PHYSIOLOGICAL RESPONSES OF RARE COASTAL SALT MARSH PLANT *TRIGLOCHIN MARITIMA* L. TO SOIL CHEMICAL HETEROGENEITY

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Triglochin maritima L. is a rare coastal marsh species. Field studies have revealed extreme spatial and temporal soil chemical heterogeneity in *T. maritima* sites, manifested by more than 30-fold concentration difference for several mineral elements, eventually causing significant mineral disbalance. The aim of the present study was to evaluate possible positive role of high substrate Ca concentration on alleviation of negative physiological impact of the presence of high (4 g kg^{-1}) Mg, Fe and Na concentration in the substrate. Plants were grown from seeds collected in nature in garden soil with optimum level of mineral nutrients for 10 weeks then transferred to fresh soil with addition of Na_2SO_4, $MgSO_4$ and $Fe_2(SO_4)_3$ with or without additional $CaCO_3$. Plants were cultivated for 6 weeks in a heated greenhouse with supplemented light (250 µmol m^{-2} s^{-1}, 16 h photoperiod). Substrate acidification was seen in Mg and Fe treatments, but additional Ca resulted in increase of pH up to 7.5. EC was highest in Na treatment (300%) followed by Mg (270%) and Fe (220%). Highest stimulation of both leaf and root growth was evident in Na treatment, reaching 140 and 170%, respectively, but addition of Ca completely abolished this response. Plants in Mg treatment tended to have higher root mass and this was partially reversed by Ca. Fe treatment resulted in increased leaf mass and this effect was further stimulated by Ca. As Ca treatment significantly lowered Na concentration in leaves, it is reasonable to suggest that high Na uptake is a prerequisite for growth stimulation of *T. maritima*.

Key words: chlorophyll *a* fluorescence, chlorophyll concentration, growth, Na, soil heterogeneity, *Triglochin maritima*.

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INTRODUCTION

It is well established that mineral nutrient availability and competition for them represents an important factor in determination of plant community structure (Fitter 1982, Koutroubas et al. 2000, Wijesinghe et al. 2005). From the other hand, mineral stress due to both low availability of mineral nutrients as well as ion toxicity has been emphasized as one of more important interacting factors in respect to global climate change (Lynch, St.Clair 2004). However, soil chemical heterogeneity in natural habitats represents an environmental factor often neglected in ecophysiological studies. It is usually thought as a purely technical problem related to soil analysis, which can be dealt with by multiple soil sampling in a particular plot. Nevertheless, limited number of studies has shown that soil chemical heterogeneity both in spatial and temporal terms can represent an important factor for consideration (reviewed in Hinsinger et al. 2005, Hodge 2004). Plant responses to soil nutrient heterogeneity can be analyzed at the level of root morphological plasticity, as having direct implications for nutrient acquisition (Fransen et al. 1998, Hodge 2004). In addition, sub-optimality of particular mineral nutrient concentrations in soil can have more far-reaching physiological effect on plant growth and development.

Triglochin maritima L. is a rare coastal marsh euhalophyte species characterized by succulent almost cylindrical leaves (Breckle 2002). Field studies have revealed extreme spatial and temporal soil chemical heterogeneity in *T. maritima* sites, manifested by more than 30-fold concentration difference for several mineral elements, eventually causing significant mineral disbalance (Karlsons et al. 2011). In natural conditions, concentration of several elements (sodium, magnesium, iron) can reach near-toxic concentrations. Calcium concentration in the soil of temporarily flooded wetland on coast of Lake Liepajas where *T. maritima* is present can reach as high as 14 g kg^{-1}. The aim of the present study was to evaluate a possible positive role of high substrate Ca concentration on alleviation of putative negative physiological impact of the presence of high substrate Mg, S, Fe and Na concentration in *T. maritima* plants cultivated in controlled conditions.

MATERIAL AND METHODS

T. maritima plants were grown from stratified (water-imbibed conditions, 4 °C, six weeks) seeds collected in nature in garden soil (Biolan) with optimum level of mineral nutrients for 10 weeks then transferred to a fresh soil in 1.2 l plastic containers with addition of Na_2SO_4, $MgSO_4$ and $Fe_2(SO_4)_3$ (4 g of a particular metal ion per kg of dry soil) with or without additional $CaCO_3$ (14 g Ca^{2+} kg^{-1}). Plants were cultivated for 6 weeks in a heated greenhouse (day/night temperature 15/20 °C) with supplemented light (350 µmol m^{-2} s^{-1}, 16 h photoperiod). Seven plants per treatment were used. Plants were watered with deionized water when necessary.

Throughout the experiment, soil pH and electrical conductivity (EC), leaf chlorophyll concentration

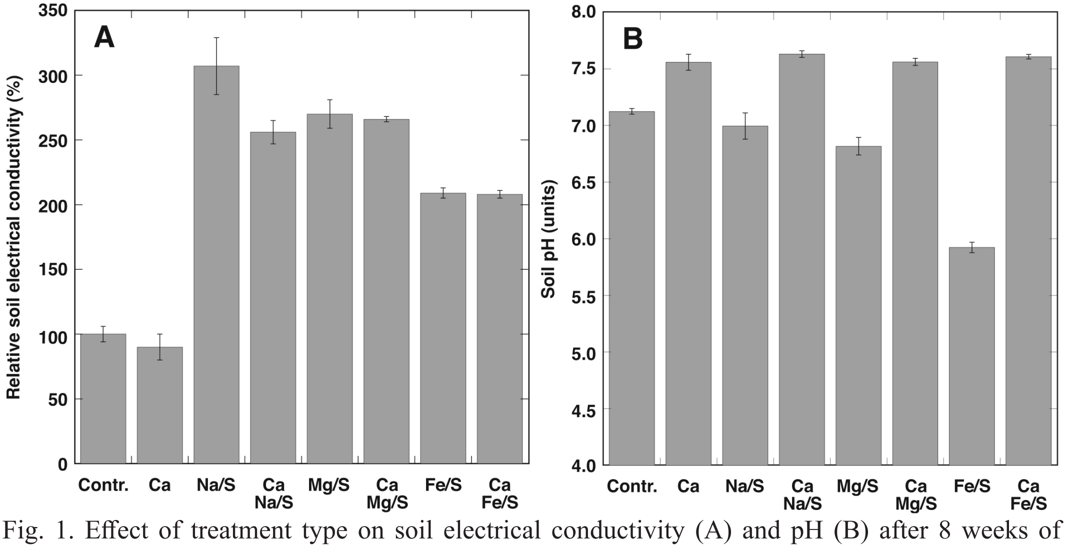

Fig. 1. Effect of treatment type on soil electrical conductivity (A) and pH (B) after 8 weeks of cultivation.

and chlorophyll *a* fluorescence, as well as concentration of Na, Ca, K, NO_3 ions in leaves were monitored weekly. Soil pH was measured by pH 3000 meter (Step Systems, Germany) making four measurements in each container. Soil EC was measured by Moisture Meter HH2 equipped with a sensor WET-2 (Delta-T Devices, USA) making four measurements in each container. Leaf chlorophyll concentration was measured by chlorophyll meter CCM-300 (Opti-Sciences, USA), five randomly selected plants per treatment once a week. Chlorophyll *a* fluorescence parameters were analyzed once a week in dark-adapted leaves by Handy PEA fluorometer (Hansatech Instruments, UK), five randomly selected plants per treatment. Electrical conductivity and Na^+, K^+, Ca^{2+} concentration in leaf water extracts and root tissue extracts (50 ml deionized water per 1 g dry weight) were analyzed by LAQUAtwin compact meters (Horiba, Japan).

RESULTS

Soil treatment with 4 g kg^{-1} Na, Mg and Fe in a form of respective sulfate salts resulted in significant increase in soil EC, which was pronounced throughout the experiment (Fig. 1A). The highest increase was in the case of Na (300%), followed by Mg (270%) and Fe (220%). Addition of 14 g kg^{-1} Ca decreased soil EC in the case of Na, but not in the case of Mg or Fe. Soil pH was significantly lowered by Mg, and, especially, by Fe addition, but additional Ca treatment resulted in significant alcalinization of the substrate up to pH 7.5 (Fig. 1B).

Fig. 2. A typical morphology of *T. maritima* plants grown in different substrates for 7 weeks.

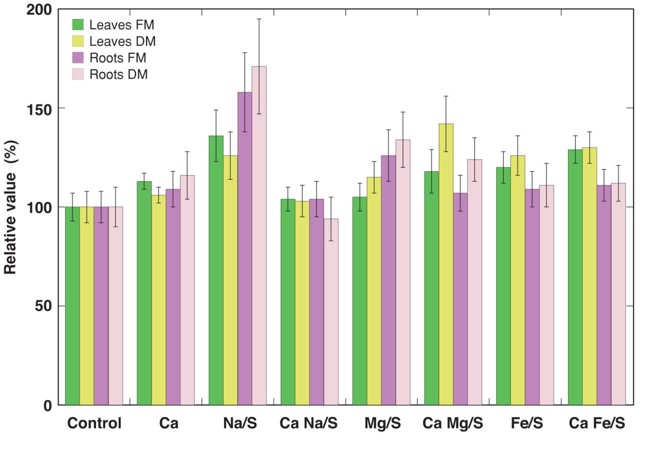

Fig. 3. Effect of treatment type on shoot and root fresh (FM) and dry (DM) mass of *T. maritima* after 8 weeks of cultivation.

There were no morphological signs of disturbance in any of the treatments (Fig. 2). The most pronounced morphological difference between the treatments was the presence of larger number of smaller leaves in Fe and Ca + Fe treatments (Fig. 2). Growth of both shoots and roots was significantly stimulated by Na treatment, but addition of Ca completely abolished this response (Fig. 3). Mg significantly stimulated only root growth with no significant further effect of Ca, but in the case of shoots, stimulation was evident by Ca + Mg treatment. There was a slight stimulation of shoot growth by Fe with additional positive effect of Ca treatment.

Chlorophyll *a* fluorescence parameter Performance Index was significantly higher in Fe-treated plants in comparison to all other treatments throughout the experiment (Fig. 4A). Ca-treated plants had lower Performance Index values than the respective treatments. Leaf chlorophyll concentration was less affected by the treatments than chlorophyll *a* fluorescence, but there was a tendency that it significantly increased in all treatments at the early stages of the experiment as well as after five weeks in comparison to control plants (Fig. 4B). Also, there was a tendency that Ca-treated plants had lower chlorophyll concentration in comparison to the respective treatments. No treatment resulted in a significant decrease of chlorophyll concentration, but significantly lower levels of Performance Index in comparison to control were evident for plants in Ca + Na and Ca + Mg treatments from the 3rd week and Ca and Ca + Fe treatments from the 4th week.

Leaves of *T. maritima* plants in all treatments accumulated relatively high concentration of Na^+ (Fig. 5A). In Na-treated plants, the maximum Na^+ level was reached after four weeks. Addition of Ca drastically inhibited Na^+ accumulation. During the first weeks of growth, K^+ concentration in leaves of treated plants was higher than control

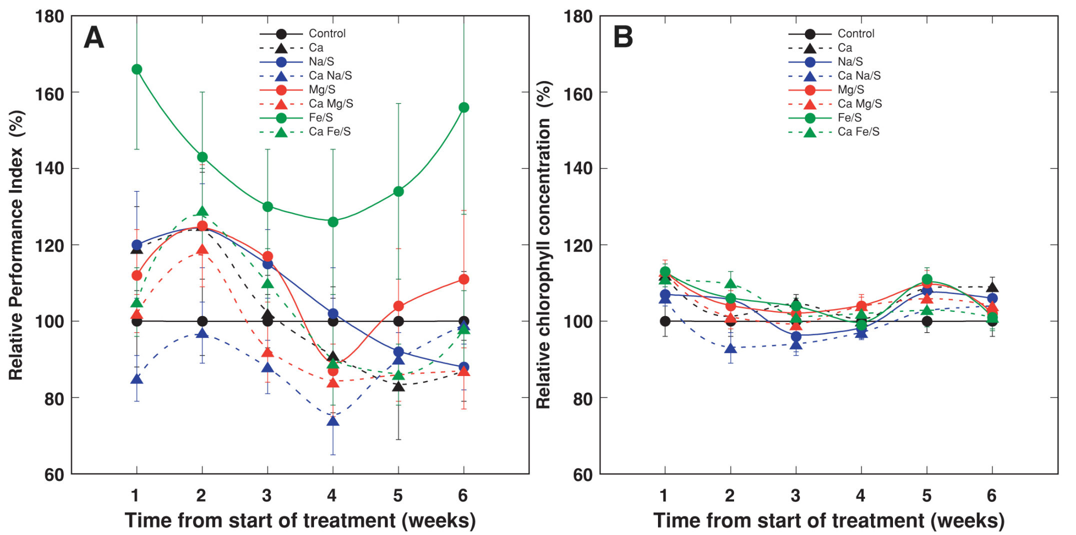

Fig. 4. Time-course of Peformance Index (A) and chlorophyll concentration (B) in leaves of *T. maritima* plants grown in different substrates.

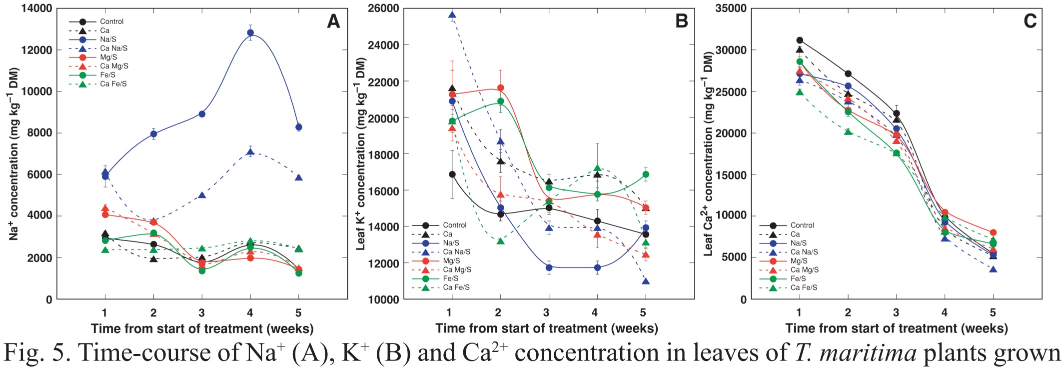

Fig. 5. Time-course of Na^+ (A), K^+ (B) and Ca^{2+} concentration in leaves of *T. maritima* plants grown in different substrates.

in all treatments, especially, Mg- and Fe-treated plants (Fig. 5B). Tissue concentration of Ca^{2+} was negatively affected by all treatments (Fig. 5C). On the later stages, it was stimulated in Fe-treated plants.

Final Na^+ concentration in root tissue was significantly lower than that in leaves (Fig. 6A). The highest Na^+ concentration was evident in Na-treated plants, followed by Fe, control, and Mg. There was no effect of Ca on root Na+ concentration. In contrast, Ca treatment lowered root K^+ concentration in control, Mg- and Fe-treated plants but increased it in Na-treated plants (Fig. 6B).

Summary concentration of Na^+ and K^+ positively correlated with EC of leaf extracts (R^2 = 0.91; Fig. 7A). There was also a highly significant positive correlation between summary concentration of Na^+ plus K^+, and EC in root extracts (R^2 = 0.94; Fig. 7B). This indicated that both Na^+ and K^+ were the main ions contributing to electrical conductivity in tissues.

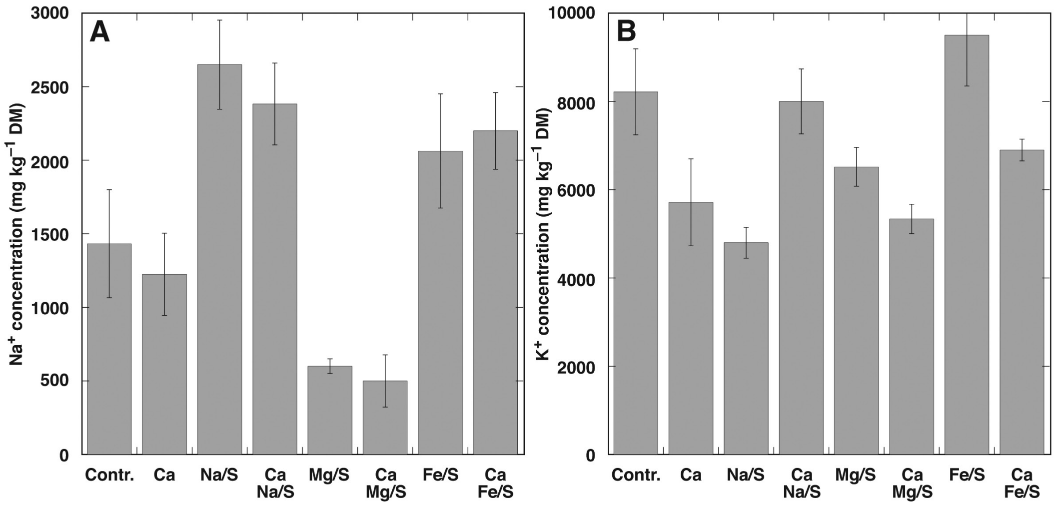

Fig. 6. Effect of treatment type on Na^+ (A) and K^+ (B) concentration in roots of *T. maritima* plants after 8 weeks of cultivation.

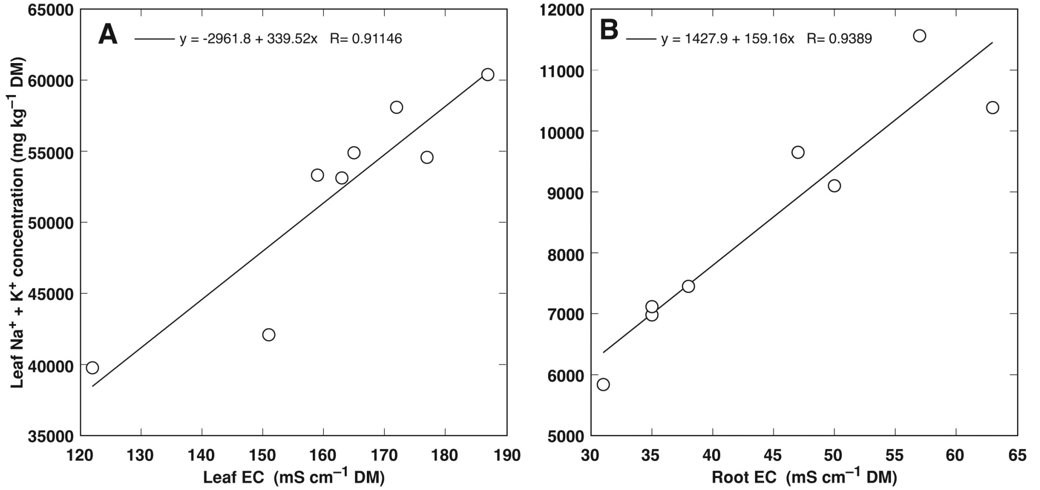

Fig. 7. Correlation between summary Na + K concentration and extract EC in leaves (A) and roots (B) of *T. maritima* plants.

DISCUSSION

It was initially expected that high concentration of Na, Mg and Fe in substrate would result in physiologically negative consequences and even growth inhibition of *T. maritima* plants. Ca^{2+} treatment was used as a possible means to reduce this high salt-induced toxicity. However, as indicated by time course of chlorophyll *a* fluorescence parameters as well as leaf chlorophyll concentration, no toxicity was evident in treated plants, but plant growth was even stimulated by Na (both shoots and roots), Mg (roots) and Fe (shoots). This is in contrast with a previous study with *T. maritima*, where a shift from "optimum" to "natural" soil with a high potentially toxic concentration of P, Ca, Mg, S, Fe, Mn, Zn, Cu, Na and Cl was accompanied by a decrease of leaf mass by 52% (Karlsons et al. 2011). It seems that combination of mineral constituents at surplus concentration can cause toxic effect on halophte growth more easily in comparison to single mineral influence.

Similar to other typical euhalophyte species, *T. maritima* accumulated higher concentration of Na^+ in leaves in comparison to that in roots. In a semi-natural soil with 0.9 g l^{-1} Na^+ added, *T. maritima* accumulated as high as 17.5 g Na^+ in leaves and 3.8 g Na^+ per kg dry mass in leaves and roots, respectively, but even in a substrate without added NaCl these values were 2.9 and 1.2 g Na^+ per kg (Karlsons et al. 2011). In the present study Na^+ level in leaves and roots reached comparable but relatively slightly lower concentration (13.0 and 2.7 g, respectively).

Salinity significantly inhibits both uptake and transport of Ca^{2+} (Rengel 1992), and this effect was seen also in the present study in *T. maritima* plants. Interestingly, high Ca^{2+} in the substrate further inhibited Ca^{2+} uptake or/and transport. In another halophytic species, *Plantago maritima*, high NaCl significantly stimulated Ca^{2+} uptake both in leaves and roots (Sleimi et al. 2015).

Tissue concentration of Na^+ decreased in *Triticum aestivum* seedlings cultivated in the presence of high Ca^{2+} (Kinraide 1999). In spite of that, Ca^{2+} was able to ameliorate negative effect of NaCl only on root elongation but not that in respect to shoot growth. It is suggested that the mechanism of Ca^{2+}-dependent decrease in Na^+ toxicity could be associated either to blocking of Na^+ entry in cells (Roberts & Tester 1997) or facilitating efficient compartmentation of Na^+ in tissues (Kinraide 1999). In contrast to that, in the present study Ca^{2+} affected Na^+ concentration at the level of uptake in shoots without any effect on Na^+ concentration in roots. However, even partial blocking of Na^+ entry in shoots had significant physiological consequences, as no growth stimulation occurred in Na-treated *T. maritima* plants in the presence of high soil Ca^{2+}.

CONCLUSION

Growth and physiological characteristics of euhalophytic plant *T. maritima* was not adversely affected by high substrate concentration of Na, Mg and Fe as well as sulfate. Elevated substrate Na_2SO_4 resulted in growth stimulation of shoots and roots of *T. maritima* plants. As Ca treatment significantly lowered Na concentration in leaves, it is reasonable to suggest that high Na uptake in shoot tissues is a prerequisite for growth stimulation of *T. maritima*.

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