



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

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21 **Abstract**

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

36 **Keywords:** Agrogeophysics, Spectral induced polarization, Salt stress, Maize roots, *Brachypodium* roots

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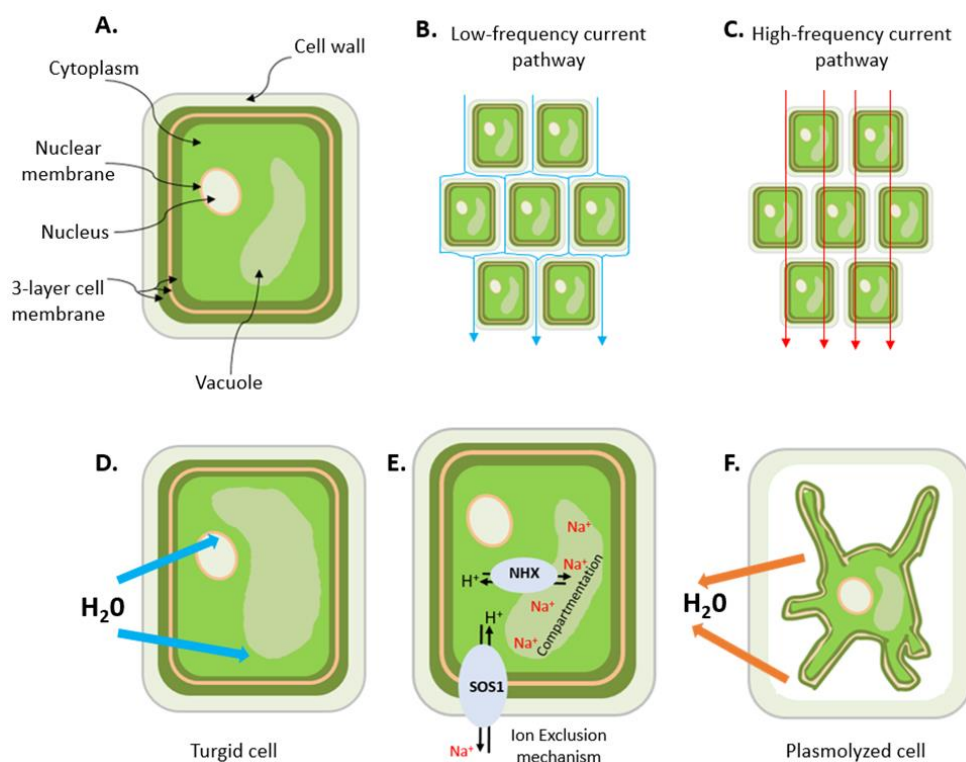


38 **1. Introduction**

39 Sustainable global crop production is challenged by several unfavorable environmental factors such as drought,  
40 extreme temperatures, salinity, nutrient deficiency, and soil contamination among others. For example, more than  
41 800 million ha of land globally is affected by salinity and excessive sodium content (FAO 2005; Munns 2005).  
42 High salt concentrations in soils induce plant stress due to low external water potential, ion toxicity ( $\text{Na}^+$  and/or  
43  $\text{Cl}^-$ ) or nutrient deficiency by interfering with the uptake and transport of various essential nutrients (Munns et al.  
44 2006; Läuchli and Grattan 2012; Hussain et al. 2013; Negrao et al. 2017; Isayenkov and Maathuis 2019). Stress  
45 magnitude depends on the species, duration of salinity exposure, the growth stage and environmental conditions  
46 (Munns and Tester 2008). Accumulation of sodium and chloride ions at toxic levels in plant tissue damages  
47 biological membranes and subcellular organelles, reducing plant growth and development (Davenport et al. 2005;  
48 Zhao et al. 2010; Farooq et al. 2015; Isayenkov and Maathuis 2019). Sodium may also displace calcium from the  
49 binding site of the cell membrane which can result in membrane leakiness (Cramer et al. 1988). Geophysical  
50 electrical methods have extensively been used to study root water uptake in soils (e.g. Michot et al. 2003; Garré et  
51 al. 2011; Beff et al. 2013) and soil salinity (e.g. Rhoades et al. 1999; Bennett et al. 2000; Doolittle et al. 2001; Ben  
52 Hamed et al. 2016; Shahnazaryan et al. 2018). Due to their sensitivity to salinity, they provide a natural means to  
53 non-invasively study salt stress impact on roots given the analogy between water flow and electrical current flow  
54 in roots. Spectral induced polarization (SIP), also known as electrical impedance spectroscopy (EIS), has been  
55 successfully used to study various plant physiological processes, such as growth (Ozier-Lafontaine and Bajazet  
56 2005; Repo et al. 2005), mycorrhizal colonization (Cseresnyés et al. 2013; Repo et al. 2014), cold acclimation  
57 (Repo et al. 2016), nutrient deprivation (Weigand and Kemna 2017, 2019), and the effects of salt stress on growth  
58 (Ben Hamed et al. 2016). In the interpretation of these SIP measurements, it is assumed that current pathways in  
59 the extracellular (apoplast) and intercellular (plasmodesmata) spaces play an important role in electrical charge  
60 migration and storage (Kinraide, 2001; Kinraide and Wang, 2010, Weigand and Kemna, 2019; Kessouri et al.,  
61 2019) (Fig. 1). In particular, current conduction is assumed to depend on the electrical properties of the apoplast  
62 and the ionic composition of the extracellular fluid (ECF), whereas polarization is assumed to occur at the cell  
63 membrane interface because charged particles such as  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  ions and amino acids cannot diffuse  
64 directly across the cell membrane. Instead, they can only cross the membrane through ion pumps and ion channels,  
65 whose opening and closing are regulated by the membrane potential difference. Polarization is also expected to  
66 occur at the outer root surface (i.e. the root-soil interface), where the charge distribution that determines  
67 polarization depends on the concentration of ions in the external fluid (Weigand and Kemna 2017, 2019).



68 Conduction and polarization mechanisms are frequency dependent (see current pathways in Fig. 1b and 1c) and  
69 can be assessed simultaneously by measuring the frequency dependent electrical impedance of a biological tissue  
70 using SIP. The suitability of this method for investigating root responses to salt stress is not well known and has  
71 rarely been studied (Ben Hamed et al. 2016).



72

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

80

81 Plants respond to salt stress by adaptive mechanisms such as root exclusion of excess sodium in the surrounding  
82 water or compartmentation, removing toxic ions from the cytoplasm where sensitive metabolic processes occur  
83 (Hasegawa et al. 2000; Munns and Tester 2008; Zhao et al. 2020) into the vacuole (Neubert et al. 2005; Farooq et



84 al. 2015; Isayenkov and Maathuis 2019). These two adaptive mechanisms are independent but their effectiveness  
85 varies across species (Grieve et al. 2012; Acosta-Motos et al. 2017). They modify the ionic composition of the  
86 extracellular and intracellular fluids (Fig. 1e), which suggests that these adaptive mechanisms can possibly also be  
87 detected by SIP. For example, Ben Hamed et al. (2016) investigated the use of EIS to non-invasively assess salt  
88 resistance and the signaling and short-term (0-240 minutes) response of Sea rocket (*Cakile maritima*) to salinity.  
89 Sea rocket was used as a model for salt-tolerant plants as it can survive extended contact with solute concentrations  
90 up to 500 mM NaCl. It accumulates salt ions preferentially in its leaves without dehydration and nutritional  
91 disorders (Debez et al. 2013). Ben Hamed et al. (2016) found that the frequency-dependent impedance of leaves  
92 changed with increasing salinity as well as the duration of stress for plants grown in sand and hydroponic culture  
93 conditions. In particular, it was observed that for a group of 10 plants exposed to increasing salinity, the electrical  
94 resistance of the leaves increased in the presence of 50-100 mM NaCl, but decreased for salinity above 100 mM  
95 NaCl, with the lowest value observed at 400 mM NaCl. For another group of 10 plants exposed to a 400mM NaCl  
96 treatment over 240 minutes, the electrical resistance increased at early stages of salt stress and reached a maximum  
97 after 180 minutes before declining rapidly. They concluded that the increasing electrical resistance within the  
98 tolerable range of salinity for growth (50–100 mM NaCl) indicated low salt movement in leaf cells due to  
99 compartmentation of salt ions in the leaf vacuoles, as reported in previous studies (e.g. Debez et al. 2004; Ellouzi  
100 et al. 2011). The decrease in electrical resistance at salinities above 100 mM NaCl was interpreted as an indication  
101 of increased movement of salt ions in the leaf cells, most probably in the apoplastic space. They suggested that at  
102 these higher salinities, leaf cells seemed to lose their ability to compartment all salt ions in the vacuoles. Therefore,  
103 ions may have accumulated in the apoplast and caused osmotic and nutritional imbalances that led to stunted  
104 growth. Similarly, Ellouzi et al. (2011) reported rapid accumulation of Na<sup>+</sup> in the vacuole and re-establishment of  
105 osmotic homeostasis shortly after salt treatment (400 mM NaCl for 4 h). They also observed a decrease in the  
106 electrical resistance of leaves of salt-treated plants, which was closely correlated with the increased accumulation  
107 of Na<sup>+</sup> in the vacuole. These studies suggest that the electrical resistance of salt-stressed plants varies with degree  
108 of salinity and the duration of salt stress. This implies that that the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in the  
109 cytoplasm and apoplast will take a long time to reach toxic levels when the salt concentration is low. At very high  
110 salt concentrations, it is expected that toxic level will be attained much faster, this could happen in a couple of  
111 minutes (e.g. Ben Hamed et al. 2016).

112 Despite these interesting studies, the suitability of SIP as a tool to study plant response to salinity has not been  
113 thoroughly investigated and few existing studies focused mainly on plant leaves. More studies are still needed to



114 better understand how roots respond to salt stress. Therefore, the aim of this study is to evaluate the SIP response  
115 of *Brachypodium* and *Maize* primary roots subjected to different levels of salinity and to link the observed changes  
116 in electrical properties with the salt adaptation mechanisms of plants to obtain further insights into the ability of  
117 SIP to detect salt stress in plant roots.

## 118 2. Materials and methods

119

### 120 2.1. Investigated plants and salt solutions

121 *Brachypodium* (*Brachypodium distachyon* L.) and *Maize* (*Zea mays* L.) were studied under different salinity  
122 treatments. *Brachypodium distachyon* L. is a salt-sensitive plant that can tolerate salt stress below 200 mM NaCl  
123 (e.g. Lv et al. 2014; Guo et al. 2020). *Zea mays* L. is moderately sensitive to salt stress (Kaddah and Ghowail  
124 1964; Farooq et al. 2015) and can tolerate relatively high salinity up to 400 mM NaCl (e.g. de Azevedo Neto et al.  
125 2004), depending on the genotype. Plants of both species were grown in the laboratory under daylight conditions  
126 (without artificial light), normal humidity and an average temperature of 23.2°C. They were grown in plastic tubes  
127 (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  
128 (Ehosioko et al. 2023). The plants were watered with tap water at 2-day intervals and were sampled at 20 days  
129 after sowing (DAS). The average diameter of the *Brachypodium* and *Maize* primary roots were 0.22 mm and 0.89  
130 mm, respectively. Both plant types were in the 3-leaves stage at the time of measurement. Before each SIP  
131 measurement, the plant was removed from the growth tube and the sand particles on the roots were removed gently.

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

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	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

## 139 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; Ehosioko 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 Ehosioko et al. (2023).

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

$$149 Z_{\omega}^* = Z'_{\omega} + jZ''_{\omega} \quad (1)$$

150 where  $Z_{\omega}^*$  is the complex impedance,  $\omega$  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

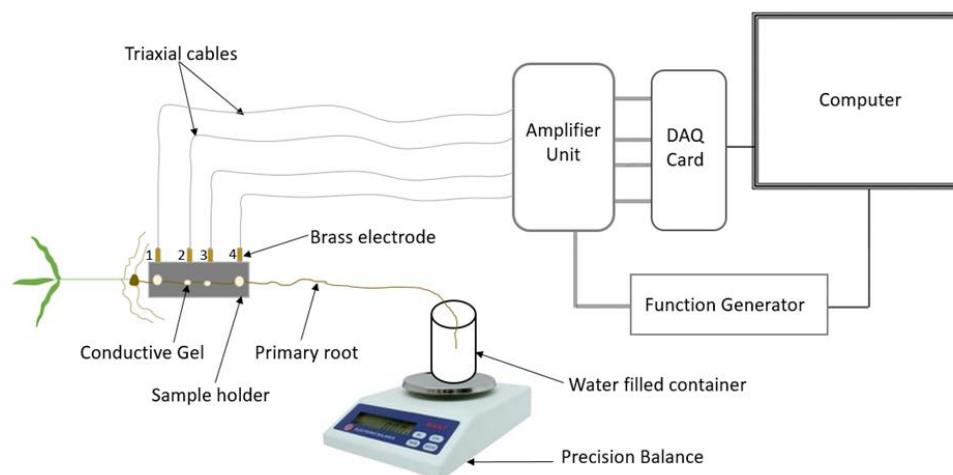
153 factor ( $K = \frac{\pi d^2}{4l}$  where  $d$  is the root diameter and  $l$  is the root length):

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

155 where  $\varphi$  is the phase shift and  $|\rho|$  is the resistivity magnitude. The relationship between complex conductivity  $\sigma_{\omega}^*$

156 and complex resistivity  $\rho_{\omega}^*$  is:

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



158

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

160

161 **2.3. Measurement protocol**

162 First, preliminary SIP measurements were performed on roots of Maize and Brachypodium plants in air to  
 163 investigate the effect of root drying on the SIP response. For this, one plant of each species was sampled. The root  
 164 was mounted in the sample holder and SIP measurements were taken at 5 minutes intervals for a total duration of  
 165 20 minutes with the root in the same position (see Fig. 2).

166 For water and salt uptake, the root was mounted on the sample holder and initial SIP measurement performed that  
 167 forms the baseline, before the root apex was tipped into a 50 ml demineralized water (e.g. Rewald et al. 2011; Li  
 168 et al. 2016) or saline water of known conductivity in a 60 ml sampling container (Fig. 2), and the initial weight of  
 169 the water, the container and the root tip was recorded. The weight was also recorded every 5 minutes for a total  
 170 duration of 20 minutes. Temperature and humidity were recorded at the end of the experiment. In the case of water





171 uptake, SIP measurements were acquired on one plant for each species using the same measurement strategy to  
172 serve as a 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 one plant of each species to two different salt solutions i.e salt-L and salt-H (see Table 1). The SIP  
175 measurements were performed at a 5 minutes interval over a 20 minutes duration while the root apex was tipped  
176 in salt solution. In the second experiment, the effect of varying salt concentrations on the SIP response of the roots  
177 was investigated. To achieve this, the measurement procedure described above was repeated with 7 different salt  
178 solutions for Brachypodium (1000 – 10000 ppm) and another 7 different salt solutions for Maize (16800 – 60000  
179 ppm) (see Table 1). Thus, a total of 14 plants was used in this experiment. To estimate evaporation loss during SIP  
180 measurements, a 50 ml demineralized water was left open on the balance and the mass was measured every 5  
181 minutes over a 20 minutes duration, this procedure was repeated for the salt solutions to estimate the loss of water  
182 from the container due to evaporation. The evaporation loss was found to be 40 mg in 20 minutes for both  
183 demineralized and saline water. The temperature and humidity at the time of measurement was also recorded (see  
184 Appendix: Table B1). The net amount of solution absorbed by the root during each measurement corresponds to  
185 the weight difference corrected for the estimated loss by evaporation.

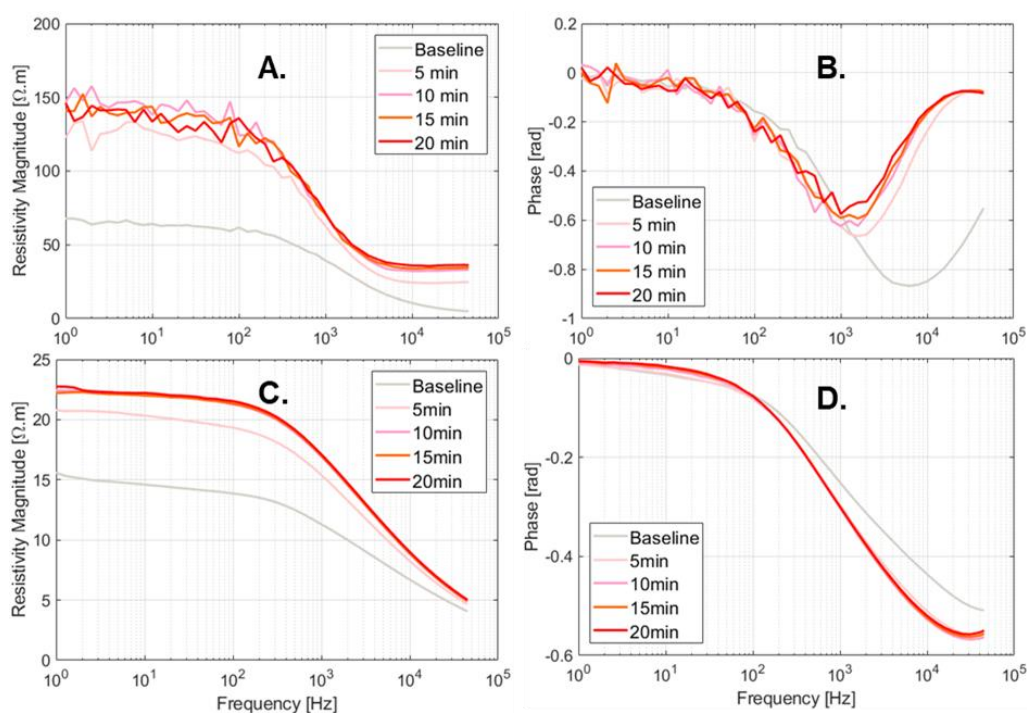
### 186 **3. Results and Discussion**

#### 187 **3.1. SIP monitoring of root desiccation**

188 The resistivity magnitude and phase of exposed Brachypodium and Maize roots are shown in Fig. 3. We can  
189 observe that the resistivity values of root segments of both species increased when the roots were exposed in the  
190 air. Water content plays a key role in maintaining the structural properties and physiological processes of the cell  
191 membrane (Crowe and Crowe 1982). Loss of water from roots may lead to a loss of turgor pressure (plasmolysis),  
192 which can result in a decrease in cell volume depending on cell wall hardness (Verslues et al. 2006; Robbins and  
193 Dinneny 2015), a decrease in cell membrane surface area, and cell membrane injury in severe cases (Lew 1996;  
194 Ando et al. 2014). Wu et al. (2008) reported an increase in total impedance during dehydration of eggplant pulp.  
195 Islam et al. (2019) also observed an increase in total impedance of onions during drying over a period of 21 days.  
196 They concluded that movement of ions due to dehydration is responsible for the increased impedance. The increase  
197 in resistivity observed in our studies for Maize and Brachypodium roots is due to loss of water from the root cells  
198 (dehydration) due to evaporation. The increase in resistivity is higher for Brachypodium (78  $\Omega\text{m}$  increase in 20  
199 minutes after the baseline measurement of 68  $\Omega\text{m}$ ) than for Maize (7  $\Omega\text{m}$  increase in 20 minutes after a baseline  
200 measurement of 16  $\Omega\text{m}$ ) both in absolute and relative values. This suggests that Brachypodium root lost water



201 faster than Maize in our experiment. We had expected that Maize would lose more water because of the larger  
202 surface area, but the result suggests that something other than surface area influenced the root dehydration, which  
203 could be the degree of saturation. Since Maize roots were observed to be more saturated than Brachypodium  
204 roots in this study, it should take longer for Maize roots to lose sufficient water and become plasmolyzed compared  
205 to Brachypodium roots. Shrinkage of Brachypodium root was clearly visible at the end of the measurement,  
206 whereas Maize appeared dry on the surface but showed no significant shrinkage. The more noisy data observed  
207 for Brachypodium is attributed to the high contact impedance of the root induced by shrinkage of Brachypodium  
208 root during drying. Polarization (phase peak) of Brachypodium showed a decrease and a shift towards lower  
209 frequencies while that of Maize first showed an increase followed by a stabilization. In a plasmolyzed cell, cell  
210 membranes shrink (see Fig. 1), which is expected to result in a decrease of the phase response. It seems that  
211 Brachypodium roots might have become plasmolyzed due to water loss (Lew 1996; Ando et al. 2014; Robbins and  
212 Dinneny 2014), while Maize roots were not plasmolyzed but rather experienced osmotic adjustment by  
213 redistribution of water to maintain equilibrium. This might explain why the phase response of Maize did not  
214 decrease. It is important to note that during the desiccation test, the leaves of both plants did not show any sign of  
215 wilting (see Appendix C, Figure C1a and C2a).



216

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



218 **3.2. SIP monitoring of roots with their tips in demineralized water**

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

223 **Table 2** Uptake of demineralized water and saline water by Brachypodium root in 20 minutes

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

224

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

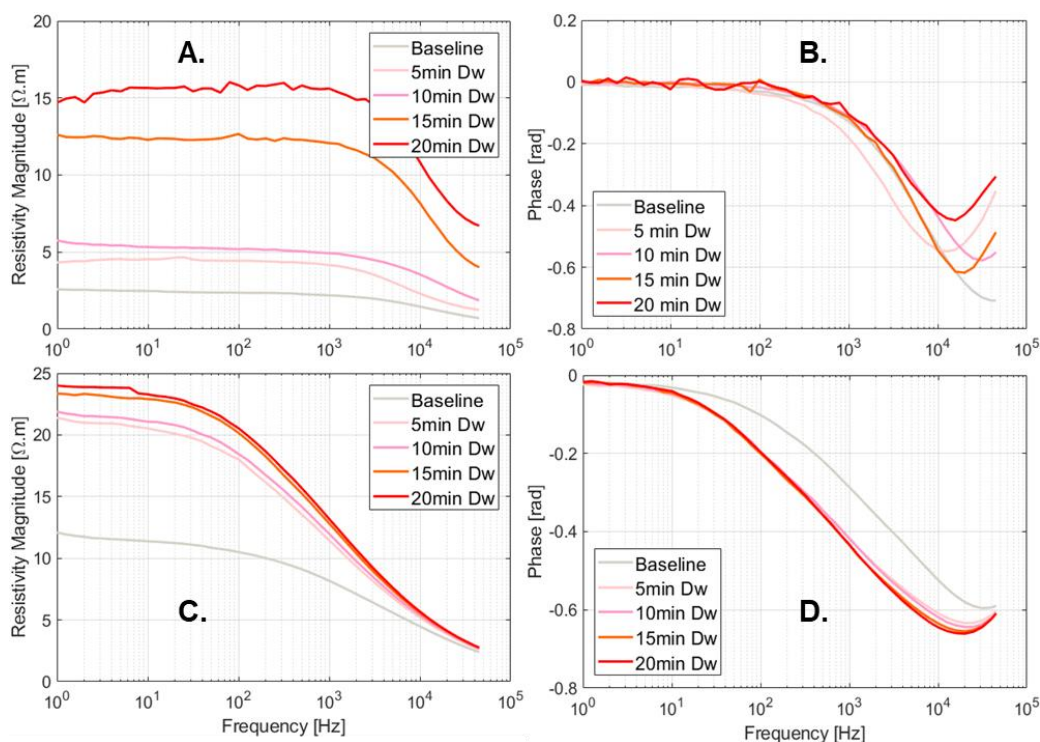
Time (min)	Mass (mg)		
	Demin water	Salt-L	Salt-H
0	-	-	-
5	20	40	30
10	30	20	30
15	30	20	30
20	30	30	20

226

227 For both species, the resistivity magnitude shows an increase with a greater effect at low frequencies (< 1 kHz)  
 228 and almost no effect at high frequencies (> 10 kHz) for Maize (Fig. 4). According to the conduction mechanisms  
 229 illustrated in Fig. 1, this suggests that extracellular fluid is diluted by DM water, which results in the observed  
 230 higher resistivity. Polarization (phase peak) of Brachypodium showed no clear trend while that of Maize remained  
 231 mostly constant after an initial increase for a broad range of frequencies (10 to 10 000 Hz), which is consistent



232 with its resistivity magnitude. Uptake of DM water may lead to dilution of cellular solutes (Schopfer 2006), which  
 233 can decrease the water potential gradient across the cell membrane that drives water movement (Robbins and  
 234 Dinneny 2015). This adjustment will be reflected in the transmembrane potential leading to the polarization effect,  
 235 and the phase peak could reflect the water redistribution and equilibrium reached as the cell regains full turgor.  
 236 The phase response of *Brachypodium* root might be linked to the adjustment of the transmembrane potential while  
 237 the steady increase in phase response of Maize suggests that its transmembrane potential might be in equilibrium.



238

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

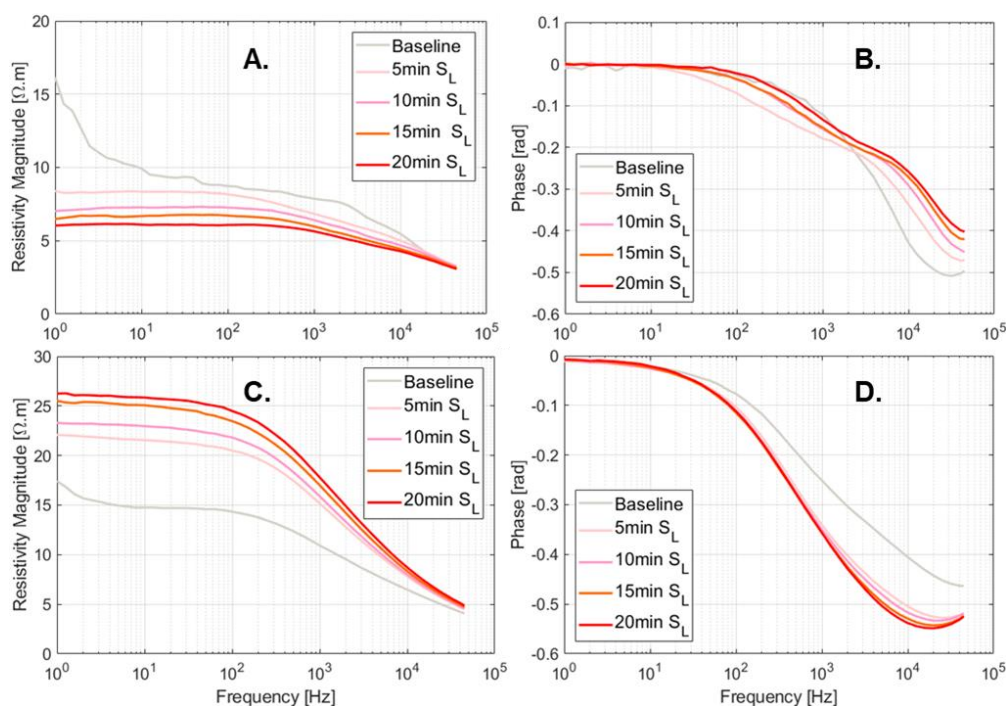
242

### 243 3.3. SIP monitoring of roots with their tips in saline water

244 The net mass of saline water (salt-L/salt-H) absorbed by the roots was similar with 40/50 and 70/70 mg for  
 245 *Brachypodium* and Maize roots, respectively (Table 2 and 3). For the low salt concentration (Salt-L), the SIP  
 246 response of Maize (Fig. 5) showed a similar response as in the case of DM water with an increasing resistivity  
 247 magnitude and phase. In contrast, the *Brachypodium* root segments showed a continuous decrease of resistivity



248 magnitude and phase. This opposite behavior may be explained in terms of salt stress tolerance. Maize is known  
249 to be moderately sensitive to salt stress (Farooq et al. 2015). Maize roots are able to take up water while excluding  
250 salts, making it more robust to salinity stress (Neubert et al. 2005; Farooq et al. 2015; Munns et al. 2020). This  
251 may explain why the SIP response of maize at this salt concentration level is similar to the response with DM  
252 water. Apparently, the concentration of the salt-L solution was already too high for Brachypodium to exclude or  
253 compartment salt in the vacuole (e.g. Lv et al. 2014) and the excess accumulation of ions in the root cell resulted  
254 in the observed decrease in resistivity and polarization (phase peak). Additionally, after 20 minutes of measurement  
255 with Brachypodium root tip in salt-L, the Brachypodium leaves showed visible signs of wilting (Appendix C:  
256 Figure C2b) which is a key sign of salt toxicity in plants (e.g. Ji et al. 2022; Plant Ditech 2023). Similar signs of  
257 wilting of leaves was observed in Maize leaves after 20 minutes of measurement with the root tip in saline water  
258 of 40000 ppm (684 mM) (see Appendix C: Figure C1b). Drought is also known to cause wilting of leaves (e.g.  
259 UCANR, 2021; Ji et al. 2022; PlantDitech 2023; Bayer 2024), however, the absence of wilting when the root tip  
260 is not in saline solution for the same duration confirms that the wilting observed in this study is a clear indication  
261 that the plants experienced salt toxicity.

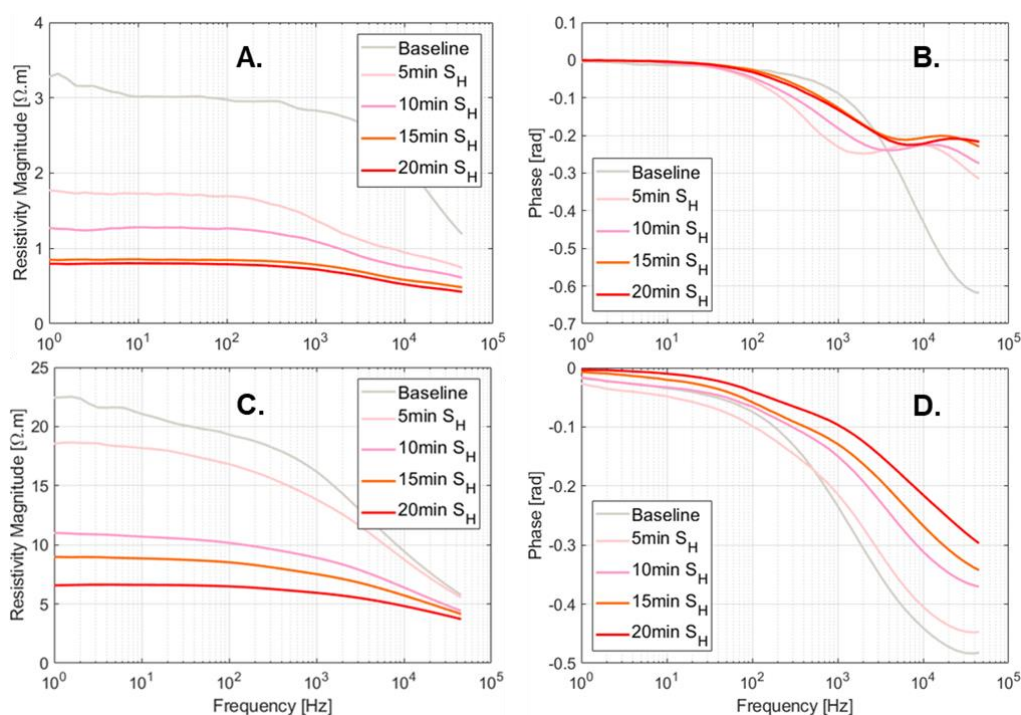


262

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



265 During high salt concentration (salt-H) uptake (Fig.6), it is interesting to see that both Maize and Brachypodium  
266 roots now have similar responses, showing a consistent decrease in both resistivity magnitude and phase. The  
267 consistent decrease in resistivity magnitude and phase for both species suggests excessive accumulation of ions in  
268 the cytoplasm and apoplast, which makes the roots more conductive (Debez et al. 2004; Ellouzi et al. 2011). At  
269 this high salt concentration (Salt-H), the plant cells apparently cannot exclude all the sodium and chloride ions or  
270 compartment them in the vacuole. This is probably the beginning of toxicity effects, although it will take time for  
271 the damage to be visible. This early detection of ion toxicity is a key advantage of SIP for root salinity studies  
272 (Ben Hamed et al. 2016).



273  
274 **Figure 6.** Changes in resistivity and phase spectra of Brachypodium (a-b) and Maize (c-d) primary roots during absorption of  
275 saline water (salt-H).

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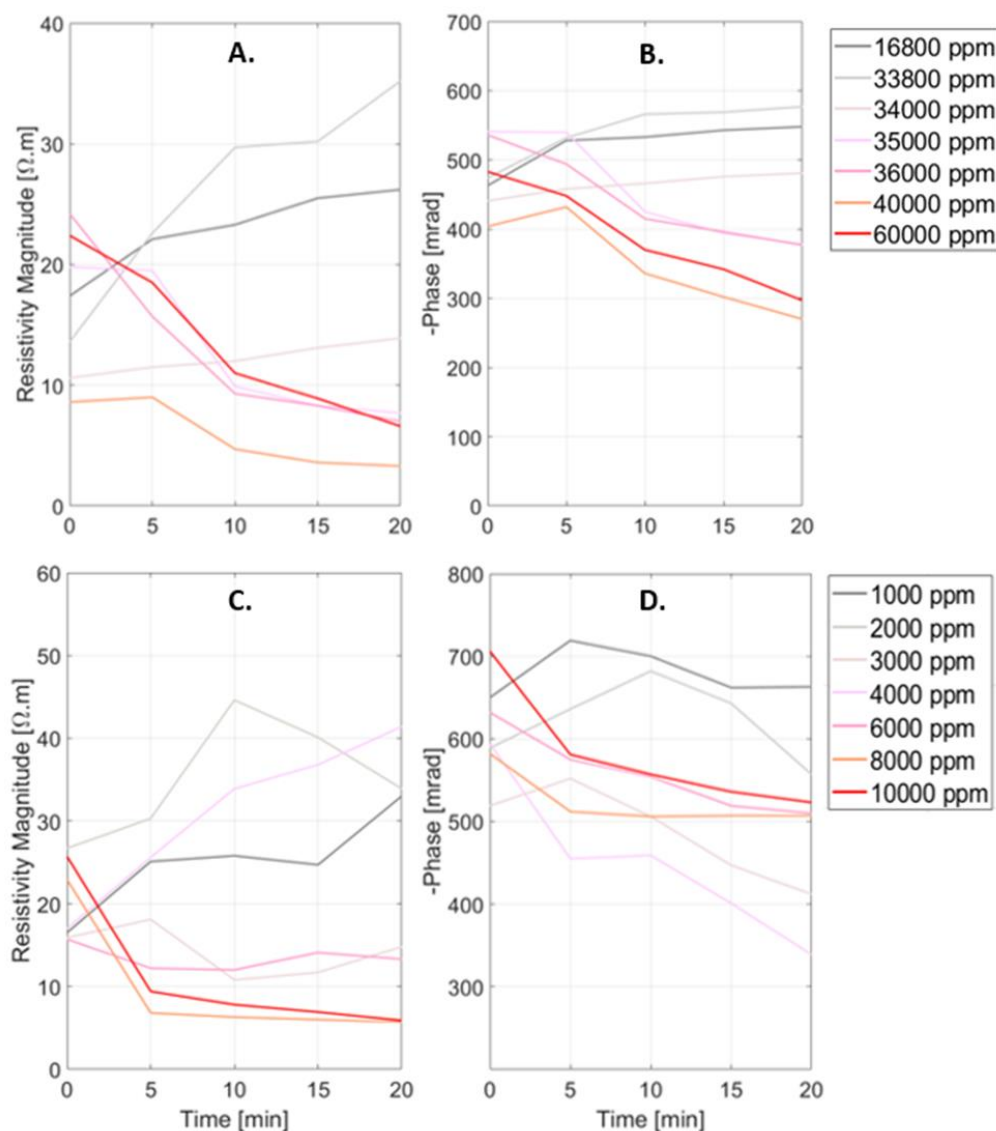
### 277 3.4. SIP monitoring of roots taking up water of gradually increasing salinity

278 The SIP response of Maize and Brachypodium roots to increasing salinity is presented in Fig. 7. Note that the  
279 range of salinity used for both species is different due to their different tolerance to salt stress. In general, a similar  
280 resistivity response was observed for both species (Fig. 7a and 7c), showing either an increase or a decrease of  
281 resistivity depending on the solute concentration, but with a different threshold due to their different salt stress





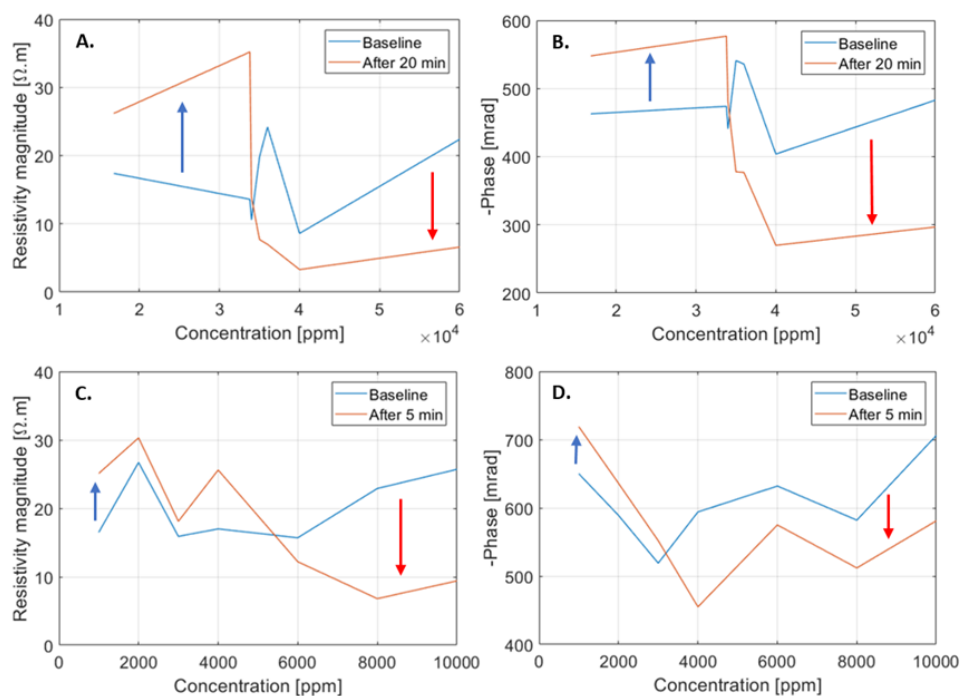
282 tolerance. For Maize roots, the phase response is similar to the resistivity response showing either an increase or  
283 decrease with concentration over time (Fig. 7b) for a concentration threshold between 34000 and 35000 ppm. For  
284 Brachypodium roots, a decrease of phase is observed at all concentrations after 10 minutes (Fig. 7d). Only at low  
285 concentration (below 4000 ppm), an initial increase in phase was observed in the first 10 minutes of the experiment.



286

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

289



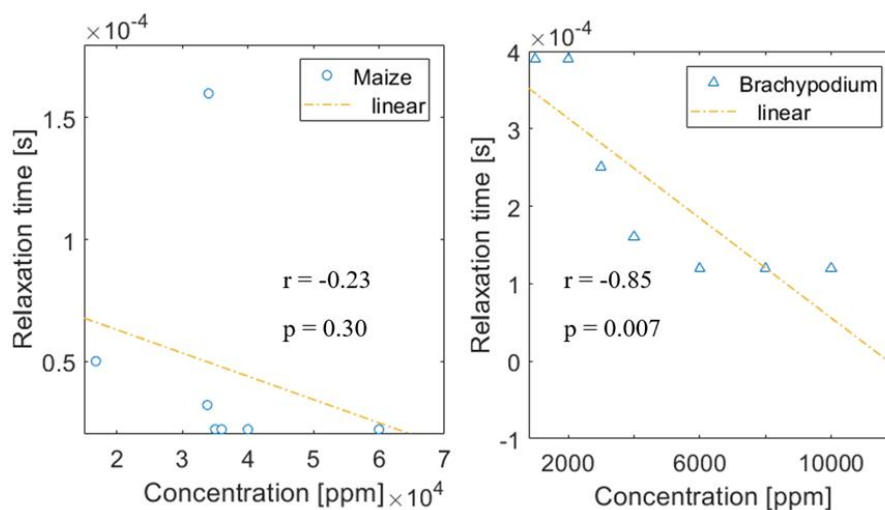
290

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

293

294 The adaptive mechanisms to salt stress may explain why the resistivity and phase response of the roots increased  
 295 at low salt concentrations and decreased at high salt concentration (Fig. 8). With increasing salt concentration,  
 296 excessive sodium accumulation in the cells occurs when the salt resistance threshold of the plant species is  
 297 exceeded (Cramer 1988; Davenport et al. 2005; Zhao et al. 2010; Farooq et al. 2015; Isayenkov and Maathuis  
 298 2019). Excess ions in the cell will increase the conductivity of the cellular fluid leading to decreased resistivity  
 299 and phase (e.g. Fig. 7 and 8). The disparity between the phase response of Maize root and Brachypodium root with  
 300 increasing salinity may be related to the salt resistance mechanisms of the species. These results seem to confirm  
 301 that Maize is more tolerant to salinity than Brachypodium, showing increasing resistivity and phase response up  
 302 to 34000 ppm before decreasing (Fig. 8a and 8b) while the Brachypodium show increasing resistivity only up to  
 303 5800 ppm before decreasing (Fig. 8c). The reversal of phase response in Brachypodium occurs at 3000 ppm but it  
 304 is only visible in the first 5 minutes (Fig. 8d). The threshold at which the reversal occurs in Maize falls within the  
 305 range of very highly saline water, while that of Brachypodium lies in the range of moderately saline water (see  
 306 Table 2).





307

308 **Figure 9.** Correlation of relaxation time with NaCl concentration for Maize and Brachypodium primary roots. The relaxation  
 309 time  $\tau_{max}$  is expressed as the inverse of  $\omega_{max}$ , where  $\omega_{max}$  is the angular frequency at which the maximum phase shift occurs.

310

311 In Figure 9, we present a trend analysis of the relaxation time ( $\tau_{max}$ ) and salt concentration during the reversal of  
 312 electrical response observed in Brachypodium (5 minutes) and Maize (20 minutes) as reported in Figure 8. Bückner  
 313 and Hördt (2013) reported that relaxation times are only weakly dependent on salinity in the case of pore radii, but  
 314 in this study we found a significant correlation between relaxation time and NaCl concentration in Brachypodium,  
 315 with Pearson's  $r = -0.85$  and  $p$  value = 0.007. This further suggests that both species respond differently to salt  
 316 stress based on their salinity tolerance.

317 Salinity tolerance varies widely across plant species and even across genotypes within a species (Grieve et al.  
 318 2012). Thus, salinity tolerance of any plant is therefore indicated by the point or range in the continuum of salt  
 319 stress where visible or quantitative adverse effects are observed (Lauchli and Grattan 2012). In this study, the  
 320 concentration at which the reversal occurs for each species could be an indication of the salt resistance threshold  
 321 of the species (Grieve et al. 2012). This implies that salt tolerant species can withstand higher degrees of salinity  
 322 over a longer period of time.

323



324 **4. Conclusions**

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

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

346

347 **Appendices**

348

349 **Appendix A: Saline water classification**

350

351 **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

352

353 **Appendix B: raw data from the experiments**

354

355 **Table B1** Evaporation estimation for demineralized water and salt solutions (salt-L and salt-H).

Time(min)	Mass (g)			Temperature (°C)			Humidity (%)		
	<i>D.water</i>	<i>Salt-L</i>	<i>Salt-H</i>	<i>D.water</i>	<i>Salt-L</i>	<i>Salt-H</i>	<i>D.water</i>	<i>Salt-L</i>	<i>Salt-H</i>
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

356

357 **Table B2** Demineralized water uptake by Maize and Brachypodium in 20 minutes

Time(min)	Mass (g)		Temperature (°C)	
	<i>Maize</i>	<i>Brachypodium</i>	<i>Maize</i>	<i>Brachypodium</i>
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

358

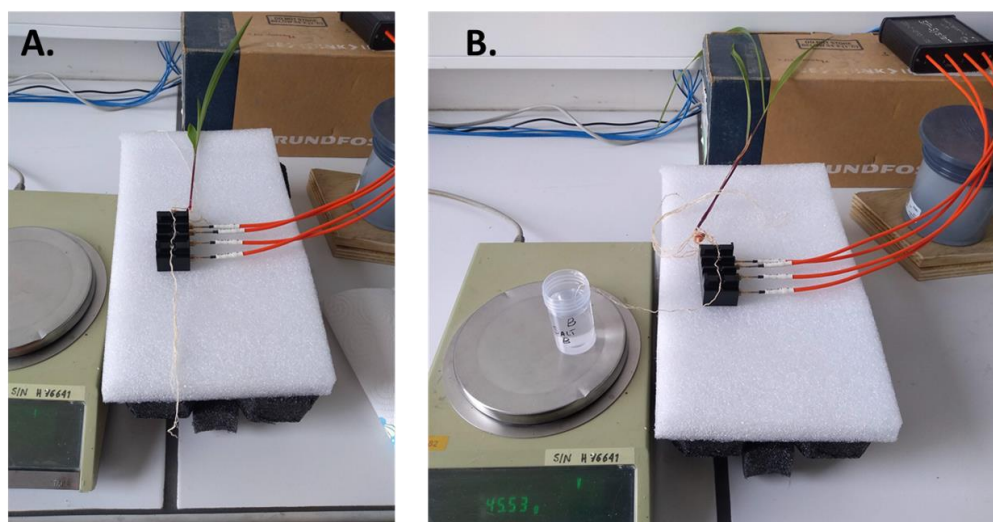
359 **Table B3** Saline water uptake by Maize and Brachypodium roots in 20 minutes

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

360

361 **Appendix C: visual inspection of plants during the experiments**

362

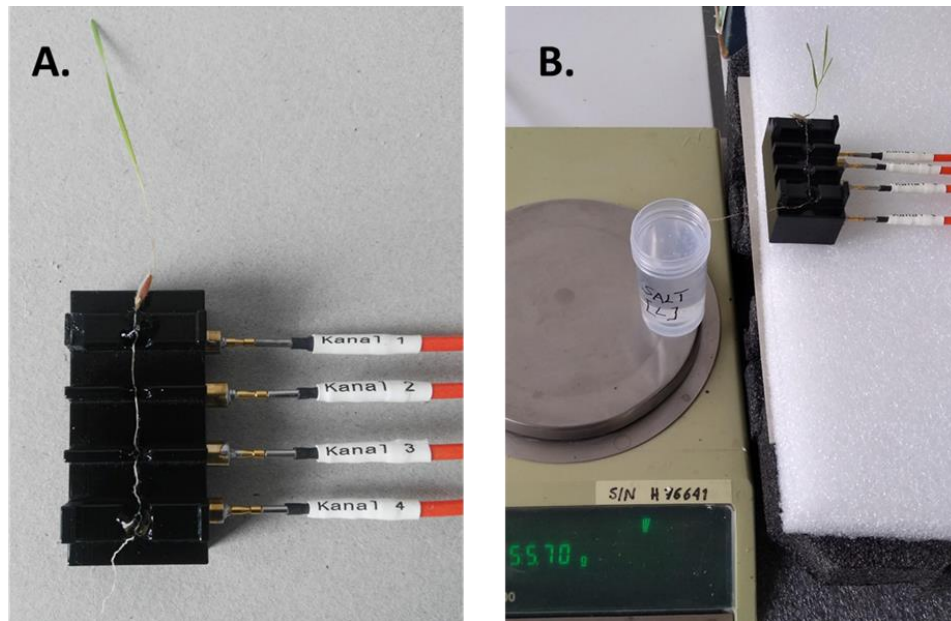


363

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

365 Maize roots exposed with the primary root tip in saline water of 40000 ppm (684 mM) concentration, the leaves showed

366 visible signs of wilting after 20 minutes of measurement.



367

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

371

#### 372 **Author Contributions**

373 Conceptualization: SE, FN, SG & MJ

374 Methodology: SE, FN, JAH, & EZ

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

376 Original draft: SE

377 Review and editing: All authors

378 Funding acquisition: SG, FN & MJ

379 Supervision: SG, FN, MJ & JAH

380

#### 381 **Conflict of Interest**

382 The authors declare no conflict of interest

383

#### 384 **Data Availability Statement**

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

386



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389

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