BSc thesis in Biology

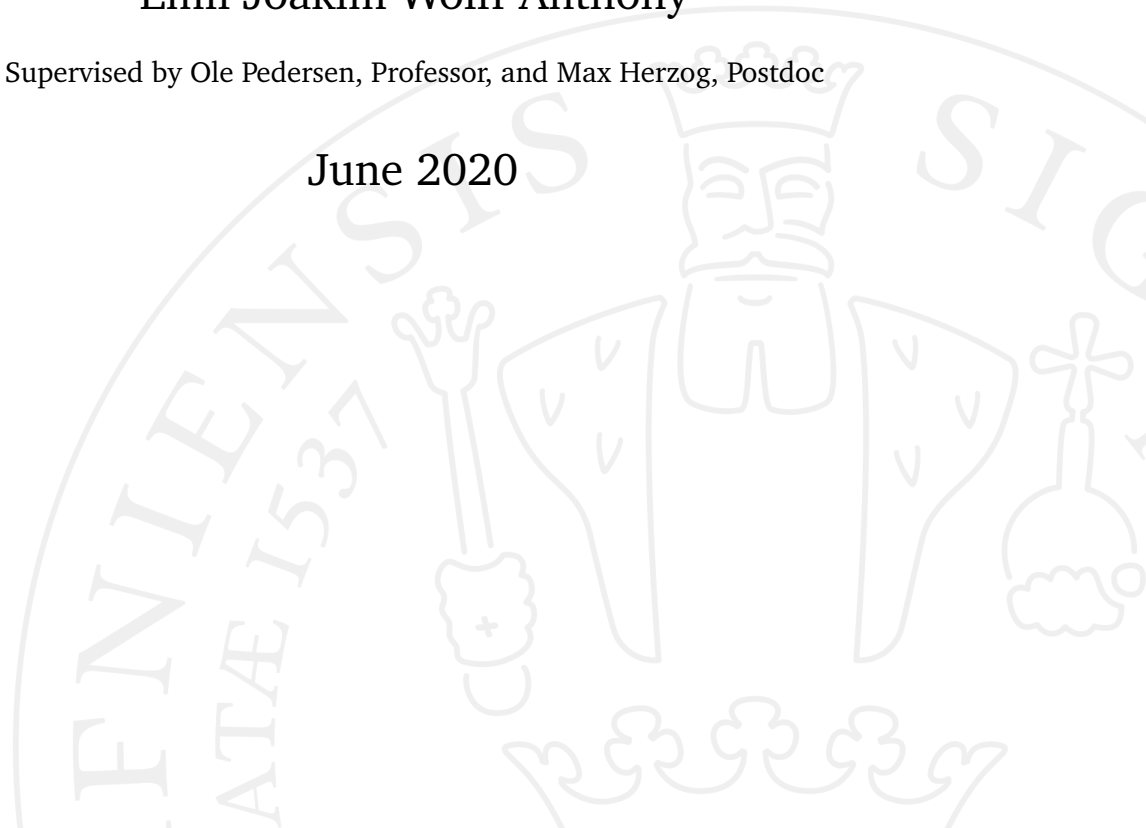# Salinity tolerances in crops and wild plants — and the effect of a root apoplastic barrier to radial oxygen loss on salt intrusion in rice

Freshwater Biological Laboratory

Emil Joakim Wolff Anthony

Supervised by Ole Pedersen, Professor, and Max Herzog, Postdoc

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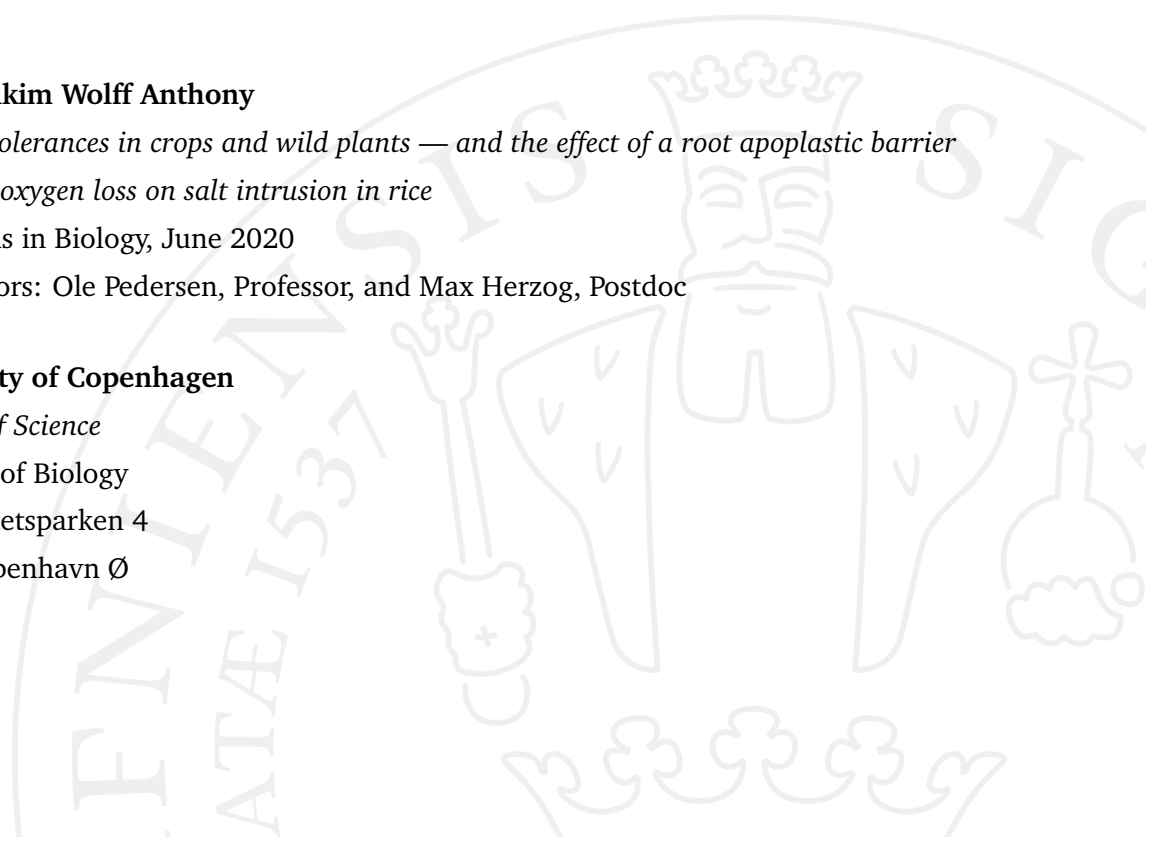Supervisors: Ole Pedersen, Professor, and Max Herzog, Postdoc

University of Copenhagen

Faculty of Science

Institute of Biology

Universitetsparken 4

2100 København Ø

Abstract

Salinity stress adversely affects plant productivity and yield and thus may represent vast losses to agricultural production worldwide. This study investigated whether wild plants are more tolerant of salinity than crops by analysing 62 published studies on responses to salinity in biomass and tissue salt concentrations. I found that increased salinity in the growth medium resulted in significantly increased accumulation of salt in shoots more so than in roots. While plants have different mechanisms to tolerate and combat the effects of salinity stress, salinity tolerances of crops and wild plants as a whole did not differ significantly. However, tolerances between several subgroups did. Halophytes had significantly higher salinity tolerances than other groups, but accumulate high concentrations of salt in the shoot, and may, therefore, be of interest for their high tissue tolerance. In contrast, rice had the lowest tolerance of all groups analysed. Through a preliminary experiment, the study identified a strong possibility of an exodermal apoplastic barrier in roots of rice contributing to ion exclusion. Ultimately, there is potential for engineering crops to be more tolerant of salinity.

Contents

Introduction

Salinity is a major source of abiotic stress to plants and a severely limiting factor to agricultural production worldwide (Yamaguchi & Blumwald, 2005). Salt-affected soils are present on all continents of the world and are estimated to affect 1030 million ha globally (FAO & ITPS, 2015). Of this large salt-affected area, 412 million ha are considered to be saline (FAO & ITPS, 2015), i.e., soils that are salt dominant (primarily NaCl) and where the electrical conductivity exceeds 4 dS/m (US Salinity Laboratory Staff, 1954). Consequently, the high amount of soluble salt in these soils adversely affects the growth and productivity of most crops (Maas & Grattan, 1999). In irrigated agriculture alone, annual losses in crop production due to salt are estimated to amount to US$ 27,3 billion (Qadir *et al.*, 2014).

Saline soils occur naturally (primary salinisation) because of geochemical processes such as mineral weathering, atmospheric deposition, and the formation of soils from marine sediments and materials. Salinity may also occur as the result of human activities (secondary salinisation) such as land clearing and poor agricultural practices. Especially, inappropriate or improper management of irrigation without adequate drainage may result in salt build-up in the root zone of plants (FAO & ITPS, 2015). Salinisation and the resulting land degradation threatens global food security as it causes a large amount of farmland to be taken out of production with estimates varying from 0,3 to 1,5 million ha annually (Munns & Tester, 2008; FAO & ITPS, 2015).

Plants generally respond to salinity in two phases: (1) a rapid osmotic phase and (2) a slower ionic phase (Munns & Tester, 2008; Munns & Gilliham, 2015). The first phase is the result of osmotic effects that start immediately after the salt concentration in the root zone reaches a threshold level. The salt decreases the difference in external and internal water potential causing osmotic imbalance that inhibits water uptake by the plant roots. The reduced water uptake causes a decrease in stomatal aperture, reduces photosynthesis, limits cell expansion, and limits cell division. This leads to reduced growth,

mainly in the shoot, and is visible in the form of wilting of leaves in dicotyledons and a reduction of tillers in cereals. The second phase of ion-specific toxicity develops over time and is a result of salt entering the transpiration stream of the plant. Ions (mainly Na^+, but also Cl^- depending on the species) gradually accumulate to toxic concentrations in the leaves as the salt remains inside the tissue while water is lost to transpiration. This accumulation causes premature senescence leading to reduced photosynthesis, protein synthesis, enzyme activity, reduced growth, and ultimately death.

To combat these effects, plants have evolved several mechanisms relating to osmotic adjustment and minimizing ionic stress. These mechanisms can be divided into three groups (Munns & Tester, 2008). (1) Osmotic stress tolerance, which aims to maintain leaf expansion and stomatal aperture. (2) Exclusion of Na^+ (and Cl^-) by the roots so that transport and subsequent accumulation in the shoot are prevented. (3) Tissue tolerance, which requires compartmentalization of Na^+ (and Cl^-) in vacuoles or particular cell types to ensure damage to metabolism is minimised.

Plants rely differently on these mechanisms of tolerance, and some species of plants are more adapted to saline environments than others. Tolerance to salinity also varies by plant growth stage and a wide range of environmental factors such as soil texture and water content (Butcher *et al.*, 2016; Ulery *et al.*, 1998). Halophytes (salt-tolerant species), generally, have high concentrations of Na^+ and Cl^- in leaves and rely on the trait of tissue tolerance by compartmentalization to avoid cytotoxicity. They further rely on the use of organic osmolytes to balance the difference in osmotic pressure (Munns & Gilliham, 2015). Glycophytes (salt-sensitive species), which include most major crops like rice and wheat, have lower leaf concentrations of Na^+ and Cl^- and instead tend to rely on ion exclusion to avoid the accumulation of salt in leaves (Munns & Gilliham, 2015).

There are major efforts in plant breeding to improve crop cultivar performance under salt stress. Breeders aim to increase salt tolerance of crops, and have been successful (e.g., Linh *et al.*, 2012), through the exploitation of natural genetic variation, for example, by marker-assisted breeding, but also through transgenic and cisgenic approaches (Yamaguchi & Blumwald, 2005). In these approaches, wild plants and wild relatives of crops may be of significant

interest as sources of possible tolerance mechanisms and genes (Palmgren *et al.*, 2015).

For instance, research indicates that apoplastic barriers in the exodermis of roots, which serve a wide array of functions (Enstone *et al.*, 2002), may also decrease the intrusion of salts into the stele (Krishnamurthy *et al.*, 2009; Krishnamurthy *et al.*, 2011; Cheng *et al.*, 2020). These barriers are in some species constitutive and in others like rice induced by abiotic stresses like waterlogged conditions (Colmer *et al.*, 1998; Ejiri & Shiono, 2019). The barriers may, therefore, contribute to salinity tolerance as a mechanism of ion exclusion. As such, this trait could be of significance for future breeding.

My main objective was to identify possible differences in salt tolerances and salt accumulation in tissues between major crops and wild plants in order to determine if there is potential for engineering crops to be more tolerant of salinity using approaches such as back-to-nature crop breeding (Palmgren *et al.*, 2015). This was done by aggregating and analysing data from 62 published studies. The study tested the hypothesis that, in general, wild plants are more tolerant of salt in their immediate environment than crops. I further experimentally explored the effects of an apoplastic barrier in roots of rice on the intrusion of salt in order to evaluate if this trait might also function as a tolerance mechanism.

Materials and Methods

<div style="text-align: right">2</div>

2.1 The salinity tolerance and tissue salt concentration database

In order to evaluate salinity tolerances of plants and their internal tissue salt concentrations when grown in saline media, a database was established. The database consisted of two datasets; (1) the salinity tolerances of various crops and wild plants, and (2) the effects of salinity treatments on tissue concentrations of Na^+ and Cl^-.

Salinity tolerance was defined as the salinity level of the growing medium resulting in a 10 percent decrease in economic or vegetative yield (primarily grain yield or dry shoot biomass; see Table A.1) relative to the control. The definition allows for the use of studies that were conducted in both an agronomic or ecological perspective and ensures comparability between the two.

2.1.1 Data source

The dataset on salinity tolerances of crops and wild plants was comprised of 297 entries compiled from 40 articles published between 1972 and 2019 in 24 different scientific journals. In this dataset, an entry defines the salinity tolerance of a specific species or cultivar. The dataset on the effect of salinity on tissue concentrations of Na^+ and Cl^- for plants was comprised of 469 entries compiled from 22 articles published between 1981 and 2016 in 16 different scientific journals. In this dataset, an entry is the Na^+ and/or Cl^- concentration of either shoot or root tissue of a specific species or cultivar at a tried salinity level. See Section A.1 for the lists of references used in the database and for a link to an online-accessible version of the database.

The species analysed were grouped into two primary categories: crops and wild plants. Crops consisted primarily of cereal grain crops (See Table A.1 and A.2 for full lists of crops included in each dataset of the database). Wild plants consisted of non-cereal plants, which were further grouped into either halophytes (i.e., high-salinity tolerant plants) or non-halophytes. The group included, among others, pasture plants, coastal plants, and wild grasses.

2.1.2 Selection criteria for articles used in the database

Articles had to fulfill two main criteria in order to be usable for the dataset on salinity tolerances; the study must (1) test the plant species at several salinity levels including a control (i.e., without added salt - the natural level of salinity for the medium), and (2) measure the effect on either economic yield (grain or ear yield; used for crops) or vegetative yield (solely dry shoot biomass; primarily used for wild plants and several crop studies). This was to ensure data was given as numerical salinity-yield pairs that could be used to derive a salinity tolerance for the species or cultivar tested (described in Section 2.1.4).

For the part of the database concerned with tissue concentrations of Na^+ and Cl^-, the studies had to fulfill similar criteria; the study must (1) test the plant species at several salinity levels and include a control, and (2) measure the resulting Na^+ or Cl^- concentrations in shoot or root tissue.

For all articles, the salinity of the imposed treatments had to be given in numerical values (and not be classified on an ordinal scale) in either molar concentration of the medium or terms of electrical conductivity of the saturated paste extract (ECe). The salinity imposed on the plant had to be mainly composed of NaCl.

2.1.3 Data description: what was recorded and how

Several general parameters were recorded in the database when a study was deemed usable. These parameters included the reference, species (common name and scientific binomial name including cultivar if applicable), group (crop or wild plant - and also non-halophytic wild plant or halophyte when applicable), and details about the study including the length of salt treatment and age of the plants when treatments were imposed.

For the dataset on tissue concentrations of Na^+ and Cl^-, the parameters, importantly, also included tissue (root or shoot), salinity level of the medium, tissue concentrations of Na^+ and/or Cl^- and their units. Here measurements on leaf tissue were grouped with those on shoot tissue and treated as one group ('shoots').

For the dataset on salinity tolerance, the additional parameters included the level of salinity tolerance, the unit, and what the tolerance was based on (grain/ear yield or shoot dry mass).

2.1.4 Deduction of salinity tolerance

In order to deduct a salinity tolerance of the species or cultivar studied in an article, I recorded all salinity levels of treatments and their corresponding yields. Yields relative to the control were then calculated and plotted in an XY-plot as a function of their salinity level. Data points were connected linearly to allow for the deduction of the salinity level when the relative yield was 90 percent of the control (i.e., the salinity tolerance).

If the study described a known salinity-yield response function such as those described by Maas and Hoffman (1977), Genuchten and Gupta (1993), or Steppuhn *et al.* (2005a), which are widely used for crop salinity studies and related indices, the parameters for the given function were used to calculate the salinity tolerance level (see Section A.2 for further details). This was the case for a large number of crops (53 entries) and wild plants (54 entries; primarily pasture grasses).

Data was extracted from tables or figures, which were digitized using the plugin FigureCalibration for IMAGEJ version 1.8.0-172 (Hessman, 2009; Schneider *et al.*, 2012).

Salinity values given in electrical conductivity of the saturated paste extract (ECe) were converted to total dissolved salts (TDS) equivalents by the following two relationships (Division of Agriculture and Natural Resources, University of California, 2020):

$$TDS = 640 \cdot EC, \text{ if } EC < 5 \tag{2.1}$$

$$TDS = 800 \cdot EC, \text{ if } EC > 5 \tag{2.2}$$

where TDS is given in mg/L and EC in dS/m. TDS (mg/L) was then converted to the molar NaCl concentration equivalent (mM NaCl) by dividing with the molar mass of NaCl (58,44 g/mol) in order to ensure comparability between studies.

2.1.5 Data analysis

GRAPHPAD PRISM 8 version 8.4.2 (GraphPad Software, La Jolla, CA, USA) for MacOS was used to analyse all data. Non-parametric Mann-Whitney U tests were performed to analyse data for Figure 3.1, Figure 3.4, and Figure 3.3 as distributions were not normal. Non-normal distributions were confirmed by visual inspection of residual plots and D'Agostino-Pearson omnibus K2 and Anderson-Darling normality tests. A non-parametric Kruskal-Wallis test was used to find significance difference in data for Figure 3.2, and a subsequent *post hoc* Dunn's multiple comparison test was used to identify which groups were significantly different. Correlation analyses (data for Figure 3.5 and Figure 3.6) were performed by use of non-parametric Spearman's rank correlations. All data analyses used a significance level of $P < 0,05$.

2.2 Pilot study of salt intrusion in roots of rice with barriers to radial oxygen loss

A one-replicate pilot study was carried out to investigate the effect of an exodermal root apoplastic barrier to radial oxygen loss (ROL) on intrusion and

accumulation of salt in rice. The aim was to evaluate whether the trait possibly contributes to the salinity tolerance of rice. Details on the pilot study and the methods and materials are given in Section A.3. In short, rice plants were cultivated hydroponically in stagnant nutrient solutions to induce a barrier to ROL and under aerated conditions for controls. Root segments from both treatments (+ROL and -ROL) were incubated in saline media at a time range of 0–4 hours. The tissue fluids were then analysed for osmolarity in a freezing point depression osmometer (The Advanced Osmometer 3250, Advanced Instruments Inc., Norwood, MA, USA).

Results

<div style="text-align: right">**3**</div>

Through an analysis of published studies on salinity-biomass response and salinity-tissue salt concentrations, this study explored the possible difference in salinity tolerance between crops and wild plants. I compared both the salinity tolerances of selected groups of plants and the effects of salinity on salt concentrations in different tissues, treatments, and groups of plants.

3.1 Salinity tolerances

The salinity tolerances of crops and wild plants were not significantly different, with a median tolerance of 40 mM NaCl for crops and 36 mM NaCl for wild plants (Figure 3.1). However, wild plants showed a much greater variation in tolerance than crops. This could indicate that some wild plants are considerably more adapted to saline environments than most major crops.

Salinity tolerances between certain groups of different crops and wild plants did, however, show significant differences (Figure 3.2). Halophytic wild plants had the broadest range of tolerances and the highest median salinity tolerance (205 mM NaCl) by more than 8-fold that of rice. Interestingly, halophytes did not differ significantly from the tolerances of barley and soybean. Barley had the highest median salinity tolerance of all selected crops (98,5 mM NaCl), while corn and rice had the lowest (36,5 mm NaCl and 25 mM NaCl, respectively). Wheat also had a comparatively low tolerance (37,5 mM NaCl) but showed a greater variation in tolerances than all the other crops. Surprisingly, wild non-halophytes had the lowest tolerance after rice (with a median tolerance of only 29 mM NaCl) and differed significantly only from halophytes and barley.

In summary, wild plants as a whole did not show higher salinity tolerance than crops, but when different subgroups of wild plants and crops were compared, there were significant differences. Specifically, halophytes had a median
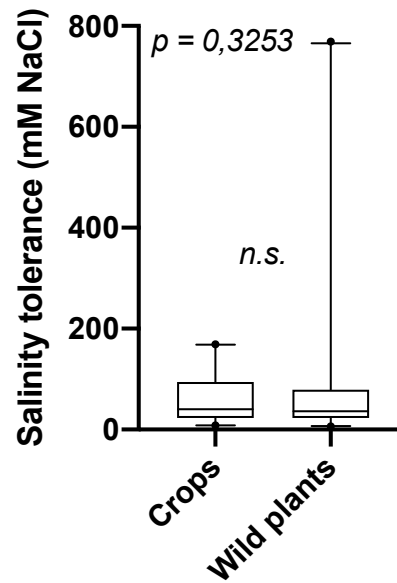
Figure 3.1.: Salinity tolerances of various crops (barley, corn, rice, rye, soybean, triticale, and wheat) ($n = 115$) and wild plants ($n = 182$, unique species $= 101$). Tolerances were based on the level of growth medium salinity resulting in a 10 percent decrease in yield or dry weight of total shoot biomass compared to that of the control. There was no significant difference (*n.s.*) between the two groups (Mann-Whitney, $P = 0,3253$). Horizontal lines in the middle of the boxes represent the median, while the boxes themselves extend to the 25th and 75th percentiles. Whiskers represent the 1st and 99th percentiles.

tolerance more than 2-fold that of barley, while rice comparably had very low tolerances.
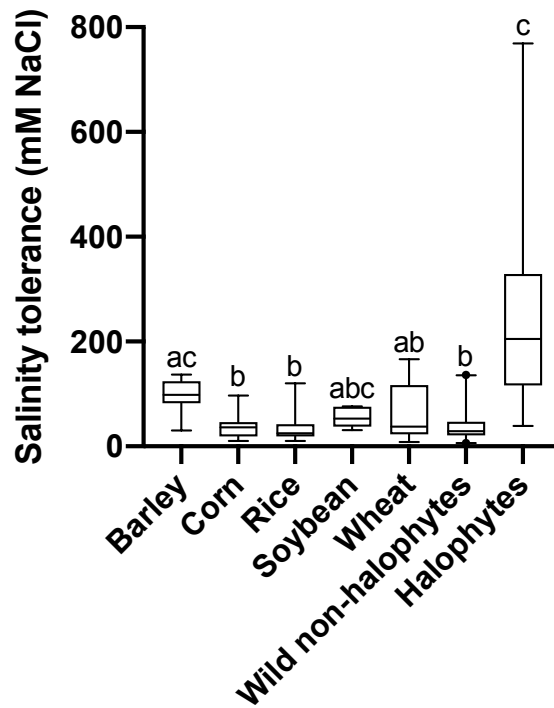
Figure 3.2.: Salinity tolerances of barley (*Hordeum vulgare*) ($n = 16$), corn (*Zea mays*) ($n = 28$), rice (*Oryza sativa*) ($n = 35$), soybean (*Glycine max*) ($n = 5$), wheat (*Triticum aestivum*) ($n = 24$), wild non-halophytes ($n = 154$, unique species = 77), and halophytes ($n = 28$, unique species = 24). Tolerances were based on the level of growth medium salinity resulting in a 10 percent decrease in yield or dry weight of total shoot biomass compared to that of the control. Different letters denote significant difference in salinity tolerance between species (Dunn's multiple comparison test, $P < 0,001$). Horizontal lines in the middle of the boxes represent the median, while the boxes themselves extend to the 25th and 75th percentiles. Whiskers represent the 1st and 99th percentiles.

3.2 Tissue salt concentrations

Overall, salinity treatments showed a significantly greater effect on shoot concentrations of both Na^+ and Cl^- than controls (without added salt) (Figure 3.3). Treatments had roughly 8–11 times greater median salt concentrations compared to controls.
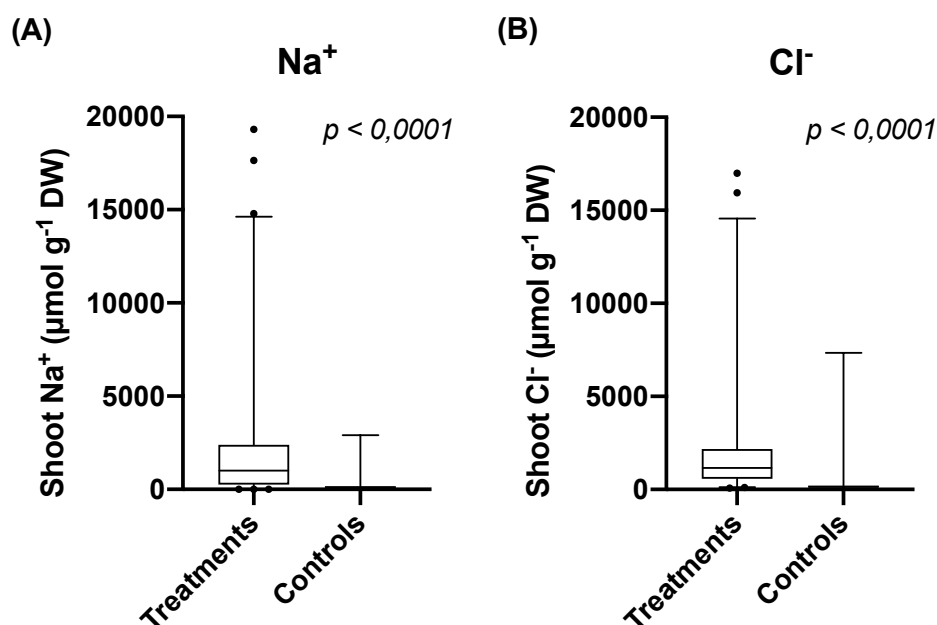

Figure 3.3.: The effect of salinity treatments (i.e., added salt to the growth medium) and controls (i.e., without salt) on salt concentration in shoot tissue of crops and wild plants. (A) The effect of salinity treatments ($n = 305$) compared to controls ($n = 87$) on shoot Na^+ concentrations. which differed significantly (Mann-Whitney, $P \leq 0,0001$). (B) The effect of salinity treatments ($n = 246$) compared to controls ($n = 71$) on shoot Cl^- concentrations, which also differed significantly (Mann-Whitney, $P \leq 0,0001$). Horizontal lines in the middle of the boxes represent the median, while the boxes themselves extend to the 25th and 75th percentiles. Whiskers represent the 1st and 99th percentiles.

Additionally, when salinity treatments were imposed, salt concentrations of Na^+ and Cl^- differed significantly from shoot to root tissue when all groups of plants were analysed as one (Figure 3.4). The median shoot Na^+ concentration was approximately 1,6-fold higher than that of roots, while for Cl^-, it was 2,4-fold higher.

When shoot salt concentrations (Na^+ and Cl^-) were plotted against their corresponding growth medium salinity, significant positive correlations with

(A) Na$^+$ *p = 0,0121*
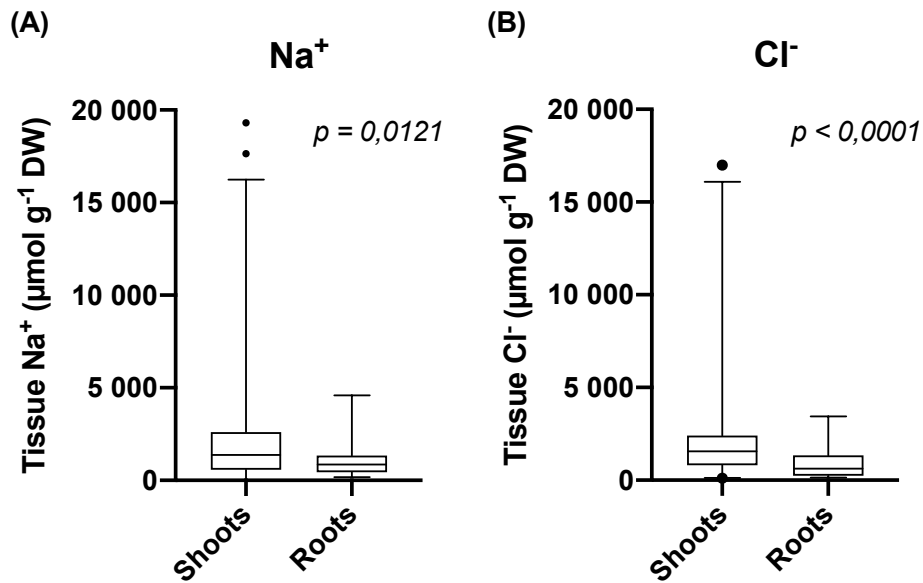
(B) Cl$^-$ *p < 0,0001*

Figure 3.4.: The effect of salinity treatments (i.e., added salt to the growth medium) on salt concentration in shoot and root tissues of crops and wild plants. (A) Na$^+$ concentrations in shoots ($n = 248$) compared to that of roots ($n = 53$). (B) Cl$^-$ concentrations in shoots ($n = 186$) compared to that of roots ($n = 51$). In both datasets (A and B), the salt concentrations of shoot and root tissues differed significantly (Mann-Whitney, respectively, $P \leq 0,0121$ and $P < 0,0001$). Horizontal lines in the middle of the boxes represent the median, while the boxes themselves extend to the 25th and 75th percentiles. Whiskers represent the 1st and 99th percentiles.

varying correlation levels were found for crops, wild plants, and when both groups were pooled as one (Figure 3.5). Thus, increased growth medium salinity correlated with increased shoot ion concentration. Wild plants had stronger correlations (as in greater r values, see figure caption) for both shoot Na$^+$ and Cl$^-$ than crops.

In contrast, rice showed a stronger correlation for shoot Na$^+$ than halophytes (Figure 3.6). Shoot Cl$^-$ were, however, significantly correlated in halophytes but not in rice. Generally, shoot salt concentrations were much higher for halophytes than for rice at a given growth medium salinity, which also seemed to be evident in the case of wild plants compared to crops. This could indicate that the two groups of halophytes and rice rely on different mechanisms of tolerance (as discussed later in Section 4).
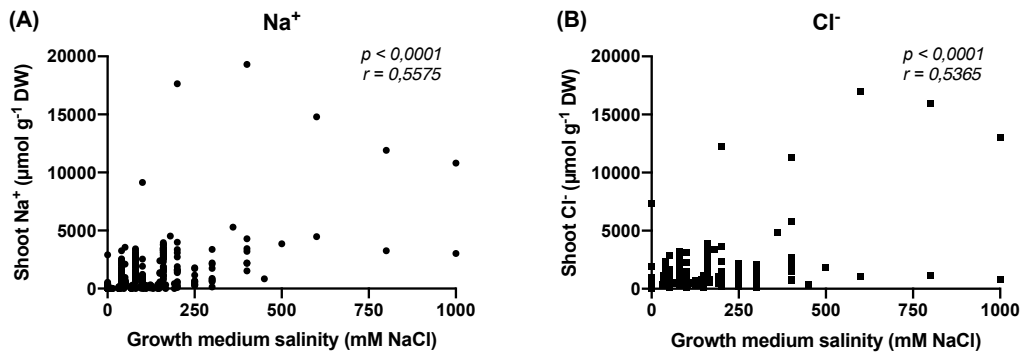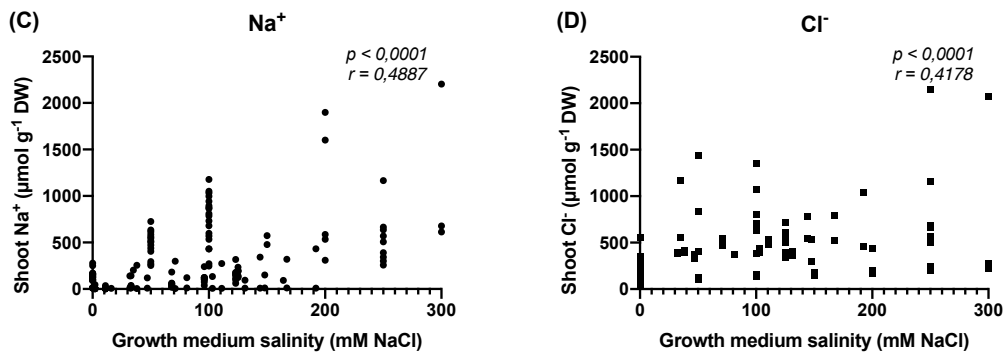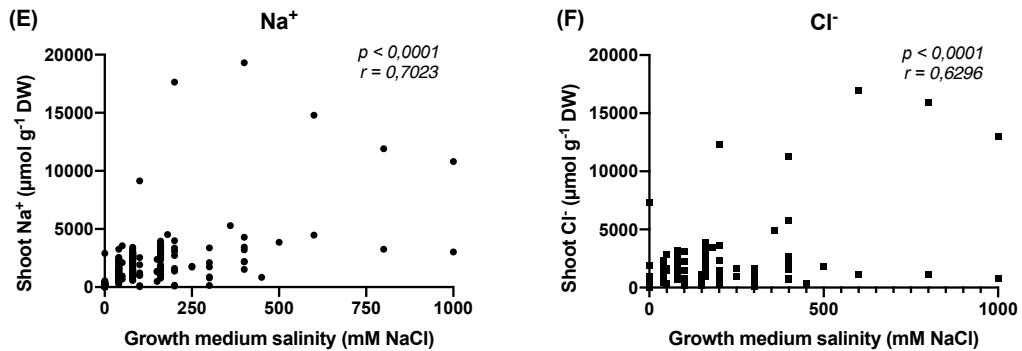
Figure 3.5.: Shoot salt concentrations of crops (C, D), wild plants (E, F), and both groups (A, B) plotted against the corresponding level of growth medium salinity. All six datasets showed significant and positive correlation between shoot ion concentration and medium salinity. P and r values refer to the results from non-parametric Spearman's rank correlation analyses. Left panels show shoot Na^+ concentrations and medium salinity for (A) both crops and wild plants ($n = 394$), (C) crops ($n = 159$), and (E) wild plants ($n = 235$). Right panels show shoot Cl^- concentrations and medium salinity for (B) both crops and wild plants ($n = 317$), (D) crops ($n = 81$), and (F) wild plants ($n = 236$).
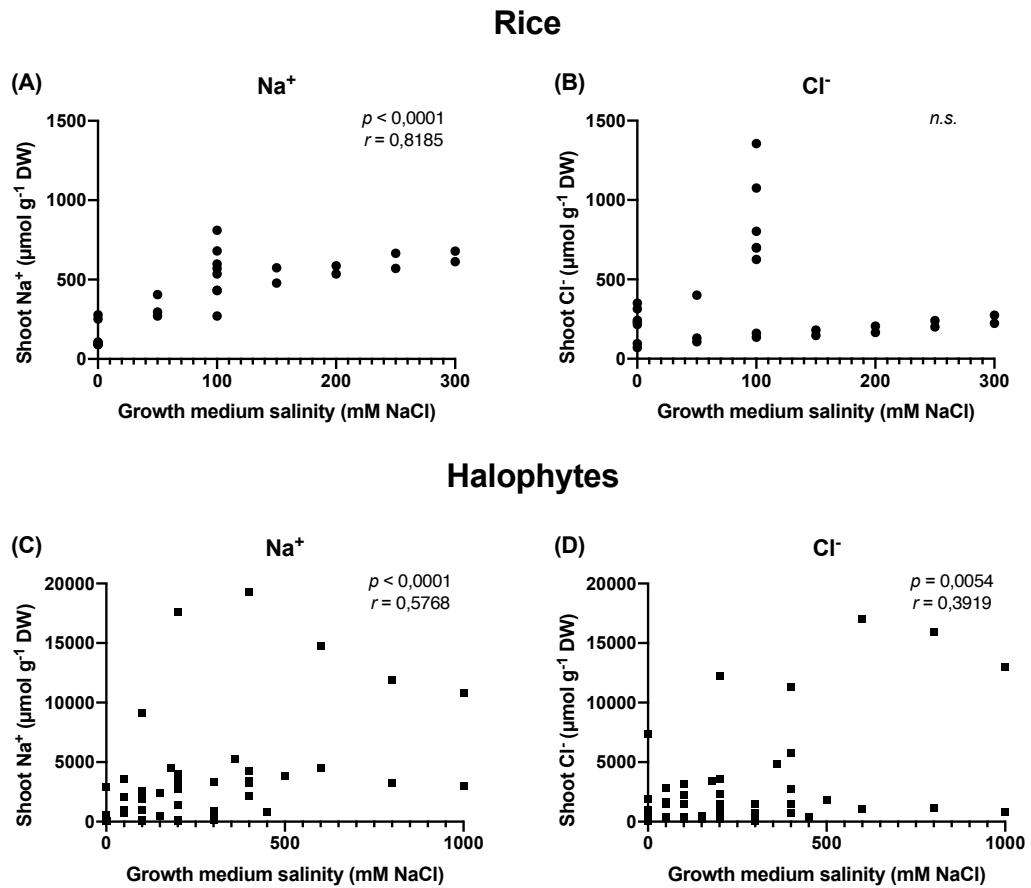
Figure 3.6.: Shoot salt concentrations of rice (A, B) and halophytes (C, D) plotted against the corresponding level of growth medium salinity. Three of the datasets (A, C, D) showed significant and positive correlation between shoot ion concentration and medium salinity. P and r values refer to the results from non-parametric Spearman's rank correlation analyses. (A) Shoot Na^+ concentrations of rice plants and the medium salinity ($n = 26$). (B) Shoot Cl^- concentrations of rice plants and the medium salinity ($n = 26$), which were not significantly correlated (Spearman's rank correlation, $P > 0,05$). (C) Shoot Na^+ concentrations of halophytic plants and the medium salinity ($n = 47$). (D) Shoot Cl^- concentrations of halophytic plants and the medium salinity ($n = 49$). See Table A.2 for references.

Results of the pilot experiment show that root segments with a barrier to radial oxygen loss (+ROL) generally had lower osmolarity than segments without (-ROL) until four hours of incubation (Figure 3.7). The difference was greatest after one hour of incubation, where the segment without a barrier (-ROL) had an osmolarity 21% greater than the segment with (+ROL). At the four hours mark, there was no noticeable difference between the two groups.
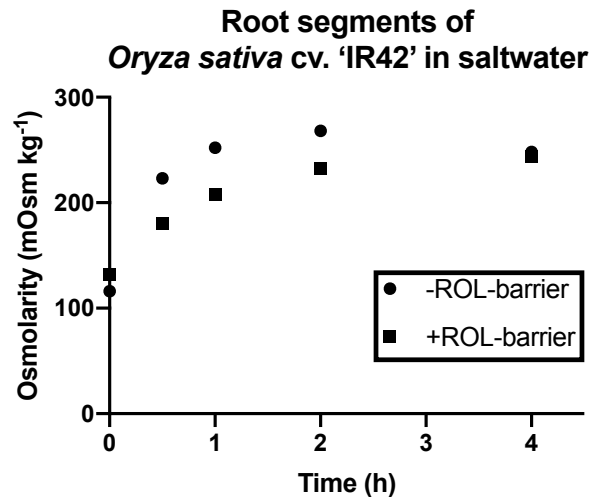

Figure 3.7.: Osmolarity of rice plant (*Oryza sativa* cv. 'IR42') root segments with a barrier to radial oxygen loss (+ROL) and without (-ROL) after incubation in 200 mM NaCl water. Data are the results of a one-replicate pilot experiment intended to study the effects of a barrier to ROL on salt intrusion in rice. Root barriers to ROL were induced by hydroponic cultivation of the rice plants in stagnant hypoxic nutrient solutions, while plants without root barriers to ROL were cultivated in aerated solutions. See Section A.3 for a detailed description of the materials and methods used in the pilot study.

3.3 Salinity-affected soils of four cereal crops

Frequency of top soils (0–30 cm soil depth) affected by salinity varied for the four cereal crops analysed (wheat, barley, rice, and African rice; Figure 3.8). For fields that were at some point during year 1950–2020 cultivated with barley and wheat, 17,2–17,6% of observations had a top soil salinity of 0,1–2 dS/m. For rice and African rice, the amount was noticeably smaller (5,1% and 2,1%, respectively). While the majority of fields for all four crops were not affected by salinity (ranging from 76,2–95,6%), a considerable amount of

observations from 2,1-4,5% were in fields with salinity exceeding 10 dS/m —
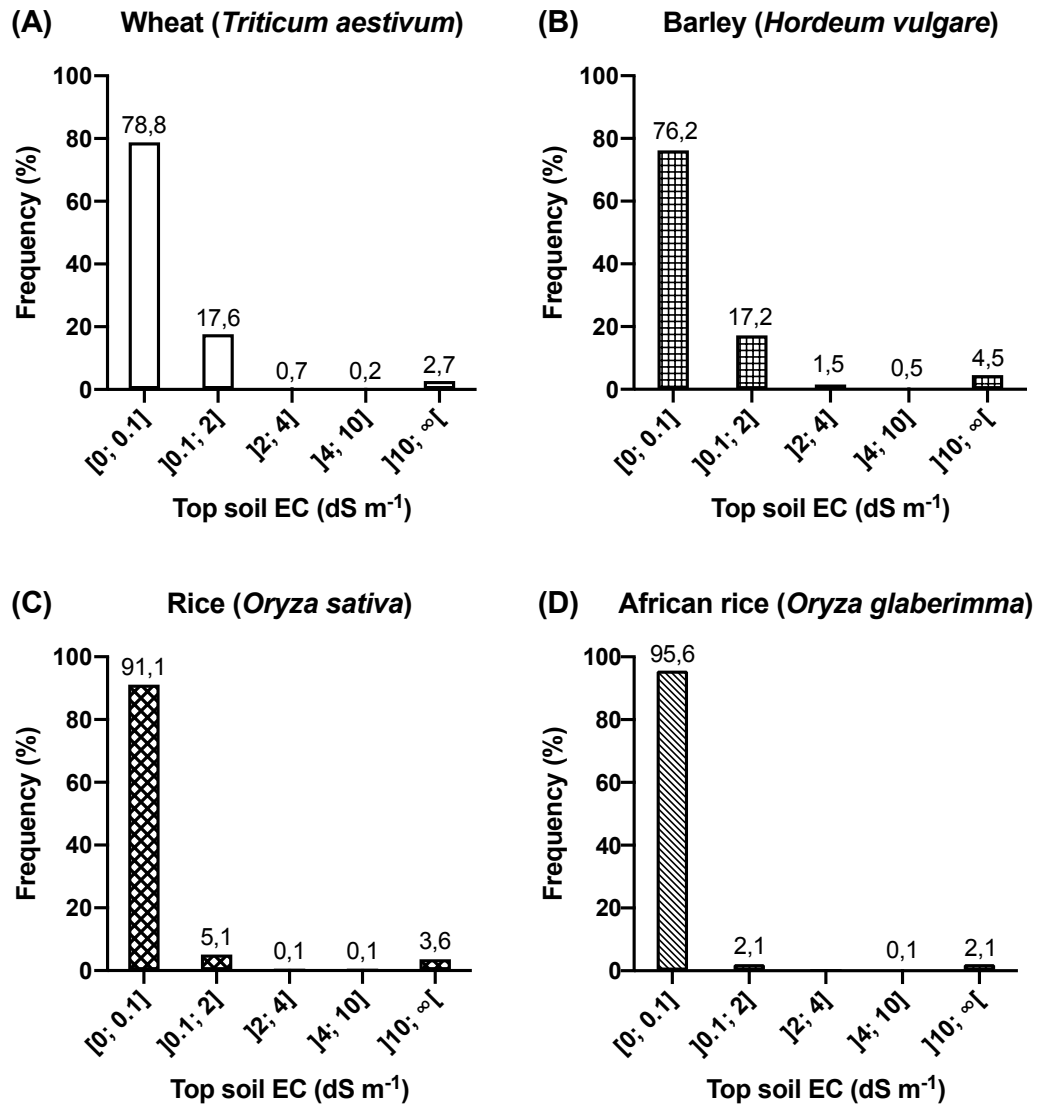for rice, 3,6% of observations.

Figure 3.8.: Frequency of salinity-affected top soils (0–30 cm soil depth) occurring on fields observed to be cultivated with wheat (A), barley (B), rice (C), or African rice (D) at some point in time during year 1950 to 2020. (A) Top soil salinity on fields cultivated with wheat (*Triticum aestivum*) ($n = 15613$) of 101 different countries. (B) Top soil salinity on fields cultivated with barley (*Hordeum vulgare*) ($n = 10659$) of 88 different countries. (C) Top soil salinity on fields cultivated with rice (*Oryza sativa*) ($n = 7921$) of 93 different countries. (D) Top soil salinity on fields cultivated with African rice (*Oryza glaberimma*) ($n = 1170$) of 17 different countries. EC values correspond to 1 mM (0,1 dS/m), 22 mM (2 dS/m), 44 mM (4 dS/m), and 137 mM (10 dS/m) of NaCl. See Section A.4 for information on data collection.

Discussion

4

4.1 Differences in salt tolerances

Contrary to the initial hypothesis, the present work found that there was no significant difference in salinity tolerances between crops and wild plants in general. However, wild plants did show considerably more variation in tolerances and included more species with very high tolerances than crops (see Figure 3.1). This variation suggests that some wild plants are indeed more adapted to high saline environments than most major crops.

The variation in salinity tolerances was evident when species were divided into subgroups (see Figure 3.2). For crops, salinity tolerances were relatively homogeneous between the various subgroups, with the notable exception of barley, which differed significantly from corn and rice. In line with other studies (Maas & Grattan, 1999; Munns & Tester, 2008), barley has the highest tolerance to salt compared to most other crops, whereas rice, a major staple food in much of the world (Fageria, 2007), is the most sensitive. The comparatively high salinity tolerance of barley has been largely attributed to its ability to tolerate high leaf tissue salt concentrations by sequestering salt in vacuoles (Munns, 1985; Munns & James, 2003; James *et al.*, 2006; Colmer *et al.*, 2005). Rice, on the other hand, is shown to rely more on low transport of salt to the shoot by its ability to exclude salts already at the root level for its mechanism of tolerance (Yeo *et al.*, 1990; Garcia *et al.*, 1995).

When wild plants were divided into non-halophytes and halophytes, wild non-halophytes had the second-lowest median tolerance of all seven subgroups analysed (see Figure 3.2). The tolerances of this group did not differ significantly from most crops. Nonetheless, this does not necessarily indicate that wild plants, in general, do not constitute a source of salt-tolerant germplasm, but may instead be the result of selection bias in the present study. The majority of salt tolerance entries for wild plants consisted of pasture plants, and

48% (74/154) of wild non-halophytes were clovers in the genus *Trifolium*, which have been classed as sensitive to salinity by some researchers (Maas & Grattan, 1999; Rogers *et al.*, 2010). This bias, and the possibly resulting skewed distribution of wild plant tolerances, is likely caused by the difficulty in finding appropriate salinity-biomass studies of non-crops and non-halophytes if they were not of particular interest to agriculture or ecology.

In comparison, halophytes differed the most from other groups and had the largest median salinity tolerance, but also large variation — e.g., two species had a growth medium tolerance of over 700 mM NaCl (550 mM NaCl is equivalent to full-strength ocean salinity). The high tolerances suggests that this group, in contrast, may contain effective salt tolerance mechanisms. For instance, a wild halophytic tropical rice was found to have leaves with salt-secreting microhairs, similar to the salt glands of mangroves (Cheng *et al.*, 2020), that contribute to an increased tissue tolerance (Flowers *et al.*, 1990). The high salinity tolerances of halophytes are often attributed to tissue tolerance by an ability to compartmentalize salts in vacuoles away from the cytosol and organelles (Munns & Gilliham, 2015; Flowers & Colmer, 2008).

4.2 Differences in tissue salt concentrations

Salinity treatments were found to result in significantly larger tissue concentrations of both Na^+ and Cl^- compared to non-saline treatments (see Figure 3.3). This indicates that plants generally are not able to exclude all salt from uptake by the roots. As a result of this salt uptake, Na^+ and Cl^- accumulated significantly more in the shoots than in the roots (see Figure 3.4). This accumulation of salt in the shoot is the result of Na^+ and Cl^- loading into the xylem through the symplastic pathway, and to a much lesser degree the apoplastic pathway, where the salts are then deposited in the leaves via the transpiration stream (for details, see extensive reviews by Plett and Møller (2010), Teakle and Tyerman (2010)). Most of the salt transported to the shoot ultimately remains there as only a small amount is recirculated back to the roots via the phloem (dependent on the species; Tester and Davenport, 2003; Munns and Tester, 2008). Na^+ in the shoot is generally thought to accumulate to toxic concentrations before Cl^-, but it is dependent on the species and its ability to regulate the

transport or compartmentalization of these ions (Tester & Davenport, 2003; Teakle & Tyerman, 2010; Munns & Tester, 2008). The metabolic toxicity of Na^+ largely results from its competition with K^+ for K^+-binding sites essential to cellular function (Tester & Davenport, 2003). High levels of Na^+ can thus disrupt enzymatic processes in the cytoplasm as well as protein synthesis.

The accumulation of salt in the shoot is not only a function of time (length of exposure), but it is also correlated with the level of salinity (see Figure 3.5). When all groups were pooled and analysed as one, there were significant positive correlations between shoot Na^+ as well as Cl^- and the corresponding salinity level of the growth medium. As expected, plants seem to accumulate more salt in shoot tissue when the level of salinity increases.

This correlation between shoot salt concentration and medium salinity was stronger in wild plants than crops (see Figure 3.5 for r values). The weaker correlation for crops might support the fact that ion exclusion plays a critical role for most glycophytes and thereby also crops (Munns & Tester, 2008). For wild plants, the stronger correlation is presumably not caused by the ability of halophytes to compartmentalize salts in the shoot (discussed in the following paragraphs). It may instead be caused by *Trifolium* spp. and their poor ion exclusion (Rogers *et al.*, 2010).

A different pattern of correlations is seen when the focus is shifted to rice and halophytes (see Figure 3.6). Here, rice had a stronger correlation for shoot Na^+ concentrations than halophytes. Shoot Cl^- concentration was, however, weakly correlated in halophytes but not significantly correlated in rice. Data may be affected by differences in experimental conditions between studies — for example, treatment duration. Even so, these differences could be indicative of the two groups' reliance on different mechanisms for salt tolerance and their effectiveness in tolerating or excluding these ions.

While halophytes rely primarily on tissue tolerance (Munns & Gilliham, 2015), it is evident that shoot salt contents are curtailed at higher levels of salinity, and there must therefore still be a need to regulate ion uptake and transport to the shoot (Tester & Davenport, 2003). This could also be influenced by the ability of some halophytes, e.g., some mangroves species and the aforementioned halophytic wild rice (Cheng *et al.*, 2020; Flowers *et al.*, 1990), to secrete salt from leaves via glands or bladders (Tester & Davenport, 2003). For rice, the

stronger correlation for shoot Na^+, but the similar curtailing of shoot salt concentration as salinity levels increase, may represent its ability to somewhat regulate Na^+ transport through exclusion at the root level. For both rice and halophytes, there is a weaker (or no) correlation for shoot Cl^- than for Na^+. This may suggest that Cl^- uptake is more effectively regulated than Na^+ uptake.

It is noteworthy that shoot salt concentrations at comparable levels of growth medium salinity (between the two groups) are noticeably higher in halophytes than rice. Halophytes may simply accumulate more salt than rice (and presumably most crops) at comparable levels of growth medium salinity. This suggests that halophytes largely do not rely on ion exclusion and supports the statement that tissue tolerance is of more importance as a tolerance mechanism for this group. This comparably lower tissue salt accumulation in rice coupled with a stronger correlation between shoot Na^+ and medium salinity indicate that the ion exclusion mechanism in rice may, to some extent, be ineffective. Importantly, apoplastic bypass flow of Na^+ into the stele and subsequent transport to the shoot is shown to constitute a large component of this salt accumulation and salt sensitivity in rice (Garcia *et al.*, 1995; Krishnamurthy *et al.*, 2011; Isayenkov & Maathuis, 2019; Plett & Møller, 2010).

Apoplastic bypass flow of Na^+ and subsequent accumulation could be restricted by apoplastic barriers in the exodermis of roots. The formation of strong apoplastic barriers (Casparian bands) would force water and salts into the symplastic pathway and through selective ion channels (Isayenkov & Maathuis, 2019). This selection could thereby work to reduce salt loading into the xylem and ultimately limit accumulation in the shoot. The pilot study found that the presence of such an apoplastic barrier (referred to as a barrier to radial oxygen loss) in root segments of rice reduced osmolarity compared to segments without the barrier until four hours of incubation in 200 mM NaCl water (see Figure 3.7). Similarly, Krishnamurthy *et al.* (2009, 2011) found that root apoplastic barriers, which were induced by conditioning plants to moderate salinity stress for one week, limited Na^+ uptake as well as bypass flow under acute salinity stress of 200 mM NaCl endured for 48 hours. The authors also noted that there were significant differences in Na^+ uptake and barrier formation between cultivars and cultivation techniques following the conditioning. The salt-tolerant cultivar tended to develop more well-defined exodermal Casparian bands, particularly at 10 mm from the root tip, and had

less Na$^+$ uptake than the salt-sensitive cultivar. Additionally, under identical stress conditions, plants grown in soil accumulated less Na$^+$ than plants grown hydroponically. Cheng *et al.* (2020) found similar results in a mangrove species, where salinity induced apoplastic barriers resulted in lower concentrations of Na$^+$ in xylem sap following ten days of exposure to 600 mM NaCl solution.

Further research is needed to establish the effects of apoplastic barriers on the influx and shoot accumulation of Na$^+$ (and Cl$^-$) in rice with certainty. Nonetheless, these findings suggest that apoplastic barriers may likely contribute to the exclusion of Na$^+$ (and possibly also Cl$^-$) and could, therefore, represent an important trait in the further improvement of salinity tolerances of crops.

Saline soils are widespread globally (FAO & ITPS, 2015), and with an increasing population, there is a need to improve crop productivity in order to ensure future food security (Qadir *et al.*, 2014). Fields cultivated with four major crops (wheat, barley, rice, and African rice) were found to be overwhelmingly non-saline. However, a considerable amount of observations (ranging from 2,1–4,5%) were highly saline with an electrical conductivity exceeding 10 dS/m (see Figure 3.8). Combined, these salt-affected areas could make up a substantial amount of agricultural land. While this may be less of an issue in the cultivation of salt-tolerant barley, it may constitute considerable problems for the productivity of the other three more sensitive cereals. In rice, 3,6% of observations were highly saline (>10 dS/m), and with the large amount of land dedicated to the cultivation of this salt-sensitive crop, these areas may represent an enormous loss in yield. This effect is likely to be even more prevalent in countries like Bangladesh that have a large rice production as well as wide-ranging salinity problems (Sinha *et al.*, 2014).

Conclusion

This study found that salinity stress adversely affects plant productivity and yield, especially in crops. The ubiquity of highly saline soils in fields of four major cereals may, therefore, represent vast losses to food production worldwide. In general, salinity treatments resulted in larger tissue salt concentrations, and this salt accumulated more so in the shoot than roots. Some groups of plants were more adapted to tolerating and combating the effects of salinity stress than others. While the salinity tolerances of wild plants and crops as a whole did not differ significantly, there were significant differences between subgroups. Particularly, halophytes had the highest tolerance and would be of interest for further studies because of their seemingly effective tissue tolerance mechanism. In contrast, rice had the lowest tolerance of all groups analysed. This was largely attributed to ineffective ion exclusion and apoplastic bypass flow. The study identified the strong possibility of a root apoplastic barrier in rice contributing to the exclusion of Na^+ (and possibly also Cl^-). While further research is needed, this trait could represent an important mechanism for improving the salinity tolerance of crops. For these reasons, there is potential for engineering crops to be more tolerant of salinity through the introduction of more effective tolerance mechanisms and traits like those found in halophytes.

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Supplementary information A

A.1 Tables of references for the salinity tolerance and tissue salt concentration database

An online-accessible version of the database of salinity tolerance and tissue concentration can be found at `https://docs.google.com/spreadsheets/d/1TNfRhyEqI-ZnUwCnf1sLuXCHGdSeQpCsA-oLqjPH1FI/edit?usp=sharing`.

Table A.1.: References for salinity tolerances grouped into crops and wild plants. Percentages indicate the number of entries of that particular species with salinity tolerance based on that type of yield.

Species	No. of entries	Tolerance based on		References
Barley *(Hordeum vulgare)*	16	Grain yield	88%	Maas and Grattan (1999), Royo *et al.* (2000), Rozema and Schat (2013), Steppuhn *et al.* (2005b).
		Shoot DW	12%	
Rye *(Secale cereale)*	4	Grain yield	75%	Maas and Grattan (1999), Steppuhn *et al.* (2005b).
		Shoot DW	25%	
Wheat *(Triticum aestivum)*	24	Grain yield	88%	Francois *et al.* (1986), Maas and Grattan (1999), Rozema and Schat (2013), Steppuhn *et al.* (2005a, 2005b), Steppuhn *et al.* (1996).
		Shoot DW	12%	
Soybean *(Glycine max)*	5	Seed yield	40%	Essa (2002), Maas and Grattan (1999), Steppuhn *et al.* (2005b).
		Shoot DW	60%	
Triticale *(×Triticosecale)*	3	Grain yield	67%	Francois *et al.* (1988), Maas and Grattan (1999), Rozema and Schat (2013).
		Shoot DW	33%	

Rice *(Oryza sativa)*	35	Grain yield Shoot DW	20% 80%	Akbar *et al.* (1972), Fageria (1985), Flowers and Yeo (1981), Hakim *et al.* (2014), Kargbo *et al.* (2019), Maas and Grattan (1999), Ologun-dudu *et al.* (2014), Steppuhn *et al.* (2005b), Zeng and Shannon (2000).
Corn *(Zea mays)*	28	Ear FW Grain yield Shoot DW	11% 4% 85%	Akram *et al.* (2007), Carpici *et al.* (2009), Hoffman *et al.* (1983), Khayatnezhad and Gholamin (2011), Mansour *et al.* (2005), Maas and Grattan (1999), Maas *et al.* (1983), Steppuhn *et al.* (2005b).
Non-halophytes	154	Shoot DW	100%	Kapulnik *et al.* (1989), Marcum (2006), Maas and Grattan (1999), Rogers *et al.* (2010), Rogers *et al.* (1996, 1997), Steppuhn *et al.* (2005b), Teakle *et al.* (2006).
Halophytes	28	Plant DW Shoot DW	4% 96%	Khan *et al.* (2000), Ashraf and Yasmin (1997), Debez *et al.* (2004), Flowers *et al.* (1990), Fowler *et al.* (1988), Gul *et al.* (2010), Gulzar *et al.* (2003a, 2003b), Karimi *et al.* (2005), Khan *et al.* (2001), Liu *et al.* (2006), Marcum (2006), Maas and Grattan (1999), Rozema and Schat (2013), Steppuhn *et al.* (2005b), Zakery-Asl *et al.* (2014).

Table A.2.: References for tissue concentrations of Na^+ and Cl^- grouped into crops and wild plants.

Species	No. of entries	Shoot entries	Root entries	Reference
Barley *(Hordeum vulgare)*	4	50%	50%	Flowers and Hajibagheri (2001)
Chickpea *(Cicer arietinum)*	6	100%	0%	Kotula *et al.* (2015)
Corn *(Zea mays)*	78	100%	0%	Akram *et al.* (2007), Maas *et al.* (1983)
Rice *(Oryza sativa)*	44	59%	41%	Flowers and Yeo (1981), Khare *et al.* (2015), Kumar and Khare (2016, 2015), Kumar *et al.* (2008)
Wheat *(Triticum aestivum)*	72	71%	29%	Ashraf and O'Leary (1996), Francois *et al.* (1986), Islam *et al.* (2007), Khan *et al.* (2009)
Non-halophytes	188	100%	0%	Rogers *et al.* (2010), Teakle *et al.* (2006)
Halophytes	77	64%	36%	Debez *et al.* (2004), Gulzar *et al.* (2003a, 2003b), Islam *et al.* (2007), Karimi *et al.* (2005), Khan *et al.* (2000), Liu *et al.* (2006), Marcum and Murdoch (1992)

A.2 Salinity-yield response functions and deduction of salinity tolerance

A large number of salinity tolerances (107 entries) in the database were derived from articles that utilized a salinity-yield response function to model the plant species response to increasing root-zone salinity. The articles, primarily on crops and pasture grasses, used either a piece-wise linear model or an S-shaped response model as described in Section A.2.1 and A.2.2. The salinity tolerances of each species, or cultivar, were derived from these functions by calculating the salinity at a 90% relative yield ($ECe_{90\%}$) from the parameters given.

A.2.1 Maas-Hoffman threshold-slope salinity-yield response function

Maas and Hoffman (1977) described the piece-wise linear model, which assumed no response in yield below a salinity threshold parameter, but above

which yields decreased linearly by a slope parameter specific to each plant species. The function is given by:

$$Y = \begin{cases} Y_m, & 0 < c < c_t \\ Y_m - Y_m \cdot s(c - c_t), & c_t < c \end{cases} \tag{A.1}$$

where Y is crop yield, c is the root zone salinity given in electrical conductivity of the saturated paste extract (ECe), Y_m is the maximum yield under non-saline conditions, c_t is the salinity threshold parameter given in dS/m, and s is the slope parameter given in relative decline in yield per one dS/m increase in salinity.

A.2.2 van Genuchten-Gupta S-shaped salinity-yield response function

Genuchten and Gupta (1993) described a Sigmoid-shaped function with the aim to better model yield response to salinity in crops. The function is given by:

$$Y_r = 1/[1 + (c/c_{50})^3] \tag{A.2}$$

where Y_r is the relative yield, c is the root zone salinity, and c_{50} a parameter specific to each species or cultivar describing the root zone salinity at which the yield has declined by 50%. Steppuhn *et al.* (2005a) described a slightly modified version of this function.

A.3 A pilot study on root barriers to radial oxygen loss and their effect on salt intrusion in rice and relatives

A pilot experiment was completed in order to study the effects of root barriers to radial oxygen loss (ROL) on salt intrusion in rice (see Figure 3.7). The intention was to later on conduct a complete experiment with more replicates and controls that would also include the use of flame photometry to more accurately assess the concentrations of Na$^+$. This was unfortunately not

possible due to the COVID-19 lockdown, which hindered access to the facilities necessary. The study also intended to prove the presence of the root barriers to ROL by the use of a periodic acid staining method. However, no experimental procedure was developed again due to the COVID-19 outbreak.

I hypothesized that the presence of an exodermal apoplastic barrier to radial oxygen loss in the roots of rice delay salt (Na^+ and Cl^-) intrusion compared to roots without a barrier when excised roots were incubated in a saline solution.

A.3.1 Experimental design

The experiment was based on osmolarity analysis of root tissues with barriers to radial oxygen loss (+ROL) and without barriers (-ROL) in rice after incubation in saline water (200 mM NaCl solution) by use of freezing point depression osmometer (The Advanced Osmometer 3250, Advanced Instruments Inc., Norwood, MA, USA). The analysis was done on root segments after 0, 30, 60, 120, and 240 min of incubation.

Plant culture The rice plant species used in the experiment, *Oryza sativa* cv. IR42, was cultivated hydroponically. Prior to use, the seeds supplied by the International Rice Research Institute (IRRI) were kept cooled at approx. 5°C in containers with silicone aggregate. Prior to imbibition, the seed containers were allowed to reach room temperature before opening to avoid water vapor condensing on the seeds. The seeds were imbibed in a Petri dish with DI water containing 0,5 mM $CaSO_4$ for three hours. They were then transferred to a Petri dish with wet paper towels, wrapped in aluminium foil to keep light out, and germinated in a temperature-controlled room (set-point 30°C during day and night) for three days. Following that, the seedlings were transferred to a mesh float in an aerated 4L black bucket containing 25% strength nutrient solution (specified below), where they were kept for a duration of eight days (from 24/2/2020 until 2/3/2020). The plants (totalling 29) were then transplanted to foam plugs sat on lids covered in aluminium foil of four aerated 4L black buckets containing 100% strength nutrient solution (specified below) for further growth. After an additional ten days (11/3/2020), two of the 4L buckets had the aeration hoses removed and then underwent hypoxic pretreatment by bubbling the nutrient solution with nitrogen for

several minutes in order to prepare for transfer to stagnant solutions. The following day, half of the plants were then transferred to stagnant nutrient solutions (with 0.1% agar and deoxygenated by flushing with N_2 for 1 h.), where they were kept for the duration of the experiment.

The composition of the nutrient solution at 100% strength was: $CaSO_4.2H_2O$, 1,5 mM; MES (buffer), 2,5 mM; $MgSO_4.7H_2O$, 0,4 mM; KNO_3, 3,75 mM; NH_4NO_3, 0,625 mM; KH_2PO_4, 0,2 mM; $Na_2O_3Si.9H_2O$, 0,1 mM; Fe-EDTA, 0,05 mM, and the following compounds for micronutrients: KCl, 50,0 μM; H_3BO_3, 25,0 μM; $MnSO_4.H_2O$, 2,0 μM; $ZnSO_4.7H_2O$, 2,0 μM; $CuSO_4.5H_2O$, 0,5 μM; $Na_2MoO_4.2H_2O$, 0,5 μM; and $NiSO_4.7H_2O$, 1,0 μM. The pH of the solution was adjusted to 6,0 by using 1M KOH. The buckets were topped with DI water to replace water lost to transpiration as needed, and the nutrient solution was refreshed periodically. The plants were kept in the same temperature-controlled growth room (set-point 30°C day/night) in Copenhagen, Denmark, from germination till the end of the experiment.

Harvesting and storage of roots Roots exceeding 6 cm were cut off by scissors a few cm from their top and placed in separate containers filled with DI water for those sourced by plants raised aerated and stagnant, where they were kept until treatment. Roots were chosen for harvest based on length and thickness, and were, importantly, also required to have an insignificant amount of laterals and root hairs that otherwise may affect the diffusion of salt into the tissue. Only roots exceeding 6 cm in length were chosen in order to ensure an adequate amount of tissue for osmolarity analysis.

Incubation of roots Roots were cut into segments of approx. 3-6 cm in a tray with DI water and had both cut ends sealed with a visible amount of lanolin to prevent intrusion of osmolytes. Half of the root segments were moved to five Petri dishes containing 200 mM NaCl solution (+NaCl) for incubation. The other half of the root segments served as controls and were incubated in DI water (-NaCl). The segments were incubated for zero to four hours in pairs of one control and one treatment.

Osmolarity After incubation, the roots were prepared for analysis by excising the ends with lanolin. The root segments were then washed in three separate containers of DI water and afterwards kept moist in between wet paper towels until all roots of the container had been prepared. The segments were then

carefully dried off in a dry paper towel. Lastly, the root segments of a treatment or control were transferred to a small piston for mechanical crushing and extraction of the tissue fluid. 50 μL of extract was then transferred to an Eppendorf tube using a pipette, where it was kept under a closed lid until all containers of treatments and controls were prepared. Samples were then transferred to osmometer-appropriate containers, where the extract was diluted with 150 μL of DI water to form a combined 200 μL solution that was then analysed in the freezing point depression osmometer (The Advanced Osmometer 3250, Advanced Instruments Inc., Norwood, MA, USA). The resulting osmolarity was recorded after correcting for the dilution factor.

Typically, a total of five-to-six root segments of 3-4 cm were needed in order to have the required amount of tissue extract for one treatment or control for the chosen dilution of 1:3 (50 μL tissue extract to 150 μL DI water).

A.4 Generating data on salinity affected soils of wheat, barley, rice and, African rice

Data on the frequency of salinity-affected top soils for wheat (*Triticum aestivum*, barley (*Hordeum vulgare*), rice (*Oryza sativa*), and African rice (*Oryza glaberrima*) as illustrated in Figure 3.8 was compiled from two online databases. Data from the *Harmonized World Soil Database* (FAO *et al.*, 2012. Downloaded from webarchive.iiasa.ac.at) on the physical and chemical characteristics of topsoils (0–30 cm soil depth) globally was downloaded and then treated in QGIS version 3.12 (QGIS Geographic Information System. Open Source Geospatial Foundation Project. `http://qgis.osgeo.org`) by vectorizing the raster files and joining to attribute data.

Data on the geographical distribution of wheat, barley, rice, and African rice fields observed at some point in time, varying from species but some observations were dated to pre-1900's, were collected from GBIF.org (GBIF: The Global Biodiversity Information Facility (2020). Downloaded from `https://www.gbif.org/`).

Datasets were cleaned for wrong and dubious coordinates in R version 3.6.3 (R Core Team, Vienna, Austria) by use of the add-on package CoordinateCleaner version 2.0-11 and its several functions listed in parenthesis (cc_val, cc_equ, cc_cap, cc_cen, cc_gbif, cc_inst, cc_sea, cc_zero, cc_outl, and cc_dupl).

The four datasets on the distributions of the selected four species compiled from GBIF.org were imported to QGIS software and joined by the HWSD data on salinity by use of the SAGA function 'Add polygon attributes to points' in order to combine data on species and topsoil salinity of a given location.

Lastly, data were filtered to only observations from the year 1950 and up in order to reduce uncertainty and error from old geographical distributions that may no longer be representative of the current situation in regards to the crop cultivated and the soil chemical properties on those particular locations. The data, however, is also affected by some degree of inaccuracy as a result of the HWSD data that is given in raster format with a resolution of approx. 1 km (30 arc-second rasters; FAO *et al.*, 2012). All observations located within that raster will, therefore, be assigned the same topsoil salinity value, even though salinity may differ locally within the area.

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