Technical note: Investigating saline water uptake by roots using spectral induced polarization

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Abstract

~~There haves been some improvements~~Developments in the methods available for root investigation in recent years ~~that~~ haves enabled many studies to be carried out on ~~the~~ root, which represents the hidden half of the plant. Despite the increased number of studies on roots, there are still knowledge gaps in our understanding of the electromagnetic properties of~~ocesses in~~ plant roots, which will be useful to quantify plant ~~properties, and~~properties and monitor plant physiological responses to dynamic environmental factors amidst climate change. In this study, we evaluated the suitability of spectral induced polarization for non-invasive assessment of root activity. We investigated the electrical properties of the primary roots of *Brachypodium distachyon L.* and *Zea mays L.* during the uptake of fresh and saline water using spectral induced polarization (SIP) measurements in a frequency range from 1 Hz to 45 kHz. The results show that SIP is able to detect the uptake of water and saline water in both species, and that their electrical signatures were influenced by the solute concentration. The resistivity and phase response of both species increased with solute concentration until a certain threshold before it decreased. This concentration threshold was much higher in m~~M~~aize than in *Brachypodium*, which implies that tolerance to salinity varies with the species, and that m~~M~~aize is more tolerant to salinity than *Brachypodium*. We conclude that SIP~~spectral induced polarization~~ is a useful tool for monitoring root ~~activity, and~~activity and could be adapted for early detection of salt stress in plants.

Keywords: Agrogeophysics, Spectral induced polarization, Electrical impedance, Phase angle, Salt stress, Maize roots, *Brachypodium* roots

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1. Introduction

Sustainable global crop production is challenged by several unfavorable environmental factors such as drought, extreme temperatures, salinity, nutrient deficiency, and soil contamination among others. For example, more than 800 million ha of land globally is affected by salinity and excessive sodium content (FAO 2005; Munns 2005). High salt concentrations in soils induce plant stress due to low external water potential, oxidative stress by excessive generation of reactive oxygen species (ROS), ion toxicity (Na^+ and/or Cl^-) or nutrient deficiency by interfering with the uptake and transport of various essential nutrients (Munns et al. 2006; Läuchli and Grattan 2012; Hussain et al. 2013; Negrao et al. 2017; Isayenkov and Maathius 2019). Stress magnitude depends on the species, duration of salinity exposure, the growth stage and environmental conditions (Munns and Tester 2008). Accumulation of sodium and chloride ions at toxic levels in plant tissue damages biological membranes and subcellular organelles, reducing plant growth and development (Davenport et al. 2005; Zhao et al. 2010; Farooq et al. 2015; Isayenkov and Maathuis 2019). Sodium may also displace calcium from the binding site of the cell membrane which can result in membrane leakiness (Cramer et al. 1988). Geophysical electrical methods have extensively been used to study root water uptake in soils (e.g. Michot et al. 2003; Garré et al. 2011; Beff et al. 2013) and soil salinity (e.g. Rhoades et al. 1999; Bennett et al. 2000; Doolittle et al. 2001; Ben Hamed et al. 2016; Shahnazaryan et al. 2018). Due to their sensitivity to salinity, they provide a natural means to non-invasively study salt stress impact on roots given the analogy between water flow and electrical current flow in roots.

Spectral induced polarization (SIP), also known as electrical impedance spectroscopy (EIS), has been successfully used to study various plant physiological processes, such as growth (Ozier-Lafontaine and Bajazet 2005; Repo et al. 2005), mycorrhizal colonization (Cseresnyés et al. 2013; Repo et al. 2014), cold acclimation (Repo et al. 2016), nutrient deprivation (Weigand and Kemna 2017, 2019), and the effects of salt stress on growth (Ben Hamed et al. 2016), and diurnal cycles in root uptake activity (Cseresnyés et al. 2024). In the interpretation of these SIP measurements, it is assumed that current pathways in the extracellular (apoplast) and intercellular (plasmodesmata and aquaporins) spaces play an important role in electrical charge migration and storage (Kinraide, 2001; Kinraide and Wang, 2010, Weigand and Kemna, 2019; Kessouri et al., 2019) (Fig. 1). In particular, current conduction is assumed to depend on the electrical properties of the apoplast and the ionic composition of the extracellular fluid (ECF), whereas polarization is assumed to occur at the cell membrane interface because charged particles such as Na^+, Ca^{2+}, K^+, Cl^- ions and amino acids cannot diffuse directly across the cell membrane. Instead, they can only cross the membrane through ion pumps and ion channels, whose opening and closing are regulated by the membrane potential difference. Polarization is also expected to occur at the outer root surface (i.e. the root-soil interface), where the charge distribution that determines polarization depends on the concentration of ions in the external fluid (Weigand and Kemna 2017, 2019). It is important to note that living tissues are equivalent to parallel resistor and capacitor (RC) circuits, which haves a characteristic phase angle that depends on alternating current (AC) frequency.~~,~~ ~~t~~Thus, ~~Conduction~~ conduction and polarization mechanisms are frequency dependent (see current pathways in Fig. 1b and 1c) and can be assessed simultaneously by measuring the frequency dependent electrical impedance of a biological tissue using SIP. The suitability of this method for investigating root responses to salt stress is not well known and has rarely been studied (Ben Hamed et al. 2016; Cseresnyés et al. 2024).

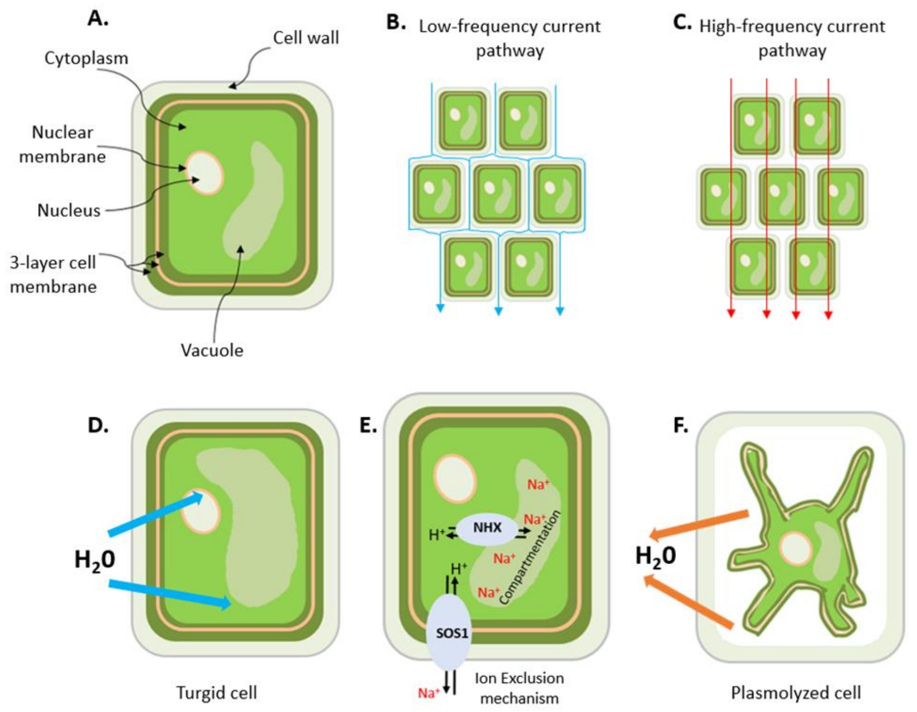


Figure 1. Schematic illustration of **a**) plant cell showing some of the organelles (vacuole, nucleus and nuclear membranes), the cell wall and the 3-layer (protein-lipid-protein) cell membrane, **b**) low-frequency current pathway, **c**) high frequency current pathway, **d**) turgid cell resulting from the uptake of water, **e**) early stage response to salt stress in a plant root cell (adapted from Deinlein et al. 2014), this involves the activation of cellular detoxification mechanisms, including NHX and SOS Na^+ transport mechanisms (NHX: Na^+/H^+ exchanger, SOS: Salt Overly Sensitive), **f**) plasmolyzed cell due to excessive loss of water. This can occur at a later stage of salt stress, when there are excess ions in the solution because the root cells can no longer exclude or compartment them into the vacuole, water leaves the cell by osmosis leading to plasmolysis.

Plants respond to salt stress by adaptive mechanisms such as root exclusion of excess sodium in the surrounding water or compartmentation, removing toxic ions from the cytoplasm where sensitive metabolic processes occur (Hasegawa et al. 2000; Munns and Tester 2008; Zhao et al. 2020) into the vacuole (Neubert et al. 2005; Farooq et al. 2015; Isayenkov and Maathuis 2019). These two adaptive mechanisms are independent, but their effectiveness varies across species (Grieve et al. 2012; Acosta-Motos et al. 2017). They modify the ionic composition of the extracellular and intracellular fluids (Fig. 1e), which suggests that these adaptive mechanisms can possibly also be detected by SIP. For example, Ben Hamed et al. (2016) investigated the use of EIS to non-invasively assess salt resistance and the signaling and short-term (0-240 minutes) response of Sea rocket (*Cakile maritima*) to salinity. Sea rocket was used as a model for salt-tolerant plants as it can survive extended contact with solute concentrations up to 500 mM NaCl. It accumulates salt ions preferentially in its leaves without dehydration and nutritional disorders (Debez et al. 2013). Ben Hamed et al. (2016)They found that the frequency-dependent impedance of leaves changed with increasing salinity as well as the duration of stress for plants grown in sand and hydroponic culture conditions. In particular, it was observed that for a group of 10 plants exposed to increasing salinity, the electrical resistance of the leaves increased in the presence of 50-100 mM NaCl, but decreased for salinity above 100 mM NaCl, with the lowest value observed at 400 mM NaCl. For another group of 10 plants exposed to a 400mM NaCl treatment over 240 minutes, the electrical resistance increased at early stages of salt stress and reached a maximum after 180 minutes before declining rapidly. They concluded that theThe increasing electrical resistance within the tolerable range of salinity for growth (50–100 mM NaCl) indicated was attributed to low salt movement in leaf cells due to compartmentation of salt ions in the leaf vacuoles, as reported in previous studies (e.g. Debez et al. 2004; Ellouzi et al. 2011), while. the decrease in electrical resistance at salinities above 100 mM NaCl was interpreted as an indication of increased movement of salt ions in the leaf cells, most probably in the apoplastic space. They suggested that at these higher salinities, leaf cells seemed to lose their ability to compartment all salt ions in the vacuoles. Therefore, ions may have accumulated in the apoplast and caused osmotic and nutritional imbalances that led to stunted growth. Similarly, Ellouzi et al. (2011) reported rapid accumulation of Na^+ in the vacuole and re-establishment of osmotic homeostasis shortly after salt treatment (400 mM NaCl for 4 h). They also observed a decrease in the electrical resistance of leaves of salt-treated plants, which was closely correlated with the increased accumulation of Na^+ in the vacuole. These studies suggest that the electrical resistance of salt-stressed plants varies with degree of salinity and the duration of salt stress. This implies that that the accumulation of Na^+ and Cl^- ions in the cytoplasm and apoplast will take a long time to reach toxic levels when the salt concentration is low. At very high salt concentrations, it is expected that toxic level will be attained much faster, this could happen in a couple of minutes (e.g. Ben Hamed et al. 2016).

Despite these interesting studies, the suitability of SIP as a tool to study plant response to salinity has not been thoroughly investigated and few existing studies focused mainly on plant leaves. However, the root cells are the first target of soil salinity and more studies are still needed to better understand how roots respond to salt stress. Therefore, the aim of this study is to evaluate the SIP response of *Brachypodium* and *Mai*ze primary roots subjected to different levels of salinity and to link the observed changes in electrical properties with the salt adaptation mechanisms of plants to obtain further insights into the ability of SIP to detect salt stress in plant roots.

2. Materials and methods

2.1. Investigated plants and salt solutions

Brachypodium (*Brachypodium distachyon L.*) and maize (*Zea mays L.*) were studied under different salinity treatments. *Brachypodium distachyon L.* is a salt-sensitive plant that can tolerate salt stress below 200 mM NaCl (e.g. Lv et al. 2014; Guo et al. 2020). *Zea mays L.* is moderately sensitive to salt stress (Kaddah and Ghowail 1964; Farooq et al. 2015) and can tolerate relatively high salinity up to 400 mM NaCl (e.g. de Azevedo Neto et al. 2004), depending on the genotype. Plants of both species were grown in the laboratory under daylight conditions (without artificial light), normal humidity and an average temperature of 23.2°C. They were grown in plastic tubes (5 x 20 cm) using a mixture of fine and coarse sand with a grain size distribution ranging from 0.1 to 1.0 mm (Ehosioke et al. 2023). The plants were watered with tap water at 2-day intervals and were sampled at 20 days after sowing (DAS). The average diameter of the *Brachypodium* and maize primary roots were 0.22 mm and 0.89 mm, respectively. Both plant types were in the 3-leaves stage at the time of measurement. Before each SIP measurement, the plant was removed from the growth tube and the sand particles on the roots were removed gently.

Salt solutions were prepared by dissolving sodium chloride (NaCl) in demineralized water. The electrical conductivity was measured using a conductivity meter (HQ14D, HACH, Mechelen, Belgium). A total of 14 salt solutions with different concentrations were prepared (Table 1). The resulting concentration is presented in ppm. The nomenclature to describe different types of saline water based on concentration and electrical conductivity is presented in Table A1 (see Appendix).

Table 1 Description of salt solutions used during the experiments.

Salt solution: mass of NaCl dissolved in 0.05 L of demineralized water (mg)	Concentration (ppm)	Concentration (mM)	Conductivity (mS/cm)	Temperature (°C)
Demineralized water (baseline)	-	-	0.0012	24.8
50	1000	17.1	1.94	22.9
100	2000	34.2	3.20	22.6
150	3000	51.3	5.46	22.6
200	4000	68.4	6.78	22.5
300	6000	102.7	9.75	22.6
400	8000	136.9	12.66	22.7
500	10000	171.1	15.47	22.6
840 *(Salt-L)*	16800	287.5	28.50	24.8
1690 *(Salt-M)*	33800	578.4	47.40	23.6
1700	34000	581.8	48.70	23.6
1750	35000	598.9	50.10	23.5
1800	36000	616	51.60	23.5
2000	40000	684.5	57.30	23.4
3000 *(Salt-H)*	60000	1,026.7	83.40	25.3

2.2. Measurement set-up

The measurement set-up consists of a precision balance (Mettler PM 2000), sampling container, SIP measurement system, and a sample holder especially designed for root segments (Fig. 2; Ehosioke et al. 2023). We used the high precision balance for a precise measurement of the uptake. The SIP measurement system is made up of a data acquisition (DAQ) card (NI USB-4431), an amplifier unit (ZEA-2-SIP04-V05), a function generator (Keysight 33511B), triaxial cables and a computer. A detailed description of the SIP measurement system and the specialized sample holder are provided in Ehosioke et al. (2023).

The SIP measurement is performed by injecting alternating current at different frequencies (1 Hz – 45 kHz), and a voltage of 5V into a sample and measuring the amplitude and phase lag of the resulting voltage, which leads to a frequency dependent electrical impedance expressed as:

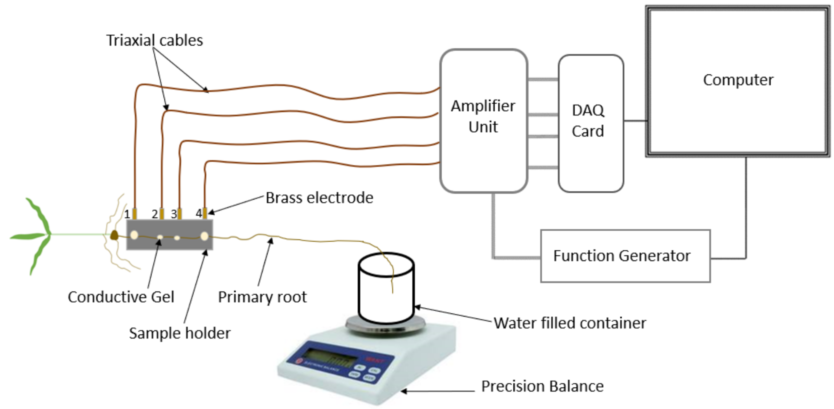
$$Z_\omega^* = Z_\omega' + jZ_\omega'' \quad (1)$$

where Z_ω^* is the complex impedance, ω is the angular frequency, Z' and Z'' are the real and imaginary parts of the complex impedance, and j is the imaginary unit. The complex impedance can be converted into the complex electrical conductivity or electrical resistivity by accounting for the dimension of the sample using a geometric factor ($K = \frac{\pi d^2}{4l}$ where d is the root diameter and l is the root length):

$$\rho_\omega^* = KZ_\omega^* = |\rho|e^{j\varphi} \quad (2)$$

where φ is the phase shift and $|\rho|$ is the resistivity magnitude. The relationship between complex conductivity σ_ω^* and complex resistivity ρ_ω^* is:

$$\sigma_\omega^* = \frac{1}{\rho_\omega^*} \quad (3)$$



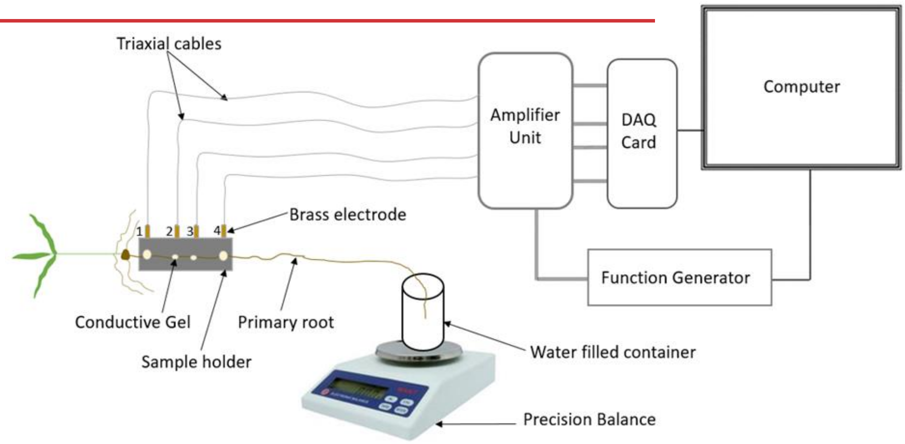


Figure 2. Measurement set-up for investigating the electrical response of roots during water uptake.

2.3. Measurement protocol

~~First, p~~Preliminary SIP measurements were performed on roots of m~~M~~aize and *Brachypodium* plants in air to investigate the effect of root drying on the SIP response. For this, one plant of each species was sampled. The root was mounted in the sample holder and SIP measurements were taken at 5 minute~~s~~ intervals for a total duration of 20 minutes with the root in the same position (see Fig. 2).

~~For~~ To investigate the response to water and salt uptake, the root was mounted on the sample holder and an initial SIP measurement was performed that forms the baseline. After this, ~~, before~~ the root apex was tipped into ~~a~~ 50 ml demineralized water (e.g. Rewald et al. 2011; Li et al. 2016) or saline water of known conductivity in a 60 ml

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sampling container (Fig. 2)~~, and~~. ~~t~~The ~~initial~~ weight of the water, the container and the root tip was recorded. ~~. The weight was also recorded~~ every 5 minutes for a total duration of 20 minutes. Temperature and humidity were recorded at the end of the experiment. In the case of water uptake, SIP measurements were acquired on one plant for each species using the same measurement strategy to serve as a reference to help interpret the electrical response of roots to the uptake of salt solutions.

The SIP response of roots in different salt solutions was investigated in two experiments. In a first experiment, we exposed one plant of each species to two different salt solutions i.e salt-L and salt-H (see Table 1). The SIP measurements were performed at a 5 minute~~s~~ interval over a 20 minutes duration while the root apex was tipped in salt solution. In the second experiment, the effect of varying salt concentrations on the SIP response of the roots was investigated. To achieve this, the measurement procedure described above was repeated with 7 different salt solutions for Brachypodium (1000 – 10000 ppm) and another 7 different salt solutions for Maize (16800 – 60000 ppm) (see Table 1). Thus, a total of 14 plants were~~was~~ ~~used~~ sampled in this experiment. To estimate evaporation loss during SIP measurements, an empty sample container with ~~a~~ 50 ml of demineralized water was left open on the balance and the mass was measured every 5 minutes over a 20 minutes duration. ~~, t~~This procedure was repeated for the salt solutions to estimate the loss of water from the container due to evaporation. The evaporation loss was found to be 40 mg in 20 minutes for both demineralized and saline water. The temperature and humidity at the time of measurement was also recorded (see Appendix B: Table B1). The net amount of solution absorbed by the root during each measurement corresponds to the weight difference corrected for the estimated loss by evaporation.

3. Results and Discussion

3.1. SIP monitoring of root ~~dessication~~desiccation

The resistivity magnitude and phase of exposed *Brachypodium* and m~~M~~aize roots are shown in Fig. 3. We can observe that the resistivity values of root segments of both species increased when the roots were exposed in the air. Water content plays a key role in maintaining the structural properties and physiological processes of the cell membrane (Crowe and Crowe 1982). Loss of water from roots may lead to a loss of turgor pressure (plasmolysis), which can result in a decrease in cell volume depending on cell wall hardness (Verslues et al. 2006; Robbins and Dinneny 2015), a decrease in cell membrane surface area, and cell membrane injury in severe cases (Lew 1996; Ando et al. 2014). Wu et al. (2008) reported an increase in total impedance during dehydration of eggplant pulp. Islam et al. (2019) also observed an increase in total impedance of onions during drying over a period of 21 days. They concluded that movement of ions due to dehydration is responsible for the increased impedance. The increase in resistivity observed in our ~~studies~~ study for ~~Maize~~ maize and *Brachypodium* roots is due to loss of water from

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the root cells (dehydration) due to evaporation. The increase in resistivity is higher for Brachypodium (78 Ωm increase in 20 minutes after the baseline measurement of 68 Ωm) than for Maize (7 Ωm increase in 20 minutes after a baseline measurement of 16 Ωm) both in absolute and relative values. This suggests that *Brachypodium* root lost water faster than ~~Maize~~ maize in our experiment. We had expected that ~~Maize~~ maize would lose more water because of the larger surface area, but the result suggests that something other than surface area influenced the root dehydration, which could be the degree of saturation. Since ~~Maize~~ maize roots were observed to be ~~more saturated~~succulent and white in color ~~than~~ while *Brachypodium* roots were dry and brownish in this study, it should take longer for m~~M~~aize roots to lose sufficient water and become plasmolyzed compared to *Brachypodium* roots. Shrinkage of *Brachypodium* root was clearly visible at the end of the measurement, whereas m~~M~~aize appeared dry on the surface but showed no significant shrinkage. The ~~more noisy~~noisier data observed for *Brachypodium* is attributed to the high contact impedance of the root induced by shrinkage of *Brachypodium* root during drying. ~~Polarization~~ Over the exposition time of 20 minutes, polarization (phase peak) of Brachypodium ~~showed a decrease~~decreased from 870 mrad at 6.3 kHz to 570 mrad at 1 kHz~~and a shift towards lower frequencies,~~ while that of m~~M~~aize first ~~showed an increase~~increased from 510 mrad at 45kHz to 560 mrad at 39.8 kHz, followed by a stabilization. In a plasmolyzed cell, cell membranes shrink (see Fig. 1), which is expected to result in a decrease of the phase response. It seems that *Brachypodium* roots might have become plasmolyzed due to water loss (Lew 1996; Ando et al. 2014; Robbins and Dinneney 2014), while m~~M~~aize roots were probably not plasmolyzed but rather experienced osmotic adjustments by redistribution of water to maintain equilibrium (e.g. Sharp et al. 1990; Ogawa and Yamauchi, 2006; Hajlaoui et al. 2010). This might explain why the phase response of ~~Maize~~ maize did not decrease. It is important to note that during the ~~dessication~~desiccation test, the leaves of both plants did not show any sign of wilting (see Appendix C, Figure C1a and C2a).

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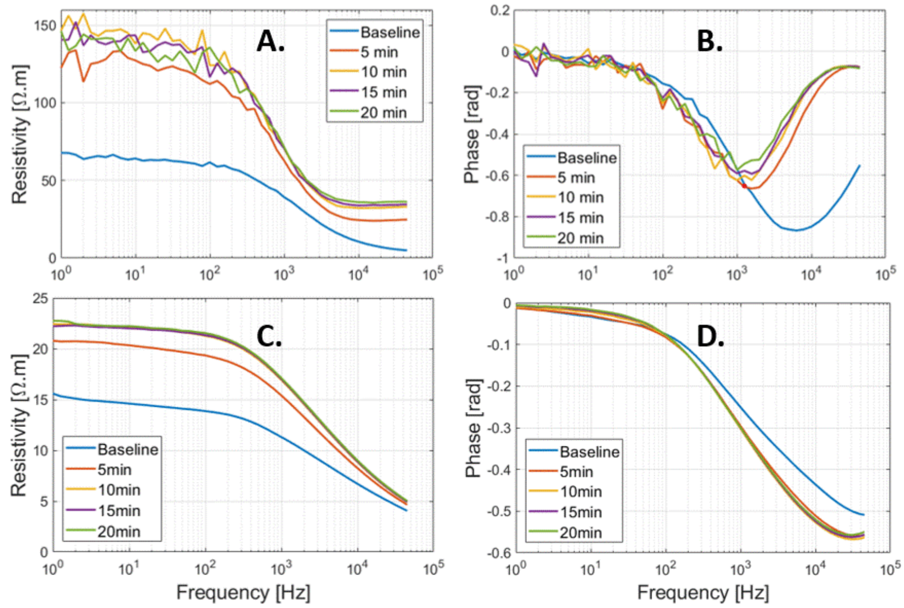

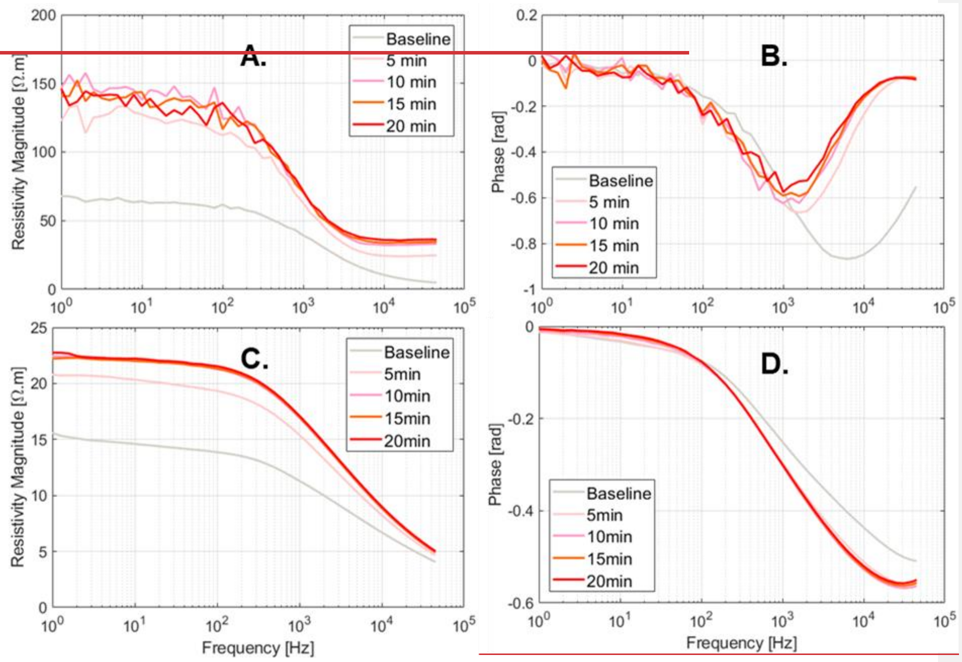

Figure 3. Resistivity and phase response of *Brachypodium* (**a-b**) and Maize maize (**c-d**) primary roots to drying.

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3.2. SIP monitoring of roots with their tips in demineralized water

The change in mass of demineralized (DM) water during SIP measurements on *Brachypodium* and Maize roots is shown in Table 2 and 3 respectively. The net mass of water uptake by the roots after correcting for evaporation loss were 40 mg and 70 mg for Brachypodium and Maize maize root, respectively (see Table2). The Maize maize absorbed more water compared to *Brachypodium* since its leaf surface area is larger and thus has a larger canopy transpiration pull.

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Table 2. Uptake of demineralized water and saline water by *Brachypodium* and maize roots in 20 minutes

Brachypodium Mass (mg)			**Maize** Mass (mg)		
Demin water	Salt-L	Salt-H	Demin water	Salt-L	Salt-H
40	50	40	70	70	70

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Time (min)	Mass (mg)		
	Demin water	Salt-L	Salt-H
0	-	-	-
5	20	20	20
10	20	20	20
15	20	20	20
20	20	30	20

Table 3 Uptake of demineralized water and saline water by Maize root in 20 minutes

Time (min)	Mass (mg)		
	Demin water	Salt-L	Salt-H
0	-	-	-
5	20	40	30
10	30	20	30
15	30	20	30

~~20~~	~~30~~	~~30~~	~~20~~

For both species, the resistivity magnitude shows an increase with a greater effect at low frequencies (< 1 kHz) and almost no effect at high frequencies (> 10 kHz) for Maize (Fig. 4). According to the conduction mechanisms illustrated in Fig. 1, this suggests that extracellular fluid is diluted by DM water, which results in the observed higher resistivity. Polarization (phase peak) of *Brachypodium* showed ~~no clear~~ a temporal trend over the measurement duration, while that of m~~M~~aize remained mostly constant after an initial increase for a broad range of frequencies (10 to 10 000 Hz), which is consistent with its resistivity magnitude. Uptake of DM water may lead to dilution of cellular solutes (Schopfer 2006), which can decrease the water potential gradient across the cell membrane that drives water movement (Robbins and Dinneny 2015). This adjustment will be reflected in the transmembrane potential leading to the polarization effect, and the phase peak could reflect the water redistribution and equilibrium reached as the cell regains full turgor. The phase response of *Brachypodium* root might be linked to the adjustment of the transmembrane potential while the steady increase in phase response of m~~M~~aize suggests that its transmembrane potential might be in equilibrium.

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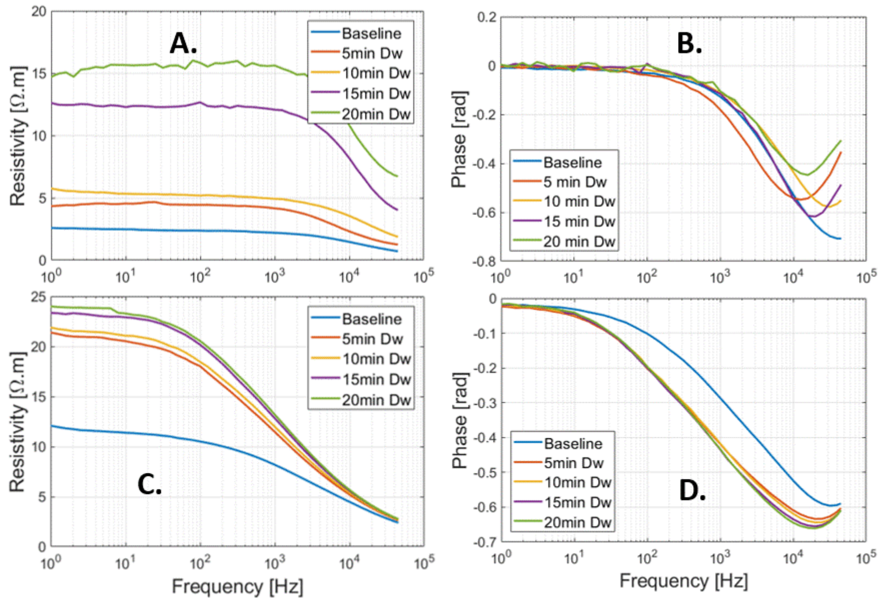
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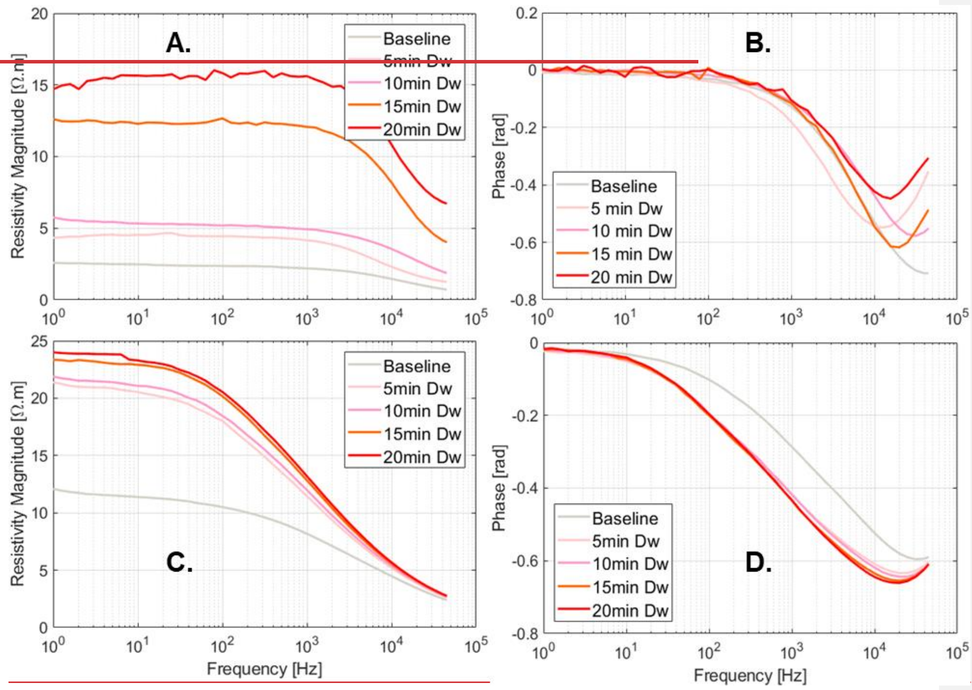
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Figure 4. Resistivity magnitude and phase spectra of *Brachypodium* (**a-b**) and ~~Maize~~ maize (**c-d**) primary roots during absorption of demineralized water. The variable temporal development of the resistivity magnitude might be due to high contact impedance of the *Brachypodium* root.

3.3. SIP monitoring of roots with their tips in saline water

The net mass of saline water (salt-L/salt-H) absorbed by the roots was similar with 40/50 and 70/70 mg for *Brachypodium* and ~~Maize~~ maize roots, respectively (Table 2~~ and 3~~). For the low salt concentration (Salt-L), the SIP response of Maize (Fig. 5) showed a similar response as in the case of DM water with an increasing resistivity magnitude and phase. In contrast, the *Brachypodium* root segments showed a continuous decrease of resistivity magnitude and phase. This opposite behavior may be explained in terms of salt stress tolerance. Maize is known to be moderately sensitive to salt stress (Farooq et al. 2015). Maize roots are able to take up water while excluding salts, making it more robust to salinity stress (Neubert et al. 2005; Farooq et al. 2015; Munns et al. 2020). This may explain why the SIP response of maize at this salt concentration level is ~~similar to the response with~~like that of DM water. Apparently, the concentration of the salt-L solution was already too high for *Brachypodium* to exclude or compartment salt in the vacuole (e.g. Lv et al. 2014) and the excess accumulation of ions in the root cell resulted in the observed decrease in resistivity and polarization (phase peak). Additionally, after 20 minutes of measurement with *Brachypodium* root tip in salt-L, the Brachypodium leaves showed visible signs of wilting (Appendix C: Figure C2b) which is a key sign of salt toxicity in plants (e.g. Ji et al. 2022; Plant Ditech 2023). Similar signs of wilting of leaves was observed in ~~Maize~~ maize leaves after 20 minutes of measurement with the root tip in saline water of 40000 ppm (684 mM) (see Appendix C: Figure C1b). Drought is also known to cause wilting of leaves ~~(e.g. UCANR, 2021; Ji et al. 2022; PlantDitech 2023; Bayer 2024)~~. ~~, ho~~However, the absence of wilting when the root tip is not in saline solution for the same duration confirms that the wilting observed in this study is a clear indication that the plants experienced salt toxicity.

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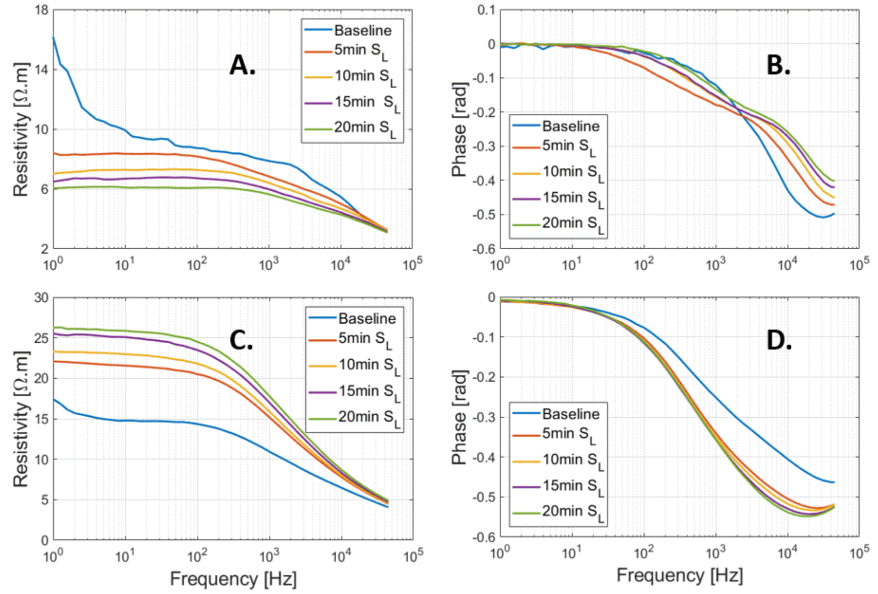

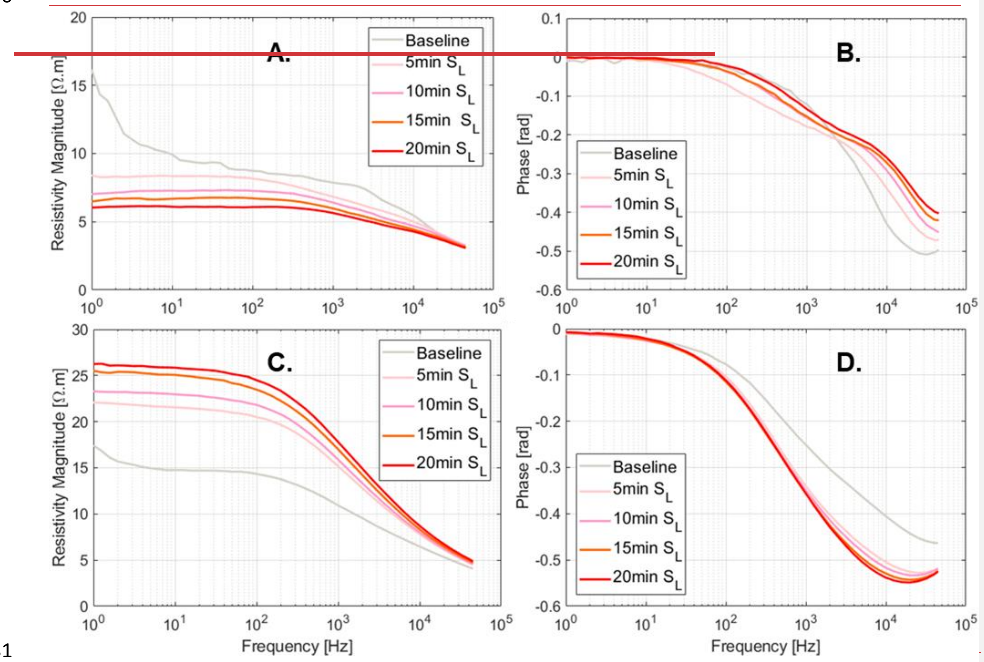

Figure 5. Changes in resistivity magnitude and phase spectra of *Brachypodium* (**a-b**) and ~~Maize~~ maize (**c-d**) primary roots during absorption of saline water (salt-L).

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During uptake of water with high salt concentration (salt-H) ~~uptake~~ (Fig.6), it is interesting to see that both ~~Maize~~ maize and *Brachypodium* roots now have similar responses, showing a consistent decrease in both resistivity magnitude and phase. Thise consistent decrease ~~in resistivity magnitude and phase~~ for both species suggests excessive accumulation of ions in the cytoplasm and apoplast, which makes the roots more conductive (Debez et al. 2004; Ellouzi et al. 2011). At this high salt concentration (Salt-H), the plant cells apparently cannot exclude all the sodium and chloride ions or compartment them in the vacuole. This is probably the beginning of toxicity effects, although it will take time for the damage to be visible. This early detection of ion toxicity is a key advantage of SIP for root salinity studies (Ben Hamed et al. 2016). Additionally, salinity can lead to membrane damage with increased permeability (e.g. Cseresnyés et al. 2018), which might have contributed to the changes observed in this study.

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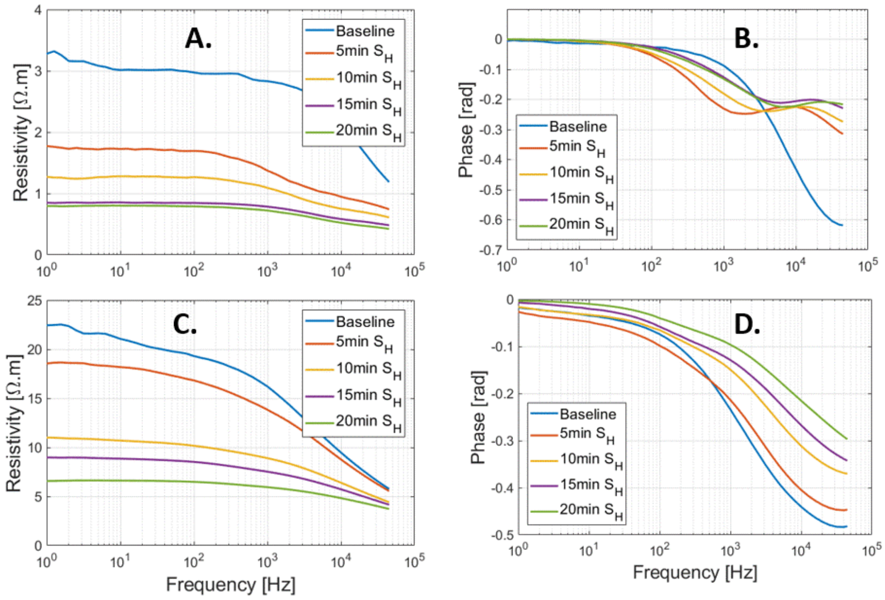

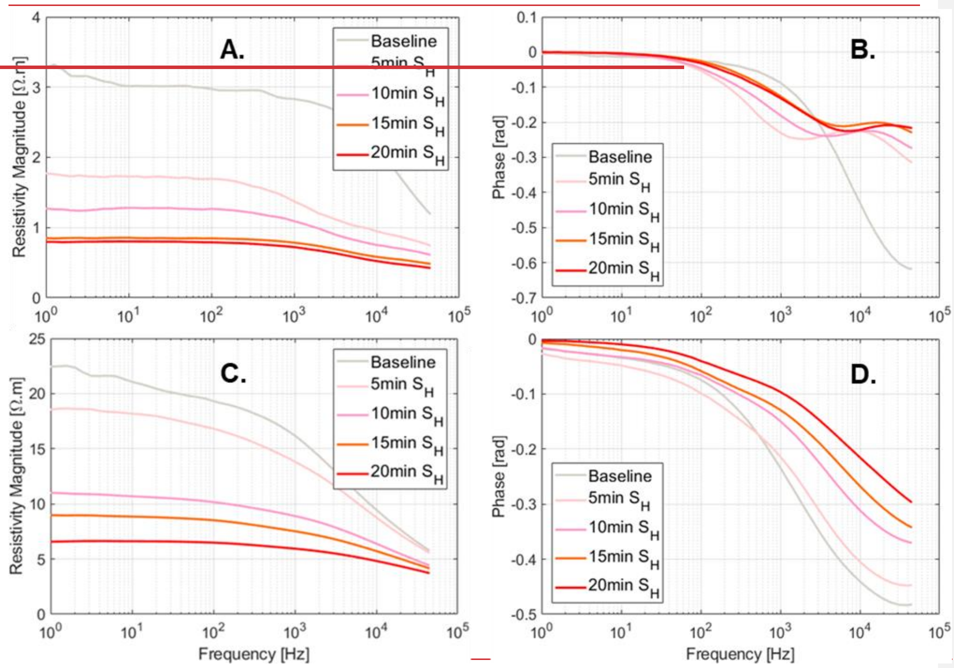

Figure 6. Changes in resistivity and phase spectra of *Brachypodium* (**a-b**) and ~~Maize~~ maize (**c-d**) primary roots during absorption of saline water (salt-H).

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3.4. Replicate measurements on maize and Brachypodium roots

Several replicate measurements on maize and *Brachypodium* roots were performed prior to the results reported in Fig. 3-6, to ensure consistency of our observations in both species. The root tips were exposed in the air for 5 minutes after the baseline measurement (to observe the effect of desiccation) before putting the root tip in demineralized water and saline water. We observed that the response to desiccation, water and saline water uptake were similar across the replicates (see Appendix D: Figure D1 and D2). Saline water (Salt-L) uptake by maize root was monitored for 60 minutes, both resistivity and phase showed consistent increase (see Appendix D: Figure D3a-b). A different saline water with a higher concentration of 33800 ppm (Salt-M) showed an increase in resistivity and phase only in the first 15 minutes (see Appendix D: Figure D3c-d). These results confirm the reproducibility of our observations.

3.54. SIP monitoring of roots taking up water of gradually increasing salinity

The SIP response of Maize maize and *Brachypodium* roots to increasing salinity is presented in Fig. 7. Note that the range of salinity used for both species is different due to their different tolerance to salt stress. In general, a similar resistivity response was observed for both species (Fig. 7a and 7c), showing either an increase or a decrease of resistivity depending on the solute concentration, but with a different threshold due to their different salt stress tolerance. For Maize maize roots, the phase response is similar tolike the resistivity response showing either an increase or decrease with concentration over time (Fig. 7b) for a concentration threshold between 34000 and 35000 ppm. For *Brachypodium* roots, a decrease of phase is observed at all concentrations after 10 minutes (Fig. 7d). Only at low concentration (below 4000 ppm), an initial increase in phase was observed in the first 10 minutes of the experiment.

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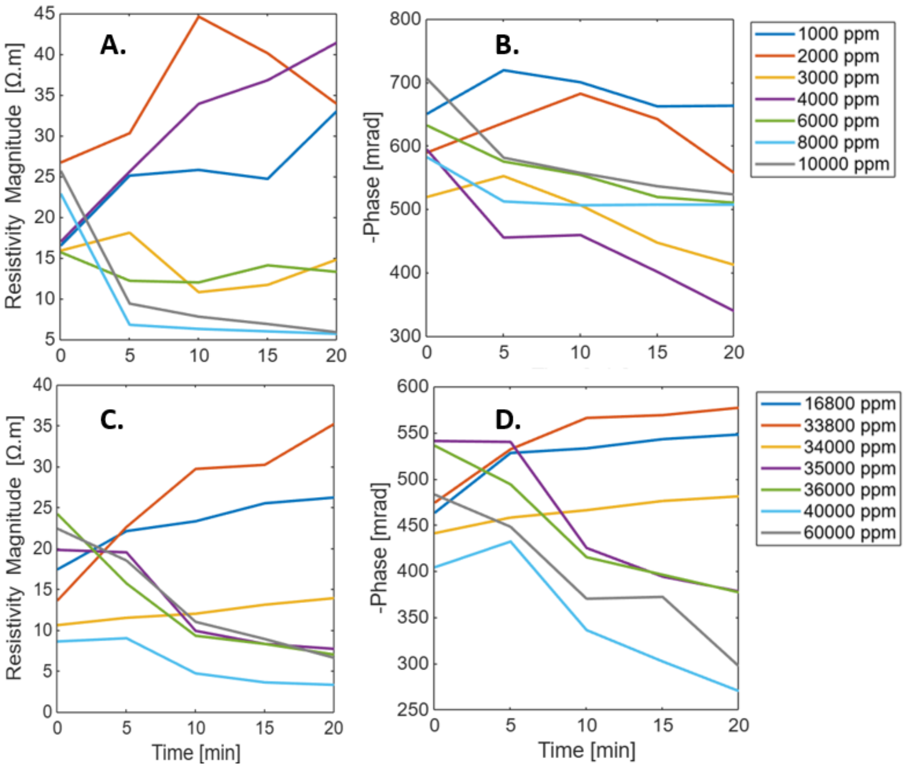
A.
B.
C.
D.
Resistivity Magnitude [Ω.m]
-Phase [mrad]
Time [min]
1000 ppm
2000 ppm
3000 ppm
4000 ppm
6000 ppm
8000 ppm
10000 ppm
16800 ppm
33800 ppm
34000 ppm
35000 ppm
36000 ppm
40000 ppm
60000 ppm

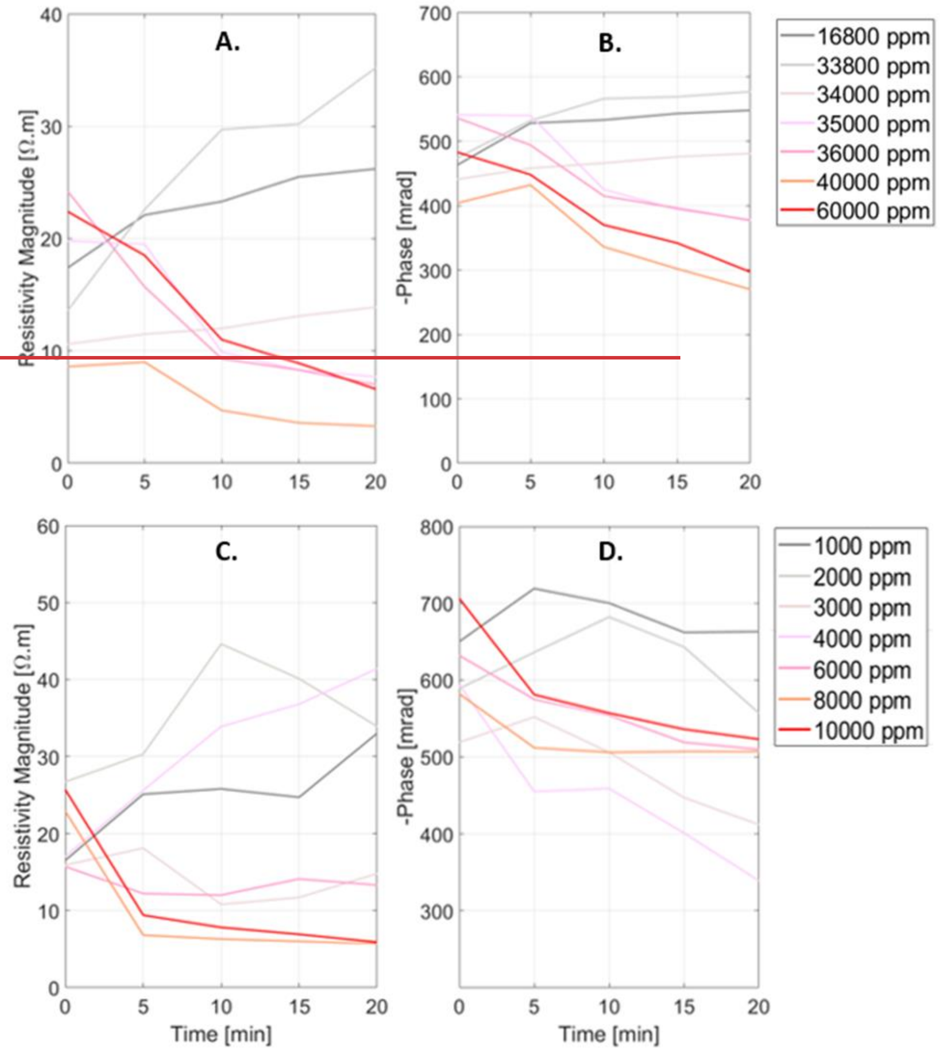

Figure 7. Changes in resistivity magnitude and phase peak of primary roots of ~~Maize~~ *Brachypodium* (**a-b**) and ~~Brachypodium~~ maize (**c-d**) with concentration over time.

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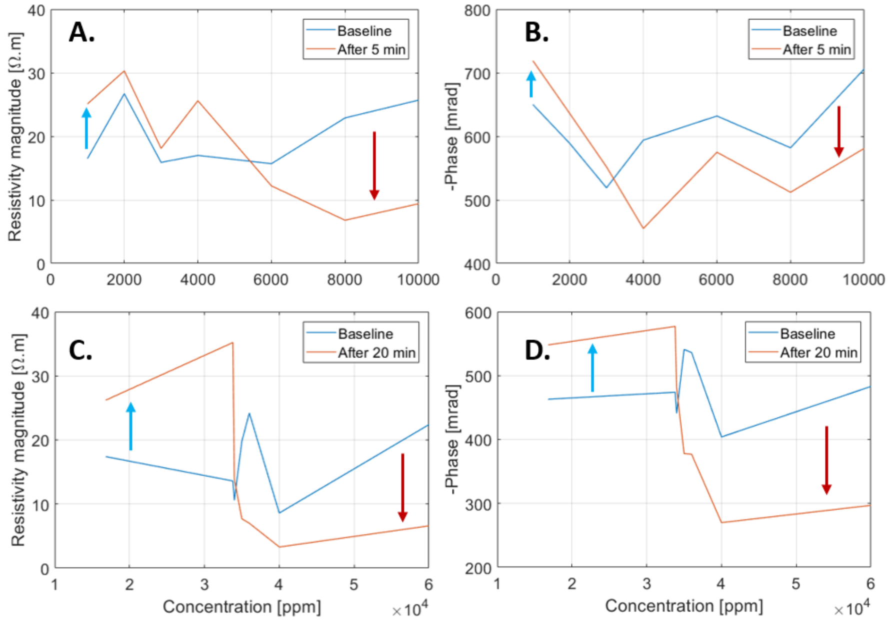
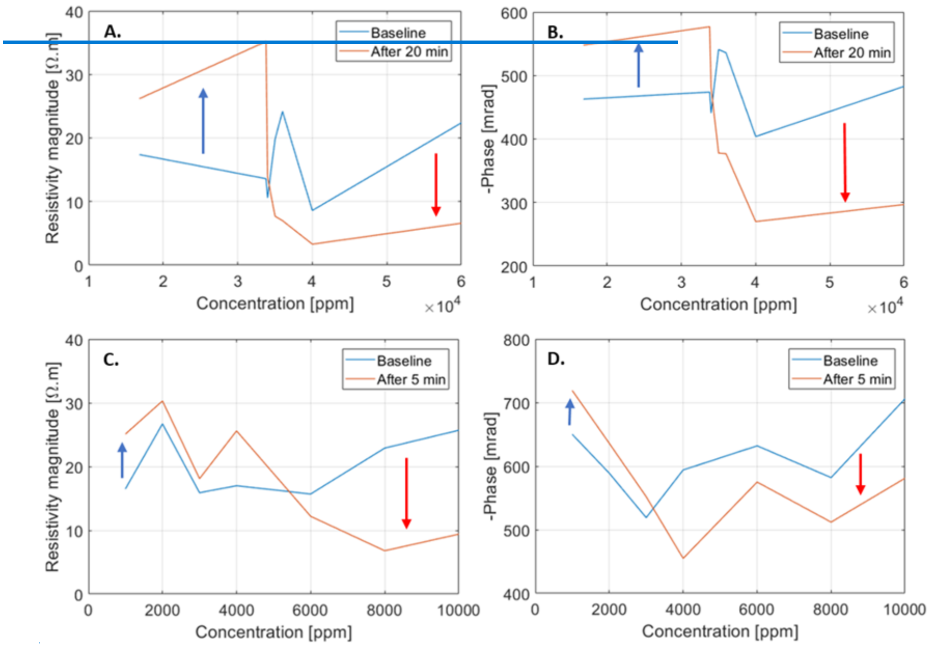


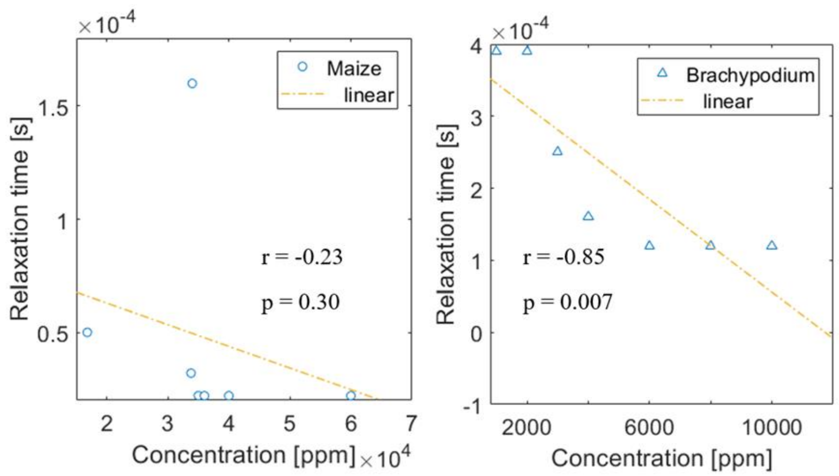
A.
B.
C.
D.
Baseline
After 5 min
After 20 min
Resistivity magnitude [Ω.m]
-Phase [mrad]
Concentration [ppm]
×10^4



A.
B.
C.
D.
Baseline
After 20 min
After 5 min
Resistivity magnitude [Ω.m]
-Phase [mrad]
Concentration [ppm]
×10^4

Figure 8. Reversal of resistivity magnitude and phase peak of ~~Maize~~ *Brachypodium* (**a-b**) and ~~Brachypodium~~ maize (**c-d**) primary roots as concentration increases.

The adaptive mechanisms to salt stress may explain why the resistivity and phase response of the roots increased at low salt concentrations and decreased at high salt concentration (Fig. 8). With increasing salt concentration, excessive sodium accumulation in the cells occurs when the salt resistance threshold of the plant species is exceeded (Cramer 1988; Davenport et al. 2005; Zhao et al. 2010; Farooq et al. 2015; Isayenkov and Maathuis 2019). Excess ions in the cell will increase the conductivity of the cellular fluid leading to decreased resistivity and phase (e.g. Fig. 7 and 8). The disparity between the phase response of ~~Maize~~ maize root and *Brachypodium* root with increasing salinity may be related to the salt resistance mechanisms of the species. For example, some maize genotypes c~~ould~~an tolerate ~~salt~~high salinity up to 400 mM NaCl (e.g. Azevedo Neto et al. 2004), while *Brachypodium* can tolerate salinity stress below 200 mM NaCl (e.g. Guo et al. 2020). These results seem to confirm that ~~Maize~~ maize is more tolerant to salinity than *Brachypodium* (see section 2.1), showing increasing resistivity and phase response up to 34000 ppm before decreasing (Fig. 8a and 8b) while the *Brachypodium* show increasing resistivity only up to 5800 ppm before decreasing (Fig. 8c). The reversal of phase response in *Brachypodium* occurs at 3000 ppm but it is only visible in the first 5 minutes (Fig. 8d). The threshold at which the reversal occurs in ~~Maize~~ maize falls within the range of very highly saline water, while that of Brachypodium lies in the range of moderately saline water (see appendix, Table A1~~2~~).



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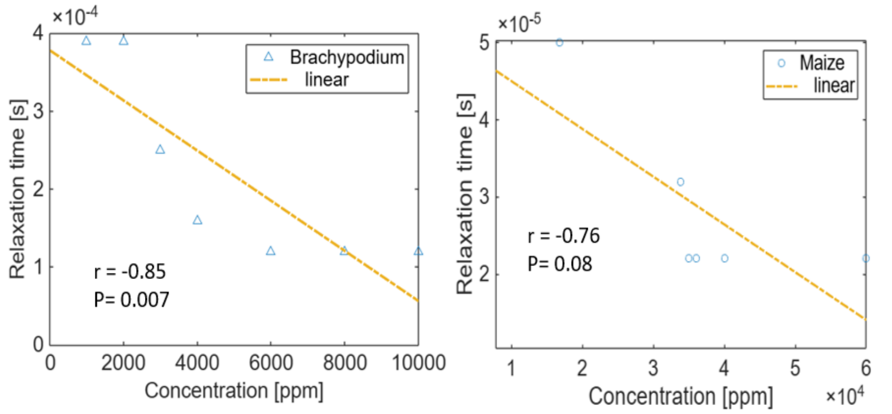

Figure 9. Correlation of relaxation time with NaCl concentration for *Brachypodium*~~Maize~~ and maize~~Brachypodium~~ primary roots. The relaxation time τ_{max} is expressed as the inverse of ω_{max}, where ω_{max} is the angular frequency at which the maximum phase shift occurs.

In Figure 9, we present a trend analysis of the relaxation time (τ_{max}) and salt concentration during the reversal of electrical response observed in Brachypodium (5 minutes) and Maize (20 minutes) as reported in Figure 8. Bücker and Hördt (2013) reported that relaxation times are only weakly dependent on salinity in the case of pore radii, but in this study we found a significant correlation between relaxation time and NaCl concentration in *Brachypodium*, (with Pearson's r = -0.85 and p value = 0.007) and maize (with Pearson's r = -0.76 and p value = 0.08). The difference in slope~~is~~ further suggests that both species respond differently to salt stress based on their salinity tolerance.

Salinity tolerance varies widely across plant species and even across genotypes within a species (Grieve et al. 2012). Thus, salinity tolerance of any plant is therefore indicated by the point or range in the continuum of salt stress where visible or quantitative adverse effects are observed (Lauchli and Grattan 2012). In this study, the concentration at which the reversal occurs for each species could be an indication of the salt resistance threshold of the species (Grieve et al. 2012). This implies that salt tolerant species can withstand higher degrees of salinity over a longer period of time.

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4. Conclusions

We showed that SIP is able to detect the uptake of water and saline water in both ~~Maize~~ maize and *Brachypodium* roots, and that the conduction and polarization of ~~Maize~~ maize and *Brachypodium* roots were influenced by the degree of salinity. Plants respond to salt stress by excluding the ions from entering the cells (ion exclusion) and by removing the sodium and chloride ions from the cytoplasm and accumulating them in the vacuole (ion compartmentation). At relatively low salt concentration, the plants activate these salt resistance mechanisms leading to osmotic adjustment which helps the cells to maintain ionic balance, turgor and volume so that the plant can function optimally, which we observe as increasing resistivity and phase in the SIP signal. At very high salt concentration, there are more ions in the solution than the plant can exclude or compartment, which leads to excess sodium and chloride ions in the cytoplasm and apoplast (ion toxicity) which we observed as decreasing resistivity and polarization. The duration of salt stress and the salt concentration determine how long it takes for ion accumulation in plants to reach toxic levels. At very low concentrations, it might take days to weeks, but at very high concentrations it takes minutes only.

More studies should focus on testing the use of SIP method for early detection of salt stress in field grown crops. Future studies should be carried out with halophytes with a clear salt tolerance threshold. For example,~~;~~ it would be interesting to know if the reversal of electrical properties at certain salt concentrations will match clearly with the salt tolerance threshold of the plants. In this study, we focused on single root segments (primary roots) in the laboratory. For field measurement, we ~~sugest~~suggest the use of an electrode set up that can be used to perform SIP measurements directly on the crop stem, which will solve the problem of current leakage through the soil-root interface in the case of stem-soil electrodes set up where the soil is more conductive than the roots (e.g. in a salty soil). Since the measurement at the root collar in this study detected uptake of saline water by the root tip, we expect that measurement at the root stem will also detect uptake of salt by the roots under field conditions.

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Appendices

Appendix A: Saline water classification

Table A1. Classification of saline water modified after Rhoades et al. (1992).

Water classification	Salt concentration (ppm)	Electrical conductivity (mS/cm)
Non-saline	< 500	0.7
Slightly saline	500 - 1500	0.7 - 2
Moderately saline	1500 - 7000	2 - 10
Highly saline	7000 - 15000	10 - 25
Very highly saline	15000 - 35000	25 - 45
Brine	> 35000	> 45

Appendix B.: Rraw data from the experiments

Table B1. Changes in mass of sample container duringEvaporation evaporation estimation for demineralized water and salt solutions (salt-L and salt-H).

Time(min)	Mass of sample container (g)			Temperature (°C)			Humidity (%)		
	D.water	*Salt-L*	*Salt-H*	*D.water*	*Salt-L*	*Salt-H*	*D.water*	*Salt-L*	*Salt-H*
0	54.08	55.24	57.27	26.7	26.5	26.2	36	32	30
5	54.07	55.23	57.27	26.5	26.5	26.6	36	32	31
10	54.06	55.22	57.25	26.9	26.5	27.0	36	32	30
15	54.05	55.21	57.24	27.1	26.6	27.4	36	32	30
20	54.04	55.20	57.23	27.3	26.6	28.2	36	32	28

Table B2. Demineralized water uptake by m~~M~~aize and *Brachypodium* in 20 minutes.

Time(min)	Mass (g)		Temperature (°C)	
	Maize	*Brachypodium*	Maize	*Brachypodium*
0	54.82	54.98	28.1	27.7
5	54.80	54.96	28.1	27.8
10	54.77	54.94	28.2	27.9
15	54.74	54.92	28.2	27.9
20	54.71	54.90	28.3	28.0

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Table B3. Saline water uptake by ~~Maize~~ maize and Brachypodium roots in 20 minutes.

Time (min)	Salt-L				Salt-H			
	Maize		Brachypodium		Maize		Brachypodium	
	Mass (g)	Temp (°C)	Mass (g)	Temp (°C)	Mass (g)	Temp (°C)	Mass (g)	Temp (°C)
0	55.54	26.1	55.71	26.2	57.66	26.4	57.79	26.8
5	55.50	26.6	55.69	26.6	57.63	26.4	57.77	26.8
10	55.48	26.7	55.67	26.9	57.60	26.6	57.75	26.8
15	55.46	26.8	55.65	27.0	57.57	26.9	57.73	26.9
20	55.43	26.7	55.62	26.9	57.55	27.1	57.71	26.9

Appendix C: ~~visual~~ Visual inspection of plants during the experiments

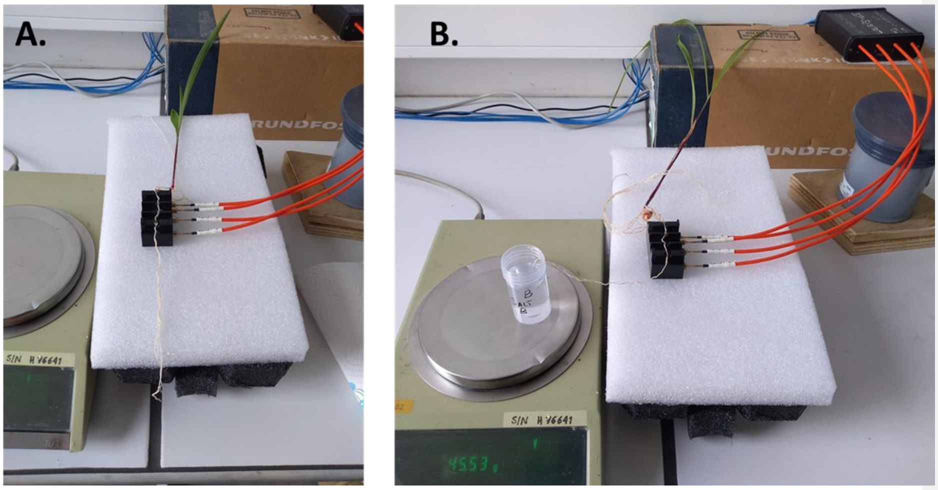

Figure C1. (a) Maize roots exposed during ~~dessication~~desiccation test over 20 minute duration, the leaves showed no sign of wilting. (b) Maize roots exposed with the primary root tip in saline water of 40000 ppm (684 mM) concentration, the leaves showed visible signs of wilting after 20 minutes of measurement.

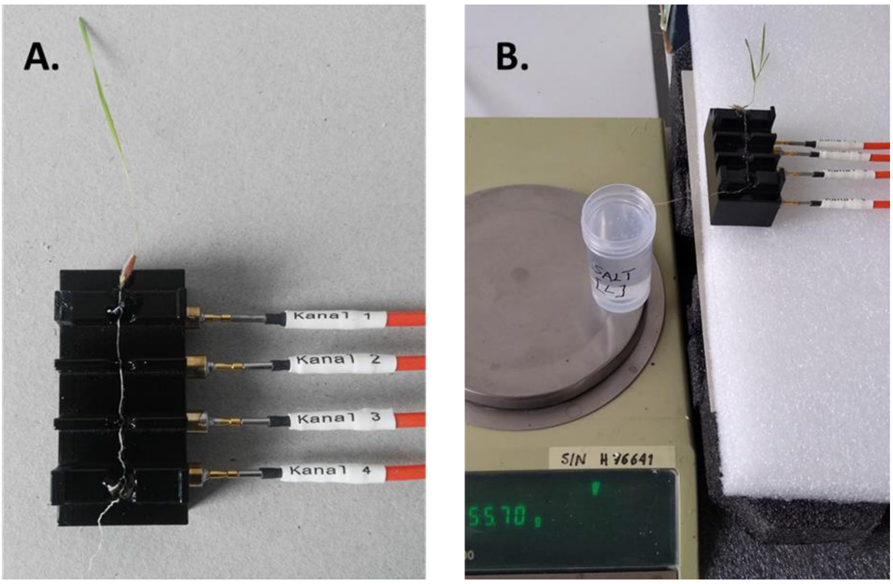

Figure C2. (a) *Brachypodium* root exposed during ~~dessication~~desiccation tests over 20 minute duration, the leaves showed no sign of wilting. (b) *Brachypodium* roots exposed with the primary root tip in salt-L solution of 16800 ppm (287 mM) concentration, the leaves showed visible signs of wilting after 20 minutes of measurement.

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Appendix D: Replicate measurement on Brachypodium and maize roots s

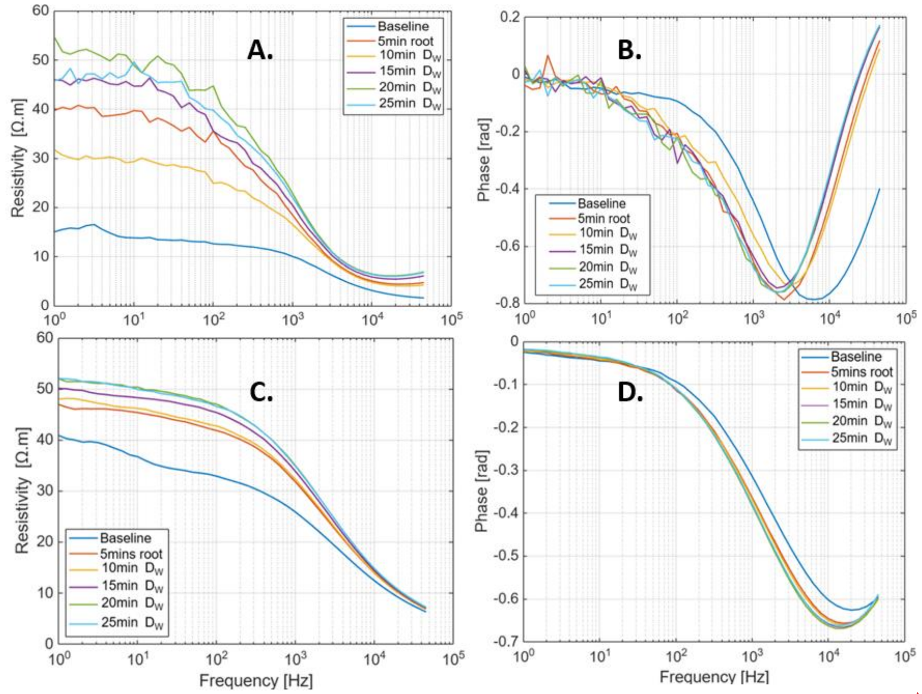

Figure D1. Resistivity and ~~P~~phase spectra of *Brachypodium* (a-b) and maize (c-d) primary roots during demineralized water uptake for 25 minutes. Measurement at 0 minute represents the baseline, measurement was repeated after 5 minutes (to observe drying effect) before putting the root tip in water at 10, 15, 20 and 25 minutes.

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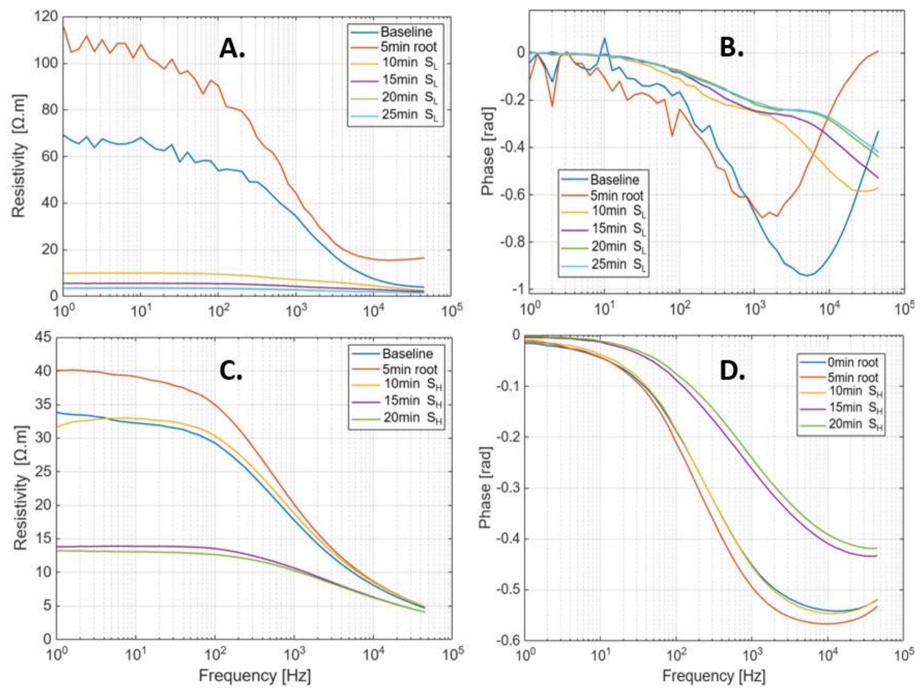

Figure D2. Resistivity and Pphase spectra of *Brachypodium* (a-b) during the uptake of saline water (salt-L) for 25 minutes, and maize (c-d) during saline water (salt-H) uptake for 20 minutes. Measurement at 0 minute represents the baseline, measurement was repeated after 5 minutes (to observe drying effect) before putting the root tip in saline water at 10, 15, 20 and 25 minutes.

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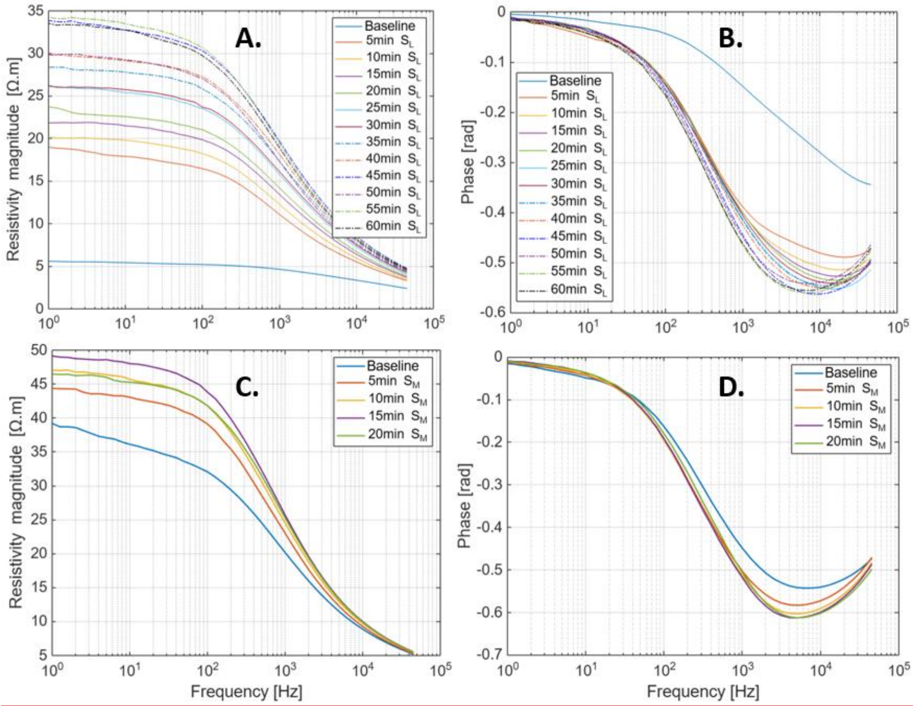

Figure D3. Resistivity and phase spectra of maize (a-b) during the uptake of saline water (salt-L) for 60 minutes, and (c-d) during saline water (salt-M) uptake for 20 minutes. Measurement at 0 minute represents the baseline, before putting the root tip in saline water.

Author Contributions

Conceptualization: SE, FN, SG & MJ

Methodology: SE, FN, JAH, & EZ

Data curation, analysis and visualization: SE, JAH, FN, & EZ

Original draft: SE

Review and editing: All authors

Funding acquisition: SG, FN & MJ

Supervision: SG, FN, MJ, EZ & JAH

Conflict of Interest

The authors declare no conflict of interest

Data Availability Statement

Data associated with this study will be made available on request.

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References

Ackmann, J.: Complex Bioelectric Impedance Measurement System for the Frequency Range from 5 Hz to 1 MHz. *Annals of Biomedical Engineering, 21*, 135–146, 1993.

Acosta-Motos, J. R., Ortuño, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J., and Hernandez, J. A.: Plant responses to salt stress: Adaptive mechanisms. *Agronomy*, *7*(1), 1–38. https://doi.org/10.3390/agronomy7010018, 2017.

Albacete, A., Ghanem, M. E., Martínez-Andújar, C., Acosta, M., Sánchez-Bravo, J., Martínez, V. … Pérez-Alfocea, F.: Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (Solanum lycopersicum L.) plants. *Journal of Experimental Botany*, *59*(15), 4119–4131. https://doi.org/10.1093/jxb/ern251, 2008.

Ando, Y., Mizutani, K., and Wakatsuki, N.: Electrical impedance analysis of potato tissues during drying. *Journal of Food Engineering*, *121*, 24–31. https://doi.org/10.1016/J.JFOODENG.2013.08.008, 2014.

~~Bayer Crop Science: Disease guide-Salt toxicity. https://www.vegetables.bayer.com/gb/en-uk/knowledge-centre/disease-guides/pepper-eggplant/salt-toxicity.html, 2024.~~

Beff, L., Günther, T., Vandoorne, B., Couvreur, V., and Javaux, M.: Three-dimensional monitoring of soil water content in a maize field using Electrical Resistivity Tomography. *Hydrology and Earth System Sciences, 17(2),* 595–609. https://doi.org/10.5194/hess-17-595-2013, 2013

Ben Hamed, K., Zorrig, W., and Hamzaoui, A. H.: Electrical impedance spectroscopy: A tool to investigate the responses of one halophyte to different growth and stress conditions. *Computers and Electronics in Agriculture*, *123*, 376–383. https://doi.org/10.1016/j.compag.2016.03.006, 2016

Bennett, D. L., George, R. J., and Whitfield, B.: The use of ground EM systems to accurately assess salt store and help define land management options, for salinity management. *Exploration Geophysics*, *31*(2), 249–254. https://doi.org/10.1071/EG00249, 2000.

Bera, T. K., Bera, S., Kar, K., and Mondal, S.: Studying the Variations of Complex Electrical Bio-Impedance of Plant Tissues During Boiling. *Procedia Technology*, 23, 248–255. https://doi.org/10.1016/J.PROTCY.2016.03.024, 2016a.

Bera, T. K., Nagaraju, J., and Lubineau, G.: Electrical impedance spectroscopy (EIS)-based evaluation of

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biological tissue phantoms to study multifrequency electrical impedance tomography (Mf-EIT) systems. *Journal of Visualization*, 19(4), 691–713. https://doi.org/10.1007/s12650-016-0351-0, 2016b

Corwin D.L.: Past, present, and future trends of soil electrical conductivity measurement using geophysical methods. In *Agricultural Geophysics* (pp. 17-44). Boca Raton, FL. CRC Press, 2008.

Cosgrove D. J.: Growth of the plant cell wall. *Nature Reviews Molecular Cell Biology* 6, 850–861, 2005.

Couvreur, V., Faget, M., Lobet, G., Javaux, M., Chaumont, F., and Draye, X. : Going with the flow: Multiscale insights into the composite nature of water transport in roots. *Plant Physiology*, 178(4), 1689–1703. https://doi.org/10.1104/pp.18.01006, 2018.

Cramer, G., Epstein, E., and Lauchli, A.: Kinetics of root elongation of maize in response to short term exposure to NaCl and elevated calcium concentration. *Journal of Experimental Botany* 39:1513–1522. doi:10.1093/jxb/ 39.11.1513, 1988.

Crowe, J. H., & Crowe, L. M.: Induction of anhydrobiosis: Membrane changes during drying. *Cryobiology*, *19*(3), 317–328. https://doi.org/10.1016/0011-2240(82)90160-2, 1982.

Cseresnyés, I., Rajkai, K., and Takács, T.: Indirect monitoring of root activity in soybean cultivars under contrasting moisture regimes by measuring electrical capacitance. *Acta Physiologiae Plantarum, 38(5)*, 121. https://doi.org/10.1007/s11738-016-2149-z, 2016.

Cseresnyés, I., Takács, T., Végh, K. R., Anton, A., and Rajkai, K.: Electrical impedance and capacitance method: A new approach for detection of functional aspects of arbuscular mycorrhizal colonization in maize. *European Journal of Soil Biology, 54,* 25–31. https://doi.org/10.1016/J.EJSOBI.2012.11.001, 2013.

Cseresnyés, I., Füzy, A., Kabos, S. *et al.* Monitoring of plant water uptake by measuring root dielectric properties on a fine timescale: diurnal changes and response to leaf excision. *Plant Methods* **20**(5), https://doi.org/10.1186/s13007-023-01133-8, 2024.

Cseresnyés, I., Rajkai, K., Takács, T., and Vozáry, E.: Electrical impedance phase angle as an indicator of plant root stress. Biosystems Engineering, 169 (5), 226-232, https://doi.org/10.1016/j.biosystemseng.2018.03.004, 2018.

Davenport, R., James, R. A., Zakrisson-Plogander, A., Tester, M., and Munns, R.: Control of sodium transport in durum wheat. *Plant Physiology, 137(3),* 807–818. https://doi.org/10.1104/pp.104.057307, 2005.

de Azevedo Neto, A.D., Prisco, J. T., Enéas-Filho, J., de Lacerda, C. F., Silva, J. V., da Costa P. H. A., and Gomes-Filho, E.: Effects ofsalt stress on plant growth, stomatal response and solute accumulation of different maize genotypes. Braz J Plant Physiol 16:31–38, 2004.

Debez, A., Ben Hamed, K., Grignon, C., & Abdelly, C.: Salinity effects on germination, growth, and seed production of the halophyte Cakile maritima. *Plant and Soil*, *262*(1–2), 179–189. https://doi.org/10.1023/B:PLSO.0000037034.47247.67, 2004.

Debez, A., Ben Rejeb, K., Ghars, M.A., Gandour, M., Megdiche, W., Ben Hamed, K., Ben Amor, N., Brown, S.C.,

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Savouré, A., and Abdelly, C.: Ecophysiological and genomic analysis of salt tolerance of Cakile maritima. *Environmental and Experimental Botany* 92, 64– 72, 2013.

Doolittle, J., Petersen, M., and Wheeler, T.: Comparison of two electromagnetic induction tools in salinity appraisals. *Journal of Soil and Water Conservation*, *56*(3), 2001.

Ehosioke, S., Nguyen, F., Rao, S., Kremer, T., Placencia-Gomez, E., Huisman, J.A., Kemna, A., Javaux, M., and Garré, S.: Sensing the electrical properties of roots: a review. *Vadose Zone J.* 2020; 19: e20082. https://doi.org/10.1002/vzj2.20082, 2020.

Ehosioke, S., S. Garré, J.A. Huisman, E. Zimmerman, E. Placencia-Gomez, M. Javaux, and Nguyen, F.: Spectroscopic approach towards unraveling the electrical signature of roots, JGR: Biogeosciences. https://doi.org/10.1029/2022JG007281, 2023.

Ellouzi, H., Ben Hamed, K., Cela, J., Munné-Bosch, S., and Abdelly, C.: Early effects of salt stress on the physiological and oxidative status of Cakile maritima (halophyte) and Arabidopsis thaliana (glycophyte). *Physiologia Plantarum*, *142*(2), 128–143. https://doi.org/10.1111/j.1399-3054.2011.01450.x, 2011.

FAO: Global network on integrated soil management for sustain- able use of salt-affected soils. FAO Land and Plant Nutrition Management Service, Rome, Italy, http://www.fao.org/ag/agl/agll/ spush, 2005.

Farooq, M., Hussain, M., Wakeel, A., and Siddique, K. H. M.: Salt stress in maize: effects, resistance mechanisms, and management. A review. *Agronomy for Sustainable Development, 35(2)*, 461–481. https://doi.org/10.1007/s13593-015-0287-0, 2015.

Franco, J. A., Fernández, J. A., Bañón, S., and González, A.: Relationship between the effects of salinity on seedling leaf area and fruit yield of six muskmelon cultivars. *Horticultural Science*. https://doi.org/10.21273/hortsci.32.4.642, 1997.

Garré, S., Javaux, M., Vanderborght, J., Pagès, L., and Vereecken, H.: Three-Dimensional Electrical Resistivity Tomography to Monitor Root Zone Water Dynamics. *Vadose Zone Journal*, *10(1)*, 412. https://doi.org/10.2136/vzj2010.0079, 2011.

Gómez-Bellot, M. J., Álvarez, S., Castillo, M., Bañón, S., Ortuño, M. F., and Sánchez-Blanco, M. J.: Water relations, nutrient content and developmental responses of Euonymus plants irrigated with water of different degrees of salinity and quality. *Journal of Plant Research*, *126*(4), 567–576. https://doi.org/10.1007/s10265-012-0545-z, 2013.

Grieve, C. M., Grattan, S. R., and Maas, E. V.: Plant Salt Tolerance. In *Agricultural Salinity Assessment and Management* (pp. 405–459). Reston, VA: American Society of Civil Engineers. https://doi.org/10.1061/9780784411698.ch13, 2012.

Hajlaoui, H., El Ayeb, N., Garrec, J.P., and Denden, M. : Differential effects of salt stress on osmotic adjustment and solutes allocation on the basis of root and leaf tissue senescence of two silage maize (Zea mays L.) varieties. Industrial Crops and Products, 31, 122-130, https://doi.org/10.1016/j.indcrop.2009.09.007, 2010.

Hasegawa P., Bressan R., Zhu J., and Bohnert H.: Plant cellular and molecular response to high salinity. *Annual*

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Review of Plant Physiology and Plant Molecular Biology 51:463–499 doi:1040-2519/00/0601-0463, 2000.

Hopmans, J. W., Lazarovitch, N., Skaggs, T., Kisekka, I., Grattan, S. R., Javaux, M., … Taleisnik, E.: Chapter one - Critical Knowledge Gaps and Research Priorities to Address Global Soil Salinity, In Advances in Agronomy, Vol. 169, pp 1–191, 2021.

Hördt, A., Bairlein, K., Bielefeld, A., Bücker, M., Kuhn, E., Nordsiek, S., and Stebner, H.: The dependence of induced polarization on fluid salinity and pH, studied with an extended model of membrane polarization. *Journal of Applied Geophysics*, 135, 408–417. https://doi.org/10.1016/j.jappgeo.2016.02.007, 2016.

Formatted: English (United States)

Hussain, M., Park, H. W., Farooq, M., Jabran, K., Lee, D. J., Hussain, M., … Lee, D.-J.: Morphological and physiological basis of salt resistance in different rice genotypes Morphological and Physiological Basis of Salt Resistance in Different Rice Genotypes. *International journal of agriculture & biology*, 15, 113–118, 2013.

Isayenkov, S. V, and Maathuis, F. J.: Plant Salinity Stress: Many Unanswered Questions Remain, *Frontiers in Plant Science*, *10*, 80. https://doi.org/10.3389/fpls.2019.00080, 2019.

Islam, M., Wahid, K. A., Dinh, A. V., and Bhowmik, P.: Model of dehydration and assessment of moisture content on onion using EIS. *Journal of Food Science and Technology*, *56*(6), 2814–2824. https://doi.org/10.1007/s13197-019-03590-3, 2019.

Ji, X.; Tang, J.; and Zhang, J.: Effects of Salt Stress on the Morphology, Growth and Physiological Parameters of Juglans microcarpa L. Seedlings. Plants, 11, 2381. https://doi.org/10.3390/plants11182381, 2022

Kaddah, M. T., and Ghowail, S. I.: Salinity Effects on the Growth of Corn at Different Stages of Development. *Agronomy Journal*, *56*(2), 214–217. https://doi.org/10.2134/agronj1964.00021962005600020028x, 1964.

Kessouri, P., Furman, A., Huisman, J. A., Martin, T., Mellage, A., Ntarlagiannis, D., … Placencia-Gomez, E.: Induced polarization applied to biogeophysics: recent advances and future prospects. *Near Surface Geophysics*, 17, 595–621. https://doi.org/10.1002/nsg.12072, 2019.

Kinraide, T. B.: Ion fluxes considered in terms of membrane-surface electrical potentials. *Australian Journal of Plant Physiology* (Vol. 28, pp. 605–616). CSIRO. https://doi.org/10.1071/pp01019, 2001.

Kinraide, T. B., and Wang, P.: The surface charge density of plant cell membranes (σ): An attempt to resolve conflicting values for intrinsic σ. *Journal of Experimental Botany*, *61*(9), 2507–2518. https://doi.org/10.1093/jxb/erq082, 2010.

Läuchli, A., and Grattan, S. R.: Plant Responses to Saline and Sodic Conditions. In *Agricultural Salinity Assessment and Management* (pp. 169–205). Reston, VA: American Society of Civil Engineers. https://doi.org/10.1061/9780784411698.ch06, 2012.

Formatted: English (United States)

Lew, R. R.: Pressure regulation of the electrical properties of growing Arabidopsis thaliana L. Root hairs. *Plant Physiology*, *112*(3), 1089–1100. https://doi.org/10.1104/pp.112.3.1089, 1996.

Li, S., Mou, P., and Hu, F.: Is root nutrient uptake a modular function? A test using Solidago canadensis. *Journal* *of Forestry Research*, *27*(2), 321–328. https://doi.org/10.1007/s11676-015-0151-8, 2016.

Lv, D. W., Subburaj, S., Cao, M., Yan, X., Li, X., Appels, R., … Yan, Y. M.: Proteome and phosphoproteome characterization reveals new response and defense mechanisms of brachypodium distachyon leaves under salt stress. *Molecular and Cellular Proteomics*, *13*(2), 632–652. https://doi.org/10.1074/mcp.M113.030171, 2014.

Michot, D., Benderitter, Y., Dorigny, A., Nicoullaud, B., King, D., and Tabbagh, A.: Spatial and temporal monitoring of soil water content with an irrigated corn crop cover using surface electrical resistivity tomography. *Water Resources Research*, 39(5). https://doi.org/10.1029/2002WR001581, 2003.

Munns, R.: Comparative physiology of salt and water stress. *Plant, Cell and Environment*, 25(2), 239–250. https://doi.org/10.1046/j.0016-8025.2001.00808.x, 2002.

Munns, R., and Tester, M.: Mechanisms of Salinity Tolerance. *Annual Review of Plant Biology*, *59*(1), 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911, 2008.

Munns, R., Passioura, J. B., Colmer, T. D., and Byrt, C. S.: Osmotic adjustment and energy limitations to plant growth in saline soil. *New Phytologist*, *225*(3), 1091–1096. https://doi.org/10.1111/nph.15862, 2020

Munns, Rana, James, R. A., and Lä Uchli, A.: Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany*, 57(5), 1025–1043. https://doi.org/10.1093/jxb/erj100, 2006.

Munns, R.: Genes and salt tolerance : bringing them together. *New Phytologist*, (167), 645–663, 2005.

Negrão, S., Schmöckel, S. M., and Tester, M.: Evaluating physiological responses of plants to salinity stress. *Annals of Botany*, *119*(1), 1–11. https://doi.org/10.1093/aob/mcw191, 2017.

Neubert AB, Zörb C, and Schubert S.: Expression of vacuolar Na+/H+ antiporters (ZmNHX)and Na+ exclusion in roots ofmaize (Zeamays L.) genotypes with improved salt resistance In: Li CJ et al. (eds) Plant nutrition for food security, human health and environmental protection, *Tsinghua University Press*, Bejing, China, pp 544–545, 2005.

Ogawa, A., and Yamauchi, A. : Root Osmotic Adjustment under Osmotic Stress in Maize Seedlings 1. Transient Change of Growth and Water Relations in Roots in Response to Osmotic Stress. Plant Production Science, 9(1), 27-38, https://doi.org/10.1626/pps.9.27, 2006.

Formatted: English (United States)

Ozier-Lafontaine, H., and Bajazet, T. : Analysis of Root Growth by Impedance Spectroscopy (EIS). *Plant and Soil*, 277(1–2), 299–313. https://doi.org/10.1007/s11104-005-7531-3, 2005.

Formatted: English (United States)

PlantDitech.: Salinity Stress in Plants. https://www.plant-ditech.com/news-events/blog/241-salinity-stress-in-plants-physiological-biochemical-effect-plant-ditech, 2023.

Repo, T., Cao, Y., Silvennoinen, R., and Ozier-Lafontaine, H.: Electrical Impedance Spectroscopy and Roots. In Measuring Roots (pp. 25–49). Berlin, Heidelberg: *Springer Berlin Heidelberg*. https://doi.org/10.1007/978-3-642-22067-8_2, 2012.

Repo, T., Korhonen, A., Laukkanen, M., Lehto, T., and Silvennoinen, R.: Detecting mycorrhizal colonisation in

Scots pine roots using electrical impedance spectra. *Biosystems Engineering*, 121, 139–149. https://doi.org/10.1016/j.biosystemseng.2014.02.014, 2014.

Repo, T., Korhonen, A., Lehto, T., and Silvennoinen, R.: Assessment of frost damage in mycorrhizal and non-mycorrhizal roots of Scots pine seedlings using classification analysis of their electrical impedance spectra. *Trees*, 30(2), 483–495. https://doi.org/10.1007/s00468-015-1171-x, 2016.

Repo, T., Laukkanen, J., and Silvennoinen, R.: Measurement of the Tree Root Growth Using Electrical Impedance Spectro- scopy. *Silva Fennica*, 39(2), 159–166, 2005.

Revil, A., Karaoulis, M., Johnson, T., and Kemna, A.: Review: Some low-frequency electrical methods for subsurface characterization and monitoring in hydrogeology. *Hydrogeology Journal*, 20(4), 617–658. https://doi.org/10.1007/s10040-011-0819-x, 2012.

Rewald, B., Ephrath, J. E., and Rachmilevitch, S. (2011). A root is a root is a root? Water uptake rates of Citrus root orders. *Plant, Cell & Environment*, *34*(1), 33–42. https://doi.org/10.1111/j.1365-3040.2010.02223.x, 2011.

Rhoades, J.D., Kandiah, A., and Mashali, A. M.: The use of saline waters for crop production, FAO Irrigation and Drainage Paper #48, Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 1–145, 1992.

Formatted: English (United States)

Rhoades, J.D., Chanduvi, F., and Lesch, S. : Soil salinity assessment: Methods and interpretation of electrical conductivity measurements, FAO Irrigation and Drainage Paper #57, Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 1–150, 1999.

Formatted: English (United States)

Robbins, N. E., and Dinneny, J. R.: The divining root: Moisture-driven responses of roots at the micro- and macro-scale. *Journal of Experimental Botany*. Oxford University Press. https://doi.org/10.1093/jxb/eru496, 2015.

Rodríguez, P., Torrecillas, A., Morales, M. A., Ortuño, M. F., and Sánchez-Blanco, M. J. : Effects of NaCl salinity and water stress on growth and leaf water relations of Asteriscus maritimus plants. *Environmental and Experimental Botany*, *53*(2), 113–123. https://doi.org/10.1016/j.envexpbot.2004.03.005, 2005.

Formatted: English (United States)

Schopfer P.: Biomechanics of plant growth. American Journal of Botany 93, 1415–1425, 2006.

Shahnazaryan, E., Lutz, W., Garre, S., Lazarovich, N., and Vanderborght, J.: Monitoring salt accumulation in the root zone by electrical resistivity tomography. *Geophysical Research Abstracts* (Vol. 20), 2018.

Sharp, R.E., Hsiao, T.C., and Silk, W.K.: Growth of the Maize Primary Root at Low Water Potentials: II. Role of Growth and Deposition of Hexose and Potassium in Osmotic Adjustment. Plant Physiology, 93(4), 1337-1346, https://doi.org/10.1104/pp.93.4.1337, 1990.

~~UCANR.: Nutrient and Mineral excesses, salinity and salt toxicity. https://ipm.ucanr.edu/PMG/GARDEN/ENVIRON/salttoxicity.html, 2021.~~

Valdez-Aguilar, L., Grieve, C., Razak-Mahar, A., McGiffen, M., and Merhaut, D.: Growth and Ion Distribution Is Affected by Irrigation with Saline Water in Selected Landscape Species Grown in Two Consecutive

Growing Seasons: Spring–summer and Fall–winter in, *Horticultural Science*, *46*(4), 2011.

Verslues P., Agarwal M., Katiyar-Agarwal S., Zhu J., and Zhu, J.K.: Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant Journal 45, 523–539*

Wang, P., Kinraide, T. B., Zhou, D., Kopittke, P. M., and Peijnenburg, W. J. G. M.: Plasma membrane surface potential: Dual effects upon ion uptake and toxicity. *Plant Physiology*, 155(2), 808–820. https://doi.org/10.1104/pp.110.165985, 2011.

Weigand, M.: Monitoring structural and physiological properties of crop roots using spectral electrical impedance tomography. Univerity of Bonn, 2017.

Weigand, M., and Kemna, A.: Multi-frequency electrical impedance tomography as a non-invasive tool to characterize and monitor crop root systems. *Biogeosciences, 14, 921–939. https://doi.org/10.5194/bg-14-921-2017, 2017.*

Weigand, M., and Kemna, A.: Imaging and functional characterization of crop root systems using spectroscopic electrical impedance measurements. *Plant and Soil*, 435(1–2), 201–224. https://doi.org/10.1007/s11104-018-3867-3, 2019.

Wu, L., Ogawa, Y., and Tagawa, A.: Electrical impedance spectroscopy analysis of eggplant pulp and effects of drying and freezing–thawing treatments on its impedance characteristics. *Journal of Food Engineering*, *87*(2), 274–280. https://doi.org/10.1016/j.jfoodeng.2007.12.003, 2008.

Zekri, M., and Parsons, L. R.: Growth and root hydraulic conductivity of several citrus rootstocks under salt and polyethylene glycol stresses. *Physiologia Plantarum*, *77*(1), 99–106. https://doi.org/10.1111/j.1399-3054.1989.tb05984.x, 1989

Zhao, K., Song, J., Fan, H., Zhou, S., and Zhao, M.: Growth response to ionic and osmotic stress of NaCl in salt-tolerant and salt-sensitive maize. *Journal of Integrated Plant Biology. 52, 468–475*. Doi: 10.1111/j.1744-7909.2010.00947.x, 2010.