Fertilization positively affects plants performance but reduces seed viability in seashore mallow (Kosteletzkya pentacarpos): implication for biomass production and species conservation

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Fertilization positively affects plants performance but reduces seed viability in seashore mallow (*Kosteletzkya pentacarpos*): implication for biomass production and species conservation

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Abstract

Kosteletzkya pentacarpos (L.) Ledeb. (seashore mallow) is recognized as an important plant species for several industrial applications, especially in North America and Asia. In Europe, seashore mallow is highly threatened and subject of conservation actions (e.g. reintroduction). In order to define appropriate multipurpose cultivation protocols, suitable for biomass production and for conservation purposes, we investigated the effect of varying levels of soil salt content and nutrient availability on seashore mallow. As expected, seashore mallow had the best performance in terms of growth rate, flowering and fruit production when fertilization was applied, while salt had only limited effects. Interestingly, seeds produced by fertilized plants showed a lower germination performance and higher mortality than seeds developed form control and non-fertilized plants. Our results highlight a trade-off between parental plant growth and seed performance, the former being enhanced by fertilizer addition and the latter being reduced by fertilization. The causes of this trade-off are related to a transgenerational maternal effect of fertilizer that has important implications for seashore mallow cultivation. From a practical point of view, biomass production benefits from fertilization but if the quality of seeds and the fitness of the next generation are major objectives (e.g. seed production and species conservation), fertilization is not recommended.

Keywords

Cultivation protocol, Fertilization, Reproduction cost, Seed germination, Salt stress

Introduction

Seashore mallow [*Kosteletzkya pentacarpos* (L.) Ledeb.] is a perennial halophytic herb, belonging to Malvaceae. Until recently, the North American populations of *K. pentacarpos* were placed in *K. virginica* (L.) C. Presl ex A. Gray (Alexander *et al.*, 2012). However, Blanchard (2008) turned the name into synonymy within *K. pentacarpos* because of the weak morphological difference between European and American populations. So, this species is native of southeastern U.S.A., Western Asia and Southern Europe, where it grows in brackish to saline coastal wetlands (Pino & De Roa, 2007; Blanchard, 2012; Ercole *et al.*, 2013). This species rapidly grows up to 2 m high (Pino & De Roa, 2007), producing a large number of stems with excellent fiber properties that can be used for novel textiles (Fan *et al.*, 2011). It can also be used for restoration of saline soils (Qin *et al.*, 2015). Nevertheless, one of the most interesting applications of seashore mallow is in the field of bioenergy. The search for alternative fuel sources stimulates the demand for plant-based energy production (Markevicius *et al.*, 2010). Biofuels are alternative, renewable, nontoxic and biodegradable energy sources obtained from plant carbohydrates that make up about 75% of plant dry weight . Since seashore mallow produces high amounts of seeds (up to 1500 kg/ha) with high protein (32%) and oil (22%) content, it can be used as an oilseed/bioenergy crop (Moser *et al.*, 2013; Knothe & Moser, 2015).

Seashore mallow can grow on saline mesotrophic to eutrophic soils thanks to both cellular-level and whole plant-level mechanisms (Li *et al.*, 2006). So, it has been suggested as a grain crop for seawater-based agricultural systems with high salt content in the soil (Ruan *et al.*, 2008). Halophyte domestication, either for biomass production or as potential crops is an emerging approach in agriculture, which enhances rehabilitation of salinized lands (Flowers, 2004). Indeed, seashore mallow can represent an effective tool for improving the productivity of marginal areas, such as saline–alkaline soils, also considering future rising of sea level due to climate change (Nicholls & Cazenave, 2010). Importantly, the widespread distribution of seashore mallow may prevent concerns related to the introduction of non-native plants into new areas. In fact, intentional introductions of non-native plants can boost the invasion of local ecosystems with severe negative ecological and economic consequences (Reichard & White, 2001; Simberloff, 2008; Quinn *et al.*,

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2015). Besides their potential economic interest, wild seashore mallow populations are locally threatened, especially in Europe where this species deserved the designation of special conservation areas listed in the Habitat Directive 92/43/CEE (Annex II) or in the Berne Convention (Annex I). Seashore mallow was evaluated as 'critically endangered' in Italy (Ercole *et al.*, 2013), 'vulnerable' in Europe (Bilz *et al.*, 2011) and 'least concern' globally (Matchutadze, 2014). Whatever the goal, agriculture or conservation, cultivation of seashore mallow is receiving increasing attention (Pino & De Roa, 2007; Qin *et al.*, 2015; Voutsina *et al.*, 2015). However, consolidated cultivation protocols are still lacking. Experimental cultivation of seashore mallow in China has shown that plant biomass and seed yield vary within the same plantation, which suggests a great potential for genetic improvement of seashore mallow (He *et al.*, 2003), but also a possible effect of microsite conditions on plant performance.

In particular, interactions between salinity and nutrient load in brackish meso-eutrophic habitats can result in trade-off between biomass yield and seed quality, the latter in turn affecting offspring performance (Obeso, 2002). High nutrient availability increases root biomass and capsule production in cultivated seashore mallow (Halchak *et al.*, 2011). However, salt stress has been found to depress seed production and seed viability in the halophyte *Cakile maritima* Scop. (Debez *et al.*, 2008). *Cicer arietinum* L. possesses different sensitivity to salt stress depending on life stage and timing of stress events (Saminemi *et al.*, 2011). Vegetative growth, reproduction and offspring performance in seashore mallow may thus be differently affected by interactions between salt stress and nutrient load.

This study aims to investigate the response of *K. pentacarpos* to varying levels of soil salt content and nutrient availability. In particular, we focused on possible trade-offs between vegetative growth and reproductive performance, and their consequences on offspring vitality. Our final aim was to set up appropriate protocols for seashore mallow cultivation suitable for multiple purposes such as biomass production, seed harvest and species conservation.

Materials and methods

Plant material and experimental cultivation

Mature seeds were collected in September 2013 from capsules of wild seashore mallow plants in the Po Delta region (Natural Reserve of Volano, 44°48' N, 12°16' E). The material originated from four different populations in a 3 ha area. All plants were located in dune slacks in reed communities with *Phragmites australis* as the dominant species, and *Juncus acutus* and *J. maritimus* as the most

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abundant companion species. The seeds were bulked in order to ensure as much genetic diversity as possible which can improve the adaptive potential in the germinated plants (Godefroid *et al.*, 2016). The seeds were immediately carried to the Seed Bank of the University of Pavia for germination and subsequent pre-cultivation. The seeds of seashore mallow are characterized by physical dormancy due to a thick seed coat (Poljakoff-Mayber *et al.*, 1992), that has to be broken or removed to allow germination. Manual scarification is often used to break the hard tegument of seeds like those of *K. pentacarpos*. As manual scarification may damage the embryo, we applied percussion scarification for five minutes using a pneumatic paint shaker (CycloneTM, Broncorp Mfg. Co., Denver, CO, USA), modified as described in Mondoni *et al.* (2013). After germination, seedlings with fully open cotyledons were transferred to pot trays containing a commercial soil mixture (perlite and peat; Terflor s.r.l., Capriolo, Brescia, Italy). The transplanted seedlings (one seedling per pot) were pre-cultivated for two months in a greenhouse (20±4°C, natural light).

In April 2014 the seedlings were carried to the Botanical Garden of the University of Ferrara for the experimental cultivation. The seedlings were transferred to plastic pots (14×14×16 cm) containing the same commercial soil mixture used in the pre-cultivation period and regularly watered at 2-3 days intervals until October. Data on air temperature, precipitation and soil temperature can be found in Tables S1 and S2 (Supporting information). Two months after starting the experimental cultivation, the plants were sprayed with 2 ml of nitrogen (N free insecticide and acaricide). During the whole cultivation period, the plants were subjected to experimental addition of three levels of salt and three levels of fertilizer. The treatments were carried out by spraying the plants 25 times, at two-week intervals, with 25 ml of low (LS) or high (HS) concentration of salt (NaCl dissolved in distilled water: 7 g of NaCl and 20 g of NaCl, respectively) and 25 ml of a universal liquid fertilizer (Cifo N8, P5, K10), at low (LF) or high (HF) concentration (5 g of fertilizer and 10 g of fertilizer, respectively). The plants receiving no salt (0S) or no fertilizer (0F) were sprayed with 25 ml of distilled water, respectively. The experimental design (full-factorial with 15 replicates for each of the nine factorial combinations) is summarized in Table 1. Data on soil chemistry in the cultivation pots are summarized in Table S3 (Supporting information).

Response variables

The plants were monitored at weekly intervals for acquiring, whenever appropriate, data on vegetative growth and reproductive performance as described below.

Vegetative growth. Throughout the cultivation period we measured height (using a manual meter) and diameter (using a manual caliper) of all plants. On the same occasions, we also counted the number of branches in each plant. From 25 July to 26 September, we determined net CO_2 exchange

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rates in three sound leaves from three different individuals for each treatment. To this aim we used an open infrared gas analysis system (LCA-4, ADC Co., Hoddesdon, UK) by enclosing the leaves in a broad-type leaf chamber (PLC4B). All measurements were made at saturating photon flux density (>1000 µmol photons m^{-2} s^{-1}) and (sub)optimal temperature (25 to 35 °C; Lin *et al.*, 2012). From 20 August to 23 October, we visually assessed plant senescence according to the BBCH phenology scale (BBCH 90-99) that records senescence in relation to leaf abscission (Meier, 2001).

Reproductive performance. From 26 June to 2 October, we counted the total number of flower buds, from 13 August to 25 September the number of flowers and from 13 August to 23 October the number of fruits in each plant. At the end of the cultivation period, seeds harvested from plants that experienced the nine combinations of salt addition and fertilizer addition were used for germination tests after percussion as described before. Seed germination tests involved sowing three replicates of 30 seeds each in Petri dishes (90 mm diameter) filled with 1% agar in distilled water. The germination tests were conducted at 25°C in temperature and light-controlled incubators (LMS Ltd, Sevenoaks, UK), with 12-h daily photoperiod. Light was provided by cool white fluorescent tubes, with photosynthetically active radiation of 40-55 µmol photons m^{-2} s^{-1}. We scored the seeds for germination at daily intervals during a 15-day period. By the end of this period, most seeds germinated or died (i.e., moldy seeds). At the end of the germination tests, we performed a cut-test in order to identify ungerminated but viable seeds. Soft, empty or damaged seeds were considered non-viable and excluded from the total number of sown seeds. Ungerminated seeds were dissected with a scalpel to check if they were viable or not. Percentages of seed germination and seed mortality were recorded.

Statistics

Maximum plant height, maximum plant diameter and maximum number of branches and duration of senescence (phenophases 90-99; Meier, 2001) were statistically analyzed by two-way factorial ANOVAs with salt addition and fertilizer addition as fixed factors. Net CO_2 exchange rates were also analyzed with two-way factorial ANOVAs as above. To this aim, we analyzed separately maximum net CO_2 exchange rates, that can be regarded as a proxy of maximum photosynthetic capacity, and mean net CO_2 exchange rates calculated throughout the experimental period for each individual plant.

The periodic data on number of buds, number of flowers and number of fruits were statistically analyzed by repeated-measure two-way ANOVAs with salt addition and fertilizer addition as between-subject factors and time as within-subject factor. All ANOVA computations were carried out using the package Statistica 6.0 (StatSoft©; Version 6; StatSoft Inc., Tulsa, OK, USA). Fisher's

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post-hoc LSD tests were applied, where appropriate, for assessing significance of differences between means. Homogeneity of variance was tested, for all variables, by the Levene's test. Log transformations were applied to heteroscedastic data.

Effects of salt addition and fertilizer addition on seed germination and seed mortality were statistically assessed by two different Generalized Linear Models (GLM, full models) with binomial error and logit-link function, with salt addition and fertilizer addition as fixed factors and replicate as covariate. Significance of differences between treatments were assessed by Bonferroni's pairways contrast in SPSS 21.0 (IBM, SPSS Statistics, Chicago, IL, USA).

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Results

Vegetative growth

Both height and diameter of seashore mallow plants were significantly affected by fertilizer addition (Table 2). Salt addition did not affect either plant height or diameter (Table 2). Indeed, seashore mallow grew more both in height and in diameter when adding fertilizer (Fig. 1A,B). Fertilization enhanced growth independent of fertilizer concentration when no salt was added. Conversely, adding fertilizer at low concentration exerted stronger effects when salt was added at both concentrations (Fig. 1A,B). Both salt addition and fertilizer addition promoted ramification (Table 2). Ramification, even more than growth, responded differently to fertilization depending on levels of salt addition. Fertilization increased the number of branches when fertilizer was applied at both concentrations with no salt addition. Conversely, adding fertilizer at low concentrations exerted stronger effects on ramification when salt was added (Table 2, Fig. 1C).

Fertilizer addition also influenced the duration of senescence, with no direct effects of salt addition (Table 2) The non-fertilized plants experienced earlier leaf abscissions and longer senescence phase (59 days on average; Fig. 2) compared with the plants receiving fertilizer addition both at low concentration and at high concentration (39 days on average and 35 days on average, respectively; Fig. 2).Maximum net CO_2 exchange rate ranged between 17.09±4.59 (in 0S/LF) and 28.49±7.35 (in 0S/HF) µmol CO_2 m^{-2} s^{-1}. Mean net CO_2 exchange rate ranged between 8.61±0.64 (in 0S/0F) and 12.63±1.38 (in 0S/HF) µmol CO_2 m^{-2} s^{-1}. There were no significant differences between treatments for either maximum net CO_2 exchange rate or for mean net CO_2 exchange rate (Table 2).

Reproductive performance

Fertilizer addition enhanced the development of buds and flowers while salt addition did not (Table 3, Fig. 3A,B). However, the significant salt × fertilizer interaction (Table 3) reflected the stronger effect of fertilization at high concentration when no salt was added, as opposed to the stronger effect of fertilization at low concentration when salt was added (Fig. 3A,B). Fertilization also affected the timing of bud and flower development, as shown by the significant main effect of time and the significant time × fertilizer and time × salt × fertilizer interactions (Table 3). The plants bore flower buds all over the growing season but the flowers blossomed from early August onwards (Fig. 3C). The plants exhibited the highest number of buds and flowers in mid-August. However, fertilization generally extended the flowering period, especially when salt was added (Fig. 3C).

Fruit production was significantly affected both by salt addition and by fertilizer addition, again with a significant salt × fertilizer interaction (Table 3). Fertilization enhanced fruit production but the number of fruits was lower in the plants receiving high salt concentration unless fertilizer was added at low concentration (Fig. 3C). Significant interactions of all treatments with time mostly reflected longer persistence of fruits on the fertilized plants (Table 3, Fig. 3C).

Fertilization had a significant effect on seed germination (Table 4). The seeds produced by plants not receiving fertilizer addition exhibited higher germination percentage, with almost 100% germination in 0S/0F (Fig. 4A). In contrast, germination percentage was significantly lower in seeds produced by fertilized plants, especially when fertilizer was added in combination with salt (Fig. 4A). Instead, there was no main effect of salt addition (Table 4). Seed mortality was also significantly affected by fertilization, but not by salt addition (Table 4). The seeds produced by non-fertilized plants presented lowest mortality (<10%), while highest mortality (*ca.* 50%) was recorded in HS/LF (Fig. 4B). Considering the above mentioned results on seed germination and mortality, we performed a further germination test on remnant seeds from LS/LF, 0S/HF and HS/LF to check whether ungerminated, but alive seeds were dormant or had an intrinsic low viability. Seed tests were performed as described above, but after the last germination event ungerminated seeds were further manually scarified and transferred on Agar with addition of GA_3 (250 mg L^{-1}). The addition of GA_3 did not stimulate germination (only two out of 17 remnant seeds germinated on GA_3 in LS/LF after one week).

Discussion

Our study showed that seashore mallow performed best, in terms of growth rate, flowering performance and fruit production, when fertilization was applied. In contrast, salt addition had no or limited effects. This is in accordance with the ecological requirements of this species and with the

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results of previous studies suggesting that seashore mallow tolerates moderate soil salinity as a consequence of biochemical (Tang *et al.*, 2015) and molecular (Wang *et al.*, 2014) mechanisms preventing salt toxicity. Moreover, seashore mallow requires high levels of nutrients, especially N, to accumulate biomass and produce fruits (Halchak *et al.*, 2011; Moser *et al.*, 2013). Surprisingly, increased growth and flowering in fertilized plants was not reflected in higher photosynthetic rates. This can be explained considering that when nutrient availability is low additional carbon produced by photosynthesis could not be converted into plant tissues (Kirschbaum, 2011). Instead, when nutrient availability was increased by fertilizer addition the carbon surplus could be used productively, stimulating plant growth and biomass accumulation. In other words, it can be hypothesized that in treatments without fertilization the growth of seashore mallow was nutrient limited. Fertilizer addition also exerted a positive effect on senescence, as fertilized plants showed a reduced senescence phase compared with non-fertilized plants.

In sharp contrast to the positive response of plant growth to fertilization, seed germination and mortality were greatly reduced and increased, respectively, by fertilizer addition, that contrasts with studies showing reduced seed quality when parental plants were grown in nutrient-poor conditions (e.g., Laztel *et al.*, 2010). This represented a trade-off between parental plant growth and seed performance, the former being enhanced by fertilizer addition and the latter being reduced by fertilization. Considering that seashore mallow is typical of nutrient-rich habitats, like river deltas and estuaries (Ravetto *et al.*, 1997; Pino & De Roa, 2007; Ercole *et al.*, 2013), it is possible that treatments without fertilizer addition created a stressful condition for the plant. Under stressful conditions maternal plants can often invest more resources to reproduction and increase seed provisioning and quality as an adaptive response to ensure seedling survival (Herman & Sultan, 2011; Gan *et al.*, 2013), while under optimal environmental conditions several plant species allocate a larger proportion of resources to growth than to the offspring (Shi *et al.*, 2005).

The causes of the low seed quality in the fertilized plants are not fully supported by literature data. Previous studies found both release (Baskin & Baskin, 2014; Gutterman, 2000) and induction (Hejcman *et al.*, 2012; Karimmojeni *et al.*, 2014) of dormancy when N was provided to parental plants or directly to seeds. However, in our study GA_3 did not stimulate germination of ungerminated seeds, which demonstrated that the seeds were not dormant. Instead, high mortality suggested that seeds had a low initial viability. The low quality of the seeds produced by fertilized maternal plants could reasonably be ascribed to a transgenerational maternal effect of fertilizer addition on seed provisioning. Indeed, quantity and quality of seed provisioning are often mediated by the maternal environment (Bañuelos & Obeso, 2003; Herman and Sultan, 2011; Mondoni *et al.*, 2014). The seeds did not differ across treatments in terms of weight (data not shown), so it can be

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concluded that the low viability of seeds from fertilized maternal plants was due to the quality and not to the quantity of seed provisioning, contrary to what observed in other coastal plants (Del Vecchio *et al.*, 2012; Murru *et al.*, 2015). Surprisingly, very few studies have so far addressed the effects of parental plant treatment on seed quality, especially with respect to nutrients. However, some studies underlined the importance of such trade-off for developing optimal cultivation protocols (Peragón *et al.*, 2015).

Trade-off between maternal growth and seed quality may have important implications for cultivation protocols, considering that seashore mallow is grown for different purposes such as textiles production (Fan *et al.*, 2011), energy production (Markevicius *et al.*, 2010) and others. In these cases high biomass production rates are essential and, consequently, fertilization may be advantageous even at higher levels than those applied in our study. However, if the goal of seashore mallow cultivation is seed production, fertilizer addition should be avoided as it may result in low seed viability. From a conservation point of view, reintroduction or population reinforcement of seashore mallow populations may be required in Europe, where wild populations of this species are endangered. Because in plant translocation both the performance of released plants and the successful establishment of the next generation are extremely important, the trade-off highlighted in this study suggests that a compromise between maternal plant vigour and seed performance should be achieved in the pre-translocation nursery.

Our study provided data useful to refine protocols for cultivating seashore mallow for different purposes. However, two important questions still remain open. 1) Is there a relationship between the environmental conditions experienced by plants and oil quantity and quality in the seeds? In this case the trade-off highlighted in our study may also be of interest for oilseed production (Knothe & Moser, 2015). 2) Does the trade-off expression depend on timing of stress application? It is, indeed, known that seed quality differs if stress is applied to mother plants in prezygotic or in postzygotic phase Kochaneck *et al.* (2010). In conclusion, our study highlighted a previously unknown aspect of the reproductive biology of seashore mallow, a promising commodity in North-America and Asia and an endangered plant in Europe. The protocol developed in this study may be adopted as a reference for future cultivation of seashore mallow based on the cultivation purpose. When seashore mallow is cultivated for biomass production (e.g., biofuel), fertilization is required. If the quality of seeds and the fitness of the next generation are major objectives (e.g. seed production and species conservation), fertilizer addition is not recommended.

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For Peer Review

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Field Code Changed

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Table 1 Abbreviation of the nine treatments for different levels of salt addition and fertilizer addition

Abbreviation	**NaCl**	**NPK**
0S/0F	0g	0g
0S/LF	0g	5g
0S/HF	0g	10g
LS/0F	7g	0g
LS/LF	7g	5g
LS/HF	7g	10g
HS/0F	20g	0g
HS/LF	20g	5g
HS/HF	20g	10g

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Table 2 *F*-values and associated *P* levels resulting from two-way factorial ANOVAs for height, diameter, number of branches, duration of senescence, maximum net CO$_2$ exchange rate and mean net CO$_2$ exchange rate in seashore mallow plants subjected to nine combinations of salt addition and fertilizer addition. Significant ($P<0.05$) values in bold character

	Height	Diameter	N. of branches	Duration of senescence	Maximum net CO$_2$ exchange rate	Mean net CO$_2$ exchange rate	*df*
Salt	0.51 ($P=0.61$)	1.24 ($P=0.31$)	**5.14 ($P<0.01$)**	1.22 ($P=0.30$)	0.01 ($P=0.99$)	0.91 ($P=0.42$)	2
Fertilizer	**5.31 ($P=0.02$)**	**24.78 ($P<0.001$)**	**182.54 ($P<0.001$)**	**84.70 ($P<0.001$)**	0.69 ($P=0.51$)	2.55 ($P=0.11$)	2
Salt × Fertilizer	0.60 ($P=0.67$)	2.25 ($P=0.10$)	**10.93 ($P<0.001$)**	**2.54 ($P=0.04$)**	0.30 ($P=0.87$)	0.93 ($P=0.47$)	4

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Table 3 *F*-values and associated *P* levels resulting from repeated-measure two-way ANOVAs with salt addition and fertilizer addition as between-subject factors and time as within-subject factor for number of buds, number of flowers and number of fruits in seashore mallow plants subjected to nine combinations of salt addition and fertilizer addition. Significant ($P<0.05$) values in bold character

	N. of buds	*df*	N. of flowers	*df*	N. of fruits	*df*
Salt	0.62 (*P=0.54*)	2	0.18 (*P=0.84*)	2	**3.84 (*P<0.05*)**	2
Fertilizer	**68.58 (*P<0.001*)**	2	**38.67 (*P<0.001*)**	2	**28.90 (*P<0.001*)**	2
Salt × Fertilizer	**8.52 (*P<0.001*)**	4	**6.81 (*P<0.001*)**	4	**7.54 (*P<0.05*)**	4
Time	**134.02 (*P<0.001*)**	14	**42.58 (*P<0.001*)**	6	**101.50 (*P<0.001*)**	10
Time × Salt	0.77 (*P=0.81*)	28	1.08 (*P=0.37*)	12	**2.71 (*P<0.001*)**	20
Time × Fertilizer	**27.00 (*P<0.001*)**	28	**8.50 (*P<0.001*)**	12	**15.26 (*P<0.001*)**	20
Time × Salt × Fertilizer	**6.11 (*P<0.001*)**	56	**4.42 (*P<0.001*)**	24	**3.53 (*P<0.01*)**	40

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Table 4 Wald χ^2 and associated P levels resulting from binary logistic GLMs for seed germination percentage and seed mortality percentage in seashore mallow plants subjected to nine combinations of salt addition and fertilizer addition (replicate as the covariate). Significant ($P<0.05$) values in bold character

	Seed germination %	Seed mortality %	*df*
Salt	1.23 (*P=0.54*)	0.25 (*P=0.88*)	2
Fertilizer	**16.28 (*P<0.001*)**	**55.89 (*P<0.001*)**	2
Replicate	2.39 (*P=0.12*)	0.11 (*P=0.92*)	1
Salt × Fertilizer	3.34 (*P=0.50*)	6.50 (*P=0.16*)	4

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FIGURE CAPTIONS

Figure 1 Mean (+*SE*) values of plant height A), stem diameter (B) and number of branches (C) of seashore mallow plants subjected to nine combinations of salt addition (legend as in Table 1). Different letters indicate significant ($P<0.05$) differences between treatments. N=15.

Figure 2 Box-plot diagrams for the duration of senescence in seashore mallow plants subjected to nine combinations of salt addition and fertilizer addition (legend as in Table 1).

The box indicates the mean (inner square) ±*SE*; the bars indicate ±*SD*. The circles and the asterisks indicate outliers and extreme values, respectively. N=15.

Figure 3 Mean (+*SE*) values of number of buds (A), number of flowers (B) and number of fruits (C) recorded at different times in seashore mallow plants subjected to nine combinations of salt addition and fertilizer addition (legend as in Table 1). Different letters indicate significant ($P<0.05$) differences between treatments. N=15.

Figure 4 Mean (+*SE*) percentage of germination (A) and mortality (B) of seeds harvested from seashore mallow plants subjected to nine combinations of salt addition and fertilizer addition (legend as in Table 1). Different letters indicate significant ($P<0.05$) differences between treatments. N=30.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Maximum, minimum and mean monthly air temperatures and total monthly precipitation recorded in the meteorological station of the Botanical Garden during the cultivation period.

Table S2. Maximum, minimum and mean air temperatures within the plant canopy (10-cm height) recorded continuously by data loggers during the cultivation period.

Table S3. Mean (± SE) concentrations of Na and nutrients concentration in the pot soils for the nine combinations of salt addition and fertilizer addition. Salt addition and fertilizer addition both affected significantly (P<0.05) all variables.

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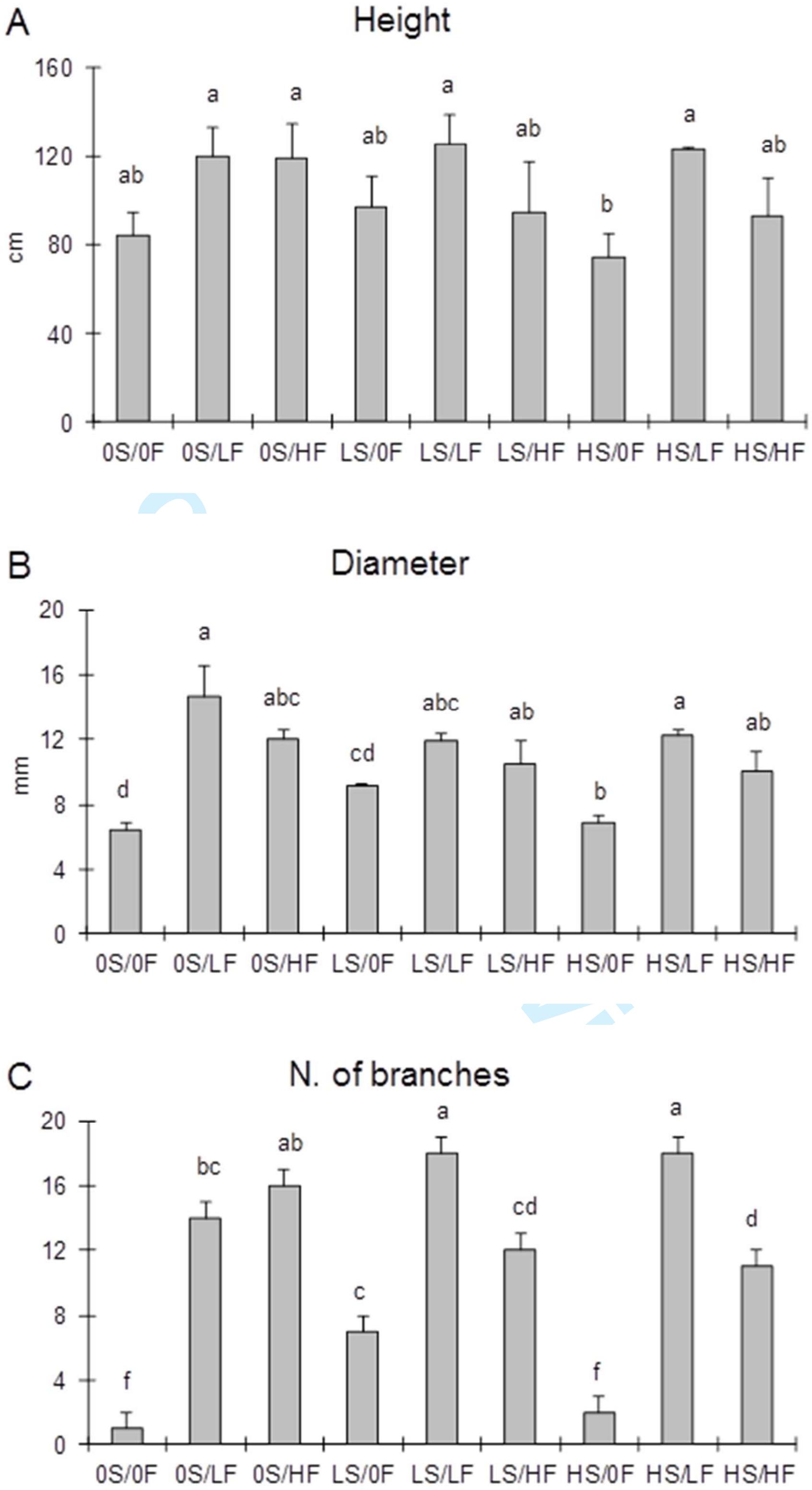
A
Height
cm
160
120
80
40
0
ab
a
a
ab
a
ab
b
a
ab
0S/0F
0S/LF
0S/HF
LS/0F
LS/LF
LS/HF
HS/0F
HS/LF
HS/HF
B
Diameter
mm
20
16
12
8
4
0
d
a
abc
cd
abc
ab
b
a
ab
0S/0F
0S/LF
0S/HF
LS/0F
LS/LF
LS/HF
HS/0F
HS/LF
HS/HF
C
N. of branches
20
16
12
8
4
0
f
bc
ab
c
a
cd
f
a
d
0S/0F
0S/LF
0S/HF
LS/0F
LS/LF
LS/HF
HS/0F
HS/LF
HS/HF

Fig. 1

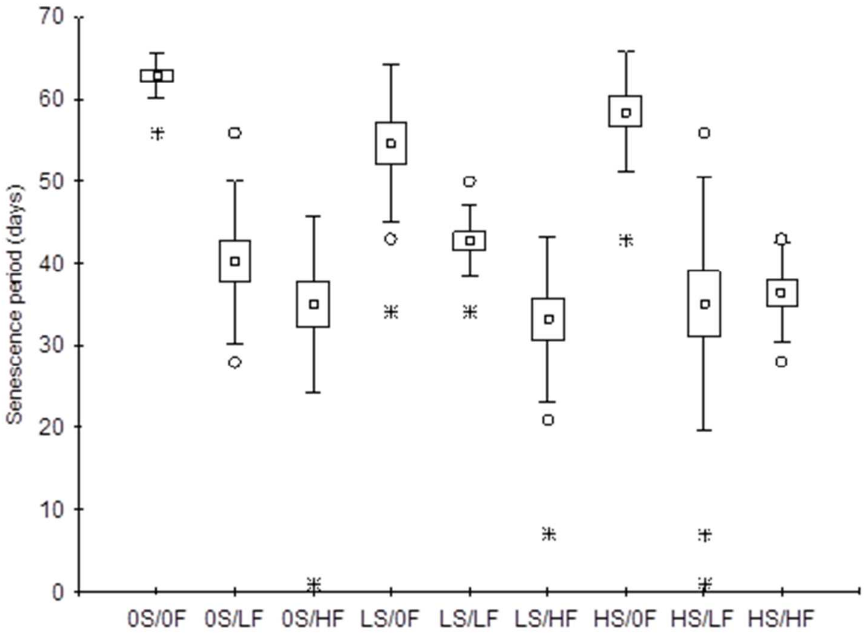

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Fig. 2

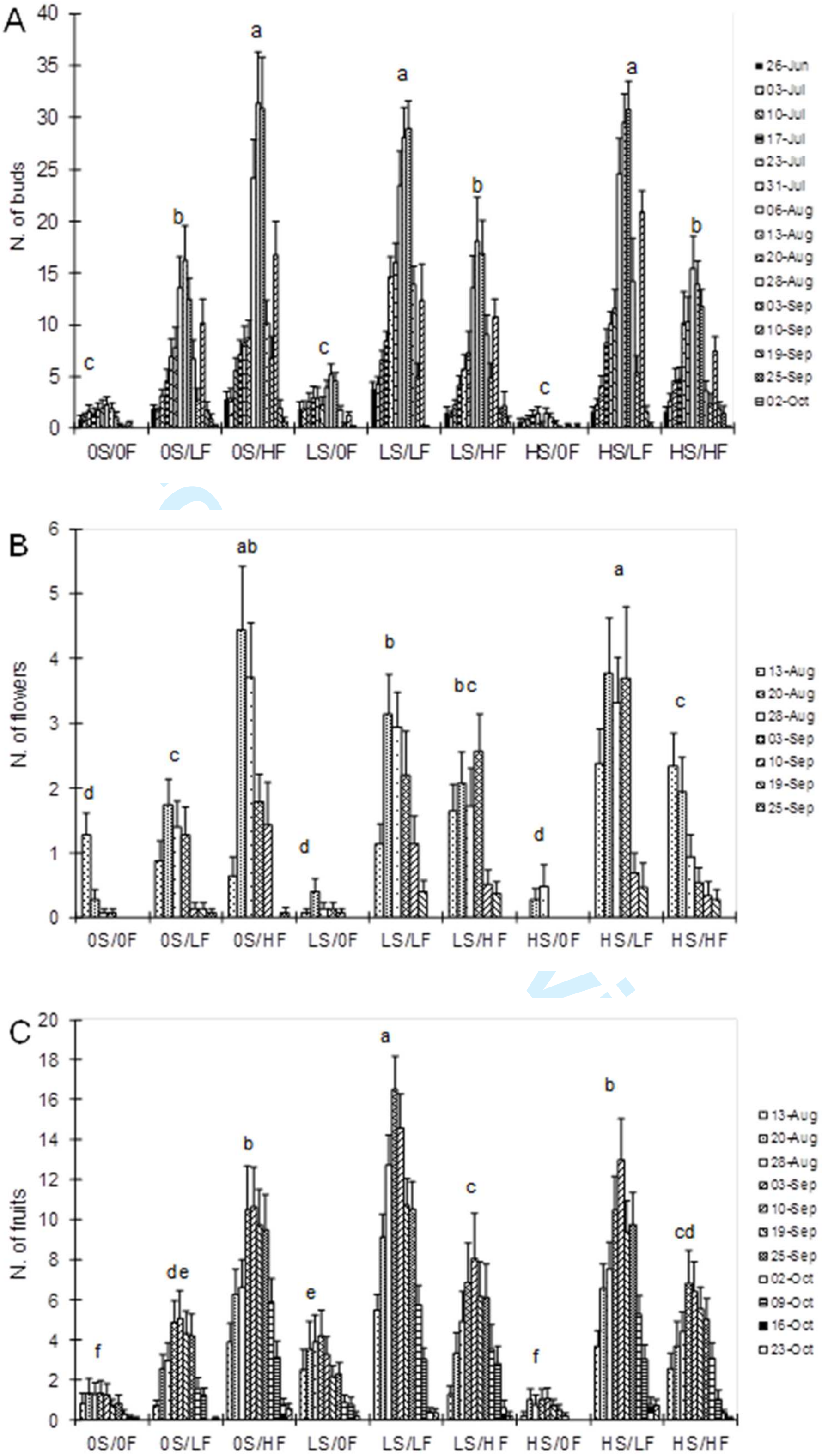
A
N. of buds
0
5
10
15
20
25
30
35
40
c
b
a
c
a
b
c
a
b
0S/0F
0S/LF
0S/HF
LS/0F
LS/LF
LS/HF
HS/0F
HS/LF
HS/HF
26-Jun
03-Jul
10-Jul
17-Jul
23-Jul
31-Jul
06-Aug
13-Aug
20-Aug
28-Aug
03-Sep
10-Sep
19-Sep
25-Sep
02-Oct
B
N. of flowers
0
1
2
3
4
5
6
d
c
ab
d
b
bc
d
a
c
0S/0F
0S/LF
0S/HF
LS/0F
LS/LF
LS/HF
HS/0F
HS/LF
HS/HF
13-Aug
20-Aug
28-Aug
03-Sep
10-Sep
19-Sep
25-Sep
C
N. of fruits
0
2
4
6
8
10
12
14
16
18
20
f
de
b
e
a
c
f
b
cd
0S/0F
0S/LF
0S/HF
LS/0F
LS/LF
LS/HF
HS/0F
HS/LF
HS/HF
13-Aug
20-Aug
28-Aug
03-Sep
10-Sep
19-Sep
25-Sep
02-Oct
09-Oct
16-Oct
23-Oct

Fig. 3

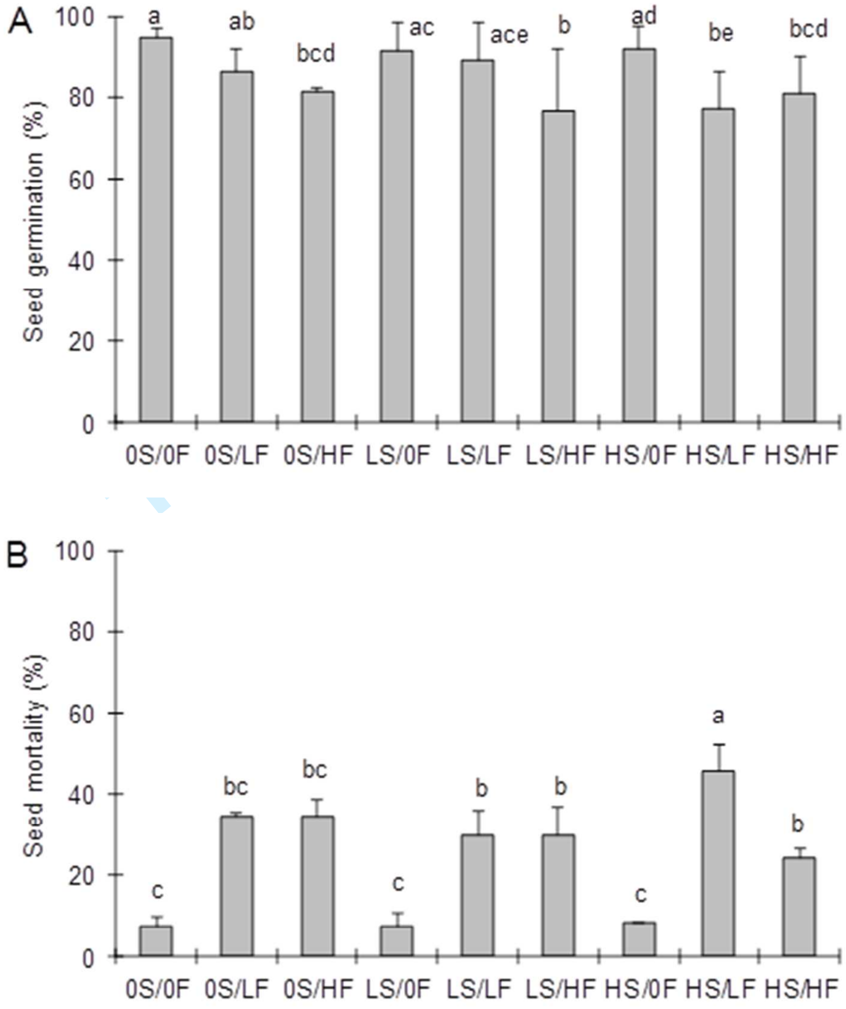
A
Seed germination (%)
0
20
40
60
80
100
a
ab
bcd
ac
ace
b
ad
be
bcd
0S/0F
0S/LF
0S/HF
LS/0F
LS/LF
LS/HF
HS/0F
HS/LF
HS/HF
B
Seed mortality (%)
0
20
40
60
80
100
c
bc
bc
c
b
b
c
a
b
0S/0F
0S/LF
0S/HF
LS/0F
LS/LF
LS/HF
HS/0F
HS/LF
HS/HF

Fig. 4

view