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Factors influencing seed germination of the pastoral plant *Retama raetam* subsp. *bovei* (Fabaceae): interactive effects of fruit morphology, salinity, and osmotic stress

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The aim of this work was to study the morphology of retama seeds and the combined effects of salt and water stress on the seeds germination of four Tunisian populations of *Retama raetam*. Seeds were harvested from Bouhedma National Park, Meknassi, Oueslatia, and Rtiba. In this study we were interested in evaluating different parameters of germination in presence of the salinity and osmotic stress. The results revealed that this species can withstand salinity up to 15 g/l of salt (the germination percentage of the Rtiba population reached 42%) and it can also tolerate severe drought resulted in significant doses of PEG_{6000} (germination percentage reached 29% to the water potential of −1.6 MPa of Oueslatia population). The morphological traits of the seeds were measured to find a relationship between the size of the seeds and their germination capacity under salinity and osmotic stress. Results have shown that for the correlation between morphological traits of seeds and the germination percentage, the seed weight is a major factor in promoting germination under stress and confirmed that saline water and sprouting inhibition have impact on the small caliber of seeds.

Keywords: *Retama raetam*, population, morphological traits, salt stress, water stress, germination

INTRODUCTION

The genus *Retama* belongs to the family Fabaceae (500 genera and 1,000 species). It includes three species (*Retama monosperma*, *Retama raetam* and *Retama sphaerocarpa*) with a large distribution in the East Mediterranean region, North Africa, and in the Canary Islands (Mahane 2009). *Retama raetam*, locally named as "R'tm", is a desert shrub native to several countries of North Africa (Algeria, Egypt, Libya, Morocco, and Tunisia), temperate Asia (Israel, Jordan, Lebanon, Palestine, and Syria), and South-eastern Europe (Sicily in Italy) (GRIN Database, 2011). In Morocco, it is largely located in desert regions and in the Middle Atlas. *Retama raetam* with steno-Mediterranean and saharo-sindic distribution mainly grows in dune and desert habitats where it can withstand extreme aridity. For this reason it is often used in dune stabilization and in

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restoration of desert areas (Boulila et al., 2009). *Retama reatam* has a very productive vertical and horizontal root system that can reach 20 m. The *Retama* species contributes to the bio-fertilisation of poor soils because of its aptitude to associate with fixing nitrogen bacteria Rhizobia (Boulila et al., 2009; Selami et al., 2014). Therefore, the genus of the *Retama* is included in a revegetation programme for degraded areas in semi-arid Mediterranean environments (Caravaca et al., 2003). *Retama raetam* is also a medicinal and aromatic plant present in humid to arid bioclimatic regions and it is a famous herbal drug in Tunisia (Saad et al., 2014). Actually, in traditional medicine, *Retama raetam* is used for the treatment of several diseases such as diabetes and hypertension, and its shoots are also used as an antidote against snake bites (Maghrani, 2005). Research undertaken on genus *Retama* showed that the aqueous extract had diuretic and hypoglycaemic effects (Maghrani, 2005). Even more, oral administration of 20 mg/kg of this aqueous extract reduced significantly the glucose levels in the blood of diabetic rats (Maghrani, 2005). Moreover, administration of *Retama raetam* aqueous extracts induces a decrease in the triglyceride concentrations in the plasma of normal and diabetic rats and leads to a significant decrease in weight (Maghrani, 2005). All the fractions from the Tunisian halophyte *Retama raetam* showed a potent antioxidant activity and some of them exhibited interesting antibacterial qualities (Saad et al., 2014). The phenolic concentration in this species is very high, especially for the ethyl acetate fraction, and is probably responsible for its important biological activities (Saad et al., 2014). The RP-HPLC data revealed a large number of phenolic acids and flavonoids in *Retama raetam* shoots, with coumarin and syringic acid as major phenolics. Also, *Retama raetam* is a source of natural antioxidants useful in medical and food industries (Saad et al., 2014). In the south-eastern region of Morocco (Tafilalet), this plant is largely recommended by traditional herbal healers for diabetes control and phytotherapy. This ethnobotanical information has been recently verified experimentally (Maghrani et al., 2003). The underlying mechanism of this pharmacological effect has been hypothesised as inhibition of renal glucose reabsorption (Maghrani et al., 2005). *Retama raetam* species has developed a particularly important root system and is adapted to arid areas. Cellulose remains the major component of the cell wall (27% for young roots and 80% for adult roots), hemicelluloses (14.3% for young roots and 3.6% for adult roots) and pectins (17.3% for young roots and 4.1% for adult roots) (Bokhari et al., 2015). Previous pharmacological studies on this plant have revealed its antibacterial, antifungal, antihypertensive, anti-oxidant, antiviral, diuretic, and hypoglycemic properties, and hepatoprotective, nephroprotective and cytotoxic effects (Maghrani et al., 2005; Eddouks et al., 2007; Hayet et al., 2007, 2008; Koriem et al., 2009; Algandaby et al., 2010; Edrizi et al., 2010). *Retama raetam* appears to be a valuable candidate as a forage resource in Sinai (Egypt). This fodder species should be considered valuable nonconventional forage in the Mediterranean arid ecosystem (Naser et al., 2013). Although present in low quantities in branches/leaves and seeds of *Retama monosperma*, lipids are an important fraction in terms of quality given their high unsaturated fatty acids, particularly oleic, linoleic and linolenic acids whose therapeutic virtues are highly recommended (El Hamdani, Fdil, 2015). The fact that the linoleic acid is considered to be beneficial in cancer and diabetic prevention provides some scientific basis for the traditional use of the plant as an antidiabetic, and for its pharmaceutical indication as antileukemic (El Hamdani & Fdil, 2015). Based on this, further pharmacological investigations to screen other potential bioactivities of fatty acids of *Retama monosperma* may be recommended (El Hamdani, Fdil, 2015). The highest mineral content is that of Al, Fe, Mg, Zn, Ca, K, Na, and P. However, the composition of the plant is mainly dependent on the composition of the soil which is influenced primarily by the nature of the rocks from which the soil is derived. In Algeria, the plant grows in Sahara and Atlas regions and is used in folk medicine to reduce blood glucose and skin inflammations (Baba, 1999) The Saharan plant *Retama raetam*

(Fabaceae family) is used as a locally available adsorbent for removal of copper ions from aqueous solution. The aerial parts of *Retama raetam* can be used as an inexpensive biosorbent and as suitable alternatives for the removal of copper ions from wastewater (Cheriti et al., 2009). Among xerophytic shrubs, *Retama raetam* has a potential economic importance. It plays a significant role in soil protection and stabilization against wind or water erosion and provides an important dietary source for livestock species such as camels, goats, and sheep (Laudadio, 2009). Additionally, this species represents a viable fuel source for humans (Cheriti et al., 2009). It also has medicinal and potential industrial values since its roots are used to treat diarrhoea, the leaves are used to help aching joints, back pain, and eye troubles (Said et al., 2002). Shrubs are key components in these ecosystems as they influence both biotic and abiotic conditions. Woody species may create "islands of fertility" by improving the availability of water and nutrients (Moro et al., 1997) or by protecting against direct irradiance and overheating (Moro et al., 1997; Lopez-Pintor et al., 2000). In addition, the legume species can increase soil fertility due to nitrogen-enriched litter deposition or direct release of nitrogen from roots (Dart, 1998). Recently, researchers have become interested in woody legumes due to their ecological importance (Ndiaye, Ganry, 1997; Dart, 1998; El-Shaer, 2000). Information on the nutritive value of forage could help range management in selecting suitable grazing sites to sustain animal life without inflicting vegetation damage (Arzani et al., 2004). Thanks to high palatability of *Retama raetam* (Laudadio, 2009), this legume may represent an important forage resource for livestock species, especially during the dry season when shortages of pasture are common in this Mediterranean region. However, very little attention has been given to the forage potential of wild leguminous species, especially to trees and shrubs (Dart; 1998). So, the ecological potentiality of *Retama raetam* can contribute to the reduction of fodder shortages in the arid areas of the Mediterranean ecosystem. *Retama raetam* has a strong potential as a forage crop with a valuable nutritional quality for browsing animals. Moreover, this species may represent an alternative feedstuff to the conventional forage and a promising substitute fodder in the Mediterranean ecosystem. (Naser et al., 2013). Concerning seed germination, literature data (Gutterman, 1993; Izhaki, Ne'eman, 1997; Seglie et al., 2012) show that the percentage of germination in *Retama* is very low due to physical dormancy caused by the impermeability of seed coat to water (Kigel, 1995). Hence, studies have also been carried out on seed morphology and seed germination under abiotic factors (salt stress and osmotic stress) to get new data on the endemic taxon of Tunisia *Retama raetam* subsp. *bovei* in view of conservation and habitat restoration.

MATERIALS AND METHODS

Plant material

Seeds of *Retama raetam* were collected in 2014 from four natural habitats, namely, Meknassi, Bouhedma, Rtiba, and Oueslatia (Fig. 1). After the collection, the seeds were stored in a cold room. The climatic conditions prevailing in each region were considered. Before the germination tests, damaged and insect-infected seeds were discarded, and the empty ones were eliminated using the method of floating in distilled water. Seeds were separated from fruits and soaked in water for 24 h. These seeds were sterilised with Benlate (1 g/l) for 20 min, then with sodium hypochlorite 50% for few minutes, and rinsed three times with distilled water.

Morphological traits

Some specific characteristics of *Retama* populations were chosen as descriptors. Three were qualitative traits: the length, the width, and the weight of the seeds. Thirty seeds for each *Retama* populations were analyzed.

Salinity and drought treatments

To determine the tolerance on the germination stage of each *Retama* population under salt stress and water stress, seeds were sown in a NaCl solution at different concentrations (0 (distilled water), 3, 6, 9, 12, and 15 g of NaCl

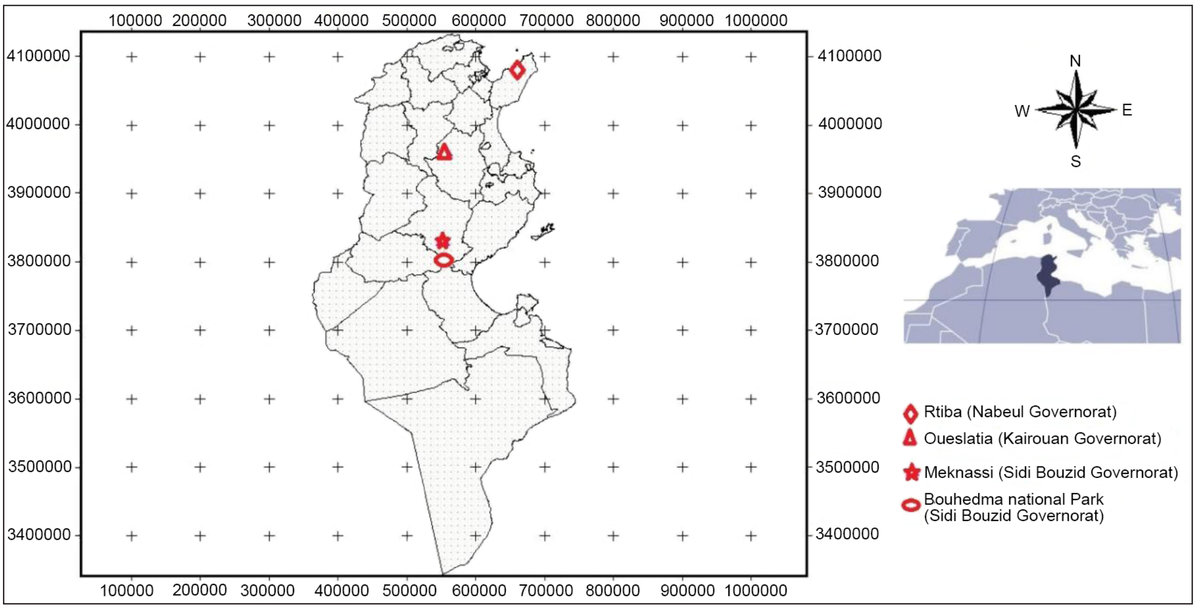

Fig. 1. Geographical location of the studied populations of *Retama reatam*

added to one litre of distilled water). The water stress treatments used were 0 (control), −0.03, −0.1, −0.7, −1 and −1.6 MPa of PEG_{6000} added to one litre of distilled water. Seeds were placed in sterile Petri dishes with two discs of filter paper saturated with distilled water for control, and NaCl solutions for treatments. NaCl solutions were renewed every 48 h under sterile conditions in order to avoid salt accumulation (Rahman et al., 2008). Five replicates of 20 seeds each were used for each treatment with 10 ml of test solution. Seeds were allowed to germinate in relative humidity of 80% at 25°C in complete darkness for 30 days (Maraghni et al., 2010). A seed was considered to have germinated when the emerging radicle elongated to 2 mm (Redondo-Gomez et al., 2007).

Methods of germination expression

The germination rate (GR) is an estimate of the viability of a population of seeds. The germination percentage was calculated using the equation below:

GR = seeds germinated/total seed × 100

The mean time of germination (MTG) and the parameters of the germination speed were also calculated for supplementary explanations. The MTG was calculated as follows:

$$MTG = \sum n_i \times d_i / n$$

where n is the total number of germinated seeds during the germination test, n_i is the number of germinated seeds on day d_i and i is the number of days during the germination period (between 0 and 30 days) (Yousheng & Sziklai 1985). Germination counts were performed daily for 30 days. The germination percentage (GP %) was evaluated daily and the final value was obtained after 30 days. The Kotowski's coefficient (CV) was calculated according to this method:

$$CV = \sum(n \times Jn)/\sum n$$

Correlation between morphological traits and germination properties

A correlation between morphological parameters and their germination rate, the average time of germination, and their velocity coefficient was determined.

Statistical analysis

Data were analysed using SPSS for Windows, version 11.5 (SPSS, 2002). A two-way analysis of variance (ANOVA) was carried out to test the effects of main factors (morphology and population/salinity and population/drought and population) and their interaction on the rate and the final percentage of germination. SNK test (Student, Newmen and Keuls) was used to estimate the least significant range between the means.

RESULTS

Morphological traits

For four *Retama* populations, the length varied from 4.08 to 6.42 mm, the weight varied from 5.16 to 8.77 g, and the width varied from 1.19 to 1.93 mm. Our results show that there is no significant variation of morphological traits of the four *Retama* seeds populations (Table 1 and Fig. 2). According to Table 2 and Fig. 3, population 2 has the longest seeds (4.65 mm) and population 1 has the maximum weight of seeds (1.68 g) and the maximum width of seeds (5.98 mm). In order to describe and gain a better understanding of variance sources among the studied *Retama* populations, principal component analysis (PCA) was carried out. A clear separation of the studied populations was observed and four main groups could be distinguished (Figs. 4, 5). The Euclidean distance assigned the four *Retama* populations into three groups (Fig. 6). The first group included population A (population 1), the second group included population B (population 2), and the third group was divided into two subgroups: the first subgroup included population C (population 3) and the second was represented by population D (population 4).

Table 1. **Descriptive analysis of seed morphology of four populations *of Retama raetam***

Variable	N	Minimum	Maximum	Mean	SD	SE (mean)	CV	F value	Pr(>F)	Signification
Length of seeds (mm)	120	4.085	6.416	4.589	0.261	0.023	0.056	1.721	0.167	NS
Weight of seeds (g)	120	5.165	8.768	1.650	0.117	0.010	0.071	1.998	0.118	NS
Width of seeds (mm)	120	1.190	1.934	5.824	0.465	0.042	0.079	1.813	0.149	NS

Significant difference from control at * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. NS = not significant ($P > 0.05$).

Table 2. **Descriptive analysis of seed morphology of the individuals of each studied population of *Retama raetam* (Population 1: Rtiba; Population 2: Oueslatia; Population 3: Meknassi; Population 4: Bouhedma National Park)**

Variable	Population	mean	SD	SE (mean)	CV	N
Length of seeds (mm)	Population 1 (Rtiba)	4.596	0.140	0.025	0.030	30
	Population 2 (Oueslatia)	4.655	0.221	0.040	0.047	30
	Population 3 (Meknassi)	4.505	0.412	0.075	0.091	30
	Population 4 (Bouhedma National Park)	4.602	0.171	0.031	0.037	30
Weight of seeds (g)	Population 1 (Rtiba)	1.683	0.157	0.028	0.093	30
	Population 2 (Oueslatia)	1.668	0.104	0.019	0.062	30
	Population 3 (Meknassi)	1.625	0.088	0.016	0.054	30
	Population 4 (Bouhedma National Park)	1.625	0.100	0.018	0.061	30
Width of seeds (mm)	Population 1 (Rtiba)	5.987	0.569	0.103	0.095	30
	Population 2 (Oueslatia)	5.813	0.215	0.039	0.036	30
	Population 3 (Meknassi)	5.760	0.666	0.121	0.115	30
	Population 4 (Bouhedma National Park)	5.737	0.184	0.033	0.032	30

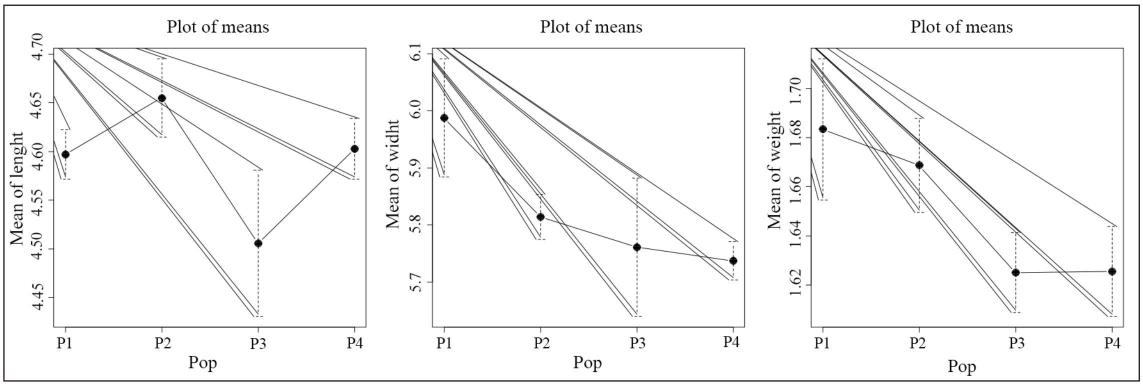

Fig. 2. Averages (±SD) of three morphological parameters of four populations (Pop) of *Retama reatam* (P1: Rtiba; P2: Oueslatia; P3: Meknassi; P4: Bouhedma National Park)

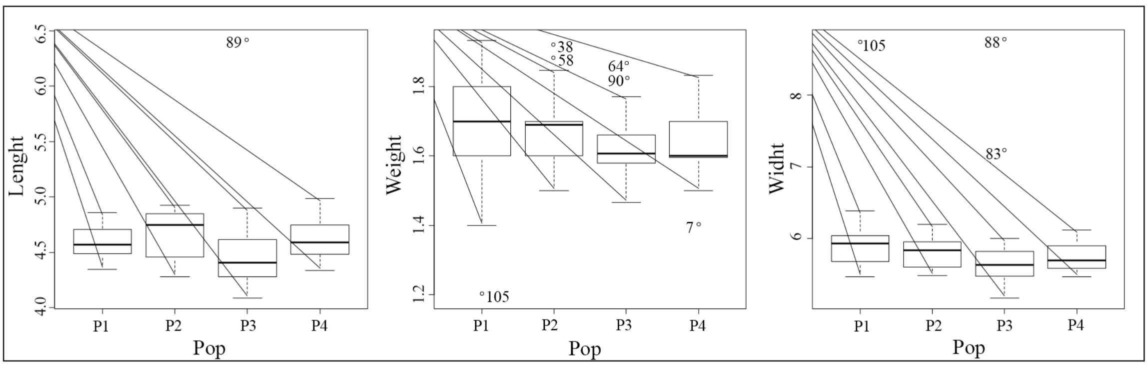

Fig. 3. Box dispersion of three morphological parameters of four populations (Pop) of *Retama reatam* (P1: Rtiba; P2: Oueslatia; P3: Meknassi; P4: Bouhedma National Park)

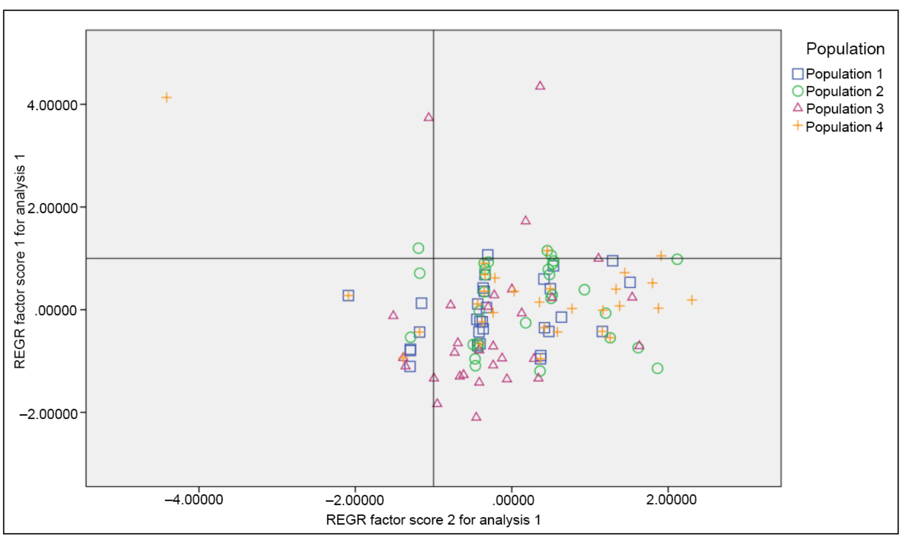

Fig. 4. Principal components analysis of seeds morphology of each studied population individuals of *Retama reatam* using the SPSS program (Population 1: Rtiba; Population 2: Oueslatia; Population 3: Meknassi, Population 4: Bouhedma National Park)

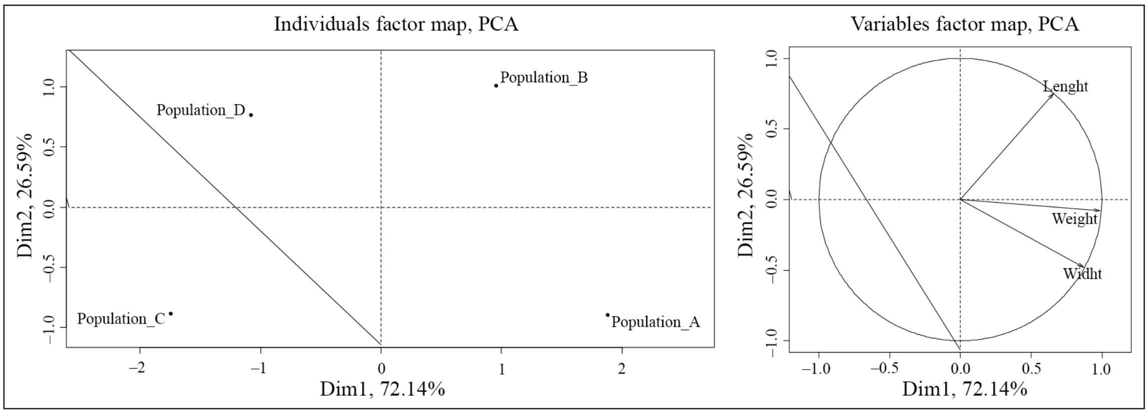

Fig. 5. Principal components analysis of *Retama reatam* populations using the R program; Individuals factor map and variables factor map (Population_A: Rtiba; Population_B: Oueslatia; Population_C: Meknassi, Population_D: Bouhedma National Park)

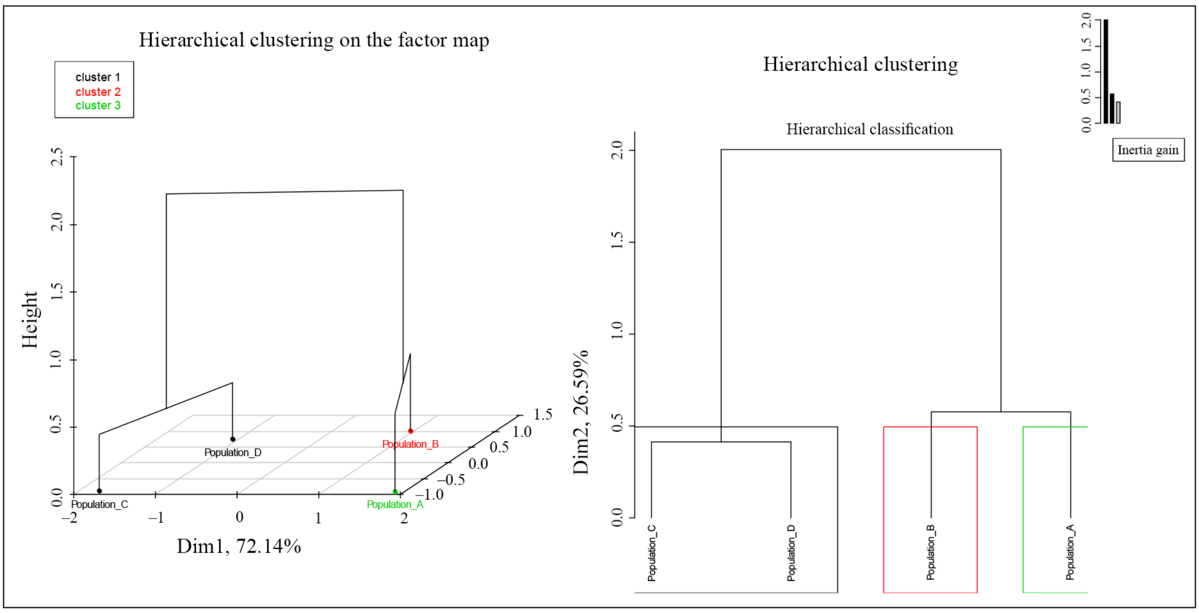

Fig. 6. Euclidean distance between the four *Retama reatam* population susing the R program (Population_A: Rtiba; Population_B: Oueslatia; Population_C: Meknassi, Population_D: Bouhedma National Park)

Effects of salt stress on seed germination

A two-way ANOVA indicated a significant interaction between population and salinity from Kotowski's coefficient (Table 3). The germination percentage varied significantly between the four *Retama* populations ($P < 0.0001$). Salinity had a significant effect on the germination percentage, Kotowski's coefficient and the mean time of germination ($P < 0.0001$). The effects of salinity constraint (NaCl treatment) on seed germination are reported in Table 4. The results of ANOVA showed that NaCl treatments had a significant effect on the germination percentage and mean germination time (Table 4). However, salinity significantly affected the germination percentage of Retama populations (Table 4). Germination in distilled water was the highest, it varied between 81% at 73%. However, it decreased significantly with increased NaCl concentrations (Table 4). Population 1 is the most resistant to salinity (42% of germination percentage at 15 g/l), population 4

Table 3. **A two-way ANOVA of the effects of salinity, population, and their interaction on germination characteristics of germination of four populations of *Retama reatam* seeds**

Source	Dependent Variable	F-value	P-value	Signification
Population	Germination percentage	20.489	**0.000**	***
	Kotowski's coefficient	0.906	0.441	NS
	Mean time of germination	2.706	0.050	NS
Concentration	Germination percentage	30.127	**0.000**	***
	Kotowski's coefficient	35.144	**0.000**	***
	Mean time of germination	26.403	**0.000**	***
Population * Concentration	Germination percentage	0.911	0.554	NS
	Kotowski's coefficient	1.832	0.**041**	**
	Mean time of germination	0.969	0.493	NS

Significant difference from control at * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ by SNK test. NS = not significant ($P > 0.05$).

Table 4. **Characteristic of germination of four populations of *Retama reatam* after their transfer from 0, 3, 6, 9, 12, and 15 g/l of salinity at 25°C. Data represented means ± SD. Different letters indicate significant differences between treatments (Salinity) at $P < 0.05$ according to the SNK test. (Population 1: Rtiba; Population 2: Oueslatia; Population 3: Meknassi; Population 4: Bouhedma National Park)**

Population	Characteristic of germination	Salinity (g/l)					
		0	3	6	9	12	15
Population 1 (Rtiba)	Germination percentage	81 ± 12.45^{c}	71 ± 14.47^{bc}	64 ± 8.21^{abc}	60 ± 15.41^{abc}	53 ± 15.24^{ab}	42 ± 20.18^{a}
	Kotowski's coefficient	13.41 ± 3.13^{d}	10.15 ± 0.77^{c}	8.86 ± 1.54^{bc}	5.85 ± 1.23^{ab}	7.24 ± 0.55^{ab}	5.94 ± 0.34^{a}
	Mean time of germination	7.92 ± 2.53^{a}	9.89 ± 0.76^{ab}	11.58 ± 2.15^{bc}	14.95 ± 2.62^{de}	13.87 ± 1.07^{cd}	16.87 ± 0.99^{e}
Population 2 (Oueslatia)	Germination percentage	86 ± 9.61^{b}	50 ± 16.95^{a}	42 ± 23.61^{a}	39 ± 25.43^{a}	29 ± 20.43^{a}	28 ± 27.74^{a}
	Kotowski's coefficient	14.66 ± 4.09^{b}	8.41 ± 2.07^{a}	7.57 ± 0.60^{a}	6.44 ± 1.33^{a}	7.03 ± 2.08^{a}	5.18 ± 1.21^{a}
	Mean time of germination	7.25 ± 1.96^{a}	12.48 ± 3.09^{b}	13.26 ± 1.06^{b}	16.02 ± 3.03^{bc}	15.46 ± 5.38^{bc}	20.19 ± 4.92^{c}
Population 3 (Meknassi)	Germination percentage	73 ± 15.24^{b}	47 ± 25.64^{a}	45 ± 17.32^{a}	30 ± 10.60^{a}	27 ± 18.23^{a}	19 ± 11.93^{a}
	Kotowski's coefficient	18.32 ± 5.35^{b}	8.97 ± 3.23^{a}	8.32 ± 2.27^{a}	6.06 ± 1.57^{a}	4.70 ± 0.84^{a}	4.45 ± 0.57^{a}
	Mean time of germination	5.84 ± 1.56^{a}	12.88 ± 6.31^{b}	12.58 ± 2.66^{b}	17.24 ± 3.59^{bc}	21.78 ± 3.54^{c}	22.76 ± 2.94^{c}
Population 4 (Bouhedma National Park)	Germination percentage	75 ± 5.00^{d}	47 ± 4.47^{c}	23 ± 11.51^{b}	17 ± 10.36^{ab}	10 ± 7.07^{a}	08 ± 4.47^{a}
	Kotowski's coefficient	13.93 ± 1.88^{b}	09.39 ± 3.67^{ab}	13.16 ± 5.58^{b}	8.24 ± 5.12^{ab}	5.69 ± 1.70^{a}	5.39 ± 1.87^{a}
	Mean time of germination	7.28 ± 0.98^{a}	12.71 ± 7.16^{ab}	9.14 ± 4.83^{ab}	15.90 ± 8.47^{ab}	18.75 ± 5.16^{b}	20.26 ± 6.62^{b}

is the most sensitive to salinity (8% of the germination percentage at 15 g/l). There was a strong negative relationship between germination and salinity. The index of the germination velocity calculated using Kotowski's coefficient showed that the rate decreased with an increase in salt concentration (Table 4). The germination speed expressed by the mean time of germination decreased with the decrease in NaCl potentials. The germination was significantly reduced by high NaCl levels and there were no great differences in the final germination percentage between 3 and 9 g/l, so the germination percentage was reduced with increasing NaCl to levels above 15 g/l (SNK test).

Correlation between morphological traits and germination properties

Table 5 shows that there is a correlation between the germination percentage and Kotowski's coefficient, the germination percentage and the mean time of germination, and between Kotowski's coefficient and the mean time of germination. As for the correlation between morphological traits and germination properties, we noticed a correlation between the weight of seeds and the germination percentage, and between the weight and the length of seeds.

Effects of the osmotic potential on seed germination

A two-way ANOVA of the germination percentage, Kotowski's coefficient and the mean time of germination indicated a significant effect of the osmotic potential concentrations and high interaction between the osmotic potential and population (Table 6). The germination characteristics varied significantly between *Retama* populations. The effects of water stress (PEG treatment) on seed germination are reported in Table 7. The osmotic potential significantly affected the percentage of germination of *Retama* populations (Table 7). The germination percentage reached 94% to 99% in control seeds (0 MPa). However, it decreased significantly with an increase of the osmotic potential (Table 7). Population 2 was the most resistant to the osmotic potential (29% of the germination percentage at –1.6 MPa), population 1 was the most sensitive one (10% of the germination percentage). The mean time of germination increased with increasing osmotic potential concentra-

Table 5. **Correlation between characteristic of germination and morphology of seeds of four populations of *Retama reatam* after their transfer of salinity**

Variable	Correlation	Germination percentage	Kotowski's coefficient	Mean time of germination	Length of seeds	Width of seeds	Weight of seeds
Germination percentage	Pearson Correlation	1	**0.528****	**–0.508****	–0.212	–0.064	**0.376***
	Sig. (2-tailed)		0.003	0.004	0.261	0.737	0.041
Kotowski's coefficient	Pearson Correlation	**0.528****	1	**–0.953****	–0.198	–0.233	–0.276
	Sig. (2-tailed)	0.003		0.000	0.295	0.216	0.139
Mean time of germination	Pearson Correlation	**–0.508****	**–0.953****	1	0.160	0.217	0.269
	Sig. (2-tailed)	0.004	0.000		0.399	0.250	0.151
Lenght of seeds	Pearson Correlation	–0.212	–0.198	0.160	1	0.180	**0.822****
	Sig. (2-tailed)	0.261	0.295	0.399		0.340	0.000
Widht of seeds	Pearson Correlation	–0.064	–0.233	0.217	0.180	1	0.225
	Sig. (2-tailed)	0.737	0.216	0.250	0.340		0.233
Weight of seeds	Pearson Correlation	**–0.376***	–0.276	0.269	**0.822****	0.225	1
	Sig. (2-tailed)	0.041	0.139	0.151	0.000	0.233	

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Table 6. **A two-way ANOVA of the effects of Osmotic potential, population, and their interaction on germination characteristics of *four population of Retama raetam***

Source	Dependent Variable	*F*-value	*P*-value	Signification
Population	Germination percentage	6.951	**0.000**	***
	Kotowski's coefficient	7.398	**0.000**	***
	Mean time of germination	15.855	**0.000**	***
Concentration	Germination percentage	116.391	**0.000**	***
	Kotowski's coefficient	13.313	**0.000**	***
	Mean time of germination	9.111	**0.000**	***
Population * concentration	Germination percentage	0.841	0.631	NS
	Kotowski's coefficient	4.767	**0.000**	***
	Mean time of germination	3.915	**0.000**	***

Significant difference from control at * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ by SNK test. NS = not significant ($P > 0.05$).

Table 7. **Characteristic of germination of four populations of Retama reatam after their transfer from 0, –0.03, –0.1, –0.7, –1 and –1.6 MPa of osmotic potential at 25°C. Data represent means ± SD. Different letters indicate significant differences between treatments (Osmotic potential) at P < 0.05 according to the SNK test. (Population 1: Rtiba; Population 2: Oueslatia; Population 3: Meknassi; Population 4: Bouhedma National Park)**

Population	Characteristic of germination	Osmotic potential (MPa)					
		0	–0.03	–0.1	–0.7	–1	–1.6
Population 1 (Rtiba)	Germination percentage	96 ± 4.18^{c}	46 ± 17.10^{b}	27 ± 12.04^{a}	24 ± 8.21^{a}	$14 \pm 4{,}18^{a}$	10 ± 6.18^{a}
	Kotowski's coefficient	9.10 ± 1.62^{b}	7.27 ± 1.23^{b}	8.21 ± 8.48^{b}	7.6 ± 1.48^{b}	7.81 ± 2.29^{b}	14.35 ± 6.26^{c}
	Mean time of germination	11.29 ± 2.2^{a}	14.05 ± 2.29^{a}	13.30 ± 4.18^{a}	13.66 ± 3.37^{a}	14.05 ± 5.50^{a}	17.85 ± 2.66^{a}
Population 2 (Oueslatia)	Germination percentage	99 ± 2.23^{b}	52 ± 17.88^{a}	50 ± 19.68a	33 ± 7.58^{a}	32 ± 17.17^{a}	29 ± 15.57^{a}
	Kotowski's coefficient	20.27 ± 4.33^{b}	8.58 ± 1.28^{b}	6.92 ± 1.01^{b}	6.30 ± 1.40^{b}	6.33 ± 1.44^{b}	5.94 ± 0.33^{c}
	Mean time of germination	5.10 ± 1.01^{a}	11.86 ± 1.72^{b}	14.69 ± 2.20^{bc}	16.41 ± 3.23^{c}	16.35 ± 3.19^{c}	16.87 ± 1.03^{c}
Population 3 (Meknassi)	Germination percentage	96 ± 2.23^{b}	41 ± 15.57^{a}	33 ± 15.24a	33 ± 12.55^{a}	30 ± 07.07^{a}	21 ± 04.18^{a}
	Kotowski's coefficient	17.08 ± 5.65^{b}	11.15 ± 2.63^{ab}	11.15 ± 2.56^{ab}	9.47 ± 1.15^{b}	11.52 ± 4.77^{ab}	8.69 ± 3.78^{b}
	Mean time of germination	6.38 ± 2.02^{a}	9.42 ± 2.44^{ab}	9.50 ± 2.71^{ab}	10.77 ± 1.77^{ab}	09.75 ± 3.47^{ab}	12.90 ± 4.20^{b}
Population 4 (Bouhedma National Park)	Germination percentage	94 ± 4.18^{a}	37 ± 09^{b}	30 ± 16.95^{b}	27 ± 8.36^{b}	25 ± 11.72^{b}	22 ± 05.70^{b}
	Kotowski's coefficient	15.23 ± 2.54^{a}	10.50 ± 2.61^{a}	9.98 ± 1.91^{a}	12.17 ± 4.80^{a}	13.60 ± 1.92^{a}	9.64 ± 3.29^{a}
	Mean time of germination	6.72 ± 1.22^{a}	10.00 ± 2.46^{a}	10.33 ± 2.12^{a}	09.35 ± 3.85^{a}	07.46 ± 1.04^{a}	11.22 ± 3.21^{a}

tions (Table 7). There was a strong negative relationship between the germination percentage and the osmotic potential. The index of the germination velocity calculated using Kotowski's coefficient showed that the rate decreased with an increase in osmotic potential concentrations.

Correlation between morphological traits and germination properties

Table 8 shows that there was a correlation between the germination percentage and Kotowski's coefficient, the germination percentage and the mean time of germination, and between Kotowski's coefficient and the mean time of germination. As regards the correlation between morphological traits and germination properties, we noticed a correlation between the weight of seeds and the germination percentage.

DISCUSSION

Morphological traits

In this study, we used morphological traits to assess the variation among four Tunisian *Retama* populations. For all traits analysed, significant differences between the studied populations were found. In fact, a substantial variation and important heterogeneity between these populations were observed for qualitative traits. Furthermore, this study showed that the qualitative morphological characters differed significantly among the four *Retama* populations and that they were influenced by environmental factors. Principal component analysis for qualitative morphological characteristics of these populations showed that a reduced number of descriptors could be used efficiently to discriminate among them. Diallo et al., (2008), in a study of the change in biometric characteristics of seeds and seedlings of nine provenances of *Tamarindus indica* L. (Caesalpinioideae) proposed that differences between variables were highly significant. In the seed study, Diallo et al., (2008) obtained a group linked to Indian and East African provenances, another linked to West African origins, and a middle group from Thailand. The dendrogram obtained from phenotypic similarities (seed and seedling variables) shows a structure by geographical area. The phenotypic

Table 8. **Correlation between characteristics of germination and morphology of seeds of four populations of *Retama* after their transfer of osmotic potential**

Variable	Correlation	Germination percentage	Kotowski's coefficient	Mean time of germination	Length of seeds	Width of seeds	Weight of seeds
Germination percentage	Pearson Correlation	1	0.391**	−0.350**	0.072	−0.023	0.186*
	Sig. (2-tailed)		0.000	0.000	0.432	0.803	0.042
Kotowski's coefficient	Pearson Correlation	0.391**	1	−0.895**	0.043	0.119	0.018
	Sig. (2-tailed)	0.000		0.000	0.644	0.197	0.847
Mean time of germination	Pearson Correlation	−0.350**	−0.895**	1	−0.006	−0.139	−0.053
	Sig. (2-tailed)	0.000	0.000		0.948	0.131	0.566
Length of seeds	Pearson Correlation	0.072	0.043	−0.006	1	0.283**	0.008
	Sig. (2-tailed)	0.432	0.644	0.948		0.002	0.931
Width of seeds	Pearson Correlation	−0.023	0.119	−0.139	0.283**	1	−0.053
	Sig. (2-tailed)	0.803	0.197	0.131	0.002		0.564
Weight of seeds	Pearson Correlation	0.186*	0.018	−0.053	0.008	−0.053	1
	Sig. (2-tailed)	0.042	0.847	0.566	0.931	0.564	

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

inter-provenance variability of seedlings and seeds combined with the correlations between variables could be used to select early provenances according to the objectives defined for the selection (Diallo et al., 2008). Kouyaté, Van Damme (2002), showed that there was variability between *Detarium microcarpum* populations, which related to the characteristics measured on the fruit, the seed and the leaf. Three shapes of mature fruit and one shape of seed were identified. Results obtained do not make it possible to confirm the existence of different varieties within the species *Detarium microcarpum* (Kouyaté, Van Damme, 2002). Kouyaté et al. (2011) showed that there was significant variability which was observed according to the provenance of the baobab tree (*Adansonia digitata* L.) in Mali. The discriminating morphological descriptors for the baobab in Mali are the fruit length and width, the fruit peduncle length, the leaf length and width, and the number of lobes. Assogbadjo et al., 2005, showed that the morphometric variables made it possible not only to make a rather precise typology of various forms of baobab tree capsules (*Adansonia digitata* L.) in Benin, but also to estimate their production starting from predictive models. The variability of the baobab capsule production in various climatic zones could be a useful parameter for genetic improvement of the species answering the needs and the means of the rural populations. The seed number and the total seed mass were the best fruit characteristics for the prediction of the tree type (Cooper et al., 2003). Seed weights of *Metrosideros excelsa* differed between trees within categories (filled and unfilled seeds). Fertile seeds had a lower length/width ratio than unfilled seeds. Measurements of length and width varied less than their allometric ratio (Gabriele et al., 2002). Components analyses show that some populations are opposed to others in relation to some characteristics of pods and/or seeds. In the case of the weakest values (pods and seeds), the populations come from the regions of high altitude and/or with small rainfall; and on the contrary, populations with large seeds and pods come from low regions and/or with high rainfall (Senhandri Maamri et al., 2000). Therefore, the morphological approach continues to be the initial step for the classification of many plants: for example, the olive (Rotondi et al., 2003), the cotton (Campbell et al., 2009), wheat (Pagnotta et al., 2009). Lahoz et al. (2011), showed that the cluster analysis is an efficient method for grouping cultivated cultivars, facilitating effective management. Indeed, the analysed Tunisian *Retama* populations fell into different clusters according to the characterization method used. In conclusion, this paper represents the first study of the genetic diversity of Tunisian *Retama* populations. This genetic diversity will be furthermore highlighted using molecular markers, although, the morphological descriptors of *Retama* must be completed by a molecular analysis using RAPD, SSR, or AFLP to understand the genetic organisation of this species in Tunisia.

Salinity and drought treatments

Salinity stress can affect seed germination through osmotic effects (Welbaum et al., 1990) or by ion toxicity (Huang and Reddman, 1995). In this context, the term "salt stress" during seed germination is used only to refer to situations where the seeds germinate rapidly under salt stress conditions. No distinction is made between osmotic and ionic effects of salinity stress (Bayuelo-Jiménez et al., 2002). Our study indicated that the effect of salt stress on germination was weakly pronounced in the case of the castor bean for the four *Retama* populations studied and compared to control. The highest germination of *Retama raetam* was obtained in distilled water. Increasing the concentration of NaCl significantly reduced the germination percentage. The results obtained in the present experiments corroborate several other studies (Jaouadi et al., 2010; Makhlouf et al., 2015). These results are consistent with those obtained in such other species as *Reaumuria vermiculata* (Gorai & Neffati, 2007), *Diplotaxis harra* (Tlig et al., 2008). In Tunisia, salinity affects large areas mainly in central and south regions where the arid climate increases the proliferation of these territories. The effect of salinity on germination may be explained by osmotic and/or toxic effects (Song et al., 2005; Tlig et al., 2008). Seeds of some species are reported to tolerate high salinity during the period when they are dormant in the soil and subsequently germinate when soil salinities are reduced (Khan, Ungar, 1997). Our data showed that seeds of *Retama reatam* behaved in two characteristic ways according to salinity. First, germination was reduced, indicating that germination was inhibited by salt. Second, seeds showed the phenomenon of "salt stimulation". *Retama reatam* seeds have the ability to tolerate moderate salinity and osmotic potential, faster and higher germination was recorded. This can be attributed to both ionic and osmotic effects (Song et al., 2005; Gorai, Neffati, 2007; Tlig et al., 2008). High NaCl significantly affected germination patterns. Although higher salinity generally decreases germination, the detrimental effect of salinity is less severe at the optimum germination osmotic potential. Salt stress decreased both the rate and the percentage of germination of *Retama reatam*. This result corroborates several other studies revealing that halophytes, as glycophytes, are sensitive to salt during the germination stage (Ungar, 1995; Katembe et al., 1998; Khan et al., 2002; Gorai, Neffati, 2007; Gorai et al., 2011). In saline and dry soils, water potential is not very different to that of desiccated seeds. Therefore, at low osmotic potentials water does not enter the seeds and induce germination. Desert shrubs vary in their ability to germinate in the presence of moisture stress. In our experiments, it should be noted that moisture stress was tested at the most suitable temperature found (25°C). Results showed that provenance 2 had a higher germination percentage of the stressed seeds at –0.03 MPa compared with non-stressed seeds. Seed germination percentage and Kotowski's coefficient generally decrease as soil water potential decreases (Evans, Etherington, 1990; Oberbauer, Miller, 1982), either by drought or by higher salinity. By increasing water stress, similar results were found on germination of *Diospyros texana* seeds that decreased from about 95% to 45% at 0 and –0.6 MPa, respectively (Everitt, 1984), whereas germination of three deciduous semi-shrubs of genus Artemisia was inhibited severely in PEG_{6000} solutions at –1.2 MPa (Tobe et al., 2006). Seed imbibition rate, germination percentage and germination rate generally decrease as soil water potential decreases (Song et al., 2005), either by drought or by higher salinity. Our data show that an increase in osmolality of PEG_{6000} solutions results in decreasing both the percentage and the rate of germination in *Retama* populations, indicating that water stress inhibits germination. At low osmotic potentials, water does not induce germination. This was in agreement with the germination behaviour of most species (Tobe et al., 2006; Gorai et al., 2009; Maraghni et al., 2010). From the present study it can be concluded that *Retama* populations have the ability to tolerate salt stress and recover after exposure to NaCl solutions and osmotic potential concentrations. Further investigations are necessary to understand the early establishment of this species under field conditions and to determine if there are differences between the seed germination stage and early seedling growth in their responses to salinity and drought stresses. In our study, we showed a strong correlation between the seed weight and the germination percentage of *Retama* populations under salt stress and water stress. In this direction, Ndiaya et al., 2014, find that the most important for germination are seeds of large caliber, while small and medium sizes have low percentages of germination, therefore saline inhibition concerns germination of seeds of small and medium sizes more. Salinity inhibits seed germination of *Gossypium hirsutum* and the seed size improves the germination ability facing salt stress. It would be more useful to use the larger caliber of seeds in a saltwater environment (Ndiaya et al., 2014).

CONCLUSIONS

This work was performed as part of the evaluation of the behaviour of *Retama raetam* populations under water and salt stress in the germination stage. The morphological parameters were measured to identify a relationship between the size of the seeds and their germination capacity under salinity and osmotic stress. The results showed that the seed weight is the major factor in promoting germination under stress and confirm that saline water and sprouting inhibition have impact on the smaller caliber of seeds. Also, the results of our study show that salt and osmotic stress affected seed germination of *Retama raetam* populations by decreasing the germination percentage and the average time for germination and Kotowski's coefficient.

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Conflict of interest

The authors declare that they have no conflict of interest.

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VEIKSNIAI, TURINTYS ĮTAKOS PAŠARINIŲ AUGALŲ (*RETAMA RAETAM* SUBSP. *BOVEI* (FABACEAE) SĖKLŲ DAIGUMUI: INTERAKTYVUS POVEIKIS VAISIŲ MORFOLOGIJAI DRUSKINGUMO IR OSMOSINIO STRESO SĄLYGOMIS

Santrauka

Retama gentis priklauso pupinių (Fabaceae) šeimai. *Retama raetam* rūšies augalai daugiausia auga kopose ir dykumose; čia jie pakenčia ypač didelę sausrą, todėl dažnai yra naudojami kopoms stabilizuoti ir dykumoms atkurti. Šio tyrimo metu buvo analizuojama keturių augalo *Retama raetam* populiacijų sėklų morfologija, druskingumo ir osmosinio streso poveikis daigumui Tunise. Sėklos buvo surinktos iš Bouhedma nacionalinio parko Meknassi, Oueslatia ir Rtiba populiacijų. Rezultatai rodo, kad ši rūšis gali pakęsti iki 15 g/l druskingumą (Rtiba populiacijos daigumas siekė 42 %) ir toleruoti didelę sausrą PEG_{6000} (Oueslatia populiacijos daigumas siekė 29 % esant −1,6 MPa vandens potencialui). Buvo išmatuotos sėklų morfologinės savybės siekiant nustatyti ryšį tarp sėklų dydžio ir jų daigumo druskingumo ir osmosinio streso sąlygomis. Rezultatai atskleidė koreliaciją tarp sėklų morfologinių savybių ir daigumo procento. Sėklų svoris yra pagrindinis daigumą skatinantis veiksnys streso metu, taip pat patvirtina, kad sėklų dydis priklauso nuo sūraus vandens ir ūglių augimo slopinimo.

Raktažodžiai: *Retama raetam*, populiacija, morfologinės savybės, druskingumo stresas, osmosinis stresas