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Salinity tolerance traits in three grass species

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CHAPTER 3

Root architecture and productivity of three grass species under salt stress

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ABSTRACT

Salinity not only directly affects above-ground plant growth and development, but also root system architecture and functioning. The mature root system of grasses composes of primary and seminal roots with their laterals, and crown roots. The root structure strongly influences the plant's ability to take up water and nutrients, thereby affecting plant health and tolerance to abiotic stress. Three grass species, *Lolium perenne*, *Festuca rubra* and *Puccinellia maritima* from habitats with different salinities were screened for salt-induced changes in root architecture. Scans of roots were used to quantify root structural architectural traits using the RootNav software package. The results show that: 1) salinity affects root system architecture differently in the three-grass species studied: in *Festuca rubra* and *Puccinellia maritima*, salinity promoted primary root growth: 2) seminal root length of *L. perenne* and *P. maritima* was stimulated significantly by salinity, whereas in *F. rubra* no stimulation occurred up to 150 mM NaCl and seminal root length was inhibited at 200 mM. And 3) salinity inhibited lateral root growth in *L. perenne*, but promoted lateral root growth in both *F. rubra* and *P. maritima*.

Key word: Root system architecture (RSA), salt stress, salinity tolerance, *Lolium perenne*, *Festuca rubra*, *Puccinellia maritima*

INTRODUCTION

Soil salinity severely limits the productivity and quality of crops and, therefore, poses a threat to food security throughout the world. Among the various abiotic stress factors, soil salinization is one of the most serious land degradation threats to modern agriculture. Increasing and more extensive salinization of arable land, combined with the projected growth of the human population, necessitates using more salt-tolerant crop plants.

The main functions of plant roots are anchoring the plant and the uptake of nutrients and water. Root system architecture (RSA) alludes to the spatial configuration of the root system and combines several structural features such as the length of primary roots and their spread, the number, angle and length of lateral roots and responds to external environmental conditions such as water availability, nutrition and ion concentrations in the soil (Khan *et al.*, 2016). Grasses (Poaceae) have seminal roots, which consist of primary axes, formed during embryogenesis and already present in the un-germinated caryopsis, and the laterals which emerge from these with time (Leszek, 2012). Water and nutrient capture depend largely on laterals of seminal roots and rhizosphere properties (Ahmed *et al.*, 2018; Carminati *et al.*, 2017; Boudiar *et al.*, 2020). Under salt stress, the RSA of grasses can be reshaped in various ways. Salt stress has been shown to block primary root meristem division and inhibit cell elongation, causing inhibition and alterations to cell morphology, in combination with changing root length and the number of primary, lateral and seminal roots (Potters *et al.*, 2007).

As RSA is highly variable and responds plastically to environmental conditions, it is considered a new target for breeding efforts. An efficient RSA improves plant growth and health by improving nutrient and water uptake under stress conditions, including salinity (Li *et al.*, 2018; Berg *et al.*, 2018). Salt-marsh grasses offer natural examples of plants with high salt tolerance and understanding their properties and strategies could yield critical parameters to be used in screening for crop plant varieties with potentially high salt tolerance. Native salt-marsh species have adapted to frequent flooding with sea water and are considered to be potentially highly salt tolerant (Rouger and Jump, 2015). *Puccinellia maritima*, or common salt-marsh grass, is one of the dominant grass species on salt marshes in Western Europe and is an important food source for migrating geese in early spring (Fokkema *et al.*, 2016). *Festuca rubra* is another dominant species on saltmarshes but can also be found on non-saline soils (Gray and Scott 1977; Rouger *et al.*, 2014).

In this study, we compared these two salt marsh grass species to *Lolium perenne*, a perennial pasture grass, widely used as fodder for cows and sheep, that can potentially also be used for biofuel purposes (Jauhar, 1993). To identify the relevant traits for salt-stress tolerance, we measured the development of the root systems and productivity of the three species under both control and saline conditions.

MATERIALS AND METHODS

Caryopses of grasses were germinated in 5-liter containers filled with vermiculite moistened with tap water. After germination, uniform seedlings with one leaf were selected and trans-

ferred to plastic pots filled with vermiculite and placed in a culture room (temperature 22/18°C, light/dark: 14 h/8h, 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The seedlings were watered with tap water every day till the third leaf reached 3 cm. After that, the seedlings were watered with a 25% Hoagland solution for 4 days. At day 18 after germination, the salt treatment started. The 25% Hoagland solution was supplemented with NaCl. To avoid salt shock, the salinity was applied gradually with stepwise increases of 25 mM NaCl, every 2–3 days, until the final concentrations of 50, 100, 150 and 200 mM were reached. Plant samples were taken at 3, 6, 9 and 12 days after the final NaCl concentration was reached. Each pot contained three plants, and for each treatment three replicates were used.

When sampling, whole plants were carefully removed from the pots and rinsed under running tap water to remove any adhering vermiculite, avoiding any damage to the roots. Subsequently, the roots were stained in a solution of 0.5 g/L Neutral Red for 10 min and rinsed 5 times in distilled water. The roots were spread out to reduce crossing roots as much as possible on an Epson 10000 flatbed scanner controlled by WinRhizo, Arabido 2009 software. The images of the scanner were cropped to size and color inverted with Paint and then analyzed with RootNav 2.0 software, making distinctions between primary roots, seminal roots and lateral roots (Figure 3.1 for a schematic representation of the root architecture). Since there is no option for seminal roots in this software, the primary roots were marked as 1st primary and the other seminal roots were marked as sequential lower order primaries. The statistical analysis of ANOVA using the Tukey test was performed using Prism (version 8 for MacOs, GraphPad) and R studio.

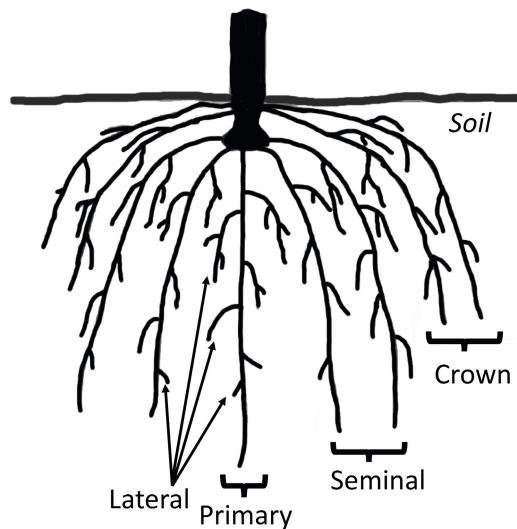


Figure 3.1 Schematic representation of the fibrous root system architecture common in graminaceous species. The root types (primary, seminal and lateral) that were measured are indicated (adapted after Sparks and Benfey, 2017). Under the culture conditions used in this study no crown roots were formed. Fresh weight of roots and shoots were measured directly after scanning of the root system. Three replicate seedlings in one pot were weighed together to increase accuracy since individual seedlings were still small.

For determination of the RSA parameters, the 12-day old plants of *L. perenne* were too large to be analyzed by RootNav. The analysis of *Lolium* plants could, therefore, only be followed up to day 9. To determine the effect of salinity on growth rate and RSA, root and shoot weight and its ratio denoted as R/Sh, the relative ratios of results under 100 or 200 mM NaCl and control treatments were calculated according to formulas 3.1 and 3.2

$$\frac{(\text{value}_{100\text{mM}} - \text{value}_{0\text{mM}}) / \text{value}_{0\text{mM}} \times 100\%}{\text{value}_{0\text{mM}}} \quad (3.1)$$

$$\frac{(\text{value}_{200\text{mM}} - \text{value}_{0\text{mM}}) / \text{value}_{0\text{mM}} \times 100\%}{\text{value}_{0\text{mM}}} \quad (3.2)$$

RESULTS

EFFECT OF SALINITY ON ROOT LENGTH

Salinity affected the primary root length (PRL) differently in the three-grass species studied (Figure 3.2). The PRL of *Lolium perenne* and *Puccinellia maritima* was increased when exposed to NaCl, but *Festuca rubra* showed a significant increase ($P = 0.05$) at intermediate salt concentrations, a stimulation that was no longer apparent at 200 mM NaCl.

The seminal root length (SRL) of the three-grass species also showed an increase with salinity, but this difference was not significant. Compared with the PRL, the SRL of *L. perenne* was much shorter. The SRL ranged from 6% to 45% of the PRL in *L. perenne* and *F. rubra* and was not affected significantly by salinity. In contrast, the SRL of *P. maritima* was very low at 0 mM NaCl (4%) but increased strongly (by 50–62%) when exposed to salinity.

The effect of salinity on lateral root length (LRL) differed significantly between the three species. The LRL of *L. perenne* was highest in the control plants and was reduced significantly at the higher salinity levels. In contrast, the LRL of *F. rubra* was highest under high salinity (100, 150 and 200 mM NaCl) and was lower at 0 and 50 mM NaCl. In *P. maritima* the LRL was relatively high under all salinity conditions but was not strongly affected by high salinity levels.

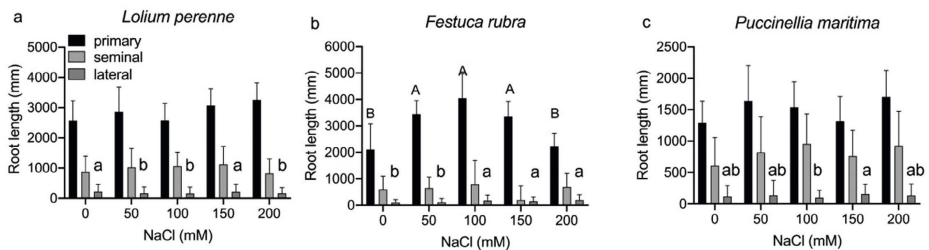


Figure 3.2 Effect of salinity on root length after 9-day of exposure to NaCl. Samples were taken at 3-day, 6-day, 9-day and 12-day after start of the salt treatment. Five salt concentrations were used: 0mM (control) 50mM, 100mM, 150mM, 200mM NaCl (n= 9). Labels of letters above the bar indicate the significant difference at level $P < 0.05$ with turkey test. The bars without letters are not significant different.

EFFECT OF SALINITY ON ROOT ARCHITECTURE

Root architecture was strongly affected by salinity stress as shown in Table 3.1. The total SRL increased in *L. perenne* at 100 mM NaCl compared to control, but the contribution of the lateral roots decreased (lateral root total length by -32% and -36% at 100 and 200 mM NaCl, respectively). *F. rubra* showed the most positive increases among three grasses. Apart from the seminal root number (a moderate decrease of -3% and -13% at 100 and 200 mM NaCl, respectively) all the other 6 parameters of root architecture increased at both 100 and 200 mM NaCl, the highest being the lateral root total length by 39%. However, for primary root, lateral root and seminal root (except for number of seminal root), an inhibition by 200 mM compared to 100 mM NaCl was apparent.

Each treatment has 9 replicates. Indicated is the percentage change after 9 days of exposure to NaCl in the nutrient solution compared to the control treatment.

Contrary to *F. rubra*, with *P. maritima* the highest stimulation was observed at 200 mM: both primary and seminal total root length of *P. maritima* increased at both 100 and 200 mM relative to the control. The seminal total length increased by 126% at 100 mM NaCl. Although the primary and seminal roots showed an increase at 100 mM, the lateral roots showed a slight decrease. This trend continues at 200 mM: the 7 root traits consistently increased in *P. maritima* especially with the seminal total length (87%), while the lateral length was not affected (1 to 2% in lateral mean length).

■ **Table 3.1. Percentage NaCl-induced change in root architecture parameters**

	NaCl (mM)	Primary length	Seminal number	Seminal mean length	Seminal total length	Lateral number	Lateral mean length	Lateral total length
<i>L. perenne</i>	100 ¹⁾	0	1	1	37	-6	-19	-32
<i>F. rubra</i>	100	86	-3	11	8	134	92	279
<i>P. maritima</i>	100	16	34	1	126	-6	-20	-12
<i>L. perenne</i>	200 ²⁾	27	-5	-5	9	-13	-14	-36
<i>F. rubra</i>	200	6	-13	15	0	46	75	128
<i>P. maritima</i>	200	29	11	11	86	25	1	60

¹⁾ comparison of 100 mM NaCl to control

²⁾ comparison of 200 mM NaCl to control

Each treatment has 9 replicates. Indicated is the percentage change after 9 days of exposure to NaCl in the nutrient solution compared to the control treatment.

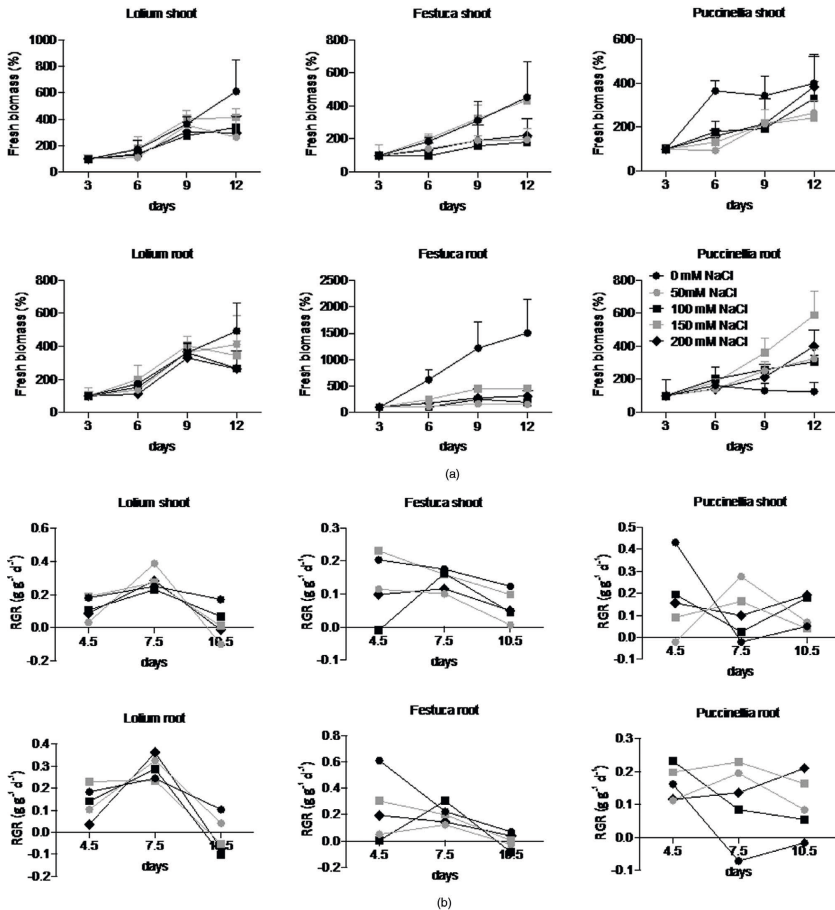


Figure 3.3 Effect of salinity on root and shoot productivity of three grasses. Uniform germinated seedlings with one leaf of *F. rubra*, *P. maritima*, and *L. perenne* were transferred to pots with vermiculite and watered with 25% Hoagland solution. Salt treatments started ~17-18 days after germination. Salt was gradually added to plants to avoid the salinity shock. End concentration NaCl were 0, 50, 100, 150, 200 mM, samples were taken at 3, 6, 9, 12 days after start of the salinity treatment ($n=3$). The top 6 panels indicate the increase in biomass in shoot and root in % (biomass at day 3 = 100%). The bottom 6 panels indicate the calculated relative growth rate (RGR) based on the data in the top panels.

P. maritima increased at both 100 and 200 mM relative to the control. The seminal total length increased by 126% at 100 mM NaCl. Although the primary and seminal roots showed an increase at 100 mM, the lateral roots showed a slight decrease. This trend continues at 200 mM: the 7 root traits consistently increased in *P. maritima* especially with the seminal total length (87%), while the lateral length was not affected (1 to 2% in lateral mean length).

EFFECT OF SALINITY IN SHOOT AND ROOT PRODUCTIVITY

Figure 3.3 shows the development of root and shoot fresh weight relative to the fresh weight present at day 3. Comparing the overall pattern of biomass increase for the three species, we observed very distinct differences. Although in all species growth was highest in the control (with the interesting exception of *P. maritima*, where prolonged exposure did show more or

equal growth in the presence of NaCl), comparing the immediate (day 6) and post-acclimation (day 12) does yield some interesting differences. In *L. perenne*, the exposure to salt did not result in a significant inhibition of growth. Up to day 9, there was identical growth in both shoot and root in all treatments. However, at day 12 the high NaCl treatments did lead to such a strong effect that the root biomass showed a negative trend. In *F. rubra*, exposure to salinity induced an immediate growth reduction in both root and shoot and this growth reduction was present at a constant level throughout the whole 12 days period. A third pattern is observed in *P. maritima* where NaCl did have an instant effect leading to growth inhibition at day 6. However, this effect was transient, and at day 12 the growth in the presence of NaCl was as vigorous as under control conditions. In all three species, the observed patterns were most manifest in root biomass, but were also visible in the shoot development.

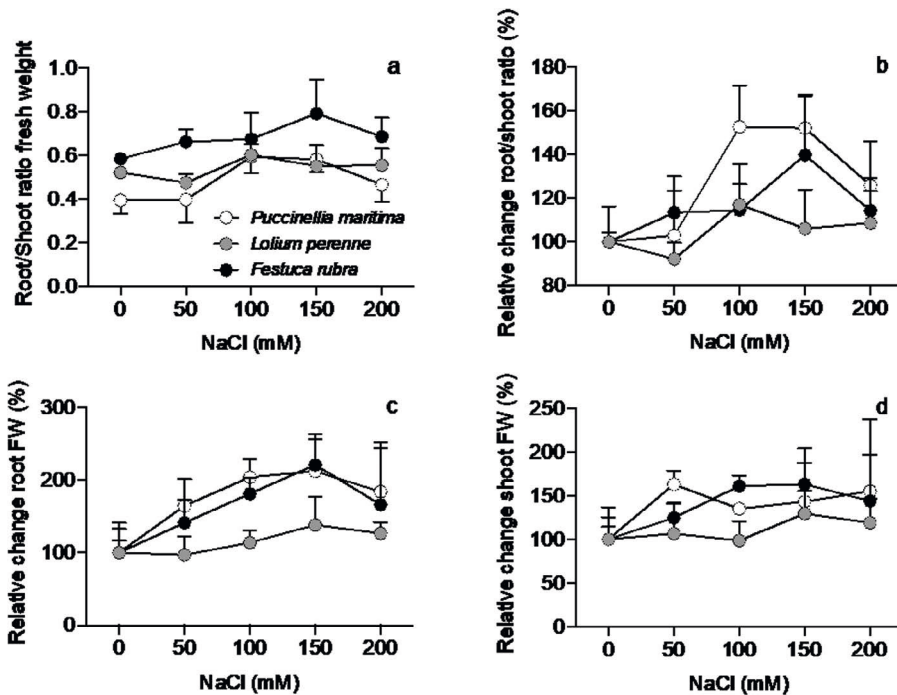


Figure 3.4 Effect of salinity on root shoot ratio after 9 days of growth. See legend to Figure 3.3 for details. Fresh Root shoot (R/Sh) ratio, salt to control Fresh R/Sh ratio and salt to control fresh root and fresh shoot were calculated based on fresh root and shoot weight. (a) Fresh Root Shoot weight ratio. (b- d) The R/Sh, Root weight and Shoot divided by the control, respectively (n=9).

EFFECT OF SALINITY ON ROOT TO SHOOT RATIO

The fresh weight data at day 9 were used to analyze the effect of increasing salinity on the root the shoot ratio (R/Sh). In Figure 3.4a, the ratios are directly plotted and although already differences the effect of salinity can be discerned, the difference became more apparent when we calculated the relative (compared to control) salinity induced change in R/Sh (Figure 3.4b).

The relative R/Sh of *L. perenne* did not significantly change, whereas in both *F. rubra* and *P. maritima* the R/Sh increased with salinity, only to drop again at the highest NaCl concentration. This increase in R/Sh in the two salt marsh species was caused by a relative increase in the root fresh weight with salinity, while the shoot biomass was not affected. In *L. perenne*, NaCl did not induce a similar increase in root biomass, resulting in a constant R/Sh.

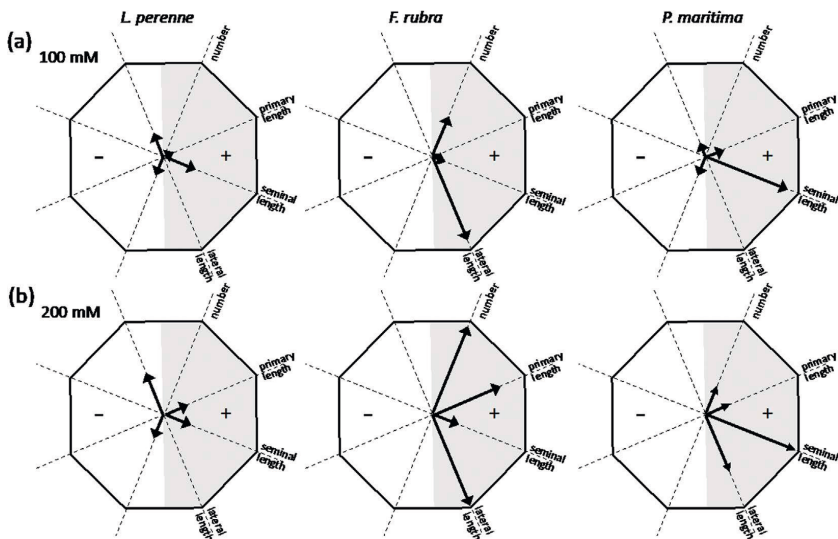


Figure 3.5 The effect of 100 and 200 mM NaCl on the following root architecture parameters: root number, primary root length, seminal root length and lateral root length. An arrow pointing to the white (left hand) half indicates a decrease in the indicated parameter, an arrow pointing to the grey (right hand) half indicates an increase in the parameter value. (a) The effect of 100 mM NaCl compared to the control conditions, (b) the effect of 200 mM NaCl. The changes are relative to the control (no salt) values and calculated by: $(index_{100mM} - index_{0mM}) / index_{0mM} \times 100\%$ for 100m compared to control and $(index_{200mM} - index_{0mM}) / index_{0mM} \times 100\%$ for 200mM compared to control.

DISCUSSION

Salinity had a significant effect on RSA in all three species in this study. However, the way the root system architecture was affected differed between species. The patterns are summarized in Table 3.2. In the comparison between the species, we have mainly taken data of day 9 of the salinity treatment, a time point when the detrimental effects of high salinity were not yet apparent in *L. perenne*, the most salinity sensitive species. At the higher salinity levels, the *L. perenne* showed a reduction in biomass (negative growth) at day 12 and although the patterns were even more pronounced, it is likely that from that day on physiological processes were not within the normal operational range and would have introduced an unwanted bias.

Most of the results we obtained confirm that *L. perenne* is a moderately salt sensitive species. In short-term experiments, the plant behaves as an excluder, maintaining growth when

exposed, probably by excluding Na⁺ from entering the plant. This strategy fails at longer exposure periods as is evident from the reduction in root biomass at day 12.

F. rubra and *P. maritima* exhibit radically different patterns, both different from *L. perenne*, but also from each other. At moderate levels of salinity, *F. rubra* increases strongly in the number and length of its lateral roots (Figure 3.2 and Tables 3.1, 3.2), reduces its shoot growth (Figure 3.3) and increases R/Sh ratio. All these adjustments to the growth pattern indicate an acclimation to a more restricted uptake of water and nutrients (Koevoets *et al.* 2016). Effectively, the *F. rubra* invests in the root system, increasing its uptake potential, while simultaneously decreasing the demand for nutrients (reduction of shoot growth) and water (smaller shoot leads to less evaporating leaf surface).

■ **Table 3.2. Salinity-induced changes in root architecture after 9-day of exposure**

	100mM NaCl ¹				200mM NaCl ²			
	PL	SL	LL	LN	PL	SL	LL	LN
<i>L. perenne</i>	+	+	--	--	++	+	--	-
<i>F. rubra</i>	+++	++	++++	+++	+	+	++++	++
<i>P. maritima</i>	++	++++	--	-	++	+++	+++	++

¹) comparison of 100 mM NaCl to control $(\text{index}_{100\text{mM}} - \text{index}_{0\text{mM}}) / \text{index}_{0\text{mM}} * 100\%$

²) comparison of 200 mM NaCl to control $(\text{index}_{200\text{mM}} - \text{index}_{0\text{mM}}) / \text{index}_{0\text{mM}} * 100\%$

PL represent Primary root length; SL represent Seminal root Length; LL represent lateral root length and LN represent lateral root number.

The relatively percentage were calculated by Salt effect: $(\text{salt-control})/\text{control} * 100\%$

0~10%: +; 10%~50%: ++; >50%: +++; >100%: ++++; >200%: +++++

0~10%: -; -10%~-50%: --; <-50%: ---; <-100%: ----; <-200%: -----

P. maritima also increases its effective root system, but does so by increasing its primary, seminal and lateral root system. Remarkable is the stepwise increase: increasing primary and seminal roots at moderate salinity and mainly lateral root at the highest NaCl concentration. The overall growth of *P. maritima* (both shoot and root growth) is not significantly affected after an acclimation period of 3 to 6 days, making *P. maritima* in our study the most salinity tolerant species.

Although both *F. rubra* and *P. maritima* were effective in coping with salinity, the differences do confirm the relatively higher salinity tolerance of *P. maritima*, true to its ecological niche on the lower salt marsh. This comparative study clearly shows that grasses that are (highly) adapted to saline conditions are capable of restructuring their architecture by specifically investing in the development of lateral and seminal roots, increasing their capacity to take up water and nutrients. In breeding programs, these traits could be exploited, with the caveat that these traits could come at a cost, as they are in our examples associated with (temporary) reduced above ground productivity.

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