PLANTS
IN URBAN AREAS AND LANDSCAPE

# EFFECT OF SUBSTRATE SALINITY ON GROWTH OF JUVENILE PLANTS *PYRUS PYRASTER* (L.) BURGSD.

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The responses of *Pyrus pyraster* L. Burgsd. to salinity stress in the juvenile stage of growth were studied, particularly the influence of different salt concentrations on seedlings growth, content of assimilation pigments and sodium ion distribution. Two years old seedlings were subjected to the salt treatment by addition of 60 and 120 mM NaCl solution to the substrate for 92 days. Control plants were saturated by water. The WinRhizo REG 2009 system was used for the analytical processing of the plant root system. The stem increment, leaf area, content of assimilatory pigments and allocation of the dry matter in the plant organs were also determined. The obtained data documented significant reduction in dry weight of the leaves (-36%) stems (-32%) and roots (-22%) in the treatment with higher concentration of NaCl (120 mM). Under low salinity treatment (60 mM NaCl) *P. pyraster* invested more dry matter to roots and maintained a balanced total dry weight per plant. In the response to salt treatments seedlings created coarser roots, maintained higher water content in the leaves and roots and changed ion balance in the plant organs.

**Keywords:** adaptability, salt stress, seedlings, roots, woody plants

## 1 Introduction

The application of de-icing salts in urban areas affects the chemical composition of urban soils and has a complex effect on trees. Salinity of soil and water is caused by the presence of excessive amounts of salts. Most commonly, high $Na^+$ and $Cl^-$ cause salt stress.

Salt stress reduces water potential and causes ion imbalance or disturbances in ion homeostasis and toxicity (Parida and Das, 2005; Galvani-Ampudia and Testerink, 2011). Salinity affects woody plants by inducing injury, inhibiting growth, and altering plant morphology and anatomy (Kozlowski, 1997). Negative impact of salinity stress is observed at the whole-plant level as death of plants or decrease in productivity.

Suppression of growth occurs in all plants, but there is quite large variability among species in their tolerance levels and rates of growth reduction at lethal concentrations of (Flowers et al., 1977; Greenway and Munns, 1980; Hernandez et al., 1995; Cherian et al., 1999; Takemura et al., 2000).

The variations in salt tolerance of species and genotypes of woody plants are well documented (Holmes, 1961; Monk and Peterson, 1962; Braun et al., 1978; Dirr, 1978; Francois and Clark, 1978; Tal, 1986). Only some tree species originated in central Europe tolerate salt stress. Generally, conifers, fruit trees (stone fruits) and any young trees are more prone to be damaged by salt than deciduous trees and trees older than 3–5 years (Šerá, 2017). Most fruit trees are sensitive to salinity, including *Malus domestica*, *Pyrus* ssp., *Prunus domestica*, *Prunus persica* and *Citrus* ssp. (Mc Kersie and Leshem, 1994). Most of the forest tree species growing close to roads are described as salt sensitive (Thornton et al., 1988; Sehmer et al., 1995; Alaoui-Sossé et al., 1998; Epron et al., 1999).

Tolerance of woody plants to salinity is changing in the course of life as they are relatively tolerant during seed germination, more sensitive during the emergence and young seedling stages and become progressively more tolerant with increasing age (Shannon et al., 1994). The variation in salinity tolerance may also depend on salinity level, or phenological stage of a plant (Tatar et al., 2010).

Munns and Tester (2008) reported 3 adaptation mechanisms of plants exposed to salinity stress: osmotic stress tolerance, $Na^+$ or $Cl^-$ exclusion and tissue tolerance to accumulated $Na^+$ or $Cl^-$. In the literature, the

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Plants in Urban areas and Landscape | 2020 | pp. 66–73 |
https://doi.org/10.15414/PUAL/2020.66-73

impact of salinity on plants and their responses to salt stress, as well as biochemical pathways of salt tolerance are widely discussed (Parida and Das, 2005; Tuteja, 2007; Munns and Tester, 2008; Craig and Moller, 2010). However, adaptations of roots and their relevance to salt tolerance especially in woody plant species are less recognized.

The aim of the present research was to assess the adaptive responses of *Pyrus pyraster* to salinity stress in the juvenile stage of growth. There is evaluated the influence of different salt concentrations in the substrate on seedlings growth, content of assimilation pigments and sodium ion distribution.

*P. pyraster* is a light-demanding tree tolerant to fluctuations in the soil water content (Paganová, 2003). The lifespan and aesthetic properties of *P. pyraster* are comparable with other urban tree species. This woody plant can increase the species diversity in urban conditions, and evaluation of the plasticity to abiotic stresses, including soil salinity is an important precondition of its wider utilization.

## 2 Material and methods

### 2.1 Plant material and growth conditions

Two-year old plants of *Pyrus pyraster* were grown from seeds collected in the location Tŕnie in Slovakia (for location characteristics see Table 1). The seeds were extracted manually after harvest and were subjected to a cold stratification treatment with temperatures ranging from -10 °C to +5 °C.

During the phenological growth stage "beginning of bud swelling", the seedlings were placed in plastic pots (90 mm in diameter, volume 0.47 l) with a fertilized peat-based growth substrate (20% black peat and 80% white peat moss, 0–5 mm fraction, pH of 5.5–6.5, enriched with nutrients 1.0 kg/m$^3$ NPK 14 : 16 : 18).

There were applied two salt treatments by addition of 60 or 120 mM NaCl solution to the substrate. In the salt treatments, the experimental plants were regularly saturated by the salt solution in the amount 3.5 ml per plant and day. Within the experiment a total amount 320 ml of the saline solution was applied per plant. Control plants were saturated by water. There were 20 replications for each treatment. The proposed sample size maintained sufficient data homogeneity.

The water content in the growth substrate was calculated based on wet weight (Trautmann and Richard, 1996) and maintained at 60% water as per the weight of the fully saturated substrate. The water regime was maintained by regularly weighing the pots on a Kern SD digital scale (max. = 8,000 g, standard deviation = 0.05 g) at 2-day intervals.

Potted plants were placed in the growth chamber PolEko KK 1450. The photoperiod of the growth chamber was set to a long light period of 14 h and a dark period of 10 h; the irradiation density on the surface of the uppermost leaves was 99.54 µmol/m$^2$/s. Air humidity was 65%; the temperature was maintained at 24 °C during the light period and 14 °C during the dark period. After 29 days of acclimatization, the plants were maintained under salt treatment for 90 days from March to June.

### 2.2 Measurement and analysis of plant parameters

The WinRhizo REG 2009 system (Regent Instruments, Canada, SK0410192) was used for the analytical processing of the plant root system. The following root parameters were measured: root length (mm), root surface (mm$^2$), root volume (mm$^3$), average root diameter (mm), number of root tips, and volume of particular root fractions (mm$^3$). The length of the primary stem of the experimental plants was also measured, and the total leaf area (*LA*) was determined by scanning fresh leaves using the ImageJ software.

The dry weight ($DW_R$) of the roots was determined after the plant material was dried at 105 °C until it has reached a constant weight. Other parameters calculated were the leaf water content (*LWC*), the specific root length (*SRL*), the specific leaf area (*SLA*), the root to shoot ratio (*R* : *S*), and the fine-to-coarse root volume ratio (*F*/*C*). Fine roots were defined as roots with $\phi$ <2 mm and coarse roots with $\phi$ >2 mm. The chlorophyll content (*CC*) in the leaves was measured with a spectrophotometer (model Lange DR 3900), following the procedures described by Lichtenthaler and Buschmann (2001). The chlorophyll content was expressed in relation to leaf

**Table 1** Climatic-geographic description of the original stand of mother plants

| Taxon | Location | Altitude (m) | Exposure | TI. (°C) | TVII. (°C) | Precipitation (mm) | Type | Subtype |
|---|---|---|---|---|---|---|---|---|
| ***Pyrus pyraster*** | Tŕnie | 540 | S | -3 | 18 | 750 | MW | W6 |

Source: Lapin et al., 2002
TI.– the average temperature in January; TVII. – the average temperature in July; MW – moderately warm region; W6 – moderately warm, humid, highland climate

area, as plants usually respond to salt stress by reducing the size of leaf area.

### 2.3 Statistical analysis

Mathematical and statistical data analysis was performed using the Statgraphics Centurion XVII software (StatPoint Technologies, USA, XVIII, license number: B480-E10A-00EA-P00S-60PO). The data were checked for normality (Shapiro-Wilk's test at significance level $\alpha = 0.001$) and homogeneity (Leven's test at a significance level $\alpha = 0.05$). All analyzed data showed a normal data distribution and met the assumption of homogeneity.

## 3 Results and discussion

According to the literature, *Pyrus* is regarded a plant with weak tolerance to salinity. The results presented here document a significant impact of salt treatment on growth of *P. pyraster* samplings and allocation of dry mater in plant organs (Table 2). The null hypothesis was adopted for the stem increment, *SLA*, Chl *b*, Chl *a*/*b* ratio, the root to shoot ratio and the fine-to-coarse root ratio.

The salt stress of plants often results in a considerable decrease in the fresh and dry weights of leaves, stems, and roots (Hernandez et al., 1995; Ali-Dinar et al., 1999; Chartzoulakis and Klapaki, 2000). In the presented experiment with *P. pyraster* seedlings, the total dry weight per plant was significantly reduced only under the treatment with 120 mM NaCl solution (-27%) compared to the Control. The reduction of dry weight was -32% for stem and -36% for leaves compared to the Control. In this treatment there were observed burned leaves and leaf tips on several seedlings and recorded plant mortality up to 35% due to excessive accumulation of salts, which can lead to death of tissues, organs and whole plants (Munns and Termaat, 1986; Munns, 1993).

*P. pyraster* invested more dry matter to roots (R : S = 1.02) and maintained a balanced total dry weight per plant at the low salinity treatment (60 mM NaCl). A similar growth reaction to salinity is referred by Kurban et al. (1999) for halophytic plant *Alhagi seudoalhagi* exposed to different treatments of NaCl solution, when total plant weight increased at low salinity (50 mM NaCl), but decreased at high salinity (100 and 200 mM NaCl) treatments. In agreement with the findings of Flowers et al. (1977), Greenway and Munns (1980) the growth suppression of *P. pyraster* seedlings was directly related to concentration of soluble salts in the substrate.

There were reduced the leaf area (-36%) and total chlorophyll content (-14%) of the *P. pyraster* seedlings under the higher salinity treatment (120 mM NaCl). Especially the Chl *a* content was negatively affected, while the Chl *b* and Chl *a*/*b* ratio remained stable even under the

**Table 2** One way ANOVA for selected aboveground and root parameters of two-year old seedlings of *P. pyraster* grown after 90 days of treatment in control and NaCl treatments (60 and 120 mM)

| Parameter | Treatment | |
|---|---|---|
| | ***F* value** | ***p*-value** |
| **Stem increment (mm)** | 1.70 | 0.1927 |
| **Leaf area (mm$^2$)** | 12.75 | 0.0000 |
| **Specific leaf area (mm$^2$/mg)** | 2.34 | 0.1064 |
| **Dry weight of stem (mg)** | 6.35 | 0.0034 |
| **Dry weight of leaves (mg)** | 9.42 | 0.0003 |
| **Dry weight (mg)** | 6.50 | 0.0031 |
| **Leaf water content (%)** | 17.46 | 0.0000 |
| **Chl *a*, *b* (mg/mm$^2$)** | 4.44 | 0.0166 |
| **Chl *a* (mg/mm$^2$)** | 5.37 | 0.0076 |
| **Chl *b* (mg/mm$^2$)** | 2.60 | 0.0843 |
| **Chl *a*/*b* ratio** | 2.19 | 0.1219 |
| **Total carotenoids (mg/mm$^2$)** | 7.25 | 0.0017 |
| **Root length (mm)** | 16.68 | 0.0000 |
| **Specific root length (mm/mg)** | 6.12 | 0.0042 |
| **Root surface (mm$^2$)** | 16.94 | 0.0000 |
| **Root volume (mm$^3$)** | 7.59 | 0.0013 |
| **Average root diameter (mm)** | 1.99 | 0.1476 |
| **Number of root tips** | 5.70 | 0.0060 |
| **Dry weight of root (mg)** | 8.83 | 0.0005 |
| **Root water content (%)** | 6.63 | 0.0027 |
| ***R* : *S* (root to shoot ratio)** | 2.29 | 0.1116 |
| **Volume of fine roots (mm$^3$)** | 15.28 | 0.0000 |
| **Volume of coarse roots (mm$^3$)** | 4.92 | 0.0112 |
| ***F*/*C* (fine to coarse root ratio)** | 1.87 | 0.1654 |

treatment with higher concentration (120 mM) of the salt solution. The reduction in the rate of leaf surface expansion is considered an immediate response to salt stress leading to cessation of expansion as salt concentration increases (Wang and Nii, 2000; Munns and Tester, 2008; Zörb et al., 2015). The reduction of leaf area has also been recorded by other authors. The decrease of total chlorophyll, Chl *a* and β carotene content by NaCl stress in the leaves of tomato is referred by Khavari-Nejad and Mostofi (1998). According to Parida and Das (2005) the chlorophyll content and total carotenoid contents of leaves decrease in general under salt stress. The development of chlorosis and fall of the oldest leaves with prolonged period of salt stress are referred by several authors (Hernandez et al., 1995; 1999; Gadallah, 1999; Agastian et al., 2000).

The salt treatment in both concentrations (60 mM and 120 mM) of the NaCl solution had a significantly negative effect on root length and root surface of the pear seedlings. The decrease in the root length was (-33%) and (-45%) with the application of 60 and 120 mM NaCl solution compared to the control. The root surface was reduced (-19%) and (-41%) by application of 60 and 120 mM NaCl solution compared to the control. Salt treatments inhibited the root elongation and growth of the lateral roots. It is documented by a significant decrease of the specific root length (-26%, -31%) of the seedlings grown under salt treatments, as well as a significant decrease in the number of root tips (-25%, 28%) compared to the control.

Under the salt treatment, the volume of the root system of *Pyrus* seedlings (Table 3) was negatively affected, especially in the fine root fraction. The volume of fine root fraction was significantly reduced by 25% and 45% with 60 and 120 mM NaCl, respectively. The seedlings created coarser roots, what is documented by a weak,

**Table 3** Plant parameters of *P. pyraster* seedlings after 90 days of salt treatment in the Control and NaCl treatments (60 and 120 mM). Presented data are means ±SE ($n$ = 20). Values followed by different letters differ significantly ($p$ <0.05)

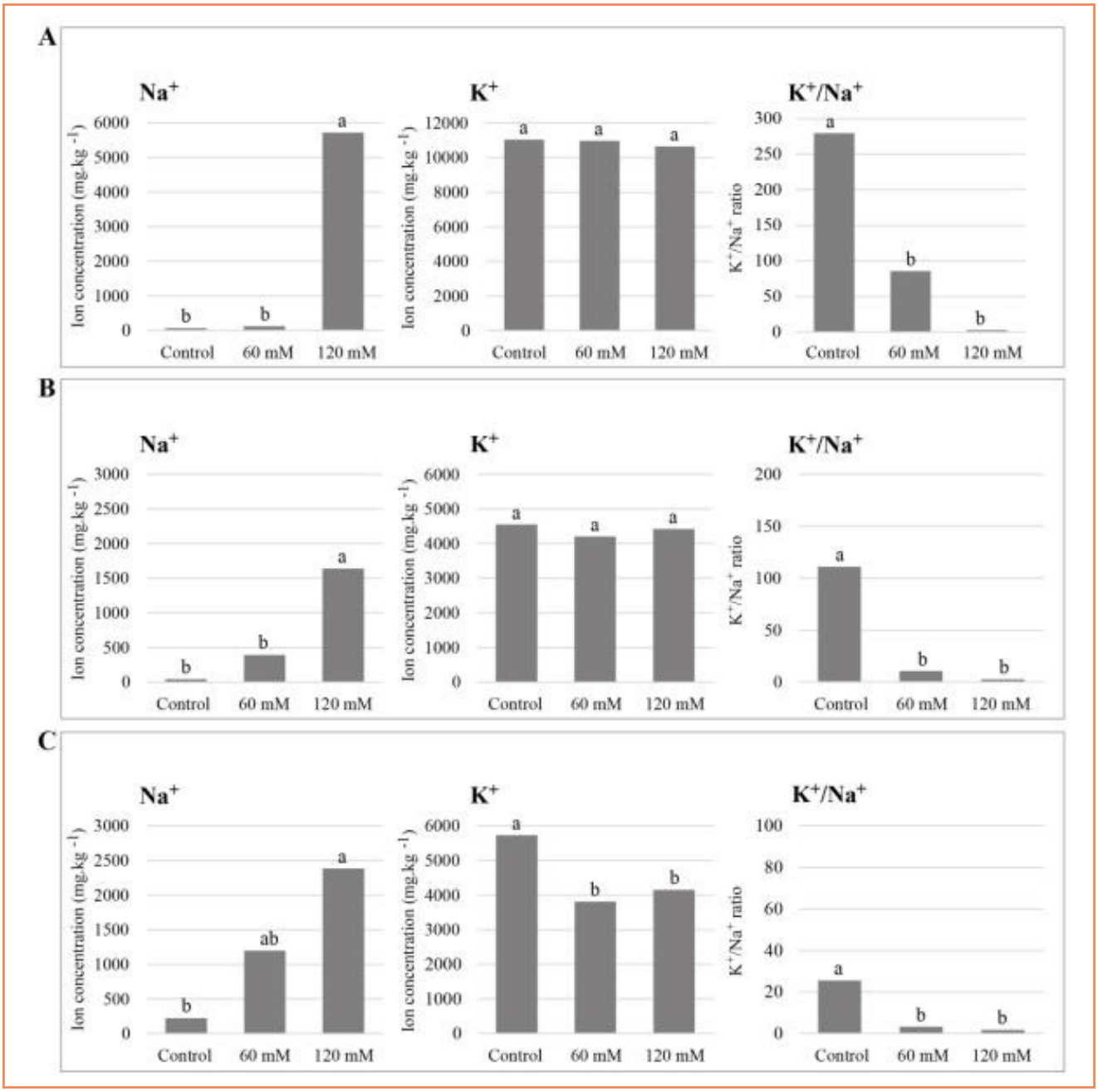
| Parameter | Control | 60 mM NaCl | 120 mM NaCl |
|---|---|---|---|
| **Stem increment (mm)** | 107.55 ±35.22 a | 101.65 ±30.72 a | 85.46 ±36.96 a |
| **Leaf area (mm²)** | 12,854.00 ±2,603.99 a | 10,868.50 ±2,734.66 a | 8165.97 ±2,421.67 b |
| **Specific leaf area (mm²/mg)** | 16.13 ±1.49 a | 14.03 ±4.88 a | 15.67 ±1.03 a |
| **Dry weight of stem (mg)** | 1,259.85 ±363.52 a | 1,219.10 ±420.82 a | 856.86 ±228.55 b |
| **Dry weight of leaves (mg)** | 810.66 ±202.54 a | 703.99 ±200.17 a | 519.21 ±140.81 b |
| **Dry weight (mg)** | 2,070.52 ±551.47 a | 1,923.09 ±592.19 a | 1,407.06 ±356.10 b |
| **Leaf water content (%)** | 41.76 ±1.90 b | 43.39 ±1.43 b | 46.42 ±3.39 a |
| **Chl *a* . *b* (mg/mm²)** | 681.19 ±77.52 a | 665.03 ±101.59 ab | 584.39 ±121.85 b |
| **Chl *a* (mg/mm²)** | 504.68 ±55.23 a | 487.00 ±71.94 a | 429.29 ±81.46 b |
| **Chl *b* (mg/mm²)** | 176.66 ±24.55 a | 178.19 ±30.12 a | 155.24 ±42.53 a |
| **Chl *a*/*b* ratio** | 2.87 ±0.21 a | 2.74 ±0.11 a | 2.81 ±0.25 a |
| **Total carotenoids (mg/mm²)** | 141.43 ±15.13 a | 129.96 ±13.97 ab | 121.77 ±17.40 b |
| **Root length (mm)** | 4,614.68 ±1,041.58 a | 3,609.08 ±1,019.65 b | 2,531.69 ±978.70 c |
| **Specific root length (mm/mg)** | 2.72 ±0.77 a | 2.02 ±0.74 b | 1.89 ±0.77 b |
| **Root surface (mm²)** | 11,033.40 ±2,079.68 a | 8,897.25 ±2,186.23 b | 6,549.64 ±2,295.67 c |
| **Root volume (mm³)** | 6,239.55 ±1,696.34 a | 5,794.70 ±1,787.16 a | 3,991.47 ±1,409.78 b |
| **Average root diameter (mm)** | 0.85 ±0.12 a | 0.91 ±0.13 a | 0.96 ±0.19 a |
| **Number of root tips** | 749.20 ±198.10 a | 562.83 ±211.52 b | 537.00 ±205.27 b |
| **Dry weight of root (mg)** | 1,753.00 ±347.86 a | 1,887.35 ±449.55 a | 1,360.87 ±289.39 b |
| **Root water content (%)** | 48.37 ±2.69 ab | 46.12 ±3.09 b | 50.87 ±5.59 a |
| ***R* : *S* (root to shoot ratio)** | 0.88 ±0.19 a | 1.02 ±0.23 a | 0.99 ±0.21 a |
| **Volume of fine roots (mm³)** | 1,533.25 ±359.10 a | 1,146.10 ±329.68 b | 838.86 ±404.28 b |
| **Volume of coarse roots (mm³)** | 4,706.30 ±1,568.81 a | 4,648.61 ±1,664.85 a | 3,152.60 ±1,178.69 b |
| ***F*/*C* (fine to coarse root ratio)** | 0.35 ±0.12 a | 0.28 ±0.13 a | 0.27 ±0.15 a |

but gradual increase of the average root diameter from 0.85 mm (control) to 0.96 mm (120 mM NaCl).

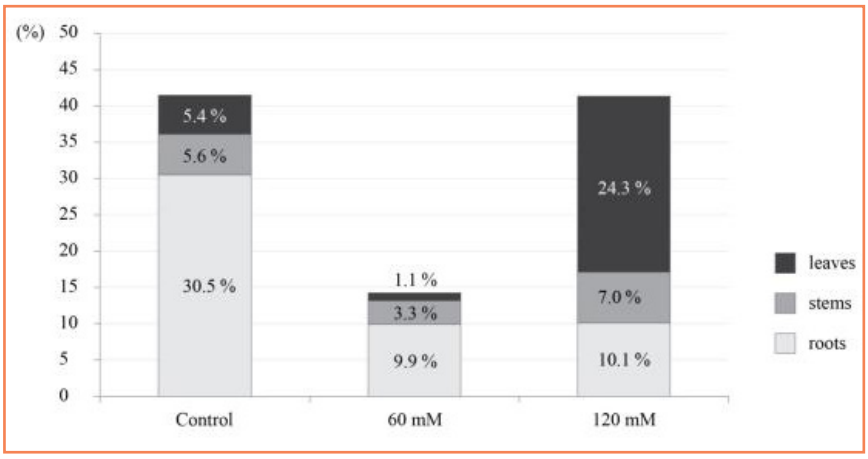
Despite the mentioned reduction of the absorptive surface of the roots, under salt stress, *P. pyraster* significantly increased the water content in roots and leaves (Table 2), what is probably related to the need maintain the physiological processes in the plant body.

Under the low salt treatment (60 mM), the accumulation of dry mass in the root did not change significantly compared to the control, but at a higher concentration (120 mM) the decrease of the $DW_R$ parameter was significant (-22%), the $R : S$ ratio did not change significantly under different treatments (Table 3).

The presented data for root and shoot dry matter of *P. pyraster* are in agreement with findings of Laffray et al. (2018), who reported stronger reduction in aerial biomass than in root system of *Q. robur* seedlings under different salt treatments. However, *P. pyraster* significantly reduced the absorptive root surface, root length and volume of fine root fraction even under




■ **Figurere 1:** The ion content ($Na^+$, $K^+$, $K^+/Na^+$) in the leaves (A), stems (B) and roots (C) of two-year old seedlings of *P. pyraster* after 90 days of treatment with $NaCl^-$ solutions (60 and 120 mM) and in the control. Values followed by different letters differ significantly ($p$ <0.05)




■ **Figurere 2:** Distribution of the ion content $Na^+$ in plant organs (roots, stems and leaves) of *P. pyraster* seedlings calculated from the total amount of $Na^+$ that was delivered in the substrate within 90 days of the treatment by NaCl solution (60 and 120 mM)

low salt concentrations. These data indicate quite high sensitivity of the fine root structures of *P. pyraster* seedlings to salt stress.

After 90 days of salt treatment, the accumulation of $Na^+$ the roots *P. pyraster* seedlings grown with 60 and 120 mM NaCl was 5 and 10 times higher than the control content (Figure 1). The statistically significant difference in the ion content was found in the stem between the control plants and salt treatment with 120 mM NaCl. A significant difference in $Na^+$ content in the leaves was found only in treatment with 120 mM NaCl. The significant increase in $K^+$ content was recorded in both salt treatments. The values of $K^+/Na^+$ ratio decreased in the root growing zone from 25.49 in control to 3.19 and 1.74 in 60 and 120 m M NaCl treated roots respectively.

In a study by Huffaker and Wallace (1959) it was found that $Na^+$ appears to be excluded at or below the shoot-root transition zone and this may account for the relatively later appearance of $Na^+$-induced salinity symptoms.

The distribution of $Na^+$ in the plant organs (Figure 2) clearly indicates retention of the salt ions in the roots and stems of *P. pyraster* seedlings. The relative distribution of the salt ions was calculated from the total amount of $Na^+$, that was delivered in the substrate within 90 days of the treatment of *P. pyraster* seedlings by NaCl solution (60 and 120 mM). In both salt treatments, the functional retention of $Na^+$ ions is evident in the roots and stems. The control plants did not retain $Na^+$ ions in the root and stem tissues (Figure 2). The mentioned retention was effective under treatment with low concentration (60 mM) NaCl, when only 1.1% of the total amount of $Na^+$ delivered in the substrate was transported to the leaves. These data support the opinion that differences between leaf and woody tissues in $Na^+$ concentrations suggest a functional role of wood in preventing $Na^+$ accumulation in leaves (Ziska et al., 1991). However, the retention of $Na^+$ ions in wood is effective only up to the certain level of the salt concentration in the substrate. This barrier is finally overcome in salt stressed species, and the $Na^+$ accumulation in leaves which later ensues is typified by either mottling or by tipburn in stone fruit trees (McKersie and Leshem, 1994).

## 4 Conclusion

The obtained data documented the ability of *P. pyraster* to cope with moderate salt stress (60 mM NaCl) via morphological adaptations of the root system (created coarser roots at the expense of the root elongation and fine root fractions). Despite reduction of the absorptive surface of the roots under salt stress, seedlings significantly increased the water content in roots and leaves, and maintained balanced growth of the aboveground organs and chlorophyll content in the leaf tissues.

The mentioned root adaptive responses, as well as retention of sodium ions in the wood tissues (roots and stems) were insufficient to maintain adequate water uptake and prevent transport of the sodium to leaves in conditions of sever salinity treatment (120 mM NaCl). Therefore, salt stress induced a strong reduction in growth of *P. pyraster* seedlings.

This study should be completed within a more detailed evaluation of the responses of *P. pyraster* to salinity. The topic has to be explored in more detail before utilization of this woody species in urban areas where it is often exposed to the stress from application of de-icing salts.

## Acknowledgements

This work was supported by the AgroBioTech Research Centre (ITMS 26220220180). The research was supported by a grant from the Cultural and Educational Grant Agency of the Ministry of Education, Science, Research, and Sport of Slovak Republic (KEGA):

"Development and implementation of the standards for urban greenery management." Project registration number 003SPU-4/2017.

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