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Assessment of Salt Stress Tolerance of Evergreen Ferns

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ABSTRACT

Ferns are an essential group of forest plants, however their salt tolerance is poorly understood. This study aimed to evaluate the effect of 50 or 100 mM sodium chloride (NaCl) on the growth, biomass, and ionic potassium (K^+), calcium (Ca^{2+}) and sodium (Na^+) concentrations in the fronds of evergreen ferns: *Cyrtomium fortunei* var. *clivicola*, *Polystichum setiferum,* and *Polystichum setiferum* 'Proliferum'. Visual quality of the plants immediately after the stress application and after the overwintering period was also carried out. Salinity resulted in the relative chlorophyll content reduction with exposure to increasing NaCl for all ferns. In *C. fortunei* var. *clivicola* and *P. setiferum,* salt stress led to a decrease in plant height, frond length, and weight of the above-ground part of plants as well as visual quality. For *P. setiferum* 'Proliferum' there was not a significant reduction in fresh and dry weight. Moreover, *P. setiferum* 'Proliferum' grown under salt stress maintained stable K^+ concentrations in the fronds and high aesthetic appearance both immediately after the stress factor and after several months. In conclusion, *P. setiferum* 'Proliferum' was relatively salt-tolerant and *Cyrtomium fortunei* var. *clivicola* and *P. setiferum* were sensitive to soil salinity.

Keywords: abiotic stress, Pteridophytes, salt, mineral ion, landscape plants.

INTRODUCTION

In urban areas, many factors limit the proper growth and development of plants. One of them is the excessive soil salinity (Francini et al., 2022). Salinization can be caused by, among other things, the use of salt to remove snow from streets and sidewalks in winter under a temperate climate (Jamshidi et al., 2020). Salinity leads to impaired uptake of water and nutrients, which translates into stunted growth and development of plants, browning, and death of tissues, individual organs, and eventually whole plants (Safdar et al., 2019). Saline soil is a particularly hostile environment for plants for landscape use. The search continues for ornamental plants that retain their aesthetic appearance despite stressful conditions (Sun et al., 2022). The negative effects of salt stress on ornamental plants can be attempted to some extent by using seed priming, application of organic and mineral amendments, inoculation with mycorrhizae, application of compatible solutes phytohormones, antioxidant molecules, plant growth regulators, mineral salts, nanoparticles, and biostimulants (García-Caparrós and Lao 2018; Byczyńska et al., 2023). However, one of the best method that can prevent salinization is the cultivation of salinity-tolerant species and cultivars (Guo et al., 2022).

Ferns are a valuable and sought-after group of landscape plants that have become increasingly important in recent years. They are characterized by great diversity in size, habit, shape, and color (Pomatto et al., 2023). Hardy ferns, due to their unique aesthetic qualities, have recently become increasingly popular perennials recommended for landscaped areas and urban gardens. Lush growth, low maintenance requirements, and longevity characterize garden ferns (Salachna and Piechocki, 2021). Ferns grow well in shady places, are an excellent part of mixed beds under trees and are helpful in covering large areas. Particularly valuable are species and cultivars of ferns with evergreen leaves, as they are ornamental all year round. There is still little ecological research on the response of ferns to urban disturbance and the use of ferns as a bioindicators (Bergeron and Pellerin, 2014). In general, ferns belong to glycophytes, i.e., plants adapted to growth under conditions of low salt concentration (Anderson, 2022). Under excess salinity, ferns exhibit abnormalities in their life processes (Bogdanović et al., 2012; Anderson, 2021), but their sensitivity to salt stress varies by genotype and is still very poorly understood. This study aimed to evaluate the effect of salinity stress on the growth of three evergreen ferns: *Cyrtomium fortunei* var. *clivicola*, *Polystichum setiferum* and *Polystichum setiferum* 'Proliferum.' The response of the fern taxa selected for the study to salinity stress is currently unknown. The study evaluated the morphological and physiological response of the above-described fern taxa to salt stress induced by watering the plants with NaCl solutions of varying concentrations. Visual quality of the plants immediately after the stress application and after the overwintering period was also carried out.

MATERIAL AND METHODS

One-year plants of the hardy ferns *C. fortunei* var. *clivicola*, *P. setiferum,* and *P. setiferum* 'Proliferum' (Dryopteridaceae) were used in the experiment. Each plant had several (5–7) properly formed leaves, on average 10 cm high and a very well-formed root ball. The plants were planted on June 15, 2020, into black PVC pots of 1.7 dm^3 filled with TS1 peat substrate (pH 6.0) with the addition of PG Mix fertilizer in the amount of 1 g dm^{-3}. The composition of the fertilizer was as follows (%): 5.5 $N\text{-}NO_3$, 8.5 $N\text{-}NH_4$, 16 P_2O_5, 18 K_2O, 0.8 MgO, 19 SO_3, 0.03 B, 0.12 Cu, 0.09 Fe, 0.16 Mn, 0.20 Mo and 0.04 Zn. Starting on July 13, 2020, the plants were watered four times every five days with sodium chloride (NaCl) solution at concentrations of 50 or 100 mM, using 100 ml of solution per plant. The control was plants watered with tap water with an electrolytic conductivity of 0.63 mS cm^{-1}. NaCl concentrations were chosen based on previous studies (Bogdanović et al., 2012). The findings of these studies demonstrated that ferns tolerated 50 and 100 mM of NaCl-enriched media, quite well. Each treatment (control, 50 and 100 mM NaCl) had a total of 27 plants per fern taxon, with three replicates of nine plants each in a randomized design. Plants were grown in a plastic tunnel under 75% shade until spring 2021. The temperaturea in the tunnel was controlled by automatically opening the upper roof vent. The average air temperature was as follows in 2020: June 20.5 °C, July 19.5 °C, August 22.2 °C, September 17.1 °C, October 11.8 °C, November 7.2 °C December 2.9 °C, and in 2021: January 0.5 °C, February 3.6 °C and March 8.5 °C. Thirty days after the last NaCl treatment, the plant height (the distance from the substrate surface to the top of the plant), and length of the longest frond were measured in 10 plants from each treatment, and the relative chlorophyll content (leaf greening index) was measured with a Chlorophyll Meter SPAD-502 optical apparatus (Minolta, Japan). Measurements in SPAD (Soil Plant Analysis Development) units were taken on three aligned fronds without necrosis located in the center of each plant, taking three readings on each frond. In 4 plants from each treatment, the aboveground part of the plants was cut and weighed, and after drying in the dark at 65 °C, the dry weight was determined. The dried and crushed leaf samples to a particle diameter of less than 1 mm were mineralized for one hour using 17 cm^3 of concentrated 96–97% sulfuric acid per 2 g of weight. Mineral contents of K^+, Ca^{2+} and Na^+ were determined by flame photometry on an AFP-100 flame photometer (Biotech Engineering Management UK) according to the methods of (Ostrowska, 1991), and each determination was performed in triplicate. To assess the visual score of the plants, a rating of all plants was carried out on September 1, 2020 by five people on a scale of 1 to 5 points, where a value of 1 meant low attractiveness, expressed in poor foliage, growth, and habit, and insufficient tolerance to salinity stress, 2 meant moderate foliar damage, 3 meant slight foliage damage, 4 meant good quality with minimal foliar salt damage (acceptable as landscape performance) and 5 meant maximum decorative effect, expressed in even growth, attractive habit, healthy foliage, and very good tolerance to salinity. The results were analyzed by analysis of variance (ANOVA) for a single classification separately for each taxon using the computer program Statistica, version 13.0 (StatSoft). The significance of differences between the obtained means ± standard deviation was evaluated using the Tukey HSD test at $\alpha = 0.05$.

RESULTS

Salt stress impacted plant morphological traits with various responses among fern taxon (Table 1). In *C. fortunei* var. *clivicola,* a higher dose of NaCl (100 mM) caused a significant reduction in plant height compared with the control.

Table 1. The effect of NaCl concentration on the plant height, frond length and relative chlorophyll content of evergreen ferns

Treatments	Plant height [cm]	Frond length [cm]	Relative chlorophyll content [SPAD]
C. fortunei var. *clivicola*			
Control	27.3 ± 2.82 a	29.7 ± 3.01 a	44.5 ± 2.11 a
50 mM NaCl	25.2 ± 1.63 a	26.3 ± 2.23 ab	40.3 ± 2.33 b
100 mM NaCl	20.5 ± 1.90 b	22.5 ± 1.36 b	37.7 ± 1.46 c
P. setiferum			
Control	18.5 ± 1.01 a	22.0 ± 1.67 a	33.0 ± 2.07 a
50 mM NaCl	15.6 ± 0.45 b	19.2 ± 1.15 ab	22.9 ± 1.15 b
100 mM NaCl	13.7 ± 0.26 c	17.3 ± 1.37 b	17.8 ± 1.55 c
P. setiferum 'Proliferum'			
Control	23.5 ± 1.10 a	28.9 ± 1.95 a	39.6 ± 0.59 a
50 mM NaCl	19.9 ± 1.29 b	23.6 ± 1.05 b	26.3 ± 1.57 b
100 mM NaCl	18.2 ± 0.31 b	24.0 ± 0.55 b	23.0 ± 1.13 c

Note: Within taxa, means with the same letters within a column are significantly different among treatments tested by Tukey's HSD test at α = 0.05.

In *P. setiferum* and *P. setiferum* 'Proliferum,' both doses of NaCl (50 and 100 mM) led to a reduction in plant height, while in *P. setiferum* 'Proliferum', the height of plants treated with 50 or 100 mM NaCl did not differ significantly. In *C. fortunei* var. *clivicola* and *P. setiferum,* salt stress induced by 100 mM NaCl resulted in shorter frond length compared to control plants. On the other hand, in *P. setiferum* 'Proliferum,' both applied NaCl concentrations significantly affected frond length shortening. In all fern taxa, the relative chlorophyll content (SPAD) decreased significantly with increasing NaCl concentration. At NaCl concentration of 100 mM, ferns had the lowest SPAD values. Compared to control plants, treatment with 100 mM NaCl resulted in a decrease in SPAD values by 15.3% (*C. fortunei* var. *clivicola*), 46.1% (*P. setiferum*) and 58.1% (*P. setiferum* 'Proliferum').

The fresh and dry weight of ferns subjected to salinity stress are presented in Table 2. *C. fortunei* var. *clivicola* treated with 100 mM NaCl showed a significant decrease in fresh and dry weight accumulation by 45.3% and 41.3%, respectively, compared to the control. In *P. setiferum,* a decrease in fresh and dry weight was recorded both at 50 mM NaCl (by 17.0 and 20.3%, respectively) and at 100 mM NaCl concentration (by 24.9%, respectively). In *P. setiferum* 'Proliferum,' there

Table 2. The effect of NaCl concentration on the fresh and dry weight of the above-ground part of evergreen ferns

Treatments	Fresh weight [g]	Dry weight [g]
C. fortunei var. *clivicola*		
Control	34.7 ± 4.14 a	9.56 ± 0.82 a
50 mM NaCl	29.5 ± 2.44 a	9.08 ± 1.12 a
100 mM NaCl	19.0 ± 3.78 b	5.61 ± 0.69 b
P. setiferum		
Control	29.5 ± 2.30 a	10.4 ± 0.34 a
50 mM NaCl	23.5 ± 0.61 b	8.23 ± 0.21 b
100 mM NaCl	24.5 ± 2.06 b	7.81 ± 0.70 b
P. setiferum 'Proliferum'		
Control	30.8 ± 2.06 a	11.6 ± 1.75 a
50 mM NaCl	32.3 ± 2.01 a	11.4 ± 0.67 a
100 mM NaCl	28.7 ± 0.55 a	9.32 ± 0.49 a

Note: Within taxa, means with the same letters within a column are significantly different among treatments tested by Tukey's HSD test at α = 0.05.

was no significant effect of salt stress on plant fresh and dry weight.

Under salt stress, leaf damage in the form of desiccation of individual leaves was observed in *C. fortunei* var. *clivicola* and *P. setiferum*; mainly when NaCl was applied at a concentration of 100 mM (Figure 1a,b). The deleterious effect of NaCl on plant quality is reflected by the significantly lower visual score obtained by *C. fortunei* var. *clivicola* and *P. setiferum* grown under stress conditions (Table 3). In *P. setiferum* 'Proliferum,' no severe leaf damage was observed as a result of salinity; however, at 100 mM NaCl, plants had a reduced visual score. Evaluating *P. setiferum* 'Proliferum' plants in spring showed that salinity stress had no significant effect on their visual score.

Table 4 shows the results of chemical analyses of the evaluated fern taxa exposed to salt stress. The salinity had no significant effect on K^+ content of ferns except for *P. setiferum*. In this taxon, treatment with 100 mM NaCl caused a significant increase in K^+ content (by 38.0%) compared to the control. As a result of salinity stress, *C. fortunei* var. *clivicola* showed no significant fluctuations in Ca^{2+} content. In contrast, *P. setiferum* and *P.* setiferum 'Proliferum' plants exposed to 100

Table 3. Visual scores assessed two times during the experiment of evergreen ferns as affected by the NaCl treatments

Treatments	Visual score	
	September 1, 2020	March 18, 2021
C. fortunei var. *clivicola*		
Control	5.00 ± 0.00 a	5.00 ± 0.00 a
50 mM NaCl	3.33 ± 0.58 b	3.67 ± 0.58 b
100 mM NaCl	2.33 ± 0.58 c	3.00 ± 0.00 b
P. setiferum		
Control	5.00 ± 0.00 a	5.00 ± 0.00 a
50 mM NaCl	3.67 ± 0.58 b	3.33 ± 0.58 b
100 mM NaCl	2.67 ± 0.58 b	2.33 ± 0.58 b
P. setiferum 'Proliferum'		
Control	5.00 ± 0.00 a	5.00 ± 0.00 a
50 mM NaCl	4.67 ± 0.58 ab	4.67 ± 0.58 a
100 mM NaCl	4.00 ± 0.00 b	4.33 ± 0.58 a

Note: Within taxa, means with the same letters within a column are significantly different among treatments tested by Tukey's HSD test at $\alpha = 0.05$.

Table 4. The effect of NaCl concentration on K^+, Ca^{2+} and Na^+ content (expressed in % dry weight) in fronds of evergreen ferns

Treatments	K^+	Ca^{2+}	Na^+
C. fortunei var. *clivicola*			
Control	1.30 ± 0.06 a	0.64 ± 0.06 a	0.30 ± 0.01 c
50 mM NaCl	1.17 ± 0.10 a	0.49 ± 0.07 a	0.48 ± 0.07 b
100 mM NaCl	1.33 ± 0.15 a	0.56 ± 0.04 a	0.58 ± 0.05 a
P. setiferum			
Control	1.21 ± 0.12 b	0.30 ± 0.03 b	0.16 ± 0.03 c
50 mM NaCl	1.16 ± 0.03 b	0.42 ± 0.10 b	0.37 ± 0.06 b
100 mM NaCl	1.67 ± 0.15 a	0.61 ± 0.07 a	1.03 ± 0.22 a
P. setiferum 'Proliferum'			
Control	1.13 ± 0.03 a	0.30 ± 0.08 b	0.33 ± 0.06 c
50 mM NaCl	1.19 ± 0.05 a	0.42 ± 0.03 ab	0.66 ± 0.04 b
100 mM NaCl	1.26 ± 0.07 a	0.45 ± 0.04 a	0.86 ± 0.10 a

Note: Within taxa, means with the same letters within a column are significantly different among treatments tested by Tukey's HSD test at $\alpha = 0.05$.

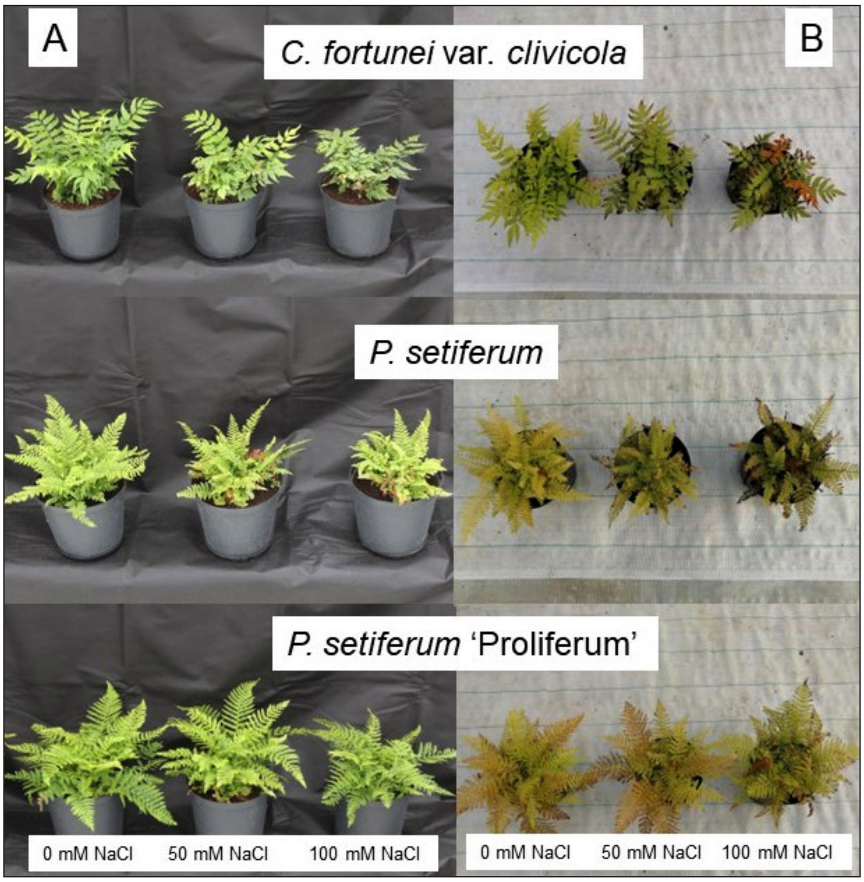

Figure 1. External appearance of the investigated fern taxa immediately after salt stress application (a) and after the overwintering period (b)

mM NaCl showed a marked increase in Ca^{2+} ions content by 103% and 50.0%, respectively, compared to the control. The increasing concentration of NaCl caused a significant increase in the accumulation of Na^+ in the tested ferns. In *C. fortunei* var. *clivicola*, *P. setiferum,* and *P. setiferum* 'Proliferum' as a result of the application of 50 mM NaCl, the content of Na^+ was increased by 0.6, 1.3, and 1.0 times, respectively, while at 100 mM NaCl, it was increased by 1.9, 6.4 and 2.6 times, respectively, compared to nonsalted plants.

DISCUSSION

Although many studies have been published on the response of plants to salt stress, most of these results concern seed plants (García-Caparrós and Lao, 2018). There is very little information on the salt tolerance of evergreen ferns. The present study evaluated the morph-physiological responses of evergreen ferns *C. fortunei* var. *clivicola*, *P. setiferum,* and *P. setiferum* 'Proliferum' to salt stress imposed by NaCl treatment. The evaluated taxa were characterized by different degrees of sensitivity to NaCl. At higher salinity (100 mM NaCl), the ferns *C. fortunei* var. *clivicola* and *P. setiferum* were characterized by a marked reduction in growth parameters such as height, frond length, and plant weight. In *C. fortunei* var. *clivicola* and *P. setiferum,* excessive salinity also led to the drying of leaves, which significantly reduced the aesthetic appearance of the plants. Visual foliage salt damage appeared immediately after the induction of stress in mature leaves and persisted until spring. Reduced height and reduction in the weight of the aboveground part as a result of salt stress were also observed in the evergreen ferns *Dryopteris affinis*, *Dryopteris atrata*, *Dryopteris filix-mas* 'Linearis-Polydactylon' (Salachna and Piechocki, 2021), *Dryopteris erythrosora* (Pietrak et al., 2023) and in the deciduous ferns *Dryopteris filix-mas* (Salachna and Piechocki, 2021), and *Athyrium nipponicum* 'Red Beauty' (Pietrak et al., 2023). Leaf damage in the form of necroses caused by NaCl salinity was shown in earlier studies in *D. affinis* and *D. atrata*, while no leaf damage was observed in *D.*

filix-mas, D. filix-mas 'Linearis-Polydactylon,' *A. nipponicum* 'Red Beauty' and *D. erythrosora* (Salachna and Piechocki, 2021; Pietrak et al., 2023). Reduced plant growth, inhibition of cell division, tissue death, and the appearance of necrotic spots on leaves are often typical harmful effects of salinity resulting from physiological and biochemical changes caused by high levels of salt (García-Caparrós and Lao, 2018; Sun et al., 2022). On the other hand, reduced growth and stunted cell growth under salt stress may not only be a symptom of damage but also one of the defense mechanisms against salinity since cells of smaller sizes are more resistant to stress (Safdar et al., 2019). The evergreen fern *P. setiferum* 'Proliferum' evaluated in the study, despite salt stress, was characterized by high visual scores and no signs of toxicity both immediately after the stress factor and after several months. Moreover, no significant decrease in fresh and dry weight was observed in P. *setiferum* 'Proliferum' plants grown under salt stress conditions.

Excessively high concentrations of mineral salts in the substrate lead to disturbances in physiological processes and ionic balance in plants (Arif et al., 2020). The consequence of salinity stress is often a decrease in the content of assimilatory pigments, which is confirmed by the results obtained in this study. A markedly lower chlorophyll concentration in leaves was also shown in studies on the evergreen ferns *Asplenium viride*, *Ceterach officinarum*, and *Phyllitis scolopendrium* exposed to high doses of NaCl (Bogdanović et al., 2012). The decrease in chlorophyll content in plants exposed to salinity could be due to the inhibition of CO_2 assimilation and a decrease in the intensity of photosynthesis (Hameed et al., 2021). Under NaCl salinity, there is an excessive accumulation of toxic Na^+ and Cl^- ions in plant tissues (García-Caparrós and Lao, 2018). In this study, increasing NaCl concentrations caused a marked increase in the accumulation of Na^+ for all tested ferns. The highest value of leaf Na^+ concentration (1.03% dry weight) was found in *P. setiferum* treated with 100 mM NaCl, which may explain the most remarkable slowdown in the growth and the occurrence of leaf damage in this species. High leaf Na^+ concentration (from 0.89 to 1.53% dry weight) as a result of 100 mM NaCl treatment was also previously shown in *D. atrata*, *D. affinis*, *D. filix-mas,* and *D. filix-mas* 'Linearis-Polydactylon' (Salachna and Piechocki, 2021). In the present study, despite salt stress, *C. fortunei* var. *clivicola* maintained homeostasis of K^+ and Ca^{2+} ions. Similarly, the absence of changes in the content of K^+ and Ca^{2+} was shown in *D. affinis* and *D. filix-mas* ferns treated with 50 and 100 mm NaCl (Salachna and Piechocki, 2021). In *P. setiferum,* at the higher dose of NaCl (100 mM), changes in the level of macronutrients involving an increase in the content of K^+ and Ca^{2+} were noted. On the other hand, in *P. setiferum* 'Proliferum' under salinity, tissue K^+ concentration did not change; only the content of Ca^{2+} increased slightly. Stable levels of K^+ or increased accumulation of K^+ and Ca^{2+} in leaves of ferns exposed to salinity may indicate the involvement of these ions in the activation of defense mechanisms (Amin et al., 2021). Since ion homeostasis is crucial for tolerance to salinity stress (Hussain et al., 2021), further extensive studies are needed to better understand the effects of NaCl on mineral accumulation in individual fern organs.

CONCLUSIONS

In summary, the evaluated taxa of evergreen ferns were characterized by different degrees of sensitivity to salt stress. The presence of NaCl in the substrate significantly reduced plant growth and weight of the above-ground part of plants in *C. fortunei* var. *clivicola* and *P. setiferum*. In both of these taxa, NaCl stress caused foliage damage. Plants of *P. setiferum* 'Proliferum,' despite salt stress, were characterized by no decrease in weight of the above-ground part of plants, high visual score, and stable leaf K^+ content. Among the evaluated fern taxa, *P. setiferum* 'Proliferum' should be considered relatively salt-tolerant.

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