

Coastal plant species as electrolytophytes: effect of NaCl and light intensity on accumulation characteristics of *Atriplex glabriuscula* from coastal drift lines

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Abstract

The aim of the present study was to analyze growth response and ion accumulation characteristics in *Atriplex glabriuscula*, a species from coastal drift lines, as affected by increased concentration of NaCl in substrate and light intensity in controlled conditions. In the first experiment, different regimes and concentration of NaCl were used for treatment of *A. glabriuscula* plants, resulting in a Na⁺ concentration gradient in substrate (0.88 to 11.9 g kg⁻¹). In the second experiment, plants were subjected to different light intensity (25, 50, 75 and 100%), and half of the plants in each light treatment were constantly irrigated with 200 mM NaCl. Na⁺-treated *A. glabriuscula* plants showed a concentration-independent significant increase in shoot fresh and dry mass, but root mass was not significantly affected. The characteristic increase of Na⁺ concentration in shoots due to increasing substrate Na⁺ corresponded to a relationship typical for Na⁺-accumulating species. In the second experiment, dry mass of leaves and stems of control plants linearly increased with increasing light intensity, root mass increased relatively less, but a more pronounced increase was evident for flowers. Increasing light intensity stimulated NaCl-dependent Na⁺ accumulation in leaves, but decreased accumulation in stems and flowers. Absolute values of electrical conductivity in shoots were extremely high, with the maximum level of 520 mS cm⁻¹ in stems of plants at 25% light intensity. In *A. glabriuscula* plants, tissue electrical conductivity is tightly regulated by accumulation/exclusion of both Na⁺ and K⁺, confirming a special status of the species as an extreme coastal electrolytophyte.

Key words: *Atriplex glabriuscula*, coastal habitats, electrolyte accumulation, K⁺, Na⁺, salinity.

Abbreviations: DM, dry matter; EC, electrical conductivity.

Introduction

Understanding of salinity tolerance mechanisms of plants adapted to natural salt-affected habitats is of large theoretical and practical importance. Firstly, new physiological traits related to salt tolerance need to be identified in wild plants with an aim to further introduce them in crop species through breeding programmes (Shabala 2013). Secondly, many wild salt-tolerant species are potential crop species for food or biomass production on salt-affected soils (Geissler et al. 2014) or can be used for reclamation of degraded lands (Suaire et al. 2016).

Annual vegetation on drift lines (EUH code 1210) is of major interest for functional studies of salt-tolerant wild plant species, because of their relative rarity and both floristic and environmental significance of this protected habitat. Spatial segregation and temporal instability of drift line communities in nature make them especially vulnerable and difficult to assess experimentally. The total area of this habitat in Latvia is only 26 ha, and it is the only place of existence for several rare plant species of special protection

status (i.e., *Atriplex glabriuscula*, *Atriplex longipes*, *Atriplex prostrata* subsp. *calotheca*; Laime 2013).

Many well-studied *Atriplex* species are characterized as xero-halophytes, being adapted also to semi-arid conditions and heavy metals in addition to salinity (Manousaki, Kalogerakis 2009; Martínez-Fernández, Walker 2012). Several of them can grow both in coastal habitats as well as in inland dry areas, especially, in the Mediterranean region, but the majority of investigated species are components of desert vegetation (Mooney et al. 1997; Khan et al. 2000; Martinez et al. 2003; Qiu et al. 2014; Bendaly et al. 2016). It appears that less attention has been given to studies of salt tolerance of coastal *Atriplex* species of temperate zone.

High accumulation of inorganic ions, mainly Na⁺ and K⁺, has been suggested as one of osmotic adjustment-related salt-tolerance mechanisms of *Atriplex* species (Nedjimi 2014). However, a largely unexplored aspect of salinity tolerance is related to electrolyte accumulation in salt tolerant plant species. As K⁺ is not incorporated in any structures and acts as a main cationic species that maintains ionic strength necessary for enzyme activation

and protein synthesis (Leigh, Wyn Jones 1984), as well as because of chemical similarity between K^+ and Na^+ , it is logical to assume that both ions significantly contribute to electrolytical activity of the liquid cellular environment. In contrast to the common approach of estimation of Na^+ and K^+ concentration on a dry matter basis, it would be thoughtful to analyze concentration of these ions on tissue water basis, as tissue water content can differ between various plant parts and due to changes in substrate salinity (Benzarti et al. 2014). Only some studies so far have explored both aspects, including measurement of ion concentration also on tissue water basis (Silveira et al. 2009). Accumulating types of halophytes need to possess a general ability to tolerate high tissue electrolytical activity due to higher cytoplasmic tolerance or efficient accumulation of electrolytically active species in the vacuole, or both. During a field study, *Atriplex glabriuscula* plants from wet sandy beach driftlines on the coast of the Baltic Sea showed exceptional electrolyte accumulation ability, with leaf Na^+ and K^+ concentration reaching 96 and 50 g kg⁻¹ on dry matter (DM) basis, respectively (Ievinsh et al. 2019).

Plant adaptation to specific light conditions in general is known to affect plant abiotic stress responses, both through changes in photosynthetic rate and high light-induced production of reactive oxygen species (Gururani et al. 2015; Mullineaux et al. 2018). Interaction of light and salinity has been studied in the annual salt marsh species *Aster laurentianus*, but it appeared that high light had no additional adverse effect on plant growth together with salinity (Reynolds et al. 2001). In contrast, a synergistic negative effect of high light intensity and salinity on photosynthetic capacity was found for *Mesembryanthemum crystallinum* plants (Broetto et al. 2006). From the other side, the shade tolerant species *Hydrocotyle vulgaris* showed significant stimulation of both leaf and clonal growth by 100 mol L⁻¹ NaCl treatment in low light conditions, and by 25 mol L⁻¹ NaCl even in moderate light conditions, but salt treatment was strongly inhibitory in high light conditions (Samsone et al. 2020).

The aim of the present study was to analyze growth response and ion accumulation characteristics in *A. glabriuscula* plants from coastal drift lines in controlled conditions as affected by increased concentration of NaCl in substrate and light intensity.

Materials and methods

Several flowering individuals of *A. glabriuscula* were taken from a native coastal drift line habitat in the territory of Jūrmala city near the estuary of river Lielupe, Latvia. The plants were transplanted in 5 L plastic containers in a mixture of commercial garden soil (Biolan, Finland) and quartz sand (1:1, v/v) and kept in a greenhouse until seedlings had established sporadically from fallen seeds. Individual seedlings were transplanted to 200 mL plastic containers filled with the same substrate as previously.

Plants were cultivated in an experimental automated greenhouse (HortiMax, Netherlands) with supplemented light from Master SON-TPIA Green Power CG T 400 W (Philips, Netherlands) and Powerstar HQI-BT 400 W/D PRO (Osram, Germany) lamps (380 μmol m⁻² s⁻¹ at the plant level), 16 h photoperiod, day/night temperature 23/15 °C, relative air humidity 60 to 70%.

Two separate experiments were performed. In the first experiment, different regimes and concentration of NaCl were used for treatment of *A. glabriuscula* plants, resulting in increasing concentration of Na^+ in a substrate, reaching from 0.88 g kg⁻¹ Na^+ up to 11.9 g kg⁻¹ Na^+ in the highest treatment (Table 1). Fertilization was performed once a week with a Kristalon Green Label fertilizer (NPK 18-18-18 with micronutrients; Yara International, Norway) solubilized in deionized water (150 g L⁻¹), with 5 mL of stock solution per L, 50 mL of the final fertilizer per container. Four weeks after the start of the treatment, the experiment was terminated, plants were separated in roots and shoots, weighed and dried in a thermostat at 60 °C until a constant mass, and then dry mass was measured. Final substrate electrical conductivity (EC) and Na^+ concentration was measured in soil water extracts, using 50 mL H₂O g⁻¹ dry soil by LAQUAtwin compact meters (Horiba Scientific, Japan). Dry plant material was used for analysis of EC and Na^+ and K^+ concentration. Tissue water content was calculated as g H₂O per g dry mass.

In the second experiment, *A. glabriuscula* plants were subjected to different light intensity by placing containers with plants in larger black plastic containers of various height, resulting in approximately of 25, 50, 75 and 100% light intensity from maximum. Half of the plants in each light treatment were continuously irrigated with 200 mM NaCl instead of deionized water. Five individual plants per treatment were used. Fertilization was performed as in the first experiment. Six weeks after the start of the treatment, the experiment was terminated and plants were separated in roots, leaves, stems, and flowers (including fruit and bracteoles). All parts were dried in a thermostat at 60 °C until a constant mass, then dry mass was measured. Dry plant material was used for analysis of EC and Na^+ and K^+ concentration.

For analysis, tissues were homogenized by crushing to small pieces and a sample (0.2 g) was taken for analysis of electrical conductivity (EC), Na^+ concentration and K^+ concentration in water extract by LAQUAtwin compact meters as described previously (Samsone, Ievinsh 2018). Ion analysis was performed separately for each individual plant. The transfer factor of Na^+ was calculated as a ratio between concentration of Na^+ in roots and that in shoots. The bioconcentration factor was calculated as a ratio between Na^+ concentration in substrate and that in shoots.

Statistical significance of differences between treatments was evaluated by a t test using Prism (GraphPad Software, USA).

Table 1. Final soil electrical conductivity and Na⁺ concentration in different treatments for *Atriplex glabriuscula* plants. Values are means \pm SE from the same number of soil samples as that for individual plants. Means with identical letters are not statistically significantly different ($P < 0.05$)

Treatment No.	Soil extract electrical conductivity on DM basis (mS cm ⁻¹)	Soil Na ⁺ concentration (g kg ⁻¹ DM)	Apparent soil salinity (mmol kg ⁻¹)	Number of plants
1	3.8 \pm 0.2a	0.84 \pm 0.03a	37	11
2	6.8 \pm 0.6b	1.51 \pm 0.11b	66	5
3	10.3 \pm 0.6c	2.39 \pm 0.09c	104	3
4	13.9 \pm 0.4d	3.26 \pm 0.06d	142	8
5	16.4 \pm 1.1e	4.01 \pm 0.09e	174	5
6	19.2 \pm 0.6f	4.72 \pm 0.06f	205	9
7	23.9 \pm 1.0g	5.38 \pm 0.05g	234	4
8	24.8 \pm 0.8g	5.92 \pm 0.08h	257	6
9	28.5 \pm 0.8h	7.00 \pm 0.16i	304	4
10	30.1 \pm 1.9h	7.77 \pm 0.07j	338	3
11	31.9 \pm 2.1h	8.50 \pm 0.10k	370	2
12	39.8 \pm 1.7i	11.90 \pm 0.20l	517	2

Results

Experiment 1: effect of different substrate Na⁺

Shoot fresh mass of *A. glabriuscula* plants tended to increase near linearly with increasing soil Na⁺ concentration up to 4.01 g kg⁻¹, but the first statistically significant difference was at 2.39 g kg⁻¹ Na⁺ (Fig. 1A). Shoot fresh mass tended to decrease with further increase in soil salinity, but it was still significantly higher (up to 7.00 g Na⁺ kg⁻¹) than that in control. At the highest soil salinity levels, fresh mass of plants did not differ significantly from that in control plants. A similar character of changes in dependence on soil salinity was evident for shoot dry mass (Fig. 1B). However, both fresh and dry mass of roots changed very little in respect to soil salinity (Fig. 1A, B). Only for plants cultivated in a Na⁺ concentration range from 5.92 to 7.77 g kg⁻¹ root fresh mass was significantly lower than that in control plants.

Leaf water content did not change much with increasing

soil salinity, probably due to relatively large variation between individual plants (Fig. 1C). There was a tendency to have higher water content in a treatment range from 3.26 to 8.50 g Na kg⁻¹, but significant effect was found only for plants grown at 4.72 g Na kg⁻¹.

Na⁺ concentration on a dry matter basis in shoots of *A. glabriuscula* was relatively high already for plants grown in substrate with no added NaCl (0.84 g kg⁻¹ Na), but it further significantly increased with increase in soil Na⁺ concentration, reaching maximum value at 7.77 g Na⁺ kg⁻¹ (Fig. 2A). When molar concentration of Na⁺ was expressed on a tissue water basis, there was a pronounced significant increase of the concentration in plants from 0.84 to 1.51 g kg⁻¹ Na⁺ treatment, but a more variable trend was evident with further increase in soil salinity (Fig. 2B). In contrast, root Na⁺ concentration both on dry mass and tissue water basis was only negligibly affected by increase in soil Na⁺, with a tendency to somewhat increase, but with statistically significant effect only in some treatments (Fig. 2).

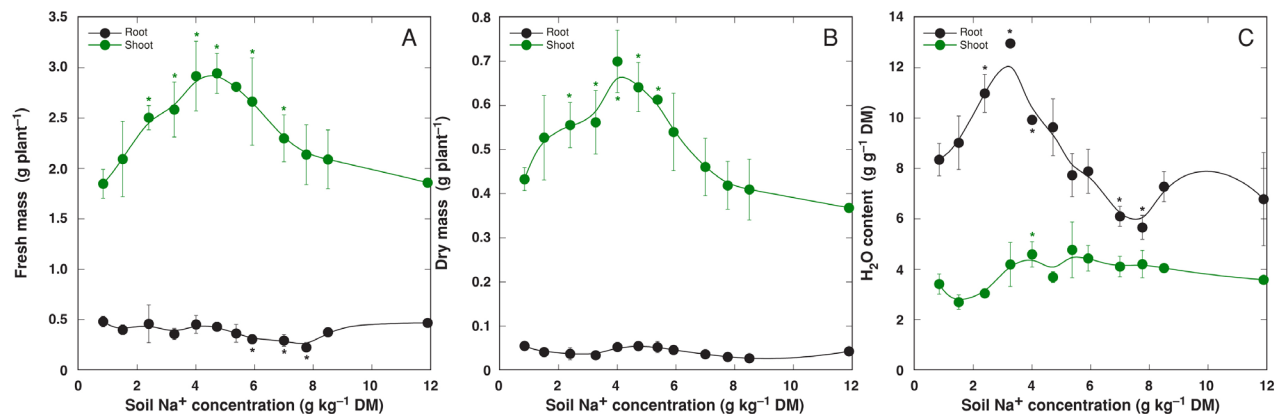


Fig. 1. Effect of increasing substrate Na⁺ concentration on fresh mass (A), dry mass (B) and H₂O content of shoots and roots of *Atriplex glabriuscula* plants. Asterisks indicate statistically significant difference from the respective control ($P < 0.05$).

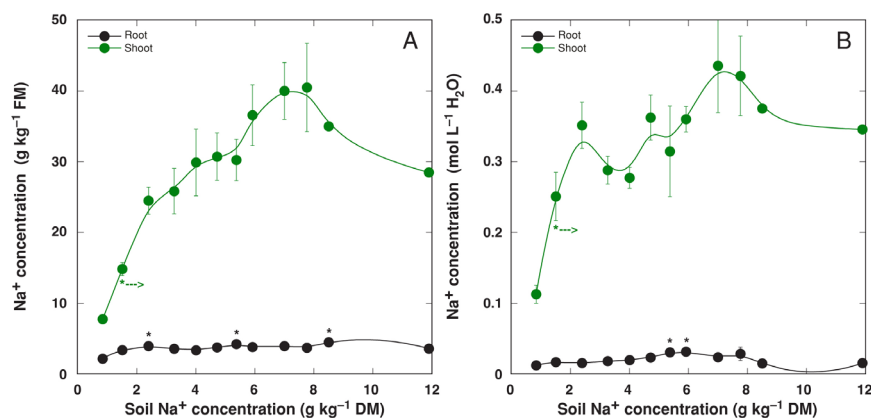


Fig. 2. Effect of increasing substrate Na⁺ concentration on Na⁺ concentration on dry mass (A) and cellular water (B) basis in shoots and roots of *Atriplex glabriuscula* plants. Asterisks indicate statistically significant difference from the respective control ($P < 0.05$).

Increased soil Na⁺ concentration resulted in significant decrease of shoot K⁺ concentration irrespective of the soil salinity level both on a dry mass (Fig. 3A) and tissue water (Fig. 3B) basis. In roots, K⁺ concentration on dry mass basis also tended to decrease in saline substrate, but a statistically significant effect was evident only for plants in separate treatments (Fig. 3A).

Electrical conductivity, measured in plant extracts and expressed on a dry mass basis, showed statistically significant increase in *A. glabriuscula* plants from 1.51 to 2.39 g Na⁺ kg⁻¹ and remained largely unchanged with further increase in soil salinity, but with some tendency to increase from a middle to high range of soil salinity, followed by significant decrease at 11.9 g Na⁺ kg⁻¹ (Fig. 4A). The parameter increased also in roots by added NaCl, irrespective of the particular soil salinity level. In contrast, electrical conductivity expressed on a tissue water basis showed relatively high variability and some tendency to increase, but statistically significant increase was evident only for plants cultivated in substrate with 2.39 g Na⁺ kg⁻¹ (Fig. 4B). There was no statistically significant effect of soil salinity on electrical conductivity in plant roots on a tissue water basis.

Shoots and roots of control plants differed in the K⁺/Na⁺ molar concentration ratio (2.7 vs 1.5, respectively), but the ratio was similar with addition of NaCl to substrate and did not change significantly with increase in substrate salinity (Fig. 5A). An ability to concentrate Na⁺ in shoots and roots in relation to soil Na⁺ concentration was evaluated by means of the bioconcentration factor, which was significantly higher for shoots, but gradually decreased in both parts with increase in substrate salinity (Fig. 5B). The relationship between shoot and root Na⁺ accumulation ability depending on soil salinity was evaluated by the Na⁺ transfer factor (Fig. 5C). When calculated on a dry mass basis, the transfer factor increased with increasing soil Na⁺ concentration up to intermediate levels, and then further remained nearly constant. The transfer factor on a tissue water basis showed relatively large variability with a tendency to remain higher than that in control plants.

When tissue electrical conductivity was correlated with ion concentration on a dry mass basis, it showed a relatively tight degree of correlation with Na⁺ concentration in shoots (Fig. 6A), but in roots correlation was moderate both for Na⁺ and K⁺ (Fig. 6B). A similarly tight correlation was evident between electrical conductivity on a tissue water basis and

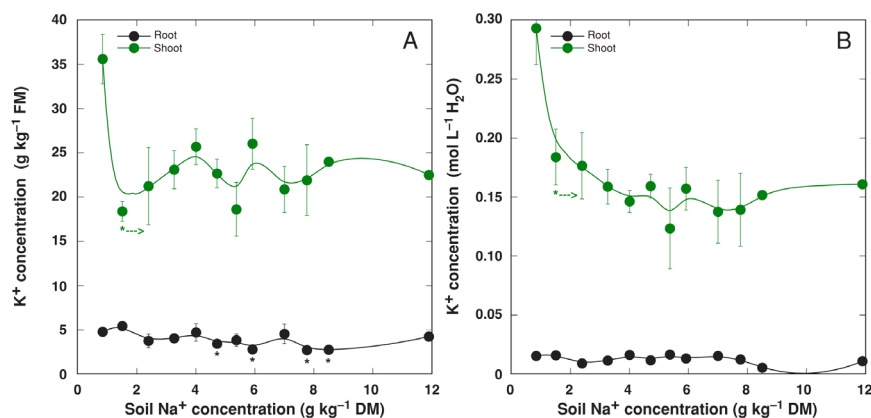


Fig. 3. Effect of increasing substrate Na⁺ concentration on K⁺ concentration on dry mass (A) and tissue water (B) basis in shoots and roots of *Atriplex glabriuscula* plants. Asterisks indicate statistically significant difference from the respective control ($P < 0.05$).

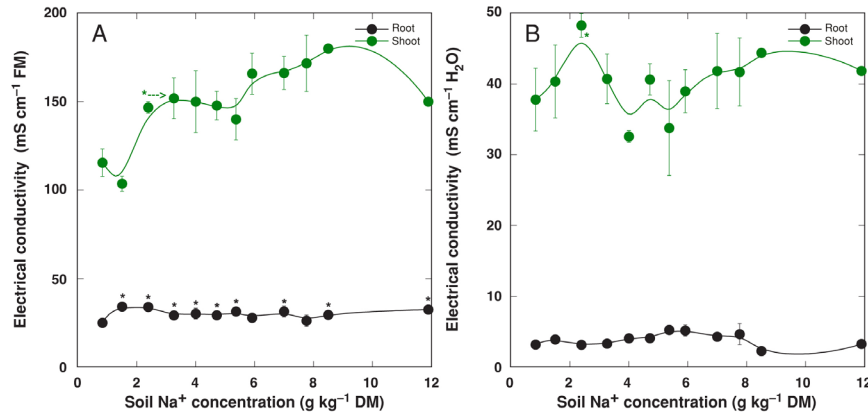


Fig. 4. Effect of increasing substrate Na^+ concentration on tissue electrical conductivity on dry mass (A) and cellular water (B) basis in shoots and roots of *Atriplex glabriuscula* plants. Asterisks indicate statistically significant difference from the respective control ($P < 0.05$).

summed molar concentration of $\text{Na}^+ + \text{K}^+$ in shoots (Fig. 6C) and a very tight correlation between these parameters was evident in roots (Fig. 6D).

Experiment 2: effect of different light and Na^+

Decrease in light intensity during cultivation had dramatic inhibitory effect on growth of *A. glabriuscula* plants (Fig. 7). At 25% light intensity, dry mass of control plant leaves, stems, flowers and roots was only 14, 14, 1, and 13%, respectively, from these in plants grown at 100% light intensity. The comparable degree of inhibitory effect of low light on growth was evident also in NaCl-treated plants. Dry mass of all plant parts gradually increased with increase in light intensity, but the most pronounced effect was seen for growth of generative structures. Stem and leaf growth was significantly stimulated by NaCl treatment only at full light intensity, with 37 and 36% increases over the control, respectively. Root growth was significantly stimulated by NaCl at both highest light intensities (57 and 46% increase over the control).

In control plants, increase in light intensity resulted in significantly increased accumulation of Na^+ only for leaves,

but it somewhat decreased for flowers and roots (Fig. 8A). Similarly, increase in light intensity resulted in increased accumulation of Na^+ also in leaves of NaCl-treated plants, with some decrease in stems and flowers. For K^+ , there was a pronounced decrease of the ion concentration with increasing light intensity in all aboveground plant parts (Fig. 8B). NaCl treatment significantly decreased K^+ concentration in all parts, and the inhibitory effect was higher at low light intensity. As a result of changes in concentration of individual ions, the summed molar concentration of Na^+ and K^+ was similar between leaves and stems of control plants (Fig. 8C).

In NaCl-treated plants, the summed concentration increased in stems, but only at 75 and 100% light intensity also in leaves. Changes in tissue electrical conductivity as a result of changes in light intensity and NaCl treatment followed the same trend as observed for summed Na^+ and K^+ concentration (Fig. 9A). As expected, the summed molar concentration of Na^+ and K^+ showed extremely tight correlation with tissue electrical conductivity both for control and NaCl-treated plants (Fig. 10). Ratio of molar K^+/Na^+ concentration in control plants significantly varied

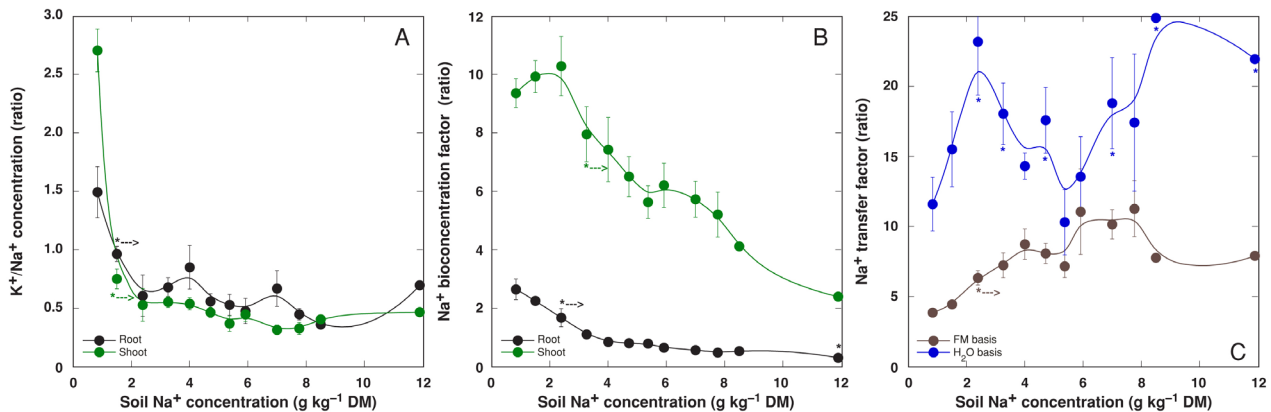


Fig. 5. Effect of increasing substrate Na^+ concentration on K^+/Na^+ concentration ratio (A) and Na^+ bioconcentration factor (B) in shoots and roots, and Na^+ transfer factor (C) on DM and tissue water basis in *Atriplex glabriuscula* plants. Asterisks indicate statistically significant difference from the respective control ($P < 0.05$).

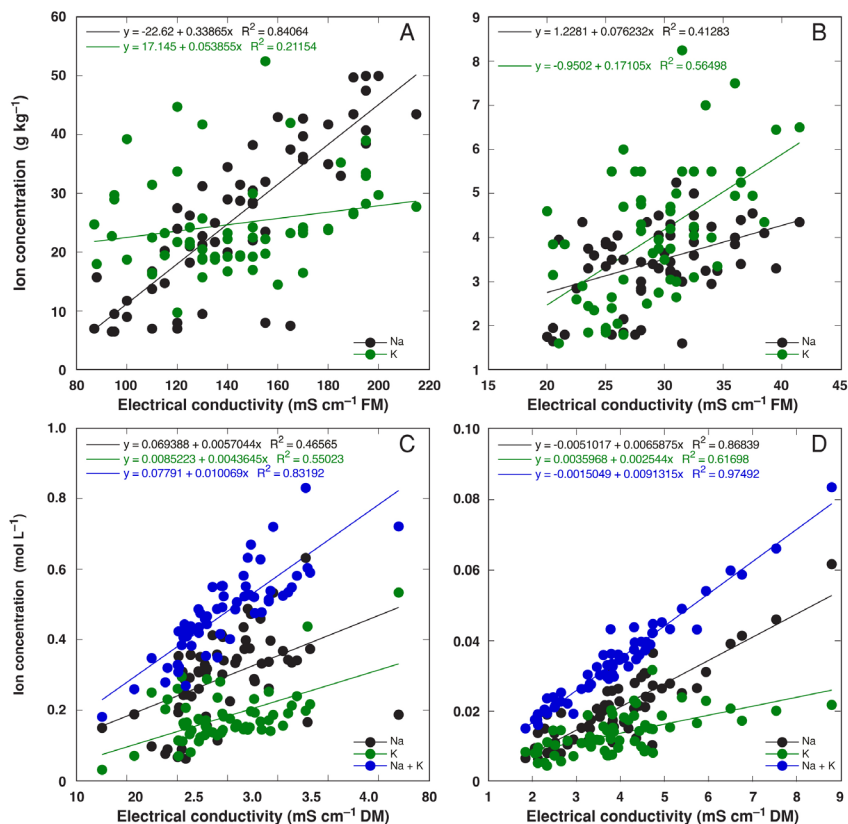


Fig. 6. Relationship between plant tissue electrical conductivity and Na⁺ and K⁺ concentration on dry mass basis in shoots (A) and roots (B), and tissue electrical conductivity and molar Na⁺, K⁺ and Na⁺ + K⁺ concentration on tissue water basis in shoots (C) and roots (D) of *Atriplex glabriuscula* plants cultivated at different substrate salinity.

between plant parts and also changed in response to light intensity (Fig. 9B). In all parts of NaCl-treated plants, the K⁺/Na⁺ ratio was below 1, but it was the highest in generative structures, followed by stems, leaves and roots. Differences in the K⁺/Na⁺ ratio due to changes in light intensity were not statistically significant.

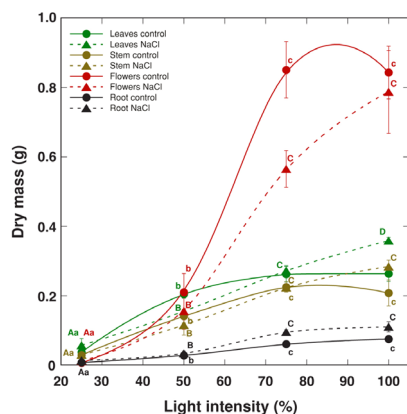


Fig. 7. Effect of light intensity and NaCl treatment on dry mass of different parts of *Atriplex glabriuscula* plants. Means with identical letters are not statistically significantly different ($P < 0.05$).

Discussion

Species of the genus *Atriplex* are among a limited number of salt tolerant plants showing significant increase of shoot growth at moderate salinity, in addition to more or less pronounced biomass accumulation tolerance at high salinity. Thus, maximum growth rate of *Atriplex glabriuscula* in hydroponics was found at 50 mmol L⁻¹ NaCl, with average stimulation of dry matter accumulation by 40%, but growth was completely inhibited at 600 mmol L⁻¹ NaCl (Lee, Ignaciuk 1985). Similarly, for *Atriplex portulacoides*, leaf growth was stimulated by 200 mol L⁻¹ NaCl by about 68%, but it was inhibited by about 70% at 400 mmol L⁻¹ NaCl (Redondo-Gómez et al. 2007). High salinity tolerance was reported for the xerohalophyte *Atriplex halimus*, with optimum growth at 100 mmol L⁻¹ NaCl and growth inhibition at 400 mol L⁻¹ only by about 25% (Bendaly et al. 2016). Exceptional salinity tolerance was found in *Antriplex centralasiatica*, with small but statistically significant growth stimulation even at 400 mol L⁻¹ NaCl (Qiu et al. 2014).

In the present study, dry matter accumulation for *A. glabriuscula* plants increased by 67% with increase of Na⁺ from 0.84 to 4.01 g L⁻¹ (Fig. 1A), and there was no growth reduction even at the highest soil Na⁺ concentration (11.9 g L⁻¹ or 517 mmol L⁻¹). It is interesting that there was no

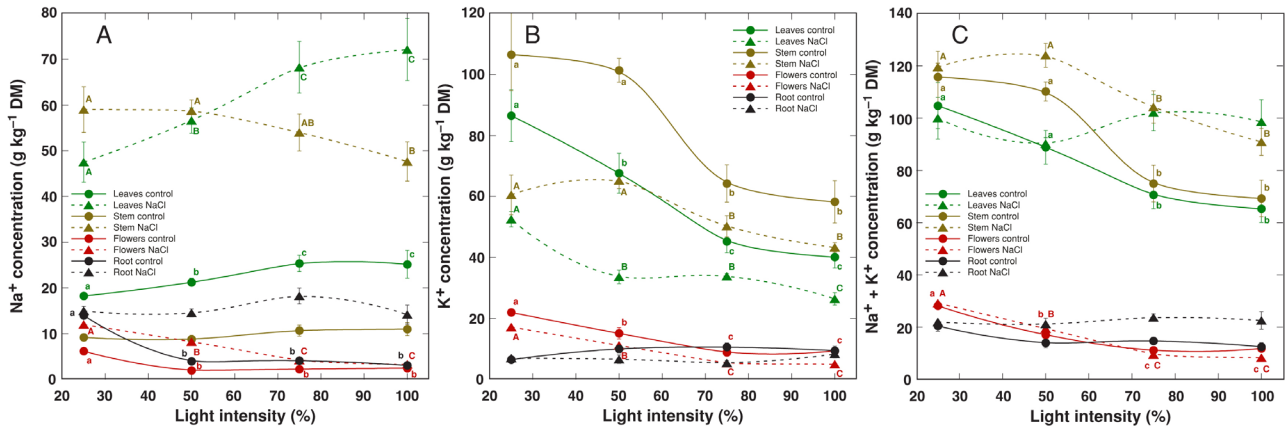


Fig. 8. Effect of light intensity and NaCl treatment on Na^+ (A), K^+ (B) and $\text{Na}^+ + \text{K}^+$ concentration in different parts of *Atriplex glabriuscula* plants. Means with identical letters are not statistically significantly different ($P < 0.05$).

stimulation of water content in leaf tissues by increasing salinity, but root water content significantly increased by treatment up to $3.26 \text{ g Na}^+ \text{ kg}^{-1}$ (Fig. 1C). However, salinity-induced increase in leaf water content has often been reported for experiments with various *Atriplex* species (Benzarti et al. 2014; Bendaly et al. 2016), but reduction of leaf water content by salinity has also been shown, especially, at plant maturity stage (Khan et al. 2000). In contrast to the above, *Atriplex prostrata*, a facultative halophyte, showed decreased growth with increasing salinity, in parallel to accumulation of Na^+ and decrease in K^+ concentration both in leaves and stems (Wang et al. 1997). Therefore, it was suggested that growth inhibition is associated with lower K^+ levels in growing tissues.

The accumulation potential of *A. glabriuscula* for Na^+ on a dry mass basis found in the present study was comparable to that reported for other *Atriplex* species, typically reaching 70 g kg^{-1} or 3 mol kg^{-1} in leaves (Redondo-Gómez et al. 2007; Masters et al. 2009; Bendaly et al. 2016), but also higher values have been reported, such as 6 mol kg^{-1} in leaves of *Atriplex portulacoides* (Benzarti et al. 2014). Several accessions of *Atriplex canescens* were

able to accumulate 2 mol kg^{-1} Na^+ in leaves of control plants, growing in substrate with no added NaCl, further increasing up to 5 mol kg^{-1} under 1 mol L^{-1} rootzone salinity (Glenn et al. 1996). However, there is only a limited number of studies available on salinity tolerance with *Atriplex* species reporting ion concentration estimated on a tissue water basis. In a study with *Atriplex nummularia*, native to Australian deserts, Na^+ accumulated up to 0.8 mol L^{-1} in leaves and 0.1 mol L^{-1} in roots (Silveira et al. 2009). In the present study, only 0.44 mol L^{-1} was reached in leaves, with root Na^+ concentration being only up to 0.03 mol L^{-1} (Fig. 2B).

Cellular compartmentation of ions under salinity has been rarely estimated in *Atriplex* species, but limited evidence suggests that vacuoles are the main site for accumulation of both Na^+ and Cl^- , as vacuoles isolated from leaves of *Atriplex gmelinii* contained 569 mmol L^{-1} Na^+ , in comparison to 582 mmol L^{-1} found in whole protoplasts and 500 mmol L^{-1} in leaf tissue water (Matoh et al. 1987). In addition, many *Atriplex* species have been shown to have vesiculated hairs on the leaf surface, acting as extreme sinks of salts (Breckle et al. 1990). Presence of

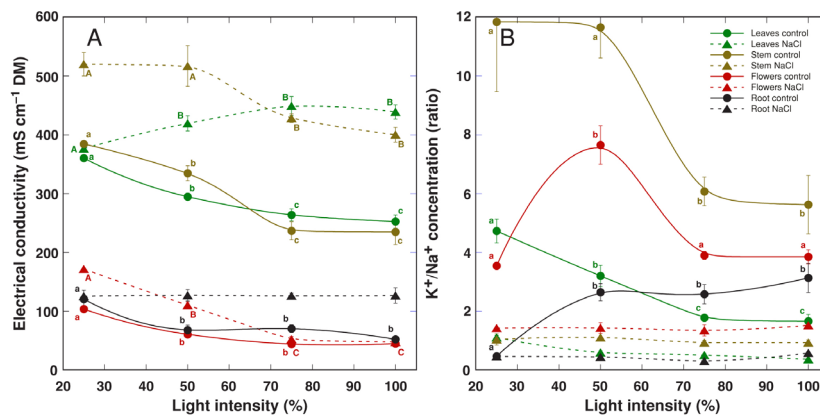


Fig. 9. Effect of light intensity and NaCl treatment on tissue electrical conductivity (A) and K^+/Na^+ molar concentration ratio (B) in different parts of *Atriplex glabriuscula* plants. Means with identical letters are not statistically significantly different ($P < 0.05$).

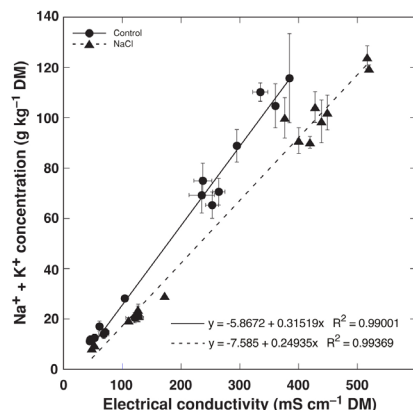


Fig. 10. Relationship between plant tissue electrical conductivity and molar summed Na^+ and K^+ concentration on dry mass basis in shoots and roots of *Atriplex glabriuscula* plants cultivated at different light intensity.

numerous trichomes on the surface of leaves and ability to form new layers of hair underneath after bursting and depositing salts on leaf surface represent a salt tolerance mechanism through avoidance, as described for *Atriplex halimus* (Mozafar, Goodin 1970).

Preferential accumulation of Na^+ in shoot parts over roots was a characteristic feature of *A. glabriuscula*, with a root-to-shoot transfer factor up to 10 (Fig. 5C). A similar trend was noted also for leaves of *A. portulacoides* (Benzarti et al. 2014), but woody stem tissues of this species can have even lower Na^+ concentration than that in roots (Redondo-Gómez et al. 2007). However, some *Atriplex* species were clear Na^+ excluders, accumulating majority of Na in root tissues, as an accession of *Atriplex halimus* (Belkheiri, Mulas 2013).

Decrease of shoot K^+ concentration due to increased substrate NaCl seems to be a characteristic response of *Atriplex* species. In *Atriplex portulacoides* from a coastal salt marsh grown in hydroponics, leaf K^+ concentration decreased from $2.0 \text{ mol kg}^{-1} \text{ DM}$ in control conditions to 1.6 mol kg^{-1} in plants treated with $0.02 \text{ mol L}^{-1} \text{ NaCl}$ (20% decrease) and remained relatively constant with further increase in salinity (Redondo-Gómez et al. 2007). In the present study, K^+ concentration in shoots decreased from 36 g kg^{-1} (0.9 mol kg^{-1}) by 30 to 45% at increased substrate salinity $\geq 66 \text{ mmol kg}^{-1}$ (Fig. 3A). It seems that this clearly reflects an ability of certain *Atriplex* species to maintain a stable cellular K^+ level, irrespective of fluctuating substrate salinity (Ramos et al. 2004).

It has been shown that in some xerophytic *Atriplex* species, shoot K^+ concentration continuously decreases with increasing NaCl concentration (Khan et al. 2000; Wang et al. 1997; Nedjimi 2014; Bendaly et al. 2016). It appears that in many *Atriplex* species, with characteristic general prevalence for inorganic adjustment of osmotic balance, Na^+ ions contribute to osmotic adjustment more

efficiently in comparison to K^+ ions (Ramos et al. 2004; Pan et al. 2016). For xerohalophytic *Atriplex* species, both ions participate in osmotic adjustment, with K^+ increasing in low moisture-induced osmotic stress, but being replaced by Na^+ during salinity episodes (Khan et al. 2000). There is also contradicting evidence available from studies with *Atriplex halimus*, showing that both Na^+ and K^+ concentration increased at a similar rate in leaves of drought-stressed plants (Martinez et al. 2003). In a desert accession of *Atriplex canescens*, plant leaves accumulated high concentration of both Na^+ (3 mol kg^{-1}) and K^+ (2 mol kg^{-1}), and there was no significant decrease in K^+ concentration at $400 \text{ mol L}^{-1} \text{ NaCl}$ (Pan et al. 2016).

It is evident that sometimes contradicting effects have been described regarding ion accumulation characteristics even for the same model species of the genus. One possible explanation could be related to high genetic variation of *Atriplex*. Indeed, existence of high genetic diversity with several varieties and numerous ecotypes is known for some species. In *Atriplex canescens* a link has been established between genotype, its habitat preference, including salt tolerance characteristics, and particular morphophysiological traits (Glenn et al. 1996).

Effects of light have not been frequently assessed in studies with *Atriplex*. *Atriplex* species from desert areas are well adapted to high light conditions mostly because of high reflectance properties of aerial plant parts (Mooney et al. 1977). Considering the effect of light on growth and salinity responses of *A. glabriuscula* plants, it needs to be stressed that competition for light is suggested to be an important factor affecting plant distribution in coastal salt marshes (Ungar 1998). Drift line habitats have not been assessed in this respect, but it is logical to propose that especially juvenile plants in densely overgrown drift line vegetation could exhibit low light conditions for a prolonged period of time. It appears that low light conditions not only strongly inhibited vegetative and generative growth of *A. glabriuscula*, but also did not allow for salt-dependent growth increase during episodes of high salinity.

For *Hydrocotyle vulgaris*, growth stimulation by NaCl in low light conditions was not related to the degree of low light-induced growth inhibition, indicating that different regulation mechanisms for these two light-dependent effects exist (Samsone et al. 2020). For *A. glabriuscula*, NaCl-dependent stimulation of vegetative growth was evident only at full light intensity (Fig. 7), but Na^+ accumulation in leaves significantly increased from low (25%) to moderate (75%) light intensity, independent of the substrate NaCl level (Fig. 8A). This might be related to the fact that transport of salt ions to epidermal salt bladder cells in *Atriplex* is a light-dependent process (Lüttge 1971), but decreased light intensity usually causes reduction of salt hair density (Karimi, Ungar 1989).

In contrast to other parameters, K^+ concentration significantly decreased in all aboveground plant parts with

increasing light intensity (Fig. 8B). It appears that also for *A. glabriuscula* plants, several light intensity-dependent regulatory pathways could be involved in controlling salinity-induced morphological and biochemical responses. In addition, increased K^+ concentration in plant tissues due to younger age, their lower growth rate or slower development has been shown for a number of species, especially, in conditions of K^+ deficiency (Coïc, Lesaint 1971; Zörb et al. 2014).

In the context of the present study, both Na^+ and K^+ , usually regarded only as osmotically active ions, are primarily considered as electrolytically active species. Electrical conductivity measurements in studies aiming at understanding ion accumulation characteristics of salt-adapted plant species seems to be a promising complement to measurement of particular ion concentrations. While it is logical to assume that the amount of information from results from electrical conductivity measurements in plant extracts would increase when expressed on a tissue water basis, this was only partially fulfilled with *A. glabriuscula* in conditions of the present experiments. When tissue electrical conductivity in plants grown at different soil salinity was correlated to monovalent cation concentration, relationship between the parameters were only moderately tight when all parameters were expressed on a dry mass basis, especially for root tissues (Fig. 6A, B). As expected, tightness of correlation increased when electrical conductivity and ion concentrations were expressed on a tissue water basis (Fig. 6C, D). However, in respect to particular soil salinity values, while electrical conductivity on a dry mass basis showed significant increase at soil salinity $\geq 104 \text{ mmol kg}^{-1}$ (Fig. 4A), no significant differences between treatments were evident for this parameter expressed on a tissue water basis, mostly due to high variability between individual plants (Fig. 4B).

There are not much data available for comparison, but in a study with 105 wild plant species from salt-affected coastal habitats it was established that for leaf tissue electrical conductivity on a tissue water basis, an intermediate 50% value range was 24.0 to 37.7 mS cm^{-1} (Ievinsh, unpublished results). Consequently, *A. glabriuscula* in the present study showed electrolytical activity level in tissue water (40 mS cm^{-1} in average) corresponding to a highly electrolytophytic species, with high concentration of both Na^+ and K^+ as the main putative components of electrolytic activity. The results are consistent with previous studies showing that some salt-adapted *Atriplex* species have exceptional ability to accumulate both K^+ and Na^+ (Ramos et al. 2004). It is obvious that also other soluble ions in plant tissue extracts contribute to their electrical conductivity, and in the case of NaCl-treated plants, also Cl^- effects needs to be considered. However, there was no difference in control vs NaCl-treated *A. glabriuscula* plants in respect to correlation between tissue extract electrical conductivity and summed molar concentration of Na^+ and K^+ (Fig. 10), indicating that

putative accumulation of Cl^- in the treated plants had no apparent effect on tissue electrical conductivity.

In salt tolerance studies in controlled conditions, usually hydroponic cultivation systems with mineral nutrient solution are preferred due to the possibility to tightly control nutrient and salinity levels. However, these systems tend to be highly unrealistic in respect to plants growing natively in salt-affected soils. Therefore, in our studies, we tried to adopt a cultivation system for wild plant species using a soil-based substrate (Andersone-Ozola et al. 2017; Ievinsh et al. 2020). The main problem in the use of substrate-based systems seems to be control of optimal mineral nutrition regime and accurate and comparable dosing of the salt treatment. In contrast to hydroponic systems, which allow for full regular replacement of fertilizer solutions, simple irrigation in soil-based systems with a solution containing nutrients and other chemical factors of interest could lead to excessive or simply uncontrolled accumulation of certain elements. As an alternative, in the first experiment of the present study, groups of *A. glabriuscula* plants received NaCl treatment by different schemes, involving variation in both salt concentration and timing of treatment. Treatment groups for further analysis of measured parameters were selected on the basis of instrumental analysis of substrates for individual plants after termination of the experiment.

In conclusion, the results of the present study with the extremely salt tolerant electrolyte-accumulating species *A. glabriuscula* emphasized an important aspect of salinity tolerance related to tissue electrolyte tolerance, indicating both Na^+ and K^+ as major electrolytically active species. Additional comparative data from experiments using salt-adapted species from saline coastal habitats, including coastal drift lines, are necessary to generalize the relationship between species electrolyte accumulation capacity in shoot tissues at the level of both Na^+ and K^+ , and its ability to successfully grow and reproduce in highly saline conditions.

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References

- Andersone-Ozola U., Gaile L., Ievinsh G. 2017. Physiological responses of rare coastal salt marsh species *Triglochin maritima* L. to soil chemical heterogeneity. *Acta Biol. Univ. Daugavpiliensis* 17: 149–155.
- Belkheiri O., Mulas M. 2013. The effects of salt stress on growth, water relations and ion accumulation in two halophyte *Atriplex* species. *Environ. Exp. Bot.* 86: 17–28.
- Bendaly A., Messedi D., Smaoui A., Ksouri R., Bouchereau A., Abdelly C. 2016. Physiological and leaf metabolome changes in the xerohalophyte species *Atriplex halimus* induced by

- salinity. *Plant Physiol. Biochem.* 103: 208–218.
- Benzarti M., Rejeb K.B., Messedi D., Mna A.B., Hessini K., Ksontini M., Abdelly C., Debez A. 2014. Effect of high salinity on *Atriplex portulacoides*: Growth, leaf water relations and solute accumulation in relation with osmotic adjustment. *South Afr. J. Bot.* 95: 70–77.
- Broetto F., Duarte H.M., Lüttge U. 2006. Responses of chlorophyll fluorescence parameters of the facultative halophyte and C₃-CAM intermediate species *Mesembryanthemum crystallinum* to salinity and high irradiance stress. *J. Plant Physiol.* 164: 904–912.
- Geissler N., Lieth H., Koyro H.W. 2014. Cash crop halophytes: the ecologically and economically sustainable use of naturally salt-resistant plants in the context of global changes. In: Ahmad P., Wani M. (eds) *Physiological Mechanisms and Adaptation Strategies in Plants Under Changing Environment*. Springer, New York, NY, pp. 145–162.
- Glenn E., Pfister R., Brown J.J., Thompson T.L., O'Leary J. 1996. Na and K accumulation and salt tolerance of *Atriplex canescens* (Chenopodiaceae) genotypes. *Am. J. Bot.* 83: 997–1005.
- Gururani M.A., Venkatesh J., Phan Tran L.-S. 2015. Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Molec. Plant* 8: 1304–1320.
- Ievinsh G., Dišlere E., Karlsons A., Osvalde A., Vikmane M. 2020. Physiological responses of wetland species *Rumex hydrolapathum* to increased concentration of biogenous heavy metals Zn and Mn in substrate. *Proc. Latv. Acad. Sci. B* 74: 35–47.
- Ievinsh G., Ieviņa S., Samsone I., Andersone-Ozola U. 2019. Functional chemistry of coastal plant species: towards a definition of Na and K metallophytes (electrolytophytes). *Environ. Exp. Biol.* 17: 63–64.
- Karimi S.H., Ungar I.A. 1998. Development of epidermal salt hairs in *Atriplex triangularis* Willd. in response to salinity, light intensity, and aeration. *Bot. Gaz.* 150: 68–71.
- Khan M.A., Ungar I.A., Showalters A.M. 2000. Effects of salinity on growth, water relations and ion accumulation of subtropical halophyte, *Atriplex griffithii* var. *stooksii*. *Ann. Bot.* 85: 225–232.
- Laime B. 2013. 1210 Annual vegetation on drift lines. In: Auniņš A. (ed) *European Union Protected Habitats in Latvia. Interpretation Manual*. Riga, Latvian Fund for Nature, Ministry of Environmental Protection and Regional Development, 41–44 p.
- Lee J.A., Ignaciuk R. 1985. The physiological ecology of strandline plants. *Vegetatio* 62: 319–326.
- Leigh R.A., Wyn Jones R.G. 1984. A hypothesis relating critical potassium concentrations for growth to the distribution and function of this ion in the plant cell. *New Phytol.* 97: 1–13.
- Lüttge U. 1971. Structure and function of plant glands. *Annu Rev. Plant Physiol.* 22: 23–44.
- Manousaki E., Kalogerakis N. 2009. Phytoextraction of Pb and Cd by the Mediterranean saltbush (*Atriplex halimus* L.): metal uptake in relation to salinity. *Environ. Sci. Pollut. Res.* 16: 844–854.
- Martinez J.P., Ledent J.F., Bajji M., Kinet J.M., Lutts S. 2003. Effect of water stress on growth, Na and K accumulation and water use efficiency in relation to osmotic adjustment in two populations of *Atriplex halimus* L. *Plant Growth Regul.* 41: 63–73.
- Martínez-Fernández D., Walker D.J. 2012. The effects of soil amendments on the growth of *Atriplex halimus* and *Bituminaria bituminosa* in heavy metal-contaminated soils. *Water Air Soil Pollut.* 223: 63–72.
- Masters D., Tiong M., Norman H., Vercoe P.E. 2009. The mineral content of river saltbush (*Atriplex amnicola*) changes when sodium chloride in the irrigation solution is increased. In: Papachristou T.G., Parissi Z.M., Ben Salem H., Morand-Fehr P. (eds) *Nutritional and Foraging Ecology of Sheep and Goats*. CIHEAM/FAO/NAGREF, Zaragoza. Options Méditerranéennes: Série A. Séminaires Méditerranéens. No. 85, pp. 153–157.
- Matoh T., Watanabe J., Takahashi E. 1987. Sodium, potassium, chloride and betaine concentrations in isolated vacuoles from salt-grown *Atriplex gmelini* leaves. *Plant Physiol.* 84: 173–177.
- Mooney H.A., Ehleringer J., Björkman O. 1977. The energy balance of leaves of the evergreen desert shrub *Atriplex hymenelytra*. *Oecologia* 29: 301–310.
- Mozafar A., Goodin J.R. 1970. Vesiculated hairs: a mechanism for salt tolerance in *Atriplex halimus*. *Plant Physiol.* 45: 62–65.
- Mullineaux P.M., Exposito-Rodriguez M., Laissue P.P., Smirnov N. 2018. ROS-dependent signalling pathways in plants and algae exposed to high light: Comparisons with other eukaryotes. *Free Radic. Biol. Med.* 122: 52–64.
- Nedjimi B. 2014. Effects of salinity on growth, membrane permeability and root hydraulic conductivity in three saltbush species. *Biochem. Syst. Ecol.* 52: 4–13.
- Pan Y.-Q., Guo H., Wang S.-M., Zhao B., Zhang J.-L., Ma Q., Yin H.-J., Bao A.-K. 2016. The photosynthesis, Na⁺/K⁺ homeostasis and osmotic adjustment of *Atriplex canescens* in response to salinity. *Front. Plant Sci.* 7: 848.
- Qiu N., Zhou F., Wang Y., Peng X., Hua C. 2014. The strategy of Na⁺ compartmentation and growth of *Atriplex centralasiatica* in adaptation to saline environments. *Rus. J. Plant Physiol.* 61: 238–254.
- Ramos J., Lopez M.J., Benlloch M. 2004. Effect of NaCl and KCl salts on the growth and solute accumulation of the halophyte *Atriplex nummularia*. *Plant Soil* 259: 163–168.
- Redondo-Gómez S., Mateos-Naranjo E., Davy A.J., Fernández-Muñoz F., Castellanos E.M., Luque T., Figueroa M.E. 2007. Growth and photosynthetic responses to salinity of the salt-marsh shrub *Atriplex portulacoides*. *Ann. Bot.* 100: 555–563.
- Reynolds C.E., Houle G., Marquis C. 2001. Light and salinity affect growth of the salt marsh plant *Aster laurentianus*. *New Phytol.* 149: 441–448.
- Samsone I., Andersone-Ozola U., Karlsons A., Ievinsh G. 2020. Light conditions affect NaCl-induced physiological responses in a clonal plant species *Hydrocotyle vulgaris*. *Proc. Latv. Acad. Sci. B* 74: 00–00. /accepted paper, in press/
- Samsone I., Ievinsh G. 2018. Different plant species accumulate various concentration of Na⁺ in a sea-affected coastal wetland during a vegetation season. *Environ. Exp. Biol.* 16: 117–127.
- Shabala S. 2013. Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. *Ann. Bot.* 112: 1209–1221.
- Silveira J.A.G., Araújo S.A.M., Lima J.P.M.S., Viégas R.A. 2009. Roots and leaves display contrasting osmotic adjustment mechanisms in response to NaCl-salinity in *Atriplex nummularia*. *Environ. Exp. Bot.* 66: 1–8.
- Suaire R., Durickovic I., Framont-Terrasse L., Leblain J.-Y., De Rousk A.-C., Simonnot M.-O. 2016. Phytoextraction of Na⁺ and Cl⁻ by *Atriplex halimus* L. and *Atriplex hortensis*

- L.: A promising solution for remediation of road runoff contaminated with deicing salts. *Ecol. Eng.* 94: 182–189.
- Ungar I.A. 1998. Are biotic factors significant in influencing the distribution of halophytes in saline habitats? *Bot. Rev.* 64: 176–199.
- Wang L.-W., Showalter A.M., Ungar I.A. 1997. Effect of salinity on growth, ion content, and cell wall chemistry in *Atriplex prostrata* (Chenopodiaceae). *Am. J. Bot.* 84: 1247–1255.