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Response of macrophyte *Berula erecta* to low concentrations of NaCl *in vitro*

Odziv vrste *Berula erecta* na nizke koncentracije NaCl *in vitro*

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Abstract: Macrophyte *Berula erecta,* grown in tissue culture, was exposed to various low concentrations of NaCl in the water (1–100 mg L^{-1}). Added NaCl had a positive effect on plant's growth and development. The number of shoots increased, as well as the length of the roots. The lowest concentration (1 mg L^{-1}) increased photochemical efficiency of photosystem II (Fv/Fm) while the highest (100 mg L^{-1}) slightly decreased it. Chlorophyll content was negatively affected by NaCl addition after 3 weeks. Carotenoid and anthocyanin levels were firstly raised and later lowered in NaCl treatment comparing to control. Overall, added NaCl had no negative effect on plants morphology, while decreased amount of pigments was observed.

Keywords: NaCl, *Berula erecta*, photochemical efficiency, growth parameters, pigments

Izvleček: Makrofit ozkolistni koščec (*Berula erecta*), gojen v tkivni kulturi, smo izpostavili različnim koncentracijam NaCl v vodi (1–100 mg L^{-1}). Dodani NaCl je pozitivno vplival na rast in razvoj rastline. Število poganjkov se je povečalo v primerjavi s kontrolo, prav tako so bile korenine rastlin, izpostavljene NaCl, daljše kot pri kontrolnih rastlinah. Nizka koncentracija NaCl (1 mg L^{-1}) je povišala vrednost fotokemične učinkovitosti, medtem ko je visoka koncetracija (100 mg L^{-1}) rahlo znižala vrednost tega parametra. Vsebnost klorofilov se je ob koncu poskusa občutno znižala pri rastlinah, izpostavljenim NaCl. Vsebnost karotenoidov in antocianinov se je na začetku povišala in nato nižala proti koncu poskusa. Zaključimo lahko, da dodan NaCl ni negativno vplival na morfologijo rastlin, medtem ko je znižal vsebnost barvil v rastlinah.

Ključne besede: NaCl, *Berula erecta*, fotokemična učinkovitost, rastni parametri, barvila

Introduction

The evolution of a physiology of plants that utilized K^+/H^+ rather than Na^+ was a vital step in the colonization of fresh water, and provided the basis for colonization of the land (Willey 2016). Therefore K^+ and not Na^+ (as in animals), is the primary osmoticum and the electrochemical gradients of H^+ the primary energizer of ion transport in plants (Willey 2016).

The role of Na^+ in plants is not fully understood, but trace amounts are required for the growth of plant species with C_4 and CAM photosynthetic pathways (Willey 2016). Small amounts of Na^+ are also essential, and they benefit the growth of some terrestrial plants, but in contrast to animals, the majority of them have no enzymatic requirements for Na^+ (Willey 2016).

For most plants even mild salinity is highly toxic. Salinity induces osmotic, ionic and oxidative stresses, inhibits plant growth, and disturbs photosynthesis and metabolism (Shabala and Munns 2017). Na^+ is physico-chemically similar to K^+ and Na^+ competes with K^+ in cell metabolism. It can be used as a partial replacement for K^+ and aids in the opening and closing of stomata, which helps regulate internal water balance. The chloride is a component of the water-splitting system of photosystem II and is involved in the stomatal regulation of many species and is therefore an essential micronutrient. Sodium competes with cations potassium, calcium, magnesium and ammonium for its uptake by the plant (Shabala and Munns 2017). Chloride can compete with anions nitrate, phosphate and sulfate uptake. Therefore, if concentration of sodium or chloride is high in the growing medium, while other beneficial elements are at low or normal levels, the plant increase acceptance of what is in excess, and this can lead to a lack of another elements. Therefore, the plant may not acquire sufficient levels of a required beneficial elements and this can lead to its deficiency in the tissue (Shabala and Munns 2017).

In the studies of the effects of various elements on plants, the elements are very often added in the form of salts. Therefore, the purpose of our study was to investigate the plant response to sodium salt in the form of NaCl. The objective of our study was to investigate the effect of salts, below the concentrations featured for saline soil, which can be promote and not inhibitory for plants. We tested the influence of various low concentrations of NaCl on species *Berula erecta*. To reach these, we measured several parameters describing growth and development, physiology and biochemistry of the plants.

Materials and methods

Plants and growth conditions

Detached shoots of *Berula erecta* L. were placed on 20 mL of solid Murashige and Skoog (1962) medium (MS) without growth regulators. The MS medium was supplemented with 0.8% Difco Bacto agar, with 3% sucrose, and adjusted to pH 5.7–5.8 before autoclaving. Two shoots were placed on the surface of the MS medium in a culture vessel for root induction and after three weeks the vessels were filled with an additional 20 mL of sodium chloride (NaCl) (98%, Sigma–Aldrich®, Taufkirchen, Germany) aqueous solutions at concentrations of 1, 10, and 100 mg L^{-1} for another three weeks. Controls consisted of plants that were treated with water. The vessels were incubated under controlled conditions at 23 ± 2 °C, with a photoperiod of 16 h at 38–50 mol $m^{-2}s^{-1}$ (Osram L 58W/77 – Fluora) and at 50% relative humidity. All experiments were repeated twice.

Measurements of selected parameters

In order to monitor the growth and developmental parameters, the dry and fresh weight, the length of plants and roots and number of shoots were monitored weekly.

Photochemical efficiency (maximum quantum yield; Fv/Fm) was measured weekly on 10-12 plants from each concentration treatment, using a fluorometer (Handy PEA, Hansatech, Kings Lynn, UK). The measurements of chlorophyll *a* fluorescence were made after 10 min of darkness, pro-vided by dark-adaptation clips. Fluorescence was excited with a saturating beam of 'white light' (PPFD = 8000 mol $m^{-2}s^{-1}$, 0.8 s) (Schreiber et al. 1995).

For content of chlorophylls *a* and *b,* carotenoids and anthocyanins, leaves of 4–9 plants from each treatment were selected. The amounts of pigments were determined as described by Lichtenthaler and Buschmann (2001). The total anthocyanin content was measured as described by Drumm and Mohr (1978).

Statistical analysis

The statistical package SPSS® 24.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The level of statistical significance (p) among different treatments was determined by the analysis of variance (ANOVA) using the post hoc Duncan test. Differences at $p < 0.05$ were considered statistically significant. Different letters indicate significant differences. The number of replicates was from 10-24 for growth and developmental parameters, 10-12 for photochemical efficiency and 4-9 for pigments.

Results and discussion

The fresh weight of *B. erecta* was slightly increased in NaCl exposed plants compared to control at the beginning. More pronounced positive effect of NaCl was observed in the end of an experiment, the average values being 2.45, 5.26, 3.31 and 3.76 g for control, 1, 10 and 100 mg L^{-1} respectively. Dry weight was statistically increased in the end of an experiment, the average values ranged from 0.14 to 0.20 g for treated plants and 0.12 g for control. Many studies have shown that the fresh and dry weights of the shoot system are affected, either negatively or positively, by changes in salinity concentration, type of salt present, or type of plant species (Amira and Qados 2011, Al-Karaki 2000). Beneficial effects of salts usually occurs at concentrations around 50 mg L^{-1} (Willey 2016) which is also evident from our study.

Plant height was statistically positively affected by 100 mg L^{-1} in all weeks of an experiment, average values being between 4.77 and 6.45 cm while to control height reached 4.29 to 5.80 cm. The other two concentrations only increased plant height in week 3 compared to the control. The general trend of increasing the length of the bean plants exposed to 2 mg L^{-1} NaCl was observed (Amira and Qados 2011). Generally speaking, the elongation of the stem when treated with low concentrations of salts may induce osmotic adjustment activity in the plants which may improve growth. On the other hand, plant height of tomato and *Atriplex lentiformis* decreased with increasing NaCl in the nutrient solution (up to 5 mg L^{-1}) (Al-Karaki 2000, Smit et al. 2017).

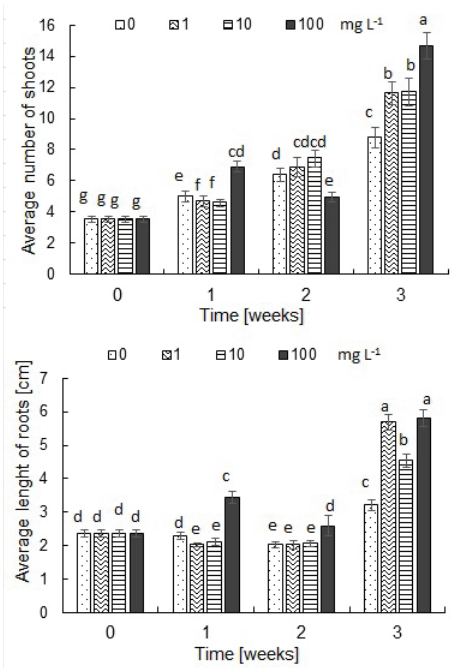

Figure 1: The number of shoots and length of roots in *Berula erecta* at low concentrations of NaCl *in vitro* (means ± SD, n=10-24).

Slika 1: Število poganjkov in dolžina korenin pri vrsti *Berula erecta*, izpostavljeni nizkim koncentracijam NaCl *in vitro* (povprečne vrednosti ± SD, n=10-24).

The promotion effect of low concentrations of NaCl was demonstrated also after determination of the average length of roots and the average number of shoots (Fig. 1). The highest concentration positively affected root length from the beginning, while the other two NaCl concentrations increased the length of roots towards the end of an experiment compared to the control (Fig. 1). The shoots were positively affected by 100 mg L^{-1} in week 1 and 3, while in week 2 this concentration decreased the number of shoots. In week 3 concentrations 1 and 10 mg L^{-1} also increased the number of shoots (Fig. 1). In *Atriplex lentiformis,* concentration of 5 mg L^{-1} NaCl reduced the number of shoots (Al-Karaki, 2000).

It looks that take up of salts, which can reduce the growth of plants (Shabala and Munns 2017), in our experimental system did not reduce the ability of plants to grow.

Photochemical efficiency (Fv/Fm) in all three treatments ranged from 0.81 to 0.83, which shows, that added NaCl did not affect the process of photosynthesis, since these values indicate that plants are in good condition (Schreiber et al. 1995). However, there were some statistical significant differences between treatments. The concentration of 1 mg L^{-1} increased photochemical efficiency towards the end of an experiment, while 100 mg L^{-1} decreased it (data not shown). In concentration of 10 mg L^{-1} the differences were observed between weeks, with the highest overall value in week 2 (data not shown). NaCl an especially Na^+ can negatively affects photosynthesis processes, photosystems or pH homeostasis metabolism due to H^+-coupled Na^+ efflux mechanisms (Shabala and Munns 2017).

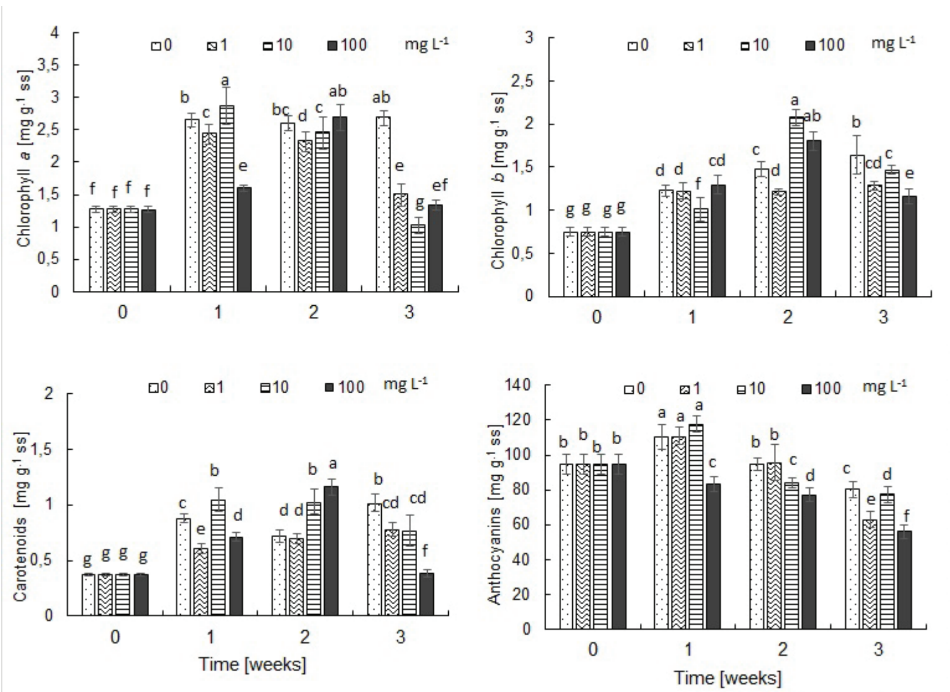

Figure 2: The content of pigments in leaves of NaCl treated *Berula erecta* (means ± SD, n=4-9).

Slika 2: Vsebnost pigmentov v listih vrste *Berula erecta*, izpostavljene nizkim koncentracijam NaCl *in vitro* (povprečne vrednosti ± SD, n=4-9).

The average content of chlorophyll *a* and *b* and carotenoids in *B. erecta* increased in weeks after the NaCl addition comparing to the content before the addition (Fig. 2). The highest NaCl addition decreased the content of chlorophyll *a* in week 1, while in week 3 the addition of NaCl decreased the amount of this pigment regardless concentration (Fig. 2). The similar decrease was observed with NaCl treatment in *Chrysanthemum* species (Lee and van Iersel 2008, Chen et al. 2003), *Atriplex lentiformis* (Smit et al. 2017) and onion (Hanci et al. 2016).

Significant decrease in chlorophyll *b* was observed in 1 and 100 mg L^{-1} in week 3, when all treatments were lower as control (Fig. 2). In week 2 the highest two concentrations increased the content of this pigment. Tort and Turkyilmaz (2004) reported that the exposure of barley to 7 and 14 mg L^{-1} NaCl led to the decrease in chlorophyll *a* and *b*. Also in *Atriplex lentiformis* exposed to 2-5 mg L^{-1} the content of chlorophylls decreased (Smit et al. 2017).

Reduction in chlorophyll content is commonly observed phenomena as salinity increases and plants are subjected to salt stress. The determination of chlorophylls is therefore usual way to determine salt tolerance.

The peak concentration of carotenoids was measured in week 2. The amount of carotenoids then lowered towards the end of an experiment, with the lowest carotenoids content in 100 mg L^{-1} treatment. The same was observed in bean plants to salt stress where the formation of carotenoids was inhibited and a decrease was observed as well (Amira and Qados 2011). The amount of anthocyanins first increased but towards the end decreased. Since the content of carotenoids and anthocyanins was lower we presume that plants were not under stress because these pigments start to accumulate in less favourable conditions (Fargašová 1998, Winkel-Shirley 2002).

Conclusions

Sodium chloride in low concentrations that were used in our experiments, had no negative effect on plant morphology and no severe effect on the process of photosynthesis although it lowered chlorophyll *a* content in the end. The stress was absent since the protective pigments (carotenoids, anthocyanins) were not increased and values of photochemical efficiency showed that plants are in good condition at all treatments. We can conclude that chosen concentrations had not yet triggered stress in the selected species.

Povzetek

V različnih študijih vpliva elementov na rastline so elementi pogosto dodani v obliki soli. Cilj našega poskusa je bil ugotoviti, kako se rastline odzivajo na natrijeve soli v obliki NaCl, v koncentracijah nižjih od tistih, ki so značilne za slana tla. Makrofit ozkolistni koščec (*Berula erecta*) smo v tkivni kulturi izpostavili različnim nizkim koncentracijam NaCl v vodni raztopini (1–100 mg L^{-1}). Dodane nizke koncentracije NaCl, uporabljene v poskusu, so pozitivno vplivale na rast in razvoj rastlin. Povečalo se je število poganjkov in dolžina korenin. Najnižja koncentracija NaCl (1 mg L^{-1}) je povečala, največja (100 mg L^{-1}) pa rahlo zmanjšala maksimalno fotokemično učinkovitost fotosistema II (Fv/Fm). Dodani NaCl je znižal vsebnost klorofilov na koncu poskusa. Vsebnost karotenoidov in antocianinov se je najprej povečala, nato nekoliko zmanjšala proti koncu poskusa pri rastlinah tretiranih z NaCl v primerjavi s kontrolo. Zaključimo lahko, da izbrane nizke koncentracije NaCl niso negativno vplivale na morfologijo rastlin in fotosintezo. Pri rastlinah pri nobeni obravnavi nismo opazili stresa, saj se zaščitni pigmenti niso akumulirali, izmerjene vrednosti fotokemične učinkovitosti pa so pokazale na dober fitnes rastlin. To pomeni, da izbrane koncentracije NaCl še niso sprožile stresa pri izbrani rastlinski vrsti.

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