Evaluation of direct and transgenerational influences of salinity on germination and early seedling growth in an edible halophyte, *Crithmum maritimum*

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Abstract

Background and Aims: *Crithmum maritimum* is a wild, edible halophyte with large potential as a cash crop for salinized soils. However, the tolerance during seed germination appears to be highly site-specific and contradictory, whereas little is known on salinity tolerance during early seedling growth. This study was aimed at characterizing variation in the responses of germination and early seedling growth in diverse *C. maritimum* populations along the Iberian Southwest coast. Specifically, we sought to distinguish between direct salinity effects and those influenced by the salinity of maternal environments.

Methods: Physicochemical properties, including salinity of maternal environments, were assessed across diverse habitats. A total of 3480 seeds from 58 mother plants were utilized. Seeds were subjected to germination assays under various salinity treatments (0-500 mM NaCl), with subsequent monitoring of germination parameters. Non-germinated seeds were tested for recovery germination, and viability was assessed using the tetrazolium test. Of germinated seeds, 1160 seedlings were monitored for survival and early growth metrics. General Linear Models were employed to analyze the effects of salinity and maternal environmental influence on germination and early growth.

Key Results: Despite reduced and delayed germination under salinity, seeds showed remarkable tolerance up to 150 mM, surpassing prior reports, with consistent viability up to 500 mM, indicating substantial salinity-induced dormancy. Seedling growth was more sensitive to continued treatment; no plants survived above 150 mM. The salinity experienced by maternal plants had only a marginal effect on germination but significantly contributed to reduce seedling biomass production, both above and below ground.

Conclusions: This study highlights the significance of maternal salinity on early growth in *C. maritimum*, emphasizing the species' resilience to salt stress during germination and recovery. These insights are crucial for optimizing cultivation techniques and informing research on other halophytes in saline environments.

Keywords: sea fennel, salinity tolerance, maternal effects, soil salinity, seed growth, halophytic response

INTRODUCTION

Salinization of soil has emerged as a substantial worldwide concern, steadily reducing the area of arable lands and threating agricultural sustainability (FAO, 2023). Increased soil salt content significantly impairs nutrient availability and induces osmotic stress, limiting water uptake and causing the accumulation of Na^+ and Cl^- ions into plant tissues. The resulting physiological disturbances of increased soil salinization (Munns and Tester, 2008; Talbi Zribi et al., 2020), leads to reduced crop yields and thus threatens global food security. The cultivation of halophytes is a promising sustainable strategy for addressing salt-affected lands (Karakas et al., 2020). These plants exhibit natural adaptations to saline environments (Flowers and Colmer, 2008; Shabala, 2013) and offer a high versatility of uses, including food production, forage, energy crops and medicinal applications (Panta et al., 2014).

Halophytes display a wide range of physiological, metabolic and anatomical strategies to accommodate and even benefit from a saline environment, including ion exclusion by the roots, specialized salt-secreting glands, succulence and accumulation of compatible osmotic substrates (Rozema and Schat, 2013; Flowers and Colmer, 2015). However, these mechanisms are less effective during seed germination, a crucial process in the plant life cycle (Gul et al., 2013), and many studies have revealed a great sensitivity of halophytes to salt during seed germination and seedling emergence (Debez et al., 2004). However, limited information exists regarding salt tolerance in halophyte seedlings, despite their critical importance for successful crop establishment (Ruiz-Carrasco et al., 2011).

Other plant strategies have evolved to maintain seed and seedling viability under environmental conditions of high salinity, such as seed dormancy mechanisms, an increase in seed size, and seed heteromorphism (Gul et al., 2013; Liu et al., 2018). In highly saline habitats, many species, such as *Suaeda vermiculata*, show enforced dormancy delaying germination until water availability is more favorable for seedling survival and growth (Al- Shamsi et al., 2018). Moreover, substantial differences in salinity tolerance have been observed within species, especially for germination (Ruiz-Carrasco et al., 2011), likely associated with the provenance of populations and adaptation to their local habitat conditions. These transgenerational effects may represent a further form of adaptation in halophytes (Redondo-Gómez et al., 2008; Wang et al., 2021), by altering the phenotypes expressed by offspring and leading to their improved performance under stressed environmental conditions (Herman and Sultan, 2011; Caño et al., 2016). However, maternal environmental influence shows evident contrasts between species. For instance, seeds matured in highly saline environments may display enhanced salinity tolerance during germination in halophytes such as *Festuca rubra* (Bülow- Olsen, 1983) and *Suaeda aralocaspica* (Wang et al., 2012). In contrast, seeds from facultative halophytes, including *S. vermiculata*, *S. aegyptiaca* and *Anabasis setifera*, that had matured in non-saline soils, demonstrated significantly better germination under lower salinity conditions than seeds matured in saline environments, whereas no differences were observed at higher salinity levels (El-Keblawy et al., 2016, 2018, 2020). Additionally, other halophytes, such as *Zygophyllum coccineum*, exhibit similar germination rates between seeds matured in non-saline habitats and those matured in saline soils when exposed to moderate salinities (Mohamed et al., 2020). These findings underscore the intricate relationships between maternal conditions, transgenerational effects and germination adaptation, which play a key role in the survival of halophytes in saline environments. However, the full extent of their impact seems to be largely context-dependent and remains poorly understood.

Crithmum maritimum L., commonly known as sea fennel, is a wild, edible halophyte with multiple applications in the food and pharmaceutical industries (Karkanis et al., 2022; Martins-Noguerol et al., 2022). It thrives in different habitats (sandy beaches, shingle beaches and cliffs) where it is subjected to a great variety of environmental conditions, including contrasting salinities (Martins-Noguerol et al., 2023); in consequence it is a promising sustainable crop for saline and salinized soils (Renna et al., 2018). While many studies have investigated the salinity tolerance of *C. maritimum* (reviewed in Karkanis et al., 2022), the findings have been contradictory. For instance, Atia et al. (2006, 2011*a*) reported no reduction in seed germination at 50 mM NaCl but a 90% reduction in germination at 100 mM NaCl. In contrast, Meot-Duros and Magne (2008) reported a 98% reduction in the presence of 50 mM NaCl. Such conflicting results may reflect local adaptations associated with geographical origin or habitat diversity (Meot-Duros and Magne, 2008). The extent to which maternal or transgenerational influences of salinity could influence germination and early growth in halophyte species is a question that remains largely unexplored. Understanding such mechanisms is an imperative for advancing the cultivation of *C. maritimum* under saline environments.

The present work aimed to (i) establish the salinity tolerance thresholds of *C. maritimum* seeds across individuals from six wild populations exposed to varying habitats and salinity conditions; (ii) evaluate the survival and early growth of seedlings under different salinity regimes; and (iii) distinguish between potential effects of maternal salinity environments on the germination and early growth of *C. maritimum* seedlings from direct effects of salinity. We hypothesize that maternal exposure to salinity enhances the salinity tolerance of *C. maritimum* seedlings, affecting both germination and early growth. This is the first comprehensive analysis of the influence of maternal effects on the salinity tolerance of *C. maritimum* during both germination and early growth. Apart from providing insights into its ecology, this study potentially establishes a basis for optimizing its cultivation in saline environments, offering a promising pathway for its sustainable development as a crop.

MATERIALS AND METHODS

Study sites and plant material

We selected six wild populations of *C. maritimum*, each with a minimum of 30 adult plants, at sites along the southern coasts of the Iberian Peninsula that represent its different habitat types: sandy beaches, shingle beaches, and cliffs (Figure 1; Supplementary Table S1; Atia et al., 2011*b*). From each population, we selected 8-10 adult reproductive plants that were at least 2 meters apart. Seven umbels per plant, bearing the single-seeded mature fruits, were collected and transported to the laboratory in mid-September 2020 for fruit counts and germination experiments. Each fruit contained a single dry seed measuring 4-10 mm in length.

Characterization of maternal traits

To account for maternal effects, we made several measurements on each mother plant: the vegetative and reproductive heights, the number of umbels per plant, and the number of seeds per umbel and per plant. Plant volume was estimated by measuring the maximum diameter, minimum diameter, and maximum height and calculating the volume of a semispheroid. We also weighed the collected seeds and fruits using a precision balance. To assess the water status of the plants, we measured the leaf relative water content (RWC) in the laboratory (in one leaf per plant). Leaves were collected in the field at the same time of day across all populations (typically between 10:00-12:00 AM) to ensure consistency in the physiological state of the plants. The leaves were transported in a cooler with ice and they were promptly processed upon arrival at the laboratory. The RWC was calculated as the ratio of fresh water content to water content at saturation and expressed as a percentage, using the following equation:

$$\mathrm{RWC} = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{SW} - \mathrm{DW})} \times 100$$

where, FW is fresh weight; DW is dry weight and SW is saturated weight of leaf.

From each population, we collected 8-10 topsoil samples (0-30 cm depth) from the vicinity of the selected plants. These samples were used to characterize soil properties of maternal plants and to assess their transgenerational effects on germination and seedling establishment. Soil pH was determined using a digital meter (Crison pH-25, Spain) in a 1:2.5 (w/v) soil:water suspension. Soil organic matter content was determined by calcination in a muffle furnace at 450 °C for 4 h. To estimate soil salinity, electrical conductivity was measured using a conductivity meter (Crison-522, Spain) in a 1:5 (w/v) soil:water suspension. Coarse elements were removed from the soil fractions by sieving (>2 mm), and the percentage of gravel was determined. Soil texture analysis was performed on the <2 mm soil fraction using the Bouyoucos method (Bouyoucos, 1962).

Seed germination and viability

Seeds were surface-sterilized by immersion in 1% sodium hypochlorite solution in sterilised distilled water for 2.5 minutes with gentle mixing. Then, they were rinsed three times with sterile distilled water. Subsequently, the sterilized seeds were germinated in 9-cm Petri dishes on a double layer of Whatman filter paper (10 seeds per dish, all from the same mother plant) moistened with 3 mL of the corresponding saline solution (50, 100, 150, 300 and 500 mM NaCl). Sterilised distilled water served as the control treatment. To minimize evaporation, the dishes were wrapped with Parafilm. The experiment included a total of 8-10 maternal plants from each wild population, with 10 seeds per dish per plant, resulting in a total of 3,480 experimental seeds (10 seeds/dish x 8-10 maternal plants x 6 populations x 6 salinity treatments). The dishes were randomly distributed in a growth chamber where they were rerandomized every 3 days. The growth chamber maintained a light regime of 16 h of light and a day/night temperature of 28/22 °C, providing a maximum light flux of 1000 µmol m^{-2} s^{-1}, and a relative humidity of 40-60%. Germination was monitored by recording the number of newly germinated seeds in each dish every day for 104 days. Germination was considered to have occurred when the radicle was at least 2 mm long. To maintain consistent salinity levels throughout the experiment, the solution in each Petri dish and the filter paper were replenished on a weekly basis.

Germination percentage was calculated daily for each Petri dish, and the final germination percentage was determined at the end of the experiment. The germination index (GI), which represents both the percentage and rate of germination (Kader, 2005; Javaid et al., 2018), was calculated as described by the Association of Official Seed Analysis (1993) as follows:

$$\mathrm{GI} = \frac{\text{Number of germinated seeds}}{\text{Days of the first count}} + \ldots + \frac{\text{Number of germinated seeds}}{\text{Days of final count}}$$

The speed of germination was assessed as the reciprocal of the time to 50% of final germination ($1/T_{50}$; Al-Turki et al., 2022). The time to first germination (lag time) was also calculated in each Petri dish. Mean germination time (MGT) was calculated following the equation:

$$\mathrm{MGT} = \frac{\Sigma(n1\,T1 + n2\,T2 + \cdots + nk\,Tk)}{\Sigma(n1 + n2 + \cdots + nk)}$$

where n is number of newly germinated seeds and T is the time from the beginning of the experiment.

To assess the viability of the non-germinated seeds, they were first transferred to distilled water and germination was recorded after 25 days (recovery percentage); all remaining non-germinated seeds were tested for viability using a 1% (w/v) solution of 2,3,5-triphenyltetrazolium chloride (Tetrazolium viability percentage; Bradbeer, 2013). The overall percentage of viable seeds in each Petri dish was calculated as follows:

$$\% \text{ Viability} = \frac{Ns + Nr + Nz}{Nt} x \ 100$$

where *Ns* is the number of germinated seeds in the saline solution, *Nr* is the number of recovered seeds (i.e., germinated in distilled water), *Nz* is the number of seeds positive for tetrazolium test, and *Nt* is the total number of sampled seeds.

Seedling early growth

To assess how seedling growth and establishment were influenced by salinity, five seedlings from each Petri dish (approximately 14-17 days from the start of germination) were transplanted into sand-filled pots arranged in trays, maintaining their corresponding salinity treatments. The salt treatments were prepared by combining 20% Hoagland's solution with NaCl at the appropriate concentration. To maintain consistent salinity levels throughout the growth experiment, the volume of salinity solution in the trays was carefully monitored (4 L; 4 cm depth), following the methodology described in Martins-Noguerol et al. (2021). Greenhouse conditions were maintained under natural daylight (200 µmol m^{-2} s^{-1} as the minimum and 1000 µmol m^{-2} s^{-1} as the maximum light flux), temperature between 28/22 °C and 40–60% relative humidity. After approximately 5 months (with a ±3 days range to account for the variability in germination times ensuring consistency and accuracy), the maximum height of each plant was measured, and the leaf relative water content was calculated as described previously. The final dry biomass of the aboveground (AGB) and the belowground (BGB) parts was determined by harvesting the plants, drying both fractions at 80 °C for 48 h, and weighing them with a precision balance. The relative growth rate (RGR) of the entire plants was calculated as follows:

$$\mathrm{RGR} = \frac{\ln \mathrm{Bf} - \ln \mathrm{Bi}}{\mathrm{D}}$$

where *Bf* is the final dry mass, *Bi* the initial dry mass and *D* is the duration of experiment (days).

Statistical analyses

The mother plants were categorized on the basis of the salinity of the soil from which they had been sampled, classifying them into low salinity (<100 mS cm^{-1}, 15 individuals), intermediate salinity (100-200 mS cm^{-1}, 15 individuals), high salinity (201-450 mS cm^{-1}, 15 individuals) or hypersalinity (>450 mS cm^{-1}, 13 individuals), regardless of the population they belonged to (Figure 2). Individuals were categorized by salinity ranges instead of by populations because a preliminary analysis showed that salinity differences were greater at the intrapopulation level (among individuals) than at the interpopulation level (Supplementary table S1). However, to account for differences between populations, this factor was used as a random variable. To test the influence of salinity category on fitness of maternal plants, General Linear Mixed Models (GLMM) and post hoc Tukey's tests were performed using five response variables related to reproductive fitness (seed weight, fruit weight, number of infructescences per plant, number of seeds per infructescence, and number of seeds per plant) and four variables related to plant growth and status (volume, vegetative height, reproductive height and RWC). Maternal salinity was considered a fixed factor, with population as a random factor. GLMMs were also used to model effects of maternal salinity and salinity treatment (0, 50, 100 and 150 mM NaCl) on germination (germination, recovery from salinity, tetrazolium dormancy, total viability, GI, MGT, $1/T_{50}$, lag time) and seedling growth (maximum height, RWC, AGB, root biomass and RGR) of their offspring. Maternal salinity and salinity treatment were considered as fixed factors, with population as a random factor and seed mass as a covariate to account for its potential effects on germination (Agrawal, 2001).

Differences in environmental characteristics (conductivity, pH, organic matter and gravel, sand, silt and clay contents) among the sampling sites were also analyzed using GLMs, with population as the only fixed factor. Prior to the analyses, data were tested for homoscedasticity using Levene's test and for normality using Shapiro–Wilk test for the model residuals. In order to meet these assumptions of parametric tests, some response variables were transformed using ln(x), 1/x, √x and logit. When homoscedasticity was not achieved after data transformation, univariate differences were analyzed using the γ generalized linear mixed model (GLMM) with Wald's χ^2 (Ng and Cribbie, 2017), as all variables were continuous and positive (AGB, BGB, RWC, RGR). Additionally, pairwise correlations between variables were examined, and highly correlated response variables (Kendall's coefficient >0.9) were removed from the analyses. All statistical analyses were performed using R-software (R Core Team, 2022), using the packages *DHARMa* (Hartig et al., 2016) and *lme4* (Bates et al., 2015).

RESULTS

A total of 3480 seeds obtained from 58 mother plants were sown for their use in the experiments of germination and seedling growth. From these, 1160 seedlings were selected and 983 of them (those that survived the transplantation process) were monitored for survival and early growth.

Maternal traits

The maternal environments exhibited a wide range of soil physicochemical properties, with mean levels of soil salinity per geographical population ranging from 138 to 492 mS cm^{-1} (Supplementary table S1). In addition, we encountered considerable intra-population variation in the salinities experienced by individual maternal plants (9 to 1511 mS cm^{-1}); hence, the classification of maternal plants based on their salinities was made at the individual level, irrespective of population (Figure 2). Despite the significant variation in soil maternal salinity, there were no significant differences in vegetative and reproductive yields among the maternal plants (Table 1). The vegetative volume of plants ranged from 0.53 to 0.78 m^3, with a vegetative height of 0.26-0.31 m and a reproductive height of 0.37-0.48 m. All the plants produced between 83,366-88,441 seeds per plant, and there were no significant differences in the weight of seeds or fruits, which ranged between 2.00-2.23 mg and 1.35-1.52 mg, respectively (Table 1).

Germination, seedling survival and growth in response to salinity

Salinity treatment significantly affected all the germination and seed viability parameters quantified in this study, whereas seed weight had no significant effects on any of these response variables ($p>0.05$; Table 2). We found a slight reduction of the final germination percentages for seeds germinated at 50 mM and 100 mM NaCl compared with non-saline solutions, a 22% reduction at 150 mM NaCl and a drastic 95% reduction at 300 mM; there was no germination at 500 mM NaCl (Figure 3A). The lag time for germination increased progressively with salinity (Figure 3B), indicating delayed radicle emergence in saline conditions that was reflected in the MGT (Figure 3E). Accordingly, GI was progressively reduced with increasing salinity (Figure 3D) and 1/T_{50} decreased in all salinity treatments (Figure 3C), indicating a slowed germination rate in those seeds subjected to increasing saline conditions. Salinity conditions starting from 50 mM to 150 mM NaCl significantly reduced seed viability in 6-12% comparing with the non-saline solutions, whereas 300 mM and 500 mM decreased it by 26% and 13% respectively (Figure 3F). A large fraction of seeds that had failed to germinate under saline treatments, particularly at 300 and 500 mM, were able to germinate when transferred to distilled water (Figure 3G). Among the seeds that did not recover, 1-11% of the original samples remained viable according to the tetrazolium test, a fraction showing a clear tendency to increase with salinity, if with only limited significant differences between treatments (Figure 3H).

When seedlings were grown under the same salinity conditions that they had initially germinated in (0-300 mM NaCl), none was able to survive when exposed to a salinity of 300 mM NaCl, whereas 90% survived in non-saline conditions, 91% at 50 mM, 89% at 100 mM, and 69% at 150 mM NaCl. All parameters related with early seedling growth were affected by salinity. RGR was not significantly reduced by 50 mM NaCl but was progressively lowered by 7% and 25% at 100 and 150 mM NaCl, respectively (Figure 4A). The contributions of aboveground and belowground biomass to this response were rather different: belowground biomass was not significantly affected by 50 mM NaCl but declined steeply with greater salinity (Figure 4B), whereas aboveground biomass simply declined linearly with increasing salinity (Figure 4C). The aboveground biomass response was replicated in the linear decline of plant height with salinity (Figure 4D). All salinity treatments reduced RWC significantly by 3.3-4.5% relative to the control (Figure 4E), with no differences among them.

Maternal effects on germination and seedling growth

Effects of maternal salinity during seed development on subsequent germination characteristics were subtle and complex. Averaged over all salinity treatments, maternal salinity had no discernible impact on the germination percentage, any of the measures of the timing/speed of germination or seed viability (Figure 5; Supplementary figure S1). However, GLMs separated the effects of maternal and treatment salinities and accounting for potential effects of variation in seed weight did detect certain responses to maternal salinity (Table 2). There was a marginally significant effect of maternal salinity on GI ($p=0.0527$), indicating a slight delay in germination in those seeds coming from mother plants exposed to higher soil salinity (Table 2). There was no indication of interactive effects between maternal and treatment salinities.

In contrast, maternal salinity had a greater impact on progeny seedling growth (Figure 6). Seedlings derived from mothers that had experienced salinities <100mS cm^{-1} had a slightly but significantly higher RGR than those from mothers growing at higher salinities (Figure 6A). This pattern was also reflected in their significantly higher aboveground and belowground biomasses (Figure 6B-C). In terms of the GLMs, the separate response of RGR to maternal salinity was marginally significant (p=0.051) but the interactive (maternal salinity x treatment salinity) effect was highly significant for both aboveground (p=0.022) and belowground (p=0.0063) biomasses. No effects of maternal salinity on plant height (Figure 6D) or RWC (Figure 6E) were detected.

DISCUSSION

The six populations of *C. maritimum* that were sampled for this study spanned the entire southern coast of the Iberian Peninsula, including climatic conditions influenced by the Atlantic Ocean and the Mediterranean Sea. Previous studies have suggested that climatic factors may contribute to variation in genome size in *C. maritimum* along the coasts of mainland Portugal and its Atlantic islands (Roxo et al., 2023). However, the physicochemical characteristics of the soils supporting our populations were remarkably similar on average, emphasising the distinctive niche (Fernández-Pascual et al., 2017) of this species on coastal cliffs and sands. Despite this apparent uniformity, there was substantial within-population heterogeneity in the salinity experienced by individual plants, which could have contributed to the lack of differentiation in salinity responses. Another likely factor is efficient long-distance dispersal by hydrochory, which has been attributed to weak genetic structuring elsewhere in the species' range (Latron et al., 2020). Our broad base of populations, however, provides a robust characterization of the salinity tolerance of *C. maritimum* during both seed germination and early seedling growth stages.

Direct effects of salinity on germination and early growth

C. maritimum seeds exhibited high viability, with close to 100% germination in distilled water. The ability to germinate under saline conditions was remarkably high for a species that typically does not experience seawater inundation during growth (Woodell, 1985; Gul et al., 2013). Germination percentage diminished by 6-22% at 50-150 mM NaCl, with little germination at 300 mM and none at 500 mM NaCl. This result is broadly consistent with findings for material from the SW coast of England (Okusanya, 1977). However, it suggests significantly greater tolerance than that reported for populations from the coasts of Brittany, NW France (Meot-Duros and Magne, 2008), Sardinia (Marchioni-Ortu and Bocchieri, 1984), SW Italy (Strumia et al., 2020) and Croatia (Nimac et al., 2018). Results from NW Tunisia have varied somewhat but agree that there is little or no germination by a concentration of c. 200 mM NaCl (Atia et al., 2006, 2009*a*, 2009*b*, 2011*c*, 2012). Meot-Duros and Magne (2008) also reported almost complete inhibition of germination at 50 mM NaCl. Differences in genetic or phenotypic responses to local environmental conditions at diverse geographical locations may have contributed to the observed variations (Adolf et al., 2013; Bhatt et al., 2020). However, our findings from the southern Iberian coast cover a broader geographical gradient than any previous studies.

Increasing salinity also progressively delayed radicle emergence and slowed germination, as measured by all three velocity metrics employed. This response is typical of the germination of virtually all salt-tolerant plants studied (Baskin and Baskin, 1998) and has been reported in most studies of the germination of *C. maritimum* at relatively low salinities (e.g. Atia et al., 2006, 2011*a*, 2012). However, the ability to maintain germination velocity can be a further indicator of tolerance. In our material from the Iberian coast, severe effects were only evident above 150 mM NaCl, suggesting a greater tolerance to high salt concentrations than previously reported.

The fact that most seeds that had failed to germinate when treated with NaCl up to 500 mM were able to germinate when subsequently transferred to distilled water indicates that dormancy was enforced reversibly by the osmotic effects of salinity. It is well known that halophyte seeds often experience salinity-enforced dormancy (e.g. Gul et al., 2013), and similar good recovery has been reported repeatedly for *C. maritimum* (e.g. Marchioni-Ortu and Bocchieri, 1984; Meot-Duros and Magne, 2008; Atia et al., 2011*a*; Strumia et al., 2020). The ability of our seeds to recover from a salt concentration similar to that of seawater (500 mM NaCl) suggests that there are no toxic effects of ion accumulation during the incubation period. A previous study reported good recovery from seawater treatment (Strumia et al., 2020), which our findings support, despite their experiment's treatment phase being only 10 days. We also report for the first time a smaller fraction of seeds that experience deeper, physiological dormancy induced by salinity. Considering that the corky, buoyant seeds of this species rely on hydrochory in seawater for long-distance dispersal (Latron et al., 2020), these findings highlight the importance of understanding variations in their ability to survive immersion in seawater.

Our results from the seedling growth experiment suggests that salinity is more limiting during seedling establishment and growth than during germination, as survival was reduced above 50 mM, and no seedlings survived above 150 mM NaCl. However, RGR was unaffected at 50 mM and only depressed by 25% at 150 mM relative to the control, indicating significant salt tolerance at this crucial stage. In the only comparable study known to us, Amor et al. (2005) found significant increase in seedling growth at 50 mM NaCl compared to controls, although all growth aspects measured were dramatically reduced at 200 mM NaCl. The maintenance of RGR in our Iberian material involved interesting growth adjustments to salinity, with aboveground biomass and height decreasing linearly with salinity, while belowground biomass was maintained preferentially at 50 mM NaCl. A shift in biomass allocation to roots rather than shoots could be an effective response to water stress arising from the lower external osmotic potential. Comparable reductions in seedling survival and biomass with salinity were reported by Woo and Takekawa (2012) on *Sarcocornia pacifica*, a more extreme halophyte, that was able to grow in salinities up to that of seawater (30 psu).

All salinity treatments decreased the RWC of plants by only 3.5% relative to the control, with no significant differences across salinity treatments. This is likely due to effective osmoregulation within the range of 50-150 mM NaCl, associated with increased uptake of inorganic ions (Amor et al., 2005) and accumulation of compatible organic osmolytes (Hamdani et al., 2017). The sensitivity of seedlings to salinity is clearly much greater than that of adult plants, as mature individuals were able to maintain photosynthesis and growth even when exposed to a salinity of 500 mM NaCl for 60 days (Castillo et al., 2022).

Maternal effects on germination and early growth in saline conditions

In the apparent absence of differentiation between populations in salinity exposure, the focus of attention turns to distinguish possible maternal (phenotypic) effects at the individual level. By characterising the salinity experienced by individual mother plants in the field, it was possible to analyse the salt tolerance in their particular progenies. This analysis reveals subtle transgenerational influences of maternal salinity on *C. maritimum* performance for the first time. There were no apparent effects on the capacity for seeds to germinate or their potential (seed viability) when different provenances were compared. Consistent with this finding, Mohamed et al. (2020) also found no significant variations in germination percentages between seeds of the halophyte *Zygophyllum coccineum* originating from saline and non-saline habitats. However, studies on the halophytes *Suaeda aegyptiaca* and *Anabasis setifera* have reported that seeds matured in non-saline environments have a better ability to germinate under saline conditions (El-Keblawy et al., 2016, 2018).

The marginally significant effect of maternal salinity on GI may suggest an association with increased velocity of germination, as previous research has reported faster germination in halophyte seeds maturing under high salinity, thus potentially providing them advantageous access to water and nutrient resources (Van Zandt and Mopper, 2004; Lázaro-Lobo et al., 2020; Yao et al., 2010). However, this was not substantiated in our other measures of germination velocity, 1/T50 and MGT.

The high germination ability of *C. maritimum* seeds at low salinity suggests that maternal salinity does not affect innate or induced dormancy, which is consistent with previous findings in *Suaeda vermiculata* seeds (Al-Shamsi et al., 2018). However, in more extreme halophyte species, seeds matured in saline environments may display greater dormancy as an ecological survival strategy in salt-affected environments (El-Keblawy et al., 2020). The varying transgenerational germination responses of different species to salinity suggest that generalisations can only be clarified by further context-dependent or species-specific investigation.

The evidence for an influence of maternal salinity on early seedling growth was more compelling, with highly significant interactions between maternal salinity and salinity treatment for both above and below ground biomass, and a marginally significant main effect on RGR in the GLMs. These effects were negative, with maternal salinity reinforcing the inhibition imposed by salinity treatment. Although positive adaptive transgenerational responses to maternal salinity on seedling growth have been observed in other species (Caño et al., 2016), this is not a universal trend and maternal effects can reduce offspring fitness (Marshall and Uller, 2007), which aligns with our results. Mother plants grown in saline environments may decrease resource provision for the seeds, leading to reduced seedling growth (Lázaro-Lobo et al., 2020). Moreover, maternal effects are often related to increased seed size and production or earlier flowering (Caño et al., 2014; Guo et al., 2020). Halophytes that produce dimorphic seeds are known to show adaptive, transgenerational effects. For instance, the black seeds of *Suaeda splendens* tend to delay germination at high salinity and produce seedlings that are more sensitive to salinity, in contrast to the brown ones (Redondo-Gómez et al., 2008). However, *C. maritimum* does not produce dimorphic seeds, and our study has revealed no significant effects of seed weight. Additionally, there were no differences among mother plants in terms of seed production, seed or fruit weights, and vegetative or reproductive traits, regardless of the salinity levels in their growth environment. This suggests that, although not evident differences in seed size or dimorphism, *C. maritimum* seeds might possess physiological traits influenced by maternal salinity. These potential physiological characteristics could play a role in their adaptive responses to saline environments, likely explaining the observed influence of maternal salinity on early seedling growth.

C. maritimum is well adapted to habitats with highly variable soil physicochemical properties (Martins-Noguerol et al., 2023). Its seed output of 8336-88441 per plant indicates consistent fitness across the range of salinity in the field. Such phenotypic plasticity, rather than genetic differentiation, is likely attributable to two factors. First, rapid changes in salinity associated with exceptionally well-drained substrates exposed to salt spray and leaching by periodic rainfall can lead to disruptive selection. Second, local cross-pollination between individuals in high- and low-salinity environments by insects (Kozuharova et al., 2018) can prevent genetic differentiation at this scale. Future studies could consider multiple generations to distinguish between evolutionary responses and carry-over effects of field-grown materials (e.g. Wang et al., 2021). It is also important to consider other traits, such as the initiation of flowering, which have previously been found to have the most consistent maternal impacts on seedling fitness (Caño et al., 2016). Overall, the observed reduction in seedling growth, influenced by maternal salinity, opens an important path for further research on the complex mechanisms underlying early growth development in halophyte species such as *C. maritimum.*

This study distinguishes between the direct effects of salinity on *C. maritimum* and those associated with its maternal environment. We highlight, for the first time, a significant impact of maternal salinity on early growth of *C. maritimum* progeny that reinforces the negative direct effects of salinity on plant fitness. No clear similar effect was detected for germination. However, the high tolerance of *C. maritimum* to salt stress during germination and recovery appears to be higher than previously reported. This result has important implications for the development of cultivation techniques relevant to the commercial valorisation of the species. We also establish a previously undocumented sensitivity of biomass accumulation in the early stages of seedling growth to varying salinity conditions, which is critical in the evaluation of *C. maritimum* as a future crop. This highlights the importance of further investigations to uncover comprehensively the mechanisms underlying early growth under saline conditions and the role of transgenerational effects on the adaptative ability of halophytes to persist under changing environments.

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FIGURE CAPTIONS

Figure 1. Map showing the location of study populations on the southern Iberian coast.

Figure 2. Topsoil conductivity (0–30 cm depth) measured at 10–20 cm from the projected canopy of each *C. maritimum* plant from sampled populations. Dashed lines represent the threshold values used to categorize maternal salinity: low saline (<100 mS cm^{-1}), intermediate saline (101-200 mS cm^{-1}), high saline (201-450 mS cm^{-1}) and hypersaline soils (>450 mS cm^{-1}).

Figure 3. Germination parameters and viability of *C. maritimum* seeds in different salinity treatments (50-500 mM) and non-saline solution (0 mM NaCl). **(A)** Final germination percentage at the end of the experiment (104 days). **(B)** Lag time (days). **(C)** Mean Germination Time (days). **(D)** Germination Index. **(E)** Reciprocal of the time to 50% of the final germination percentage (1/T_{50}). **(F)** Percentage of total viable seeds (including seeds that germinated in saline solutions, seeds that recovered and germinated after being transferred to distilled water, and seeds that were viable according to the tetrazolium test). **(G)** Percentage of seeds germinated in distilled water following exposure to saline conditions (recovery). **(H)** Percentage of viable seed according to tetrazolium test. Different letters indicate significant differences ($p < 0.05$). Data represent mean and standard error of 58 independent replicates. N/A, not applicable.

Figure 4. Responses of different parameters related to early growth and plant water status depending on the salinity treatments where they germinated (50-500 mM NaCl or non-saline solution). **(A)** Relative growth rate (g/ g day). **(B)** Belowground biomass (dry weight/ plant).

(C) Aboveground biomass (g dry weight/ plant). **(D)** Maximum vegetative height (cm). **(E)** Relative water content (%). Different letters indicate significant differences ($p < 0.05$). Data represent means and standard errors of 201-264 independent replicates.

Figure 5. Maternal effects on the germination and viability of *C. maritimum* seeds assessed based on the maternal salinity (<100 mS cm^{-1}, 100-200 mS cm^{-1}, 201-450 mS cm^{-1}, >450 mS cm^{-1}). **(A)** Final germination percentage at the end of the experiment (104 days). **(B)** Lag time (days). **(C)** Mean Germination Time (days). **(D)** Germination Index. **(E)** Reciprocal of the time to 50% of the final germination percentage ($1/T_{50}$). **(F)** Percentage of total viable seeds (including seeds that germinated in saline solutions, seeds that recovered and germinated after being transferred to distilled water, and seeds that were viable according to the tetrazolium test). **(G)** Percentage of seeds germinated in distilled water following exposure to saline conditions (recovery). **(H)** Percentage of viable seed according to tetrazolium test. Different letters indicate significant differences ($p < 0.05$). Data represent mean and standard error of 78-90 independent replicates.

Figure 6. Maternal effects on different parameters related to early seedling growth and plant water status in *C. maritimum*. **(A)** Relative growth rate (g/ g day). **(B)** Belowground biomass (dry weight/ plant). **(C)** Aboveground biomass (g dry weight/ plant). **(D)** Maximum vegetative height (cm). **(E)** Relative water content (%). Different letters indicate significant differences ($p < 0.05$). (*) indicates marginally significant effects. Data represent mean and standard error of 222-259 independent replicates.

TABLES

Table 1. Maternal traits of *C. maritimum* plants categorized on the basis of the salinity levels of their soil environment (low saline, intermediate saline, high saline, and hypersaline). Data represent the mean and standard error of 13-15 independent replicates. No significant differences were detected in the variables based on GLM analyses.

Maternal plant traits	**Low salinity** (<100 mS cm^{-1})	**Intermediate salinity** (101-200 mS cm^{-1})	**High salinity** (201-450 mS cm^{-1})	**Hypersalinity** (>450 mS cm^{-1})	**F**	**p**
Seed weight (mg)	2.12 ± 0.174	2.11 ± 0.172	2.36 ± 0.18	2.00 ± 0.18	1.5709	0.2075
Fruit weight (mg)	1.46 ± 0.08	1.35 ± 0.08	1.40 ± 0.08	1.52 ± 0.08	1.9235	0.1371
Infructescences per plant	220 ± 46.7	218 ± 48.9	172 ± 45.9	202 ± 47.3	1.1332	0.3441
Seeds per infructescence	427 ± 72	389 ± 73.3	423 ± 71.5	452 ± 72.4	1.3146	0.2797
Seeds per plant	88,441 ± 24485	83,366 ± 25523	86,847 ± 24106	85,904 ± 24777	0.6869	0.564
Volume (m^3)	0.72 ± 0.19	0.78 ± 0.20	0.53 ± 0.18	0.70 ± 0.19	1.0334	0.3855
Vegetative height (m)	0.30 ± 0.04	0.31 ± 0.04	0.26 ± 0.04	0.28 ± 0.04	0.8117	0.4932
Reproductive height (m)	0.44 ± 0.05	0.48 ± 0.05	0.37 ± 0.05	0.45 ± 0.05	0.5528	0.6485
Leaf water content (%)	84.8 ± 3.66	89 ± 3.9	90.9 ± 3.61	86.6 ± 3.71	0.5987	0.6186

Table 2. General Linear Mixed Models (GLMM) testing the effects of salinity treatments, maternal salinity, the interaction between salinity treatments and maternal salinity, and seed weight on different parameters related with seed germination and early seedling growth. F values and associated significance levels (P) are shown. Population was included as a random factor. Those factors that exerted significant or marginally significant effects are highlighted in dark bold: (***) p<0.001, (**) p<0.01, (*) p<0.05. GI, germination index; MGT, mean germination time; RGR, relative growth rate; BGB, belowground biomass; AGB, aboveground biomass; RWC, relative water content.

			Salinity treatments	**Maternal salinity**	**Salinity treatments *x* Maternal salinity**	**Seed weight**
Germination	Germination %	F	380.1722	0.1221	0.7238	1.4808
		P	**<0.001 *****	0.9470	0.7598	0.2254
	GI	F	18.7584	2.6078	0.7113	1.4344
		P	**<0.001 *****	**0.0527**	0.6982	0.2328
	MGT	F	82.9125	2.0908	0.8536	3.4968
		P	**<0.001 *****	0.1024	0.5677	0.0629
	Lag time	F	32.8147	1.6642	1.1549	2.0933
		P	**<0.001 *****	0.1758	0.3257	0.1495
	$1/T_{50}$	F	5.8162	0.5281	0.5181	2.2516
		P	**<0.001 *****	0.6642	0.8606	0.14228
	Total viability %	F	16.9108	1.4523	0.8038	3.6098
		P	**<0.001 *****	0.2279	0.6732	0.0585
	Recovery %	F	61.7825	1.0868	0.8395	1.0574
		P	**<0.001 *****	0.3576	0.6098	0.3083
	Tetrazolium viability %	F	3.0623	1.2851	0.9179	2.1760
		P	**0.0185 ***	0.2821	0.5308	0.1441
Seedling growth	RGR	F	213.2055	7.7658	11.8517	0.7221
		P	**<0.001 *****	**0.0511**	0.2218	0.3955
	BGB	F	154.9518	3.5828	22.9626	1.9676
		P	**<0.001 *****	0.310187	**0.0063 ****	0.1607
	AGB	F	215.1787	5.5678	19.4381	1.9567
		P	**<0.001 *****	0.1346	**0.0217 ***	0.1619
	Height	F	289.9754	1.9645	1.4358	2.2516
		P	**<0.001 *****	0.1179	0.1680	0.1341
	RWC	F	60.7550	6.1555	14.0510	1.4562
		P	**<0.001 *****	0.1043	0.1205	0.2275

Figure 1


○ Sandy
● Cliffs
● Shingle
Falesia
Conil
Bolonia
El Toyo
Los Muertos
Calblanque

Figure 2

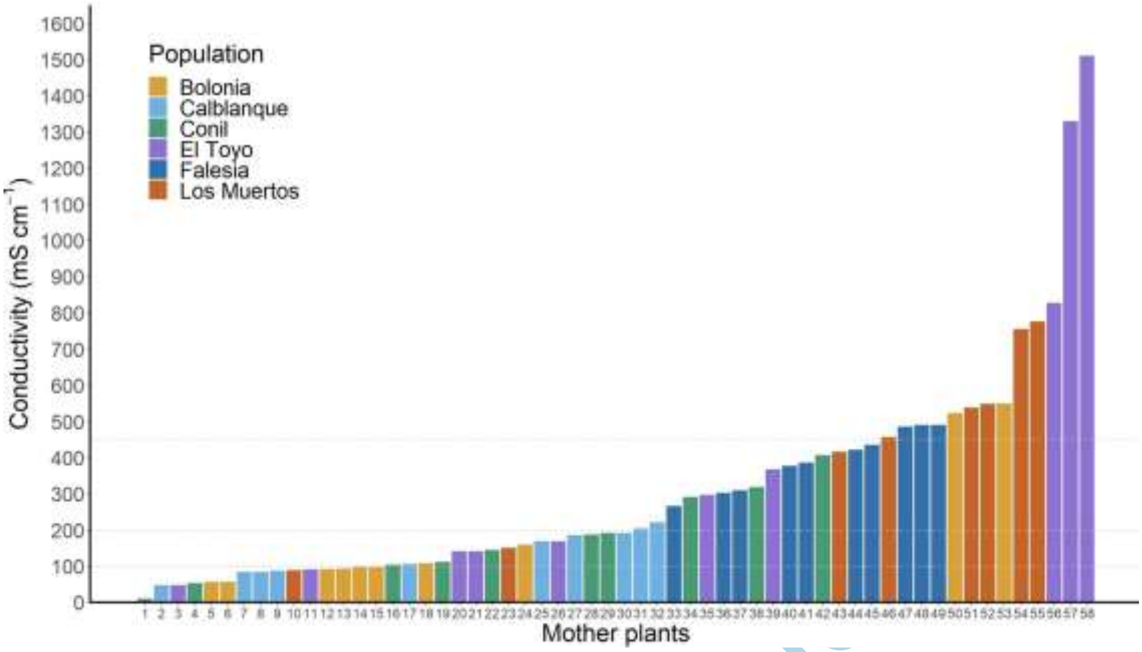
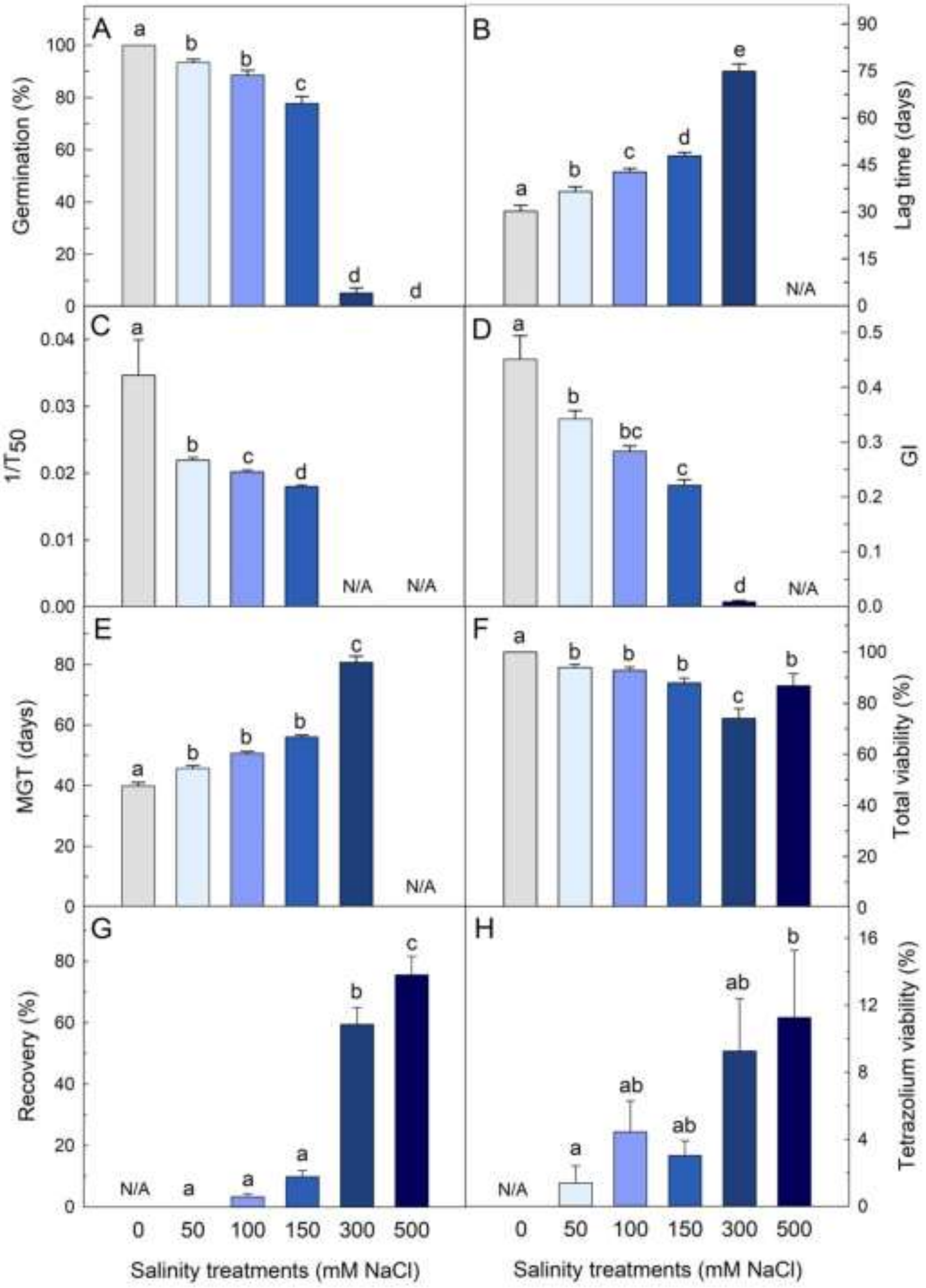

Population
Bolonia
Calblanque
Conil
El Toyo
Falesia
Los Muertos
Conductivity (mS cm^{-1})
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
Mother plants
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58

Figure 3



A
Germination (%)
0
20
40
60
80
100
a
b
b
c
d
d
B
Lag time (days)
0
15
30
45
60
75
90
a
b
c
d
e
N/A
C
1/T50
0.00
0.01
0.02
0.03
0.04
a
b
c
d
N/A
N/A
D
GI
0.0
0.1
0.2
0.3
0.4
0.5
a
b
bc
c
d
N/A
E
MGT (days)
0
20
40
60
80
a
b
b
b
c
N/A
F
Total viability (%)
0
20
40
60
80
100
a
b
b
b
c
b
G
Recovery (%)
0
20
40
60
80
N/A
a
a
a
b
c
H
Tetrazolium viability (%)
0
4
8
12
16
N/A
a
ab
ab
ab
b
0
50
100
150
300
500
Salinity treatments (mM NaCl)
0
50
100
150
300
500
Salinity treatments (mM NaCl)

Figure 4

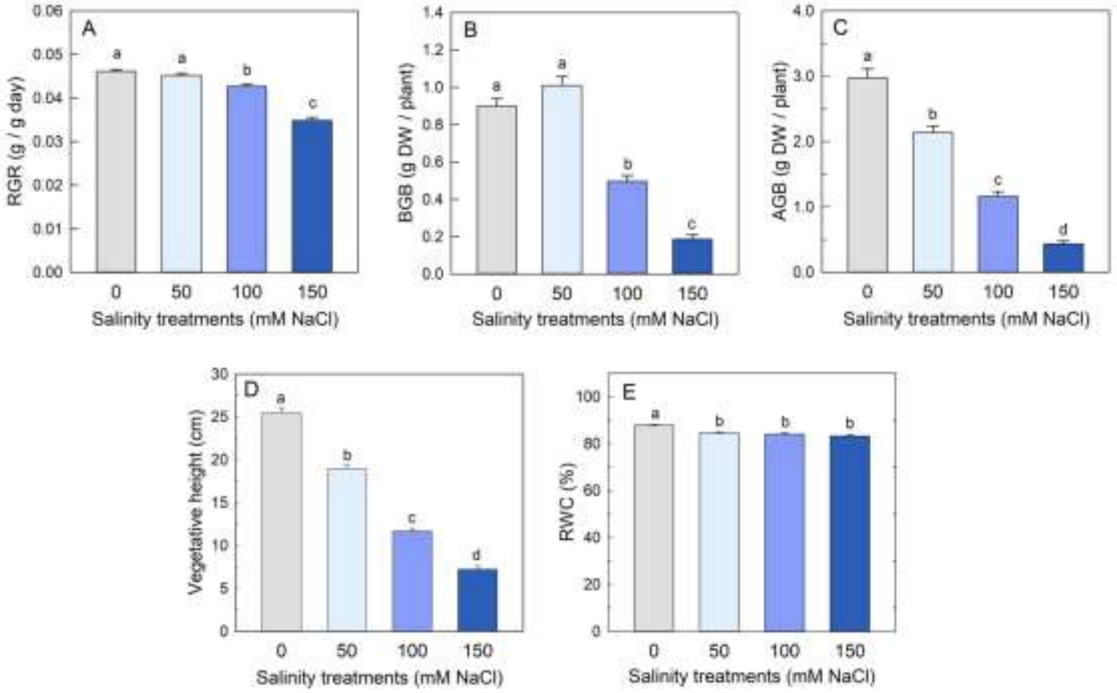
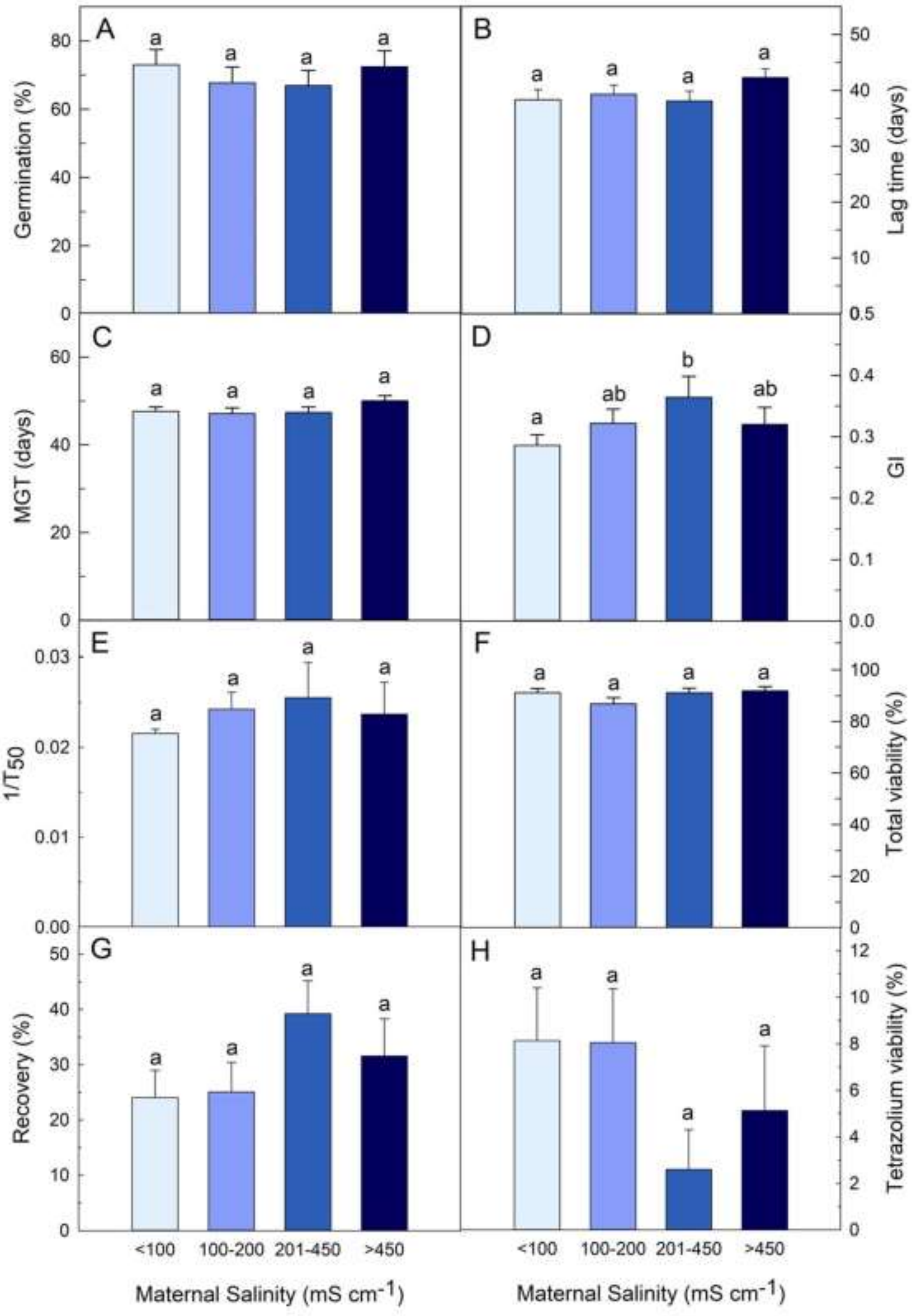
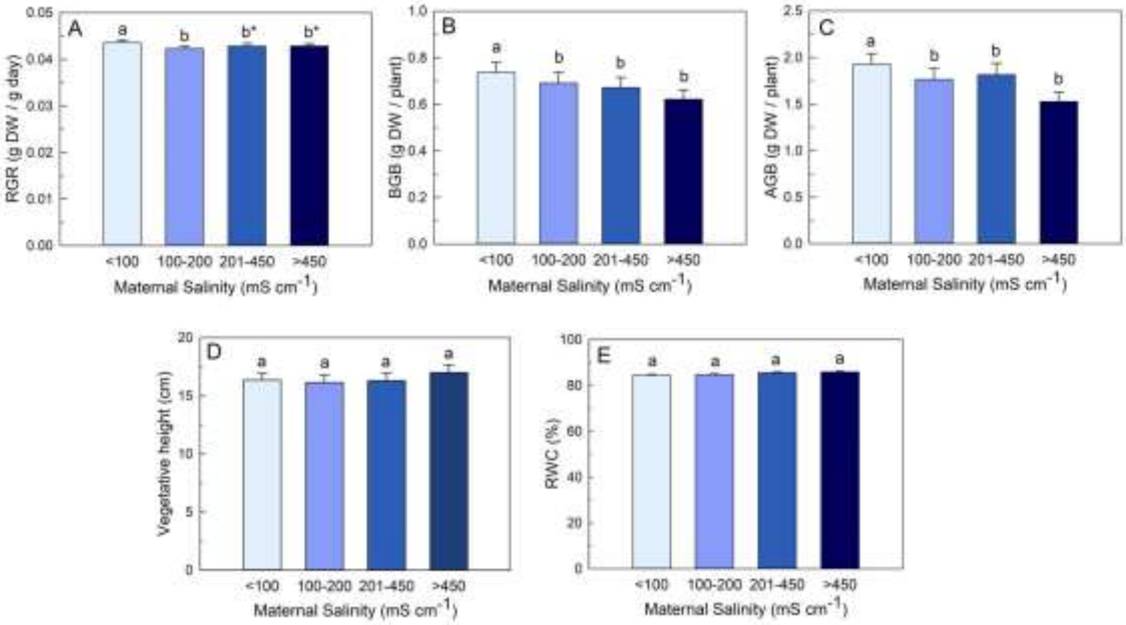

A
RGR (g / g day)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
a
a
b
c
0
50
100
150
Salinity treatments (mM NaCl)
B
BGB (g DW / plant)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
a
b
c
0
50
100
150
Salinity treatments (mM NaCl)
C
AGB (g DW / plant)
4.0
3.0
2.0
1.0
0.0
a
b
c
d
0
50
100
150
Salinity treatments (mM NaCl)
D
Vegetative height (cm)
30
25
20
15
10
5
0
a
b
c
d
0
50
100
150
Salinity treatments (mM NaCl)
E
RWC (%)
100
80
60
40
20
0
a
b
b
b
0
50
100
150
Salinity treatments (mM NaCl)

Figure 5



A
Germination (%)
0
20
40
60
80
a
a
a
a
B
Lag time (days)
10
20
30
40
50
a
a
a
a
C
MGT (days)
0
20
40
60
a
a
a
a
D
GI
0.0
0.1
0.2
0.3
0.4
0.5
a
ab
b
ab
E
1/T50
0.00
0.01
0.02
0.03
a
a
a
a
F
Total viability (%)
0
20
40
60
80
100
a
a
a
a
G
Recovery (%)
0
10
20
30
40
50
a
a
a
a
H
Tetrazolium viability (%)
0
2
4
6
8
10
12
a
a
a
a
<100
100-200
201-450
>450
Maternal Salinity (mS cm-1)
<100
100-200
201-450
>450
Maternal Salinity (mS cm-1)

Figure 6


A
RGR (g DW / g day)
0.00
0.01
0.02
0.03
0.04
0.05
a
b
b*
b*
<100
100-200
201-450
>450
Maternal Salinity (mS cm^{-1})
B
BGB (g DW / plant)
0.0
0.2
0.4
0.6
0.8
1.0
a
b
b
b
<100
100-200
201-450
>450
Maternal Salinity (mS cm^{-1})
C
AGB (g DW / plant)
0.0
0.5
1.0
1.5
2.0
2.5
a
b
b
b
<100
100-200
201-450
>450
Maternal Salinity (mS cm^{-1})
D
Vegetative height (cm)
0
5
10
15
20
a
a
a
a
<100
100-200
201-450
>450
Maternal Salinity (mS cm^{-1})
E
RWC (%)
0
20
40
60
80
100
a
a
a
a
<100
100-200
201-450
>450
Maternal Salinity (mS cm^{-1})