

RESPONSE OF SEED GERMINATION OF TUNISIAN *ALLIUM AMPELOPRASUM*  
TO TEMPERATURE AND SALT STRESSESChedia GUENAOU<sup>1,\*</sup>, Saleh KSIKSI TAOUFIK<sup>2</sup>, Samira SMITI<sup>3</sup> & Mohamed NEFFATI<sup>1</sup>

RÉSUMÉ.— *Réponse à la température et à la salinité de la germination des semences d'Allium ampeloprasum en Tunisie.*— La salinité et les températures élevées sont les principaux problèmes inhibant la germination des semences et menaçant le couvert végétal dans les environnements arides comme c'est le cas dans certaines régions de Tunisie. Ainsi, pour réhabiliter ces régions, des espèces adaptées peuvent être utilisées pour contourner ces contraintes environnementales. Dans la présente étude, on a évalué l'effet du sel et de la température sur la germination des semences d'*Allium ampeloprasum* de deux îles tunisiennes (Kneiss & Djerba). Trois températures (15° C, 23° C & 30° C) et quatre niveaux de salinité (0, 75, 150 & 225 mM NaCl) ont été testés à une photopériode de 10 h. Les deux populations étudiées ont montré des comportements similaires. L'optimum thermique pour la germination a été de 15° C. La germination a été inhibée par une augmentation de température et de salinité. Le sel a diminué le taux de germination finale et le pourcentage de germination (PG%). Le délai de germination ( $T_0$ ) et le temps pour 50 % de germination ( $T_{50}$ ) ont augmenté significativement en réponse à une augmentation de la température et de la salinité. Une interaction entre la salinité et la température a complètement inhibé la germination à 30° C avec 225 mM NaCl. Les semences transférées à l'eau distillée ont montré un pourcentage de recouvrement variant de 0 à 40,6 %. Le test de viabilité a montré que toutes les semences non germées étaient viables, prouvant ainsi que *A. ampeloprasum* adopte une stratégie d'inhibition temporaire de germination pour survivre à des hautes températures et salinités. *A. ampeloprasum* peut être classifié comme une espèce modérément tolérante.

SUMMARY.— High salinity and temperature are major problems inhibiting seeds germination and threatening plant cover in arid environment such as the conditions in some regions in Tunisia. Thus, the use of adapted species to rehabilitate these regions could be an alternative to overcome these environmental constraints. In the present study, we intended to evaluate the effect of salt and temperature on seed germination of the wild leek (*A. ampeloprasum* L.) from two different Tunisian islands (Kneiss & Djerba). Three temperatures (15° C, 23° C & 30° C) and four salinity levels (0, 75, 150 & 225 mM NaCl) were tested in a 14 h dark:10 h light photoperiod. The two studied populations showed a similar behaviour. The optimum temperature for seed germination was 15° C. Germination was inhibited by an increase in both temperature and salinity. Salt stress decreased both the final germination rate and the final germination percentage (PG%). Delay of germination ( $T_0$ ) and the time to half of germination ( $T_{50}$ ) significantly ( $p < 0.05$ ) increased in response to salt and temperature increasing. An interaction between salinity and temperature completely inhibited germination in 225 mM NaCl at 30° C. Seeds transferred from salt solution to distilled water showed a recovery percentage (R%) varying from 0 to 40.6 %. Viability test showed that all non-germinated seeds were alive proving that *A. ampeloprasum* adopted a strategy of temporal inhibition of germination to survive under salt and temperatures increasing. As such it could be classified as a moderately salt tolerant species.

---

<sup>1</sup> Arid Regions Institute, Range Ecology Laboratory, 4119 Medenine, Tunisia. Tel: +216 75 663 005; Fax: +216 75 663 006; e-mails: chedia\_g@yahoo.fr & Neffati.Mohamed@ira.rnrt.tn

<sup>2</sup> Faculty of Sciences, Biology Department, United Arab Emirates University, 17551, Al Ain, United Arab Emirates. E-mail: tksiksi@uaeu.ac.ae

<sup>3</sup> University of El-Manar II, Faculty of Sciences, Unit of Plant Ecology Research, Campus Academy, 2092 Tunis, Tunisia. E-mail: Samira.smiti@planet.tn

\* Corresponding author

The genus *Allium*, family of Alliaceae, includes more than 700 species (Stearn, 1980; Hanelt *et al.*, 1992). The adaptation to the wide array of geo-climatic conditions resulted in a considerable polymorphism (Hanelt *et al.*, 1992) and complicated the taxonomic analysis within the genus. The latest intrageneric classification divided the genus *Allium* into 15 subgenera and 72 sections (Friesen *et al.*, 2006). The subgenus *Allium* is the largest, comprising around 280 species (Hanelt *et al.*, 1992), 114 of which compose its largest section, *Allium* (Mathew, 1996). Many vegetables in *Allium* genus are economically interesting and used mainly for their unique flavours (Havey, 1999). They produce health-enhancing compounds such as flavonoids (Havey, 1999) and they have been known as rich sources of secondary metabolites with interesting biological activities (Augusti, 1990; Block, 1992). Hsing *et al.* (2002) affirmed that the consumption of *Allium* vegetables reduced the risk of prostate cancer. *Allium ampeloprasum* L., locally known as “kur-rat” in Tunisia, a wild leek species, originates from the Mediterranean region and southwest Asia (Figliuolo & Mang, 2010). It includes wild cytotypes phenotypically homogeneous along with their domesticated relatives (Figliuolo & Di Stefano, 2007). It has a high economic value (Havey, 1999). Figliuolo & Mang (2010) indicated that *A. ampeloprasum* has economic significance for direct use and breeding. It is rich in flavonoids such as myrecitin, kaemferol and quercitin (Horbowicz & Kotlińska, 2000), responsible for anticarcinogenic and antithrombotic activities (Havey, 1999). Many *Allium* species (*Allium roseum* L., *Allium cepa* L., *Allium sativum* L.) were well studied in the world as well as in Tunisia (Najjaa *et al.* 2007; Leporatti & Ghedira, 2009; Zammouri *et al.* 2009; Guetat *et al.* 2010). However, the main interest given to *Allium ampeloprasum* L., in the world, was for morphological, cytological and molecular analysis (Figliuolo & Di Stefano, 2007; Figliuolo & Mang, 2010). In Tunisia, no work has been published on *Allium ampeloprasum* L., although it is widely distributed and commonly used in local foods. Herein, we report the effect of temperature and salt stresses on the germination behaviour of *Allium ampeloprasum* L. seeds, collected from two different Tunisian islands, Kneiss and Djerba. These two regions are dominated by arid conditions and salt soils and thus native species are suggested to be resistant to such environment. Philips *et al.* (2010) noted that the aspects of germination characteristics among *Allium* species are suggested to closely reflect their particular environment.

The successful establishment of plants in arid regions is often limited by temperature (Oberbauer & Miller, 1982; Jordan & Haferkamp, 1989; Evans & Etherington, 1990). The loss of vegetation always results in soil erosion and loss of wildlife habitat and food resources (Ksiksi *et al.*, 2006). Seed germination is one of the most salt-sensitive plant growth stages (Vicente *et al.*, 2004). The whole seed germination period includes imbibition, protrusion, germination, and seedling establishment stages, and at any stage salt injury is disadvantageous to seed germination (Parida & Das, 2005). Increase in salt level inhibits seeds germination (Sosa *et al.* 2005) and provokes a reduction in seeds germination percentage and a delay in the time to germinate (Ungar, 1982; Phillipupillai & Ungar, 1984; Khan & Ungar, 1996; Keiffer & Ungar, 1997). Khan & Ungar (1998) considered this delay of germination as an adaptation strategy that avoids seedling mortality under high salinities.

Moreover, when salt concentrations overpass the tolerance limits of the species they can completely inhibit seed germination (Ungar, 1991). Seed germination tolerance to salinity can be determined by the temperature-salinity interaction and in some species optimal temperature generally reduces the detrimental effect of salinity contrary to higher temperatures (El-Keblawy & Al-Rawai, 2005). Following high precipitation, soil salinity decreases and changes in osmotic potential of soils narrow the range of temperature favourable for seeds germination (Hegarty, 1978). Moreover, different species approved that responses were significantly affected by changes in temperature regimes to which seeds were exposed (Khan & Ungar, 1996, 1997, 1998a, b, 1999; Khan & Gul, 1998).

The knowledge of germination behaviour may be useful to evaluate the germination characteristics or the establishment potential of *Allium ampeloprasum* L. The purpose of our investigation was to examine the effects of temperature and salt on seed germination of *Allium ampeloprasum* L. so as to determine the upper level of salt and temperature tolerance, which can be considered as a benchmark for irrigation with seawater and would help in developing possible methodology of introduction of *A. ampeloprasum* species to rehabilitate saline and arid regions.

## MATERIAL AND METHODS

### PLANT MATERIAL

Seeds of *Allium ampeloprasum* L. were collected from plants installed in the experimental field of the Institute of Arid Regions of Medenine, Tunisia. These plants were transplanted from two different islands in Tunisia, Kneiss and Djerba (Tab. I).

### EXPERIMENTAL PROCEDURE

Once matured, seeds of *Allium ampeloprasum* were separated from inflorescences and stored at ambient temperature (25-30° C). Germination was carried out in 90 mm Petri dishes containing two disks of Whatman No. 1 filter papers with 5 ml of test solution of four different NaCl concentrations (0 mM; 75 mM, 150 mM and 225 mM), separately. Four replicates of 25 seeds each were used for each treatment. Seeds were germinated in incubators at constant temperature of either 15° C, 23° C or 30° C with 10/14-h day/night. Seeds were considered to be germinated with radicle emergence. Every two days the number of germinated seeds on each Petri dish was observed and the final germination percentage was determined. For each treatment, we calculated the number of days taken for the first seed to germinate, delay of germination ( $T_0$ ) and the time (in days) taken for half of those seeds that germinated by the end of the experiment to germinate ( $T_{50}$ ), as an estimate of the speed of germination (Barnea *et al.*, 1991; Traveset *et al.*, 2001). The rate of germination was estimated by using modified Timson's index of germination velocity which is as follows:  $\Sigma G/t$ , where G is percentage of seed germination at 2-day intervals, and t is total germination period (Khan & Ungar, 1984). The higher the value the more rapid the germination is. Subsequently, non-germinated seeds were transferred to distilled water to study the recovery of germination, which was also recorded at 2-day intervals for 14 additional days. The recovery percentage was determined following Pujol *et al.* (2000), using the formula:  $[(a - b)/(c - b)] \times 100$ , where a is the total number of seeds germinated after being transferred to distilled water, b is the total number of seeds germinated in saline solution and c is the total number of seeds. By the end of the experiment, non-germinated seeds were tested for viability via Tetrazolium (TZ) test using 2,3,5,- triphenyltetrazolium chloride at 0.5% (v/v). This colourless solution is taken up by seeds and then reacts with respiratory enzymes (dehydrogenases) to form an insoluble light pink (magenta) precipitant. Tissues that are alive and respiring will stain, and those that are not alive will not (AOSA, 2000).

### STATISTICAL ANALYSIS

Germination data were analysed using SPSS for Windows release 17 (SPSS, 2008). A Duncan test was used to determine significant differences ( $p < 0.05$ ) between means.

## RESULTS

### EFFECTS OF TEMPERATURE AND SALINITY ON GERMINATION

Optimal germination of *Allium ampeloprasum* in 10/14 hr day/night occurred in distilled water. Over 80 % (80.8 for Djerba and 90.4 % for Kneiss) of seeds in the control sample (0 mM NaCl) had already germinated after 10 days at 15° C (Fig. 1). Differences in final germination percentage (PG%) between 0 and 75 mM NaCl were not significant ( $p > 0.05$ ) for both Djerba and Kneiss at 15° C. One-way ANOVA showed significant effect of temperature ( $p < 0.05$ ) on germination of the two studied populations. Seed germination was higher and more rapid at 15° C in comparison to the other two tested temperatures (Fig. 1 & 2; Tab. II). In non-salt condition, the delay of germination ( $T_0$ ) was about 5 days for both Djerba and Kneiss at 15° C and increased to 20 days and 15 days at 30° C for Djerba and Kneiss, respectively (Tab. II).  $T_{50}$  was about 7 days for both Djerba and Kneiss at 15° C and increased by 3 and 1 day at 23° C, for Djerba and Kneiss, respectively. At 30° C, seeds germination did not reach 50 %, neither for Djerba nor for Kneiss (Fig. 2). Final germination percentage decreased from 63 % in both Djerba and Kneiss populations in non-salt water to 51 % and 55.4 % in 75mM NaCl, to 48 % and 51.2 % in 150mM and to 38 % and 30 % in 225mM NaCl, for Djerba and Kneiss populations, respectively (Fig. 3). However, one-way ANOVA showed that these decreases were not significant ( $p > 0.05$ ) for the two populations. The decrease in final germination percentage under salt stress and temperature increasing was accompanied by an increase in MTG (Fig. 2 & 3). Two-way ANOVA showed a significant effect of temperature ( $p < 0.05$ ), salinity ( $p < 0.05$ ) and their interaction ( $p < 0.05$ ) on MTG for both Djerba and Kneiss.

TABLE I

Geographical coordinates, altitude, bioclimatic zones and soil type of the sampling localities of the studied populations of *A. ampeloprasum* L. in Tunisia

Origin	Genepool	Bioclimatic zone <sup>a</sup>	Latitude (N)	Longitude (E)	Altitude (m)	Soil type
Kneiss	Wild	ATW	34.2203	10.1859	0	Halomorphic salted sabkha soil
Djerba	Wild	AWW	33.4820	11.0148	2	Halomorphic salted steppe soils

<sup>a</sup> The bioclimatic zones are defined according to the Emberger's pluviothermic coefficient  $Q^2$  (Emberger, 1966):  $Q^2 = 2000 * P / (M^2 - m^2)$  where, P is the yearly average in mm of the pluviometry, M is the average of the most elevated temperatures of the hottest month and m is the average of the lowest temperature of the coldest month. ATW: arid at temperate winter, AWW: arid at warm winter.

TABLE II

Variation of  $T_0$ ,  $T_{50}$  and cumulative germination percentage of *Allium ampeloprasum* L. seeds in saline solutions of NaCl at different temperatures (15°C, 23°C & 30°C)

Independent variables	Temperature (°C)	NaCl (mM)	$T_0$		$T_{50}$		Germination (%)	
			Djerba	Kneiss	Djerba	Kneiss	Djerba	Kneiss
15	0	0	5.6 ± 3.0a	5.6 ± 2.0a	7.4 ± 1.4a	7.6 ± 1.4a	90.4 ± 4.1a	92.0 ± 4.2a
		75	7.6 ± 2.0ab	6.2 ± 2.0a	10.4 ± 1.0b	8.6 ± 1.4a	96.8 ± 2.3a	93.6 ± 2.0a
	150	10.4 ± 3.0bc	7.6 ± 2.0b	17.2 ± 1.4c	9.6 ± 1.4a	87.2 ± 2ab	92.0 ± 2.5a	
		225	13.0 ± 2.0c	10.6 ± 2.0c	21.8 ± 2.9d	16.6 ± 1.4b	83.2 ± 5.1b	68.0 ± 4.6b
23	0	0	5.6 ± 2.0a	5.0 ± 2.0a	12.4 ± 1.4a	10.0 ± 1.4a	75.2 ± 7.3a	97.6 ± 1.6a
		75	9.3 ± 2.0b	5.2 ± 1.0a	24.6 ± 1.8b	14.0 ± 1.6ab	51.2 ± 10b	72.8 ± 9.0b
	150	14.7 ± 2.0b	8.0 ± 1.3b	27.0 ± 1.8b	22.2 ± 1.6b	52.8 ± 5.2ab	61.6 ± 5.1b	
		225	21.0 ± 1.0c	17.0 ± 2.0c	. <sup>a</sup>	. <sup>a</sup>	32.8 ± 7.1b	24.8 ± 4.3c
30	0	0	20.0 ± 2.0a	15.7 ± 1.3	. <sup>a</sup>	. <sup>a</sup>	7.2 ± 4.4a	0.8 ± 0.9a
		75	21.8 ± 5.0a	. <sup>a</sup>	. <sup>a</sup>	. <sup>a</sup>	6.4 ± 3.7b	0 ± 0b
	150	22.0 ± 7.0a	. <sup>a</sup>	. <sup>a</sup>	. <sup>a</sup>	4.8 ± 2.3b	0 ± 0b	
		225	23.1 ± 3.0a	. <sup>a</sup>	. <sup>a</sup>	. <sup>a</sup>	0.8 ± 0.8b	0 ± 0b

Values are means ± S.E. (n = 5). Means within a column that have a different letter are significantly different at  $P < 0.05$ , Duncan test.

<sup>a</sup>  $T_0$  values could not be estimated because germination percentage was  $< 0\%$  and  $T_{50}$  values could not be estimated because germination percentage was  $< 0\%$ . Therefore, these treatments were excluded from ANOVA.

The data demonstrate a diminution in germination rate with the increase in temperature and NaCl concentration, for both studied populations (Fig. 4). Highest rates of germination (33.7 % for Djerba and 36.9 % for Kneiss) were obtained at 15° C in distilled water. Salt water and temperature increases, individually and their interaction, significantly ( $p < 0.05$ ) affected the rate of seeds germination of *Allium ampeloprasum* L. of the two studied populations. Lowest rate of germination was observed at 30° C in 225 mM NaCl where it was less than 1 % for both Djerba and Kneiss.

#### EFFECTS OF TEMPERATURE AND SALT TREATMENTS ON GERMINATION RECOVERY

After 26 days of salinity treatment, non-germinated seeds were transferred to distilled water to examine their ability to recover germination. Seeds germination recovery from salinity stress is shown in Table III. The percentage of recovery varied from 0 to 40.6 % within seeds from Djerba and from 0 to 26.6 % for those from Kneiss (Tab. III). These percentages decreased especially at 30° C for both Djerba and Kneiss. At 15° C, seeds from Djerba previously treated in 75 mM NaCl and those from Kneiss previously treated in 225 mM NaCl, were unable to recover (0 %). According to one-way ANOVA, the effect of salt treatment in the recovering

percentage was not significant for seeds from Kneiss ( $p > 0.05$ ). For seeds from Djerba, the differences in R (%) were significant only at 15° C and 30° C ( $p < 0.05$ ).

The test of viability via Tetrazolium test, applied by the end of the experiment showed a high seed viability (Tab. III). The salinity, temperature and their interaction have no significant effect on the seeds viability ( $p > 0.05$ ). The percentages of viability after treatments varied from 93.3 to 100 %.

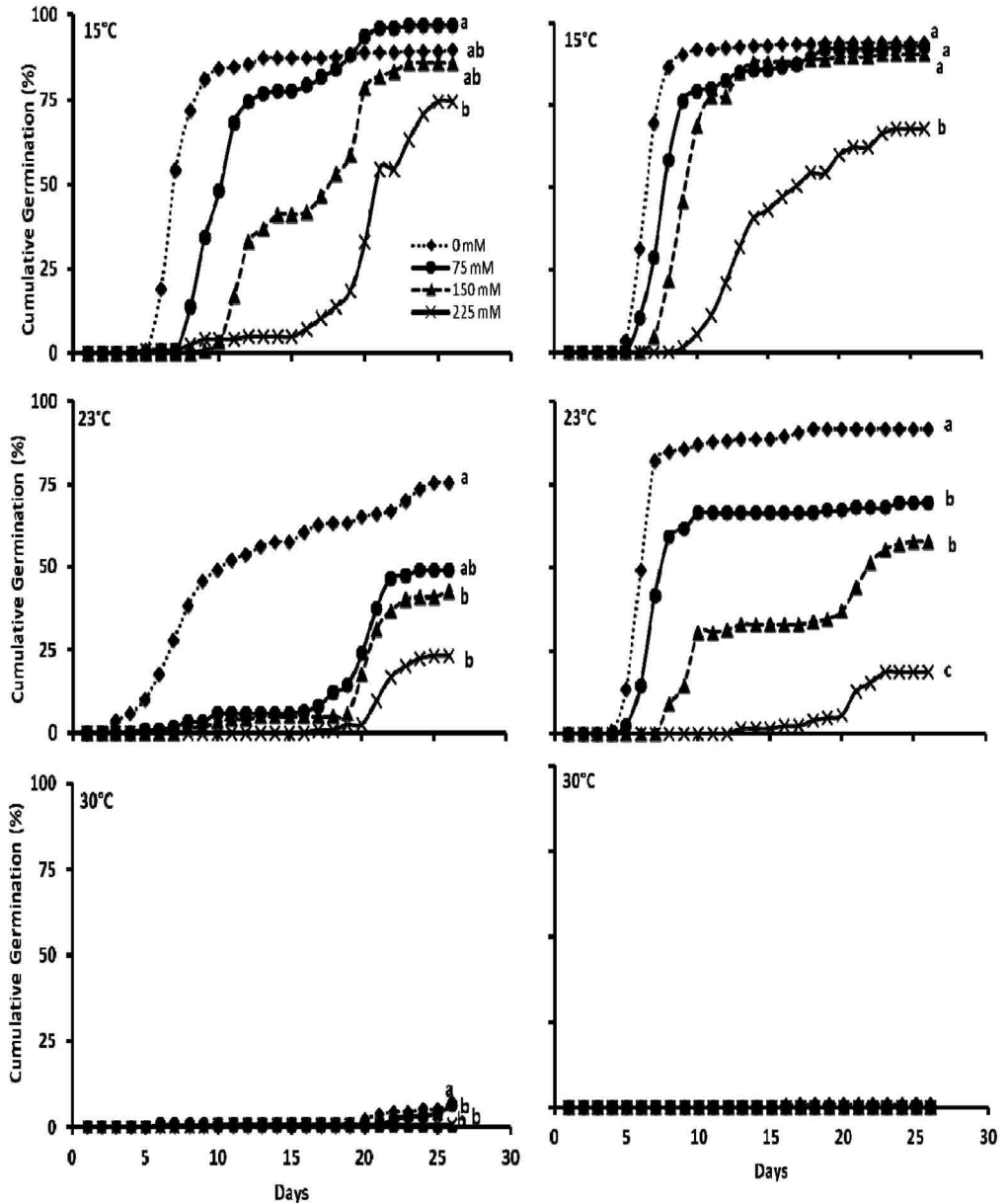


Figure 1.— Cumulative germination percentage of seeds of *Allium ampeloprasum* L. from Djerba (left) and Kneiss (right) in salt water (0; 75; 150 and 225 mM) at 15° C, 23° C and 30° C. Values of the final germination percentage (Mean  $\pm$  S.E.) having the same letter are not significantly different at  $P < 0.05$ , Duncan test. (n=5).

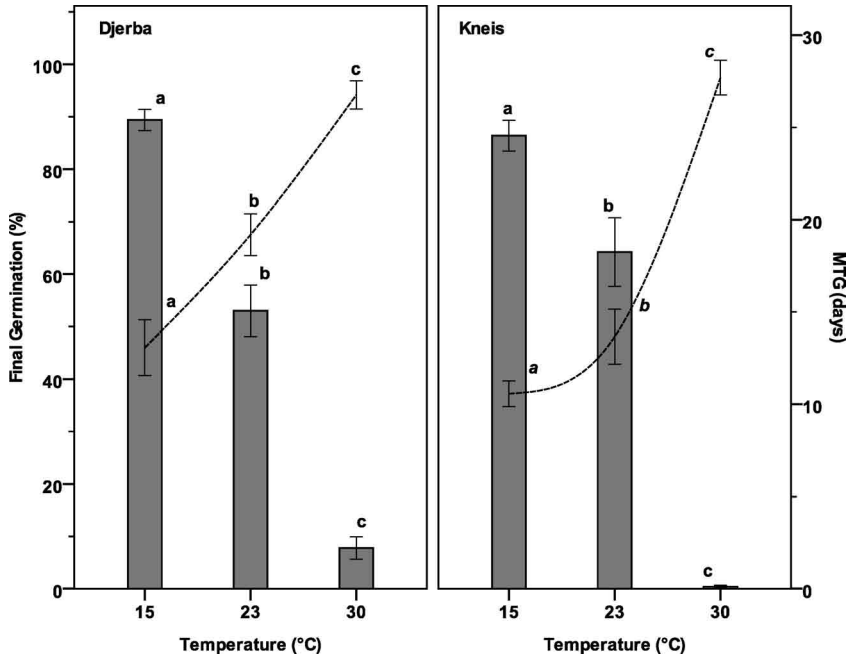


Figure 2.— Variation of final germination percentage and mean time to germinate for seeds of *Allium ampeloprasum* L. at 10°C, 23°C and 30°C. Values are means  $\pm$  SE from the NaCl treatments (0; 75; 150 and 225 mM) (n = 5). Means for each temperature treatment that have different letters are significantly different at  $P < 0.05$ , Duncan test.

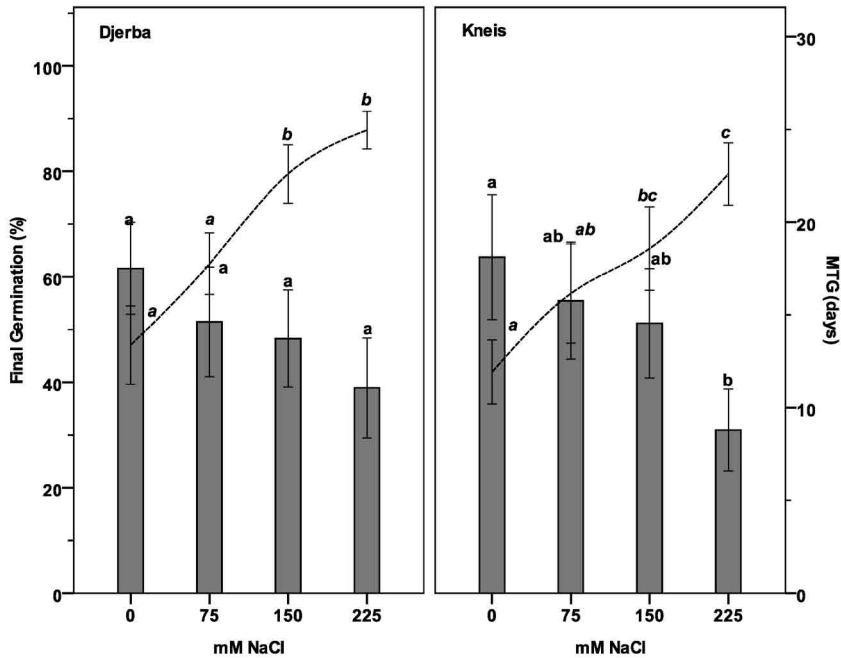


Figure 3.— Variation of final germination percentage and mean time to germinate for seeds of *Allium ampeloprasum* L. under 0 to 225 mM NaCl salinity. Values are means  $\pm$  SE from the three temperatures (15; 23 and 30°C) (n = 5). Means for each salinity concentration that have different letters are significantly different at  $P < 0.05$ , Duncan test.

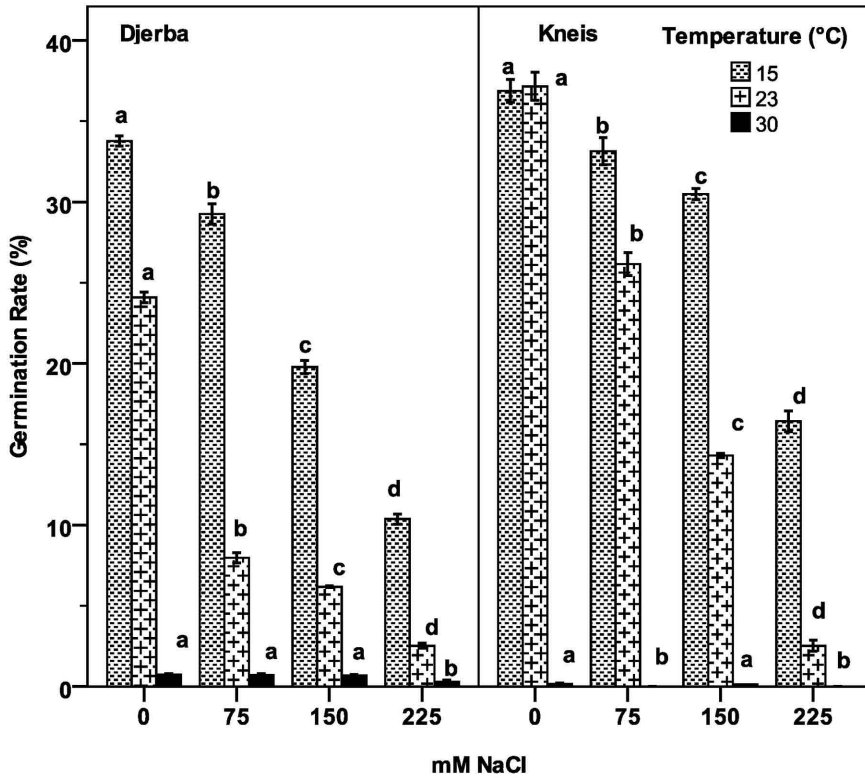


Figure 4.— Variation of germination rate (%) of seeds of *Allium ampeloprasum* L. germinated under 0 to 225 mM NaCl salinity, at 10° C, 23° C and 30° C. Means  $\pm$  SE (n = 5). For each temperature, means that have different letters are significantly different at  $P < 0.05$ , Duncan test.

TABLE III

Recovery and viability percentages of non-germinated seeds of *Allium ampeloprasum* L. at different temperatures (15; 23 & 30° C) after transfer from NaCl solutions (0; 75; 150 & 225 mM) to distilled water

Independent variables		Recovery (%)		Viability (%)	
Temperature (°C)	NaCl (mM)	Djerba	Kneiss	Djerba	Kneiss
15	0	2.8 $\pm$ 2.8	20.0 $\pm$ 20.a	96.6 $\pm$ 2.9a	93.3 $\pm$ 1.9a
	75	0 $\pm$ 0a	6.6 $\pm$ 6.6a	98.3 $\pm$ 0.5a	95.1 $\pm$ 2.9a
	150	10.6 $\pm$ 6.8a	26.6 $\pm$ 19.4a	97.0 $\pm$ 2.9a	95.5 $\pm$ 3.4a
	225	40.6 $\pm$ 15.2b	$\pm$ 0a	97.2 $\pm$ 2.1a	94.3 $\pm$ 3.9a
23	0	25.7 $\pm$ 19.3a	0 $\pm$ 0a	95.5 $\pm$ 3.1a	100.0 $\pm$ 0a
	75	3.7 $\pm$ 2.3a	7.6 $\pm$ 4.7a	96.9 $\pm$ 2.9a	96.6 $\pm$ 3.9a
	150	18.3 $\pm$ 6.0a	9.5 $\pm$ 4.0b	98.5 $\pm$ 0.1a	95.1 $\pm$ 3.9a
	225	12.9 $\pm$ 5.0a	7.7 $\pm$ 1.9ab	97.7 $\pm$ 1.9a	98.0 $\pm$ 1.9a
30	0	8.2 $\pm$ 2.7a	3.2 $\pm$ 3.2a	97.8 $\pm$ 3.9a	98.3 $\pm$ 0.9a
	75	0 $\pm$ 0a	0 $\pm$ 0a	98.2 $\pm$ 0.9a	98.4 $\pm$ 1.9a
	150	2.4 $\pm$ 2.4a	0.8 $\pm$ 0.8a	98.2 $\pm$ 0.9a	99.2 $\pm$ 0.7
	225	0 $\pm$ 0a	0 $\pm$ 0a	98.3 $\pm$ 0.2a	98.4 $\pm$ 1.9

Values are means  $\pm$  S.E. (n = 5). Means within a column that have a different letter are significantly different at  $P < 0.05$ , Duncan test.

## DISCUSSION

The result of our germination experiments showed high seed germination capacity at 15° C and 23° C. This result is in agreement with the finding of Specht & Keller (1997) who recommended testing *Allium* subgenus species at 16° C. At 15° C, germination percentage of seeds overlapped 87 % in 0 mM, 75 mM and 150 mM NaCl and the lowest percentage (68.4 %) was found in 225 mM with seeds from Kneiss (Tab. II). At 23° C, final germination overreached 50 % except in 225 mM NaCl where it was 32.8 % for Djerba and 24.8 % for Kneiss (Tab. II). These results demonstrated an adaptation of the two studied populations to the applied salt concentrations. These populations originated from two islands in South Tunisia, Djerba and Kneiss, where dominate an arid climate and a halomorphic salty soil. Thus, results strongly suggested that aspects of germination characteristics in these two populations of *Allium ampeeloprasum* closely reflect the particular environment found at each original site. Similar habitat-specific germination behaviour was reported for some intermountain *Allium* species (Phillips *et al.*, 2010) and for other species (Skoridilis & Thanos, 1995; Cavieres & Arroyo, 2000). The inhibition of germination under salt treatment at 30° C, observed especially within seeds from Kneiss, could be considered as an effect of salinity and salinity-temperature interaction. This interpretation was also given by Khan & Ungar (1998) who indicated that the inhibitory effect of salinity at higher temperatures prevents seeds from germination in salt-affected habitats.

Although they were transferred to distilled water for additional 14 days, at the same temperature, the non-germinated seeds did not completely recover their germination (Tab. III). The highest recovering (40.6 %) was observed within the population of Djerba for seeds previously treated in 225 mM NaCl at 15° C, contrarily to those previously in the same salt level but at 30° C. Equally, Khan & Ungar (1998*b*) indicated that seeds of *Suaeda fruticosa* recovered after transfer from hypersaline conditions to distilled water at all temperatures, but specified that only seeds exposed to high salinity at lower temperatures showed salt stimulation of germination. That was not the case for seeds from Kneiss when transferred from 225 to 0 mM at 15° C, and R (%) was null. This contradiction could be attributed to a habitat-specific germination behaviour. Indeed, *Allium* species from different habitats show different germination mechanisms and different responses to environmental conditions (Kamenetsky & Gutterman, 2000).

These observations demonstrate also that not only salt but even temperature is a limiting factor. Similar conclusion about temperature effect has been reported in literature (Oberbauer & Miller, 1982; Jordan & Haferkamp, 1989; Evans & Etherington, 1990). El-Keblawy & Al-Rawai (2005) showed that optimal temperature generally reduces the detrimental effect of salinity contrarily to higher temperatures. Low recovering percentages were also observed for seeds from Djerba treated at 15° C in 75 mM NaCl, and for seeds from Kneiss treated at 23° C in 0 mM NaCl. This could be attributed to the need for a longer period of recovering than we provided. In their original habitats and during the season of germination, September and October, these seeds naturally submit to a decrease in temperature and a long precipitation period contributing to a decrease in soil salinity level. These changes in osmotic potential of soils narrow the range of temperature favourable for seeds germination (Hegarty, 1978). Thus, it is likely that two weeks might not be enough to recover their germination. Likewise, Phillips *et al.* (2010) stated that twenty-four weeks of cold moist chilling were not enough to increase seeds germination of *Allium brandegei* that may naturally experience thirty weeks of continuous snow cover.

It is also interesting to note the rise and fall variations in R (%) between salt treatments at the same temperature. Although differences were not significant ( $P > 0.05$ ), only for Kneiss at 15° C ( $P < 0.05$ ) and 30° C ( $P < 0.05$ ), R (%) showed a clear trend to decrease from 0 to 75 mM NaCl, followed by an increase from 75 to 150 mM NaCl within the two populations. The recovery percentage diminished again from 150 to 225 mM NaCl, excepting seeds from Djerba at 15° C, where R (%) increased from 10.6 % (150 mM NaCl) to 40.6 % (225 mM NaCl). These fluctuations in recovery responses could be due to the difference in the temperature regime to which seeds were exposed. Many germination studies with different species approved that responses were significantly affected by changes in temperature regimes (Khan & Ungar, 1996, 1997, 1998*a, b*, 1999; Khan & Gul, 1998).



The high viability percentage (> 90 %) showed that the inhibition of germination under salt and temperature stresses is an adaptive strategy adopted by this species to survive under the unfavourable conditions. Similar behaviour has been signalled by Khan & Ungar (1998) who noted that the delay of germination under salty conditions is an adaptive strategy that avoids seedling mortality under high salinities.

These experiments afford new information concerning germination ecophysiology of Tunisian *Allium ampeloprasum*. The studied ecotypes showed an interesting ability to maintain seed viability during exposure to high saline conditions and they were able also to ensure their germination and may just need more than 2 weeks to recover once salinity stress is removed. This current understanding of the influence of habitat on germination traits in this species will aid in the development of seed specific propagation strategies that contribute to rehabilitation of arid regions.

## ACKNOWLEDGEMENTS

The investigators would like to express their sincere appreciation to the United Arab Emirates University for the financial support of this project under fund Grant # 01-04-2-11/09. The support from the Biology Department and the Faculty of Science is also much appreciated. The investigators would also like to express their gratitude to Dr. Mohamed Taher, Dr Gaber Ramadan, Ms. Latifa Al-Shamsi, Ms. Wafa Sager Aldhaheeri, Ms. Naima Al Shamsi, Ms. Raja Al-Muskari and Ms. Yusra Al-Dhaheeri for their support and their assistance.

## REFERENCES

- AOSA (2000).— *Tetrazolium testing handbook*. Contrib. 29. Handbook on seed testing. Lincoln, NE: AOSA.
- AUGUSTI, K.T. (1990).— Therapeutic and medicinal values of onions and garlic. *Onions and Allied Crops*, 3: 93-108. CRC Press Inc., Boca Raton, Florida, Etats-Unis.
- BARNEA, A., YOM-TOV, Y. & FRIEDMAN, J. (1991).— Does ingestion by birds affect seed germination? *Funct. Ecol.*, 5: 394-402.
- BLOCK, E. (1992).— The organosulfur chemistry of the genus *Allium*. Implications for organic sulfur chemistry. *Angewandte Chemie, Int. Ed. Engl.*, 31: 1135-1178.
- CAVIERES, L.A & ARROYO, M.T.K. (2000).— Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae). *Plant Ecol.*, 149: 1-8.
- EL-KEBLAWY, A. & AL-RAWAI, A. (2005).— Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw) D.C. *J. Arid Environ.*, 6: 555-565.
- EMBERGER, L. (1966).— Une classification biogéographique des climats. *Recherches et travaux des laboratoires de Géologie, Botanique et Zoologie*. Faculté des Sciences Montpellier France, 7: 1-43.
- EVANS, C. & ETHERINGTON, J.R. (1990).— The effect of soil water potential on seed germination of some British plants. *New Phytol.*, 115: 539-548.
- FIGLIUOLO, G. & DI STEFANO, D. (2007).— Is single bulb garlic *Allium sativum* or *Allium ampeloprasum*? *Sci. Hortic.*, 114: 243-249.
- FIGLIUOLO, G. & MANG, S.M. (2010).— Characterization and discrimination of Mediterranean bulb-producing garlic. Pp 181-197 In: M. Pacurar & G. Krejci (eds.), *Garlic Consumption and Health*. Nova Science Publishers, Inc., New York, USA.
- FRIESEN, N., FRITSCH, R. & BLATTNER, F.R. (2006).— Phylogeny and new intrageneric classification of *Allium* (Alliaceae) based on nuclear ribosomal DNA ITS sequences. *Aliso*, 22: 372-395.
- GUETAT, A., VILATERSANA, R., NEFFATI, M. & BOUSAID, M. (2010).— Genetic diversity in Tunisian rosy garlic populations (*Allium roseum* L.) as evidenced by chloroplastic DNA analysis: Sequence variation of non-coding region and intergenic spacers. *Biochem. Syst. Ecol.*, 38-4: 502-509.
- HANELT, P., SCHULTZE-MOTEL, J., FRITSCH, R., KRUSE, J., MAASS, J., OHLE, M. & PISTRIC, K. (1992).— Infrageneric grouping of Allium-The Gatersleben approach. Pp. 107-123 In: P. Hanelt, K. Hammer & H. Knupffer (eds).— *The Genus Allium - Taxonomic problems and genetic resources*. Proceedings of an International Symposium held at Gatersleben, Germany, 1991.
- HAVEY, M.J. (1999).— Advances in new Alliums. In: J. Janick (ed.), *Perspectives on new crops and New Uses*, ASHS Press, Alexandria, Virginia.
- HAVEY, M.J. (2007).— Allium CAP: A Plan for translational genomics of the Alliums. USDA-ARS and University of Wisconsin.
- HEGARTY, T.W. (1978).— The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. *Plant Cell Environ.*, 1: 101-119.

- HORBOWICZ, M & KOTLIŃSKA, T. (2000).— Level of flavonols in wild and cultivated *Allium* species. *Acta Hort.*, 517: 375-380.
- HSING, A.W., CHOKKALINGAM, A.P., GAO, Y.-T., MADIGAN, M.P., DENG, J., GRIDLEY, G. & FRAUMENI, JR. J.F. (2002).— Allium vegetables and risk of prostate cancer: A population-based study. *Journal of the National Cancer Institute*, 94 (21).
- JORDAN, C.L. & HAFERKAMP, M.R. (1989).— Temperature responses and calculated heat units for germination of several range grasses and shrubs. *J Range Manage.*, 42: 41-45.
- KEIFFER, C.H. & UNGAR, I.A. (1997).— The effect of extended exposure to hypersaline conditions on the germination of five inland halophyte species. *Am. J. Bot.*, 84: 104-111.
- KHAN, M.A., AHMED, M.Z. & HAMEED, A. (2006).— Effect of sea salt and l-ascorbic acid on the seed germination of halophytes. *J. Arid Environ.*, 67: 535-540.
- KHAN, M.A. & GUL, B. (1998).— High salt tolerance in germinating dimorphic seeds of *Arthrocnemum indicum*. *Int. J. Plant Sci.*, 159: 826-832.
- KHAN, M.A. & UNGAR, I.A. (1984).— The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. *Am. J. Bot.*, 71: 481-489.
- KHAN, M.A. & UNGAR, I.A. (1996).— Alleviation of seed dormancy in the desert forb *Zygophyllum simplex* L. from Pakistan. *Ann. Bot.*, 80: 395-400.
- KHAN, M.A. & UNGAR, I.A. (1997).— Germination responses of the subtropical annual halophyte *Zygophyllum simplex*. *Seed Sci. Technol.*, 25: 83-91.
- KHAN, M.A. & UNGAR, I.A. (1998a).— Seed germination and dormancy of *Polygonum aviculare* L. as influenced by salinity, temperature, and gibberellic acid. *Seed Sci. Technol.*, 26: 107-117.
- KHAN, M.A. & UNGAR, I.A. (1998b).— Germination of salt tolerant shrub *Suaeda fruticosa* from Pakistan: Salinity and temperature responses. *Seed Sci. Technol.*, 26: 657-667.
- KHAN, M.A. & UNGAR, I.A. (1999).— Seed germination and recovery of *Triglochin maritima* from salt stress under different thermoperiods. *Great Basin Naturalist*, 59: 144-150.
- KSIKSI, T.S., EL KEBLAWY, A., AL-ANSARI, F. & ALHADRAMI, G. (2006).— Artificial forest ecosystems of the UAE are hot spots for plant species. *World J. Agric. Sci.*, 2: 359-366.
- LEPORATTI, M.L. & GHEDIRA, K. (2009).— Comparative analysis of medicinal plants used in traditional medicine in Italy and Tunisia. *J Ethnobiol. Ethnomed.*, 5: 31.
- MATHEW, B. (1996).— *A review of Allium section Allium*. Royal Botanic Gardens, Kew, UK.
- NAJIAA, H., NEFFATI, M., ZOUARI, S. & AMMAR, E. (2007).— Essential oil composition and antibacterial activity of different extracts of *Allium roseum* L. - a North African endemic species. *C. R. Chim.*, 10: 820-826.
- OBERBAUER, S. & MILLER, P.C. (1982).— Effect of water potential on seed germination. *Holarctic Ecol.*, 5: 218-220.
- PARIDA, A.K. & DAS, A.B. (2005).— Salt tolerance and salinity effects on plants: a review. *Ecotox. Environ. Safe*, 60: 324-349.
- PHILIPUPALLAI, J. & UNGAR, I.A. (1984).— The effect of seed dimorphism on the germination and survival of *Salicornia europaea* L. populations. *Am. J. Bot.*, 71: 542-549.
- PHILLIPS, N.C., DROST, D.T., VARGA, W.A., SHULTZ, L.M. & MEYER, S.E. (2010).— Germination characteristics along altitudinal gradients in three intermountain *Allium* spp. (Amaryllidaceae). *Seed Technol.*, 32: 15-25.
- PUJOL, J.A., CALVO, J.F. & RAMIREZ-DIAZ, L. (2000).— Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain. *Ann. Bot.*, 85: 279-286.
- SKORDILIS, A. & THANOS, C.A. (1995).— Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis*. *Seed Sci. Res.*, 5: 151-160.
- SOSA, L., LLANES, A., REINOSO, H., REGINATO, M. & LUNA, V. (2005).— Osmotic and specific ion effect on the germination of *Prosopis strombulifera*. *Ann. Bot.*, 96: 261-267.
- SPECHT, C.E. & KELLER, E.R.J. (1997).— Temperature requirements for seed germination in species of genus *Allium* L. *Genet. Res. Crop Evol.*, 44: 509-517.
- STEARNS, W.T. (1980).— *Allium* L. Pp 49-69 In: T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters & D.A. Webb (eds). *Flora Europaea*, Vol. 5. Cambridge University Press, Cambridge.
- TRAVESSET, A., RIERA, N. & MAS, R.E. (2001).— Passage through bird guts causes interspecific differences in seed germination characteristics. *Funct. Ecol.*, 15: 669-675.
- UNGAR, I.A. (1982).— Germination ecology of halophytes. Pp 143-154 In: D.N. Sen & K.S. Rajpurohit (eds). *Contribution to the ecology of halophytes*. Junk, The Hague.
- UNGAR, I.A. (1991).— *Ecophysiology of vascular halophytes*. CRC Press, Boca Raton, FL.
- VICENTE, O., BOSCAIU, M., NARANJO, M.A., ESTRELLES, E., BELLES, J.M. & SORIANO, P. (2004).— Responses to salt stress in the halophyte *Plantago crassifolia* (Plantaginaceae). *J. Arid Environ.*, 58: 463-481.
- ZAMMOURI, J., GUETAT, A. & NEFFATI, M. 2009.— Morpho-phenological characterization of *Allium roseum* L. (Alliaceae) from different bioclimatic zones in Tunisia. *AJAR*, 4 (10): 1004-1014.