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Nursery pre-treatments positively affects reintroduced plant performance via plant ~~hardening~~pre-treatment, but not ~~via transgenerational~~ maternal effects

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Abstract

Pre-release treatments have long been neglected in plant translocation science, ~~although~~despite being ~~of~~ crucial ~~importance~~ for reintroduction success. Practitioners sometimes adopt acclimation and ~~hardening~~pre-treatment to reduce environmentally-mediated shocks at the recipient site, although the effects of these techniques are unclear. ~~Indeed, t~~The conditions experienced during ~~the~~ cultivation ~~phase~~ may affect the performance of plants once released, but ~~also of~~ the offspring via transgenerational effects. ~~Direct~~ The influence of cultivation environment and ~~the~~ transgenerational maternal effects ~~produced by~~from fertilizer and salt addition treatments on post-release performance were investigated on ~~post-release performances of~~ *Kosteletzkya pentacarpos*, a threatened plant species growing in coastal wetlands. Two experimental translocation sites, representing the opposite ends of the ecological range of *K. pentacarpos* were chosen. One was~~:~~ a nutrient-rich, freshwater site and the other was a nutrient-poor, brackish water site. ~~Salt~~ The salt addition had negligible effects on performance, while fertilization positively affected the vegetative and reproductive performance of m~~other~~aternal plants~~, with durable effects~~ throughout the growing season. However, ~~hardening~~pre-treatment effects were mos~~re~~t evident at the ~~site characterized by the~~ highest nutrient ~~content~~site, suggesting that ~~hardening~~pre-treatment could be connected to memory in plants. No transgenerational maternal effects were observed. Overall, results show that ~~hardening~~pre-treatment can increase the chances of survival and improve the performance of translocated plants at recipient site. ~~Transgenerational effects seem to be less important, although further studies are needed to better understand their role in plant translocation science.~~ From an applied conservation perspective, *ex situ* cultivation and nursing conditions may play a key role in plant translocation success. Results have important implications for the ~~conservation of the species via conservation translocation as well as for the~~ use of *K. pentacarpos* for the restoration of saline wetlands, especially ~~also~~ outside of its native range, but also for the conservation of the species via conservation translocation in general.

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Keywords: ex situ cultivation, fertilization, *Kosteletzkya pentacarpos*, plant conservation, plant translocation, pre-translocation cultivation

1. Introduction

Plant translocations are increasingly used to conserve species threatened with eExtinction ~~and to allow plant populations to recover from strong decline~~ (Seddon, Armstrong, & Maloney, 2007; Maschinski & Haskins, 2012; Abeli & Dixon, 2016). Several plant translocations have been performed worldwide (Godefroid et al., 2011; Dalrymple, Banks, Stewart, & Pullin, 2012), but still ~~most~~ many ~~of them we~~are unsuccessful (Godefroid et al., 2011). The primary objective of a translocation ~~action~~ is the restoration of long-term viability ~~of~~ within target populations (IUCN, 2013;- Rossi, Amosso, Orsenigo, & Abeli, 2013). ~~Reasons for failure are manifold, but k~~Knowledge of ecological and biological requirements of the species and selection of ecologically suitable translocation sites can reduce the failure rate (Abeli & Dixon, 2016 and references therein). However, ~~knowledge of biology and ecology is poor~~ -for- many plant– species, –we still know very little, ~~which implies that~~hence most translocation projects will have to proceed ~~on the basis of~~with partial or incomplete information (Falk, Millar, & Olwell, 1996; Godefroid et al., 2011~~), potentially leading to vain conservation efforts)~~. When there is limited ~~few~~ -data ~~are~~ available for a target species, experimental translocation can help to identify suitable release sites characteristics and appropriate reintroduction techniques ~~directed~~ to improve ~~the success of the action~~establishment. Since several years of monitoring may be necessary to ~~reveal~~ determine the outcome of a translocation action, inadequate planning and preparation can seriously compromise long-term efforts (Drayton & Primack, 2012).

The existing literature on plant translocations ~~basically~~ focuses on post-translocation outcome (Colas et al., 2008; Alonso et al., 2014), but issues related to pre-release phases can ~~also~~ be ~~of~~ crucial ~~importance~~ for translocation success (Godefroid et al., 2016). The selection of appropriate source plant material and its genetic diversity is one of the aspects

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Formatted: Space Before: 3.75 pt, Line spacing: Exactly 14.35 pt that are receiving more attention in reintroduction science (see Crawford & Whitney, 2011; Breed et al., 2013; Herman et al. 2014; Basey, Fant, & Kramer, 2015). ~~Additionally,~~ ~~t~~Translocations often go through a phase of *ex situ* propagules conservation (e.g., in seed bank; Maxted & Guarino, 2003; Hoban & Schlarbaum, 2014), plant propagation and cultivation, ~~that~~ which may strongly affect translocation outcome. ~~M~~A major issue~~s~~ in the *ex situ* conservation and propagation phase ~~are~~ is the maintenance of adequate genetic variation and

Formatted: Indent: Hanging: 1.07", Space Before: 0 pt, Line spacing: Exactly 13 pt the avoidance of genetic drift due to limited source material (Basey, Fant & Kramer, 2015; Ensslin, Tschöpe, Burkart, & Joshi, 2015), even if seed collection aims to maximize sample genetic variation (ENSCONET, 2009). For~~-~~ example, plants cultivated *ex situ* (e.g., cultivation in botanical gardens), exhibit lower genetic variation than natural populations and this variation decreases with increasing duration ~~of the~~in *ex situ* cultivation (Ensslin, Sandner, & Matthies, 2011). Another important aspect is that nursery conditions are ~~unavoidably~~ different~~-~~ from ~~-~~the~~-~~ natural ~~environmental~~ conditions~~-~~ of ~~at~~ the~~-~~ recipient sites (e.g., cultivation in greenhouses; Atkinson & Lacroix, 2013), but this issue has received less attention (Dumroese, Kasten Luna, & Landis, 2009). The ~~stronger~~ larger the difference in ecological conditions between cultivation site and recipient site, the greater the potential~~ly~~ negative impact ~~of the environment~~ on survival and fitness of the translocated plants (Dumroese, Kasten Luna, & Landis, 2009). To reduce environmentally-mediated shocks, practitioners often adopt acclimation techniques ~~and (~~(hardening)~~) techniques~~ prior to relocating *ex situ* propagules to the recipient site. These include gradual acclimation to external temperatures fro~~r plant grown in a~~m greenhouse to outdoor (Atkinson & Lacroix, 2013), reduction in water availability, reduced ~~and~~ fertilization and~~,~~ decreased or increased shading, and even salt addition (this paper)~~), and so on~~ (Vallee et al., 2004; Jacobs & Landis, 2009).

However, the environmental conditions experienced during the cultivation phase may have multi-generation affect, such that the performance of the offspring ~~produced by the~~of cultivated plants ~~originally cultivated *ex situ* (henceforth, mother plants), namely the performance of the second generation~~can be impacted ~~(henceforth, offspring)~~ via transgenerational maternal effects (Saarinen, Lundell, Aaström, & Hänninen, 2011; Gesch et al., 2016). Environmentally induced ~~For example, m~~Maternal effects ~~elicited by the environmental conditions experienced by mother plants~~ are known to modify several offspring plant traits, such as biomass (Kou et al., 2011), fruit and seed production (Whittle, Otto, Johnston, & Krochko, 2009), seed germination and longevity (Galloway, 2005; Mondoni et al., 2014), thus increasing or decreasing the fitness of offspring. Recently, Caño et al. (2016) demonstrated that moderate salt stress in the maternal environment influences the performance of the invasive species *Baccharis halimifolia* L. Hence, ~~transgenerational~~maternal

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effects can play an important role in population growth rate even at sub-lethal levels of stress. ~~This aspect, as well as t~~The effect of *ex situ* cultivation treatments in translocation success, and long-term maternal effect have been ~~so far~~ overlooked in the scientific literature despite the important implications ~~that maternal effects~~they may have in translocated populations derived from *ex situ* cultivation.

This paper reports on experimental translocation (i.e. reinforcement) of seashore mallow [*Kosteletzkya pentacarpos* (L.) Ledeb.], a threatened plant species growing in coastal wetlands, with a ~~specific~~ focus on the pre-translocation effects of ~~pre-translocation~~ fertilizer and salt addition treatments on successful establishment. Seashore mallow ~~plants~~ were planted in two recipient sites ~~in the wild~~, characterized by nutrient-rich freshwater and nutrient-poor brackish water, respectively. A previous study, aiming to define appropriate ex situ cultivation protocols for seashore mallow, demonstrated that fertilizer application generates a trade-off between parental plant growth and seed performance, the former being enhanced and the latter being reduced by fertilizer application while salt application has no effects either on growth or on seed performance (Abeli et al., 2017). The aim of this study was to evaluate the direct influence of cultivation environment and the transgenerational maternal effects produced by fertilizer and salt addition treatments on the performance of seashore mallow ~~subjected to~~in experimental translocations. In particular, two ~~main~~ outstanding questions were addressed:

1. ~~If, and t~~To what extent, if at all, ~~–~~ does the pre-treatments applied during ~~ex situ~~ cultivation ~~directly~~ enhanced the performance of the plants released in the recipient sites ~~through hardening effects~~. 2. ~~If, and t~~To what extent, if at all, does the pre-treatments applied to ~~mother~~maternal plants affected ~~indirectly~~ the performance of non-treated offspring via ~~transgenerational~~ maternal effects.

Answers to these questions are expected to increase the success of restoration ~~actions based on~~for this species, which ~~that~~ is important for the rehabilitation of salinized lands (Flowers, 2004) and ~~as~~ a crop species in saline agricultural systems (Ruan et al., 2008), but also outside its native range.

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2. Material and Methods

2.1. Study species

Seashore mallow is a perennial halophytic herb belonging to Malvaceae. The species is listed both in the 92/43/CEE 'Habitat' Directive (Annex II) and in the Bern Convention (Annex I) and is ~~considered~~ classified ~~threatened with extinction~~ in Italy as 'critically endangered' and in Europe, ~~where seashore mallow~~as ~~is classified as 'critically endangered' and~~ 'vulnerable'~~,~~ ~~respectively~~ (Rossi et al., 2016; Bilz, Kell, Maxted, & Lansdown, 2011). Seashore mallow grows in brackish to saline, nutrient-rich habitats. This species occurs in coastal wetlands, river deltas and estuaries of southeastern USA, Western Asia and Southern Europe (Pino & De Roa, 2007; Ercole et al., 2013). However, its distribution range is strongly shrinking in Europe, ~~and~~ especially in Italy. At the beginning of the Twentieth century seashore mallow populations were found in six Italian regions. In 2005, its ~~occurrence~~ was restricted to three regions, and~~.~~ ~~Nowadays, seashore mallow~~currently is ~~populations are~~ documented in only two regions, Veneto and Emilia-Romagna, within the Po river delta area (Ercole et al., 2013).

~~The biological cycle of s~~Seashore mallow growing season starts in May and ends in late October – early November. Several shoots developing from rhizomes in late July ~~produce~~ producing many ~~(even 50)~~ solitary pink flowers that ~~eventually produce~~develop into a capsule with five seeds. The seeds are equipped with air sacs that ensure flotation (Poljakoff-Mayber, Somers, Werker, & Gallagher, 1992), while waterproof teguments allow the preservation ~~of the seeds~~ even in saline habitats. However, seed vitality is often jeopardized by parasites, especially insects responsible for seed abortion (eg. *Oxycarenus lavaterae* Fabricius 1787~~, responsible for seed abortion~~) and fungi living on the seed surface (Monés, 1998). Seashore mallow does not ~~present~~ reproduce via vegetative reproduction.

2.2. Study area and translocation protocol

The experimental translocations ~~of seashore mallow was~~were carried out at ~~t~~ Bosco della Mesola, Northern Italy, ~~in the~~ a nature ~~r~~Reserve, and ~~and~~ Site of Community Importance (IT4060015), ~~Bosco della Mesola, Northern Italy~~ (44°, 50' N, 12° 15' E, 1088 ha, 0-2.8 m above sea level, Fig. 1). The study area lies on a dune system ~~formed~~ consisting of ~~sand~~ dunes and ~~dune~~ slacks with North-South orientation (Fig.

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1). Bosco della Mesola is the main locality of seashore mallow in Emilia-Romagna, where the presence of seashore mallow was last confirmed in 2014 ~~after a long period without any~~

~~report~~ (L. Brancaleoni, unpublished). ~~Bosco della Mesola~~The site is mostly covered with woodlands, ~~representing~~ a remnant of ancient coastal forests that have almost totally disappeared in the North-Adriatic coastal region (Gerdol, Ferrari, & Piccoli, 1985). This area is ~~nowadays~~ located in a reclaimed territory equipped with a dense network of canals supplying freshwater to the forest ecosystem. The south-eastern peripheral sector is in a hydraulic ~~continuity~~ continuum ~~with the~~leading to brackish water ~~of~~ in a ~~a~~ nearby lagoon ~~and is therefore~~ covered with halophytic vegetation. The entire coastal area in this region is subjected to subsidence which determines increasing levels of salt-water ingression towards the inner part of the nature reserve.

The experimental translocation was carried out at two sites within Bosco della Mesola (Fig. 1). The two sites were chosen because they represent opposite ends of the ecological range of seashore mallow. The first site (Elciola) is located in a permanent 7-ha lentic pond fed with nutrient-rich freshwater inflow from the canals. Consequently, the Elciola pond mainly depends on the hydraulic management of the canals ~~so~~ hence th~~e~~at ingression of salt water from the lagoon is ~~overall poor~~low. The second site (Goara) is located in the south-eastern saltmarshes, which~~. This site~~ is naturally fed with brackish water from the lagoon, ~~with~~ and no hydraulic control. The water at Goara ha~~i~~s poorer ~~in~~ nutrients content compared with Elciola because a dense fringe of reeds effectively filter the nutrients dissolved in the lagoon water.

Seashore mallow plants ~~of~~ from two generations (~~mother~~maternal plants and offspring) were used for the translocation experiment in 2015. The year before, the ~~mother~~maternal plants had been ~~subjected to experimental~~ cultivat~~ion~~ed in the Botanical Garden of the University of Ferrara with a ~~under~~ factorial combination of three levels of salt addition (none (0S), low (LS) and high salt additions (HS)) and ~~×~~ three levels of fertilizer addition (none (OF), low (LF) and high (HF) of fertilizer added)

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~~addition~~ (see Abeli et al., 2017 for further information)~~): 0S (no salt addition), LS (low level of salt addition), HS (high level of salt addition); 0F (no fertilization), LF (low level of fertilization), HF (high level of fertilization)~~. The offspring plants were germinated from seeds collected from ~~mother~~maternal plants but received no further treatment. The 1-yr old ~~mother~~maternal plants sprouted from rhizomes and the germinated offspring seedlings were ~~pre-~~cultivated for about 1 month at the Botanical Garden in pots containing a commercial soil mixture of perlite and peat with no fertilizer or salt(Terflor s.r.l., Capriolo, Brescia, Italy). At the end of the ~~pre-~~cultivation period (4 June 2015), 45 ~~mother~~maternal plants and 45 offspring plants were translocated at each of

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2.3. Response variables

Environmental support data

Data on air temperature and precipitation were obtained from a weather station, located about 1 km apart from the study sites (Bosco Mesola). Soil temperature was recorded continuously by two data loggers (Hobo, Onset Bourne, MA, USA) placed 5 cm belowground at each of the two sites. Water-table depth was measured on seven days during the growing period. Water-table measurements were taken manually in a graduated PVC pipe placed into the soil at each of the two sites.

Five soil samples were collected from the top 5-cm layer at each of the two sites, using a stainless steel ~~cylindric~~cylindrical corer (inner diameter 6.6 cm). The soil samples were ~~carried~~ taken to the laboratory, and stored in a refrigerator for 3-4 days before the analyses. Soil pH was measured in aqueous 1 : 20 (vol/vol) solutions with a pH-meter (Hanna Edge, Villafranca Padovana, Italy). Salinity and electrical conductivity were measured in the same solutions with a conductivity-meter (Crison CM 35, L'Hospitalet de Llobregat, Spain).

About 50 mg of fresh soil was analyzed colorimetrically for available dissolved nutrients using a micro flow automated continuous-flow analyzer (Systea Flowsys, Anagni, Italy). Concentration of extractable ammonium was determined at 690 nm wavelength, on 6% KCl digests. Concentration of extractable nitrate was determined at 420 nm wavelength, on distilled water digests. Concentration of extractable phosphate was determined at 700 nm wavelength, on digests obtained using the Truog's solution (Allen, 1989).

Vegetative growth and reproductive performance

The two sites were surveyed at about 20-day intervals during the growing period (4 June to 20 October) for acquiring data on vegetative growth and reproductive performance. Plant height and plant diameter were measured several times during the growing period using a measuring tape and a manual caliper, respectively. The diameter was measured at the first (lowest) node~~. On the same occasions,~~, as well as the number of branches o~~i~~n each plant. ~~were also counted.~~ From 7 August to 20 October, plant senescence was visually assessed according to the BBCH phenology scale (BBCH 90-99) that records senescence based on leaf abscission (Meier, 2001). Three times during the growing period net CO_2 exchange rates was determined in three to five ~~sound~~ healthy leaves from one individual plant for each treatment. ~~Net~~ The net CO_2 exchange was measured ~~with~~ using an open infrared gas analysis system (LCA-4, ADC BioScientific LTD, UK) by enclosing the leaves in a broad-~~type~~ leaf chamber. All measurements were made at saturating photon flux density (>1000 µmol photons m^{-2} s^{-1}) and about 30 °C air temperature. From 17 July to 1 October presence/absence of flowers on each plant was visually assessed. The duration of the blossoming period was also recorded for each plant. From 17 July to 20 October the total number of fruits on each plant were counted.

2.4. Statistics

The data on soil chemistry were ~~statistically~~ analyzed ~~by~~ using one-way ANOVAs, while the~~.~~ ~~Growth in~~ height, ~~and~~ diameter, branching and fruit production were all ~~were statistically~~ analyzed ~~by~~ using a four-way factorial ANOVAs with site, generation salt addition and fertilizer addition as fixed factors. Duration of senescence was assessed through linear regressions of phenophas

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es 90-99 against time. The periodic data on net CO_2 exchange were ~~statistically~~ analyzed by repeated-measures four-way ANOVAs with site, generation, salt addition and fertilizer addition as between-subject factors and time as within-subject factor. Homogeneity of variance was tested, whenever appropriate, by Levene's tests. The data were log-transformed when the homoscedasticity assumption was violated. Significance of differences between treatments were assessed by Fisher's post-hoc LSD tests. All ANOVA computations and regressions were carried out using the package Statistica 7.0 (StatSoft©; Version 7; StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1.Environmental data

During the growing period, the mean air temperature and total precipitation were 20.5 °C and 124 mm, respectively (Fig. S1). The first part of the growing period was hot and dry, with mean monthly air temperature of 27.3 °C in July. ~~Afterwards, air~~ T~~t~~emperature gradually decreased over the growing season with ~~and several~~ increasing precipitation events, ~~occurred~~ especially in late September and October (Fig. S1). Soil temperatures substantially mirrored the air temperature trend (Figs. S1 and S2). Mean soil temperature during the growing period was somewhat higher at Elciola (23.4 °C) compared with Goara (22.4 °C).

The water table fluctuated during the growing period, with ~~a seasonally~~ contrasting trends at the two sites, ~~in close relationship~~associated with hydraulic management. At the beginning of the growing period ~~both sites the~~ the water-table depth was positive for both, i.e. the soil was flooded~~, at the beginning of the growing period (Fig. 2)~~however~~.~~ ~~Subsequently~~as the growing season progressed, the water table dropped below ~~the~~ ground level at Goara ~~whereas the soil~~but ~~was still~~remained flooded at Elciola~~.~~, and ~~At~~ by the end ~~of the growing period~~ the water table rose at Goara but sank ~~considerably~~ at Elciola (Fig. 2). The soil was weakly acidic at both sites, with mean pH of about 5.9. The soil at Elciola was richer in nutrients, although only nitrate concentration was significantly higher than at Goara. In contrast, salinity and electrical conductivity were both significantly higher at Goara (Table 1).

H~~Growth in h~~eight and ~~growth in~~ diameter ~~were~~ was ~~both~~ significantly ~~affected~~ different by site,

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3.2.Vegetative performance

H~~Growth in h~~eight and ~~growth in~~ diameter ~~were~~ was ~~both~~ significantly ~~affected~~ different by site,

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~~mother~~ Plant were taller and wider at overall at Elcolia, and Maternal plants ~~grew more~~were bigger than offspring at both sites ~~and growth performance was overall better at Elciola~~.

~~Growth was stimulated by f~~Fertilization during cultivation, especially at high concentration~~. –~~, produced bigger plants, h~~H~~owever, the ~~after-effects of fertilization~~ it was ~~were~~more effective on ~~mother~~maternal plants ~~only~~ (Fig. 3), as shown by significant generation × fertilizer interactions (Table 2). Branching ~~also~~ was greate~~s~~t~~r~~ in ~~mother~~maternal plants at the Elciola site, but was unaffected by fertilization (Table 2, Fig. 3).

Net CO_2 exchange rates were overall higher in ~~mother~~maternal plants (Fig. 4). Net CO_2 exchange rates differed ~~much~~ more between the ~~two generations~~offspring and maternal plants at ~~of~~ Goara ~~plants~~ compared with Elciola ~~plants~~. Net CO_2 exchange rates generally decreased across the growing period but ~~at the end of the growing period net CO_2 exchange rates~~ declined more ~~strongly~~ rapidly in Goara than in Elciola, especially in ~~mother~~maternal plants (Fig 4). Fertilization generally enhanced net CO_2 exchange, especially in the ~~mother~~maternal plants. However, the repeated-measures ANOVA revealed significant interactions of fertilization ~~with~~ for all other between-subject factors and time ~~as well~~ (Table S1), which mirrors erratic trend of fertilization across site, generation and salt addition (Table S2).

Senescence occurred at different times ~~with regard to both~~for site and generation. ~~Indeed, t~~The plants at Goara presented higher phenological codes compared to the plants at Elciola (Fig. 5~~).~~), meaning that ~~This means that the plants at~~ Goara experienced earlier leaf abscission, ~~thus~~ and show~~ing~~ed only few yellow leaves ~~at~~ by the end of the growing period. In contrast, almost

The length of the flowering period differed ~~overall~~ little between sites and among treatments (Fig. 6). However, the ~~mother~~maternal plants ~~presented somewhat~~flowered longer ~~duration~~ and half ~~50%~~ of the leaves of Elciola plants were still green ~~at~~ by the end of the growing period. ~~At both sites, the mother~~Maternal plants started senescence earlier than the offspring (Fig. 5), however. ~~While differences were found in timing of senescence initiation,~~the duration of senescence was similar ~~comparable between~~across sites, generations and treatments. The senescence period varied from 20 to 50 days, with broader ranges in the ~~mother~~maternal plants compared with the offspring (data not shown).

3.3. Reproductive performance

The length of the flowering period differed ~~overall~~ little between sites and among treatments (Fig. 6). However, the ~~mother~~maternal plants ~~presented somewhat~~flowered longer ~~duration~~ and sites (Fig. 6). Fertilization and salt addition had ~~poor~~ no influence on the length of the flowering period, although~~. Only~~ in ~~in~~ the untreated (0S/0F) ~~mother~~maternal plants the ~~was the duration of the~~ flowering period was c~~c~~onsiderably shorter ~~in the untreated (0S/0F) plants compared with fertilized plants without salt addition (0S/LF and 0S/HF) at both sites~~ (Fig. 6).

Fruit production was significantly affected by all factors (Table 2, Fig. 3). Overall higher number of fruits were recorded at Elciola~~.~~ and in ~~Mother~~maternal plants. ~~presented higher performance in terms of fruit production. However, t~~The difference between the two generations was higher at Goara (see the significant site × generation interaction; Table 2). Fertilization stimulated fruit production except at high fertilization levels. However, the difference was significant only for ~~mother~~maternal plants, as shown by the significant generation × fertilizer interaction (Table 2, Fig. 3).

4. Discussion

~~Survival~~ Aside from survival of transplanted plants, ~~is the main criterion to assess the success of a translocation.~~ a~~A~~n additional important criterion is the ability of transplanted plants to reproduce and contribute to a self-sustaining population in the long-term (Primack & Drayton, 1997; Maschinski & Haskin, 2012). Source population, ~~individual~~ genetic traits and ecological characteristics of the recipient sites are all factors affecting the outcome of a translocation ~~action~~ (Godefroid, Le Pajolec, & Van Rossum, 2016). The quality of the released plants is ~~also~~ important for establishment success, but is scarcely considered ~~so far~~ (Havens, Guerrant, Maunder, & Vitt, 2004). For example, plants propagated and grown *ex situ* under optimal common garden conditions may not perform well when exposed to selective pressure at a recipient site, and~~which~~ can lead~~s~~ to higher initial mortality (Rossi, Amosso, Orsenigo, & Abeli, 2013). For this reason, it is often considered best practices ~~suggest~~ to mimic the~~king~~ *ex situ* conditions that the plants will experience~~cope~~ ~~with~~in if plants are kept for multiple generation in cultivation, ~~the field,~~ to ~~simulate a sort of natural selection and~~ avoid the 'domestication syndrome' (Havens, Guerrant, Maunder, & Vitt, 2004; Basey, Fant & Kramer, 2015). However prior to translocation

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Comment [A2]: This is only really considered important if you are maintaining plants in multiple generations in cultivation

~~Contrary to this practice, our~~ pre-~~translocation~~ treatment of plants to produce robust larger plants can~~s~~ increase~~d~~ the performance. We found with ~~of~~ seashore mallow plant ~~plants~~ grown with fertilizer *ex situ* performed better, althoug~~.~~h s~~S~~alt addition had a negligible effect. ~~,~~ ~~while~~ F~~f~~ertilization ~~affected~~improved vegetative and reproductive performance of the ~~mother~~maternal plants, ~~with durable effects~~

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throughout the growing season. Although we found no interaction Plant response to the interactive effect ofbetween salinity and nutrients, vary across and within the sameour species, it was as found in *Spartina patens* (Aiton) Muhl (Delaune, Pezeshki, & Jugsujida, 2005; Merino, Huval, & Nyman, 2010). The lack of scant effect of salinity found in our experiment may be due to the low concentration of salt applied in common garden compared with the salinity in the experimental field sites.

More interesting is the different response of mother plants and offspring to fertilization.

Maternal plants that had been fertilized in ex situcultivation showed higher growth, net CO_2 exchange rates, and reproduction compared with non-fertilized plants. Moreover, the fertilized mothermaternal plants generally showed higher performance than the offspring. Our This confirmed our first hypothesis that pre-treatments applied during cultivation enhanced the performance of the plants released in the recipient sites. (hardening) was therefore confirmed, and this was in accordance with better field performance of plants fertilized in common garden conditions. Fertilization increased the performance of seashore mallow plants for several months, which has also been, as found for the case for example in tree species (Oliet, Puértolas, Planelles, & Jacobs, 2013; Garcia-Pérez et al., 2015). Hardening Pre-treatment induces substantial morphological and physiological changes in plants grown *ex situ*, but the type and intensity of pre-treatments affect the outcome of such modifications (Garcia-Pérez et al., 2015). In addition, it is interesting to note that hardening pre-treatment was effective only with respect to fertilization at the nutrient-rich site (Elciola). At the nutrient-poor site Goara, differences between treatments generally were non-significant suggesting that hardeningpre-treatment effects were weak less evident at this a harsher site. Such remarkable pattern is difficult to explain. However, hardeningpre-treatment seems directly connected to memory in plants (Bruce, Matthes, Napier, & Pickett, 2007). For example, Walter et al. (2011) showed that plants can 'remember' stress conditions and react steadily when a stress event is repeated. Stress memory applies to both

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Comment [A3]: I think is more to do with the fact that the differences are not as large – as the plant performed less well overall at this site hence it is harder to see a larger difference as the numbers are constrained to the lower limits. This could be corrected by making everything relative. So rather than use height or diameter you could use percent of the biggest plant at site.

abiotic and biotic factors and the ~~behaviour~~ response of ~~mother~~maternal plants in our experiment may be explained ~~through~~ by this mechanism. ~~In other words, p~~Hence plants that experienced high nutrient levels during pre-treatment may have acquired the ability to use nutrients better and faster than untreated plants. In principle, stress memory may be passed to non-treated offspring plants (Iqbal & Ashraf, 2007), but this did not happen in our study.

A previous common garden experiment, showed that fertilized seashore mallow plants produced low quality seeds, suggesting there is a trade-off between ~~mother~~maternal plant vigour and quality of the offspring (Abeli et al., 2017). This behaviour was ~~ascribed~~ attributed to different seed

Comment [A4]: Plants do not behave.

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Comment [A5]: Could it not be that larger plants respond better to translocation and establish faster?

Formatted: Body Text, Indent: Left: 0", Space Before: 5.65 pt, Line spacing: Exactly 13.35 pt, Tab stops: 1.18", Left provisioning by ~~mother~~maternal plants under various levels of stress, via ~~transgenerational~~ maternal effects (Galloway & Etterson, 2007; Kochanek, Steadman, Probert, & Adkins, 2011) and ~~was at~~ the basis of our second hypothesis. ~~Transgenerational maternal~~ Maternal effects were not observed in our study as differences in offspring performance (growth and reproduction) across ~~mother~~maternal plant treatments were not significant. Mechanisms underlying ~~transgenerational~~ maternal effects are ~~manifold~~ multiple and include ~~for example~~ seed provisioning and epigenetic effects (Feng, Jacobsen, & Reik, 2010; Kochanek, Steadman, Probert, & Adkins, 2011). However, it is possible that the treatments applied to ~~mother~~maternal plants were too weak to induce a transgenerational responses.

Nevertheless, maternal effects may strongly modify offspring performance and fitness (Mondoni et al., 2014; Gesch et al., 2016). Hence, further studies on ~~transgenerational~~ maternal effects applied to translocation are needed, especially to evaluate ~~to what~~the extent they can contribute to the low success rate of this type of conservation actions (Godefroid et al., 2011). In *Baccharis halimifolia* L. a strong positive effect of parental growth conditions (salinity) on offspring performance was found, also explaining its invasiveness (Caño et al., 2016). This has further implications for more extreme translocation actions such as introduction and assisted migration (Ricciardi & Simberloff, 2009)~~), if maternal effects lead non-native species to spread~~.

In conclusion, ~~hardening~~pre-treatment can effectively increase the chances of survival and performance of translocated plants and possibly increase their competitive ability in the recipient community. This latter aspect deserves further investigation, and open the way to new practices of ex situ propagation and seed care. A balanced *ex situ* cultivation mediating between ~~no~~ minimal care for maintain long-term and ~~controlled care~~ increased care ~~practices may~~to produce better ~~performing plants~~propagules for~~and finally~~ higher survival of transplanted plants. Although no evidences of ~~transgenerational~~ maternal effects were found in our experimental translocation of seashore mallow, we suggest that further studies with other species and ecological factors will be performed to better understand the role, if any~~, of~~ transgenerational plasticity in translocated populations.

Acknowledgements

Comment [A6]: Did you compare offspring alone (without maternal plants)> Again – since offspring performed worse than maternal plants you might not see an impact as the difference to maternal plants was so large. You could you look at relative response to see if the offspring of certain parents performed better or worse.

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We are particularly grateful to Dr. Giovanni Nobili, Mr. Mauro Menghini and the staff of the former State Forestry Corps (presently CUTFAA) for ~~the~~ permissions for release~~d~~ and the valuable support during the experiment ~~within~~ at the Riserva Naturale Bosco della Mesola. We also want to thank Mr. Fausto Molinari assisted during the fieldwork and ~~.~~ Dr. Roberta Marchesini who did the chemical analyses. ~~They all are kindly acknowledged.~~

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Table 1

Soil chemistry at the two sites. Mean (± SE) concentrations of nitrate, ammonium, phosphorus and mean (± SE) values of salinity and electrical conductivity are shown with corresponding F-values and associated *P* levels obtained by one-way ANOVAs. Significant (P<0.05) values in bold character. N = 5

	NO_3^- (µg g^{-1})	NH_4^+ (µg g^{-1})	PO_4^{3-} (µg g^{-1})	Salinity (mg l^{-1})	El. conductivity (µS cm^{-1})
Elciola	3.54±1.07	3.19±0.57	4.22±1.06	249.28±56.22	118.82±27.49
Goara	1.1±0.09	2.51±0.46	5.03±0.97	1209.6±258.17	596.2±130.53
F values and *P* levels	**5.17 (*P*=0.05)**	0.86 (*P*=0.38)	0.31 (*P*=0.59)	**0.05 (*P*<0.01)**	**0.05 (*P*<0.01)**

Table 2

F-values and associated *P* levels resulting from factorial ANOVAs for height, diameter, number of branches and number of fruits in seashore mallow plants of two generations. The year before, the ~~mother~~maternal plants had been subjected to three levels of salt addition × three levels of fertilizer, in factorial combination, during ex situ experimental cultivation. The offspring plants were germinated from seeds collected from ~~mother~~maternal plants but received no further treatment. Significant values (*P*<0.05) in bold character.

	Height	df	Diameter	df	Branches	df	Fruits	df
Site	**232.58 (*P*<0.001)**	1	**66.18 (*P*<0.001)**	1	**7.14 (*P*=0.009)**	1	**31.94 (*P*<0.001)**	1
Generation	**54.23 (*P*<0.001)**	1	**71.23 (*P*<0.001)**	1	**5.50 (*P*=0.02)**	1	**5.47 (*P*=0.02)**	1
Salt	0.59 (*P*=0.55)	2	1.82 (*P*=0.17)	2	1.16 (*P*=0.32)	2	**3.05 (*P*=0.05)**	2
Fertilizer	**5.94 (*P*=0.003)**	2	**6.46 (*P*=0.002)**	2	2.30 (*P*=0.10)	2	**4.76 (*P*=0.01)**	2
Site × generation	**3.83 (*P*=0.05)**	1	2.87 (*P*=0.09)	1	1.05 (*P*=0.31)	1	**5.03 (*P*=0.03)**	1
Site × salt	0.01 (*P*=0.99)	2	0.06 (*P*=0.94)	2	0.57 (*P*=0.56)	2	0.02 (*P*=0.97)	2
Generation × salt	0.80 (*P*=0.45)	2	**4.37 (*P*=0.01)**	2	1.90 (*P*=0.15)	2	2.01 (*P*=0.14)	2
Site × fertilizer	1.94 (*P*=0.15)	2	1.29 (*P*=0.28)	2	0.15 (*P*=0.86)	2	1.15 (*P*=0.32)	2
Generation × fertilizer	**9.69 (*P*<0.001)**	2	**7.88 (*P*=0.001)**	2	2.59 (*P*=0.08)	2	**5.10 (*P*=0.008)**	2
Salt × fertilizer	0.91 (*P*=0.46)	4	1.59 (*P*=0.18)	4	0.92 (*P*=0.45)	4	1.04 (*P*=0.39)	4
Site × generation × salt	0.20 (*P*=0.82)	2	0.18 (*P*=0.83)	2	0.58 (*P*=0.56)	2	0.39 (*P*=0.68)	2
Site × generation × fertilizer	0.95 (*P*=0.39)	2	3.04 (*P*=0.05)	2	0.73 (*P*=0.48)	2	0.42 (*P*=0.66)	2
Site × salt × fertilizer	0.38 (*P*=0.82)	4	0.23 (*P*=0.92)	4	0.70 (*P*=0.59)	4	1.11 (*P*=0.36)	4
Generation × salt × fertilizer	0.67 (*P*=0.61)	4	0.76 (*P*=0.55)	4	0.56 (*P*=0.69)	4	0.22 (*P*=0.93)	4
Site × generation × salt × fertilizer	0.85 (*P*=0.5)	4	0.76 (*P*=0.56)	4	0.40 (*P*=0.81)	4	0.45 (*P*=0.77)	4

Table 3

F-values and associated *P* levels resulting from factorial Generalized Linear Model for seed germination percentage in seashore mallow plants of two generations. The year before, the ~~mother~~maternal plants had been subjected to three levels of salt addition × three levels of fertilizer, in factorial combination, during ex situ experimental cultivation. The offspring plants were germinated from seeds collected from ~~mother~~maternal plants but received no further treatment. Significant values ($P<0.05$) in bold character.

	Germination %	df
Site	**13.580 ($P<0.001$)**	1
Generation	**12.818 ($P<0.001$)**	1
Salt	**7.801 ($P=0.020$)**	2
Fertilizer	**34.613 ($P<0.001$)**	2
Site × generation	**115.950 ($P<0.001$)**	1
Site × salt	**26.242 ($P<0.001$)**	2
Generation × salt	2.933 ($P=0.231$)	2
Site × fertilizer	**23.215 ($P<0.001$)**	2
Generation × fertilizer	1.520 ($P=0.468$)	2
Salt × fertilizer	**10.125 ($P=0.038$)**	4
Site × generation × salt	5.665 ($P=0.059$)	2
Site × generation × fertilizer	**7.714 ($P=0.021$)**	2
Site × salt × fertilizer	**11.844 ($P=0.019$)**	4
Generation × salt × fertilizer	8.490 ($P=0.075$)	4
Site × generation × salt × fertilizer	2.566 ($P=0.464$)	3

FIGURE CAPTION~~S~~

Fig. 1

Map of the study area ~~with~~ and the two experimental translocation sites.

Fig. 2

Water-table depth at the two sites across the growing period.

Fig. 3

Mean (+SE) ~~values of~~ height (A), stem diameter (B), number of branches (C) and number of fruits (D) of seashore mallow plants ~~of~~ from two generations. The year before, the ~~mother~~maternal plants had been subjected to three levels of salt addition × three levels of fertilizer, in factorial combination, during ex situ experimental cultivation.

0S/0F = no salt addition, no fertilization

0S/LF = no salt addition, low level of fertilization

0S/HF = no salt addition, high level of fertilization

LS/0F = low level of salt addition, no fertilization

LS/LF = low level of salt addition, low level of fertilization

LS/HF = low level of salt addition, high level of fertilization

HS/0F = high level of salt addition, no fertilization

HS/LF = high level of salt addition, low level of fertilization

HS/HF = high level of salt addition, high level of fertilization

Different letters indicate significant (P<0.05) differences between treatments for the ~~mother~~maternal plants. No significant differences were found for the offspring.

Fig. 4

Mean (+SE) ~~values of~~ net CO2 exchange rates recorded on three dates across the growing period in two generations of seashore mallow plants ~~of two generations~~ at the two sites. Different letters

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Formatted: Body Text, Indent: Left: 0", Space Before: 0 pt, Line spacing: single, Tab stops: 1.18", Left indicate significant ($P<0.05$) differences among dates. Lowercase letters refer to the ~~mother~~maternal plants, capital letters to the offspring. Asterisks show significant ($P<0.05$) differences between plants of the two generations on each date. Effects of fertilization and salt addition are shown in Table S2.

Fig. 5
Phenological codes across time for the BBCH9 phenophase in two generations of seashore mallow plants of two generations at the two sites. Regression lines are shown separately forper site and generation.

Elciola mothermaternal plants: $R2 = 0.80$; $y = 75.68 + 0.07x$

Elciola offspring: $R2 = 0.71$; $y = 77.91 + 0.06x$
Goara mothermaternal plants: $R2 = 0.83$; $y = 64.89 + 0.11x$

Goara offspring: $R2 = 0.79$; $y = 69.67 + 0.09x$

Fig. 6
Box-plot diagrams for length of the flowering period of seashore mallow plants of two generations. The year before, the mothermaternal plants had been subjected to three levels of salt addition × three levels of fertilizer, in factorial combination, during ex situ experimental cultivation.

Legend of abbreviations as in Fig. 3. The box indicates the mean (inner square) ±SE; the bars indicate ±SD. The circles and the asterisks indicate outliers and extreme values, respectively.

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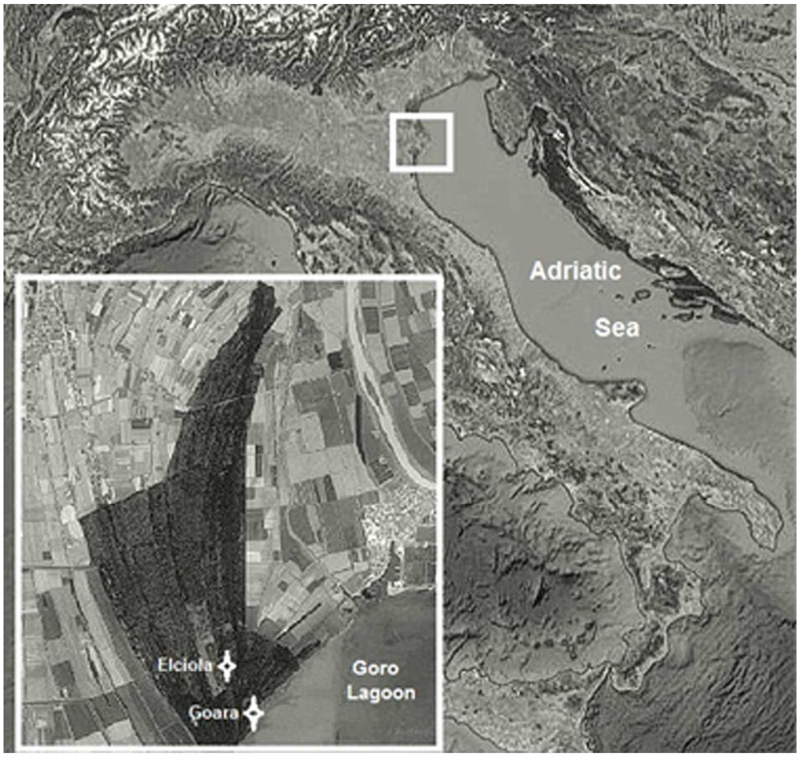

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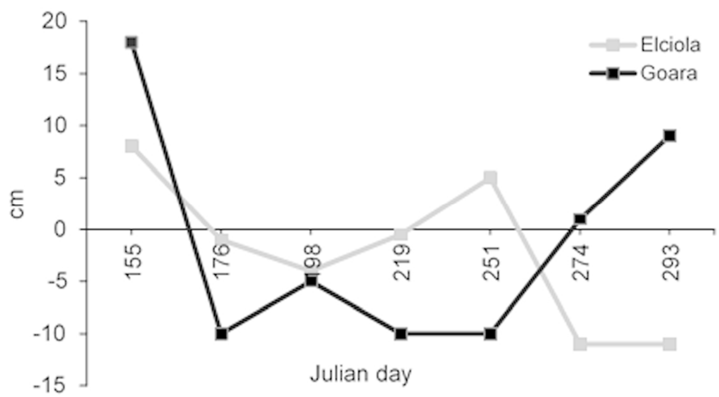

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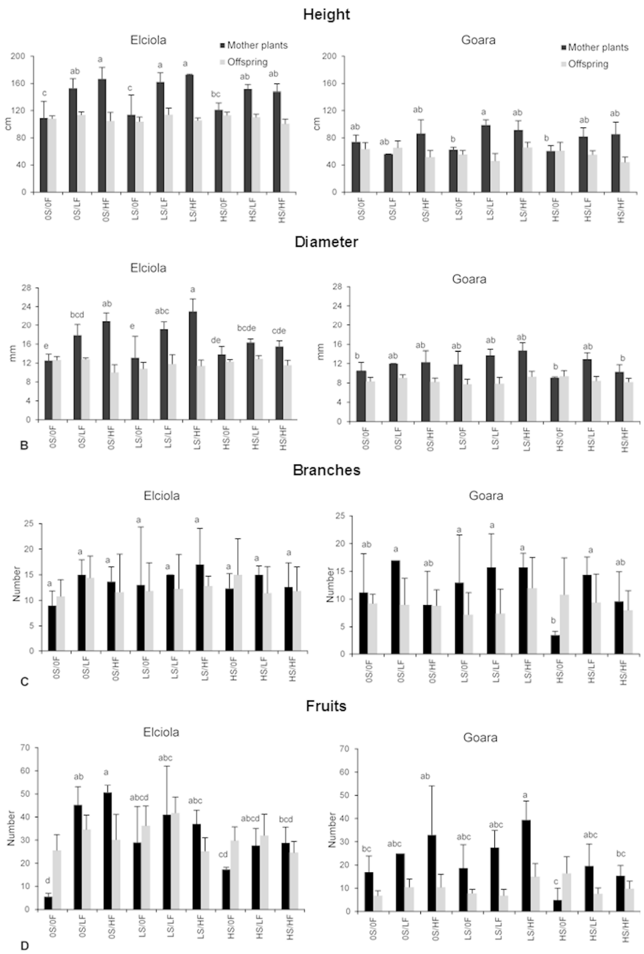

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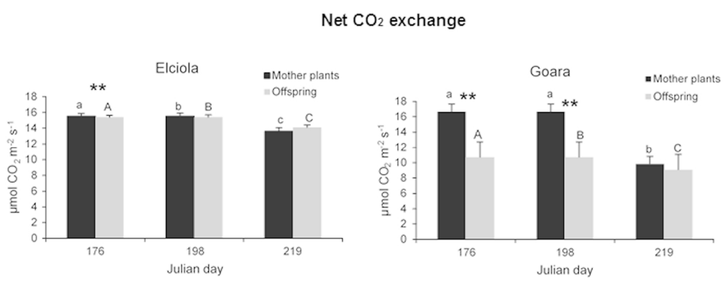

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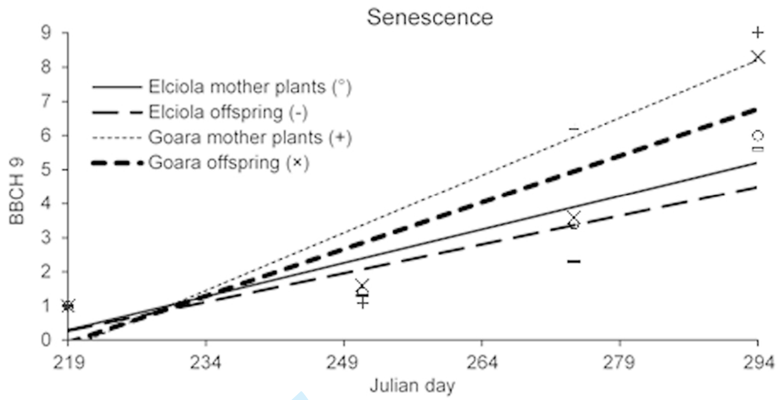

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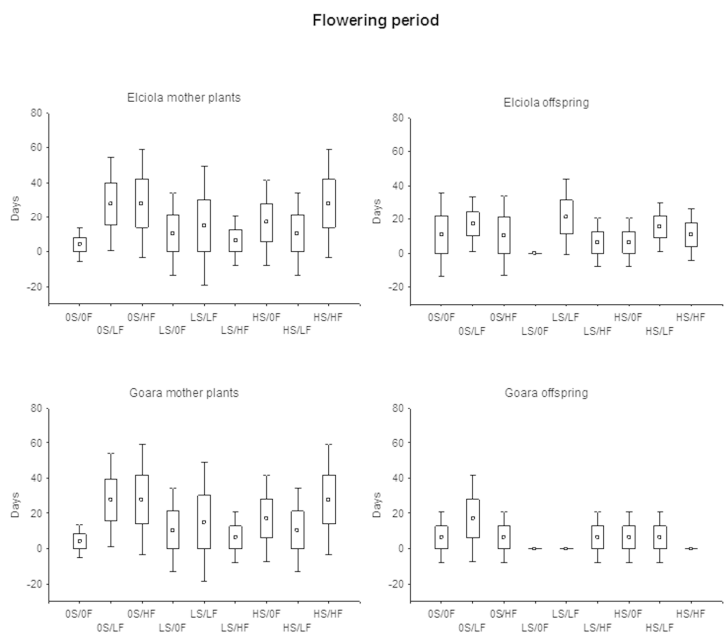

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