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EFFECT OF SALINITY ON SELECTED PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF *Spartina pectinata* (Link.) 'AUREOMARGINATA'

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ABSTRACT

The aim of the present study was to examine physiological and morphological characteristics of *Spartina pectinata* 'Auremarginata' in response to various soil salinity conditions. Investigating the plant's response and time-point of potential adjustment to salinity stress will help determine the suitability of the species for growing in the roadsides. The effect of various levels of salinity on *S. pectinata* 'Aureomarginata' was examined. The NaCl was applied at five different levels ($g \cdot dm^{-3}$): 0 (control), 15, 30, 45, 60. The plant response was analysed after 14, 28, 42 and 56 days of the experiment. The highest concentration showed the strongest negative effects, which were indicated by a decrease in net photosynthesis rate (P_N), stomatal conductance (g_s), transpiration rate (E), specific leaf area (SLA), relative water content (RWC) and the number of shoots, number of young shoots and length of mature shoots. Plants have even some types of adjustment to stress conditions, at medium levels. This was especially valid for P_N and g_s after 28 days of the experiment. Principal component analysis revealed negative relationship of salinity level with P_N, g_s, E, RWC, SLA, number of young shoots and number of shoots, whereas a positive relationship was recorded with CMS, C_i, number of young leaves and leaf chlorophyll content (SPAD).

Key words: salinity, photosynthesis activity, membrane injuries, water content, leaf area

ABBREVIATIONS

RWC – relative water content in the leaves, CMS – cell membrane stability, SLA – specific leaf area, SPAD – content of chlorophyll, P_N – net photosynthetic rate, g_s – stomatal conductance, E – transpiration rate, C_i – intercellular CO_2 concentration

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INTRODUCTION

Salinity is a very important issue on a global scale. There is an increasing number of areas affected by elevated soil salinity in almost every world continent [Munns 2002]. One of the important reasons of soil salination is the use of sodium chloride for road thawing, especially in cities. This source is usually underestimated, due to its lack of connection with crop and horticulture plant productivity. This problem might be very important due to the amount of this compound used for de-icing the roads in many countries in Europe, USA and Canada [Howard and Maier 2007, Cunningham et al. 2008]. It is estimated that in cold regions, 9 to 10 million tons of sodium chloride, 0.3 million tons of calcium chloride and 11 million tons of abrasives are used annually [Ramakrishna and Viraraghavan 2005]. Using sodium chloride for road thawing may adversely impact on the soil properties along the roads. The direct effect of salination is a decrease in value of physical, chemical and biological properties of the soil, and indirect – a significant reduction in plant growth or death [Bryson and Barker 2002, Dai et al. 2012]. Plants growing along the roads reveal visible injuries, and faster senescence is observed [Ceksterea et al. 2008].

Sodium chloride used for de-icing the roads can be very harmful to plants. It is particularly dangerous when it accumulates on above-ground parts of plants, especially green ones in winter. In addition, in winter it does not leach so quickly into the deeper layers of the soil, due to less rainfall and frozen soil. In periods of thaw, when root growth begins, high concentration of sodium chloride causes dying of many plant species [Wrochna et al. 2006]. There is a disturbance of water homeostasis, reduction of water availability for plants and inhibition of cell growth as well as ionic homeostasis, excessive accumulation of Na^+, which causes interference in the uptake of other ions. The effect of this stress is usually the inhibition of photosynthesis. Respiration may increase or decrease. Initially, the growth is inhibited, and when the stress continues, the organs begin to gradually dry up and die out. Glycophytes, plants of non-salty habitats, react to salinity by rapid yellowing and dying of leaves. The reason for this is accumulating NaCl in these organs in excessive amounts. Halophytes, plants growing in saline areas, remove excess of salts from the organism or adapt to its tolerance. However, plant reactions to salinity in laboratory studies may be different, and species even closely related to each other may react differently, as well as at different stages of cultivation [Munns 2002].

The most important plant group for urban areas are perennials, because of surviving the winter without underground part. Trees and bushes accumulate Na^+ from brine [Bryson and Barker 2002]. Particularly interesting are grasses, that are widely distributed around the world and can survive in extreme conditions, due to many developed mechanisms of resistance. There are many species representing the *Spartina* genus that grow in nature on highly saline soils [Vasquez et al. 2006, Snedden et al. 2015]. They are halophytes, and hence are adapted to high salinity conditions [Levering and Thompson 1971, Warren et al. 1985]. This feature can be used in soil and water purification from high levels of ions. Wu et al. [1998] revealed adaptation of *S. patens* to high salinity, through regulation of ion transport and the capacity for their accumulation. Moreover, some species of this genus can also clean the soil from heavy metals [Eid 2011], which can be very useful in urban conditions with many influencing factors. *Spartina pectinata* (Link.) 'Aureomarginata' might have a huge potential for cultivation in urban conditions, and simultaneously it has many features attractive as an ornamental plant. What makes it special is the yellow margin of leaves. It grows strongly, up to 1.8–2.2 m in height. It has a strong stem. During flowering, it produces panicles consisting of several spike-like branches. *S. pectinata* has a wide distribution – from Newfoundland and Quebec to Arkansas, Texas and New Mexico. This is a good adapter species to various conditions. Attempts of reintroduction of this species to the seaside and ruderal areas in Canada were successful [Montemayor et al. 2008]. *S. pectinata* has strong rhizomes, thus it can grow in very compact soils. Moreover, it is a frost-resistant species, which makes it possible to cultivate in temperate climate conditions.

The aim of the present study was to examine physiological and morphological characteristics of *S. pectinata* 'Aureomarginata' in response to various soil salinity conditions. Investigating the plant's response and time-point of potential adjustment to salinity stress will help to determine the suitability of the species for growing in the roadsides.

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 2010, 2011 and 2012 involving *Spartina pectinata* (Link.) 'Aureomarginata'. Young plants, obtained from cuttings propagated in May, were transplanted on June 21, to pots of 0.750 dm^3 capacity. In each pot, the same weight of substrate was put and placed on a bowl to prevent uncontrolled outflow of saline. Plants were grown on the substrate consisting of high-moor peat with mineral sand washed at a 20 : 1 ratio (v : v). High-moor peat produced by Klasmann at pH 3.91 was limed on the basis of the neutralization curve to pH 6.40 using $CaCO_3$ at a dose of 7.0 $g\cdot dm^3$ peat. The substrate mixtures were supplemented with 2 $g\cdot dm^3$ of the fertilizer Peters Professional PL Special (20 : 20 : 20). Young plants grown for 6 weeks in these conditions were then watered with salt solutions. Before watering plants with salt solutions, the analysis of the medium was performed. The medium contained ($mg\cdot dm^{-3}$): N-NO_3 71.3, P 25.4, K 70.0, Ca 250.7, Mg 20.8 and Cl 28.6 at pH in H_2O 6.0 and EC $mS\cdot cm^{-1}$ 2.2. Plants developed 2–3 blades and 7–8 leaves. The mean length of the flag leaf was 65–69 cm. The content of chlorophyll *a* and *b* in flag leaf measured by SPAD averaged 46.

Treatment. There were following levels of salt NaCl ($g\cdot dm^{-3}$): 0 (control), 15, 30, 45, 60. In each variant, 100 ml of saline solution were poured into the pot, and 100 ml of water in the control. Prior to watering plants with salt solutions, they were watered to keep 50–55% moisture by weight of the substrate. Watering salt solutions was done once. While growing, plants were watered with water to 55–60% moisture by weight of the substrate. On the day after watering the plants with a solution of salt, the substrate was examined by the electrical conductivity (EC) method. Surface salinity ($mS\cdot dm^{-3}$) at the beginning of the experiment was in accordance with the increasing concentrations of NaCl: 1.4, 3.7, 6.5, 7.5, 9.8. Growth in saline lasted 8 weeks.

Measurements. Plant measurements consisted in determinations of the gas exchange measurements, relative water content (RWC) in the leaves [Gonzàlez and Gonzàlez-Vilar 2001], cell membrane stability (CMS) measured with the aid of conductivity [Bandurska and Gniazdowska-Skoczek 1995], specific leaf area (SLA) [Garnier et al. 2001] and growth parameters. Gas exchange measurements, SLA, CMS, and RWC were carried out one day before saline watering and then four times at intervals of two weeks. Handheld photosynthesis system Ci 340aa (CID BIOSCIENCE Inc., Camas, USA) was used to evaluate net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 (C_i) concentration. For these purposes, constant conditions of measurements in the leaf chamber were maintained: CO_2 inflow concentration (390 μmol (CO_2)$\cdot mol^{-1}$), photosynthetic photon flux density (PPFD) 1000 μmol (photon)$\cdot m^{-2}\cdot s^{-1}$, chamber temperature 23°C, relative humidity 40 ±3%. Growth parameters were measured in the last, eighth week of cultivation. The number of mature shoots (with a mature flag leaf) and of young shoots, the length of shoots (cm; the mean of three randomly selected, fully developed shoots per plant), the length of leaves (cm; the mean of three fully developed leaves on randomly selected blades per plant) and the number of dry leaves were evaluated. The leaf chlorophyll content (SPAD) measured in young leaves using a SPAD-502 Chlorophyll Meter was determined [Gregorczyk and Raczyńska 1997, Gregorczyk et al. 1998].

Statistical analysis. The data were analysed with the statistical software STATISTICA 13.1. Factorial ANOVA was employed to analyse an effect of salinity. The experiment was repeated for three years (2010, 2011, 2012). Each treatment of the salinity consisted of three replications (years) with twelve plants. Each plant was cultivated in a separate pot. Tukey's test was employed to analyze differences between measured parameters. A graphical presentation of Tukey's test results is provided in the present study. For determination of structure and rules in relations between variables, principal component analysis was used. In this analysis, the orthogonal transformation of observed variables to a new set of non-correlated variables (components) was performed.

RESULTS AND DISCUSSION

High salinity resulted in a decrease of such parameters as P_N, g_s, E, SLA and RWC of *S. pectinata* 'Aureomarginata' (Fig. 1 and 2). Also, graphical data presentation by principal component analysis revealed a negative relation between salinity and the parameters P_N, g_s, E, RWC and SLA, whereas a positive relation was recorded for salinity, C_i and CMS (Fig. 4). However, during the experiment, some salt stress defense mechanisms were observed, including an increase in P_N, g_s, E, SLA and RWC after 14 and 28 days of the experiment. Moreover, the E decreased by 50% in comparison to the control only in plants treated with the highest salt application of 60 g NaCl·dm^{-3}. Similar results were observed by Longstreth and Strain [1977], who found that S*partina alterniflora* maintained the P_N at a high level after application of salt at the levels 10 and 30 g NaCl·dm^{-3}. Similar observations were reported for salinized glycophytes and halophytes, including other grass species of C4 type of photosynthesis, for example *Spartina anglica* in Europe [Mallot et al. 1975] and *Distichlis spicata* in the USA [Warren and Brockelman 1989]. However, these authors described the resistance features at salinity levels between 15 and 30 g NaCl·dm^{-3}. Henschke [2017] defined *S. caerulea* and *S. nutans* as a halophyte, and in those grass, RWC did not decrease under the influence of salinity. Glenn [1987] also found that *Spartina* sp. should be considered a halophyte, as it survived an experiment with salinity at the level 30 g NaCl·dm^{-3}. Our investigations are in agreement with previous findings. In spite of a decrease of gas exchange parameters (P_N, g_s, E), this decrease was not higher than 50% of control values even at a salinity level of 45 g NaCl·dm^{-3}.

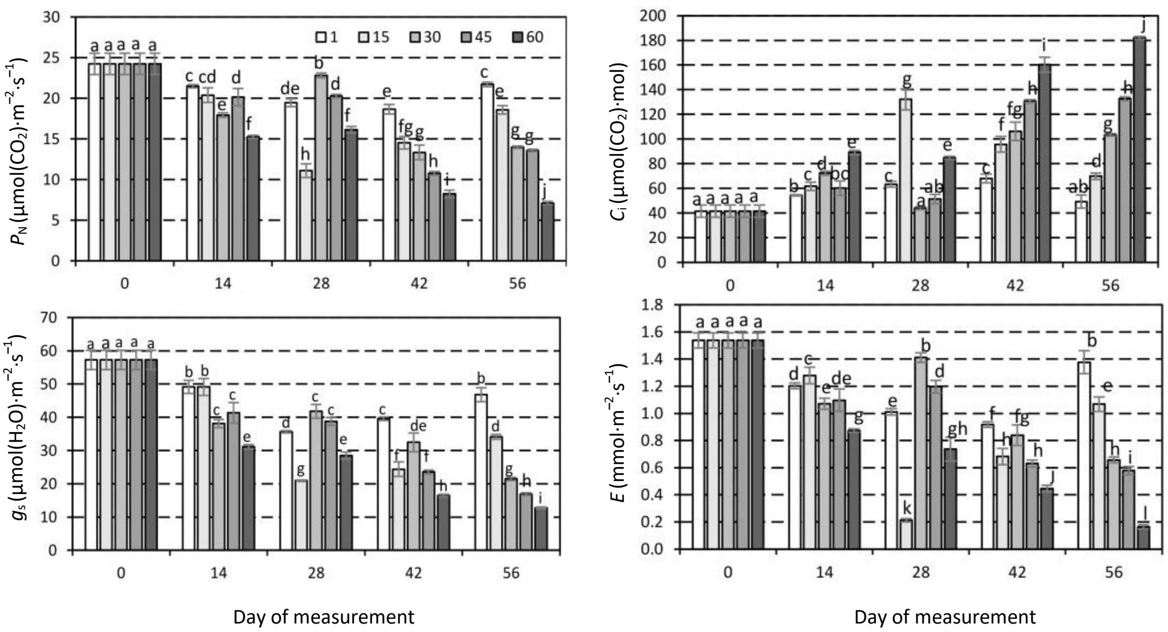

Fig. 1. Mean ± SE of net photosynthesis rate (P_N – µmol(CO_2)·m^{-2}·s^{-1}), stomatal conductance (g_s – µmol (H_2O)·m^{-2}·s^{-1}), intercellular CO_2 concentration (C_i – µmol(CO_2)·mol) and transpiration rate (E – mmol·m^{-2}·s^{-1}) of plants cultivated in different soil salinity level conditions at five times of measurement. Letters denote significant differences between means at p = 0.05

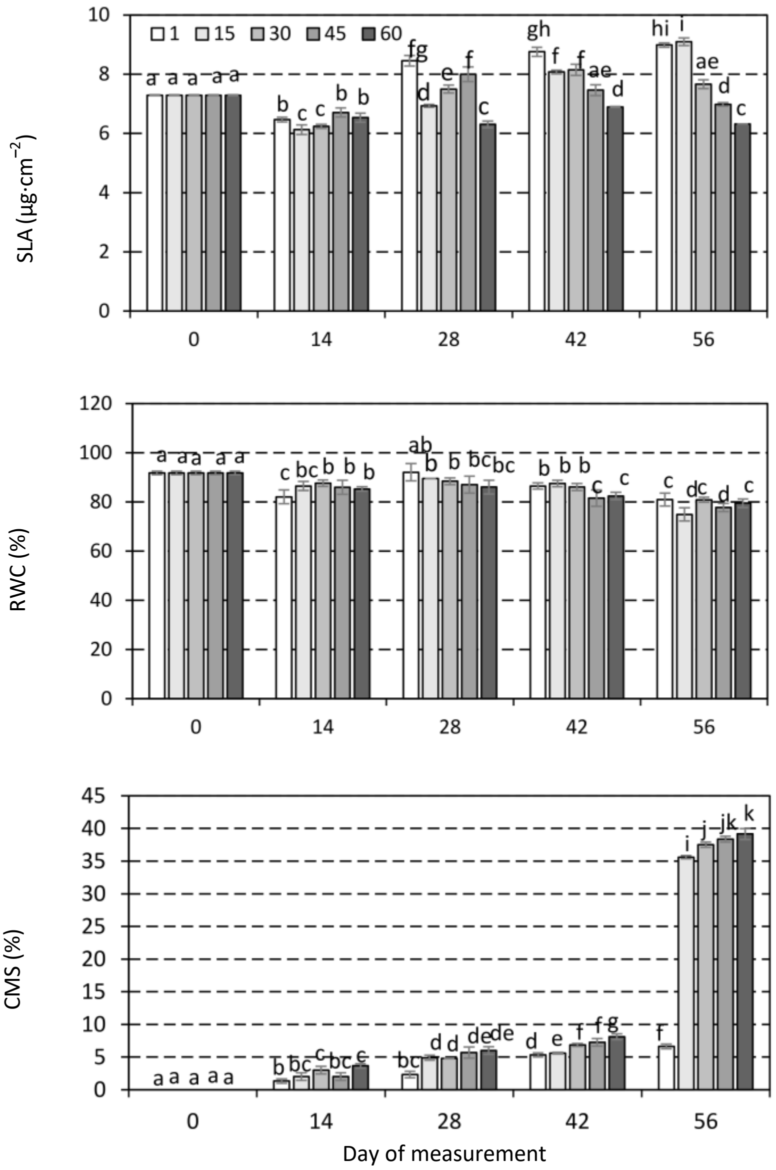

Fig. 2. Mean ± SE of specific leaf area (SLA – $\mu g \cdot cm^{-1}$), cell membrane injuries (CMS – %) and relative water content (RWC – %) of plants cultivated in different soil salinity level conditions in five times of measurements. Letters denote significant differences between means at p = 0.05

The effect of increased gas exchange parameters in the first part of the experiment might be related to adjustment to salinity stress conditions and activation of defense mechanisms. However, after a longer time of salinity stress (especially at a higher level), a gradual decrease of gas exchange parameters was observed (Fig. 1). The main reason for the negative effect of salinity on photosynthesis is limited access to water and an excessive level of such ions as Na^+ and Cl^- in the environment. Only specific adjustment systems of plants can promote survival in a highly saline environment. *Spartina* has developed a special salt gland in the leaf structure, which in the case of high salinity can discharge the excessive salt [Warren and Brockelman 1989, Maricle et al. 2009].

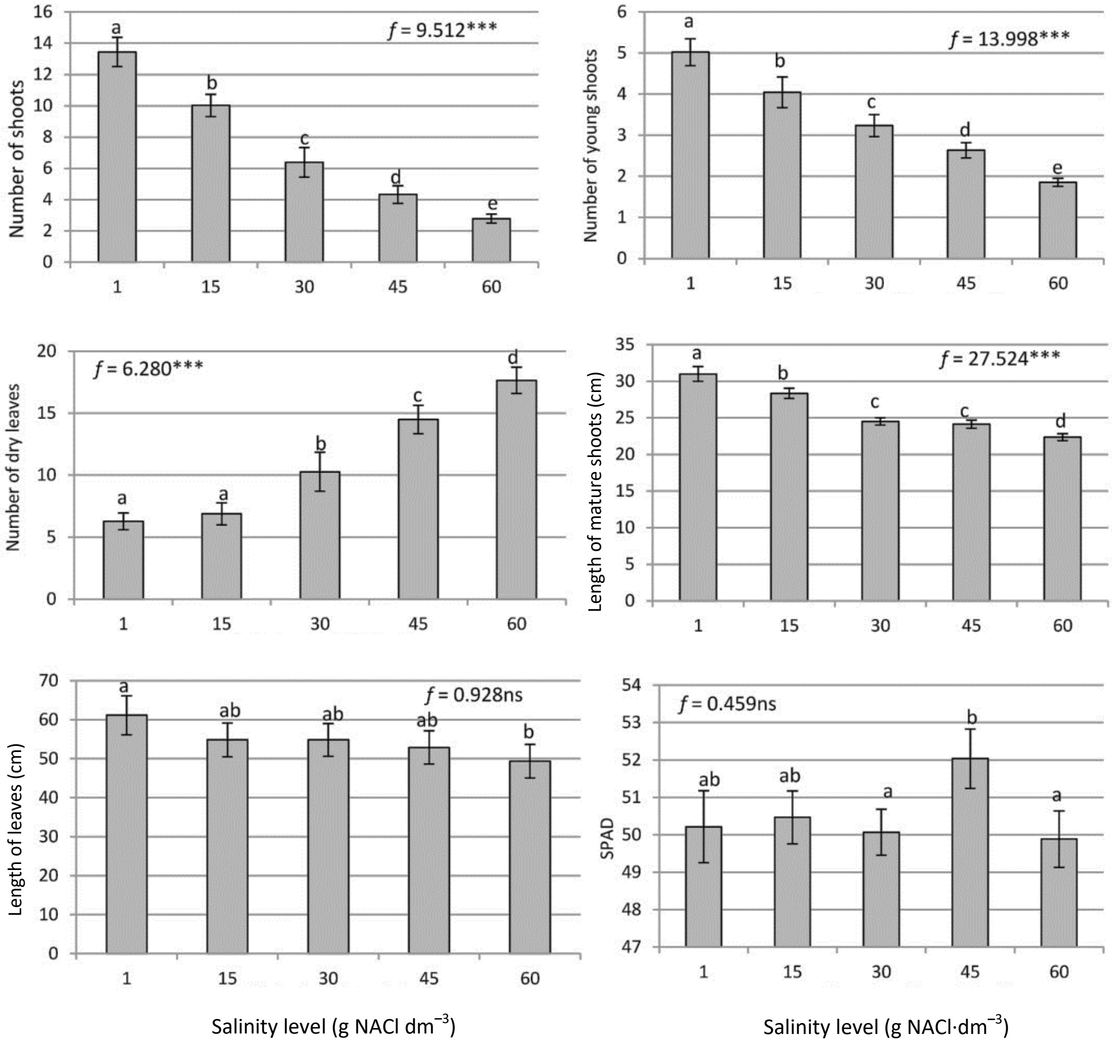

Fig. 3. Mean ± SE of morphological parameters and SPAD measured on day 52 of the experiment of plants cultivated in different soil salinity level conditions. Letters denote significant differences between means at p = 0.05

Here we found that salinity reduced the E value, but after 14 and 28 days there was an increase in plants treated with salt at a level of 15–45 g $NaCl \cdot dm^{-3}$ (Fig. 1). The plant transpiration response varies between plant species [Warren and Brockelman 1989, Qin et al. 2010]. Nabati et al. [2013], who tested resistance of *Sorghum bicolor* found changes of gas exchange parameters similarly as presented here – a decrease of P_N and E, and an increase of C_i. However, there were no differences in these parameters between various salinity levels at the end of the experiment. *S. bicolor* is treated as a species with medium level of resistance to saline stress [Maas et al. 1986]. On the other hand, Longstreth and Strain [1977] found that *S. alterniflora* at the end of an experiment with a higher salinity dose, revealed a decrease of E value. The reason for the temporary increase of E might be an increase of g_s [Radwan et al. 2000] or a decrease of water inception by roots. The second factor, in the case of our experiment, should be excluded, as RWC was noted at a relatively high level, at least at the beginning of the experiment (Fig. 2). However, the leaf structure was changed, which is revealed by the

SLA parameter. Leaf structure changes were also found by Longstreth and Strein [1977] during their experiment with *S. alterniflora*. They also found that together with leaf thickness, chlorophyll content also increases. This leaf structure change can also promote a higher photosynthesis level. In our experiment, SPAD was measured. Significantly higher levels were observed after 52 days only for the salinity level 30 g NaCl·dm^{-3} (Fig. 3). This was related to a decrease in photosynthetic parameters, although cell membrane stability (CMS) remained at a relatively low level (Fig. 2). CMS is accepted as a technique to screen for salt-tolerant wheat varieties [Farooq and Azam 2006]. The level of CMS increased along with salinity level during Na^+ accumulation and together with low values of RWC.

Plant growth was significantly lower after 52 days of the experiment in plants treated with salt (Fig. 3). Moreover, graphical data presentation by principal component analysis revealed that number of young leaves and SPAD was positively related to salinity level, whereas a negative relation was recorded for number of young shoots and number of shoots (Fig. 4). It is well known that salinity negatively affects the plants. Vasquez et al. [2006] found that salinity of the medium significantly reduces the number and length of shoots of *S. alterniflora*. On the other hand, our experiment revealed that even the highest salinity level caused a decrease in length of shoots and leaves only by 20–25% in comparison to the control. Moreover, the decrease in the number of young shoots was also above 50% in comparison to the control; this was valid only for the salinity level 45 g NaCl·dm^{-3}. A higher negative response was observed for number of mature shoots. It may suggest that an increase of P_N, g_s and E in the middle of the experiment caused a sudden increase in growth of young leaves. Based on this, we can also assume that it can cause a prolonged vitality of plants in salinity stress conditions. It is necessary to conduct additional investigations on salinity stress over a longer time.

In the present experiment, we found an increase in the number of dry leaves together with an increase of the salinity level. The values for plants treated with 60 g NaCl·dm^{-3} were three times higher in comparison to the control plants. Desiccation of leaves is one of the symptoms of saline stress. It is an effect of dehydration and accumulation of Na^+ and Cl^- ions in leaves. This was found previously for such plants as broad bean [Rabie and Almadini 2005] and grass species [Glenn 1987].

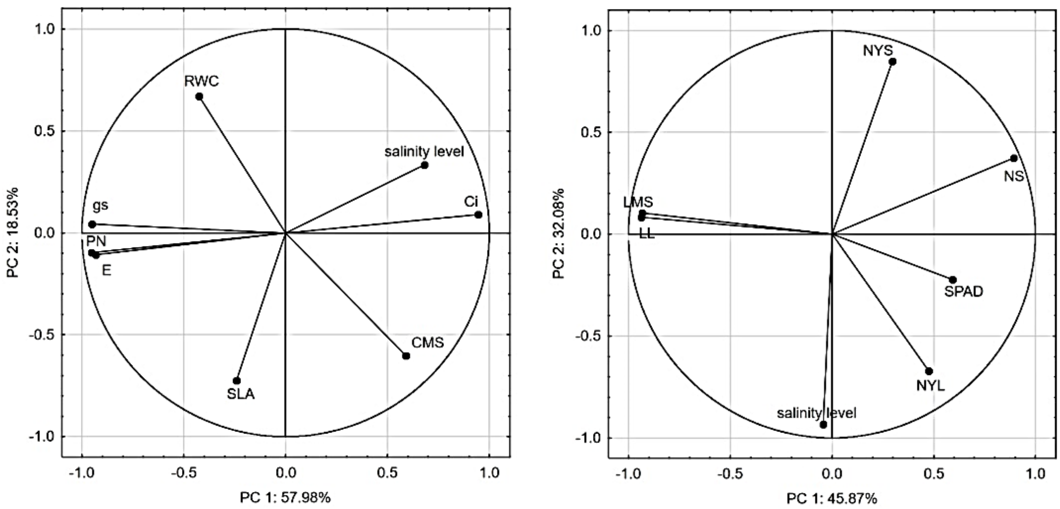

Fig. 4. Principal component analysis of gas exchange parameters, RWC, CMS and SLA (left) and morphological parameters (right). Abbreviations: NS – number of shoots; NYS – number of young shoots; NYL – number of young leaves; LMS – length of mature shoots; LL – length of leaves

CONCLUSIONS

Salinity stress caused a decrease of P_N, g_s, E, SLA and RWC in *S. pectinata* 'Aureomarginata', while a positive relation was recorded for C_i and CMS. Some defense mechanisms for salinity stress were noted during the experiment – an increase of P_N, g_s, E, SLA and RWC after 14 and 28 days of the experiment for plants with salt application at the level of 15–45 g $NaCl \cdot dm^{-3}$. Moreover, during 42 days of the experiment, P_N for plants treated with 45 g $NaCl \cdot dm^{-3}$ was recorded at a level higher than 50% in relation to control plants. After 52 days of the experiment, salinity caused a decrease of plant growth. However, even the highest dose caused a decrease not greater than 20–25% – this was valid for number and length of shoots. The number of young leaves also decreased no more than 50% in comparison to the control at a salinity level of 45 g $NaCl \cdot dm^{-3}$. This response might be a reason for longer vitality of plants in salinity stress conditions. This species can be a strong candidate for an ornamental plant growing in difficult environmental urban conditions.

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