

# Germination and recovery of heteromorphic seeds of *Atriplex canescens* (Amaranthaceae) under increasing salinity

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**Abstract** This study was conducted in order to determine the role of the two seed morphs, observed for the first time in a desert population of the widespread *Atriplex canescens* (Amaranthaceae), in regard to their germination requirements, salinity tolerance and recovery after salt exposure during the seed germination stage. Seeds of the two produced colours (brown and black) were germinated in laboratory conditions under two photoperiods (12/12-h light and continuous dark), three alternating temperature regimes (15/25, 20/30 and 25/35 °C), and several salt concentrations (0, 100, 200, 400, 600, 800 mM NaCl), in order to check the salinity tolerance of each chromatic category and its recovery. Mean seed mass of brown seeds was significantly higher than that of black ones. Brown seeds did not show dormancy on the contrary of black seeds. For both colours, light and temperature did not affect seed germination. Very few seeds germinated when they

were exposed to salinity, even at the lowest NaCl concentration; however, all seeds were able to recover their germinability once they were transferred to distilled water. Our results may indicate that both the coloured seed categories could remain viable in saline conditions and they will be able to germinate once the salinity level decreases due to rains. Seed colour variation could be a survival strategy of *A. canescens* in the unpredictable desert environment where this species may grow, although the causes of the occurrence of this phenomenon in this species only for this observed desert population need to be further deepened.

**Keywords** Amaranthaceae · Desert · Germination · NaCl · Salinity · Seed colour

## Introduction

Seed heterogeneity or heteromorphy, defined as the production of different types of seeds by a single individual, appears in many species of angiosperms (Matilla et al. 2005). Moreover, production of seeds with different morph, size, shape or testa colour within the same species (Santo et al. 2015a), especially in Amaranthaceae, Brassicaceae, Caryophyllaceae and Poaceae (Matilla et al. 2005), has been reported (Mira et al. 2011). Heteromorphy in seeds may affect physiological properties, being associated with ecological strategies that have evolutionary significance,

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including dormancy (Duràn and Retamal 1989), germination (Puga-Hermida et al. 1997) and longevity behaviour (Diederichsen and Jones-Flory 2005). Heteromorphic seeds showed also variation in dispersal ability as well as in their ability to persist in soil seed bank (El-Keblawy and Bhatt 2015). The production of heteromorphic diaspores is one of the most effective strategies for adaptation and increasing the reproductive success of desert plants, under unpredictable climatic conditions (Harper 1977). Previous studies reported that temporal and spatial environmental fluctuations favour bet-hedging strategies that allow the individuals to optimize their fitness in variable and unpredictable environmental conditions (Carter and Ungar 2003; Minissale et al. 2011; Bhatt et al. 2016).

*Atriplex* L. is one of the most important genera of Amaranthaceae and many *Atriplex* species show tolerance to salinity and drought in arid and desert regions (Busso and Bonvissuto 2009). Most of the members of the family Amaranthaceae produce dimorphic seeds, where seed morphs differ in germination characteristics and ability to tolerate salinity (Khan and Gul 1998). Consequently, seed dimorphism might help population survival by avoidance or tolerance of salinity in saline habitat conditions (Khan and Ungar 1986).

*Atriplex canescens* (Pursh) Nutt. (Amaranthaceae) is a wind-pollinated evergreen shrub native to the western and mid-western United States. This species is the most widespread North American saltbush, extending from Sonora, Mexico, to Alberta, Canada, and from sea level to 2500 m. It grows in sandy to heavy clay soils and under salinity regimes ranging from non-saline to sea-water saturated soils in the intertidal zones of desert estuaries (Glenn et al. 1996). This species is well adapted and naturalized also in the Middle East and in Egypt (Al-Turki et al. 2000). Winged fruits of *A. canescens* help seeds to be dispersed for long-distance by wind (Henrickson 1974). This species is considered an important food source for both domestic and wild animals in arid and semi-arid regions of the world due to the presence of higher protein content in its foliage (Ortíz-Dorda et al. 2005). Moreover, this species could be used as screens, hedges and barriers, especially on saline-sodic soils and has the potential to be used for conservation and recovery of eroded lands (Romero Paredes Rubio and Ramírez Lozano 2003). *A.*

*canescens* has also been recommended as an excellent phytoremediation plant in saline-alkali and heavy-metal contaminated lands (Benzarti et al. 2013).

Seed germination is one of the most important and vulnerable stages in the plant life cycle and is influenced by several factors, including light, temperature and salinity (Baskin and Baskin 2014). Light plays a crucial role in optimizing the time of seed germination (Baskin and Baskin 2014) and its presence could either inhibit germination completely (Benvenuti et al. 2004), partially (Zia and Khan 2004) or have no effect (Wei et al. 2008). Temperature can also interact with light, thereby modifying the seed sensitivity to this last factor (Sugahara and Takaki 2004). Salt stress can cause changes in the germination regulators balance, inducing a secondary physiological dormancy (Ungar 1978). In particular, salt may inhibit seed germination, either by creating a low osmotic potential, which prevents water uptake, or through the toxic effects of  $\text{Na}^+$  and  $\text{Cl}^-$  ions on the metabolic processes (Kaya et al. 2006). Seeds that are unable to germinate at high salinity levels might survive during salt exposure and maintain the ability to germinate later (recovery), when salinity decreases due to various environmental events (Baskin and Baskin 2014; Murru et al. 2015). Seeds of several species treated with high salt concentrations germinated after the transfer to distilled water. However, the ability of seeds to recover is species-specific (Song et al. 2005).

Germination responses of dimorphic seeds have been tested in some *Atriplex* species, such as *A. triangularis* Willd. (Khan and Ungar 1984), *A. patula* L. (Ungar 1996), *A. prostrata* Bouchér ex DC. (Katembe et al. 1998; Carter and Ungar 2003), *A. rosea* L. (Khan et al. 2004) and *A. sagittata* Borkh. (Mandak and Pysek 2005). Physiologic effects of activities of protective enzymes on seed germination in *A. canescens* under NaCl and  $\text{Na}_2\text{NO}_3$  stress were investigated by Wang et al. (2011), mainly focusing on seedling growth of this species. Another study (Wang et al. 2010) investigated *A. canescens* seed germination but testing frozen or 10–22 months collected seeds, differently from the present study, in which we considered only freshly collected seeds. However, the role of different seed morphs of *A. canescens* has not been investigated so far, and its occurrence, in our knowledge, was not reported before. We hypothesized that the different seed colours in this species might

have different germination requirements that may play a role in the survival of this species, in particular when it grows in harsh desert habitats. Moreover, in function of the germination response of the two seed colours, our results could give information about the potentialities for the cultivation of this species for food purpose also in hyper-salt soils, in function of seed morphs tolerance to salinity.

To test this hypothesis, we examine (1) whether the two seed chromatic categories of *A. canescens* display any difference in their germination behaviour relatively to light and temperature conditions, (2) whether the two seed morphs show differences in their salinity tolerance and (3) whether they can maintain the viability when exposed to high salinity stress and then recover their ability to germinate when incubated in distilled water.

## Materials and methods

### Seed collection and seed lot details

Fruits of *A. canescens* were collected during April 2014 from plants growing near Shahniya Nursery [Doha, Qatar ( $25^{\circ}27'39''\text{N}$ – $51^{\circ}11'22''\text{E}$ )]. Seeds were randomly collected from 49 individuals, distant at least five metres among themselves, to represent the genetic diversity of the population in the collected seed lots. Seeds were removed from their wings, cleaned by hands, then separated into brown and black seeds and immediately stored in paper bags in the laboratory at room temperature ( $20 \pm 2^{\circ}\text{C}$ ) and total darkness for less than 1 month, before the starting of germination tests. The seed mass was determined by weighing three replicates, each of 50 seeds, for each seed chromatic category.

### Effect of light and temperature on seed germination

To examine the effect of light and temperature requirements during germination, seeds of each colour were incubated in incubators (LMS, UK) at daily (12/12 h) temperature regimes of 15/25, 20/30 and 25/35 °C in 12 h dark/12 h light and continuous darkness. The lower temperature of each cycle corresponded to the night time, while the higher to the day time. Darkness was attained by wrapping,

immediately after the seeds sown, two layers of aluminium foil around the Petri dishes, to stop a light response as the seeds imbibed water. Four replicates of 25 seeds each were used for each treatment and colour. Petri dishes were sealed with parafilm (Brand parafilm, Sigma-Aldrich, UK) to minimize evaporation of water and external contaminations. Germination tests were conducted in 9-cm tight-fitting Petri dishes containing one disc of Whatman No. 1 filter paper moistened with 10 ml of distilled water. Germinated seeds were counted and removed every day per 1 month in the light treatments and at the end of the experiment (after 30 days) in the dark treatments. Seeds were considered to be germinated with the emergence of the radicle ( $\geq 2$  mm). At the end of germination tests, a cut-test with scalpel to evaluate the embryo status (living and therefore white and turgid or brown and therefore died) under a binocular microscope was carried out to evaluate the viability of ungerminated seeds.

### Effect of salinity on seed germination

To assess the effect of seed morphs on salinity tolerance during germination, both brown and black seeds were sown under six different salinity concentrations (0, 100, 200, 400, 600 and 800 mM NaCl). Four replicates of 25 seeds each were used for each treatment. Seeds were germinated in 9-cm-diameter Petri dishes on two layers of Whatman No. 1 filter paper, moistened with 10 ml of the test solution. Petri dishes were sealed with parafilm and incubated at the alternating temperature regime of 25/35 °C in 12 h dark/12 h light and continuous darkness. This temperature was chosen because it corresponded to a high germination velocity for this species during preliminary experiments. For the dark treatment, the dishes were wrapped in aluminium foil to prevent any exposure to light. Petri dishes were then incubated in 12 h dark/12 h light and monitored per 30 days and the number of germinated seeds was recorded daily.

After 30 days, all seeds that failed to germinate under light (12 h light/12 h darkness) and dark treatments after being exposed to the different NaCl concentrations were transferred to distilled water. In particular, all seeds of each individual Petri dish were transferred to another Petri dish with distilled water. Germinated seeds were recorded per 10 days

(recovery phase) of incubation in the 12/12 light photoperiod.

### Data analysis

Final germination percentages were calculated as the mean of four replicates ( $\pm 1$  standard deviation, hereafter SD), while recovery percentages (hereafter RP) according to the following equation (Pujol et al. 2000):  $RP = \{[(a-b)/(c-b)] \times 100\}$ , where  $a$  is the total number of seeds germinated in the salt solutions plus those that recovered to germination in the distilled water,  $b$  is the total number of seeds germinated in saline solutions, and  $c$  is the total number of seeds. The rate of germination was estimated by using the Timson's index of germination velocity (TI) (Santo et al. 2015b):

$TI = \sum G/t$ , where  $G$  is the percentage of seed germination at 1-day interval and  $t$  is the total germination period. Using this index, a higher value indicates more rapid germination. For all the data, the normality values were analysed by the Shapiro–Wilk test. Seed mass values, arcsine-transformed germination percentages of light and temperature experiments and  $\log_{10}$ -transformed TI were analysed by ANOVA and consequent Fisher's least significant differences (LSD) post hoc test. Germination percentages in salt conditions and RP were analysed by the non-parametric Kruskal–Wallis test, followed by a Mann–Whitney  $U$  test, due the non-satisfaction of the ANOVA assumptions neither after arcsine transformation. All graphs were made using Sigmaplot 11.0 (Systat Software Inc., London, UK), while all the statistical analyses were carried out using the statistical software Statistica 7.0 for Windows (Software Statsoft Release 7).

## Results

### Mean seed mass

The one-way ANOVA showed highly significant differences ( $p < 0.001$ ) among the two chromatic categories. Mean seed mass of brown seeds ( $1.06 \pm 0.03$  mg) was significantly higher ( $p < 0.05$ ) than that of black seeds ( $0.85 \pm 0.01$  mg).

### Effect of light and temperature on seed germination and its rate

For both photoperiods (12 h dark/12 h light and 24 h dark), brown seeds showed a significantly higher ( $p < 0.05$ ) final germination in all the three tested temperature regimes, respect to black seeds (Fig. 1). The three-way ANOVA showed a highly significant effect of seed morph ( $p < 0.001$ ) and light ( $p < 0.01$ ) on final germination, but not of temperature ( $p > 0.05$ ) to which seeds were incubated. For each seed morph, significant differences ( $p < 0.05$ ) in the final germination percentages were detected only at 25/35 °C between light- and dark-incubated seeds. However, none of the interactions (T  $\times$  C; T  $\times$  L; C  $\times$  L; T  $\times$  C  $\times$  L) among the three factors were significant ( $p > 0.05$ , Table 1).

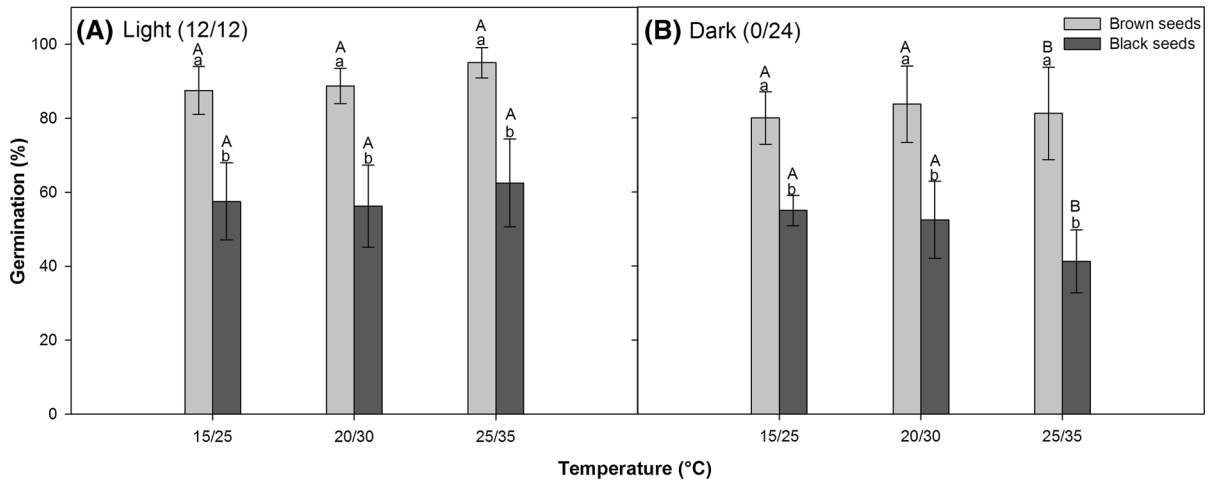
On the germination rate, both the seed colour and temperature had a significant effect ( $p < 0.01$  and  $p < 0.001$ , respectively), as well as their interaction ( $p < 0.001$ , by three-way ANOVA).

Brown seeds germinated faster respect to black ones at all the three tested alternating temperatures (Fig. 2). Moreover, the germination rate ranged from TI values of ca. 12 at 15/25 °C to ca. 18 at 25/35 °C for brown seeds. Differently, for black seeds, no differences were observed among the TI values at the three temperatures (TI of ca. 10 for all, Fig. 2). All non-germinated seeds were cutted with a scalpel at the end of the germination tests and observed under a binocular microscope, resulting imbibed and no died seeds were recorded, showing as black seeds had from 40 to 50 % of dormant seeds, while brown ones, germinating with higher percentages, did not show the phenomenon of the seed dormancy.

### Effect of salinity on seed germination

For both brown and black seeds, the highest germination ( $95.0 \pm 4.1$  and  $62.5 \pm 11.9$  % in the light, for the two colours, respectively) occurred in distilled water (0 mM NaCl) in the two tested photoperiods and significantly ( $p < 0.001$ , by Kruskal–Wallis test) decreased (germination percentages not higher than 10 % also at the lowest NaCl concentration) with increasing salinity for both colours (Figs. 3, 4).

At 0 mM NaCl, significantly higher ( $p < 0.05$ ) germination percentages were detected for brown seeds respect to the black ones, both in the 12/12



**Fig. 1** Final germination of *brown* and *black* seeds of *Atriplex canescens* **a** in *light* (12/12) and **b** *dark* (0/24) conditions. Data are the mean ( $\pm$ SD) of four replicates. Bars with the same letters are not significantly different at  $p < 0.05$  (three-way ANOVA followed by Fisher’s least significant difference (LSD) post hoc

test). Lower-case letters were used to compare final germination between the two colours at the same temperature in the same photoperiod condition (12/12 or 0/24), while capital letters were used to compare final germination between the two photoperiods (12/12 and 0/24) for the same colour

**Table 1** Effect of temperature (T), colour (C), light (L) and their interactions on the final germination (A) and germination rate (B) of *Atriplex canescens* seeds

Effect	SS	DF	MS	F	p
<b>(A)</b>					
Intercept	163600.1	1	163600.1	3805.277	***
Temperature (T)	17.3	2	8.7	0.202	ns
Colour (C)	5722.3	1	5722.3	133.098	***
Light (L)	521.8	1	521.8	12.137	**
T $\times$ C	123.0	2	61.5	1.430	ns
T $\times$ L	253.9	2	127.0	2.953	ns
C $\times$ L	16.0	1	16.0	0.372	ns
T $\times$ C $\times$ L	7.7	2	3.8	0.089	ns
Error	1547.7	36	43.0		
<b>(B)</b>					
Intercept	3668.307	1	3668.307	1919.181	***
Temperature (T)	35.106	2	17.553	9.183	**
Colour (C)	225.824	1	225.824	118.146	***
T $\times$ C	52.738	2	26.369	13.796	***
Error	34.405	18	1.911		

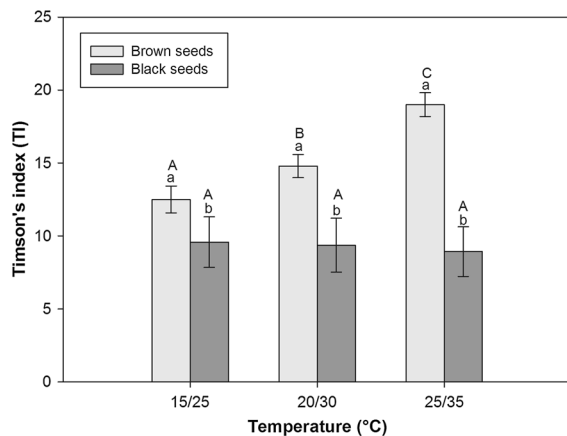
*p* values were considered not significantly ( $p > 0.05$ , ns), significantly (\*\*  $p < 0.01$ ) or highly significantly (\*\*\*)  $p < 0.001$ ) different, by three-way ANOVA. SS sum of squares, DF degrees of freedom, MS mean square, F fisher variable, *p* *p* value

photoperiod than in the total darkness. In the light, brown seeds were able to germinate up to 200 mM ( $6.25 \pm 2.5$  %), but in the dark they germinated only up to 100 mM ( $2.5 \pm 2.9$  %). Black seeds germinated up to 600 mM NaCl ( $1.2 \pm 2.5$  %) in the light, but their germination in the dark did not exceed 100 mM ( $1.2 \pm 2.5$  %) of salinity (Fig. 3). However, the germination observed at the two salt tolerance limits

for the two colours (up to 200 mM for brown seeds and 600 mM for black) was not statistically different ( $p > 0.05$ ).

**Germination recovery**

Brown seeds recovered their germination also when subjected to the highest NaCl concentration (800 mM)



**Fig. 2** Germination rate of *brown* and *black* seeds of *Atriplex canescens* (A) at the three tested alternating temperatures (15/25, 20/30 and 25/35 °C) in the *light* (12/12 h). Data are the mean ( $\pm$ SD) of four replicates. Bars with the same *letters* are not significantly different at  $p < 0.05$  (two-way ANOVA followed by Fisher's least significant difference (LSD) post hoc test). *Lower-case letters* were used to compare germination rate between the two *colours* at the same temperature, while *capital letters* were used for each colour to compare the germination rate between the three temperatures

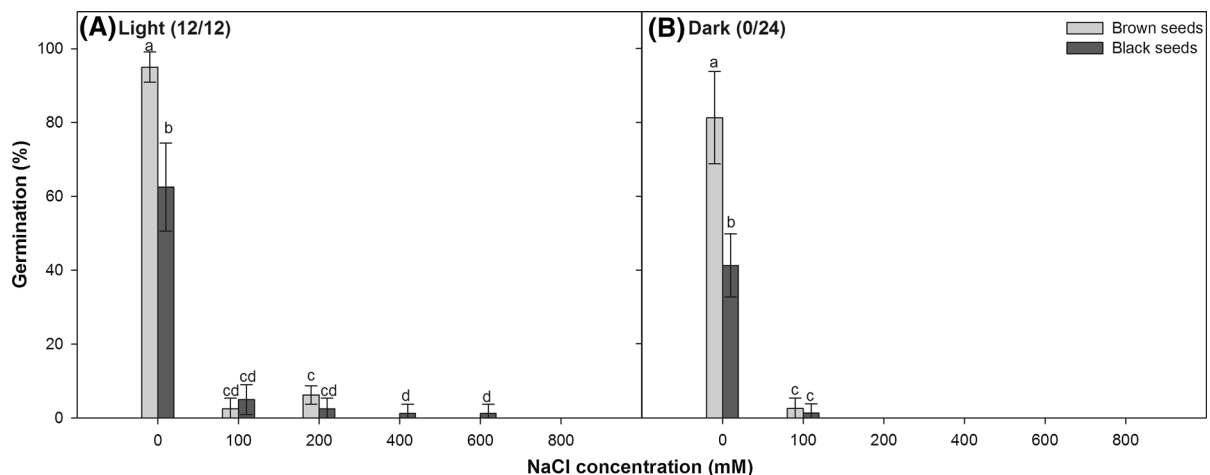
in the previous NaCl phase. Black seeds showed a recovery performance after the salt exposure similar to that detected from brown seeds, although with lower values respect to brown seeds (Fig. 4). Significant differences ( $p < 0.05$ , by Kruskal–Wallis test) were detected among the RP under different NaCl

concentrations for both brown and black seeds in the *light* (12/12; Table 2).

For each colour and under the two tested photoperiods, RP decreased with increasing salt concentration to which seeds were exposed in the previous NaCl phase. Similarly, in the total darkness (0/24), RP of each colour detected at different salinities differed significantly ( $p < 0.01$ , by Kruskal–Wallis test). In the *light* cycle (12/12), at each NaCl concentration, the recovery percentages of brown seeds were significantly higher ( $p < 0.05$ , by Mann–Whitney *U* test) respect to that of black seeds. In the darkness (0/24), significant differences among RP of the two seed colours were detected only at 100, 200 and 400 mM NaCl, while RP of the two chromatic categories were statistically similar ( $p > 0.05$ , by Mann–Whitney *U* test) at the highest salinities (600 and 800 mM NaCl; Table 2).

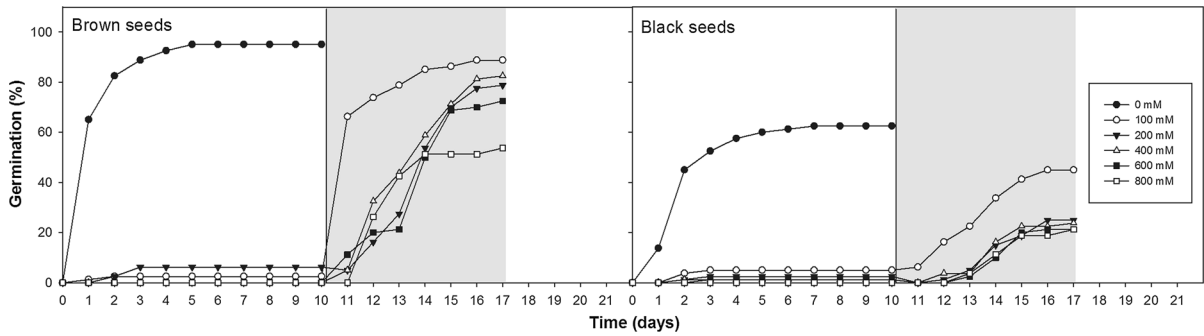
## Discussion

The present study investigated the germination requirements of the two seed morphs (*brown* and *black*) produced by *A. canescens*. These two distinct types of seeds differed in their mass, dormancy, final germination and germination rate. Seed germination of *A. canescens* was affected by seed size, indeed brown seeds (with higher seed mass) showed significantly higher germination in all the tested temperatures



**Fig. 3** Final germination of *brown* and *black* seeds of *Atriplex canescens* in different NaCl concentrations (0–800 mM), both **a** in *light* (12/12 h) and **b** *dark* (0/24 h) conditions. Kruskal–Wallis test was conducted to detect significant differences

among germination percentages of the two colours in *light* and in the *dark*. Bars with different *letters* are significantly different at  $p < 0.05$  (by Mann–Whitney *U* test). Data are the mean ( $\pm$ SD) of four replicates



**Fig. 4** Cumulative germination percentages in the light (12/12) at the tested alternating temperature regime (25/35 °C), under different saline conditions (0–800 mM NaCl) and following transfer to distilled water (recovery phase, indicated by the shaded area in the graph) for brown and black seeds of *Atriplex canescens*. Each point represents the mean ( $\pm 1SD$ ) of four

replicates. *Error bars* are not showed for clarity of the graphs and avoid overlaps, but they did not exceed 14 %. In the figure are represented, both for salt phase (white) and recovery phase (shaded) area only the period time in which variations in seed germination were recorded

**Table 2** Recovery percentages (RP) of brown and black seeds of *Atriplex canescens* in the two tested photoperiods (12/12 h and 0/24 h)

Colour	Photoperiod	NaCl concentration (mM)					
		100	200	400	600	800	
Brown	12/12	91.1 $\pm$ 8.5 aA	87.8 $\pm$ 8.3 aA	83.7 $\pm$ 7.5 aA	72.5 $\pm$ 14.4 abA	53.7 $\pm$ 13.1 bA	*
Black	12/12	47.5 $\pm$ 8.3 aB	25.7 $\pm$ 4.7 bB	24.0 $\pm$ 1.9 bB	21.4 $\pm$ 8.3 bB	21.2 $\pm$ 2.5 bB	*
		*	*	*	*	*	
Brown	0/24	87.2 $\pm$ 4.8 aA	77.5 $\pm$ 8.6 aA	51.2 $\pm$ 6.3 bA	41.2 $\pm$ 4.8 cA	22.5 $\pm$ 6.4 dA	**
Black	0/24	56.8 $\pm$ 7.5 aB	53.7 $\pm$ 7.5 aB	26.2 $\pm$ 7.5 bB	25.0 $\pm$ 10.0 bA	16.2 $\pm$ 11.1 bA	**
		*	*	*	ns	ns	

Kruskal–Wallis tests were conducted to detect the effect of the same photoperiod and salinity concentration on RP for each colour; [*p* values were considered not significantly ( $p > 0.05$ , ns) and significantly different ( $* p < 0.05$ ;  $** p < 0.01$ ), by Kruskal–Wallis test]

Data are the mean of four replicates ( $\pm 1SD$ )

Capital letters in columns are related to the same NaCl concentration of the two colours at the same photoperiod, while lower-case letters in rows to the same photoperiod of each colour at different NaCl concentrations

Values with different letters were used to indicate significant differences at  $p < 0.05$  (by Mann–Whitney *U* test)

compared to black seeds (lower seed mass). These findings suggest that increasing seed mass has a positive effect on *A. canescens* seed germination. Similar results have been reported for other species where larger seeds showed better germination respect to smaller ones (Shaukat et al. 1999; Kidson and Westoby 2000). The variation in germination response of different colours and seed masses may have ecological significance in desert conditions and it might allow the species to colonize different habitats and expand their geographic distribution limit (Mendes-Rodrigues et al. 2011; Ranieri et al. 2012). Smaller

seeds, due to their dimensions, may be facilitated in their dispersal respect to larger seeds (Kigel 1995) and may be dispersed to farther distances. In species with heteromorphic seeds is well known that differences in seed dispersal occur among seed categories in the same species (Matilla et al. 2005). In the case of *A. canescens*, black seeds may have an advantage in their dispersal by anemochory respect to brown seeds, although their final germination is lower. The difference in seed mass might help in wider distribution during the seed germination time and enhances the chances of survival under unpredictable desert



conditions by favouring the formation of soil seed banks (Baskin and Baskin 2014). Generally, small seeds tend to occupy deeper soil horizons, thereby avoiding predation and becoming more persistent in the soil seed bank (Matilla et al. 2005).

In the present study, seed dormancy was detected for about 50 % of black seeds, respect to the non-dormant brown ones. In some heteromorphic species, heterogeneity is of special relevance, since the morphs are ecologically distinct, helping the plants to cope with spatio-temporal variability of habitats (Matilla et al. 2005) and seed dormancy among different seed categories may occur as observed also for the seed categories of *A. sagittata* (Venable et al. 1995). Our results indicate that some of the black seeds may germinate when environmental conditions are favourable, while about the 50 % remains dormant and contributes in the formation of a persistent soil seed bank. On the contrary, all the brown seeds germinate when water availability and requirements for the seed germination of the species are present in the habitat. However, differences in dormancy among seed categories of heteromorphic species are not always present, as observed for *Cirsium vulgare* (Savi) Ten. (Islam et al. 2009) and *Silene diclinis* (Lag.) M.Laínz (Mira et al. 2011), in which different colours were not associated with this aspect.

Both the types of *A. canescens* seeds germinated well at all temperatures. These tested temperature ranges perhaps allow the seeds to germinate in the winter (November–March) when temperatures are slightly lower and chances of rainfalls are higher in the natural desert habitat where seeds were collected (temperatures between November and March in the collecting area vary between 20.1 and 25.9 °C) (Islam et al. 2009). In *A. canescens*, we observed a faster germination in larger seeds (brown) than in smaller (black) and this pattern was different to that detected in *A. sagittata*, in which larger seeds germinated slower respect to smaller ones (Venable 1985). Kigel (1995) asserted that germination is rapid and dormancy low in far-dispersed diaspores and vice versa, although we observed exactly the inverse in *A. canescens*, suggesting that this pattern may be species-specific.

Both coloured seeds of *A. canescens* germinated well in both light and darkness indicating that they are neutral photoblastic and therefore not photo-inhibited. This indicates that *A. canescens* seeds have equal

chance of germination if they remain on the soil surface or buried in soil seed bank. However, if they are buried too deep in soil probably they might exhaust their resources before seedlings could emerge. On the basis of the different seed dormancy detected in our study, we speculate that *A. canescens* black seeds maintain a persistent soil seed bank, while brown seeds form only a transient soil seed bank. Therefore, black seeds persist ungerminated for a longer period in the soil than brown seeds. However, only within field studies, this pattern might be fully investigated.

Both brown and black seeds germinated very fast (within a week) and better in distilled water. Many studies report that percentages of germination decreased with increased salinity stress and the highest germination occurs in the absence of NaCl in the substrate both for glycophytes and halophytes (Khan and Ungar 1984; Baskin and Baskin 2014; Santo et al. 2014a, b). In particular for halophytes, this pattern indicates as the halophilous character is an ecological avoiding mechanism to avoid the ecological competition with other species. In fact, this is an adaptive strategy of halophyte plants because the salt content of the soil is reduced only for a short duration. This characteristic is similar to other desert halophytes such as *Salsola imbricata* Forssk. (Zaman et al. 2010), *Salsola rubescens* Franch. (El-Keblawy et al. 2013), *Halocnemum strobilaceum* (Pall.) Bieb. and *Halopeplis perfoliata* (Forssk) Bunge ex Schweinf (El-Keblawy and Bhatt 2015). Very few seeds of *A. canescens* were able to germinate with NaCl in the substrate, indicating that *A. canescens* seeds cannot tolerate the high salinity during the germination stage and seeds will not able to germinate in natural conditions until the salts are dissolved by rains. Generally, salinity enforce the dormancy in seeds and therefore they remain ungerminated under saline conditions, but once the salinity stress is alleviated, they retain their capacity to germinate (Ungar 1991) and this was also the case of *A. canescens*. Seeds of several species treated with high salinity levels recovered their germination following transfer to distilled water; however, the temperature regimen to which seeds were exposed may greatly influence the recovery percentages (Pujol et al. 2000; Gulzar et al. 2001; El-Keblawy et al. 2007). The ability of a species to tolerate high salinities is reflected on the maximum salt concentration at which seeds may germinate, and to have the possibility of recovery after NaCl exposure



(Ungar 1982). Seeds of both colours were able to recover their germination once they were transferred to distilled water, however, for both the two chromatic categories, we observed a higher germination recovery for seeds exposed to lower NaCl concentrations, respect to those under higher salt concentrations. Our results indicate that although *A. canescens* seeds have the ability to remain viable in saline conditions, in the field they will be able to germinate once the salinity level decreases by rainfall. However, it is unclear whether dimorphic seeds of *A. canescens* differ in persistence in the soil seed bank. Evolution may have arrived to a trade-off with respect to seed size, since the small seeds are more suited to dispersion, whereas larger seeds favour establishment and adaptation to a particular ecosystem. The germination recovery of black seeds is largely reduced by salinity stress suggesting that the brown seeds of *A. canescens* have the ability to better maintain their viability under salinity stress, which allows the species to withstand unfavourable periods of environmental stress. However, difference in ability to recover their germination could be related with the variation in seed mass. Previous studies also reported that large seeds are less sensitive to salinity stress than the small seeds such as in *Atriplex rosea* (Khan et al. 2004), *Suaeda aralocaspica* (Bunge) Freitag and Schutze (Wang et al. 2008), *Chenopodium album* L. (Yao et al. 2010) and *Atriplex centralasiatica* (Xu et al. 2011). In conclusion, this study has allowed to enlarge the knowledge of *A. canescens* seed ecology, in particular investigating the phenomenon of heteromorphy observed in this species in a population from a desert habitat. It is not known what causes this heterogeneity in this species, but it may be an important strategy for the survival of plant species in adverse and variable ecosystems (Egli 1998). The occurrence of the heteromorphy phenomenon in one desert population of *A. canescens* is similar to that observed by Santo et al. (Santo et al. 2015a) for *Brassica insularis* Moris in a small islet in South Sardinia (Italy) which was probably due to an evolutionary divergence process. More information on chemical properties of the seed testa and genetic analysis on plant individuals of this desert population of *A. canescens* are needed in order to address the colour differences and occurrence of heteromorphy found in this population. Further studies are necessary to better investigate seedlings of the two chromatic categories and their growth in the field and

under salinity conditions, possibly evaluating also inter-population variability.

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#### Compliance with ethical standards

**Conflict of interest** None.

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