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GROWTH OF ORNAMENTAL GRASSES UNDER SALINITY STRESS

Monika HENSCHKE*
Poznań University of Life Sciences
ul. Dąbrowskiego 159, 60-594 Poznań Poland

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ABSTRACT

The aim of this study was to determine the effect of substrate salinity caused by increasing NaCl doses on growth and development of ornamental grasses: *Briza media* L., *Deschampsia cespitosa* (L.) P. Beauv., *Koeleria glauca* (Spreng.) DC., *Sesleria caerulea* (L.) Ard. and *Sorghastrum nutans* (L.) Nash. Results provide the basis for the preliminary classification of analyzed grasses in terms of their tolerance to substrate salinity. Grasses, responding negatively to substrate salinity caused by small doses of 5 and 10 g·NaCl·dm^{-3}, may be considered to be sensitive species. Within the studied genotypes, *B. media* showed a negative response to salt stress in most of the analyzed traits. Genotypes responded differently to salinity but all had a decreased tolerance index even at the lowest concentration of NaCl. The lowest percentages of dry leaves at 5 and 10 g NaCl·dm^{-3} were in *K. glauca* and *S. caerulea*. The percentage of dry matter and leaf greenness were least affected.

Key words: shoots, dry matter content, leaf chlorophyll content index, leaves, sodium chloride

INTRODUCTION

Saline soils are found on every continent and are particularly common in desert and arid regions (Siyal et al. 2002). Frequently, human activity, such as mineral fertilization and industry, contributes to soil salinity. The impact of sodium chloride used in road de-icing is considered to be less important. This source of soil salinization is often underestimated, since it does not directly affect plant production. However, in view of the amount of NaCl applied in road de-icing, this problem is gaining in importance not only in Europe, but also in North America and Canada (Howard & Maier 2007; Cunningham et al. 2008).

Under salinity stress, plants need to adapt their metabolism to environmental changes. Survival under these stress conditions depends on the capability of plants to detect a stimulus, generate and transmit signals, as well as induce biochemical changes, which will accordingly modulate metabolism (Rani 2011). If the salt content in soil exceeds the threshold level, first of all the water uptake capacity of plants is reduced, leading to osmotic stress (Xiong & Zhu 2002). Salt contained in the soil leads directly to ion stress (Munns 2002). Ions of Na^+ and Cl^- may be accumulated in leaves and cause several types of damage in plants. The basic symptoms of salt stress include growth retardation and reduction of dry matter. Ion balance is disturbed and photosynthetic pigments are degraded. As a result of stress, the catabolic processes may dominate over anabolic processes and cause accelerated ageing, shedding of leaves and withering of plants. Many species are capable of avoiding the stressor by generating barriers countering penetration of harmful ions inside the plants, regulating ion transport and capacity to accumulate them (Warren et al. 1985; Warren & Brockelman 1989; Cheong & Yun 2007). Halophytes were found to be equipped with the largest number of mechanisms of resistance to salinity. In turn, glycophytes have a limited capacity to avoid harmful ions, which are eventually accumulated in leaf cells in toxic amounts (Farooq & Azam 2006). However, sensitivity to salinity and resistance reactions vary greatly, to a considerable

*Corresponding author:
e-mail: mohen@up.poznan.pl

degree depending on the species or even a cultivar (Sudhakar et al. 2001).

Ornamental grasses are an interesting group of plants, recently gaining in importance. Many species exhibit a tolerance to adverse growth conditions (Dana 2002; Henschke 2014); thus, among them, we need to search for species resistant to salinity and in this way, broaden the currently limited assortment of plants for roadside green areas. The aim of this study was to determine the effects of substrate salinity caused by increasing the doses of NaCl on growth and development of ornamental grasses: *Briza media* L., *Deschampsia cespitosa* (L.) P. Beauv., *Koeleria glauca* (Spreng.) DC., *Sesleria caerulea* (L.) Ard. and *Sorghastrum nutans* (L.) Nash.

MATERIAL AND METHODS

Plant material

An experiment was conducted at the Marcelin Experimental Station in a greenhouse of the Poznań University of Life Sciences in the years of 2010, 2011 and 2012. Breeding clones of five species of perennial grasses were studied. *Briza media* L. comes from Eurasia, grows on meadows, frameworks and moors. Clumps of leaves grow to 15-25 cm of height; during the flowering, the height is 90 cm. *B. media*, as an ornamental plant, can be cultivated in gardens and parks. *Deschampsia cespitosa* (L.) P. Beauv., which comes from Eurasia and America, grows on wet meadows, moors, alluviums, in forests and brushwoods. Clump of leaves grows to 30-50 cm of height, during flowering the height is 150 cm. *D. cespitosa* can be cultivated in gardens and parks. *Koeleria glauca* (Spreng.) DC. comes from Europe and north Asia, grows on sandy dunes and alluviums in pine forests. Clumps of leaves grow to 20 cm of height, length of inflorescence stem is 60 cm. *K. glauca* can be planted in gardens and urban greenery. *Sesleria caerulea* (L.) Ard. comes from Eurasia, grows on wet meadows and low peat bogs. The height of plants is 20-50 cm. *S. caerulea* can grow in gardens and urban greenery. *Sorghastrum nutans* (L.) Nash. comes from North America, grows on prairies, in brushwoods and at roadside. The height of the clump of leaves is 50 cm and 150 cm during the flowering. It can be planted in gardens and parks (Foerster 1978).

The cuttings of grasses were obtained from division of rhizomes. They were grown from 4 May until 21 June in boxes under low tunnels made of white nonwoven ground cover. At that time, young plants were transplanted to pots of 0.750 dm^3. They were grown for 6 weeks in the substrate of Highmoor peat (Klasmann, pH 3,9) with washed mineral sand at a 20 : 1 ratio (v : v). The Highmoor peat was limed on the basis of neutralization curve to pH 6.40 using $CaCO_3$ at a dose of 7.0 $g \cdot dm^{-3}$ peat. The ready-to-use substrate mixtures were supplemented with 2 $g \cdot dm^{-3}$ of a Peters Professional PL Special fertilizer (20 : 20 : 20). Each pot contained the same weight of the substrate. At that stage, *B. media* developed 11 shoots and 18 leaves, and the length of the flag leaf was 29.2 cm, *D. cespitosa* developed 7 shoots and 7 leaves, and the average length of the flag leaf was 29.7 cm. *K. glauca* developed 4 shoots and 12 leaves, and the average length of the flag leaf was 13.4 cm. *S. caerulea* developed 2-3 shoots and 5-6 leaves, and the length of the average flag leaf was 28.0 cm, while *S. nutans* developed 4 shoots and 4 leaves and the average length of its flag leaf was 37.6 cm.

Treatments

In the beginning of August, the plants were treated with salt solutions. One day before, an analysis of the substrate was made, which showed that it contained ($mg \cdot dm^{-3}$): $N\text{-}NO_3$ 83.3, P 25.4, K 75.0, Ca 250.7, Mg 22.7, Cl 12.5, pH in H_2O 6.1, EC $mS \cdot cm^{-1}$ 2.0. The plants were subjected to salt stress induced by a range of NaCl concentration ($g \cdot dm^{-3}$): 0 (control), 5, 10, 15, 30 and the electrical conductivity (EC) of soil saturation extract was ($mS \cdot cm^{-1}$): 2.0, 2.5, 3.5, 4.0, 5.7. In each variant, 100 ml of saline solution was poured into the pot and 100 ml of distilled water was used in the control. Each pot was placed on a bowl to prevent an uncontrolled outflow of saline. Prior to treatment of plants with salt solutions, the substrates were watered to 50-55% moisture by weight of the substrate. Treatment with salt solutions was done only once. While growing, the plants were watered to 55-60% moisture by weight of the substrate. The experiment was conducted under natural photoperiod at quantum irradiance of 300-400 μmol·m^{-2}·s^{-1}, the average temperature and air humidity depended on the year of cultivation and it was respectively: 22-24 °C, 45-50% in August and 17-19 °C, 45-50% in September.

Measurements

The measurements, which were performed after 8 weeks of cultivation under saline conditions included the growth parameters, the percentage of dry leaves relative to the total number of leaves, the percentage of dry matter, leaf chlorophyll content (SPAD) and tolerance indices. Growth parameters included: the number of mature shoots (with at least one mature leaf – deflected into right angle from shoot) and of juvenile shoots, the length of shoots (cm) (measured from substrate surface to flag leaf) – a mean of three randomly selected mature shoots per plant and the length of leaves (cm) – a mean of three fully developed leave blades on randomly selected shoots per plant.

The leaf chlorophyll content (SPAD) measured in proximal and adaxial part of young leaf, using a SPAD-502 Chlorophyll Meter was determined according to Gregorczyk and Raczyńska (1997) and Gregorczyk et al. (1998). It was not measured in *K. glauca* as the leaves were too narrow.

Tolerance index related to salinity (%) was determined by Shetty et al. (1995) according to the formula Ti = dry mass at salinity level × 100/ dry mass at the 0.0 level of salinity.

Experimental design

The experiment was conducted as a random process. The treatments with different salt doses (0-control, 5, 10, 15, 30 g NaCl·dm^{-3}) was a factor of study. Grass species were analyzed separately. The experiment was repeated for three years (2010, 2011, 2012). One year was treated as replication in time. Each treatment consisted of three replications (years) with twelve plants for each one species. Each plant was cultivated in a separate pot.

Statistical analysis

The obtained results were analyzed statistically by means of analysis of one-way variance. The Newman–Keuls test at the significance level of p = 0.05, was employed to analyze differences between the measured parameters.

RESULTS AND DISCUSSION

Growth under salt stress inhibited the growth of all studied genotypes including the number of shoots, length of shoots and leaves, and the degree of adverse effects increased with the concentration of NaCl in water solution. The exemptions were *S. nutans* (number of shoots and length of leaves not decreased) and *S. caerulea* (the same length of shoots) (Table 1). The lowest level of salinity had the negative effect on three growth parameters of *B. media*, whereas in *D. cespitosa* only the number of mature shoots was lower. In *K. glauca* and in *S. caerulea* the number of mature shoots and the length of leaves were lower. The highest salt concentration in watering solution reduced the number of mature and young shoots of *D. cespitosa* almost 3 times, although the length of shoots and leaves decreased at the highest concentration of NaCl only 1.5 times. In the other species, the number of shoots decreased from 1.4 to 2.2 times. Salt at 5 and 10 g NaCl·dm^{-3} did not influence the length of shoots of the four species, and reduced the length of shoots significantly in *S. nutans* only. The greatest tolerance to the lowest dose of salt expressed in growth inhibition was found in *S. nutans*. In this species, the number of shoots and length of leaves were not changed.

Growth is the final effect of morphological expression of various metabolic activities taking place in plants. The negative effect of salinity inhibiting cell elongation may be manifested in the reduction of the number and length of shoots and leaves (Katerij et al. 1998). According to Glenn (1987), even grasses accounted to halophytes grow faster on non-saline substrate. The response is found in leaves and mature shoots first, while young shoots, as sites of cell differentiation, are exposed to the effects of salinity to a lesser extent. In this study, only *S. nutans* did not decrease the number of juvenile shoots and the length of leaves (Table 1). Among the examined species, *S. nutans* had the least number of shoots. Moreover, they were the longest. Thus, it may be stated that *S. nutans* showed certain adaptations to growth under salinity. Tolerance of lower salt concentrations may result solely from the inhibitory action of osmotic stress. In the case of halophytes, the salinity causes mainly osmotic stress, while glycophytes are also exposed to ion stress (Xiong & Zhu

2002). The effect of salinity on the number of grass shoots was also investigated by Vasquez et al. (2006). They showed that under the influence of increasing salt doses, the plants produced less shoots. *Phragmites australis*, a species exhibiting greater sensitivity, at the largest dose produced almost 90% fewer shoots than in the control without salinity, whereas in the resistant species *Spartina alterniflora*, the number of shoots was only 40% lower. The negative effect of salinity on shoots growth in *Spartina patens*, was reported by Wu et al. (1998), while such a negative effect on plant height in *Phragmites australis* and *Spartina alterniflora* by Vasquez et al. (2006).

In this experiment, a particularly large percentage of dry leaves under the influence of salinity was observed in *B. media* (Table 2). The rate of dry leaves increased with increasing salt concentration and at the 30 g NaCl·dm^{-3}, it was 10.2 times greater than in control plants. Considering that it is a plant of abundant growth with a large number of shoots and leaves, both dehydration and accumulation of harmful ions in that species caused withering of a large number of leaves. Many dry leaves were also formed by *K. glauca* and *S. caerulea*. The highest dose of salt (30 g NaCl·dm^{-3}) caused a significant increase in the number of dry leaves 4.8 and 5.5 times, respectively comparing with control. Withering of mature leaves is a primary symptom of salt stress. It is a consequence of dehydration and accumulation of Na^+ and Cl^- ions. Accumulation of those ions in leaves of grasses was reported by Glenn (1987). The effect of salinity on plant growth may be greatly varied depending on the species and its genotype, and produced phytohormones (Javid et al. 2001).

Table 1. The effect of salinity stress on growth parameters of ornamental grasses

Species	Salt concentration (g NaCl·dm^{-3}) (proportion to control in brackets)				
	0	5	10	15	30
Number of mature shoots					
Briza media	18.4 e*	15.6 d	13.1 c	9.7 b	7.1 a (2.6)
Deschampsia cespitosa	23.8 e	18.7 d	16.0 c	12.5 b	8.8 a (2.7)
Koeleria glauca	26.9 d	21,5 c	18.5 b	16.2 b	12.3 a (2.2)
Sesleria caerulea	10.0 d	8.6 bc	8.0 b	9.3 cd	6.2 a (1.6)
Sorghastrum nutans	5.4 c	5.6 c	4.7 b	5.2 ab	3.8 a (1.4)
Number of juvenile shoots					
Briza media	19.9 c	16.1 b	16.5 b	15.3 b	10.2 a (2.0)
Deschampsia cespitosa	5.8 d	4.7 c	3.8 b	4.5 c	2.0 a (2.9)
Koeleria glauca	4.4 c	4.2 c	3.1 b	3.4 b	2.5 a (1.8)
Sesleria caerulea	2.2 c	2.8 d	1.9 b	1.7 b	1.1 a (2.0)
Sorghastrum nutans	4.7 ab	5.3 b	4.9 ab	4.8 ab	4.3 a (1.1)
Length of shoots (cm)					
Briza media	5.5 c	5.4 c	5.1 c	4.5 b	3.6 a (1.5)
Deschampsia cespitosa	3.2 b	3.1 b	3.1 b	2.4 a	2.1 a (1.5)
Koeleria glauca	4.0 c	3.9 c	3.7 c	3.1 b	2.7 a (1.5)
Sesleria caerulea	4.1 a	4.0 a	4.0 a	4.1 a	4.1 a (1.0)
Sorghastrum nutans	15.9 d	13.4 c	12.6 b	13.6 c	11.3 a (1.4)
Length of leaves (cm)					
Briza media	30.3 d	28.4 c	27.0 c	22.8 b	15.1 a (2.0)
Deschampsia cespitosa	24.2 c	24.1 c	23.3 c	20.7 b	16.6 a (1.5)
Koeleria glauca	16.2 d	14.9 c	13.2 b	12.9 b	10.7 a (1.5)
Sesleria caerulea	24.5 d	22.5 c	21.3 b	20.5 b	17.5 a (1.4)
Sorghastrum nutans	34.5 ab	32.8 ab	32.8 ab	32.1 a	35.2 b (1.0)

*Average marked with the same letter within the species are not significantly different at the level of p = 0.05

Table 2. The effect of salinity stress on the number of dry leaves, percent of dry mass, young leaf greenness SPAD and tolerance indices of ornamental grasses

Species	Salt concentration (g NaCl·dm^{-3}) (proportion to control in brackets)				
	0	5	10	15	30
Percentage of dry leaves					
Briza media	6.0 a*	14.6 b	18.3 b	28.8 c	61.2 d (10.2)
Deschampsia cespitosa	9.4 a	12.7 a	17.2 b	26.0 c	32.2 d (3.4)
Koeleria glauca	8.1 a	8.8 a	18.3 b	29.8 c	38.6 d (4.8)
Sesleria caerulea	4.9 a	7.0 b	10.5 c	12.5 d	27.1 e (5.5)
Sorghastrum nutans	12.3 a	21.7 b	31.4 c	37.8 d	45.0 e (3.7)
Percentage of dry mass					
Briza media	33.6 a	33.4 a	35.0 a	35.6 a	48.4 b (1.4)
Deschampsia cespitosa	33.5 a	32.2 a	37.6 ab	40.9 b	46.0 c (1.4)
Koeleria glauca	37.2 a	46.5 a	49.2 a	67.5 b	82.4 c (2.2)
Sesleria caerulea	55.8 a	51.0 a	56.4 a	60.8 a	59.8 a (1.1)
Sorghastrum nutans	43.0 a	44.2 a	47.0 a	39.0 a	39.0 a (0.9)
Young leaf greenness SPAD					
Briza media	35.4 a	37.1 a	42.2 b	43.5 b	43.6 b (1.2)
Deschampsia cespitosa	31.4 a	32.5 a	35.2 b	35.3 b	36.4 b (1.2)
Koeleria glauca	-	-	-	-	-
Sesleria caerulea	44.4 b	44.7 b	44.2 b	44.4 b	41.1 a (0.9)
Sorghastrum nutans	26.5 a	25.2 a	24.5 a	24.9 a	26.1 a (1.0)
Tolerance index (%)					
Briza media	100.0 d	78.2 c	72.1 c	61.1 b	45.7 a (2.2)
Deschampsia cespitosa	100.0 c	69.4 b	62.6 b	51.5 a	42.8 a (2.3)
Koeleria glauca	100.0 d	86.5 c	76.9 b	72.1 ab	65.1 a (1.5)
Sesleria caerulea	100.0 c	72.9 b	72.8 b	74.9 b	57.3 a (1.7)
Sorghastrum nutans	100.0 e	87.6 d	70.6 c	60.1 bc	49.2 a (2.0)

* Explanation as in Table 1

Salinity results in plant dehydration, which may also be analyzed based on the percentage content of dry mass. In this experiment, an increase in dry matter was only significant at 15 g NaCl·dm^{-3} in *D. cespitosa* and *K. glauca* (Table 2). Dry mass of *S. caerulea* and *S. nutans* was not different compairing with control even at 30 g NaCl·dm^{-3}. The contents of dry mass were higher 2.2 times in *K. glauca* and 1.4 times in *B. media*, and *D. cespitosa*. Glenn (1987) showed that all the 18 investigated grass species responded to salinity (60–540 mol·m^{-3} NaCl) by losing water. Also, Farooq and Azam (2006) showed a lower water content in wheat leaves under the influence of salinity (100-250 mM NaCl). A lack of an influence of salinity on changes in dry mass in halophytes was shown by Longstreth and Strain (1977) in *Spartina alterniflora* and by Wu et al. (1998) in *Spartina patens*. This suggests resistance of these species to substrate salinity due to only a moderate water loss. Moreover, a high percentage content of dry matter in plants not irrigated with saline in both these species is typical of halophytes, which was also shown by Glenn (1987). According to that author, halophytic grasses contain less water than glycophytic grasses grown with no salinity.

A higher value of the chlorophyll index in this study, was recorded in young leaves of *B. media* and *D. cespitosa* under the influence of substrate salinity of 15 and 30 g NaCl·dm^{-3} (Table 2). Withering of old leaves and probably root system damage could have also influence on an enhancement of photosynthesis and accumulation of its products in young leaves. Comparable values of the chlorophyll index in leaves of *S. caerulea* and *S. nutans*, irrespective of salinity levels, indicate lesser disturbances in the course of basic physiological reactions. Generally, salinity causes necroses, yellowing and a decrease of chlorophyll content in mature leaves. A reduction of chlorophyll content in leaves of *Hyacinthus orientalis*, under the influence of salinity, was reported by Türkoglu et al. (2011), Longstreth and Strain (1977) in *Spartina alterniflora*, as well as Nabati et al. (2013) in *Sorghum bicolor*. The negative effects of salinity connected with a reduction of the length of leaves and the withering of mature leaves may cause a greater efficiency of photosynthesis in young leaves. A decreased content of Na^+ ions in young leaves due to a low transpiration rate, caused a short life of those leaves and defense mechanisms. This contributes to a lesser damage of photosynthesis (Singla-Pareek et al. 2003).

The tolerance index may reliably illustrate the effect of substrate salinity on plants. In this experiment, it was shown that with an increase in salinity, its values decreased in all the tested grasses, even in the species that showed resistance reactions (Table 2). In this study, *B. media*, *D. cespitosa* and *S. nutans* had lower values of the tolerance index than *K. glauca* and *S. caerulea*. *K. glauca* under the influence of salinity caused by the greatest salt doses (15 and 30 g $NaCl \cdot dm^{-3}$) exhibited a high percentage content of dry mass, which in this analysis focused on the tolerance index based on plant dry mass and is confirmed by high values of this index. *B. media*, *D. cespitosa*, and *S. nutans* naturally grow on soils poor in nutrients. For this reason, they are not adapted to large concentrations of salt in soil solution. *K. glauca* are found in dry places and sandy soils (Krechowski et al. 2012). Probably due to drought resistance, it has large values of content of dry matter. *S. caerulea* grows on wet meadows and low peat bogs. According to Misra (2003), plants which prefer moist soils, are generally fast-growing, have a high nutrient uptake and can withstand fluctuations of wet/dry periods. They are able to utilize increased solubility of nutrients more.

CONCLUSIONS

1. *Briza media*, had the strongest negative response to all NaCl concentrations, even at 5 and 10 $g \cdot dm^{-3}$, manifested in the number of shoots, length of leaves, percentage of dry leaves and tolerance index.
2. Tolerance index was decreased in all genotypes already at 5 and 10 g $NaCl \cdot dm^{-3}$, the most in *D. cespitosa* and *S. caerulea*. At 30 g $NaCl \cdot dm^{-3}$, the lowest tolerance index had *D. cespitosa* and *B. media*.
3. Dry mass and leaf greenness were under the least influence of salinity stress.

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