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Pastoral plants for rehabilitation of degraded soil in Tunisia: the case for use of *Calicotome villosa* and *Genista spachiana* (Fabaceae)

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Calicotome villosa and *Genista spachiana* are classified as vulnerable in Tunisia and they are of great interest for the rehabilitation of degraded ecosystems, food for goats and camelids, and for the use of its essential oils in phytotherapy. The aim of this study was to examine the germination characteristics of both species through analysis of the effects of the salt stress and water stress on germination. We monitored and evaluated different germination characteristics (germination percentage, mean time to germination and germination speed) in the presence of the salt and water stress. *Calicotome villosa* and *Genista spachiana* can withstand salinity of up to 15 g/l of salt (31–37% germination at 15 g/l) and also tolerate large doses of PEG_{6000} (30–40% germination at –1.6 MPa: Polyethylene glycol (PEG) is a hydrophilic polymer). The tolerance of the two species to salinity and water stress allows them to be a source of food for goats and camelids during drought.

Keywords: *Calicotome villosa*, *Genista spachiana*, arid land forage, salt stress, water stress

INTRODUCTION

Tunisia is among the countries most seriously affected by desertification. Accounting for three quarters of the country (Le Houérou, 1959), southern Tunisia is subdivided into: (i) the arid zone, which covers 5.5×10^4 km^2 with average annual precipitations between 100 and 350 mm, and (ii) the desert zone, which occupies an area of about 6.5×10^4 km^2 with an annual average rainfall less than 100 mm (PNUD/FAO, 1979). One of the promising options for restoration of decertified regions in southern Tunisia is to use native shrub and tree species that have multiple functions in the ecosystem. Several species of the legume family (Fabaceae) are of high interest due to their adaptation to arid and semi-arid environments, nitrogen fixing capacity, and ability to grow in poor soils (Ibanez, Passera, 1997). Legumes are of great importance worldwide, especially in countries with a Mediterranean climate like Tunisia, since they contribute to soil fertility and prevention of soil erosion. Several native species of legumes are potentially useful for rehabilitation of degraded ecosystems, food for goats and camelids, and dune stabilization and vegetation. *Calicotome villosa* is very common in the Mediterranean area (Gibbs, 1968; Tutin, 1972). It is a tall 50–150 cm spiny shrub that produces yellow flowers in spring. It grows especially in North Africa and Spain (Greuter et al., 1989). The species has

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intricate, angular, pubescent branches that are green when young and become greyish when mature. It has alternate leaves: the lower leaves are elongate, oval, trifoliate, and covered with sericeous down. The flowers are yellow and have a bell-shaped calyx, thickly downy legume with a protuberant upper rib, and round dark olivaceous seeds. *Genista spachiana* is a shrub that reaches 1–1.5 m and is found in depressions of deep sandy soil. This species is a dominant perennial shrub in active sand dunes and stabilized sand fields in the southern arid zone of Tunisia.

Successful establishment of plants largely depends on successful germination. Germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time (Camarada, Valsecchi, 1983). Several environmental factors including temperature, salinity, light, and soil moisture simultaneously influence germination (El-Keblawy and Al-Rawai, 2005, 2006; Huang et al., 2003; Ungar, 1995; Zia, Khan, 2004). Seed germination behaviour in relation to thermal and salt stress is a very important determinant of the colonization capacity of a species (Ungar, 1982, 1995). Tolerance to salinity during germination is critical for the establishment of plants growing in saline soil of arid regions (Khan, Gulzar, 2003; Ungar, 1995). Increased salinity leads to a reduction and/or delay in germination of seeds of both halophytes and glycophytes. Failure of germination in saline soils often is a result of high concentrations of salts in the seed-planting zone because of the upward movement of the soil solution and subsequent evaporation at the soil surface. Seed germination under saline conditions occurs after high precipitation, when soil salinity is usually reduced due to leaching and dilution (El-Keblawy, 2004; Huang et al., 2003; Khan, Ungar, 1996; Redondo et al., 2004). Although salinity and osmotic stress of arid environments decrease germination, the detrimental effect of salinity and osmotic stress are generally less severe at optimum germination temperature (Gorai, Neffati, 2007; Tlig et al., 2008; Gorai et al., 2009; Maraghni et al., 2010).

This study was conducted to better understand seed germination requirements of *Calicotome villosa* and *Genista spachianna*. The effects of a wide range of salinity and drought levels on the germination percentage, speed rate (velocity), and mean time to germination of three provenances of each species were studied to determine their individual effect and the interaction between these factors on germination.

MATERIALS AND METHODS

Plant Material

Seeds of *Calicotome villosa* were collected in 2013 from two natural habitats, namely Meknassi and Bouhedma, while seeds of *Genista spachianna* were collected from Rtiba. Seeds of the three provenances differ in morphology (Table 1). Before the germination tests, damaged and insect-infected seeds were discarded, and the empty ones were eliminated using floatation 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 and then with 50% sodium hypochlorite for a few minutes and rinsed three times with distilled water.

Salinity and drought treatments

To determine the tolerance of germination under the salt stress, seeds were sown in NaCl solution at different concentration: 0 (distilled water), 3, 6, 9, 12, and 15 g of NaCl 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 obtained by adding PEG_{6000} 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 with 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).

Table 1. **Morphological diversity of seeds and climate variability of the three provenances**

Seeds	Bioclimatic	Annual rainfall (mm)	Temperature (°C)	Type of soil	Seed morphology
Seeds of *Calicotome villosa* of Meknessi site (north of Saharan Atlas chain)	arid	200–350	25–30 °C in summer and 5 to 10 °C in winter	Calcareous soil	
Seeds of *Calicotome villosa* of Bouhedma site (south of Saharan Atlas chain)	arid	100–200	32–36 °C in summer and 4 to 7 °C in winter	Sandy soil	
Seeds of *Genista spachiana* of Rtiba site (north of Dorsal chain).	subhumid	500–700	15–25 °C in summer and 2 to 5 °C in winter	Sandy soil	

Methods of germination expression

Mean time to germination (MTG) calculated as follows:

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

where "n" is the total number of germinated seeds during the 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. Cumulative germination percentage (GP %) was evaluated daily and the final value was obtained after 30 days and Kotowski's coefficient (CV) was calculated according to this method:

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

where "n" is the total number of germinated seeds during the test, "Jn" is the number of days during the germination period (between 0 and 30 days).

Statistical analysis

Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance. 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 (effect of salinity on the provenance and effect of drought on the provenance) and their interaction on the final germination percentage. Tukey HSD test was used to estimate significant differences between means.

RESULTS

Effects of salt stress on seed germination

Salinity significantly ($P < 0.0001$) affected the percentage of germination of *Calicotome villosa* and *Genista spachianna* (Table 2).

Table 2. **Mean germination percentage of *Calicotome villosa* and *Genista spachianna* seeds after transfer from 0, 3, 6, 9, 12, and 15 g/l NaCl at 25 °C. Data are means ± SE. Different letters indicate significant differences between treatments (NaCl) at *P* < 0.05 according to the Tukey test**

Species	NaCl (g/l)					
	0	3	6	9	12	15
Calicotome villosa of Meknassi	75 ± 10.840[a]	60 ± 3.162[ab]	49 ± 7.649[ab]	50 ± 6.124[ab]	47 ± 2.550[ab]	32 ± 7.517[b]
Calicotome villosa of Bouhedma	78 ± 13.748[a]	51 ± 8.276[ab]	48 ± 6.819[ab]	41 ± 5.339[b]	35 ± 4.743[b]	31 ± 7.969[b]
Genista spachianna of Rtiba	82 ± 15.133[a]	61 ± 7.969[ab]	60 ± 4.472[ab]	56 ± 6.595[ab]	42 ± 3.742[b]	37 ± 5.385[b]

[a, b, c] Values in the same row with the same superscript are not significantly different (*P* > 0.05).

Germination in distilled water was the highest. However, it decreased significantly with an increase in NaCl concentrations (Fig. 1). Seeds germinated rapidly in distilled water during the first five days; however, germination was delayed to eight days at concentrations greater than 3 g/l. The highest germination percentage was in distilled water followed by 3, 6, 9, 12 and 15 g/l NaCl. There was a strong negative relationship between germination and salinity. The germination velocity calculated using the Kotowski coefficient showed that the rate decreased with an increase in salinity (Fig. 2). The results of the ANOVA showed that the salt stress (NaCl treatments) had a significant effect (*P* < 0.001) on the germination percentage and on mean germination time (Table 3).

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, thus the germination percentage was reduced with increasing NaCl to levels above 12 g/l (Tukey's multiple test). A two-way ANOVA of the germination rate indicated a significant effect of salinity but not an interaction between species and salinity (Table 3).

Effects of osmotic potential on germination

Osmotic potential significantly (*P* < 0.001) affected the percentage of germination of *Calicotome villosa* and *Genista spachianna* (Table 4), which was highest in distilled water. However, the germination percentage decreased significantly with an increase in osmotic potential.

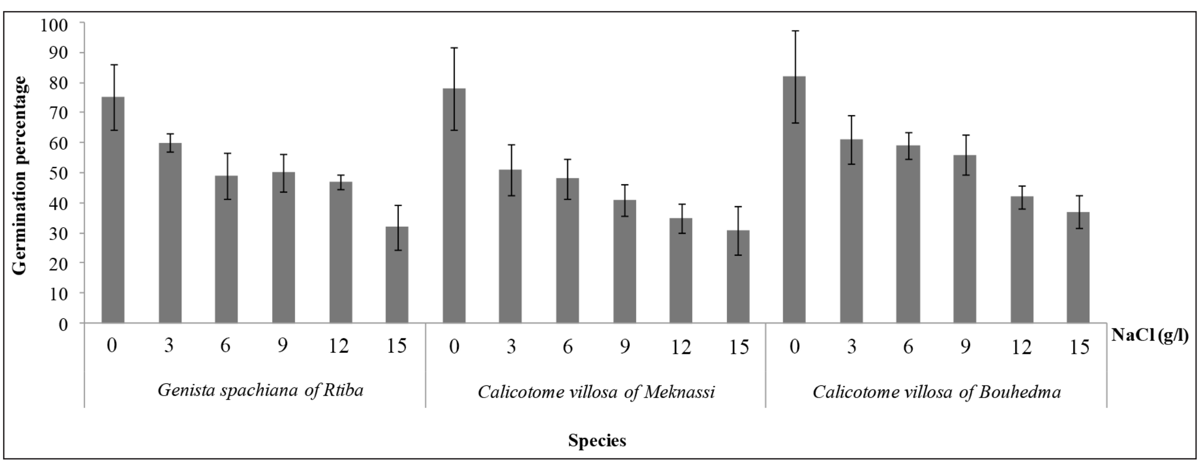

Fig. 1. Mean germination percentage of *Calicotome villosa* and *Genista spachianna* seeds in various NaCl concentrations (0, 3, 6, 9, 12, and 15 g/l) at 25 °C. Values (mean ± SEM)

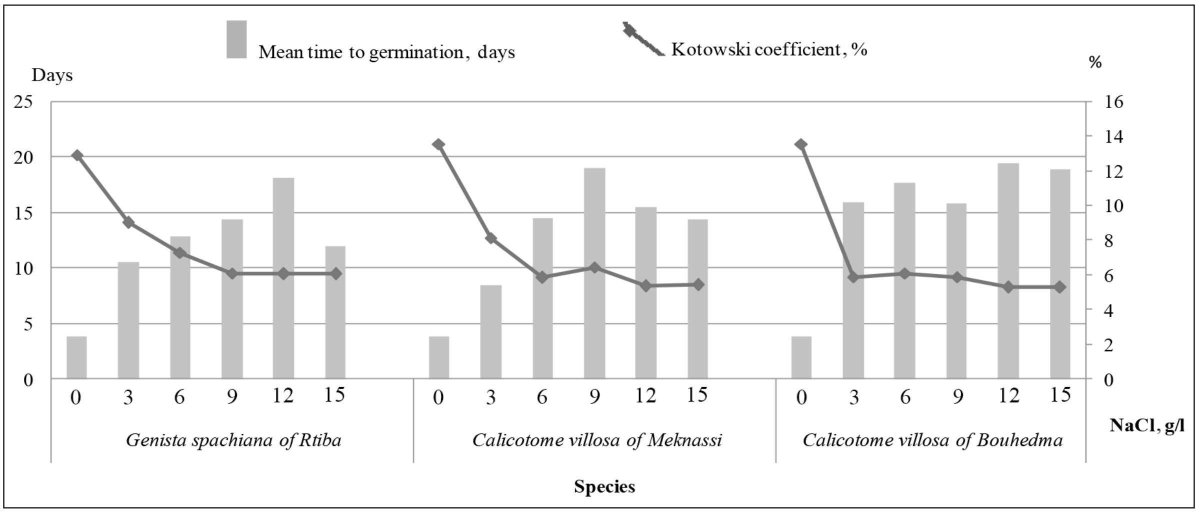

Fig. 2. Mean time to germination and Kotowski coefficient of *Calicotome villosa* and *Genista spachianna* seeds in various NaCl concentrations (0, 3, 6, 9, 12, and 15 g/l) at 25 °C

Table 3. **Two-way ANOVA of the effects of salinity (S), species (T), and their interaction on germination characteristics of *Calicotome villosa* and *Genista spachianna***

Variable	Characteristics of germination	*F*-value	*P*-value	Signification
Species	Germination percentage	1.985	0.145	NS
	Kotowski coefficient	0.591	0.557	NS
	Mean time to germination	4.976	0.009	**
Concentration	Germination percentage	11.673	**0.000**	***
	Kotowski coefficient	12.503	**0.000**	***
	Mean time to germination	29.097	**0.000**	***
Species * Concentration	Germination percentage	0.225	0.993	NS
	Kotowski coefficient	0.234	0.992	NS
	Mean time to germination	1.259	0.270	NS

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

Table 4. **Mean germination percentage of *Calicotome villosa* and *Genista spachianna* seeds after their transfer from 0, –0.03, –0.1, –0.7, –1, and –1.6 MPa at 25 °C. The data are means ± SE. Different letters indicate significant differences between treatments (osmotic potential) at *P* < 0.05 according to the Tukey's multiple test**

Species	Osmotic potential (MPa)					
	0	–0.03	–0.1	–0.7	–1	–1.6
Calicotome villosa of Bouhedma	78 ± 4.062^{a}	65 ± 10.840^{ab}	65 ± 8.944^{ab}	56 ± 3.674^{abc}	41 ± 9.407^{bc}	30 ± 4.743^{c}
Calicotome villosa of Meknassi	74 ± 4.848^{a}	79 ± 4.359^{a}	65 ± 6.892^{ab}	56 ± 6.595^{ab}	43 ± 8.746^{c}	38 ± 7.517^{c}
Genista spachianna of Rtiba	78 ± 4.062^{a}	65 ± 6.892^{ab}	55 ± 4.743^{ab}	48 ± 8.746^{ab}	42 ± 9.566^{c}	40 ± 9.618^{c}

$^{a, b, c}$ Values in the same row with the same superscript are not significantly different ($P > 0.05$).

Seeds germinated rapidly in distilled water during the initial first days; however, the start of germination was delayed for 10 days at concentrations than 3 g/l (Fig. 3).

Delay in germination increased with increasing osmotic potential (Fig. 4). The highest germination percentage was in distilled water followed by 0, –0.03, –0.1, –0.7, –1, and –1.6 MPa. However, at –0.03 MPa *Calicotome villosa* seeds from Meknassi had a germination rate higher than that at 0 MPa. There was a strong negative relationship between the germination percentage and the osmotic potential for both species. The Kotowski coefficient showed that the rate decreased with an increase in osmotic potential. For all three provenances to *Calicotome villosa* and *Genista spachianna*, the water stress (PEG) had a significant effect ($P < 0.001$) on the germination percentage, mean germination time (MGT) and the Kotowski coefficient (Table 4). The germination percentage and speed decreased with a decrease in water potential, this decrease was significant at lower –1 Mpa. The germination percentage for *Calicotome* seeds from Meknassi was the highest among all treatments (79 was at 0.03 MPa). Also, the germination percentage exceeded 50% at –1 MPa. A two-way ANOVA indicated a significant

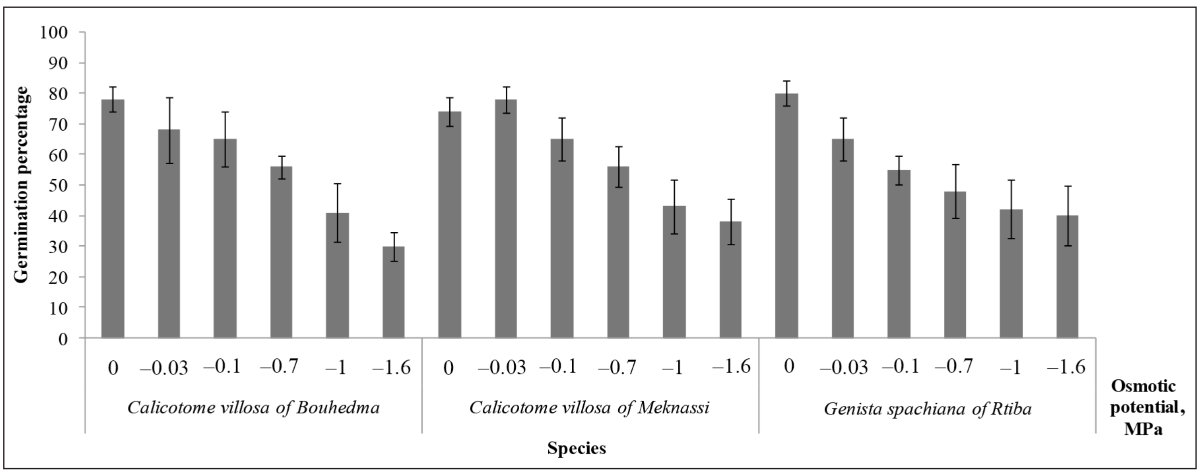

Fig. 3. Mean germination percentage of *Calicotome villosa* and *Genista spachianna* seeds in various osmotic potentials (0, –0.03, –0.1, –0.7, –1, and –1.6 MPa) at 25 °C. Values (mean ± SEM)

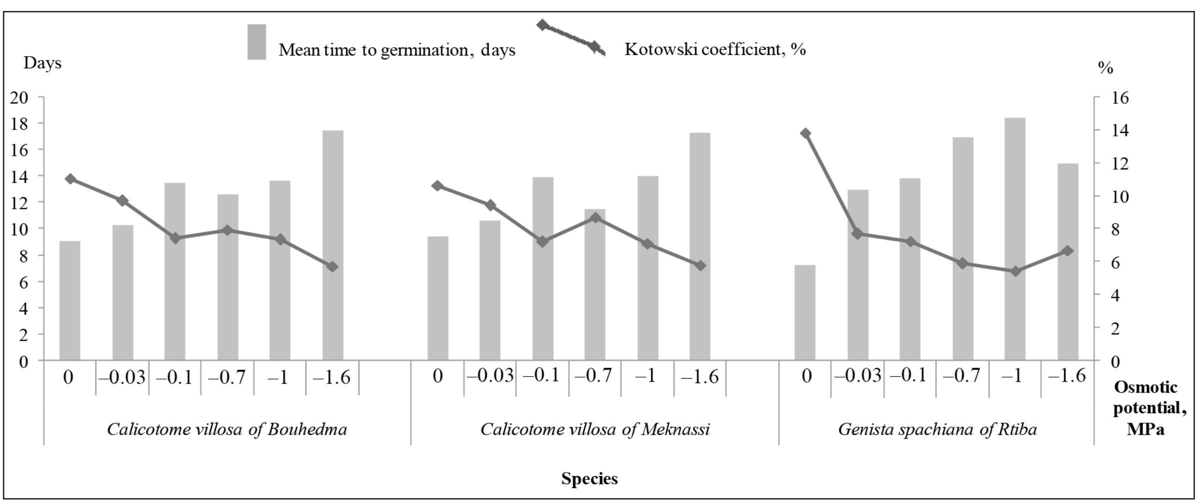

Fig. 4. Mean time to germination and Kotowski coefficient of *Calicotome villosa* and *Genista spachianna* seeds in various osmotic potentials (0, –0.3, –0.1, –0.7, –1, –1.6 MPa) at 25 °C

effect of osmotic potential on the germination rate, but not for the interaction between osmotic potential and species germination percentage (Table 5).

DISCUSSION

Salinity stress can affect seed germination through osmotic effects (Welbaum et al., 1990) and by ion-toxicity (Huang and Reddman, 1995). More than 50% of the seeds of *Calicotome villosa* and *Genista spachianna* germinated at the lowest salinity stress (9 g/l) and at the lowest water potential (–1 MPa). This suggested that this species can germinate under low water availability. Other study demonstrated that even the seeds of such desert species as *Ziziphus* lotus at a germination rate of 5% at –1 MPa. (Maraghni et al., 2010). Ibanez and Passera (1997) found that *Anthyllis cystoides* seeds germinated at –1.12 MPa (48%). Similar results were reported for other fabaceae species like *Acacia tortilis* which had no germinated seeds at –0.8 MPa (Jaouadi et al., 2010). This tolerance of water potential (–0.8 MPa) was also observed in other Fabaceae and desert species like *Retama raetam* (Youssef, 2009).

Generally, the salt stress affected the germination capacity and speed of *Calicotome* and *Genista* seeds, and these results agree with these of Lachiheb et al., (2004). In our study, seed germination percentage was higher in NaCl than in PEG at the same water potential. He et al. (2009), demonstrated that NaCl and PEG adversely affected germination, but NaCl had a less inhibitory effect on seed germination than an iso-osmotic solution of PEG. In contrast, Katembe et al. (1998) found that higher concentrations of NaCl (–1 MPa) were more inhibitory to germination of two Atriplex species (*A. halunus* and *A. numelaria*). Seeds of *Calicotome villosa* and *Genista spachianna* responded to salinity in two characteristic ways: first, germination was reduced, and second, at very low concentrations, germination was stimulated. Although higher salinity generally decreases germination, the detrimental effect of salinity is less severe at the optimum germination osmotic potential. The salt stress decreased both the rate and percentage of germination of *Calicotome villosa* and *Genista spachianna*, which agrees with several other studies revealing that halophytes, as well 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). Considering the percentage of seeds that germinated at –1 MPa and 12 g/l, we conclude that these two fabaceae species are well adapted to germinate under conditions of water and salt stresses. These abiotic stresses are typical of the environments in which they grow. The arid

Table 5. **A two-way ANOVA of the effects of Osmotic potential (S), Species (T), and their interaction on germination characteristics of *Calicotome villosa* and *Genista spachianna***

Variable	Characteristics of germination	F-value	P-value	Signification
Species	Germination percentage	0.570	0.568	NS
	Kotowski coefficient	3.782	0.027	*
	Mean time to germination	5.040	0.009	**
Concentration	Germination percentage	14.051	**0.000**	***
	Kotowski coefficient	6.330	**0.000**	***
	Mean time to germination	14.731	**0.000**	***
Species * Concentration	Germination percentage	0.436	0.924	NS
	Kotowski coefficient	0.766	0.661	NS
	Mean time to germination	2.340	0.019	*

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

lands of Tunisia are widely affected bydesertification caused particularly by the degradation of the vegetation cover, deforestation, and drought.

The high ability of *Calicotome villosa* and *Genista spachiana* to germinate over a wide range of environmental conditions provides an opportunity to contribute to future reforestation programmes. *Calicotome villosa* seeds from Meknassi had a higher rate of germination at –0.03 MPa than at 0 MPa compared with non-stressed seeds. Seed germination percentage and the Kotowski coefficient generally decreases as soil water potential decreases (Evans, Etherington, 1990; Oberbauer, Miller, 1982), either by drying or by higher salinity. By increasing water stress, similar results were found for germination of *Diospyros texana* seeds that decreased from about 95% at 0 MPa to 45% at –0.6 MPa (Everitt, 1984). On the other hand, germination of three deciduous semi-shrubs of Artemisia was inhibited severely in PEG_{6000} solutions at –1.2 MPa (Tobe et al., 2006). An increase in osmolality of PEG_{6000} solutions results in decreasing both the percentage and the rate of germination *Calicotome villosa* and *Genista spachianna*, indicating that the water stress inhibits germination, which is in agreement with the germination behaviour of most species (Tobe et al., 2006; Gorai et al., 2009; Maraghni et al., 2010). It can be concluded that seeds of *Calicotome villosa* and *Genista spachiana* have the ability to tolerate the salt stress 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 response to salinity and drought stress.

CONCLUSIONS

Our work demonstrated a relationship between germination properties of seeds and abiotic constraints in *Calicotome villosa* and *Genista spachiana*. *Genista spachiana* is most tolerant to the salt stress in a concentration of 12 g/l. The germination rate of two *Calicotome villosa* provenances and *Genista spachiana* decreases with increasing concentrations of PEG. The water stress affects the germination rate and increases the time required for seed germination of two *Calicotome villosa* and *Genista spachiana*. Even at high concentrations of PEG, seeds of the two species germinated to 30% to 40%. Thus, the species tolerate drought and harsh climatic conditions of the environment. The *Genista spachiana* provenance from Rtiba is more tolerant to water deficit than the *Calicotome villosa* provenance, with the germination rate of 40% at the water potential of –1.6 MPa.

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GANYKLINIŲ AUGALŲ NAUDOJIMAS ATKURIANT NUALINTĄ ŽEMĘ TUNISE: *CALICOTOME VILLOSA* IR *GENISTA SPACHIANA* (FABACEAE)

Santrauka

Calicotome villosa ir *Genista spachiana* yra klasifikuojami kaip pažeidžiami augalai Tunise. Jie yra labai svarbūs atkuriant nualintas ekosistemas, maitinant ožkų ir kupranugarinių šeimos gyvūnus, taip pat fitoterapijai naudojant eterinius aliejus. Buvo tiriamos abiejų rūšių daigumo savybės (daigumo procentas, vidutinis sudygimo laikas, dygimo greitis) druskingumo ir vandens stygiaus sukelto streso sąlygomis. *Calicotome villosa* ir *Genista spachiana* gali atlaikyti iki 15 g/l druskingumą (31–37 % daigumas esant 15 g/l druskos), taip pat toleruoja dideles PEG_{6000} dozes (30–40 % daigumas esant 1,6 MPa: polietilenglikolis (PEG) yra hidrofilinis polimeras). Šių dviejų augalų rūšių tolerancija druskingumo ir vandens stygiaus sukeltam stresui leidžia augalams būti ožkų ir kupranugarinių šeimos gyvūnų maisto šaltiniu sausros metu.

Raktažodžiai: *Calicotome villosa*, *Genista spachiana*, sausringos žemės pašaras, druskingumo ir vandens stygiaus sukeltas stresas