Germination responses of Mediterranean populations of Cakile maritima *to light, salinity and temperature*

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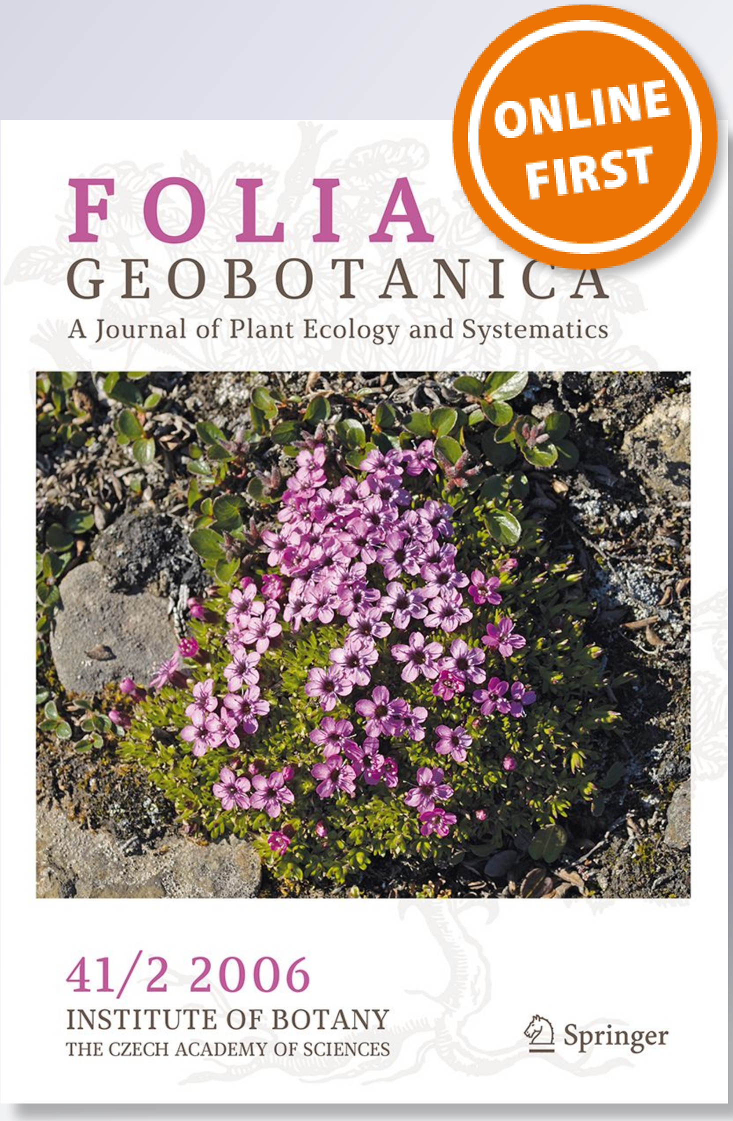

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Germination responses of Mediterranean populations of *Cakile maritima* to light, salinity and temperature

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Abstract Soil salinity is among the main factors influencing seed germination in coastal dunes, acting as a major determinant of species establishment and growth. Tolerance to salinity during the germination process is crucial especially for species of the drift line, which are exposed to high levels. *Cakile maritima* is an annual species of coastal dunes which can suffer from the effects of salt-spray and seawater inundation. We investigated the effect of light, temperature and salinity on seed germination of *C. maritima* in three populations of the Mediterranean Basin (central Italy, Sardinia and Mallorca). Results showed higher germination percentage in the dark, although the species was only weekly photoinhibited. Germination increased at 25°C and decreased under NaCl conditions. Exposure to NaCl increased mortality, especially at high temperature, suggesting a toxic effect on seeds. However, the response to salinity differed between populations, seemingly depending on the degree of aridity of collection sites: seeds from Sardinia (the most arid collection site) were the most salt-tolerant, while seeds from Mallorca (with the highest values of precipitation, especially in spring), were the least salt-tolerant. These germination patterns suggest that although being under the same macroclimate conditions, beach populations can be subjected to local conditions, such as aridity, influencing population responses at fine scale. In the context of global climate change, the investigation of germination under varying environmental factors can provide important information for understanding population dynamics, predicting the response of species to climate change and setting restoration plans.

Keywords Coastal dunes · NaCl toxicity · Population variability · Salt stress · Seed mortality

Introduction

Coastal dunes are harsh environments for the establishment and survival of plant species because of intense wind, salt-spray, occasional inundation by seawater and sand movement (Ripley and Pammenter 2008; Cogoni et al. 2013; Pinna et al. 2014; Del Vecchio et al. 2016).

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In particular, one limiting factor for species establishment and growth is represented by substrate salinity, which often affects seed germination and seedling development (Baskin and Baskin 2014; Murru et al. 2017; Santo et al. 2017).

Salinity can affect germination kinetics, delaying and slowing down the germination process, already at low concentration (100 mM; Delgado Fernández et al. 2016; Manzoor et al. 2017; Muñoz-Rodríguez et al. 2017; Santo et al. 2017). Germination usually decreases as salinity increases (Baskin and Baskin 2014). Salinity can also inhibit and suppress germination, although the intensity of the effect varies across species. Germination can be totally suppressed at higher NaCl concentrations (300–400 mM; Atia et al. 2011; Santo et al. 2014; Delgado Fernández et al. 2016), but saltmarsh halophytes can germinate up to NaCl 600 mM or 1 M (Gul et al. 2013; Muñoz-Rodríguez et al. 2017). As far as seed viability is concerned, contrasting effects have been observed. Many studies documented that high saline condition only induced secondary dormancy, as non-germinated seeds remained viable, showing complete germination recovery upon salt stress removal, or appearing viable when cut opened (e.g. Del Vecchio et al. 2012, 2013; Murru et al. 2017; Debez et al. 2018). In some halophytes, salinity exposure can even promote germination recovery (Muñoz-Rodríguez et al. 2017). In other cases, NaCl has been reported as having an irreversible effect, causing seed mortality (Saeed et al. 2011; Santo et al. 2014, 2017). However, research suggests that the effects of NaCl on seed germination depend on the species, the salt concentration, and on the interaction with several other factors such as photoperiod and temperature (Delgado Fernández et al. 2016; Murru et al. 2017; Santo et al. 2017). In particular, the toxic effect of NaCl often increases at increasing temperature (El-Keblawy and Al-Shamsi 2008; Manzoor et al. 2017; Santo et al. 2017).

The response to salinity can also vary among populations of the same species (e.g. Megdiche et al. 2007; Ghars et al. 2009; Del Vecchio et al. 2012; Santo et al. 2014, 2017). At equal concentrations of NaCl, in the range of 100–200 mM, seeds of the same species belonging to different populations showed different germination percentages. However, at higher concentrations of NaCl (400–600 mM), germination was completely inhibited regardless of the seeds' population of origin (Megdiche et al. 2007; Ghars et al. 2009; Santo et al. 2014, 2017).

Although variable depending on such environmental factors as temperature and precipitation regime (Sevink 1991), in coastal dunes the highest values of substrate salinity are normally found in the upper beach, where they can be higher than seawater concentration (Fenu et al. 2013a; Geng et al. 2016). The interaction between salinity and other environmental factors is still an open research line that needs further investigation. Thus, given the vulnerable status of coastal habitats, investigating the effect of salinity on seed germination is particularly important for those species that grow close to the coastline, i.e. the species of the drift line, which are exposed to high salt concentrations. Tolerance to salinity during the germination process is crucial especially for those species which rely on seeds for reproduction and undergo high demographic variability over time, like annual species (Fenu et al. 2013b; Huang et al. 2016).

Cakile maritima Scop. (Brassicaceae) is an annual species widely distributed in Europe, North Africa, Southwest Asia, and introduced to North America, South America and Southern Australia (Davy et al. 2006). It is a pioneer halo-nitrophilous species which grows very close to the coastline, with an average distance from the sea of 30 m (Bazzichetto et al. 2016; Silan et al. 2017). In such position, the species grows in presence of high organic matter content due to wrack deposition and has to face high salinity levels in the substrate as well as in the form of salt spray (Maun 2009). Although its growth at vegetative stage and seed production are enhanced at moderate salinity (NaCl 50–100 mM; Davy et al. 2006; Debez et al. 2006, 2012), this species is salt-sensitive during the germination phase (Debez et al. 2004, 2008, 2018; Megdiche et al. 2007; Ghars et al. 2009). While the ecology of the species has been investigated in depth (Davy et al. 2006; Megdiche et al. 2008; Debez et al. 2013; Ben Hamed-Laouti et al. 2016; Houmani and Corpas 2016; Belghith et al. 2018), the response to salinity has been mainly analysed for Tunisian populations (Debez et al. 2004, 2008, 2012, 2018; Megdiche et al. 2007; Ghars et al. 2009), while other areas of the Mediterranean Basin received less attention. Moreover, the interaction with other environmental factors such as temperature and salinity has often been overlooked, except for England, where GA_3-treated seeds were found to be less sensitive to salinity at the optimal germination temperature of 20°C (Ignaciuk and Lee 1980). Furthermore, although some authors found that *C. maritima* is strongly photoinhibited (Barbour 1970; Thanos et al. 1991; Sacheti 1998), the effect of light on the species and its interaction with temperature has been poorly investigated.

In light of these facts, the aim of this work was to investigate the response of *C. maritima* to light, temperature and salinity, and their interaction. To account for inter-population variability, we compared the behaviour of seeds coming from three different areas of the Mediterranean Basin.

Materials and methods

Seeds collection and climatic features of study sites

Seeds were collected in three sites of the Mediterranean Basin. In detail, collection sites were located along the coasts of central Italy (Torre Flavia; latitude: 41.96171366°; longitude: 12.04590248°), Sardinia (Chia: latitude: 38.88480112°; longitude: 8.86153572°) and Mallorca (Son Serra de Marina; latitude: 39.72858445°; longitude: 3.24261801°). Monthly values of temperature and precipitation (extracted from WorldClim, 1-km resolution grid; Fick and Hijmans 2017) evidenced that all the three sites have a typical Mediterranean climate with hot and dry summer and mild and rainy winter. However, Sardinia has the longest period of summer aridity (from mid-March to mid-October) whereas Mallorca has the shortest. Moreover, Mallorca is characterized by high precipitation values after winter and summer, with especially high values in spring, compared to the other sites. Central Italy shows intermediate features between the other sites. Analyses of temperature and precipitation values, and termo-pluvimetric diagrams for each site are provided in the Electronic Supplementary Material.

In each population, seeds were collected from approximately 80 randomly selected plants at the time of natural dispersal (in August), from both the upper and lower segment of the fruit. Afterwards, seeds were mixed and manually cleaned. To enable equal conditions for seeds collected in different regions, seeds were after-ripened for 90 days at room temperature (20°C and 40% relative humidity) before the experiments started.

Light and temperature requirements

To detect the best conditions for the germination of *C. maritima*, we tested the effect of temperature and light on the most abundant seed collection (central Italy). Germination tests were carried out at the Sardinian Germplasm Bank (BG-SAR) of the University of Cagliari (Italy). Four replicates of 20 seeds each were sown in Petri dishes, on 1% water agar. Seeds were incubated in growth chambers (Sanyo MLR-351, SANYO Electric Co., Ltd) at a range of constant temperatures (10, 15, 20 and 25°C), in the light (12 h irradiance per day, provided by white fluorescent lamp FL40SS.W/37 70–10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and in the dark (achieved by wrapping the dishes in two layers of aluminum foil). In the light treatment, germinated seeds were counted and removed at alternate days, while, in the dark, seeds were scored only once, at the end of the experiment, to avoid any exposure to light. The tests were ended when no additional germination occurred for 15 days, after a minimum of one month and a maximum of two months and a half from sowing. Seed viability was assessed through the cut test (Oöi et al. 2004; FAO 2014). At the end of the tests, non-germinated seeds were cut and classified as apparently viable (with firm and white embryo; hereafter 'imbibed seeds') or dead (when they collapsed if pinched gently or had grey/brownish embryo). Empty seeds were not found. Thus, the percentage of germinated, imbibed and dead seeds was calculated on the number of total seeds in each Petri dish.

The results achieved in this experiment were used to set light and temperature regimes of the next experiments, to test the effect of NaCl on seed germination and the variability among populations.

Moreover, to analyse the response to light, we calculated the 'photoinhibition of seed germination index' (Pi; Carta et al. 2017) according to the formula:

$$\text{Pi} = (\text{GD}-\text{GL})/\text{GD},$$

where GD and GL represent the final germination percentage in the dark and light, respectively. Pi varies between 0 and 1, with 0 corresponding to equivalent germination percentages in light and darkness, and 1 to germination occurring only in darkness.

Interaction of NaCl and temperature

The interaction of salinity and temperature was first analysed on the most abundant seed collections (central Italy), incubating seeds at the same temperatures (10, 15, 20 and 25°C), in the dark. Four replicates of 20 seeds each were sown in Petri dish, on 1% water agar with increasing NaCl solutions (0 mM, 125 mM and 250 mM). NaCl concentrations were selected in order to simulate seawater at 25% and 50%. Seeds were scored only once, at the end of the experiment (after a minimum of one month and a maximum of two months and a half from sowing). Non-germinated seeds were classified as imbibed or dead by the cut test.

Variability among populations

To test the effect of NaCl among populations, seeds of each site were incubated at 25°C in the dark, on 1% water agar with increasing NaCl solutions (0 mM, 125 mM and 250 mM), on the basis of the results achieved in previous experiments.

Following the same procedure used for previous tests carried out in the dark, seeds were scored only at the end of the experiment (after a minimum of one month and a maximum of two months and a half from sowing), and non-germinated seeds were classified as imbibed or dead by the cut test.

Data analysis

To analyse the germination responses of *C. maritima* to light, temperature and NaCl, we performed Generalized Linear Models (GLMs) with negative binomial error distribution and logit link function. Each model was performed on the percentages of germinated, imbibed and dead seeds as response variables, while predictors were selected according to the effect to be tested. In particular:

(I) Light and temperature were set as predictors. The models were performed on the central Italy population;
(II) Treatment (factor with three levels: Control, NaCl 125 mM and NaCl 250 mM), temperature and their interaction term (treatment × temperature) were set as predictors to test the effect of NaCl at different temperatures. The models were performed on the central Italy population;
(III) Treatment (factor with three levels: Control, NaCl 125 mM and NaCl 250 mM), population (factor with three levels: central Italy, Sardinia, Mallorca) and their interaction term (treatment × population) were set as predictors to test the effect of NaCl, population and their interactions.

When necessary, a *post hoc* Tukey test was performed to investigate if germinated, imbibed and dead seeds differed between each other within temperature, treatments and populations. All analyses were performed in R (R Core Team 2016).

Results

Light and temperature requirements

Cakile maritima germinated at all tested temperatures and photoperiods. The photoinhibition index was Pi = 0.2, showing that the species was not strongly photoinhibited. However, germination percentage was always significantly higher in the dark (Table 1), showing a mean percentage of 57.8 ± 14.8%, compared to 46.3 ± 15.5% in the light. The highest germination percentages were observed at 25°C (mean ± SD: 69.3 ± 14.6% averaged by light and darkness; $P < 0.05$ by *post hoc* Tukey tests for each comparison between results at 25°C and the other temperatures).

Light and temperature did not affect seed mortality (Table 1). Imbibed seeds significantly increased with light exposure and decreased with temperature (Table 1; percentage of imbibed seeds averaged by temperatures: 34.1 ± 17.7% in the dark and 47.5 ± 15.9% in the light).

Interaction of NaCl and temperature

Germination percentage decreased under NaCl conditions at all temperatures (Table 2; Fig. 1). Germination percentage was on average reduced by 62% at NaCl 125 mM, and by 95% at NaCl 250 mM. The effect of NaCl was greater at high temperatures, with a 78% reduction at NaCl 125mM and 100% at NaCl 250mM, at 25°C. At 15°C, seed germination at NaCl 125mM was similar to the control ($P > 0.05$ by *post hoc* Tukey tests; Fig. 1). Seed mortality increased with temperature at both NaCl concentrations and showed the highest value at 25°C, where more than 50% of non-germinated seeds were dead ($P < 0.05$ for any comparison as revealed by *post hoc* Tukey tests). At 10°C, seed mortality was not affected by NaCl ($P > 0.05$ by *post hoc* Tukey tests between control and NaCl at 125mM and 250mM; Fig. 1).

Table 1 Summary tables of GLMs testing the influence of temperature and light on the percentage of germinated, imbibed and dead seeds for the population in central Italy

	Deviance Residuals:				
Germinated seeds	**Min**	**1Q**	**Median**	**3Q**	**Max**
	−2.95	−0.5437	−0.1566	0.5996	1.4987
	Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**
	(Intercept)	3.6755	0.16543	22.218	< 0.0001 ***
	Light	−0.23099	0.09816	−2.353	0.0186 *
	Temperature	0.02157	0.00865	2.493	0.0127 *
	(Dispersion parameter for Negative Binomial (8.9601) family taken to be 1)				
	Null deviance: 40.402 on 27 degrees of freedom				
	Residual deviance: 28.641 on 25 degrees of freedom				
	AIC: 231.99				
	Number of Fisher Scoring iterations: 1				
	Theta: 21.71				
	Std. Err.: 8.37				
	2× log-likelihood: −223.995				
Imbibed seeds	**Min**	**1Q**	**Median**	**3Q**	**Max**
	−4.0494	−0.7003	0.053	0.4484	2.0111
	Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**
	(Intercept)	4.43702	0.3078	14.415	< 0.0001 ***
	Light	0.36943	0.18397	2.008	0.044631 *
	Temperature	−0.05497	0.01636	−3.359	0.000782 ***
	(Dispersion parameter for Negative Binomial (8.9601) family taken to be 1)				
	Null deviance: 47.312 on 27 degrees of freedom				
	Residual deviance: 34.963 on 25 degrees of freedom				
	AIC: 249.43				
	Number of Fisher Scoring iterations: 1				
	Theta: 4.86				
	Std. Err.: 1.65				
	2× log-likelihood: −241.428				
Dead seeds	**Min**	**1Q**	**Median**	**3Q**	**Max**
	−1.97065	−1.76258	−0.09966	0.56249	1.22992
	Coefficients:	**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**
	(Intercept)	1.01341	0.74493	1.36	0.174
	Light	−0.10982	0.43884	−0.25	0.802
	Temperature	0.05493	0.03889	1.413	0.158
	(Dispersion parameter for Negative Binomial (8.9601) family taken to be 1)				
	Null deviance: 36.855 on 27 degrees of freedom				
	Residual deviance: 34.162 on 25 degrees of freedom				
	AIC: 175.87				
	Number of Fisher Scoring iterations: 1				
	Theta: 0.857				
	Std. Err.: 0.315				
	2× log-likelihood: −167.869				

The intercept is darkness. *P*-values indicate significant differences from the intercept

Signif. Codes: 0 – ‘***’, 0.001 – ‘**’, 0.01 – ‘*’, 0.05 – ‘.’, 0.1 – ‘’

Table 2 Summary tables of GLMs testing the influence of NaCl at different temperature on the percentage of germinated, imbibed and dead seeds for the population in central Italy

	Deviance Residuals:					
Germinated seeds		**Min**	**1Q**	**Median**	**3Q**	**Max**
		−3.2643	−1.0158	−0.1141	0.2747	3.2685
	Coefficients:		**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**
	(Intercept)		3.71623	0.51248	7.251	4.12^{e-13} ***
	Temperature		0.01836	0.02574	0.713	0.4757
	NaCl 125 mM		−0.46939	0.7657	−0.613	0.53987
	NaCl 250 mM		−0.14993	0.91605	−0.164	0.86999
	NaCl 125 mM × Temperature		−0.02848	0.0403	−0.707	0.4798
	NaCl 250 mM × Temperature		−0.184	0.05441	−3.382	0.00072 ***
	(Dispersion parameter for Negative Binomial (2.3016) family taken to be 1)					
	Null deviance: 213.519 on 55 degrees of freedom					
	Residual deviance: 78.492 on 50 degrees of freedom					
	AIC: 442.83					
	Number of Fisher Scoring iterations: 1					
	Theta: 2.302					
	Std. Err.: 0.695					
	2× log-likelihood: −428.830					
Imbibed seeds		**Min**	**1Q**	**Median**	**3Q**	**Max**
		−4.0804	−0.5776	−0.0185	0.416	2.4163
	Coefficients:		**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**
	(Intercept)		5.09888	0.32345	15.764	<0.0001 ***
	Temperature		−0.0905	0.0166	−5.452	4.99^{e-08} ***
	NaCl 125 mM		−0.28786	0.47095	−0.611	0.54105
	NaCl 250 mM		−0.66238	0.46954	−1.411	0.15833
	NaCl 125 mM × Temperature		0.03891	0.02509	1.55	0.12104
	NaCl 250 mM × Temperature		0.07029	0.02492	2.821	0.00479 **
	(Dispersion parameter for Negative Binomial (6.422) family taken to be 1)					
	Null deviance: 127.277 on 55 degrees of freedom					
	Residual deviance: 72.096 on 50 degrees of freedom					
	AIC: 497.57					
	Number of Fisher Scoring iterations: 1					
	Theta: 6.42					
	Std. Err.: 1.61					
	2× log-likelihood: −483.571					
Dead seeds		**Min**	**1Q**	**Median**	**3Q**	**Max**
		−3.03661	−0.70528	−0.00535	0.46138	2.38087
	Coefficients:		**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**
	(Intercept)		0.24852	0.63278	0.393	0.6945
	Temperature		0.09838	0.03098	3.176	0.00149 **
	NaCl 125 mM		0.91164	0.87903	1.037	0.29969
	NaCl 250 mM		2.62156	0.86187	3.042	0.00235 **
	NaCl 125 mM × Temperature		0.01199	0.04503	0.266	0.79
	NaCl 250 mM × Temperature		−0.05682	0.04435	−1.281	0.20009
	(Dispersion parameter for Negative Binomial (2.1052) family taken to be 1)					
	Null deviance: 134.859 on 55 degrees of freedom					
	Residual deviance: 75.373 on 50 degrees of freedom					
	AIC: 439.36					
	Number of Fisher Scoring iterations: 1					
	Theta: 2.105					
	Std. Err.: 0.554					
	2× log-likelihood: −425.357					

The intercept is the control (NaCl = 0 mM). *P*-values indicate significant differences from the intercept. 'NaCl 125 mM' and 'NaCl 250 mM' represent the global influence of salinity at each concentration, averaged by temperature, while the interaction terms 'NaCl 125 mM × Temperature' and 'NaCl 125 mM × Temperature' represent the effect of salinity at each temperature

Signif. codes: 0 – '***', 0.001 – '**', 0.01 – '*', 0.05 – '.' 0.1 ''

Variability among populations

NaCl impaired the germination percentage in all populations, i.e. germination decreased as salinity increased (Table 3; Fig. 2). On average, germination percentage was reduced by 68% at NaCl 125 mM and by 81% at NaCl 250 mM as compared to germination in non-saline condition (control). Seed mortality increased by about 60% already at the lowest concentration of NaCl 125 mM with respect to control ($P < 0.05$ by *post hoc* Tukey test), while at NaCl 250 mM seed mortality was comparable to that observed at NaCl 125 mM (Fig. 2).

The population predictor had a significant overall effect on seed germination (Table 3; Fig. 2). In particular, seeds from Mallorca showed a significantly lower germination percentage than the other populations (11.3 ± 11.9%, compared to 30.0 ± 33.7% of central Italy and 46.7 ± 31.1% of Sardinia; $P < 0.05$ by *post hoc* Tukey test for each comparison between populations), although non-germinated seeds were imbibed, therby apparently viable (35.3 ± 24.4%). Seed mortality did not vary among populations (about 45% in all populations; $P > 0.05$ for any comparison by *post hoc* Tukey test).

The analysis of the interaction term (treatment × population) showed a different ability of seeds to tolerate salinity among populations (Table 3; Fig. 2). At NaCl 125 mM, seeds from Sardinia showed a higher germination percentage than the other two populations, with a value of 40.0 ± 10.8%, compared to 16.3 ± 8.5% of central Italy, while seeds from Mallorca did not germinate. At NaCl 250 mM, only seeds from Sardinia germinated (17.5 ± 18.9%).

Discussion

Our research showed that *C. maritima* was able to germinate at different conditions of temperature, photoperiod and salinity. The best condition for germination was in the dark, at 25°C, under non-saline condition.

Cakile maritima has been described as highly sensitive to light which suppresses or delays germination (Barbour 1970; Thanos et al. 1991; Sacheti 1998; Carta et al. 2017). Photoinhibition is a common strategy in arid and seasonal climates, and in coastal habitats (Carta et al. 2017), to prevent seed germination at the substrate surface thereby protecting seeds and seedling from dessication (Thanos et al. 1991). In our study, although the germination percentage was significantly higher in the dark, we found a very low Pi value which, according to Carta et al. (2017), allows to define *C. maritima* as a weakly photoinhibited species. In line with the literature (Ignaciuk and Lee 1980; Baskin and Baskin 2014; Fernández-Pascual et al. 2017), we found that *C. maritima* is a warm-cued germination species, with higher germination percentage at high temperature, in the range of 20–25°C. Nevertheless, it showed a germination capacity at any tested temperature, without any pre-treatment (seed scarification or cold stratification). However, in some species, seeds stored under controlled conditions of temperature and humidity for a period longer than one month can germinate at a wider range of photoperiod and temperature than fresh seeds (Probert 2000; Finch-Savage et al. 2007). The weak photoinhibition, the ability to germinate at any tested temperature and the presence of imbibed non-germinated seeds detected at the end of germination test (under no stress condition) found in this study, suggest a possible positive effect of a period of after-ripening on the germination of *C. maritima*. In light of our results, the effect of after-ripening on this species should be further investigated in order to elucidate its role in germination.

NaCl decreased the germination percentage. This is consistent with other researches showing the salt-sensitivity of *C. maritima* during seed germination (Debez et al. 2004, 2018; Megdiche et al. 2007; Ghars et al. 2009). High salinity inhibits and delays germination (Debez et al. 2018) generating unfavourable osmotic conditions which prevent seed imbibition (Ghars et al. 2009). However, several studies consistently report that moderate salt treatments inhibit germination without damaging the seeds which remain viable and able to resume germination upon stress release, with toxicity symptoms (i.e. increasing seed mortality) appearing only at severe salinity (in the range of 300–400 mM NaCl; Debez et al. 2004; Megdiche et al. 2007; Ghars et al. 2009). This would suggest osmotic constraints rather than toxicity of salinity and, most importantly, that *C. maritima* would be able to produce seeds able to tolerate high salinity levels, thereby remaining viable and germinate early in the spring, after winter rains (Debez et al. 2004; Davy et al. 2006; Ghars et al. 2009). However, as compared to non-saline conditions, we found increased seed mortality already at moderate concentration of NaCl (125 mM). The increased negative effect we detected could be a consequence of a long exposure to salinity, as suggested by Ghars et al. (2009).

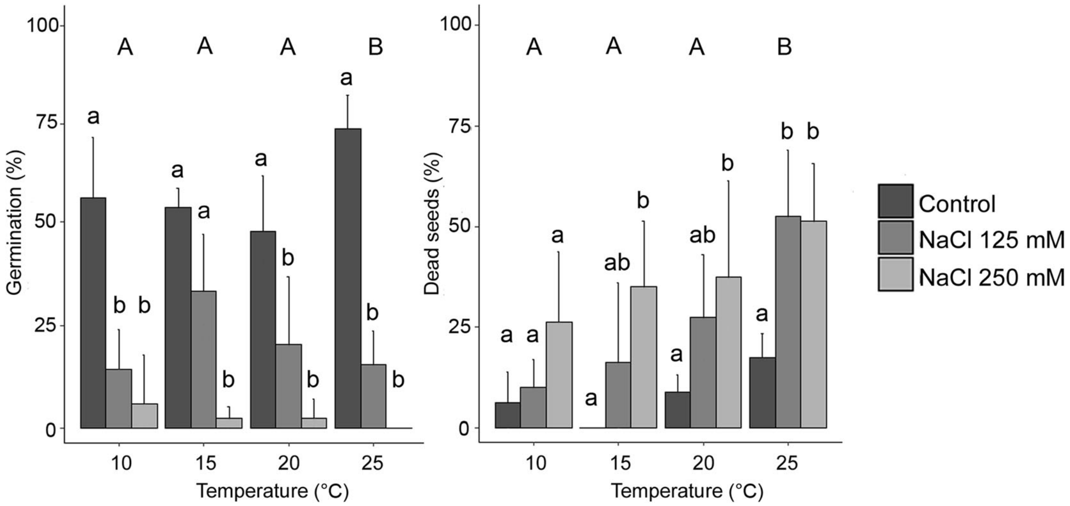

Fig. 1 Seed germination and mortality (mean ± SD) of *Cakile maritima* from the population in central Italy under increasing NaCl concentrations and at all tested temperatures. Different lowercase letters indicate significant differences between NaCl concentrations under the same temperature according to a *post hoc* Tukey test. Different uppercase letters indicate significant differences between temperatures

Indeed, non-germinated seeds exposed to NaCl were classified as viable in germination experiements that lasted for a maximum of two weeks (Megdiche et al. 2007; Ghars et al. 2009; Debez et al. 2004, 2018), while in our study the duration of the experiments had a minimum elapse of one month. Although our results were achieved in a laboratory, we cannot exclude that long exposure to salinity may represent a limiting factor in the field. Indeed, the marked seasonality of seed collection sites and *C. maritima* life cycle support this hypothesis, since germination normally occurs in the period with lower salinity levels in the substrate, i.e. in autumn and/or spring, after receiving adequate rainfall to leach out the salt (Davy et al. 2006; Contreras-Cruzado et al. 2017). Moreover, our results suggest that long aridity periods after seed dispersal may limit salt leaching thereby affecting the viability of seeds.

The interaction between salinity and temperature showed that seeds were more resistant to salinity at cool temperature (10–15°C) and that the negative effect of salinity increased with increasing temperature, a pattern also observed in other species (Santo et al. 2014, 2017; Delgado Fernández et al. 2016). In arid and semi-arid habitats, such as coastal habitats, soil salinization represents a limiting factor for species establishment and growth (Bui 2013; Parihar et al. 2015). Its negative effect on seed germination and viability when combined with high temperature can represent a further selective force for species survival, and consequently for species distribution.

The response to salt stress also differed between populations. It has been reported that *C. maritima* has a great genetic diversity due to adaptation to different ecological conditions, and, consequently, its populations may have different optimal temperatures and photoperiods for germination (Debez et al. 2013; Belghith et al. 2018) and different levels of salt sensitivity. Differences in the germination response of *C. maritima* to NaCl have been mainly attributed to different climatic conditions of collection sites. In particular, it has been suggested that salt tolerance is controlled by the degree of aridity with most salt-tolerant populations found in the most arid sites (Megdiche et al. 2007; Ghars et al. 2009). In accordance with these findings, in our study the most salt-tolerant seeds were those from Sardinia, which is characterized by the longest aridity period. Correspondingly, seeds collected in Mallorca, which has the highest values of precipitation (especially in spring), were the lowest salt-tolerant. Because of the ameliorating effect of the sea on local climate, beach species tend to have a worldwide distribution and have been described as almost azonal in their occurrence (e.g. Wiedemann and Pickart 2004; Del Vecchio et al. 2018), i.e. as linked to non-climatic environmental stress factors or extreme soil conditions (Walter

Table 3 Summary tables of GLMs testing the influence of NaCl and population on the percentage of germinated, imbibed and dead seeds

	Deviance Residuals:					
Germinated seeds		**Min**	**1Q**	**Median**	**3Q**	**Max**
		−2.30771	−0.35459	−0.00006	0.0941	2.8455
	Coefficients:		**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**
	(Intercept)		4.3007	0.1769	24.312	< 0.0001 ***
	NaCl 125 mM		−1.5126	0.2731	−5.539	< 0.0001 ***
	NaCl 250 mM		−24.6033	7,770.9319	−0.003	0.9975
	Sardinia		0.1121	0.2494	0.449	0.6531
	Mallorca		−1.4385	0.2711	−5.307	< 0.0001 ***
	NaCl 125 mM × Sardinia		0.7887	0.3737	2.11	0.0348 *
	NaCl 250 mM × Sardinia		23.0527	7,770.9319	0.003	0.9976
	NaCl 125 mM × Mallorca		−21.6522	7,770.9319	−0.003	0.9978
	NaCl 250 mM × Mallorca		1.4385	10,989.7573	0.000	0.9999
	(Dispersion parameter for Negative Binomial (8.9601) family taken to be 1)					
	Null deviance: 441.799 on 35 degrees of freedom					
	Residual deviance: 28.171 on 27 degrees of freedom					
	AIC: 210.71					
	Number of Fisher Scoring iterations: 1					
	Theta: 8.96					
	Std. Err.: 3.97					
	2× log-likelihood: −190.712					
Imbibed seeds		**Min**	**1Q**	**Median**	**3Q**	**Max**
		−2.53602	−0.55201	−0.09954	0.27273	2.17509
	Coefficients:		**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**
	(Intercept)		2.16905	0.40596	5.343	< 0.0001 ***
	NaCl 125 mM		1.27297	0.55592	2.29	0.02203 *
	NaCl 250 mM		1.71765	0.55333	3.104	0.00191 **
	Sardinia		−0.55962	0.59249	−0.945	0.34491
	Mallorca		1.74297	0.55321	3.151	0.00163 **
	NaCl 125 mM × Sardinia		−0.71335	0.81246	−0.878	0.37994
	NaCl 250 mM × Sardinia		0.07411	0.79811	0.093	0.92602
	NaCl 125 mM × Mallorca		−1.49611	0.76991	−1.943	0.05199
	NaCl 250 mM × Mallorca		−2.15616	0.76902	−2.804	0.00505 **
	(Dispersion parameter for Negative Binomial (1.8351) family taken to be 1)					
	Null deviance: 80.653 on 35 degrees of freedom					
	Residual deviance: 49.613 on 27 degrees of freedom					
	AIC: 312.96					
	Number of Fisher Scoring iterations: 1					
	Theta: 1.835					
	Std. Err.: 0.608					
	2× log-likelihood: −292.964					
Dead seeds		**Min**	**1Q**	**Median**	**3Q**	**Max**
		−4.1482	−0.5781	0.1057	0.6702	1.3126
	Coefficients:		**Estimate**	**Std. Error**	**z value**	**Pr(>|t|)**
	(Intercept)		2.8622	0.1865	15.345	< 0.0001 ***
	NaCl 125 mM		1.0986	0.2451	4.483	< 0.0001 ***
	NaCl 250 mM		1.0745	0.2453	4.38	< 0.0001 ***
	Sardinia		−0.3365	0.2744	−1.226	0.2201
	Mallorca		0.619	0.251	2.466	0.0136 *
	NaCl 125 mM × Sardinia		0.3124	0.3549	0.88	0.3788
	NaCl 250 mM × Sardinia		0.3606	0.3549	1.016	0.3096
	NaCl 125 mM × Mallorca		−0.4855	0.3361	−1.445	0.1485
	NaCl 250 mM × Mallorca		−0.3399	0.3355	−1.013	0.311
	(Dispersion parameter for Negative Binomial (12.1913) family taken to be 1)					
	Null deviance: 125.827 on 35 degrees of freedom					
	Residual deviance: 46.591 on 27 degrees of freedom					
	AIC: 311.83					
	Number of Fisher Scoring iterations: 1					
	Theta: 12.19					
	Std. Err.: 4.23					

Control (NaCl = 0 mM) and the central Italy population are the intercept. *P*–values indicate significant differences from the intercept

Signif. codes: 0 – '***', 0.001 – '**', 0.01 – '*', 0.05 – '.' 0.1 ''

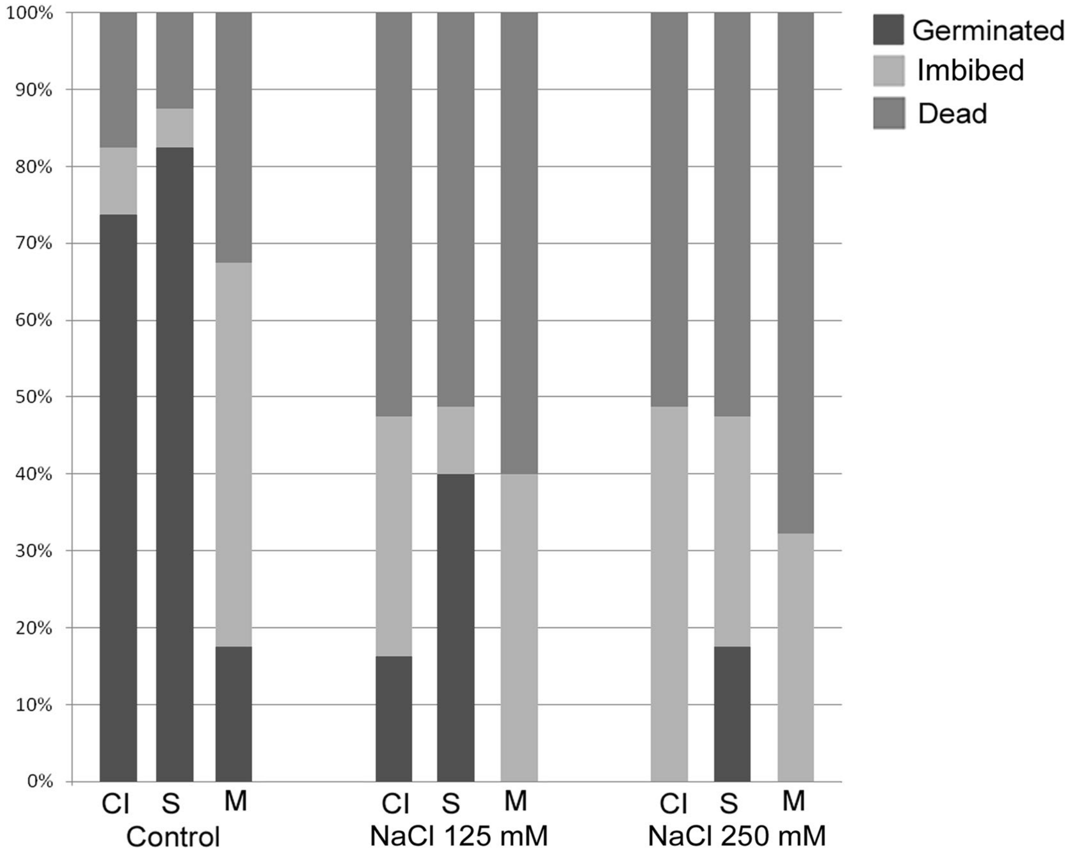

Fig. 2 Proportion (mean values) of germinated, imbibed and dead seeds for each treatment and population

1985). However, our results, which confirm at a wider extent those of Megdiche et al. (2007) and Ghars et al. (2009), suggest that although being under the same macroclimate conditions, beach populations can be subjected to local drivers, such as aridity, that can influence population responses at a finer scale.

In conclusion, our findings support the view that *C. maritima* has a higher germination percentage in the dark and at high temperature. Moreover, we found that prolonged exposure to salinity, caused e.g. by extended aridity periods, may affect seed viability and that this effect is stronger at high temperatures. Our results are particularly challenging in the context of global climate change, especially in arid and semiarid regions of the world, which are likely to be highly sensitive to climate modification. From a conservation perspective, research on germination ecology, such as that presented here, may contribute to the development of restoration plans and monitoring programmes aimed towards habitat conservation. Moreover, investigations of germination under varying environmental factors can provide important information for understanding patterns and processes in population dynamics, plant community ecology, and predicting the response of species to climate change (Jiménez-Alfaro et al. 2016; Pierce et al. 2018).

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