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Factors influencing seed germination of *Calycotome villosa* (Poir.) Link. (Fabaceae) from Southern Tunisia

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As typical arid vegetation, *Calycotome villosa* is an annual herb playing an important role in folk medicine. In this research, the influences of temperature and salinity on germination of *C. villosa* seeds gotten from southern Tunisia were investigated. The germination responses of the seeds in total darkness were determined over an extensive array of temperatures (5, 10, 15, 20, 25, 30, 35 and 40°C) as well as salinities (0, 50, 100, 150, 200, 250 and 300 mM NaCl). Seeds of *C. villosa* were able to germinate at temperatures between 5 and 40°C. Temperatures between 20 and 25°C seemed favorable to germinate these species. The minimum temperature beyond which no germination is expected was 0°C. Utmost germination percent were gotten in non-saline situations as well as arise in NaCl absorptions increasing repressed germination of seed. The rate of growth reduced with arises in salinity on all temperatures. Salt stress decreased both the percentage and the rate of germination. Percent of germination reduced with growing salinity and sternly restricted at 250 and 300 mM. Findings from this research might function as valuable information for *C. villosa* habitat establishment and renovation plans.

Key words: *Calycotome villosa*, germination, salinity, temperature, Tunisia.

INTRODUCTION

Calycotome villosa (Poir.) Linkis a shrub with spiny strong ramifications that are green when young and become greyish when mature (Pottier-Alapetite, 1981), in Tunisia, this species is known as “Gandoul”. It displays sharp spiny broom that substitute leaves; the lower leaves are elongate, oval and trifoliate. The flowers are yellow and grouped and have a bell-shaped calyx (Loy et al., 2001), flowering happens from March to April (Pottier- Alapetite, 1981). The scattering unit of this species is wavy pods comparatively large, winged inside including numerous seeds. Seeds of *C. villosa* are very smooth with a clear yellow color, a circular form, more or less flattened and having a hard tegument. The weight of 1000 seeds of *C. villosa* is 14.768 g (Neffati, 2008).

This species is very digestible by cattle (Ammar et al., 2005), and is weakly appreciated by camels (Chaieb and Boukhris 1998). However, *C. villosa* is relatively resilient to harsh environmental conditions, because of its strong spiny branches and profuse regeneration by seed (Arroyo et al., 2008). Recently the scientific communities

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became interested in seeds of *C. villosa* (Galié et al., 2015; Elkhamlichi et al., 2017). According to the same authors, similar to other Fabaceae family species, seeds of *C. villosa* contain great amounts of isoflavones, remarkably C-glycosylated isoflavones. The seeds of *Calycotome* species contain falcarinol having an important antibacterial activity (Loy et al., 2001). In the arid regions, *C. villosa* is distributed in places with precipitations varying from 100 to 200 mm (Sfax, Gabès, Gafsa, Médenine, Djerba, and Tataouine).

Seed germination, a key economic and ecological trait, is considered to be the most critical phase in the plant life cycle (Holdsworth et al., 2008; Rajjou et al., 2012). A number of ecological features for instance temperature, salinity, light, and soil moisture, concurrently impact sprouting (Ungar, 1995; Huang et al., 2003; El-Keblawy and Al-Rawai, 2005, 2006; Gorai and Neffati, 2007). Salinity and temperature are the main features influencing germination in the saline dry areas. They can interact in determining salinity tolerance during germination (Huang et al., 2003; El-Keblawy and Al-Rawai, 2005; Al-Khateeb, 2006; Zammouri and Neffati, 2010). Initial establishment of species in salt deserts is related to germination response of seeds to salinity and temperature and early establishment usually determines if a population will survive to maturity (Tobe et al., 2000; Huang et al., 2003; Song et al., 2005). Although higher salinity decreases germination, the detrimental effect of salinity is generally less severe at optimum germination temperature (Gorai et al., 2014).

The present study was carried out to determine the effect of temperature and salinity and their interactions on germination seeds of *C. villosa* under laboratory conditions and to determine the thermal time and minimum temperature for germination.

MATERIALS AND METHODS

Seed collection

C. villosa seeds were gathered in May 2003 from plants installed in the investigational field of Arid Land Institute (IRA Médenine, Tunisia: 9° 23'N, 37° 22'E). This region is arid to semi-arid with a typical Mediterranean climate, characterised by irregular rainfall events and a harsh dry summer period. This region is between arid and semi-arid with a characteristic Mediterranean climate, categorized with in even rainfall occasions as well as a tough dry summer period. Annual precipitation is 144 mm and mean evapotranspiration 1096 mm. Mean yearly temperature is 20.5°C, with the lowest temperature of 6.2°C in January and the extreme of 36.8°C in August (Gorai and Neffati, 2007). Five months old seeds sprout. Seeds were four months at the period of germination.

Germination experiments

To avoid fungus attack, the surface of the seeds were disinfected in 0.58% sodium hypochlorite solution for 1 min (Gulzar et al., 2001). The seeds were washed with distilled water and with air-dried before using them for germination experiments. 90 mm Petri dishes comprising two disks of Whatman No. 1 filter papers with 5 ml of test solution were arranged. The experiments were carried out in the dark, with incubators fixed at 5, 10, 15, 20, 25, 30, 35, and 40°C (Lumincube II, analys, Belgium; MLR-350, Sanyo, Japan). Under the optimum of germination and in dark treatments, seeds were germinated in 0, 50, 100, 150, 200, 250 and 300 mM NaCl solutions. For the germination tests, a complete randomized design was used.

For each treatment, four replicates of 25 seeds were used. The germinated seeds were counted up and removed detached every two days, for 16 days. A seed is considered germinated when the developing radicle elongates to 2 mm. Every two days, distilled water equal the mean water loss from dishes was added every two days to maintain the salt concentration close to the target levels all through the period of germination.

To assess the effect of temperature on germination rates, the reciprocal of time to 50% germination was calculated and regressed against temperature to predict the temperature at which the germination rate approaches zero (Figure 2). Based on this regression, the minimum temperature (beyond which no germination is expected) was 0°C (R^2=0.956).

Methods of germination expression

Five features of germination were determined: time to germination display (GD), first germination (TFG), mean time to germination (MTG) time to final germination (TGF) and final germination percentage (FGP). The estimation of MTG was consistent with the formula:

$$\mathrm{MTG} = \frac{\sum(n_i \times d_i)}{\mathrm{N}}$$

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 (Brenchley and Probert, 1998).

Statistical examination

The original data: the number of germinated seeds for each sample was changed to assure normal circulation and the homogeneous alterations. The alteration used was the arcsine of the square root of the fraction of germinated seeds for each sample (Sabin and Stafford, 1990; Sokal and Rohlf, 1995; Jozef et al., 2003). Non-parametric Mann–Whitney Test was conducted to compare the treatments with the control at 5 % level of significance ($P < 0.05$). Germination features data were open to two-way analysis of variance (ANOVA) and Tukey's technique was used for pair-wise contrast at a 5% level of significance ($P < 0.05$). SPSS software was used to conduct the statistical analysis (SPSS 12.5).

RESULTS

Optimal germination temperature

In responses to the tested constant temperatures, seeds of *C. villosa* were able to germinate at temperatures between 5 and 40°C. The maximum germination value (63 and 65%) occurred at two temperatures: 20 and 25°C respectively. Indeed, germination continues until a temperature of 40°C (22%) (Figure 1). The number of

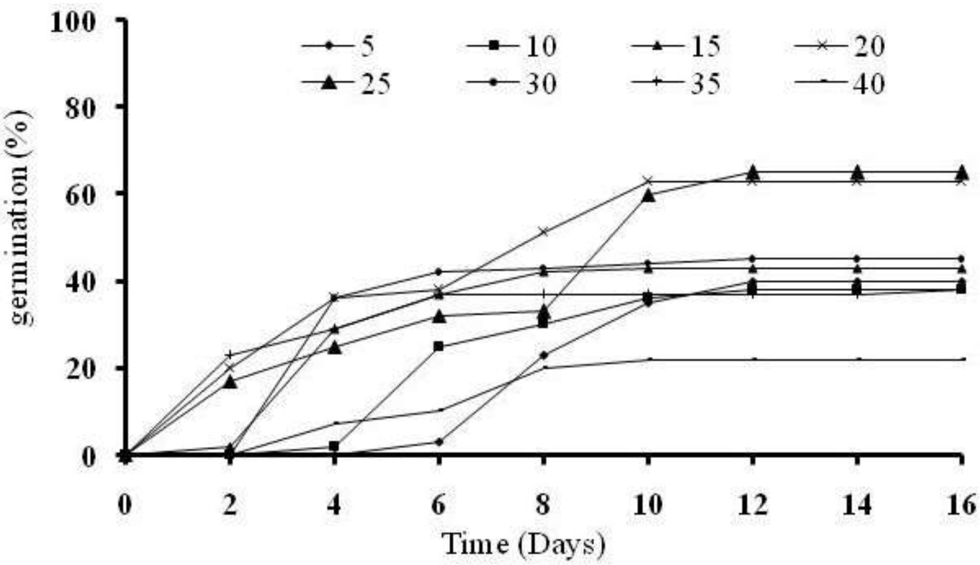

Figure 1. Cumulative germination percentage of *C. villosa seeds* during 16 days at different temperatures (n= 4 days).

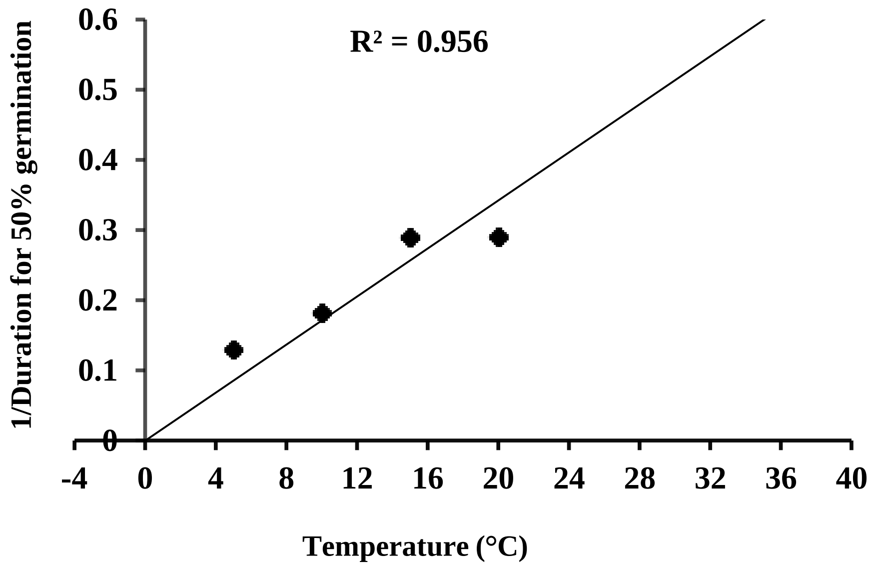

Figure 2. Relationship between temperature and the reciprocal of time to 50% germination of *C. villosa* seeds. The minimum temperature was estimated by setting *y*=0 in the linear regression equation and solving for *x*.

days to first germination (TFG) increased reduced with decreasing increasing temperature: 2 days at 20, 25 and 35°C and 7 days at 15°C were documented. The mean time to germination (MTG) reduced in the range of these temperatures. At 35°C, MTG was only 3.26 days and we recorded 7.13, 5.76 days at 25 and 20°C respectively (Table 1).

To appreciate the influence of temperature on the germination of *C. villosa* seeds, we proceeded to a classification of seeds according to their state at the end of the incubation. Four categories of seeds were identified: germinated seeds (GS), rotted seeds (RS), moistened and not germinated seeds (MNGS) and empty seeds (ES) (Figure 3). An important fraction of seeds did not germinate, and this fraction varied with temperature. At lower temperatures, we recorded 47, 60, and 39% at 5, 10, and 15°C, respectively. Above the thermal optimum (20 and 25°C), the fraction of seeds that did not

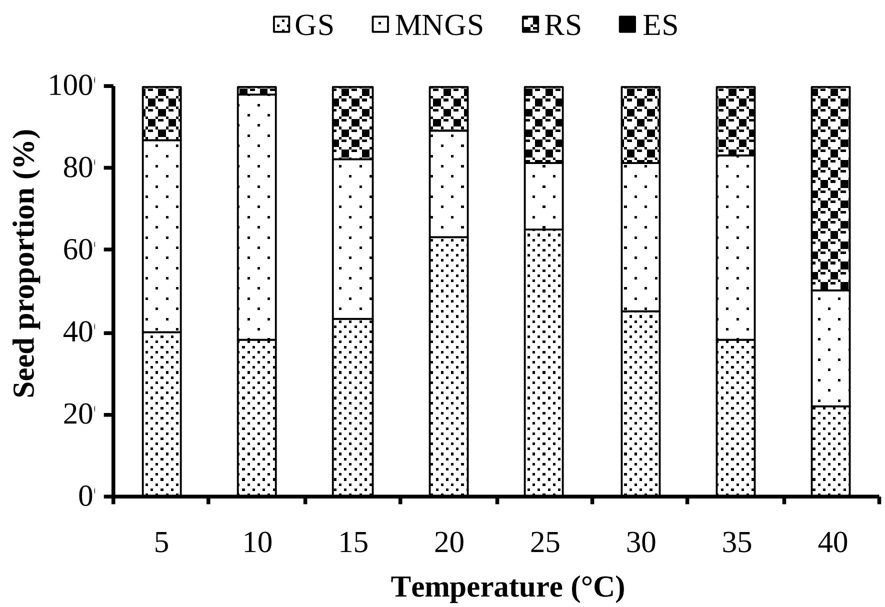

Figure 3. Proportion of the different categories of *C. villosa*seeds identified as function of temperature (at the end of incubation): germinated seeds (GS), moistened and not germinated seeds (MNGS), rotted seeds (RS) and empty seeds (ES).

Table 1. Germination characteristics of *C.villosa* seeds at various temperatures during 16 days period.

Temperature (°C)	Germination characteristics				
	FGP (%) (±Std)	**TFG (days) (±Std)**	**TGF(days) (±Std)**	**GD (days) (±Std)**	**MTG (days) (±Std)**
5	40 (11.78)[a]	7 (1.15)[a]	12 (0.00)[bc]	5 (1.15)[a]	6.56 (0.33)[a]
10	38 (8.33)[a]	5 (1.15)[b]	11 (1.15)[d]	6 (1.63)[a]	6.91 (0.37)[b]
15	43 (8.25)[b]	3 (1.15)[c]	8.5 (1.00)[a]	5.5 (1.91)[a]	4.91 (0.34)[c]
20	63 (6.00)[a]	2 (0.00)[b]	10 (0.00)[ab]	6 (0.00)[a]	5.76 (0.79)[c]
25	65 (12.38)[a]	2 (0.00)[b]	11.5 (1.00)[cd]	9.5(1.00)[a]	7.13 (0.63)[b]
30	46 (13.27)[a]	4 (0.00)[b]	9 (2.58)[d]	6 (1.63)[a]	4.65 (0.32)[b]
35	38 (10.07)[a]	2 (0.00)[a]	6.5 (1.00)[bc]	4.5 (1.00)[a]	3.26 (0.39)[a]
40	22 (7.66)[a]	4 (0.00)[a]	9 (1.15)[bc]	5 (1.15)[a]	6.58 (0.55)[b]

Note: Each value is a mean of four replicates of 25 seeds.
FGP: final germination percentage TFG: Time to first germination, TGF: Time to final germination, GD: Germination display and MTG: mean time to germination.

germinate rapidly increased and represented 26 and 16 at 20, 25% respectively. At 40°C, the fraction of rotted seeds reached 50%.

Salinity tolerance

The germination responses of *C. villosa* seeds to a variety of salinity levels under thermal optimum (25 and 20°C) are displayed in Figure 4. Seed germination reduced with increased NaCl amount in the temperatures. NaCl was discovered to obstruct development, mainly at high quantity; certainly, seeds imperiled to solutions of 200 and 150 mM NaCl have a final germination percent do not surpass 2 and 15% respectively. Germination percent decreases with increasing salinity and was inhibited (0%) at 250 and 300 mM NaCl treatments (Figure 4, Table 2).

Table 2 displays that the time to first germination (TFG) increased with increasing NaCl concentrations and was more obvious at 20°C. In contrast, the time to final germination (TGF) continues approximately unvarying. Two-way ANOVA of germination indicated a significant main effect of salinity ($F = 0.049$; $P < 0.05$) for germination

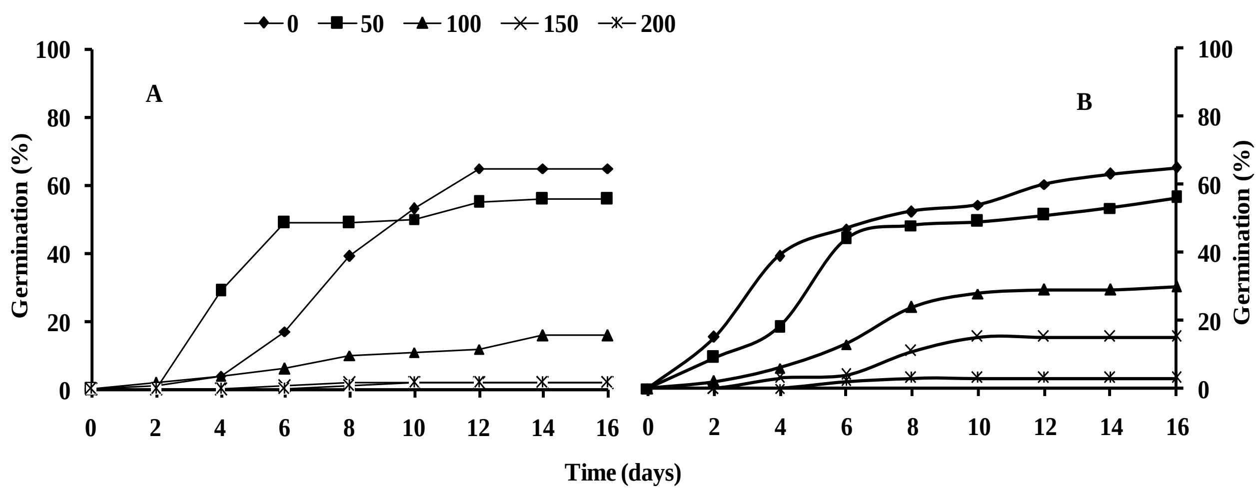

Figure 4. Germination curves of seeds at different NaCl concentrations (n=4) (A) at 20°C and (B) at 25°C.

Table 2. Final germination (%), number of days to first germination (delay of germination), mean time to-germination (MTG) of *Calycotome villosa*seeds in six salinity treatments for 16 days.

Concentration (Mm)	**Germination characteristics at 25°C**				
	FGP (%) (±Std)	**TFG (days) (±Std)**	**TGF(days) (±Std)**	**GD (days) (±Std)**	**MTG (days) (±Std)**
0 mM	65.0 (2.00)[a]	2.00 (0.00)[a]	13.5 (1.91)[b]	11.5 (1.91)[a]	5.86 (1.45)[a]
50 mM	56.0 (3.27)[b]	2.00 (0.00)[a]	14.5 (1.91)[b]	12.5 (1.91)[a]	6.17 (1.05)[a]
100 mM	30.0 (5.16)[b]	3.50 (1.00)[a]	10.5 (3.79)[b]	7.00 (3.46) [a]	7.00 (0.84)[a]
150 mM	15.0 (12.38)[b]	5.50 (3.00)[a]	10.0 (0.00)[a]	7.00 (2.00)[a]	8.00 (1.35)[a]
200 mM	3.00 (3.83)[b]	3.50 (4.12)[a]	3.50 (4.12)[b]	3.50 (4.12)[a]	3.50 (4.12)[a]
250 mM	0.00 (0.00)[b]	0.00 (0.00) [a]	0.00 (0.00)[b]	0.00 (0.00)[a]	0.00 (0.00)[a]
300 mM	0.00 (0.00)[b]	0.0 0(0.00) [a]	0.00(0.00)[b]	0.00 (0.00)[a]	0.00 (0.00)[a]

Concentration (Mm)	**Germination characteristics at 20°C**				
	FGP (%) (±Std)	**TFG (days) (±Std)**	**TGF(days) (±Std)**	**GD (days) (±Std)**	**MTG (days) (±Std)**
0 mM	65.0 (6.00)[a]	4.00 (1.63)[b]	8.0 (1.63)[ab]	8.00 (1.63)[ab]	8.59 (0.57)[b]
50 mM	56.0 (3.27)[a]	4.00 (0.00)[b]	10.5 (1.0)[a]	10.5 (1.00)[a]	6.64 (1.10)[b]
100 mM	16.0 (5.66)[a]	4.50 (2.52)[b]	6.0 (3.65)[ab]	7.00 (3.46)[ab]	6.99 (1.10)[b]
150 mM	2.00 (2.31)[a]	3.50 (4.12)[b]	3.50 (4.12)[b]	7.00 (2.00)[b]	6.99 (1.86)[b]
200 mM	2.00 (2.31)[a]	4.50 (5. 62)[a]	4.5 (5.26)[ab]	3.50 (4.12)[ab]	3.50 (4.12)[a]
250 mM	0.0 (0.00)[a]	0.0 (0.00)[b]	0.0 (0.00)[b]	0.0 (0.00)[b]	0.00 (0.00)[b]
300 mM	0.0 (0.00)[a]	0.0 (0.00)[b]	0.0 (0.00)[b]	0.0 (0.00)[b]	0.00(0.00)[b]

Note: Each value is a mean of four replicates of 25 seeds.
FGP: final germination percentage TFG: Time to first germination, TGF: Time to final germination, GD: Germination delay and MTG: mean time to germination.

display (GD) and for final germination percentage and time to final germination (F= 0.003, F=0.015 respectively). However, the interaction affects the time to final germination (P< 0.000) (Table 3).

The highest germination percentages were obtained under non-saline conditions, afterward 50 and 100 mM NaCl. The higher concentrations (150 and 200 mM NaCl) exhibited a considerable decrease in seed growth (Figure 4). Germination proportions reduced with increased salinity and were sternly restricted at 250 and 300 mM. There was a robust destructive connection with the coefficient of determination (R^2) stretching from 0.83 to 0.97 between germination and salinity (Figure 5). The linear regression examination was used to regulate the

Table 3. Analysis of variance (Two-way ANOVA) of the effects of temperature(T). salinity (S), and their interaction on germination characteristics of *Calycotome villosa* seeds.

Dependent variables	T			S			T x S		
	df	MS	P level	df	MS	P level	df	MS	P level
FGP	1	0.867	0.372^{NS}	6	4.377	0.003***	6	1.022	0.464^{NS}
TFG	1	2.454	0.867^{NS}	**6**	83.112	0.459^{NS}	6	127.211	0.209^{NS}
TGF	1	25.283	0.735^{NS}	6	661.340	0.015*	6	1323.126	0.000***
MTG	1	2.524	0.870^{NS}	6	127.255	0.254^{NS}	6	127.255	0.254^{NS}
GD	1	148.844	0.491^{NS}	6	722.147	0.049*	6	402.880	0.276^{NS}

^{NS}. not significant: * P< 0.05.**P< 0.01 and ***P<0.0001.

FGP:final germination percentage; TFG:time to first germination; TGF: time to final germination; MTG: mean time to germination; GD:germination display.

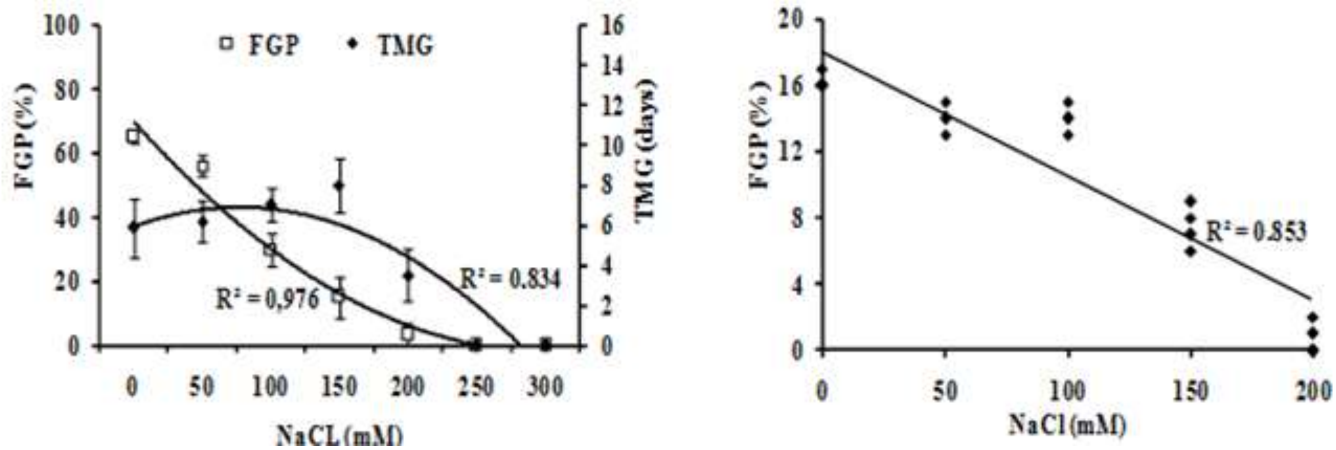

Figure 5. Variation of the final percentages (FGP) and the speed of germination (MTG, days) of *C. villosa* seeds according to the NaCl concentrations (0-200 mM). Lines describing the evolution of each parameter were obtained using fitted polynomial regression.

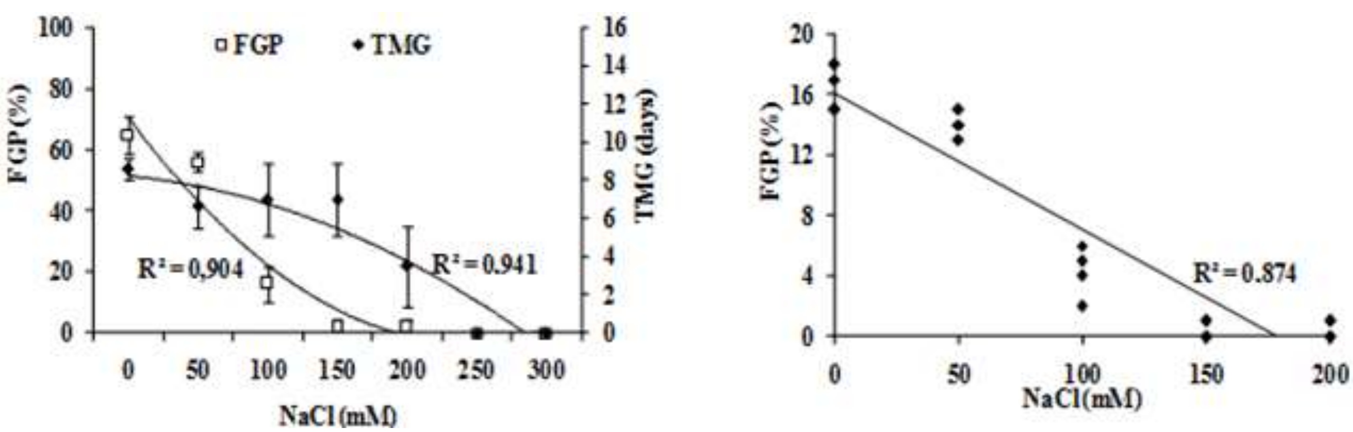

Figure 6. Regression plots for mean final germination percentages of *C. villosa* seeds at different NaCl concentrations and two incubation temperatures. Values (n=8) are from the five treatments with four replicates.

relations between FGP and salinity at different temperatures. There was a resilient negative relationship between germination and salinity (Figure 6). The restricted disparity amplitude of the coefficient of determination (R^2 = 0.85-0.87) might approve that the optimal temperature ranged from 20 to 25°C.

DISCUSSION

Temperature plays a key role in defining the periodicity of seed germination and the survival of species (Baskin and Baskin, 1988). Additionally, it is important to consider that the combination of limited water availability and high temperature is the major factor that hampers seedling survival under natural desert conditions (Ehleringer and Cooper, 1992; Valladares and Pearcy, 1997; Arvind et al., 2016). In general higher temperatures enhance physiological processes as long as the threshold temperature is not exceeded (Saxe et al., 2001). *C. villosa* ensued over a varied array of temperatures from 20 to 25°C in darkness. This performance is an archetypal plan of this shrub that develops regularly in cool places in the Mediterranean area (Gibbs, 1968; Zimowski et al., 2014).

The germination tests with *C. villosa* suggest that this species has no requirement for light to germinate. Seeds reached their highest percentage of germination at relatively low temperatures (20 and 25°C). Neffati (1994) displayed that the variant in the thermal optimum is determined by the measured species, though for the majority of southern Tunisian species germination ensued over a wide array of temperatures and that temperature of 20°C seems to improve their germination. This variant in the thermal optimum and germination speed between species institutes some adaptive approaches to severe ecological situations. It has also been suggested that high germination success recorded under high temperatures allows species to escape risks of fast desiccation of the superficial soil horizons during the period of their germination.

Linear regression analysis using the reciprocal of time to 50% germination against temperature permitted to predict the minimum temperature of *C. villosa* seeds, below which to germination is excepted, was -2.8°C. Similarly, Adam et al. (2007) showed that the minimum temperature was 1.8°C for *Lepidium sativa* and *Linum usitatissimum* and was 0°C for *Sinapsis alba*.

The fraction of seeds that did not germinate increased with the lower temperatures and above the thermal optimum. Such behavior could be considered as an adaptative strategy of leguminous to harsh environmental conditions (Torres et al., 2013). They are characterized by having coats (Lodge, 1996; Sonsa and Marcos-Filho, 2001; Siles et al., 2016) which, play an important role in germination patterns under conditions since they ensure the seed germination only occurs at optimum times for seedling growth (Lodge and Whalley, 2002). The germination of *C. villosa* seed germination was hindered in the presence of NaCl, and suggestively reserved when NaCl concentration has outdone 150 mM.

This result validates numerous other studies revealing that Fabaceae family is sensitive to salt during the timing of germination (Zammouri and al., 2009). However, some authors (Jamil et al., 2005; Patade et al., 2011; Rouhi et al., 2011 and Ansari and Sharif-Zadeh, 2012; Ibrahim, 2016) stated that cumulative salt concentration reduces the germination proportion and upsurges germination time. Läuchli and Grattan (2007) projected a general relationship between propagation proportion and time of germination after the addition of water at different salt levels. Rising salt applications not only stop the seed germination, but also cover the germination time by postponing the commencement of germination (Okcu et al., 2005; Thiam et al., 2013). Normally, low salt concentration encourages a state of latency and reductions the germination degree. Ultimately, a salt stress upsets plant development through insufficient imbibitions, hormone imbalance and metabolism alteration (Leymarie et al., 2012). Due to these disorders, salt accumulation in soils interrupts numerous development and growth stages as well as seed germination is a most censoriously susceptible phase to salinity (Cesur and Tabur, 2011). It is because of the same reason that fruitful seed growth and seedling phase under salt stress guarantee salinity-tolerant behavior of plant development.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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