

RESEARCH PAPER

Salt effects on functional traits in model and in economically important *Lotus* species

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Keywords

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ABSTRACT

A common stress on plants is NaCl-derived soil salinity. Genus *Lotus* comprises model and economically important species, which have been studied regarding physiological responses to salinity. Leaf area ratio (LAR), root length ratio (RLR) and their components, specific leaf area (SLA) and leaf mass fraction (LMF) and specific root length (SRL) and root mass fraction (RMF) might be affected by high soil salinity. We characterised *L. tenuis*, *L. corniculatus*, *L. filicaulis*, *L. creticus*, *L. burtii* and *L. japonicus* grown under different salt concentrations (0, 50, 100 and 150 mM NaCl) on the basis of SLA, LMF, SRL and RMF using PCA. We also assessed effects of different salt concentrations on LAR and RLR in each species, and explored whether changes in these traits provide fitness benefit. Salinity (150 mM NaCl) increased LAR in *L. burtii* and *L. corniculatus*, but not in the remaining species. The highest salt concentration caused a decrease of RLR in *L. japonicus* Gifu, but not in the remaining species. Changes in LAR and RLR would not be adaptive, according to adaptiveness analysis, with the exception of SLA changes in *L. corniculatus*. PCA revealed that under favourable conditions plants optimise surfaces for light and nutrient acquisition (SLA and SRL), whereas at higher salt concentrations they favour carbon allocation to leaves and roots (LMF and RMF) in detriment to their surfaces. PCA also showed that *L. creticus* subjected to saline treatment was distinguished from the remaining *Lotus* species. We suggest that augmented carbon partitioning to leaves and roots could constitute a salt-alleviating mechanism through toxic ion dilution.

INTRODUCTION

One of the most common stress sources for plants is NaCl-derived soil salinity (Strogonov 1969). Excess NaCl in soil causes hyper-osmotic stress and specific ion effects to most plants (Blumwald 2000; Zhu 2001). In addition, salinity may cause nutrient deficiencies or imbalances, due to the competition of Na⁺ and Cl⁻ with nutrients such as K⁺ and Ca²⁺ (Hu & Schmidhalter 2005). As a consequence, above- and below-ground biomass allocation may be affected by salt accumulation in plant tissues (Marschner 1995; Munns & Tester 2008).

The genus *Lotus* (Fabaceae) comprises several species that have attracted the attention of researchers for their economic, ecological and scientific relevance. Among them, two ecotypes of the species *L. japonicus* ['Gifu', derived from a riverbank on Gifu prefecture, Japan (Handberg & Stougaard 1992), and 'Miyakojima MG-20', derived from the southernmost island of Japan, (Kawaguchi 2000; Kawaguchi *et al.* 2001) were chosen as model species for legume research, whereas *L. filicaulis* (from Algeria; Brand 1898) and *L. burtii* (originally from banks of the Kabul River, Peshawar, West Pakistan; Sz.-Borsos *et al.* 1972)] have been developed as crossing partners of *L. japonicus* for genetic studies, since they produce hybrids (Somaroo & Grant

1971; O'Donoghue *et al.* 1990; Kawaguchi *et al.* 2005). In addition, *L. tenuis* and *L. corniculatus* are worldwide acknowledged for their forage value for cattle (Escaray *et al.* 2012). *L. creticus* is also used as forage in areas of the Mediterranean Basin, and for dune revegetation and reclamation of heavy metal-contaminated or burned soils in Europe (Cabot & Pagès 1997; Sánchez-Blanco *et al.* 1998; Escaray *et al.* 2012). *L. tenuis* and *L. creticus* are recognised through their high adaptability to diverse environmental constraints, in particular, soil salinity, which has encouraged their use for identification of salt tolerance determinants (Sannazzaro *et al.* 2006, 2007; Rejili *et al.* 2007; Echeverria *et al.* 2008, 2013; Sanchez *et al.* 2011b; Escaray *et al.* 2012; Paz *et al.* 2012). In addition, several studies on the genomic, ionic, metabolomic and antioxidant system responses to salinity have been carried out on these important crop and model *Lotus* species (Sanchez *et al.* 2008, 2010, 2011a; Melchiorre *et al.* 2009). However, no study has been undertaken so far in order to characterise them according to functional morphological traits.

The leaf area ratio (LAR, total leaf area per unit plant biomass), length of root per unit of plant mass (RLR; Ryser & Lambers 1995) and their components are easily measured morphological characteristics, which are regarded as functional

traits by several authors, and are of ecophysiological relevance for tolerance to saline environments in diverse plant species (Ishikawa & Kachi 2000; Rubinigg *et al.* 2003; Praxedes *et al.* 2010; Wang *et al.* 2010; Lovelli *et al.* 2012; Minden *et al.* 2012; Bompy *et al.* 2014; Xu *et al.* 2014). LAR represents the leafiness or leaf expansion of a plant, whereas RLR describes plant potential for soil resource acquisition (Marcelis *et al.* 1998; Ryser 1998). Some reports on glycophytes and halophytes showed that salinity can affect LAR and RLR (Curtis & Lauchli 1986; Shennan *et al.* 1987; He & Cramer 1993; Stevanato *et al.* 2013). In turn, LAR changes may result from alterations to its components: specific leaf area (SLA, leaf area per leaf biomass; Ishikawa & Kachi 2000; Rijkers *et al.* 2000; Evans & Poorter 2001) and leaf mass fraction (LMF, proportion of total plant mass allocated to leaves; (Lamers *et al.* 2006; Läuchli & Grattan 2007; Poorter *et al.* 2012). Similarly, changes in RLR follow fluctuations in its components: specific root length (SRL, total root length per unit root mass; (Hill *et al.* 2006; Ostonen *et al.* 2007) and root mass fraction (RMF, proportion of total plant mass allocated to roots; Poorter *et al.* 2012). Changes in LAR and RLR as a response to variations in above- and below-ground environment are mainly driven by morphological adjustments (*i.e.* SLA and SRL) or carbon allocation adjustment (LMF and RMF; Poorter *et al.* 2012; Freschet *et al.* 2015).

One factor that seems to exert an important influence on plant ability to withstand stress is the species ability to change trait values in response to environmental conditions, called 'trait plasticity' (Callaway *et al.* 2003). However, plasticity in functional traits may be neutral or adaptive (if it provides fitness benefit to the organism) or merely a response to resource limitation or physical constraints (Weiner 2004; Van Kleunen & Fischer 2005). One way to test for adaptive plasticity in a scenario of a favourable *versus* limiting environment is to determine whether the attained phenotypic variant in the limiting environment as a result of the plastic response shows a positive association with plant fitness (Pigliucci *et al.* 1996; Gianoli & González-Teuber 2005). The relationship between traits and fitness can be used as an empirical test of adaptive hypotheses (Dudley 1996).

This work was conducted in order to assess the effect of different salt concentrations on LAR and RLR traits in each species, and explore whether changes in LAR or RMF provide fitness benefit to this group of plants, and therefore may be taken as adaptive. Here we also characterise *L. tenuis*, *L. corniculatus*, *L. filicaulis*, *L. creticus*, *L. burtii* and *L. japonicus* plants grown under different salt concentrations (0, 50, 100 and 150 mM NaCl) on the basis of their growth, SLA, LMF, SRL and RMF. As the performances under salinity of all the above-mentioned *Lotus* species have not previously been addressed in a same study, we also compared their stress tolerance at three different salinity ranges using an index of stress intensity (Fort *et al.* 2015).

MATERIAL AND METHODS

Plant material and culture conditions

The experiment consisted of a randomised complete block design with two factors: (i) *Lotus* species/ecotype, with seven levels: *L. burtii*, *L. corniculatus*, *L. creticus*, *L. filicaulis*, *L. japonicus* Gifu, *L. japonicus* MG-20 and *L. tenuis*; and (ii) salinity, with four levels: 0,

50, 100 and 150 mM NaCl. Studied species were: *L. tenuis* var. Pampa INTA (provided by Estación Experimental Agropecuaria Balcarce, INTA), *L. japonicus* ecotypes MG-20 and Gifu, *L. burtii* and *L. filicaulis* (provided by Banco de Germoplasma de la Estación Experimental La Estanzuela INIA Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay) and *L. corniculatus* and *L. creticus* (sampled on the coastal sand dunes at the Devesa de la Albufera, Valencia, Spain). The *L. corniculatus* used in this work is a tetraploid, which diverges from the original description of this species as a diploid.

Seeds of *Lotus* species were treated with concentrated sulphuric acid for 2 min., rinsed ten times with sterile distilled water and germinated in Petri dishes containing agar/water (0.8%). Seedlings were transferred to 250-ml pots containing vermiculite-perlite soil mix (1:1, v/v) and cultivated in a growth chamber with a 16-h day/8-h night photoperiod (photon flux density 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by daylight and GroLux fluorescent lamps, F 40W), 24/21 ± 2 °C and 55/65 $\pm 5\%$ day/night temperature and relative humidity, respectively. There was one plant per pot (replicate) and 12 replicates per treatment. Plants were regularly irrigated with half-strength Hoagland's nutrient solution containing 3 mM KNO₃, 2 mM Ca(NO₃)₂·4H₂O, 1 mM MgSO₄·7H₂O, 0.5 mM NH₄H₂PO₄, 0.5 μM NaFeO₈EDTA·2H₂O and 0.5 mM of each of the following micronutrients MnCl₂·4H₂O, H₃BO₃, CuSO₄·5H₂O, ZnSO₄·7H₂O and Na₂MoO₄·2H₂O (Hoagland & Arnon 1950). To avoid any osmotic shock from saline treatments, 8-day-old seedlings initially received 50 mM NaCl, and this concentration was increased weekly in 25 mM portions, so that the final salt concentration (150 mM) was reached in 4 weeks. After acclimation, plants were grown under their respective treatment for another 20 days in a greenhouse, under same conditions, as above.

Trait measurement

At harvest, six plants from each treatment were divided into roots, leaves and stems, and dry weights determined by oven drying at 60 °C until constant weight. Another set of six plants per treatment was used for root length and leaf area determinations. Plant biomass (total dry weight, TDW) was estimated as the sum of all plant parts. At harvest, weighed roots and leaves were carefully extended over 20-cm \times 20-cm glass plates and scanned. Roots were previously stained with Trypan blue. Images were analysed with the Image-ProPlus version 4.1 software (Media Cybernetics, Bethesda, MD, USA).

The following leaf and root traits related to plant fitness were calculated for each plant: LAR (total leaf area per TDW, $\text{cm}^2\cdot\text{g}^{-1}$), SLA (leaf area per unit leaf mass, $\text{cm}^2\cdot\text{g}^{-1}$) and LMF (total leaf mass per TDW, $\text{g}\cdot\text{g}^{-1}$), RLR (total root length per TDW, $\text{m}\cdot\text{g}^{-1}$), SRL (total root length per root mass, $\text{m}\cdot\text{g}^{-1}$) and RMF (root mass per TDW, $\text{g}\cdot\text{g}^{-1}$).

Trait analysis

To visualise trait plasticity, reaction norms were diagrammed for each species and trait over the 0–150 mM NaCl salinity range, and observed phenotypic variation tested for each trait through one-way ANOVA for salinity effect, followed by Duncan's test ($P < 0.05$).

In species where a significant salt effect was recorded, LAR and RLR values were linearly regressed against fitness (Dudley

1996) in order to explore the adaptiveness of phenotypic plasticity in this *Lotus* species group. TDW was used as a surrogate for fitness, since biomass and overall reproductive success are often highly correlated (e.g. Murren *et al.* 2002; Murren & Pigliucci 2005)) and it has already been explicitly used as an index of fitness in several species (Moriuchi & Winn 2005; Donovan *et al.* 2009; Valluru *et al.* 2012; Chen *et al.* 2013). The occurrence of a change in association between traits and fitness between two different environments is taken as strong support for a trait's adaptive value (Wade & Kalisz 1990; Dudley 1996; Pigliucci & Schlichting 1996; Gianoli & González-Teuber 2005; Nicotra *et al.* 2010). Data were pooled in two groups, one of low and another of high salinity (0 and 50, and 100 and 150 mM NaCl, respectively). To avoid meaningless results, this analysis was performed only in species where a significant effect of salinity on these parameters was found. Comparison between regression line slopes obtained for low and high salinity environments was achieved using regressions with the dummy variables method (Di Rienzo *et al.* 2010).

To characterise *Lotus* species under the different NaCl concentrations, a principal components analysis (PCA) was conducted with functional variables SLA, LMF, SRL and RMF. Differences due to salinity in each species/ecotype were analysed using one-way ANOVA, followed by comparisons with Duncan's test (significance = $P < 0.05$). For these analysis, LAR, RLR, SLA, LMF, SRL and RMF trait data were log₂-transformed, following Freschet *et al.* (2015). The effect of salinity on each species was estimated for three ranges of salinity, 0–50, 0–100 and 0–150 mM NaCl, through an abiotic index of stress intensity (variation 0–1; Fort *et al.* 2015), based on aboveground biomass production according to:

$$\text{Index of stress intensity} = \text{SDW}(c) = \text{SDW}(s)/\text{SDW}(c)$$

where SDW(c) is mean shoot biomass of plants grown without salt addition and SDW(s) is mean shoot biomass of plants grown with 50, 100 or 150 mM NaCl (Fort *et al.* 2015).

RESULTS

Plasticity of LAR and RLR

Figure 1 shows norms of reaction for LAR and RLR as a function of salinity. Most of the species showed no significant changes in LAR and RLR when exposed to the different NaCl concentrations, compared with non-salinised controls, according to one-way ANOVA followed by Duncan's test. LAR increased with 150 mM NaCl in *L. burtii* and *L. corniculatus*, whereas this parameter showed no significant salt-induced variations in the remaining *Lotus* species. In contrast, salinity lowered RLR in *L. japonicus* Gifu, compared with non-salinised controls.

Adaptiveness analysis of LAR and RLR plasticity

As there were not significant differences in LAR and RLR between 0 and 50, and between 100 and 150 mM NaCl, data from these treatments were pooled and renamed 'low' and 'high' salinity treatments. Regression analyses indicated that total dry biomass of *L. corniculatus* presented a negative linear relationship with LAR, regardless of salinity level (Fig. 2; low salinity; $P = 0.0002$, high salinity: $P = 0.05$). However, the slope of the regression line was significantly less steep at the high than at the low salinity level. The line regressions performed on TDW versus LAR or RLR for the remaining *Lotus* species either showed no adjustment to the linear model or there were not differences between slopes corresponding to the low and high saline environments (results not shown).

Characterisation of *Lotus* species/ecotypes according to LAR and RLR components at different salinity levels

Principal components analysis (Fig. 3), computed from Log₂ of values depicted in Table 1, resulted in two axes explaining 85% of the total variance. The high co-phenetic correlation coefficient (0.981) indicated good natural grouping. Variables

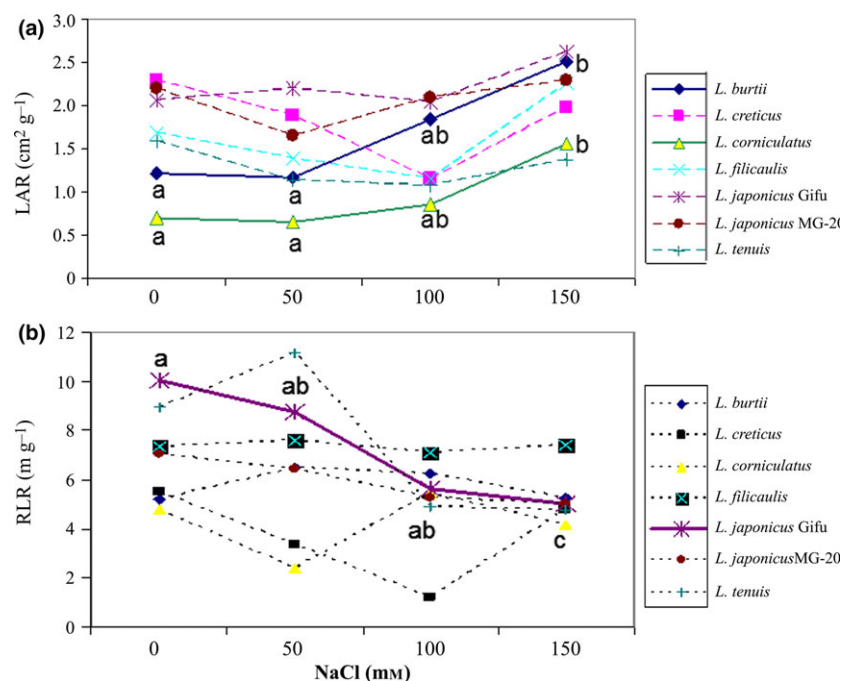


Fig. 1. Norms of reaction for LAR and RLR as a function of the amount of NaCl (0–150 mM) added to the nutrient solution. Dashed versus filled lines mean, respectively, that no significant or significant effects of salinity were detected on LAR or RLR, according with Duncan's test ($P < 0.05$).

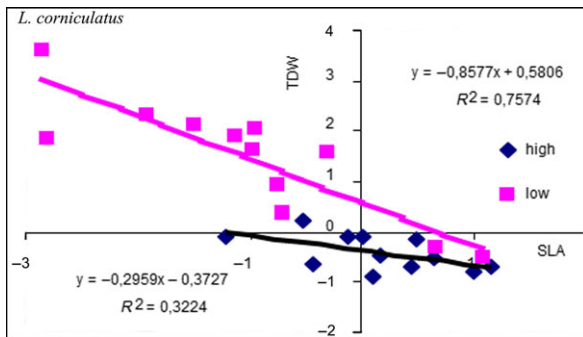


Fig. 2. Test of adaptive plasticity for *L. corniculatus* grown at low versus high salinity conditions.

correlating most with PC1, which explained 60% of the variability, were LMF (0.87), RMF (0.85) and SLA (-0.8), whereas main correlation with PC2 (explaining 25% of variability) was provided by SRL (0.8). Plants grown in the absence of NaCl were associated with higher SLA and lower LMF and RMF. As salinity level increased, plants reduced their SLA, while increasing their LMF. This last result was more obvious in *L. burtii*, *L. filicaulis* and *L. japonicus* Gifu, particularly at 150 mM salt. At 50 mM NaCl, *L. tenuis* and *L. japonicus* MG-20 differed from the remaining species/ecotypes cultivated at that salinity level by their higher SRL. In contrast, salinised *L. creticus* were separated in a group more related to higher RMF (and also LMF).

Index of stress intensity

Increasing salt concentration led to higher levels of stress intensity, being minimum and maximum at the 0–50 and 0–150 mM NaCl ranges, respectively (Fig. 4). *L. burtii* and *L. creticus* presented the highest stress intensity values, whereas *L. tenuis* and *L. corniculatus* showed the lowest. Interestingly, in the 0–50 mM NaCl range *L. tenuis* shoot growth was promoted by salinity.

DISCUSSION

The aim of this work was to assess the effect of different salt concentrations on LAR and RLR and their components in several model and economically important *Lotus* species. The observed salt-induced LAR increases in *L. burtii* and *L. corniculatus* (Fig. 1) indicate that leaf expansion was less affected than

total plant biomass in these species. The last results are in line with Nguyen *et al.* (2015), who found a significant rise in LAR with increased salinity, but disagree with results previously obtained in two *Brassica* species (He & Cramer 1993), kenaf (*Hibiscus cannabinus* (Curtis & Lauchli 1986)) and *Aster tripolium* (Shennan *et al.* 1987), where reductions in LAR were reported. In turn, the adaptiveness analysis of LAR plasticity showing a significantly less steep slope of the regression line at the high than at the low salinity level in *L. corniculatus*, suggesting that at high salt concentration, increased LAR could have adaptive value in this species.

On other hand, the RLR reduction in *L. japonicus* Gifu revealed a reduction in its capacity for soil nutrient uptake. This contrasts with findings in salinised sugar beet (Stevanato *et al.* 2013). The remaining *Lotus* species analysed did not significantly change LAR and RLR with increased salinity. Taken together, variations in the effect of salinity on LAR and RLR among *Lotus* species here studied, and among these and species of genera studied by other authors suggests a species specificity of LAR and RLR responses to salinity. However, the PCA suggested that plants optimise surfaces for light and nutrient acquisition (SLA and SRL) under favourable conditions (0 and 50 mM NaCl), whereas at higher salt concentrations (100 and 150 mM) they favour carbon allocation to leaves and roots (LMF and RMF) in detriment of their surfaces. The PCA also showed that *L. creticus* subjected to saline treatment were discriminated from the remaining *Lotus* species, suggesting that two species groups evolved different strategies to respond to a saline environment. This is supported by the fact that *L. creticus* belongs to a phylogenetically distant clade (Pedrosia) with respect to the remaining studied species (*Lotus corniculatus*; Degtjareva *et al.* 2008).

The inherent osmotic component of salinity, which may cause water deficit, could explain the inverse association of SLA values with higher NaCl concentrations, since low SLA has been reported as a general tendency for species inhabiting arid and semi-arid regions (Niinemets 2001; Wright *et al.* 2001). On the other hand, SLA was shown to negatively correlate with construction cost, whereas leaves with high SLA generally have high protein and low concentrations of other expensive compounds, such as lipids or lignin, and high concentrations of ‘cheap’ constituents, such as minerals (Villar & Merino 2001). This information could be relevant in the case of *Lotus* species used as forage for cattle production, such as *L. corniculatus* and *L. tenuis*. However, no study has been undertaken so far in order to test whether shoot proteins, lipids and other nutrient

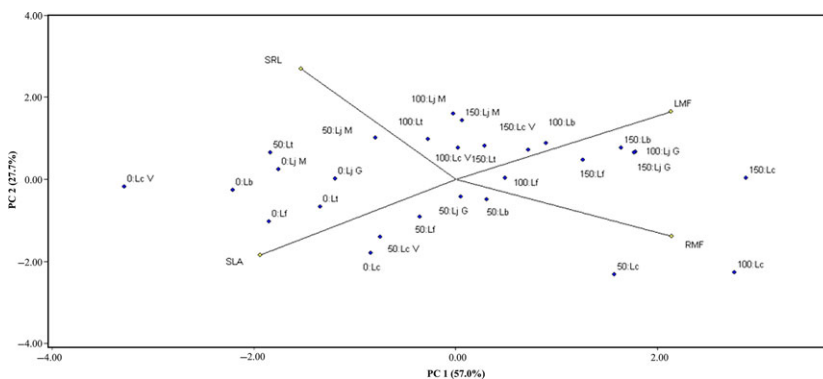


Fig. 3. Principal components analysis of specific variables leaf area (SLA), specific root length (SRL), leaf mass fraction (LMF) and root mass fraction (RMF), in *L. burtii* (Lb), *L. corniculatus* (Lc V), *L. creticus* (Lc), *L. filicaulis* (Lf), *L. japonicus* Gifu (Lj G), *L. japonicus* MG-20 (Lj M) and *L. tenuis* (Lt) grown at four different salt conditions (0, 50, 100 and 150 mM NaCl).

Table 1. Leaf area, dry weight of leaf, stem, root and total, and root length of each species at 0–150 mM salinity levels.

Species	NaCl	Leaf area (cm ²)	Leaf dry weight (mg)	Stem dry weight (g)	Root dry weight (g)	Total dry weight (g)	Root length (m)
<i>Lotus burtii</i>	0	1.64 ± 0.21 a	4.4 ± 0.6 a	1.78 ± 0.41 b	0.17 ± 0.02 a	1.96 ± 0.41 c	4.64 ± 0.21 a
	50	1.06 ± 0.11 ab	4.3 ± 0.6 a	0.84 ± 0.20 a	0.30 ± 0.13 a	1.14 ± 0.25 b	5.23 ± 2.40 a
	100	0.97 ± 0.15 a	4.8 ± 0.7 a	0.44 ± 0.05 a	0.09 ± 0.01 a	0.54 ± 0.05 a	3.08 ± 0.28 a
	150	0.95 ± 0.13 a	3.9 ± 0.8 a	0.29 ± 0.02 a	0.08 ± 0.01 a	0.38 ± 0.02 a	2.07 ± 0.50 a
<i>Lotus creticus</i>	0	1.20 ± 0.24 c	2.4 ± 0.4 a	0.52 ± 0.12 b	0.11 ± 0.01 a	0.64 ± 0.13 b	2.71 ± 0.65 b
	50	0.81 ± 0.13 bc	2.9 ± 0.7 a	0.33 ± 0.07 ab	0.15 ± 0.03 a	0.48 ± 0.08 ab	1.51 ± 0.59 ab
	100	0.58 ± 0.02 ab	3.2 ± 0.2 a	0.37 ± 0.13 ab	0.19 ± 0.09 a	0.57 ± 0.12 b	0.78 ± 0.13 a
	150	0.47 ± 0.06 a	3.0 ± 0.3 a	0.17 ± 0.02 a	0.08 ± 0.01 a	0.25 ± 0.02 a	1.15 ± 0.30 ab
<i>Lotus corniculatus</i>	0	1.74 ± 0.17 b	5.0 ± 0.5 a	4.61 ± 1.56 b	0.21 ± 0.03 ab	4.83 ± 1.57 b	6.17 ± 1.03 b
	50	1.16 ± 0.18 a	4.7 ± 1.2 a	2.06 ± 0.47 a	0.32 ± 0.07 b	2.39 ± 0.51 ab	3.68 ± 0.77 ab
	100	0.79 ± 0.13 a	4.0 ± 0.5 a	0.77 ± 0.07 a	0.14 ± 0.01 a	0.91 ± 0.07 a	5.03 ± 0.49 ab
	150	0.98 ± 0.11 a	5.1 ± 0.8 a	0.52 ± 0.03 a	0.11 ± 0.02 a	0.63 ± 0.03 a	2.55 ± 0.57 a
<i>Lotus filicaulis</i>	0	1.94 ± 0.17 b	4.6 ± 0.5 a	2.49 ± 0.81 b	0.37 ± 0.10 b	2.87 ± 0.92 b	4.07 ± 0.14 a
	50	1.74 ± 0.18 b	5.2 ± 0.3 a	1.32 ± 0.29 ab	0.32 ± 0.03 b	1.64 ± 0.31 ab	5.64 ± 0.00 a
	100	1.12 ± 0.11 a	5.0 ± 0.5 a	0.85 ± 0.17 a	0.22 ± 0.03 ab	1.08 ± 0.20 a	5.93 ± 0.99 a
	150	1.22 ± 0.14 a	5.7 ± 0.8 a	0.45 ± 0.06 a	0.14 ± 0.02 a	0.59 ± 0.07 a	4.14 ± 1.72 a
<i>Lotus japonicus</i> Gifu	0	2.41 ± 0.22 b	6.7 ± 0.9 a	1.55 ± 0.37 b	0.27 ± 0.05 a	1.83 ± 0.41 b	6.55 ± 0.34 b
	50	1.75 ± 0.27 a	5.8 ± 1.1 a	0.97 ± 0.24 ab	0.23 ± 0.06 a	1.21 ± 0.29 ab	4.45 ± 2.19 ab
	100	1.32 ± 0.09 a	7.4 ± 0.5 a	0.51 ± 0.04 a	0.14 ± 0.01 a	0.66 ± 0.04 a	3.34 ± 0.13 a
	150	1.37 ± 0.04 a	6.9 ± 0.5 a	0.39 ± 0.03 a	0.14 ± 0.04 a	0.54 ± 0.07 a	2.48 ± 0.45 a
<i>Lotus japonicus</i> MG-20	0	4.10 ± 0.73 b	10.1 ± 0.6 a	2.39 ± 0.53 b	0.27 ± 0.03 c	2.67 ± 0.53 c	7.74 ± 0.00 a
	50	2.16 ± 0.35 a	8.5 ± 0.4 a	1.60 ± 0.27 ab	0.17 ± 0.01 b	1.78 ± 0.27 b	9.43 ± 3.81 a
	100	2.14 ± 0.23 a	10.1 ± 1.3 a	0.88 ± 0.08 a	0.11 ± 0.01 a	1.00 ± 0.08 ab	5.21 ± 0.90 a
	150	1.84 ± 0.11 a	8.2 ± 0.5 a	0.73 ± 0.07 a	0.09 ± 0.01 a	0.83 ± 0.07 a	3.69 ± 1.11 a
<i>Lotus tenuis</i>	0	1.61 ± 0.25 a	4.5 ± 0.8 a	1.77 ± 0.72 ab	0.23 ± 0.05 ab	2.01 ± 0.76 b	5.16 ± 1.33 ab
	50	1.74 ± 0.26 a	6.1 ± 0.4 ab	3.00 ± 0.78 b	0.33 ± 0.05 b	3.34 ± 0.83 ab	10.8 ± 3.45 b
	100	1.36 ± 0.16 a	6.6 ± 0.7 b	1.23 ± 0.15 a	0.19 ± 0.03 a	1.42 ± 0.18 a	5.29 ± 0.73 ab
	150	1.42 ± 0.14 a	6.5 ± 0.6 b	0.87 ± 0.09 a	0.14 ± 0.02 a	1.02 ± 0.10 a	4.49 ± 0.59 a

Averages with the same letter, within each species are not significantly different ($P < 0.05$).

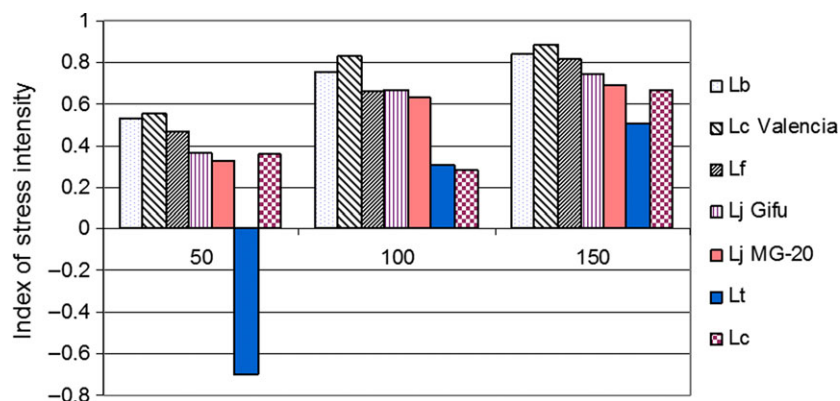


Fig. 4. Index of stress intensity for studied *Lotus* species calculated at 50, 100 and 150 mM NaCl, with respect to control conditions (0 mM). *L. burtii* (Lb), *L. corniculatus* (Lc V), *L. creticus* (Lc), *L. filicaulis* (Lf), *L. japonicus* Gifu (Lj G), *L. japonicus* MG-20 (Lj M), *L. tenuis* (Lt).

composition and content change when plants are grown under salinity.

The salt-induced rise in LMF is in accordance with slight increases in this parameter observed as result of saline treatment in moderately salt-tolerant species, e.g. lemon (*Citrus limon*; Zapata *et al.* 2003) and Mexican fan palm (*Washingtonia robusta* H.; Nieves *et al.* 2011)). There are few records regarding the influence of salinity on RMF. For example, higher root biomass allocation induced by salinity was reported in the hybrid species *Helianthus paradoxus* (Karrenberg & Widmer 2008). This result is congruent with the significant salt-induced

RMF increase here observed in *L. burtii*, *L. corniculatus* and *L. filicaulis*, along with similar trends in *L. creticus* and *L. japonicus* Gifu. Possibly, these augmented carbon partitioning to leaves and roots (at the expense of stem mass fraction; results not shown) could constitute a salt-alleviating mechanism through toxic ion dilution, as shown for orange (*Citrus sinensis*; Zapata *et al.* 2004) and Mexican fan palm (Nieves *et al.* 2011).

Finally, the negative index of stress intensity at 50 mM NaCl for *L. tenuis* suggests that although considered a glycophyte (Sanchez *et al.* 2011a,b), this species displays facultative

halophytic behaviour, as shown by Melečková *et al.* (2014). Among the remaining species, *L. corniculatus* presented the lowest index of stress intensity. Interestingly, *L. tenuis* x *L. corniculatus* hybrids were obtained from the same populations used in the present work (Escaray *et al.* 2014); these hybrids were more vigorous than parental plants. Moreover, *L. corniculatus* and hybrid plants showed higher tolerance to 45 days of 150 mM NaCl and significantly lower chloride content in older leaves than *L. tenuis*. Based on this information and our result suggesting that in *L. corniculatus* the salt-induced LAR increase might have an adaptive value, it could be hypothesised that LAR also increases in *L. tenuis* x *L. corniculatus* hybrid grown

at high salt concentrations, having adaptive value through the dilution of toxic ions. To address this hypothesis in future studies would help in gaining a deeper understanding of the basic mechanisms for salt tolerance in hybrid lines.

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REFERENCES

- Blumwald E. (2000) Sodium transport and salt tolerance in plants. *Current Opinion in Cell Biology*, **12**, 431–434.
- Bompy F., Lequeue G., Imbert D., Dulorme M. (2014) Increasing fluctuations of soil salinity affect seedling growth performances and physiology in three Neotropical mangrove species. *Plant and Soil*, **380**, 399–413.
- Brand A. (1898) Monographie der Gattung *Lotus*. *Botanische Jahrbücher für Systematik*, **25**, 166–232.
- Cabot P., Pagès J.M. (1997) Evaluación de la capacidad tapizante de cuatro especies silvestres y dos especies arbustivas ornamentales cultivadas. *Actas de Horticultura*, **17**, 153–157.
- Callaway R., Pennings S.C., Richards C.L. (2003) Phenotypic plasticity and interactions among plants. *Ecology*, **84**, 1115–1128.
- Chen L., Tiu C.J., Peng S., Siemann E. (2013) Conspecific plasticity and invasion: invasive populations of Chinese tallow (*Triadica sebifera*) have performance advantage over native populations only in low soil salinity. *PLoS One*, **8**, e74961.
- Curtis P.S., Lauchli A. (1986) The role of leaf area development and photosynthetic capacity in determining growth of kenaf under moderate salt stress. *Australian Journal of Plant Physiology*, **4**, 553–565.
- Degtjareva G.V., Kramina T.E., Sokoloff D.D., Samigullin T.H., Sandral G., Valiejo-Roman C.M. (2008) New data on nrITS phylogeny of *Lotus* (Leguminosae, Loteae). *Wulfenia*, **15**, 35–49.
- Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. (2010) InfoStat versión 2010. Universidad Nacional de Córdoba (Argentina). FCA. <http://www.infostat.com.ar>.
- Donovan L.A., Ludwig F., Rosenthal D.M., Rieseberg L.H., Dudley S.A. (2009) Phenotypic selection on leaf ecophysiological traits in *Helianthus*. *New Phytologist*, **183**, 868–879.
- Dudley S.A. (1996) Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution*, **50**, 92–102.
- Echeverría M., Scambato A.A., Sannazzaro A.I. (2008) Phenotypic plasticity with respect to salt stress response by *Lotus glaber*: the role of its AM fungal and rhizobial symbionts. *Mycorrhiza*, **18**, 317–319.
- Echeverría M., Sannazzaro A.I., Ruiz O.A., Menéndez A.B. (2013) Modulatory effects of *Mesorhizobium tianshanense* and *Glomus intraradices* on plant proline and polyamine levels during early plant response of *Lotus tenuis* to salinity. *Plant and Soil*, **364**, 69–79.
- Escaray F.J., Menéndez A.B., Gárriz A., Pieckenstain F.L., Estrella M.J., Castagno L.N., Carrasco P., Sanjuán J., Ruiz O.A. (2012) Ecological and agronomic importance of the plant genus *Lotus*. Its application in grassland sustainability and the amelioration of constrained and contaminated soils. *Plant Science*, **182**, 121–133.
- Escaray F.J., Passeri V., Babuin M.F., Marco F., Carrasco P., Damiani F., Pieckenstain F.L., Paolucci F., Ruiz O.A. (2014) *Lotus tenuis* x *L. corniculatus* interspecific hybridization as a means to breed bloat-safe pastures and gain insight into the genetic control of proanthocyanidin biosynthesis in legumes. *BMC Plant Biology*, **14**, 14–40.
- Evans J.R., Poorter H. (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environment*, **24**, 755–767.
- Fort F., Cruz P., Catrice O., Delbrut A., Luzarreta M., Stroia C., Jouany C. (2015) Root functional trait syndromes and plasticity drive the ability of grassland Fabaceae to tolerate water and phosphorus shortage. *Environmental and Experimental Botany*, **110**, 62–72.
- Freschet G.T., Swart E.M., Cornelissen J.H.C. (2015) Integrated plant phenotypic responses to contrasting above- and below-ground resources: key roles of specific leaf area and root mass fraction. *New Phytologist*, **206**, 1247–1260.
- Gianoli E., González-Teuber M. (2005) Environmental heterogeneity and population differentiation in plasticity to drought in *Convolvulus chilensis* (Convolvulaceae). *Evolutionary Ecology*, **19**, 603–613.
- Handberg K., Stougaard J. (1992) *Lotus japonicus*, an autogamous, diploid legume species for classical and molecular genetics. *The Plant Journal*, **2**, 487–496.
- He T., Cramer G. (1993) Growth and ion accumulation of two rapid-cycling *Brassica* species differing in salt tolerance. *Plant and Soil*, **153**, 19–31.
- Hill J.O., Simpson R.J., Moore A.D., Chapman D.F. (2006) Morphology and response of roots of pasture species to phosphorus and nitrogen nutrition. *Plant and Soil*, **286**, 7–19.
- Hoagland D.R., Arnon D.I. (1950) The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular*, **347**, 1–32.
- Hu Y., Schmidhalter U. (2005) Drought and salinity: a comparison of their effects on mineral nutrition of plants. *Journal of Plant Nutrition and Soil Science*, **168**, 541–549.
- Ishikawa S.I., Kachi N. (2000) Differential salt tolerance of two *Artemisia* species growing in contrasting coastal habitats. *Ecological Research*, **15**, 241–247.
- Karrenberg S., Widmer A. (2008) Ecologically relevant genetic variation from a non-Arabidopsis perspective. *Current Opinion in Plant Biology*, **11**, 156–162.
- Kawaguchi M. (2000) *Lotus japonicus* “Miyakojima” MG-20 an early-flowering accession suitable for indoor handling. *Journal of Plant Research*, **113**, 507–509.
- Kawaguchi M., Motomura T., Imaizumi-Anraku H., Akao S., Kawasaki S. (2001) Providing the basis for genomics in *Lotus japonicus*: the accessions Miyakojima and Gifu are appropriate crossing partners for genetic analyses. *Molecular Genetics and Genomics*, **266**, 157–166.
- Kawaguchi M., Pedrosa-Harand A., Yano K., Hayashi M., Murooka Y., Saito K., Nagata T., Namai K., Nishida H., Shibata D., Sato S., Tabata S., Hayashi M., Harada K., Sandal N., Stougaard J., Bachmair A., Grant W.F. (2005) *Lotus burttii* takes a position of the third corner in the *Lotus* molecular genetics triangle. *DNA Research*, **12**, 69–77.
- Lamers J., Khamzina A., Worbes M. (2006) The analyses of physiological and morphological attributes of 10 tree species for early determination of their suitability to afforest degraded landscapes in the Aral Sea Basin of Uzbekistan. *Forest Ecology and Management*, **221**, 249–259.
- Läuchli A., Grattan S.R. (2007) Plant growth and development under salinity stress. In: Jenks M.A. (Ed.), *Advances in molecular breeding toward drought and salt tolerant crops*. Springer, Berlin, Germany, pp 1–32.
- Lovelli S., Perniola M., Di Tommaso T., Bochicchio R., Amato M. (2012) Specific root length and diameter of hydroponically-grown tomato plants under salinity. *Journal of Agronomy*, **11**, 101–106.
- Marcelis L.F.M., Heuvelink E., Goudriaan J. (1998) Modelling biomass production and yield of horticultural crops: a review. *Scientia Horticulturae*, **74**, 83–111.
- Marschner H. (1995). *Mineral nutrition of higher plants* (2nd edition). Academic Press, London, UK: 889 pp.
- Melchiorre M., Quero G.E., Parola R., Racca R., Trippi V.S., Lascano R. (2009) Physiological characterization of four model *Lotus* diploid genotypes: *L. japonicus* (MG20 and Gifu), *L. filicaulis*, and *L. burttii* under salt stress. *Plant Science*, **177**, 618–628.
- Melečková Z., Dítě D., Jun P.E., Pís V., Galvánek D. (2014) Succession of saline vegetation in Slovakia after a large-scale disturbance. *Annales Botanici Fennici*, **51**, 285–296.
- Minden V., Andrantschke S., Spalke J., Timmermann H., Kleyer M. (2012) Plant trait–environment relationships in salt marshes: Deviations from predictions by ecological concepts. *Perspectives in Plant Ecology, Evolution and Systematics*, **14**, 183–192.
- Moriuchi K.S., Winn A.A. (2005) Relationships among growth, development and plastic response to

- environment quality in a perennial plant. *New Phytologist*, **166**, 149–158.
- Munns R., Tester M. (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, **59**, 651–681.
- Murren C.J., Pigliucci M. (2005) Morphological responses to simulated wind in the genus *Brassica* (Brassicaceae): allopolyploids and their parental species. *American Journal of Botany*, **92**, 810–818.
- Murren C.J., Pendleton N., Pigliucci M. (2002) Evolution of phenotypic integration in *Brassica* (Brassicaceae). *American Journal of Botany*, **89**, 655–663.
- Nguyen H.T., Stanton D.E., Schmitz N., Farquhar G.D., Ball M.C. (2015) Growth responses of the mangrove *Avicennia marina* to salinity: development and function of shoot hydraulic systems require saline conditions. *Annals of Botany*, **115**, 397–407.
- Nicotra A.B., Atkin O.K., Bonser S.P., Davidson A.M., Finnegan E.J., Mathesius U., Poot P., Purugganan M.D., Richards C.L., Valladares F., van Kleunen M. (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, **15**, 1–9.
- Nieves M., Nieves-Cordones M., Poorter H., Simón M.D. (2011) Leaf nitrogen productivity is the major factor behind the growth reduction induced by long-term salt stress. *Tree Physiology*, **31**, 92–101.
- Niinemets Ü. (2001) Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology*, **82**, 453–469.
- O'Donoghue L.S., Raelson J.V., Grant W.F. (1990) A morphological study of interspecific hybrids in the genus *Lotus* (Fabaceae). *Canadian Journal of Botany*, **68**, 803–812.
- Ostonen I., Lõhmus K., Helmisaari H.-S., Truu J., Meel S. (2007) Fine root morphological adaptations in Scots pine, Norway spruce and silver birch along a latitudinal gradient in boreal forests. *Tree Physiology*, **27**, 1627–1634.
- Paz R.C., Rocco R.A., Reinoso H., Menéndez A.B., Pieckenstain F.L., Ruiz O.A. (2012) Comparative study of alkaline, saline, and mixed saline-alkaline stresses with regard to their effects on growth, nutrient accumulation, and root morphology of *Lotus tenuis*. *Journal of Plant Growth Regulation*, **31**, 448–459.
- Pigliucci M., Schlichting C.D. (1996) Reaction norms of *Arabidopsis*. IV. Relationships between plasticity and fitness. *Heredity*, **76**, 427–436.
- Pigliucci M., Schlichting C., Jones C., Schwenk K. (1996) Developmental reaction norms: the interactions among allometry, ontogeny and plasticity. *Plant Species Biology*, **11**, 69–85.
- Poorter H., Niklas K.J., Reich P.B., Oleksyn J., Poot P., Mommer L. (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist*, **193**, 30–50.
- Praxedes S.C., De Lacerda C.F., DaMatta F.M., Prisco J.T., Gomes-Filho E. (2010) Salt tolerance is associated with differences in ion accumulation, biomass allocation and photosynthesis in cowpea cultivars. *Journal of Agronomy and Crop Science*, **196**, 193–204.
- Rejili M., Vadel A.M., Guetet A., Neffatti M. (2007) Effect of NaCl on the growth and the ionic balance K^+/Na^+ of two populations of *Lotus creticus* (L.) (Papilionaceae). *South African Journal of Botany*, **73**, 623–631.
- Rijkers T., Pons T.L., Bongers F. (2000) The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. *Functional Ecology*, **14**, 77–86.
- Rubinigg M., Posthumus F., Ferschke M., Elzenga J.T.M., Stulen I. (2003) Effects of NaCl salinity on ^{15}N -nitrate fluxes and specific root length in the halophyte *Plantago maritima* L. *Plant and Soil*, **250**, 201–213.
- Ryser P. (1998) Intra- and interspecific variation in root length, root turnover and the underlying parameters. In: Lambers H., Poorter H., Van Vuuren M.M.I. (Eds), *Inherent variation in plant growth physiological mechanisms and ecological consequences*. Backhuys, Leiden, the Netherlands, pp 441–465.
- Ryser P., Lambers H. (1995) Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. *Plant and Soil*, **170**, 251–265.
- Sanchez D.H., Redestig H., Krämer U., Udvardi M.K., Kopka J. (2008) Metabolome-ionome-biomass interactions. *Plant Signaling & Behavior*, **3**, 1–3.
- Sanchez D.H., Szymanski J., Erban A. (2010) Mining for robust transcriptional and metabolic responses to long-term salt stress: a case study on the model legume *Lotus japonicus*. *Plant, Cell and Environment*, **33**, 468–480.
- Sanchez D.H., Pieckenstain F.L., Escaray F.J., Erban A., Kraemer U., Udvardi M.K., Kopka J. (2011a) Comparative ionomics and metabolomics in extremophile and glycophytic *Lotus* species under salt stress challenge the metabolic pre-adaptation hypothesis. *Plant, Cell and Environment*, **34**, 605–617.
- Sanchez D.H., Pieckenstain F.L., Szymanski J., Erban A., Bromke M., Hannah M.A., Kraemer U., Kopka J., Udvardi M.K. (2011b) Comparative functional genomics of salt stress in related model and cultivated plants identifies and overcomes limitations to translational genomics. *PLoS One*, **6**, e17094.
- Sánchez-Blanco M.J., Morales M.A., Torrecillas A., Alarcón J.J. (1998) Diurnal and seasonal osmotic potential changes in *Lotus creticus* plants grown under saline stress. *Plant Science*, **136**, 1–10.
- Sannazzaro A.I., Ruiz O.A., Albertó E.O., Menéndez A.B. (2006) Alleviation of salt stress in *Lotus glaber* by *Glomus intraradices*. *Plant and Soil*, **285**, 279–287.
- Sannazzaro A.I., Echeverría M., Albertó E.O., Ruiz O.A., Menéndez A.B. (2007) Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza. *Plant Physiology and Biochemistry*, **45**, 39–46.
- Shennan C., Hunt R., Macrobbe E.A.C. (1987) Salt tolerance in *Aster tripolium* L. I. The effect of salinity on growth. *Plant, Cell and Environment*, **10**, 59–65.
- Somaroo B.H., Grant W.F. (1971) Interspecific hybridization between diploid species of *Lotus* (Leguminosae). *Genetica*, **42**, 353–367.
- Stevanato P., Gui G., Cacco G., Biancardi E., Abenavoli M.R., Romano A., Sorgonà A. (2013) Morpho-physiological traits of sugar beet exposed to salt stress. *International Sugar Journal*, **115**, 756–765.
- Strogonov B.P. (1969) *Physiological basis of salt tolerance of plants* (Translation). Akademi nauk, Moscow, SSSR: pp 279.
- Sz.-Borsos O., Somaroo B.H., Grant W.F. (1972) A new diploid species of *Lotus* (Leguminosae) in Pakistan. *Canadian Journal of Botany*, **50**, 1865–1870.
- Valluru R., Link J., Claupein W. (2012) Consequences of early chilling stress in two *Triticum* species: plastic responses and adaptive significance. *Plant Biology*, **14**, 641–651.
- Van Kleunen M., Fischer M. (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist*, **166**, 49–60.
- Villar R., Merino J. (2001) Comparison of leaf construction costs in woody species with differing leaf life-spans in contrasting ecosystems. *New Phytologist*, **151**, 213–226.
- Wade M.J., Kalisz S. (1990) The Causes of Natural Selection. *Evolution*, **44**, 1947–1955.
- Wang L., Mu M., Li X., Lin P., Wang W. (2010) Differentiation between true mangroves and mangrove associates based on leaf traits and salt contents. *Journal of Plant Ecology*, **1**, 1–10.
- Weiner J. (2004) Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **6**, 207–215.
- Wright I.J., Reich P.B., Westoby M. (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology*, **15**, 423–434.
- Xu G., Yu D., Xie J., Tang L., Li Y. (2014) What makes *Haloxylon persicum* grow on sand dunes while *H. ammodendron* grows on interdune lowlands: a proof from reciprocal transplant experiments. *Journal of Arid Land*, **6**, 581–591.
- Zapata J.M.C., Nieves M., Cerdá A. (2003) Improvement in growth and salt resistance of lemon (*Citrus limon*) trees by an interstock-induced mechanism. *Tree Physiology*, **23**, 879–888.
- Zapata J.M.C., Cerdá A., Nieves M. (2004) Interstock-induced mechanism of increased growth and salt resistance of orange (*Citrus sinensis*) trees. *Tree Physiology*, **24**, 1109–1117.
- Zhu J. (2001) Plant salt tolerance. *Trends in Plant Science*, **6**, 66–71.