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

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Abstract

Developments in the methods available for root investigation in recent years have enabled many studies to be carried out on 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 plant roots, which will be useful to quantify plant 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 maize than in *Brachypodium*, which implies that tolerance to salinity varies with the species, and that maize is more tolerant to salinity than *Brachypodium*. We conclude that SIP is a useful tool for monitoring root 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

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), 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),

69   where the charge distribution that determines polarization depends on the concentration of ions in the external

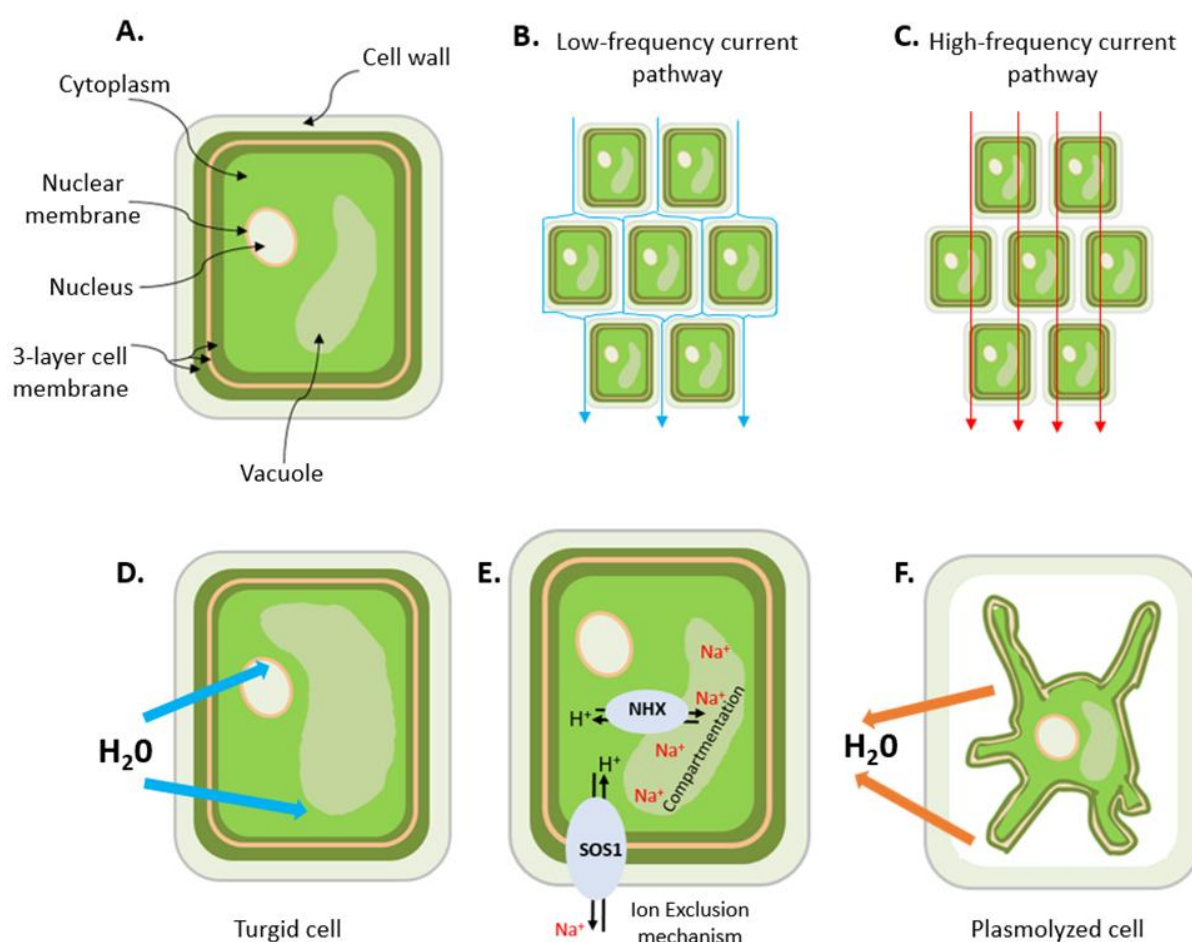
70   fluid (Weigand and Kemna 2017, 2019). It is important to note that living tissues are equivalent to parallel resistor

71   and capacitor (RC) circuits, which have a characteristic phase angle that depends on alternating current (AC)

72   frequency. Thus, conduction and polarization mechanisms are frequency dependent (see current pathways in Fig.

73   1b and 1c) and can be assessed simultaneously by measuring the frequency dependent electrical impedance of a

74   biological tissue using SIP. The suitability of this method for investigating root responses to salt stress is not well

75   known and has rarely been studied (Ben Hamed et al. 2016; Cseresnyés et al. 2024).

77   **Figure 1.** Schematic illustration of **a**) plant cell showing some of the organelles (vacuole, nucleus and nuclear membranes),
78   the cell wall and the 3-layer (protein-lipid-protein) cell membrane, **b**) low-frequency current pathway, **c**) high frequency current
79   pathway, **d**) turgid cell resulting from the uptake of water, **e**) early stage response to salt stress in a plant root cell (adapted from
80   Deinlein et al. 2014), this involves the activation of cellular detoxification mechanisms, including NHX and SOS Na^+ transport
81   mechanisms (NHX: Na^+/H^+ exchanger, SOS: Salt Overly Sensitive), **f**) plasmolyzed cell due to excessive loss of water. This
82   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
83   or compartment them into the vacuole, water leaves the cell by osmosis leading to plasmolysis.

85 Plants respond to salt stress by adaptive mechanisms such as root exclusion of excess sodium in the surrounding

86 water or compartmentation, removing toxic ions from the cytoplasm where sensitive metabolic processes occur

87 (Hasegawa et al. 2000; Munns and Tester 2008; Zhao et al. 2020) into the vacuole (Neubert et al. 2005; Farooq et

88 al. 2015; Isayenkov and Maathuis 2019). These two adaptive mechanisms are independent, but their effectiveness

89 varies across species (Grieve et al. 2012; Acosta-Motos et al. 2017). They modify the ionic composition of the

90 extracellular and intracellular fluids (Fig. 1e), which suggests that these adaptive mechanisms can possibly also be

91 detected by SIP. For example, Ben Hamed et al. (2016) investigated the use of EIS to non-invasively assess salt

92 resistance and the signaling and short-term (0-240 minutes) response of Sea rocket (*Cakile maritima*) to salinity.

93 They found that the frequency-dependent impedance of leaves changed with increasing salinity as well as the

94 duration of stress for plants grown in sand and hydroponic culture conditions. In particular, it was observed that

95 for a group of 10 plants exposed to increasing salinity, the electrical resistance of the leaves increased in the

96 presence of 50-100 mM NaCl, but decreased for salinity above 100 mM NaCl, with the lowest value observed at

97 400 mM NaCl. For another group of 10 plants exposed to a 400mM NaCl treatment over 240 minutes, the electrical

98 resistance increased at early stages of salt stress and reached a maximum after 180 minutes before declining

99 rapidly. The increasing electrical resistance within the tolerable range of salinity for growth (50–100 mM NaCl)

100 was attributed to low salt movement in leaf cells due to compartmentation of salt ions in the leaf vacuoles, as

101 reported in previous studies (e.g. Debez et al. 2004; Ellouzi et al. 2011), while the decrease in electrical resistance

102 at salinities above 100 mM NaCl was interpreted as an indication of increased movement of salt ions in the leaf

103 cells, most probably in the apoplastic space. Similarly, Ellouzi et al. (2011) reported rapid accumulation of Na^+ in

104 the vacuole and re-establishment of osmotic homeostasis shortly after salt treatment (400 mM NaCl for 4 h). They

105 also observed a decrease in the electrical resistance of leaves of salt-treated plants, which was closely correlated

106 with the increased accumulation of Na^+ in the vacuole. These studies suggest that the electrical resistance of salt-

107 stressed plants varies with degree of salinity and the duration of salt stress. This implies that that the accumulation

108 of Na^+ and Cl^- ions in the cytoplasm and apoplast will take a long time to reach toxic levels when the salt

109 concentration is low. At very high salt concentrations, it is expected that toxic level will be attained much faster,

110 this could happen in a couple of minutes (e.g. Ben Hamed et al. 2016).

111 Despite these interesting studies, the suitability of SIP as a tool to study plant response to salinity has not been

112 thoroughly investigated and few existing studies focused mainly on plant leaves. However, the root cells are the

113 first target of soil salinity and more studies are still needed to better understand how roots respond to salt stress.

114 Therefore, the aim of this study is to evaluate the SIP response of *Brachypodium* and *Maize* primary roots subjected

115 to different levels of salinity and to link the observed changes in electrical properties with the salt adaptation

116 mechanisms of plants to obtain further insights into the ability of SIP to detect salt stress in plant roots.

117

118 **2. Materials and methods**

119 **2.1. Investigated plants and salt solutions**

120 *Brachypodium* (*Brachypodium distachyon L.*) and maize (*Zea mays L.*) were studied under different salinity

121 treatments. *Brachypodium distachyon L.* is a salt-sensitive plant that can tolerate salt stress below 200 mM NaCl

122 (e.g. Lv et al. 2014; Guo et al. 2020). *Zea mays L.* is moderately sensitive to salt stress (Kaddah and Ghowail 1964;

123 Farooq et al. 2015) and can tolerate relatively high salinity up to 400 mM NaCl (e.g. de Azevedo Neto et al. 2004),

124 depending on the genotype. Plants of both species were grown in the laboratory under daylight conditions (without

125 artificial light), normal humidity and an average temperature of 23.2°C. They were grown in plastic tubes (5 x 20

126 cm) using a mixture of fine and coarse sand with a grain size distribution ranging from 0.1 to 1.0 mm (Ehosioke

127 et al. 2023). The plants were watered with tap water at 2-day intervals and were sampled at 20 days after sowing

128 (DAS). The average diameter of the *Brachypodium* and maize primary roots were 0.22 mm and 0.89 mm,

129 respectively. Both plant types were in the 3-leaves stage at the time of measurement. Before each SIP

130 measurement, the plant was removed from the growth tube and the sand particles on the roots were removed gently.

131 Salt solutions were prepared by dissolving sodium chloride (NaCl) in demineralized water. The electrical

132 conductivity was measured using a conductivity meter (HQ14D, HACH, Mechelen, Belgium). A total of 14 salt

133 solutions with different concentrations were prepared (Table 1). The resulting concentration is presented in ppm.

134 The nomenclature to describe different types of saline water based on concentration and electrical conductivity is

135 presented in Table A1 (see Appendix).

136

137 **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

138

2.2. Measurement set-up

140 The measurement set-up consists of a precision balance (Mettler PM 2000), sampling container, SIP measurement

141 system, and a sample holder especially designed for root segments (Fig. 2; Ehosioke et al. 2023). We used the high

142 precision balance for a precise measurement of the uptake. The SIP measurement system is made up of a data

143 acquisition (DAQ) card (NI USB-4431), an amplifier unit (ZEA-2-SIP04-V05), a function generator (Keysight

144 33511B), triaxial cables and a computer. A detailed description of the SIP measurement system and the specialized

145 sample holder are provided in Ehosioke et al. (2023).

146   The SIP measurement is performed by injecting alternating current at different frequencies (1 Hz – 45 kHz), and

147   a voltage of 5V into a sample and measuring the amplitude and phase lag of the resulting voltage, which leads to

148   a frequency dependent electrical impedance expressed as:

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

150   where Z_ω^* is the complex impedance, ω is the angular frequency, Z' and Z'' are the real and imaginary parts of

151   the complex impedance, and j is the imaginary unit. The complex impedance can be converted into the complex

152   electrical conductivity or electrical resistivity by accounting for the dimension of the sample using a geometric

153   factor ($K = \frac{\pi d^2}{4l}$ where d is the root diameter and l is the root length):

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

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

156   and complex resistivity ρ_ω^* is:

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

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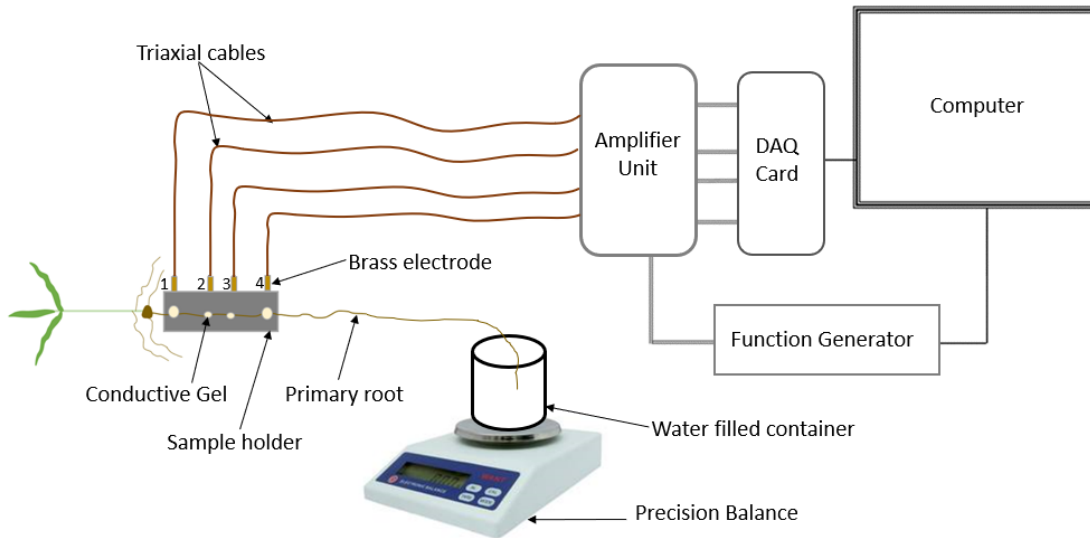
159   **Figure 2.** Measurement set-up for investigating the electrical response of roots during water uptake.

160

161   **2.3. Measurement protocol**

162   Preliminary SIP measurements were performed on roots of maize and *Brachypodium* plants in air to investigate

163   the effect of root drying on the SIP response. For this, one plant of each species was sampled. The root was

164   mounted in the sample holder and SIP measurements were taken at 5 minute intervals for a total duration of 20

165   minutes with the root in the same position (see Fig. 2).

166   To investigate the response to water and salt uptake, the root was mounted on the sample holder and an initial SIP

167   measurement was performed that forms the baseline. After this, the root apex was tipped into 50 ml demineralized

168   water (e.g. Rewald et al. 2011; Li et al. 2016) or saline water of known conductivity in a 60 ml sampling container

169   (Fig. 2). The weight of the water, the container and the root tip was recorded every 5 minutes for a total duration

170   of 20 minutes. Temperature and humidity were recorded at the end of the experiment. In the case of water uptake,

171   SIP measurements were acquired on one plant for each species using the same measurement strategy to serve as a

172   reference to help interpret the electrical response of roots to the uptake of salt solutions.

173   The SIP response of roots in different salt solutions was investigated in two experiments. In a first experiment, we

174   exposed two plants of each species to two different salt solutions i.e salt-L and salt-H (see Table 1), sampling a

175   total of 4 plants. The SIP measurements were performed at a 5 minute interval over a 20 minutes duration while

176   the root apex was tipped in salt solution. In the second experiment, the effect of varying salt concentrations on the

177   SIP response of the roots was investigated. To achieve this, the measurement procedure described above was

178   repeated with 7 different salt solutions for Brachypodium (1000 – 10000 ppm) and another 7 different salt solutions

179   for Maize (16800 – 60000 ppm) (see Table 1). Thus, a total of 14 plants were sampled in this experiment. To

180   estimate evaporation loss during SIP measurements, an empty sample container with 50 ml of demineralized water

181   was left open on the balance and the mass was measured every 5 minutes over a 20 minutes duration. This

182   procedure was repeated for the salt solutions to estimate the loss of water from the container due to evaporation.

183   The evaporation loss was found to be 40 mg in 20 minutes for both demineralized and saline water. The

184   temperature and humidity at the time of measurement was also recorded (see Appendix B: Table B1). The net

185   amount of solution absorbed by the root during each measurement corresponds to the weight difference corrected

186   for the estimated loss by evaporation.

187   **3. Results and Discussion**

188   **3.1. SIP monitoring of root desiccation**

189   The resistivity magnitude and phase of exposed *Brachypodium* and maize roots are shown in Fig. 3. We can

190   observe that the resistivity values of root segments of both species increased when the roots were exposed in the

191   air. Water content plays a key role in maintaining the structural properties and physiological processes of the cell

192   membrane (Crowe and Crowe 1982). Loss of water from roots may lead to a loss of turgor pressure (plasmolysis),

193   which can result in a decrease in cell volume depending on cell wall hardness (Verslues et al. 2006; Robbins and

194   Dinneny 2015), a decrease in cell membrane surface area, and cell membrane injury in severe cases (Lew 1996;

195   Ando et al. 2014). Wu et al. (2008) reported an increase in total impedance during dehydration of eggplant pulp.

196   Islam et al. (2019) also observed an increase in total impedance of onions during drying over a period of 21 days.

197   They concluded that movement of ions due to dehydration is responsible for the increased impedance. The increase

198   in resistivity observed in our study for maize and *Brachypodium* roots is due to loss of water from the root cells

199   (dehydration) due to evaporation. The increase in resistivity is higher for Brachypodium (78 Ωm increase in 20

200   minutes after the baseline measurement of 68 Ωm) than for Maize (7 Ωm increase in 20 minutes after a baseline

201   measurement of 16 Ωm) both in absolute and relative values. This suggests that *Brachypodium* root lost water

202   faster than maize in our experiment. We had expected that maize would lose more water because of the larger

203   surface area, but the result suggests that something other than surface area influenced the root dehydration, which

204   could be the degree of saturation. Since maize roots were observed to be succulent and white in color while

205   *Brachypodium* roots were dry and brownish in this study, it should take longer for maize roots to lose sufficient

206   water and become plasmolyzed compared to *Brachypodium* roots. Shrinkage of *Brachypodium* root was clearly

207   visible at the end of the measurement, whereas maize appeared dry on the surface but showed no significant

208   shrinkage. The noisier data observed for *Brachypodium* is attributed to the high contact impedance of the root

209   induced by shrinkage of *Brachypodium* root during drying. Over the exposition time of 20 minutes, polarization

210   (phase peak) of Brachypodium decreased from 870 mrad at 6.3 kHz to 570 mrad at 1 kHz, while that of maize

211   first increased from 510 mrad at 45kHz to 560 mrad at 39.8 kHz, followed by a stabilization. In a plasmolyzed

212   cell, cell membranes shrink (see Fig. 1), which is expected to result in a decrease of the phase response. It seems

213   that *Brachypodium* roots might have become plasmolyzed due to water loss (Lew 1996; Ando et al. 2014; Robbins

214   and Dinneney 2014), while maize roots were probably not plasmolyzed but rather experienced osmotic

215   adjustments by redistribution of water to maintain equilibrium (e.g. Sharp et al. 1990; Ogawa and Yamauchi, 2006;

216   Hajlaoui et al. 2010). This might explain why the phase response of maize did not decrease. It is important to note

217   that during the desiccation test, the leaves of both plants did not show any sign of wilting (see Appendix C, Figure

218   C1a and C2a).

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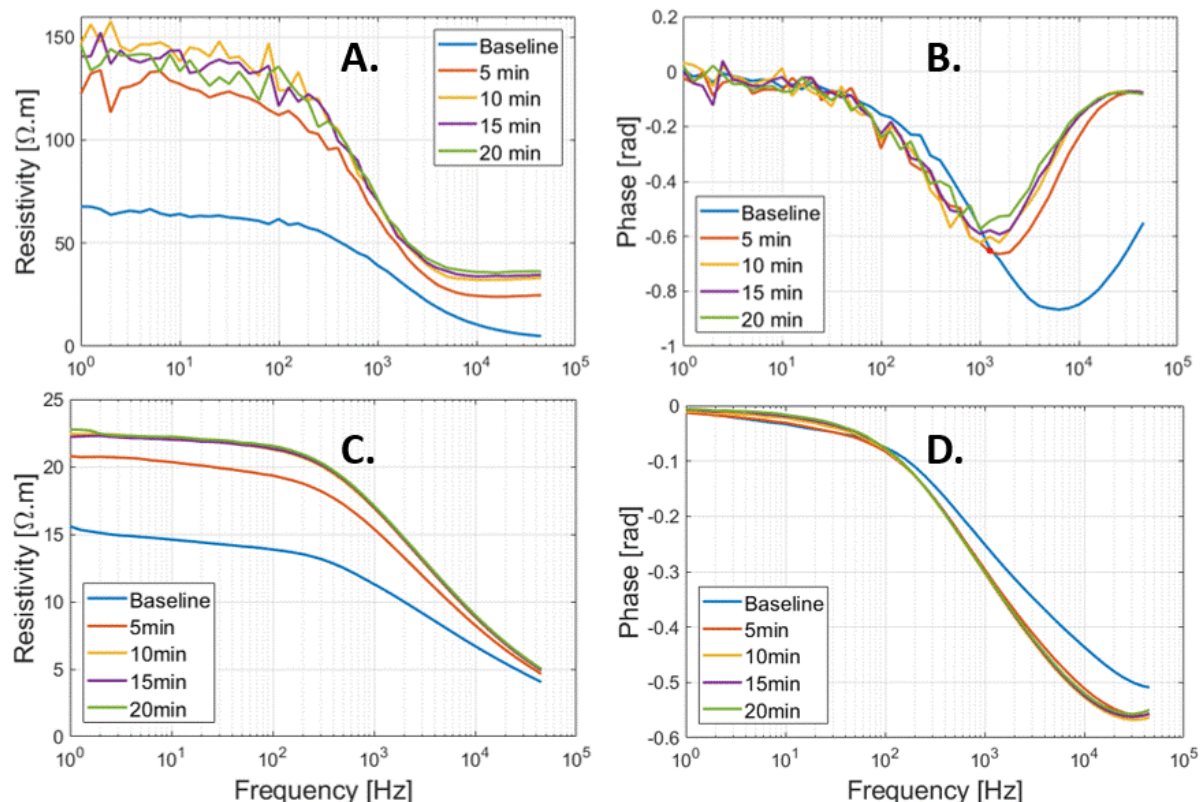
220   **Figure 3.** Resistivity and phase response of *Brachypodium* (**a-b**) and maize (**c-d**) primary roots to drying.

221   **3.2. SIP monitoring of roots with their tips in demineralized water**

222   The net mass of water uptake by the roots after correcting for evaporation loss were 40 mg and 70 mg for

223   Brachypodium and maize root, respectively (see Table2). The maize absorbed more water compared to

224   *Brachypodium* since its leaf surface area is larger and thus has a larger canopy transpiration.

225   **Table 2.** Net 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

226

227

228   For both species, the resistivity magnitude shows an increase with a greater effect at low frequencies (< 1 kHz)

229   and almost no effect at high frequencies (> 10 kHz) for Maize (Fig. 4). According to the conduction mechanisms

230   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 a temporal trend over the measurement duration, while that of maize 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 maize suggests that its transmembrane potential might be in equilibrium.
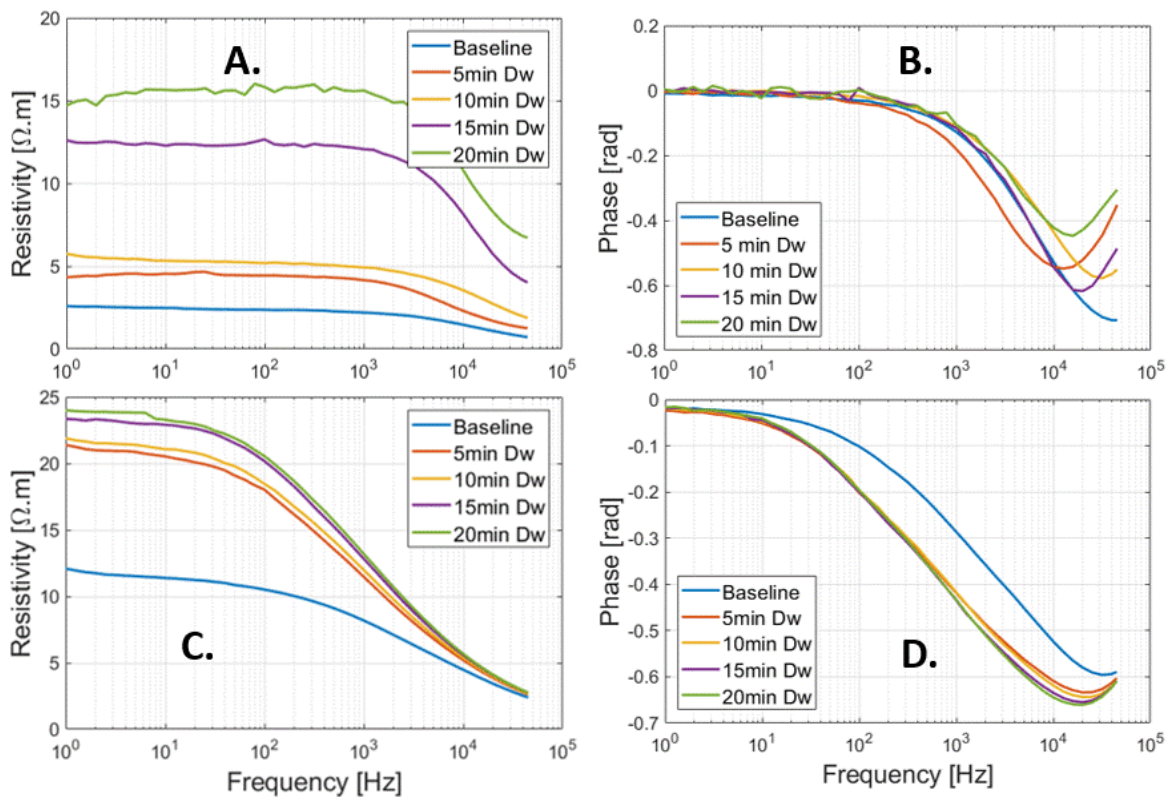
Figure 4. Resistivity magnitude and phase spectra of *Brachypodium* (**a-b**) and maize (**c-d**) primary roots during absorption of demineralized water. The variable temporal development of the resistivity magnitude might be due to high contact impedance of the *Brachypodium* root.

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 50/40 and 70/70 mg for *Brachypodium* and maize roots, respectively (Table 2). 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 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 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. 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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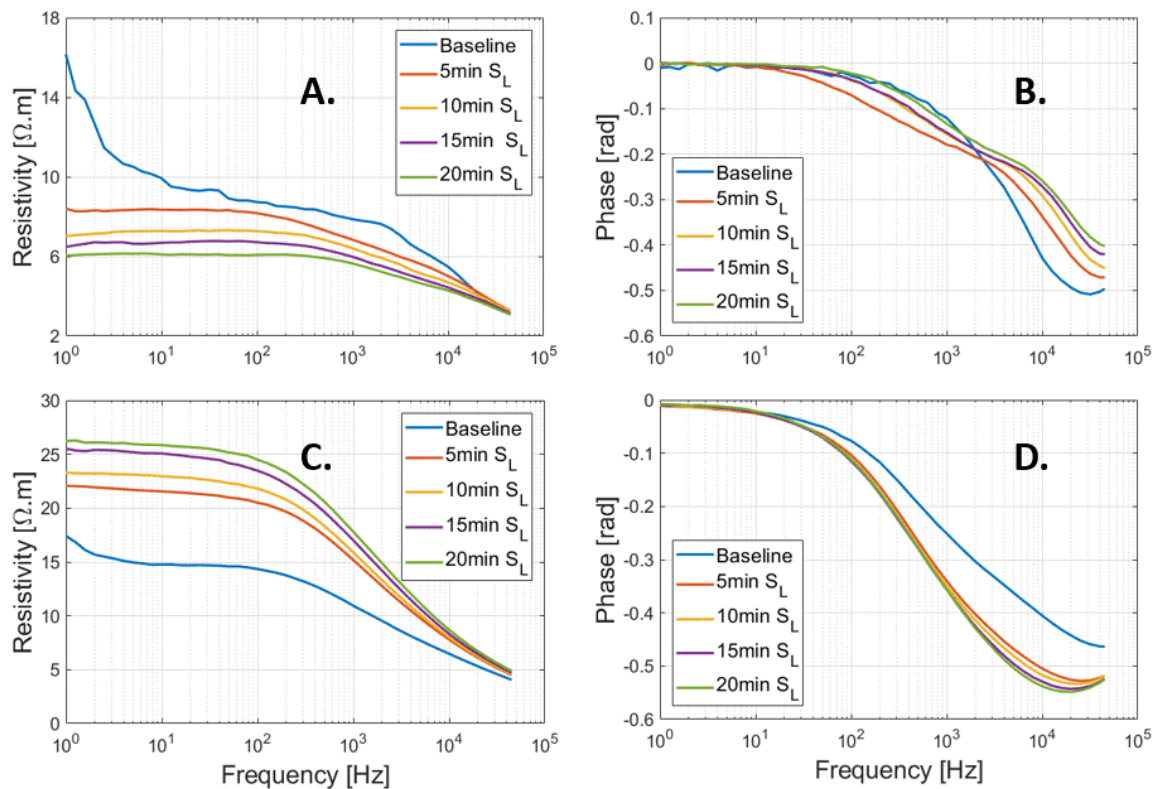


Figure 5. Changes in resistivity magnitude and phase spectra of *Brachypodium* (**a-b**) and maize (**c-d**) primary roots during absorption of saline water (salt-L).

During uptake of water with high salt concentration (salt-H) (Fig.6), it is interesting to see that both maize and *Brachypodium* roots now have similar responses, showing a consistent decrease in both resistivity magnitude and phase. This consistent decrease 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.

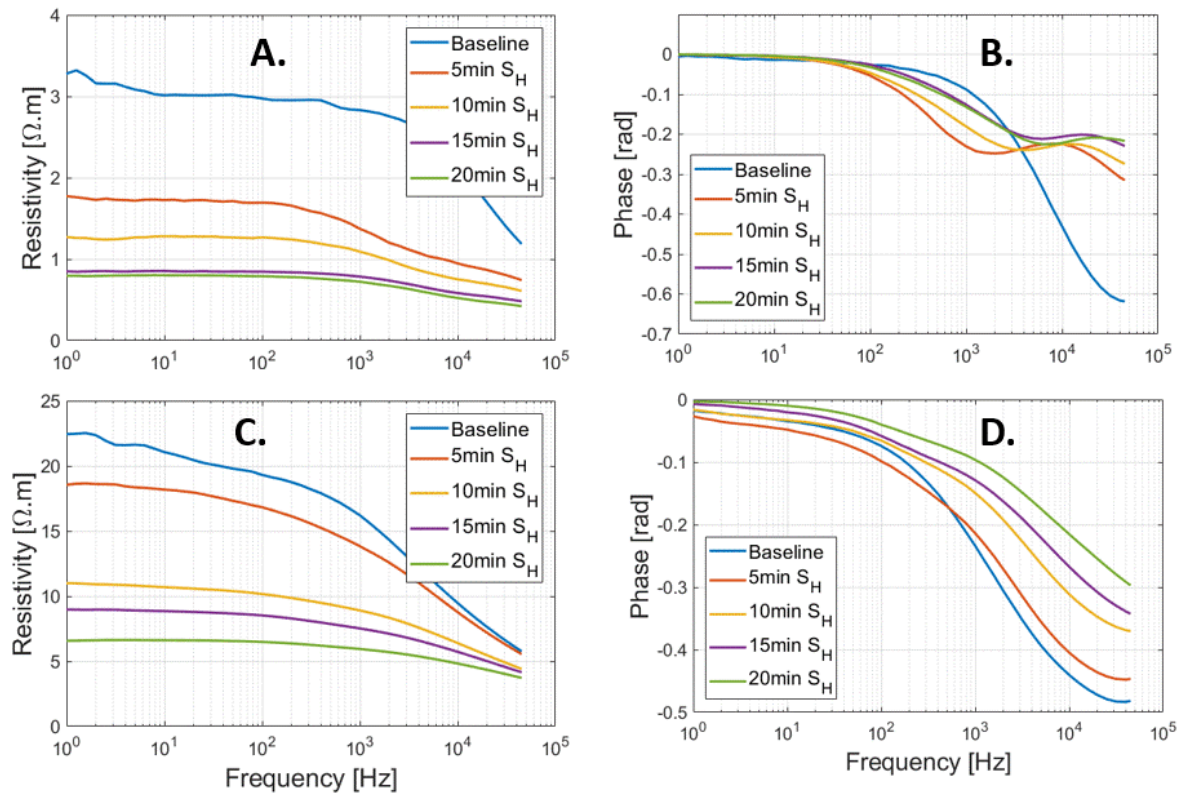



Figure 6. Changes in resistivity and phase spectra of *Brachypodium* (**a-b**) and maize (**c-d**) primary roots during absorption of saline water (salt-H).

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.

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

The SIP response of 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

293   resistivity depending on the solute concentration, but with a different threshold due to their different salt stress

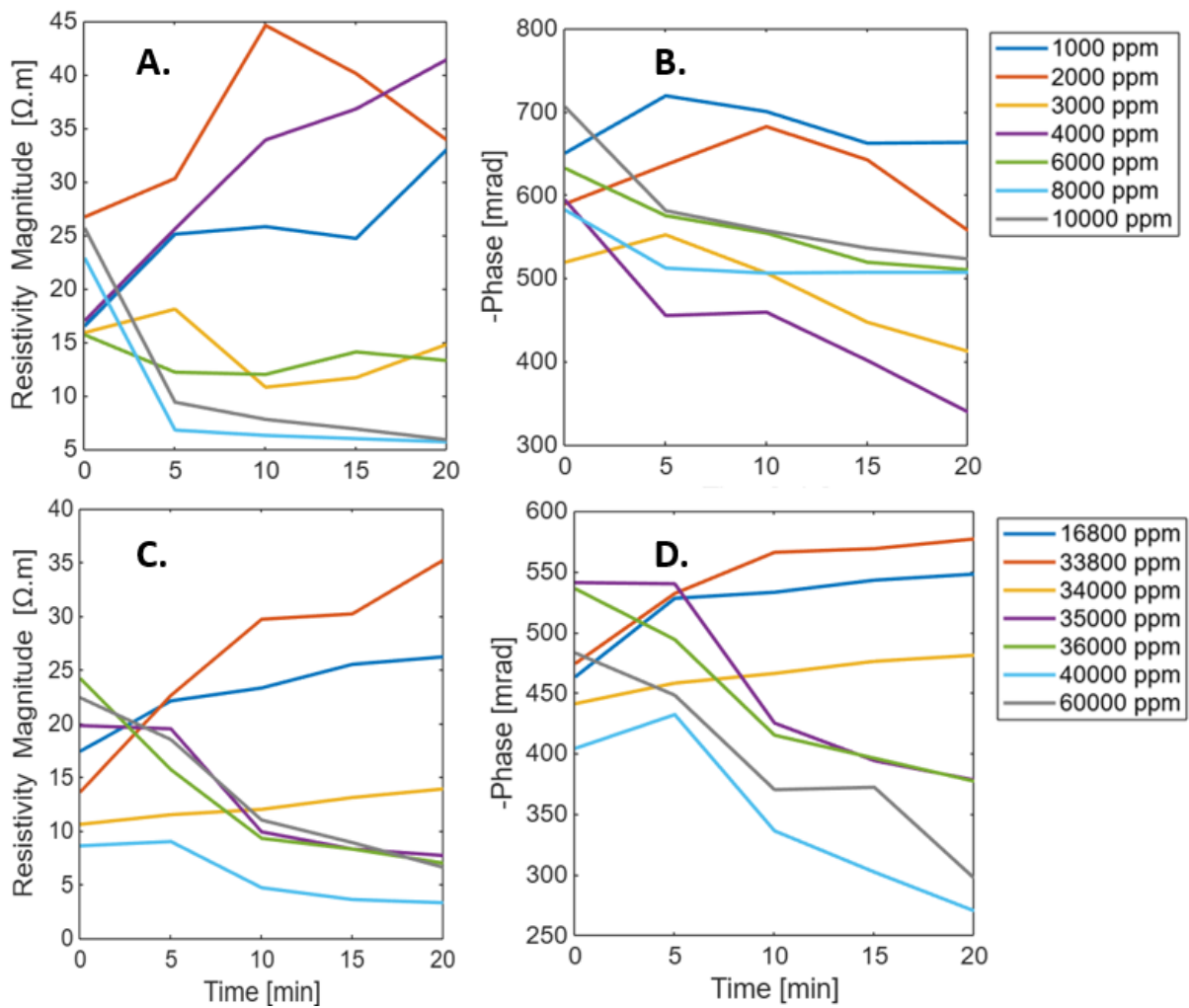
294   tolerance. For maize roots, the phase response is like the resistivity response showing either an increase or decrease

295   with concentration over time (Fig. 7b) for a concentration threshold between 34000 and 35000 ppm. For

296   *Brachypodium* roots, a decrease of phase is observed at all concentrations after 10 minutes (Fig. 7d). Only at low

297   concentration (below 4000 ppm), an initial increase in phase was observed in the first 10 minutes of the experiment.

298   In this experiment, each salinity level was tested on a single root which might have limited statistical robustness,

299   further studies should consider replicate measurement for each salinity level.

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

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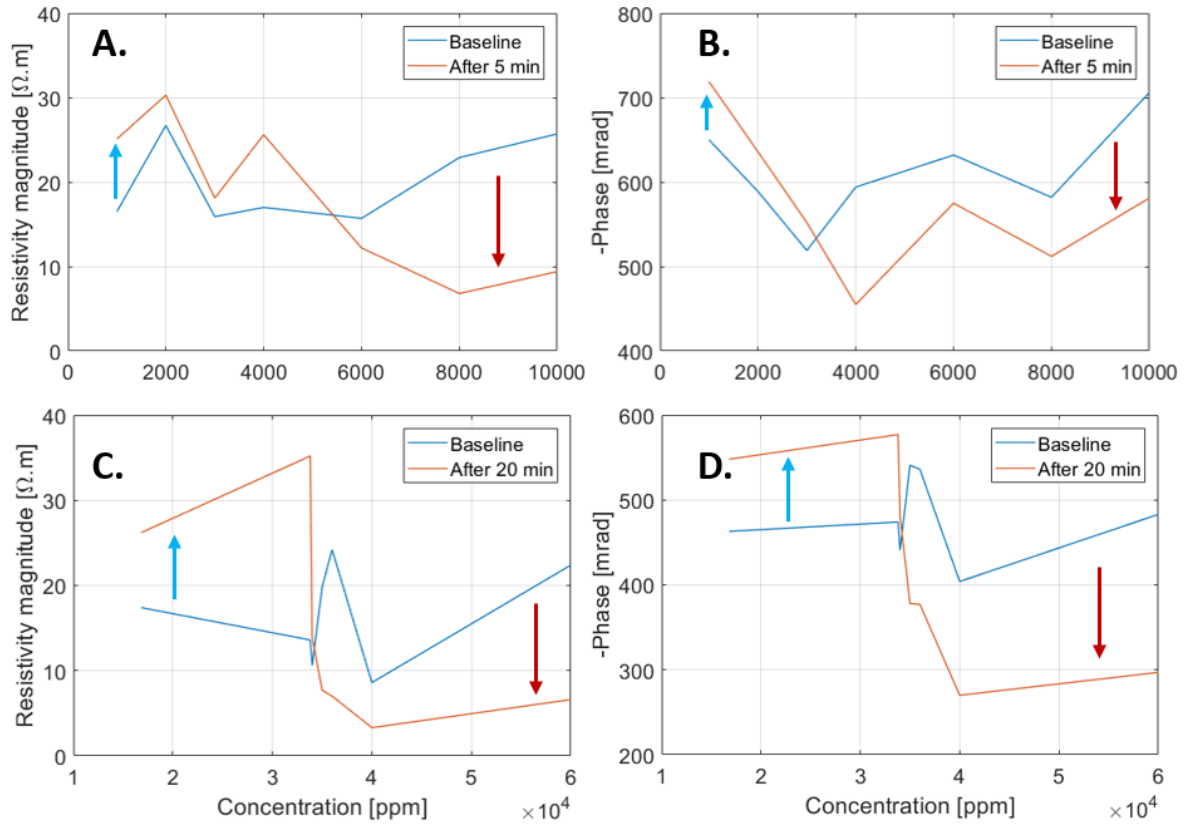
Figure 8. Reversal of resistivity magnitude and phase peak of *Brachypodium* (**a-b**) and maize (**c-d**) primary roots as
concentration increases.

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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 root and *Brachypodium* root with

increasing salinity may be related to the salt resistance mechanisms of the species. For example, some maize

genotypes can tolerate 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 is

more tolerant to salinity than *Brachypodium* (see section 2.1), showing increasing resistivity and phase response

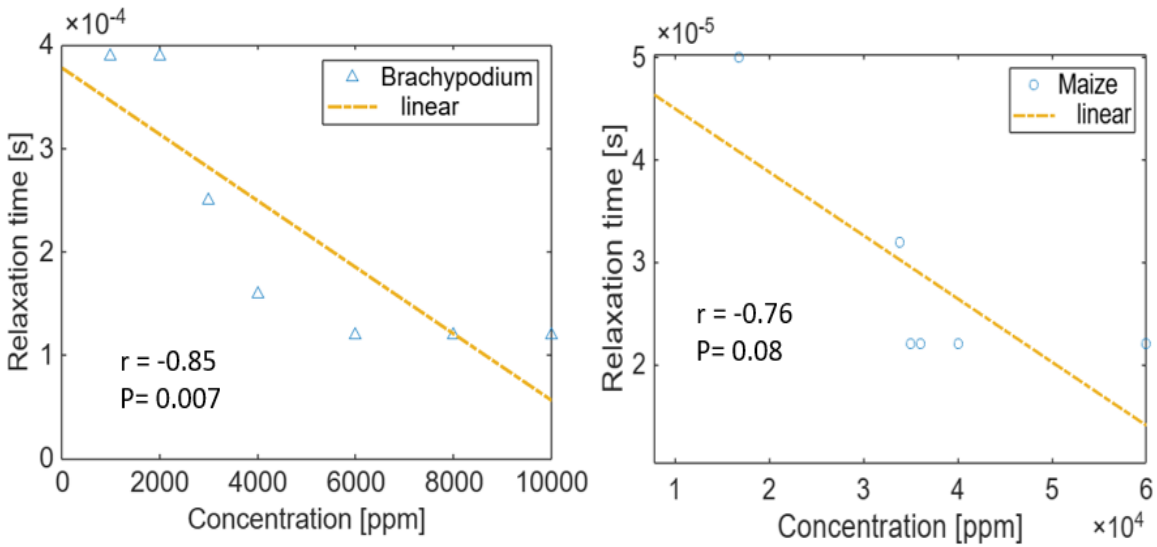
up to 34000 ppm before decreasing (Fig. 8a and 8b) while the *Brachypodium* show increasing resistivity only up

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 falls within

321 the range of very highly saline water, while that of Brachypodium lies in the range of moderately saline water (see

322 appendix, Table A1).

323

324 **Figure 9.** Correlation of relaxation time with NaCl concentration for *Brachypodium* and maize primary roots. The relaxation

325 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.

326

327 In Figure 9, we present a trend analysis of the relaxation time (τ_{max}) and salt concentration during the reversal of

328 electrical response observed in Brachypodium (5 minutes) and Maize (20 minutes) as reported in Figure 8. Bücker

329 and Hördt (2013) reported that relaxation times are only weakly dependent on salinity in the case of pore radii, but

330 in this study we found a significant correlation between relaxation time and NaCl concentration in *Brachypodium*,

331 (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

332 difference in slope further suggests that both species respond differently to salt stress based on their salinity

333 tolerance.

334 Salinity tolerance varies widely across plant species and even across genotypes within a species (Grieve et al.

335 2012). Thus, salinity tolerance of any plant is therefore indicated by the point or range in the continuum of salt

336 stress where visible or quantitative adverse effects are observed (Lauchli and Grattan 2012). In this study, the

337 concentration at which the reversal occurs for each species could be an indication of the salt resistance threshold

338 of the species (Grieve et al. 2012). This implies that salt tolerant species can withstand higher degrees of salinity

339 over a longer period of time.

340

4. Conclusions

We showed that SIP is able to detect the uptake of water and saline water in both maize and *Brachypodium* roots, and that the conduction and polarization of 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 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.

365   **Appendices**

366

367   **Appendix A: Saline water classification**

368

369   **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

370

371   **Appendix B. Raw data from experiments**

372

373   **Table B1.** Changes in mass of sample container during evaporation estimation for demineralized water and salt
374   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

375

376

377 **Table B2.** Demineralized water uptake by maize 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

378

379 **Table B3.** Saline water uptake by 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

380

381

Appendix C: Visual inspection of plants during the experiments

383

384

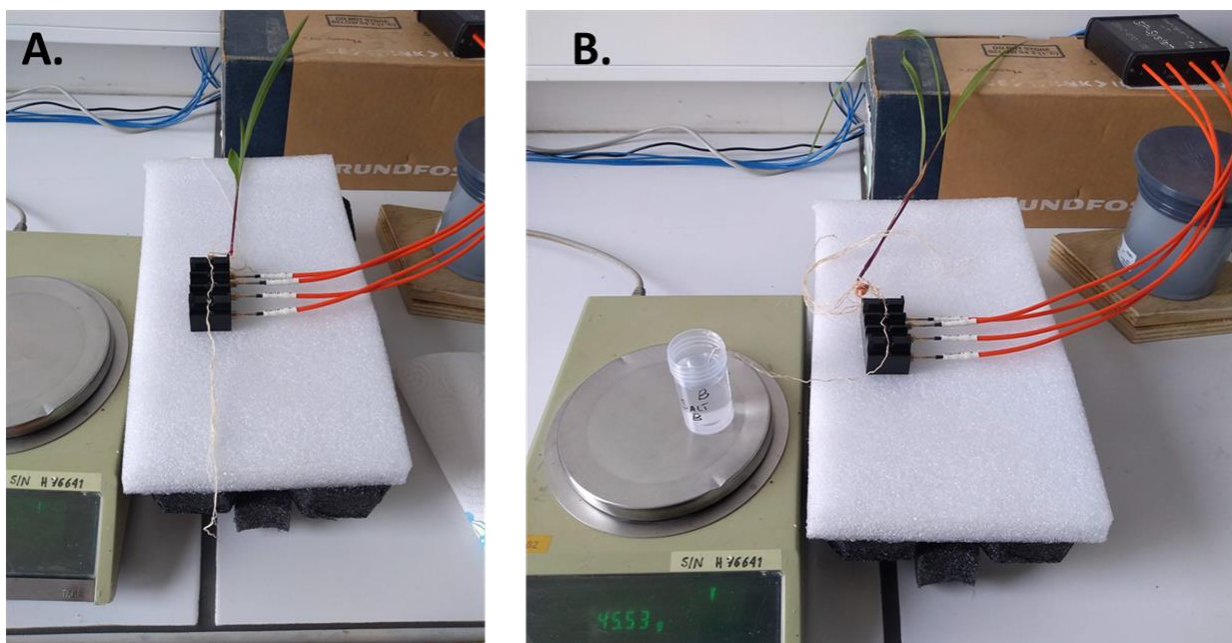



Figure C1. (a) Maize roots exposed during desiccation test over 20 minute duration, the leaves showed no sign

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.

388

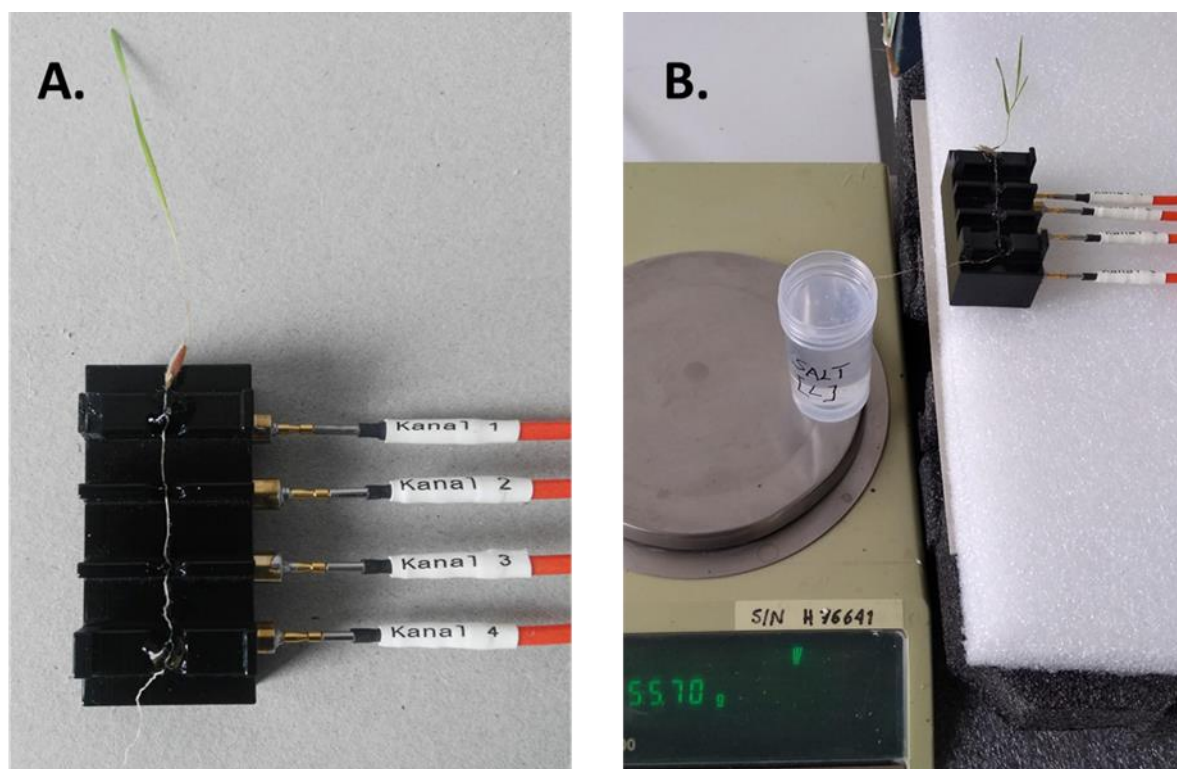
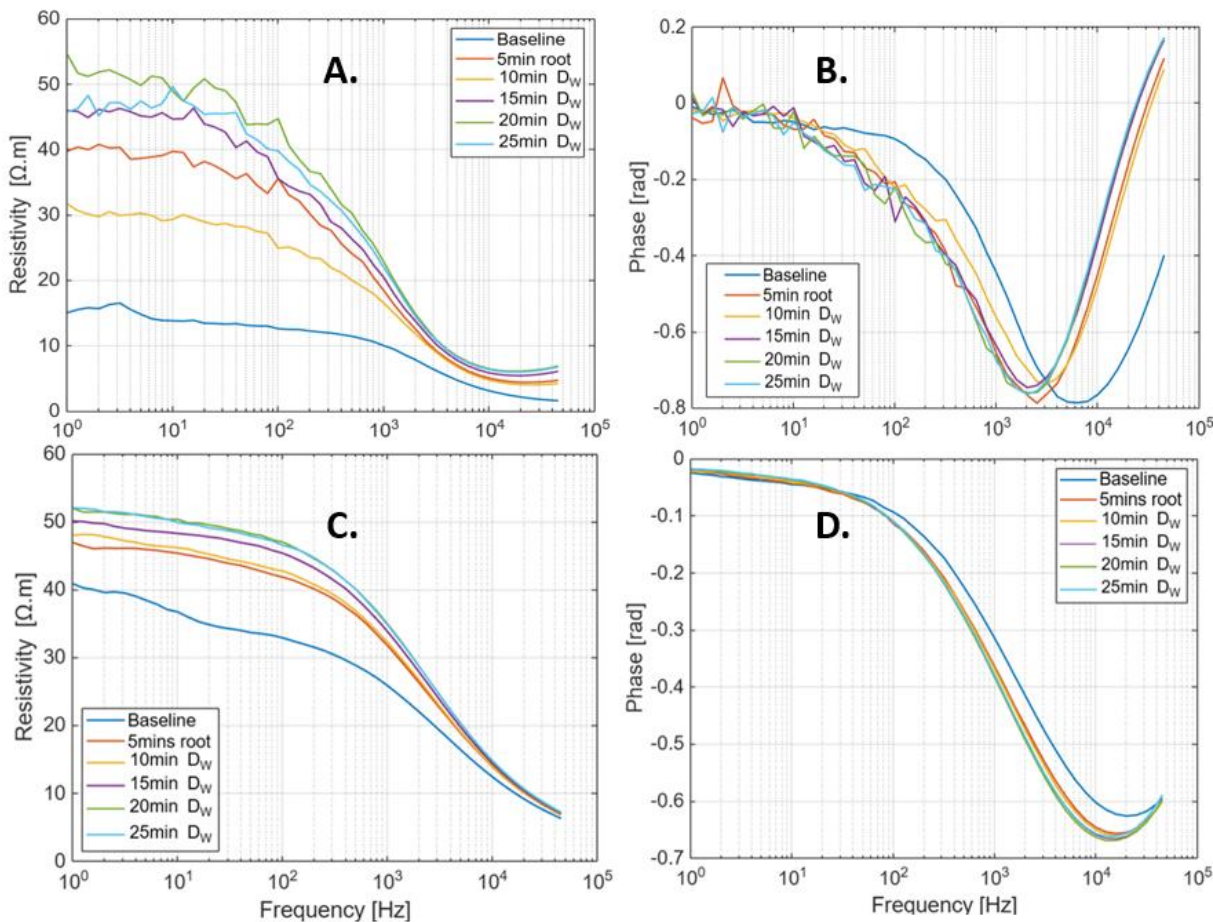



Figure C2. (a) *Brachypodium* root exposed during desiccation tests over 20 minute duration, the leaves showed

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.

 Appendix D: Replicate measurement on Brachypodium and maize roots

393

394 **Figure D1.** Resistivity and phase spectra of *Brachypodium* (a-b) and maize (c-d) primary roots during

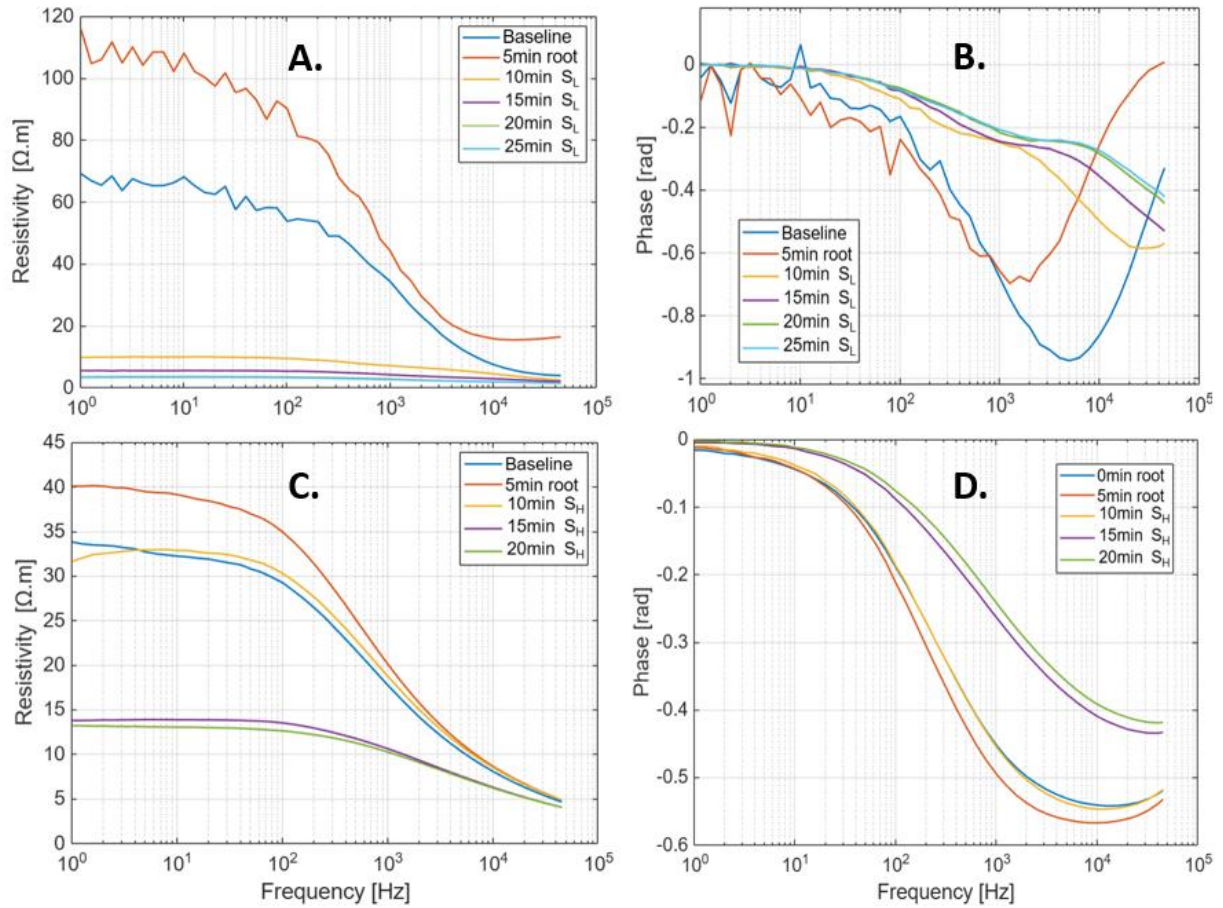
395 demineralized water uptake for 25 minutes. Measurement at 0 minute represents the baseline, measurement was

396 repeated after 5 minutes (to observe drying effect) before putting the root tip in water at 10, 15, 20 and 25 minutes.

397

398

399

400

401 **Figure D2.** Resistivity and phase spectra of *Brachypodium* (a-b) during the uptake of saline water (salt-L) for 25

402 minutes, and maize (c-d) during saline water (salt-H) uptake for 20 minutes. Measurement at 0 minute represents

403 the baseline, measurement was repeated after 5 minutes (to observe drying effect) before putting the root tip in

404 saline water at 10, 15, 20 and 25 minutes.

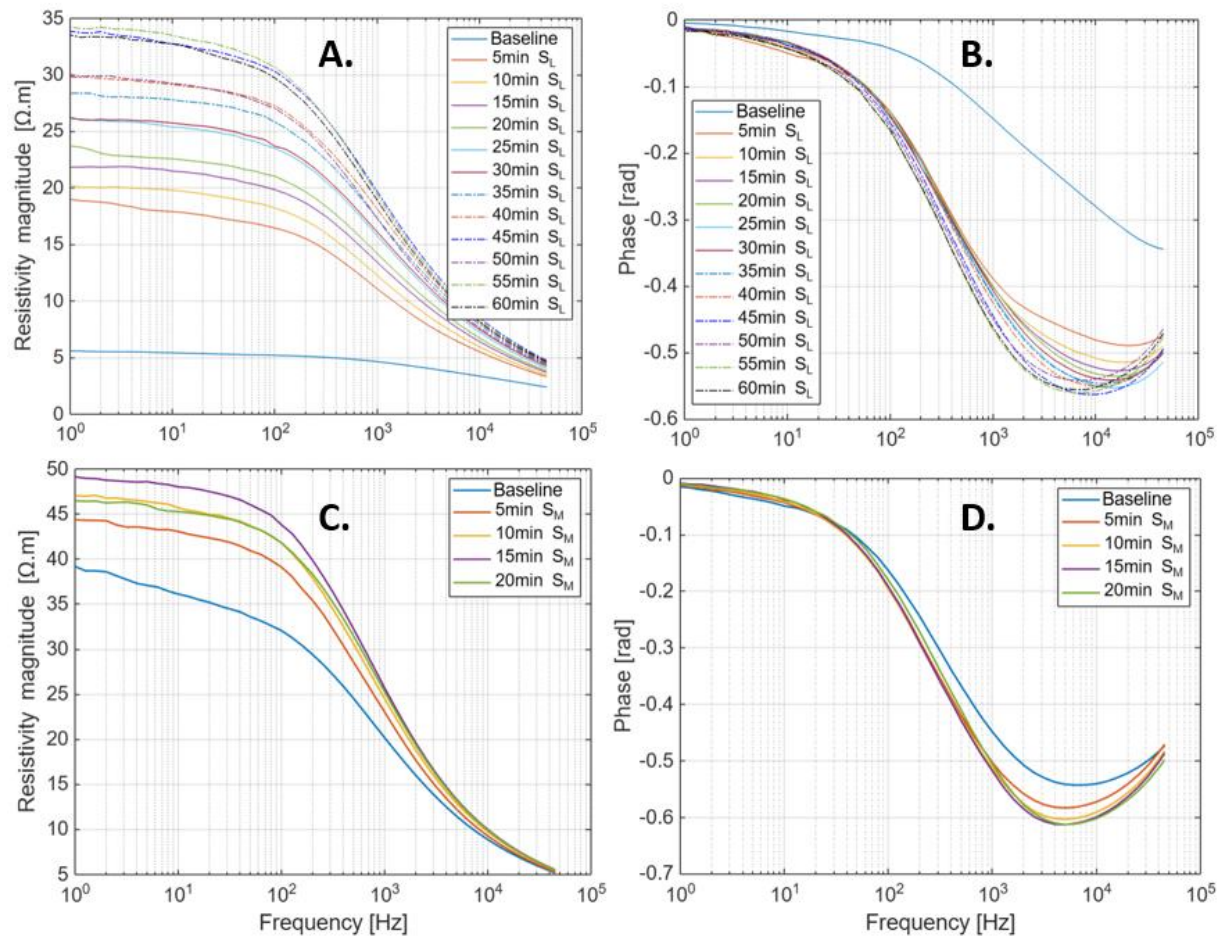



Figure D3. Resistivity and phase spectra of maize (a-b) during the uptake of saline water (salt-L) for 60 minutes, and (c-d) during saline water (salt-M) uptake for 20 minutes. Measurement at 0 minute represents the baseline, before putting the root tip in saline water.

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

421 **Data Availability Statement**

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

423

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426

427 **References**

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

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

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

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

439 Beff, L., Günther, T., Vandoorne, B., Couvreur, V., and Javaux, M.: Three-dimensional monitoring of soil water
440   content in a maize field using Electrical Resistivity Tomography. *Hydrology and Earth System Sciences,*
441   *17(2),* 595–609. https://doi.org/10.5194/hess-17-595-2013, 2013

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

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

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

451 Bera, T. K., Nagaraju, J., and Lubineau, G.: Electrical impedance spectroscopy (EIS)-based evaluation of
452   biological tissue phantoms to study multifrequency electrical impedance tomography (Mf-EIT) systems.
453   *Journal of Visualization*, 19(4), 691–713. https://doi.org/10.1007/s12650-016-0351-0, 2016b

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

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

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

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

463 Crowe, J. H., & Crowe, L. M.: Induction of anhydrobiosis: Membrane changes during drying. *Cryobiology*, *19*(3),
464   317–328. https://doi.org/10.1016/0011-2240(82)90160-2, 1982.

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

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

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

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

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

479 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-
480   Filho, E.: Effects ofsalt stress on plant growth, stomatal response and solute accumulation of different maize
481   genotypes. Braz J Plant Physiol 16:31–38, 2004.

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

485 Debez, A., Ben Rejeb, K., Ghars, M.A., Gandour, M., Megdiche, W., Ben Hamed, K., Ben Amor, N., Brown, S.C.,
486   Savouré, A., and Abdelly, C.: Ecophysiological and genomic analysis of salt tolerance of Cakile maritima.
487   *Environmental and Experimental Botany* 92, 64– 72, 2013.

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

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

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

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

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

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

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

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

510 Gómez-Bellot, M. J., Álvarez, S., Castillo, M., Bañón, S., Ortuño, M. F., and Sánchez-Blanco, M. J.: Water
511   relations, nutrient content and developmental responses of Euonymus plants irrigated with water of different
512   degrees of salinity and quality. *Journal of Plant Research*, *126*(4), 567–576. https://doi.org/10.1007/s10265-
513   012-0545-z, 2013.

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

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

520 Hasegawa P., Bressan R., Zhu J., and Bohnert H.: Plant cellular and molecular response to high salinity. *Annual*
521   *Review of Plant Physiology and Plant Molecular Biology* 51:463–499 doi:1040-2519/00/0601-0463, 2000.

522 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.

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.

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.

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.

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

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

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

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

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

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

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

572   Neubert AB, Zörb C, and Schubert S.: Expression of vacuolar Na+/H+ antiporters (ZmNHX)and Na+ exclusion
573   in roots ofmaize (Zeamays L.) genotypes with improved salt resistance In: Li CJ et al. (eds) Plant nutrition
574   for food security, human health and environmental protection, *Tsinghua University Press*, Bejing, China, pp
575   544–545, 2005.

576   Ogawa, A., and Yamauchi, A. : Root Osmotic Adjustment under Osmotic Stress in Maize Seedlings 1. Transient
577   Change of Growth and Water Relations in Roots in Response to Osmotic Stress. Plant Production Science,
578   9(1), 27-38, https://doi.org/10.1626/pps.9.27, 2006.

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

581   Repo, T., Cao, Y., Silvennoinen, R., and Ozier-Lafontaine, H.: Electrical Impedance Spectroscopy and Roots. In
582   Measuring Roots (pp. 25–49). Berlin, Heidelberg: *Springer Berlin Heidelberg*. https://doi.org/10.1007/978-
583   3-642-22067-8_2, 2012.

584   Repo, T., Korhonen, A., Laukkanen, M., Lehto, T., and Silvennoinen, R.: Detecting mycorrhizal colonisation in
585   Scots pine roots using electrical impedance spectra. *Biosystems Engineering*, 121, 139–149.
586   https://doi.org/10.1016/j.biosystemseng.2014.02.014, 2014.

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

590   Repo, T., Laukkanen, J., and Silvennoinen, R.: Measurement of the Tree Root Growth Using Electrical Impedance
591   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.

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.

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.

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.

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.

626 Weigand, M., and Kemna, A.: Multi-frequency electrical impedance tomography as a non-invasive tool to
627 characterize and monitor crop root systems. *Biogeosciences, 14, 921–939. https://doi.org/10.5194/bg-14-*
628 *921-2017, 2017.*

629 Weigand, M., and Kemna, A.: Imaging and functional characterization of crop root systems using spectroscopic
630 electrical impedance measurements. *Plant and Soil*, 435(1–2), 201–224. https://doi.org/10.1007/s11104-
631 018-3867-3, 2019.

632 Wu, L., Ogawa, Y., and Tagawa, A.: Electrical impedance spectroscopy analysis of eggplant pulp and effects of
633 drying and freezing–thawing treatments on its impedance characteristics. *Journal of Food Engineering*,
634 *87*(2), 274–280. https://doi.org/10.1016/j.jfoodeng.2007.12.003, 2008.

635 Zekri, M., and Parsons, L. R.: Growth and root hydraulic conductivity of several citrus rootstocks under salt and
636 polyethylene glycol stresses. *Physiologia Plantarum*, *77*(1), 99–106. https://doi.org/10.1111/j.1399-
637 3054.1989.tb05984.x, 1989

638 Zhao, K., Song, J., Fan, H., Zhou, S., and Zhao, M.: Growth response to ionic and osmotic stress of NaCl in salt-
639 tolerant and salt-sensitive maize. *Journal of Integrated Plant Biology. 52, 468–475*. Doi: 10.1111/j.1744-
640 7909.2010.00947.x, 2010.

641