

# Is chloride toxic to seed germination in mixed-salt environments? A case study with the coastal halophyte *Suaeda maritima* in the presence of seawater

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## ABSTRACT

Most salt tolerant plants, halophytes, use seed germination for natural regeneration. However, germination in mixed-salt environments such as seawater is poorly understood and ion toxicity by  $\text{Cl}^-$ , the most highly concentrated ion in seawater, is rarely considered over  $\text{Na}^+$ . Here, we investigate  $\text{Cl}^-$  toxicity in the germination of the halophyte *Suaeda maritima* in the presence of artificial seawater (ASW). Seeds were germinated at 15/5 °C in dilutions of ASW and at concentrations of NaCl,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$  and KCl as found in ASW. Solutions of polyethylene glycol (PEG) were used for osmotic comparison. Germination percentage and normal seedlings were quantified. Non-germinated seeds were tested for recovery on water. Germination rate ( $1/t_{50}$ ) was used in a halotime model to quantify the maximum concentration of  $\text{Cl}^-$  ( $\text{Cl}^-_{\text{max}}$ ) and  $\text{Na}^+$  ( $\text{Na}^+_{\text{max}}$ ) for germination. Germination was most negatively affected when all salts were combined in the concentrations found in ASW. Recovery of non-germinated seeds from all salt treatments on water was low, but all germinated seeds formed normal seedlings. Germination on ASW was higher than on iso-osmotic solutions of PEG. The  $1/t_{50}$  decreased with increasing  $\text{Cl}^-$  and  $\text{Na}^+$  concentration, indicating maximum thresholds to germination at 1381 mM ( $\text{Cl}^-_{\text{max}}$ ) and 1262 mM ( $\text{Na}^+_{\text{max}}$ ). The results indicate that ASW does not produce an osmotic limitation to the germination of *S. maritima*, and exposure to salt ions can even promote germination. However, ion toxicity is the major limitation, with  $\text{Cl}^-$  similarly as toxic as  $\text{Na}^+$ . In mixed-salt environments such as seawater,  $\text{Cl}^-$  toxicity should not be overlooked.

## 1. Introduction

Coastal habitats such as salt marshes, sea-grass beds and mangroves are some of the most biologically productive ecosystems across the world (Ramsar (2018)). These habitats support a number of ecosystem services including food and fuel, recreation and tourism, biodiversity, carbon storage and pollution control, as well as providing a natural buffer against coastal erosion. With sea level rise of up to 2 m predicted by 2100 (Bamber et al., 2019), the distribution of coastal habitats will change. Currently, one third of the plant species from coastal and marine intertidal habitats are already considered under threat by the IUCN (IUCN, 2018), and a high level of uncertainty exists as to how coastal species will respond to environmental change in the future.

The plants that occupy coastal habitats have evolved the ability to tolerate salinity, and for those that occupy the lower marsh, tolerance to flooding as well. These salt tolerant plants, termed halophytes, are highly productive in coastal habitats, and can also ameliorate the environmental impact of salinity; they could extend the area of cultivation into marginal areas, such as inland salt flats or coastal salt marshes, which are currently unsuitable for common crop species (Glenn et al., 1999; Panta et al., 2014). With the majority of halophytes relying on natural propagation through seed, the germination and early seedling establishment phases are critical to successful plant reproduction but are often considered bottlenecks due to their higher salt sensitivity than the adult plant (Kranter and Seal, 2013; Lombardi and Bedini, 2020).

Abbreviations: ASW, artificial seawater; PEG, polyethylene glycol.

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Salinity can both delay germination and decrease the final portion of seeds that germinate due to osmotic and/or ionic stress (Ungar, 1978; Song et al., 2005; Kranner and Seal, 2013). The effect of salinity may also be modulated by temperature as germination in saline environments often occurs during cool seasons when soil salinity levels are at their lowest (Ungar, 1978; Khan et al., 2000). In comparison to the seeds of salt-sensitive species, some halophytes can maintain viability and even some germination under extreme salinity or osmotic stress, recovering and germinating when the water potential of the growth medium increases (Gul et al., 2013). Whereas the mechanisms by which halophytic plants tolerate salt are widely reported (e.g., water uptake maintained by ion accumulation to lower the internal water potential and the synthesis of osmolytes; selectivity of essential nutrients such as  $K^+$  over  $Na^+$ ; compartmentalisation of  $Na^+$  and  $Cl^-$  to avoid toxicity; for a review see Flowers and Colmer, 2008 and Flowers et al., 2015), less is known about the underlying mechanisms of salt tolerance in seeds. Faced with the same challenges of osmotic and ionic stress as plants, seeds of some halophytes are reported to utilise tolerance mechanisms such as ion compartmentalisation into the seed coat (Khan et al., 1985; Song et al., 2005; Guja et al., 2013; Song et al., 2017), which may lower the internal osmotic potential whilst protecting the embryo from ion toxicity, or avoidance mechanisms such as dormancy to delay germination until environmental conditions are favourable (e.g. Wetson et al., 2008; Kranner and Seal, 2013). Differences in germination behaviour may also be observed within a species that produces dimorphic seeds as a bet-hedging strategy. For example, *Suaeda salsa* produces a brown morph that is non-dormant and more salt tolerant than the dormant black morph Song and Wang (2015), and *Arthrocnemum indicum* produces small seeds that are more salt tolerant than larger seeds (Nisar et al., 2018).

As the prevalent salt in saline soils and seawater, NaCl has been the focus of the majority of studies of the germination of halophytes (e.g., Khan and Ungar, 1997; Song et al., 2005; Wang et al., 2008; Debez et al., 2018; Nisar et al., 2018; Seal et al., 2018; Wijayasinghe et al., 2019) and features in over 70% of published records on halophyte seed germination (Table S1). In contrast, fewer studies (Table S1) have addressed the effects of the complexity of different salts or mixed-salt solutions on halophyte germination (e.g., Tobe et al., 2002; Tobe et al., 2004; Vicente et al., 2007; Wetson et al., 2008; Orlovsky et al., 2016; Rasool et al., 2016; Manzoor et al., 2017; Gao et al., 2018; Seal et al., 2018; Zhang et al., 2018; Ahmed et al., 2020). From these studies, evidence suggests that whereas  $Na^+$  concentration may be the best ionic correlate with germination in glycophytes (Zhang et al., 2018), ions in addition to  $Na^+$  may be influential on halophyte seed germination. For example,  $K^+$  was found to be more toxic than  $Na^+$  to the germination of *Zygophyllum propinquum* (Manzoor et al., 2017),  $Mg^{2+}$  was more toxic than  $Na^+$  to the germination of *Kalidium capsicum* while  $Ca^{2+}$  partially alleviated this toxicity (Tobe et al., 2002), and a mixed salt solution containing  $Mg^{2+}$  and  $Ca^{2+}$  mitigated against poor germination with high concentrations of NaCl in *Suaeda salsa* (Gao et al., 2018). Moreover, chloride salts tended to be more detrimental to germination than sulphate salts in the salt-marsh species *Arthrocnemum macrostachyum*, *Juncus acutus* and *Schoenus nigricans* (Vicente et al., 2007) and *Halopeplis perfoliate* (Rasool et al., 2016).  $Cl^-$  is the predominant anion in salt-affected soils (Teakle and Tyerman, 2010) and is found at a higher concentration than  $Na^+$  in seawater (0.55 M and 0.47 M respectively; DOE, 1994). It is therefore surprising that more germination studies do not consider  $Cl^-$  independently of  $Na^+$ , especially in relation to seawater environments.

To address this neglected feature of the halophyte germination literature, the objective of this study was to investigate the toxicity of  $Cl^-$  on the germination of the coastal halophyte *Suaeda maritima* in the presence of seawater and its major salt components, NaCl,  $MgCl_2$ ,  $CaCl_2$  and KCl. *Suaeda maritima* is an annual plant that is native to the tidal zone of the northern hemisphere saltmarshes, with optimal plant growth at 170–340 mM NaCl (Flowers, 1972; Thiyagarajah et al., 1996). Germination typically occurs in the spring where daytime temperatures

**Table 1**

Concentrations of artificial seawater (Harvey, 1966) and equivalent concentrations of NaCl,  $MgCl_2$  and  $CaCl_2$  used for the experiments, with pH values (full strength was measured for pH at  $21 \pm 1$  °C).

Salt	Concentration					pH
ASW	0	25%	50%	75%	full strength	6.18
NaCl (mM)	0	102.75	200.50	308.25	411.00	7.22
$MgCl_2$ (mM)	0	13.38	26.76	40.14	53.52	6.86
$CaCl_2$ (mM)	0	2.54	5.08	7.62	10.16	6.64
KCl (mM)	0	2.25	4.50	6.75	9.00	7.25

are low (ca. 15 °C) and salinity is diluted by rainfall; plants are fully established by the summer and produce new seed six months after germination (see Table 2 of Wetson et al., 2008). We used artificial seawater (ASW) and these salts at concentrations found in seawater, which vary both in chloride concentration and osmotic potential. For the first time, we use a halotime model approach to quantify the maximum concentration of  $Cl^-$  for germination to occur and compared this to  $Na^+$ . We address the hypothesis that an increasing concentration of  $Cl^-$  is associated with a decline in germination (percentage and rate) in ASW. We also investigate the effect  $Cl^-$  concentration has on the frequency of conversion from germinated seed into seedling, a critical phase of seedling establishment.

## 2. Materials and methods

### 2.1. Habitat and seed material

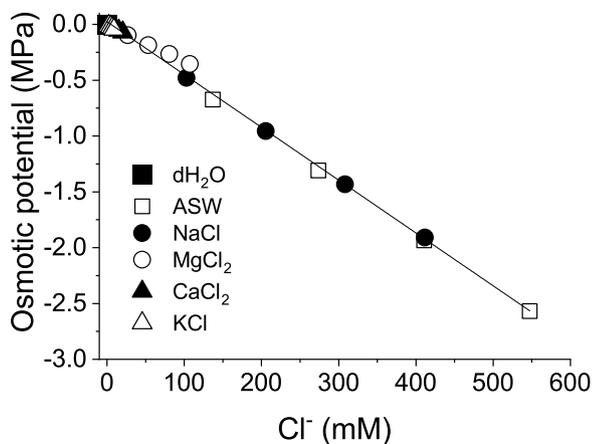
Mature fruits of *Suaeda maritima* (L). Dumort were collected in the autumn of 2010 from Cuckmere Haven, East Sussex, UK (TQ515978). Seeds were removed from the fruits by hand and stored at 15% relative humidity and 15 °C until experiments were performed in 2017. Wetson et al., (2008) found that dry storage for at least 6 months was sufficient for dormancy release.

A tetrazolium test was performed as a measure of initial seed viability (ISTA, 2019). Fifty seeds were hydrated over water for 24 h at 20 °C and then transferred to Petri dishes containing 1% water-agar for 2 days at 20 °C to fully imbibe. A small portion of the seed coat was removed and seeds were placed in 1% 2,3,5-triphenyl tetrazolium chloride in the dark for 48 h at 30 °C (ISTA, 2019). Seeds were then rinsed and cut longitudinally to assess the embryo for staining, where seeds were considered viable when stained red (uniformly) and non-viable when unstained.

To investigate whether this population of *S. maritima* has dimorphism in seed mass, as previously found in a population using seed mass as a proxy for seed size (Wetson et al., 2008), seed mass was measured by individually weighing 100 seeds on a balance recording to 7 decimal places.

### 2.2. Preparation of germination solutions

Artificial seawater (ASW) was prepared containing 411 mM NaCl, 53.52 mM  $MgCl_2$ , 28 mM  $Na_2SO_4$ , 10.16 mM  $CaCl_2$  and 9 mM KCl Harvey (1966) and dilutions made to 25, 50 and 75% of this. Concentrations of NaCl,  $MgCl_2$ ,  $CaCl_2$  and KCl equivalent to those found in the ASW dilutions were also prepared (Table 1). The pH (Metrohm, UK) of the full strength ASW and the individual salts at their concentration in undiluted ASW is also shown in Table 1. The osmotic potentials were determined using a freezing-point micro-osmometer (Roebing, Germany), and the units (mOsmol) converted into MPa using the Van't Hoff Equation (Angulo-Brown and Arias-Hernández, 1996). Solutions of polyethylene glycol (PEG) 8000 (Fisher BioReagent, Fisher Scientific, UK) were prepared to the same osmotic potential as the ASW dilutions (-0.67, -1.31, -1.94 and -2.57 MPa) according to the equation of Michel (1983) adjusting for the germination temperature (15/5 °C with a 12 h



**Fig. 1.** Correlation between the osmotic potential (measured at  $21 \pm 1$  °C) and  $\text{Cl}^-$  concentrations of the germination solutions. Solutions were 25%, 50%, 75% and full strength concentrations of artificial seawater (ASW) and equivalent concentrations of NaCl,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$  and KCl as well as  $\text{dH}_2\text{O}$  (see Table 1 for details). The equation of the linear regression (solid line) is  $y = 0.0249 - 0.005x$ ,  $R^2 = 0.997$ ,  $P < 0.05$

photoperiod) which averaged 10 °C. Osmotic potentials were confirmed using the micro-osmometer.

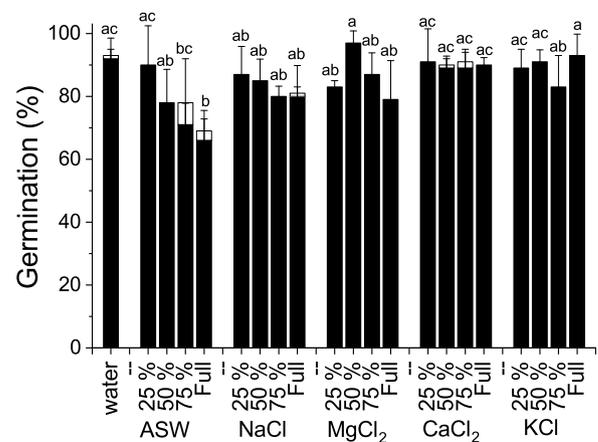
### 2.3. Germination experiments

Four replicates of 25 seeds were placed onto two layers of 90 mm diameter germination test paper (Fisherbrand, Fisher Scientific, UK) moistened with 7 ml of solution (distilled water, salt (see Table 1) or iso-osmotic PEG), so that the ratio of solution volume to germination test paper weight was  $>12$  as recommended when using PEG (Hardegee and Emmerich, 1990). Seeds were incubated at 15/5 °C to reflect the day/night temperature when seeds germinate in nature (Wetson et al., 2008) using a 12 h photoperiod (the higher temperature coinciding with the light period from warm-white fluorescent light at a photon flux density of  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Germination was recorded every 1 to 2 d and defined as radicle emergence of at least 2 mm. Germinated seeds remained in the Petri dish until normal seedlings (i.e., the development of cotyledons, hypocotyls and roots, without visible abnormalities; ISTA, 2019) were produced. After 35 d, when no further germination was observed, seeds which did not germinate (termed 'non-germinated') were washed in distilled water and transferred to fresh distilled water (7 ml) for recovery for a further 35d. Percentages of germination recovery of non-germinated seeds on distilled water and the development of normal seedlings were calculated on the basis of the number of seeds sown in salinity.

### 2.4. Statistical analysis

Values of seed mass were tested for normality using the Shapiro–Wilk test (in Origin 9.0, OriginLab Corporation). Percentages of germination, germination recovery and normal seedlings were calculated as the mean of four replicates  $\pm$  standard deviation. Generalised linear models (GLM) with binomial error structure and logit link function were used to compare germination percentages under the different treatments. A post-hoc analysis was performed within each treatment using a pairwise comparisons t-test (with Bonferroni adjustment). Statistical analyses were performed in Genstat (version 11, VSN International) or R (Version 3.5.0).

The germination rate was calculated by plotting the cumulative germination for each salt and temperature treatment which gave a sigmoidal curve and could be fitted using the Boltzmann distribution (Seal et al., 2018). From this, the time (t) taken to reach 50% of maximal



**Fig. 2.** Germination in different salt solutions (closed bars) and subsequent recovery of non-germinated seeds in distilled water (open bars) at 15/5 °C. Values are mean  $\pm$  standard deviations (four replicates of 25 seeds). Letters indicate significant differences ( $P < 0.05$ ) in germination on salt, using a pairwise comparisons t-test (with Bonferroni adjustment).

**Table 2**

GLM results for the effect of salt (S), dilution (D) and their interaction on germination percentage.

Predictor	d.f.	Deviance ( $\chi^2$ )	P value
S	5	40.659	<0.05
D	1	12.680	<0.05
S x D	4	9.513	0.461

germination of the seed population ( $t_{50}$ ) was estimated. Following the halotime model principle (Seal et al., 2018),  $1/t_{50}$  was regressed against the concentration of  $\text{Cl}^-$  and  $\text{Na}^+$ . The maximum concentration of  $\text{Cl}^-$  ( $\text{Cl}^-_{\text{max}}$ ) and  $\text{Na}^+$  ( $\text{Na}^+_{\text{max}}$ ), above which there is no germination, were calculated by extrapolation of the respective regression line to  $y = 0$ . Our study follows on from an earlier, preliminary study, using the same seed lot after a shorter time in storage, where we also observed a significant negative correlation between germination percentage and chloride concentration (data not presented). Statistical analyses were performed in Origin 9.0 (OriginLab Corporation).

## 3. Results

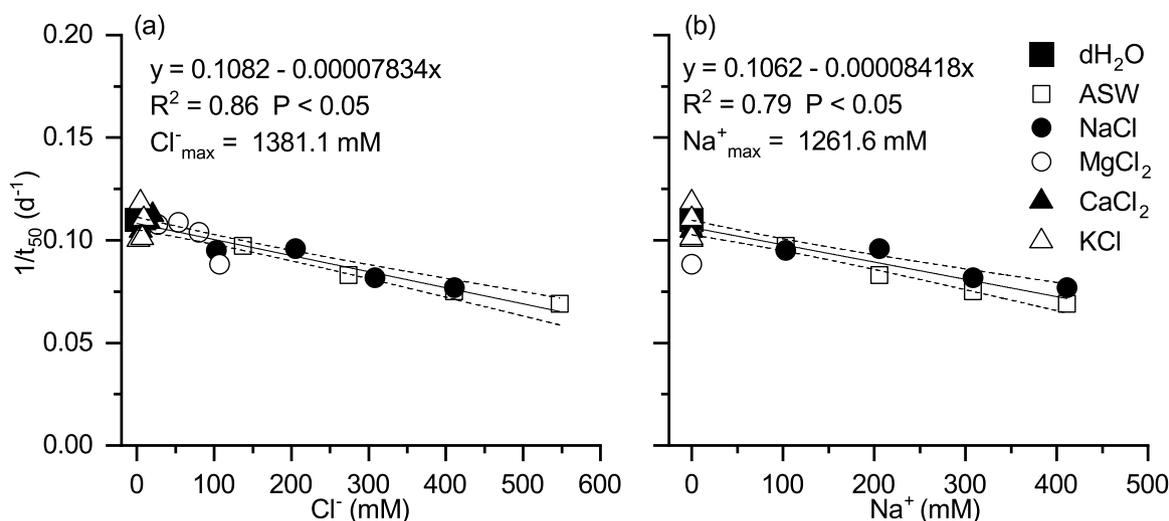
### 3.1. Salt solutions

The osmotic potential of the solutions varied considerably, with values in full-strength ASW of -2.57, and -1.91, -0.36, -0.07 and -0.05 MPa in full strength solutions of NaCl,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$  and KCl, respectively (Fig. 1). The  $\text{Cl}^-$  concentration in full-strength ASW was 547 mM, and was 411, 107, 20 and 9 mM in full strength solutions of NaCl,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$  and KCl, respectively. There was a significant correlation ( $R^2 = 0.997$ ,  $P < 0.05$ ) between  $\text{Cl}^-$  concentration and the osmotic potential of the solutions (Fig. 1). The pH of the salt solutions was close to neutral, ranging from pH 6.18–7.25 (Table 1).

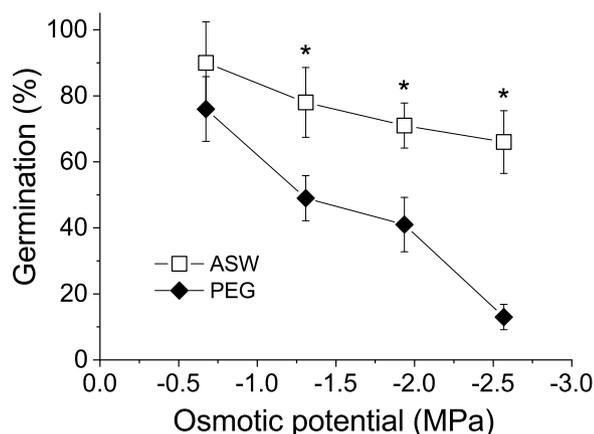
### 3.2. Seed germination and development of normal seedlings

The tetrazolium test showed that  $92 \pm 4.5\%$  of seeds were initially viable, of which 14% even germinated whilst submerged in the tetrazolium solution. Dissections of non-stained seeds revealed there were no empty seeds. Variation in seed mass was normally distributed (Shapiro-Wilk,  $P > 0.05$ ) and did not indicate dimorphism (Fig. S1).

In  $\text{dH}_2\text{O}$ , 92% germination was obtained with over 80% germination at all salt solutions except ASW (Fig. 2). This was supported by the GLM



**Fig. 3.** Germination rate, shown as the reciprocal of  $t_{50}$ , in solutions with different concentrations of (a)  $Cl^-$  and (b)  $Na^+$  at 15/5 °C. Solutions were 25%, 50%, 75% and full strength concentrations of artificial seawater (ASW) and equivalent concentrations of NaCl, MgCl<sub>2</sub>, CaCl<sub>2</sub> and KCl, and dH<sub>2</sub>O. For each treatment, four replicates of 25 seeds were tested. Data are shown as mean values. Dashed lines are 95% confidence limits of the linear regression.  $Cl^-_{max}$  and  $Na^+_{max}$  are the maximum concentrations of  $Cl^-$  and  $Na^+$ , respectively, to achieve 50% germination.



**Fig. 4.** Germination under iso-osmotic solutions of ASW and PEG 8000 at 15/5 °C. Four replicates of 25 seeds were tested with data shown as mean values  $\pm$  standard deviation. \* $P < 0.05$ , using a pairwise comparisons t-test (with Bonferroni adjustment).

analysis, where germination percentage was affected by both the salt and the dilution of salt, but not their interaction (Table 2). This was predominately due to 66% germination achieved in full strength ASW being significantly lower than several treatments including water, and full strength CaCl<sub>2</sub> and KCl (Fig. 2). NaCl permitted an intermediate germination response, with percentage values statistically similar to those achieved in water and full strength ASW. Few non-germinated seeds recovered when replaced in dH<sub>2</sub>O with the highest percentage being 7% recovery from 75% ASW. All germinated seeds converted into normal seedlings (Fig. S2).

The germination rate was defined by the reciprocal of  $t_{50}$  (i.e., time to obtain 50% of the maximal germination of the seed population). Germination became significantly slower ( $P < 0.05$ ) as the concentration of  $Cl^-$  (Fig. 3a) and  $Na^+$  (Fig. 3b) increased. The  $Cl^-_{max}$  and  $Na^+_{max}$  were estimated to be 1381 mM and 1262 mM respectively.

Germination was also evaluated on solutions of PEG equivalent in osmotic potential to the dilutions of ASW (Fig. 4). At the highest osmotic potential, germination was similar on ASW and PEG, but germination was significantly higher in ASW than in PEG at all other osmotic potentials ( $P < 0.05$ ).

#### 4. Discussion

For many halophytic plants, including *S. maritima*, optimal growth is achieved under saline conditions (Flowers et al., 1977; Flowers and Colmer, 2008; Guo et al., 2020). However, for the seeds of most halophytic species, germination is best in water (for review see Kranner and Seal, 2013). Supporting this notion, seeds of *S. maritima* achieved the highest germination in water and progressively lower germination was observed with increasing concentration of ASW (Fig. 2). Solutions of NaCl, MgCl<sub>2</sub>, CaCl<sub>2</sub> and KCl at their concentrations in full strength ASW also permitted similar levels of germination to dH<sub>2</sub>O. The concentrations of these salts in ASW (Table 1) are lower than previously reported as being detrimental to germination, with thresholds of  $> 53$  mM MgCl<sub>2</sub> and  $> 45$  mM CaCl<sub>2</sub> in seeds of *Suaeda fruticosa* (Jhamd and Sen, 1984),  $> 100$  mM MgCl<sub>2</sub> in seeds of *Suaeda salsa* (Duan et al., 2007), and  $> 630$  mM MgCl<sub>2</sub> and  $> 420$  mM CaCl<sub>2</sub> in seeds of *Haloxylon ammodendron* (Tobe et al., 2004). The combination of salts in ASW is more detrimental to germination of *S. maritima* than the separate effects of individual salts.

Osmotic stress can inhibit seed germination under salinity due to difficulties associated with the uptake of water from solutions with a low osmotic potential (Song et al., 2005; Kranner and Seal, 2013). However, osmotic stress is often reversible when alleviated, as indicated by the recovery of non-germinated seeds on water (Gul et al., 2013). Here, the poor recovery in dH<sub>2</sub>O to germination inhibited by ASW suggests it was caused by ion toxicity.

Higher germination in ASW than equivalent osmotic solutions of PEG have been reported for other plants. For example, seeds of the halophytes *Haloxylon ammodendron* (Tobe et al., 2004; Song et al., 2005), *Sarcobatus vermiculatus* (Dodd and Donovan, 1999), and *Zygophyllum xanthoxylon* (Tobe et al., 2001), as well as in non-halophytic species such as *Lespedeza davurica* and *Hordeum vulgare* (Zhang et al., 2010a; Huang and Redmann, 1995), have also reported this response in comparison with the effects of NaCl – although not previously in *Suaeda maritima* (Wetson et al., 2008). Zhang et al., (2010a) argued that their results were consistent with their hypothesis that “sodium was absorbed by seeds, facilitating water uptake and allowing germination under osmotic conditions which would otherwise prevent germination”. Certainly, PEG does not penetrate cell walls as readily as salts such as NaCl and can reduce the water content of cells killed by freezing and thawing (Flowers and Dessimoni Pinto, 1970). The water content was lower in seeds of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum*, and seedlings of *Haloxylon ammodendron*, when grown in PEG

than isosmotic solutions of NaCl at 20°C (Tobe et al., 2004; Song et al., 2005). It is reported that PEG itself may be associated with reduced oxygen availability (Mexal et al., 1975) and contain traces of metal ions which can be toxic (Greenway et al., 1968; Plaut and Federman, 1985). For seed germination, Hardegee and Emmerich (1994) argued that as long as the seed was not immersed in the PEG solution, restricted oxygen availability was an unlikely problem; here, we used 7 ml of solution which moistened the two layers of 90 mm germination test paper, but did not immerse the seeds. Any toxicity from trace metals is unlikely because of the small volume of PEG used. The results of this study are therefore consistent with Tobe et al., (2004), Song et al., (2005) and Zhang et al., (2010a) who all agree that salt ions can have beneficial effects on the seed germination of some halophytes.

If seeds are osmotically adjusting and taking up sufficient water, then the observed decrease in germination (and subsequent number of seedlings) with increasing ASW must be due to the accumulation of ions resulting in toxicity (Ungar, 1978; Song et al., 2005; Kraner and Seal, 2013).  $\text{Cl}^-_{\text{max}}$  and  $\text{Na}^+_{\text{max}}$  were of similar values, indicating that  $\text{Cl}^-$  and  $\text{Na}^+$  were similarly toxic to germination. The reasons for the toxicity of  $\text{Na}^+$  in germination have been considered previously, such as penetration and toxicity in outer seeds tissues where the effects can be alleviated by  $\text{Ca}^{2+}$  (Tobe et al., 2002; Song et al., 2017; Gao et al., 2018). However,  $\text{Cl}^-$  is often overlooked.  $\text{Cl}^-$  is a major ion in seawater and at higher concentration than any other ion, including  $\text{Na}^+$  (547 mM in full-strength ASW, compared to 411 mM  $\text{Na}^+$ ).  $\text{Cl}^-$  is an essential micronutrient (but not macronutrient) that regulates enzyme activities in the cytoplasm, acts as a counter anion to stabilise membrane potential and is involved in turgor and pH regulation White and Broadley (2001).  $\text{Cl}^-$  is also an essential co-factor in photosynthesis White and Broadley (2001) and several halophytes, including *S. maritima*, have chlorophyllous seeds that can initiate photosynthesis during germination (Zhang et al., 2010b), earlier than non-chlorophyllous species that need to synthesise chlorophyll first (Puthur et al., 2013). However, there is also evidence for the toxicity of high concentrations of  $\text{Cl}^-$ .

$\text{Cl}^-$  is reported to be as, or even more, inhibitory to protein synthesis than  $\text{Na}^+$  in halophytes (see Flowers et al., 2015) and is associated with decreased protein synthesis in cells (Weber et al., 1977). Protein synthesis is one part of the complex series of molecular and biochemical processes comprising germination, the end point of which is marked as the emergence of the radicle Bewley (1997). Therefore, the regulation of  $\text{Cl}^-$  uptake and distribution may be as critical for germination as the regulation of  $\text{Na}^+$  under salinity. In plants,  $\text{Na}^+$  may be transported by  $\text{K}^+$  channels (e.g.,  $\text{K}^+$  inward-rectifying channels), by  $\text{Na}^+/\text{H}^+$  antiporters such as encoded by *SOS1* or through non-selective cation channels and voltage-independent channels, whereas  $\text{Cl}^-$  may be transported through cation- $\text{Cl}^-$  cotransporters with  $\text{K}^+$  or  $\text{Na}^+$  or by  $\text{Cl}^-/2\text{H}^+$  symporters (Bazihizina et al., 2019; Flowers et al., 2019). This suggests that the transport of  $\text{Na}^+$  and  $\text{Cl}^-$  into cells is not always co-dependent and may occur via different transporters, such that  $\text{Na}^+$  and  $\text{Cl}^-$  could accumulate to different subcellular concentrations (Flowers et al., 2015). Under this scenario, either  $\text{Na}^+_{\text{max}}$  or  $\text{Cl}^-_{\text{max}}$  could be exceeded.

In conclusion, the germination of the halophyte *S. maritima* was most negatively affected when all salts were combined in the concentrations found in ASW. ASW did not produce an osmotic limitation to germination and exposure to salt ions even promoted germination when compared to germination on iso-osmotic solutions of PEG. Ion toxicity was the major limitation, with  $\text{Cl}^-$  similarly as toxic as  $\text{Na}^+$ . In mixed-salt environments such as seawater,  $\text{Cl}^-$  toxicity during germination and seedling establishment should not be overlooked.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Author contribution statement

CES, TJF and AM designed the experiments; AM, CE and MY conducted the experiments; CES analysed the data; and CES, TJF and AM wrote the manuscript.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2021.100030.

#### References

- Ahmed, MZ, Parveen, F, Gulzar, S, Gul, B, Khan, MA, 2020. Effects of chloride and sulfate salts on seed germination of halophytes from dry alpine climate. *J. Plant Nutr.* 43, 2299–2310.
- Angulo-Brown, F, Arias-Hernández, LA, 1996. Van't Hoff's equation for endoreversible chemical reactions. *J. Phys. Chem.* 100, 9193–9195.
- Bamber, JL, Oppenheimer, M, Kopp, RE, Aspinall, WP, Cooke, RM, 2019. Ice sheet contributions to future sea-level rise from structured expert judgement. *PNAS* 116, 11195–11200.
- Bazihizina, N, Colmer, TD, Cuin, TA, Mancuso, S, Shabala, S, 2019. Friend or foe? Chloride patterning in halophytes. *Trends Plant Sci.* 24, 142–151.
- Bewley, JD, 1997. Seed germination and dormancy. *Plant Cell* 9, 1055–1066.
- Debez, A, Belghith, I, Pich, A, Taamalli, W, Abdely, C, Braun, HP, 2018. High salinity impacts germination of the halophyte *Cakile maritima* but primes seeds for rapid germination upon stress release. *Physiol. Plant.* 164, 134–144.
- Dodd, GL, Donovan, LA, 1999. Water potential and ionic effects in germination and seedling growth of two cold desert shrubs. *Am. J. Bot.* 86, 1146–1153.
- DOE, 1994. Physical and thermodynamic data. In: Dickson, AG, Goyet, C (Eds.), *Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water*. Version 2.13, ORNL/CDIAC-74. U.S. Department of Energy, USA, pp. 1–22.
- Duan, D-Y, Li, W-Q, Liu, X-J, Ouyang, H, An, P, 2007. Seed germination and seedling growth of *Suaeda salsa* under salt stress. *Ann Botanic Fennici* 44, 161–169.
- Flowers, TJ, 1972. Salt tolerance in *Suaeda maritima* (L.) Dum. *J. Exp. Bot.* 23, 310–321.
- Flowers, TJ, Colmer, TD, 2008. Salinity tolerance in halophytes. *New Phytol.* 179, 945–963.
- Flowers, TJ, Dessimoni Pinto, CM, 1970. The effects of water deficits on slices of beetroot and potato tissue. 2. Changes in respiration and permeability to solutes. *J. Exp. Bot.* 21, 754–767.
- Flowers, TJ, Glenn, EP, Volkov, V, 2019. Could vesicular transport of  $\text{Na}^+$  and  $\text{Cl}^-$  be a feature of salt tolerance in halophytes? *Ann. Bot. (Lond.)* 123, 1–18.
- Flowers, TJ, Munns, R, Colmer, T, 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Ann. Bot. (Lond.)* 115, 419–431.
- Flowers, TJ, Troke, PF, Yeo, AR, 1977. The mechanism of salt tolerance in halophytes. *Ann. Rev. Plant Physiol.* 28, 89–121.
- Flowers, TJ, Munns, R, Colmer, T, 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Ann. Bot. (Lond.)* 115, 419–431.
- Gao, R, Wei, X, He, Z, Zhao, R, Wang, K, Yang, X, Walck, JL, 2018. Soil salt and NaCl have different effects on seed germination of the halophyte *Suaeda salsa*. *J. Plant Nutr. Soil Sci.* 181, 488–497.
- Glenn, EP, Brown, JJ, Blumwald, E, 1999. Salt tolerance and crop potential of halophytes. *Crit. Rev. Plant Sci.* 18, 227–255.
- Greenway, H, Hiller, RG, Flowers, TJ, 1968. Respiratory inhibition in *Chlorella* produced by 'purified' polyethylene glycol 1540. *Science* 159, 984–985.
- Guja, L, Wuhler, R, Moran, K, Dixon, KW, Wardell-Johnson, G, Merritt, DJ, 2013. Full spectrum X-ray mapping reveals differential localization of salt in germinating seeds of differing salt tolerance. *Botanical J. Linnean Soc.* 173, 129–142.
- Gul, B, Ansari, R, Flowers, TJ, Khan, MA, 2013. Germination strategies of halophyte seeds under salinity. *Environ. Exp. Bot.* 92, 4–18.
- Guo, J, Du, M, Tian, H, Wang, B, 2020. Exposure to high salinity during seed development markedly enhances seedling emergence and fitness of the progeny of the extreme halophyte *Suaeda salsa*. *Front. Plant Sci.* 11, 1291.

- Hardege, SP, Emmerich, WE, 1990. Partitioning water potential and specific salt effects on seed germination of four grasses. *Ann. Bot. (Lond.)* 66, 587–595.
- Hardege, SP, Emmerich, WE, 1994. Seed germination response to polyethylene glycol solution depth. *Seed Sci. Technol.* 22, 1–7.
- Harvey, H, 1966. *The Chemistry and Fertility of Seawaters*. Cambridge University Press, Cambridge, UK.
- Huang, J, Redmann, RE, 1995. Salt tolerance of *Hordeum* and *Brassica* species during germination and early seedling growth. *Can. J. Plant Sci.* 75, 815–819.
- ISTA, 2019. *International Rules for Seed Testing*. International Seed Testing Association, Bassersdorf, Switzerland.
- IUCN (2018) *The IUCN Red List of Threatened Species*. <http://www.iucnredlist.org>. Accessed on 12 June 2018.
- Jhamd, RB, Sen, DN, 1984. Seed germination behaviour of halophytes in Indian desert: 1. *Suaeda frutescens* (Linn.) Forsk. *Curr. Sci.* 53, 100–101.
- Khan, MA, Weber, DJ, Hess, WM, 1985. Elemental distribution in seeds of the halophyte *Salicornia pacifica* var. *utahensis* and *Atriplex canescens*. *Am. J. Bot.* 72, 1672–1675.
- Khan, M, Ungar, I, 1997. Effects of thermoperiod on recovery of seed germination of halophytes from saline conditions. *Am. J. Bot.* 84, 279, 279.
- Khan, MA, Gul, B, Weber, DJ, 2000. Germination responses of *Salicornia rubra* to temperature and salinity. *J. Arid. Environ.* 45, 207–214.
- Kranner, I, Seal, CE, 2013. Salt stress, signalling and redox control in seeds. *Function. Plant Biol.* 40, 848–859.
- Lombardi, T, Bedini, S, 2020. Seed germination strategies of Mediterranean halophytes under saline condition. In: Grigore, MN. (Ed.), *Handbook of Halophytes*. Springer, Cham [doi.org/10.1007/978-3-030-17854-3\\_119-1](https://doi.org/10.1007/978-3-030-17854-3_119-1).
- Manzoor, S, Hameed, A, Khan, MA, Gul, B, 2017. Seed germination ecology of a medicinal halophyte *Zygophyllum propinquum*: responses to abiotic factors. *Flora* 233, 163–170.
- Mexal, J, Fisher, JT, Osteryoung, J, Reid, CP, 1975. Oxygen availability in polyethylene glycol solutions and its implications in plant-water relations. *Plant Physiol.* 55, 20–24.
- Michel, BE, 1983. Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiol.* 72, 66–70.
- Nisar, F, Gul, B, Khan, MA, Hameed, A, 2018. Germination and recovery responses of heteromorphic seeds of two co-occurring *Arthrocnemum* species to salinity, temperature and light. *S. Afr. J. Bot.* 121, 143–151.
- Orlovsky, N, Japakova, U, Zhang, H, Volis, S, 2016. Effect of salinity on seed germination, growth and ion content in dimorphic seeds of *Salicornia europaea* L. (Chenopodiaceae). *Plant Divers.* 38, 83–189.
- Panta, S, Flowers, T, Lane, P, Doyle, R, Haros, G, Shabala, S, 2014. Halophyte agriculture: Success stories. *Environ. Exp. Bot.* 107, 71–83.
- Plaut, Z, Federman, E, 1985. A simple procedure to overcome polyethylene glycol toxicity on whole plants. *Plant Physiol.* 79, 559–561.
- Puthur, JT, Shackira, AM, Saradhi, PP, Bartels, D, 2013. Chloroembryos: a unique photosynthesis system. *J. Plant Physiol.* 170, 1131–1138.
- Ramsar, 2018. *Ramsar Convention on Wetlands. Global Wetland Outlook: State of the World's Wetlands and their Services to People*. Ramsar Convention Secretariat, Gland, Switzerland.
- Rasool, SG, Hameed, A, Ahmed, MZ, Khan, MA, Gul, B, 2016. Comparison of seed germination and recovery responses of a salt marsh halophyte *Halopeplis perfoliata* to osmotic and ionic treatments. *Pak. J. Bot.* 48, 1335–1343.
- Seal, CE, Barwell, LJ, Flowers, TJ, Wade, EM, Pritchard, HW, 2018. Seed germination niche of the halophyte *Suaeda maritima* to combined salinity and temperature is characterised by a halothermal time model. *Environ. Exp. Bot.* 155, 177–184.
- Song, J, Feng, G, Tian, C, Zhang, F, 2005. Strategies for adaptation of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* to a saline environment during seed-germination stage. *Ann. Bot. (Lond.)* 96, 399–405.
- Song, J, Shi, W, Liu, R, Xu, Y, Sui, N, Zhou, J, Feng, G, 2017. The role of the seed coat in adaptation of dimorphic seeds of the euhalophyte *Suaeda salsa* to salinity. *Plant Species Biol.* 32, 107–114.
- Song, J, Wang, B, 2015. Using euhalophytes to understand salt tolerance and to develop saline agriculture: *Suaeda salsa* as a promising model. *Ann. Bot. (Lond.)* 115, 541–553.
- Teakle, NL, Tyerman, SD, 2010. Mechanisms of Cl<sup>-</sup> transport contributing to salt tolerance. *Plant Cell Environ.* 33, 566–589.
- Thiyagarajah, M, Fry, SC, Yeo, AR, 1996. In vitro salt tolerance of cell wall enzymes from halophytes and glycophytes. *J. Exp. Bot.* 47, 1717–1724.
- Tobe, K, Li, X, Omasa, K, 2004. Effects of five different salts on seed germination and seedling growth of *Haloxylon ammodendron* (Chenopodiaceae). *Seed Sci. Res.* 14, 345–353.
- Tobe, K, Zhang, LP, Qiu, GYY, Shimizu, H, Omasa, K, 2001. Characteristics of seed germination in five non-halophytic Chinese desert shrub species. *J. Arid. Environ.* 47, 191–201.
- Tobe, K, Li, X, Omasa, K, 2002. Effects of sodium, magnesium and calcium salts on seed germination and radicle survival of a halophyte, *Kalidium caspicum* (Chenopodiaceae). *Aust. J. Bot.* 50, 163–169.
- Ungar, IA, 1978. Halophyte seed germination. *Botanical Rev.* 44, 233–264.
- Vicente, MJ, Conesa, E, Ivarez-Rogel, JA, Franco, JA, Martinez-Sanchez, JJ, 2007. Effects of various salts on the germination of three perennial salt marsh species. *Aquatic Botany* 87, 167–170.
- Wang, L, Huang, Z, Baskin, CC, Baskin, JM, Dong, M, 2008. Germination of dimorphic seeds of the desert annual halophyte *Suaeda aralocaspica* (Chenopodiaceae), a C4 plant without Kranz anatomy. *Ann. Bot. (Lond.)* 102, 757–769.
- Weber, LA, Hickey, ED, Moroney, PA, Baglioni, C, 1977. Inhibition of protein synthesis by Cl<sup>-</sup>. *J. Biol. Chem.* 252, 4007–4010.
- Wetson, AM, Cassaniti, C, Flowers, TJ, 2008. Do conditions during dormancy influence germination of *Suaeda maritima*? *Ann. Bot. (Lond.)* 101, 1319–1327.
- White, PJ, Broadley, MR, 2001. Chloride in soils and its uptake and movement within the plant: a review. *Ann. Bot. (Lond.)* 88, 967–988.
- Wijayasinghe, MM, Jayasuriya, KG, Gunatilleke, CVS, Gunatilleke, IAUN, Walck, JL, 2019. Effect of salinity on seed germination of five mangroves from Sri Lanka: use of hydrotimic modelling for mangrove germination. *Seed Sci. Res.* 29, 55–63.
- Zhang, HX, Irving, LJ, McGill, C, Matthew, C, Zhou, DW, Kemp, P, 2010a. The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. *Ann. Bot. (Lond.)* 106, 1027–1035.
- Zhang, S, Song, J, Wang, H, Feng, G, 2010b. Effect of salinity on seed germination, ion content and photosynthesis of cotyledons in halophytes or xerophyte growing in Central Asia. *J. Plant Ecol.* 3, 259–267.
- Zhang, H, Tian, Y, Guan, B, Zhou, D, Sun, Z, Baskin, CC, 2018. The best salt solution parameter to describe seed/seedling responses to saline and sodic salts. *Plant Soil* 426, 313–325.