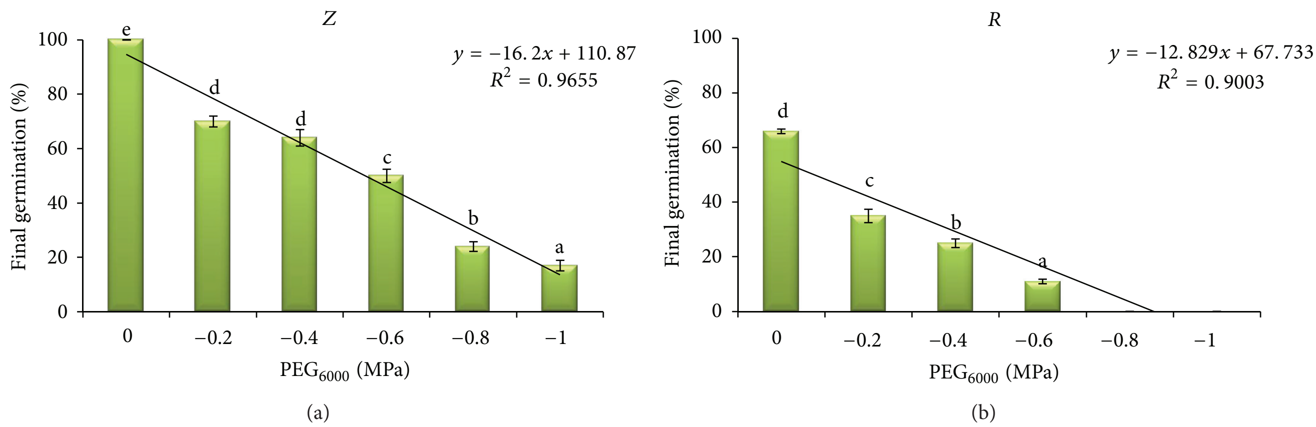


Figure 3: Variation of the final germination percentages (%) of *Ziziphus lotus* (Z) and *Rhus tripartitum* (R) seeds at different PEG_{6000} solutions of different osmotic potentials (0 to −1 MPa) ($n = 5$).

Table 1: Results of a two-way ANOVA showing effect of water stress on the germination rate in both species: *Ziziphus lotus* and *Rhus tripartitum*.

Source of variation	ddl	Mean square	F ratio	P
Species	1	2699,51	304,46	0,000***
Treatment	5	92,87	10,47	0,000***
Interaction Sp*Trt	5	89,79	10,13	0,000***
Error	48	8,87		
Total	59			

Numbers representing the value of F. $^{***}P < 0.0001$ effect very highly significant.

Table 2: Comparison of the speed of germination by Duncan test of two species of *Ziziphus lotus* and *Rhus tripartitum* as a result of water stress (species effect).

Species	Mean	Group
Z. lotus	20,03	A
R. tripartitum	6,62	B

Different letters indicate significant differences between species.

3.2. Final Germination Percentage. Final germination percentage of *Z. lotus* seeds were recorded in controls with (100%) and only 66% of *R. tripartitum*. The germination capacity is greatly affected by water stress (Figure 3), analysis of variance showed that there is a highly significant difference between treatments and species ($P < 0.0001$) (Table 1).

Test ANOVA with two factors, calculated at 5% level, presented a treatment effect and species very highly significant ($P < 0.0001$) and the interaction between them (Table 1).

Duncan test (Table 2) discriminates two homogeneous groups under species effect: G1 = A to *Z. lotus* and G2 = B to *R. tripartitum*.

Duncan's expresses homogeneous groups according to the order of decreasing water potential (Table 3). It is found that upon application of −0.2 MPa, the germination capacity is significantly reduced especially for *R. tripartitum* where it exceeds 50%.

3.3. Critical Limits of Germination. Critical limits of germination of both species were highly different. Seed germination of *Z. lotus* vanishes up there from −1 MPa but at −0.8 MPa for *R. tripartitum*. So, the critical limit of germination of *Z. lotus* are the order of −1 MPa while it was −0.6 MPa for *R. tripartitum* (Figure 3).

3.4. Influence of Water Stress on the Final Percentage and Mean Germination Time (MTG). To better understand the effect of water stress on germination of *Z. lotus* and *R. tripartitum*, we showed the relationship between mean germination time and the final percentage. In fact, in *Z. lotus*, a water potential of −0.2 MPa causes a decrease in germination capacity of around 30%. This decrease becomes important to −0.8 MPa (76%) and even more accentuated at −1 MPa (83%).

Whereas *R. tripartitum*, a potential of −0.2 MPa, causes a 65% reduction of germination capacity, this reduction increases (89%) at −0.6 MPa and reached 100% at −0.8 MPa.

The results obtained for GMT of *Z. lotus* range from ≈5 to 7 days respectively, for 0 and −1 MPa, whereas *Rhus tripartitum*, varies from 9.67 to 11 days, respectively, to 0 and −0.6 MPa. So, the lowering of the water potential leads to an increase in the mean germination time.

The comparison of both species responding to water stress shows that *Z. lotus* is the most effective (tolerant) for most treatments compared to *R. tripartitum* (Figures 4 and 5).

4. Discussion

The main objective of our paper was to assess the germination characteristics of *Z. lotus* and *R. tripartitum* under abiotic constraints. The most important result showed that germination capacity of studied species under the environmental constraints and factors is sufficiently assured to consider these species for a reforestation program and the extend consequently their area of distribution. Additionally, studied species responded differently to stress induced by PEG_{6000}. However, *Z. lotus* manifested better adaptation to drought conditions compared to *R. tripartitum*.

Under arid bioclimate, the successful establishment of plants largely depends on the success of germination. The

Table 3: Mean comparison (Duncan test $\alpha = 0.05$) of final percentage and mean time to germination of *Ziziphus lotus* (Z) and *Rhus tripartitum* (R) seeds under osmotic stress condition.

		Germination characteristics			
	Water potentiel (MPa)	Final germination percentage (FG)	Decrease (%)	CV (%)	MTG (days)
Ziziphus lotus	0	100 ± 0[f]	0	20,35 ± 2,16[a]	4,96 ± 0.55
	−0.2	70 ± 2[d]	30	19,27 ± 2,63[a]	5,27 ± 0,74
	−0.4	64 ± 3,03[d]	36	19,15 ± 3,91[a]	5,4 ± 1,12
	−0.6	50 ± 2,44[c]	50	21,66 ± 5,19[a]	4,85 ± 1,22
	−0.8	24 ± 1,78[b]	76	24,28 ± 5,49[a]	4,27 ± 0,83
	−1	17 ± 1,94[a]	83	15,48 ± 4,29[a]	6,87 ± 1,87
Rhus tripartitum	0	66 ± 0,84[a]	34	10,40 ± 0,85[b]	9,67 ± 0,83[b]
	−0.2	35 ± 2,45[b]	65	12,05 ± 1,02[a]	8,35 ± 0,70[c]
	−0.4	25 ± 1,58[c]	75	8,24 ± 0,8[c]	12,22 ± 1,14[a]
	−0.6	11 ± 0,84[d]	89	9,01 ± 1,28[c]	11,27 ± 1,46[a]
	−0.8	0[e]	100	0[d]	0[d]
	−1	0[e]	100	0[d]	0[d]

The values followed by the same letter are not significantly different at $P > 0.05$ each value is a mean of five replicates of 20 seeds.
CV: kinetics of germination; FG: final germination percentage; MTG: mean time to germinate.

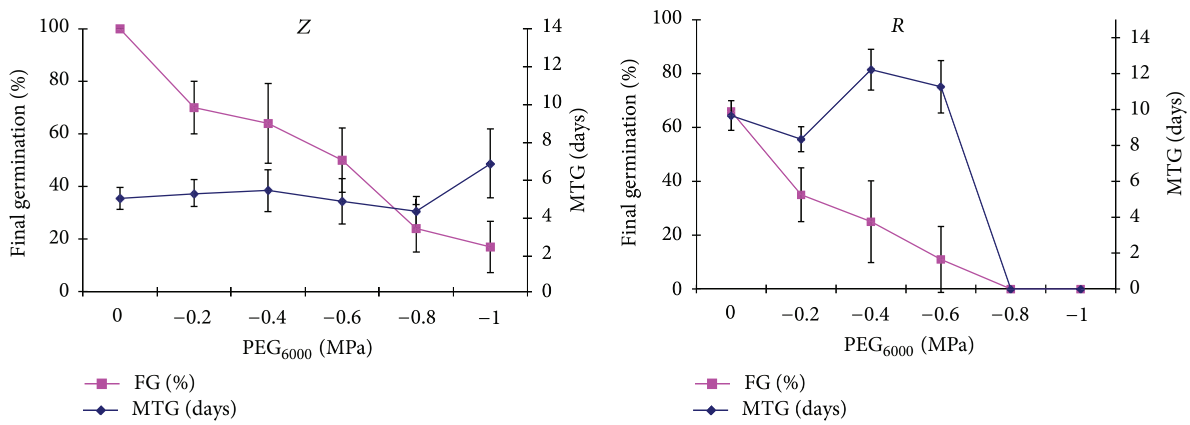


Figure 4: Variation of the final germination percentages (%) and the mean time to germination (MTG, days) of *Ziziphus lotus* (*Z*) *and Rhus tripartitum* (*R*) seeds ($n = 5$) at different PEG_{6000} solutions of different osmotic potentials (0 to −1 MPa).

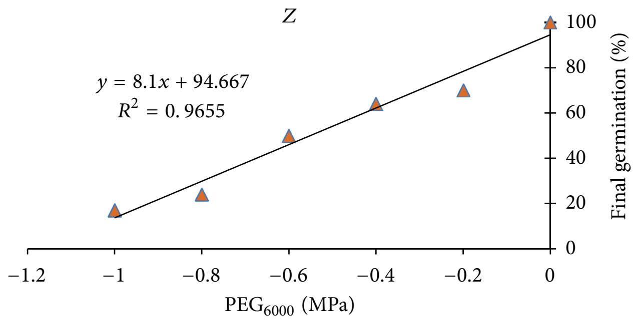
success of shrubs under warm and dry conditions is primarily dependent on optimal conditions (25/35°C and 12 h night/12 h day) for germination and recruitment [22].

Our results suggested that germination of *Z. lotus* seeds continued despite the increase of osmotic potential (Table 1). Up to −0.6 MPa, germination rate was superior to 50% for *Z. lotus* but completely inhibited for *R. tripartitum* (Table 1). The higher germination percentage of *Z. lotus* compared to *R. tripartitum* may be related to its better adaptation to water deficits. Our results are consistent with the results of Ne'eman et al. [23] who showed that germination of *Rhus coriaria* was reduced by 80% at water potential of −0.26 MPa. In the same way, Noumi et al. [24] showed that up to −0.7 MPa seeds germination of *R. tripartitum* is totally inhibited.

Under controlled conditions, the highest germination percentage of studied species was obtained for non-stressed seeds. The variation of PEG_{6000} concentration has significantly affected germination rate.

Overall, our results showed that the increase of water stress introduces the decrease in germination rate of studied species. This finding is consistent with the results of Ne'eman et al. [23] who showed that germination of *Rhus coriaria* was reduced by 80% at water potential of −0.26 MPa. In the same way, Chuang et al. [25] showed that the critical value of *Periploca sepium* was −1 MPa and the maximum value was −1.4 MPa under the PEG. This finding corroborate with results from *Lotus creticus ssp eucreticus, Plantago albicans* L. *Subsp.albicans.L.H* and *Rhanterium suaveolens Desf.* subsp.*suaveolens* (Desf) [26].

In contrast to our results, the critical threshold of germination of *Z. lotus* and *R. tripartitum* is very highest compared to others species growing under arid and Saharan climates such as *Lotus creticus* (−0.7 MPa), *Plantago albicans* (−0.5 MPa), and *Rantherium suaveolens* (−0.3 MPa) [27]. In the same context, Sharma [28] showed that germination of *Atriplex vesicaria* Heward and *Atriplex nummularia* Lindl



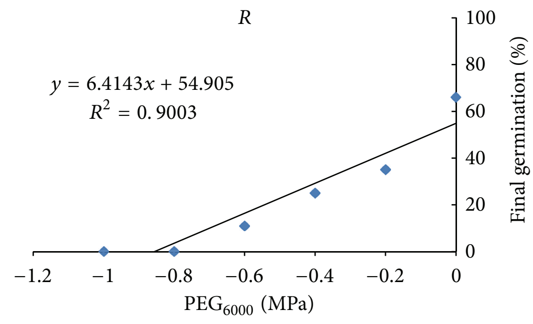


Figure 5: Regression plots for mean final germination percentages of *Ziziphus lotus* (*Z*) and *Rhus tripartitum* (*R*) seeds at different PEG_{6000} concentrations. Values ($n = 100$) are from the six treatments with five replicates.

was totally inhibited up to −0.2 and −0.4 MPa, respectively.

In Tunisia, several attempts have been made to restore degraded rangelands [7–9, 13, 14]. One important technique for the rehabilitation of degraded ecosystems is to improve the dispersal of seeds, which is labour saving when compared to transplanting nursery seedlings [15]. Arid zones in Tunisia, which cover more than 70% of the total area [2], are characterized by drought and high temperature and more or less general salinity. In addition many shrub species such as *Z. lotus*, *R. tripartita*, *Periploca angustifolia*, *Retama raetam, Acacia raddiana*, *Ceratonia siliqua*, and *Lycium shawii* are valuable species for afforestation in arid area of Tunisia as they withstand the harsh climatic conditions, improve the stability of ecosystems where they are present and contribute to reduce the risks of desertification, and are helpful in restoring degraded ecosystems [6, 13–16].

In the field, establishment of *Z. lotus* and *R. tripartitum* varies according to the season (cool/warm) and the geographical and ecological distribution as well as the amount of rainfall. Seasonal fluctuations of rain in deserts of Tunisia explain the variable establishment pattern of this plant during the course of a year, governed by seasonally different temperatures and the rainfall events. From the present results, it is evident that germination percentage decreases with increasing water stress and comes to the limit at −1 MPa.

5. Conclusion

Some demographic studies [29, 30] suggest that the establishment of many woody species from arid and semi-arid areas is a rare event, which is mainly due to the occurrence of excessive water stress caused by low rainfall and/or high evapotranspiration rates. However, the establishment of these woody species would be possible only after years of above normal and well-distributed rainfall [31].

In conclusion, *Z. lotus* and *R. tripartitum* species manifested different levels of adaptation to drought. Increasing the concentration of PEG_{6000} progressively inhibited germination for both species. Only *Z. lotus* can be considered tolerant to water stress, since, to −1 MPa, seeds germinate at a rate of 17%. As a result, *Z. lotus* presents an adaptive capacity to aridity much larger than that observed for *R. tripartitum*. Therefore, these findings form the basis for future trials involving the use of indigenous shrubs in the restoration of rangelands.

Acknowledgments

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