Final Author comments (ACs)

RC2: 'Comment on egusphere-2024-2628', Anonymous Referee #2, 05 Dec 2024

This manuscript addresses the suitability of Spectral Induced Polarization (SIP) for detecting salt stress in plant roots, using Brachypodium and Maize as model species. The topic is innovative, exploring a less-studied method for assessing root responses to salinity stress. While the study presents promising results, there are significant methodological and interpretative limitations that need to be addressed to enhance the robustness of the conclusions.

Reply: Thanks for your comment, we will revise the manuscript to address the limitations where necessary.

Main Comments

- The preliminary SIP measurements were performed on single plants for each species. Given the variability in biological systems, triplicate measurements are necessary to provide statistically meaningful baseline data.

Reply: Thanks for your comment. The baseline in figure 3-6 & 8 refers to the initial measurement on each plant before the root was tipped in water or saline water (L166-170), this enabled us to observe the change in SIP spectra due to the uptake of water or saline water for a duration of 20 minutes. It is therefore not necessary to measure several plants to establish the baseline in this case.

In general, we agree that triplicate measurement is useful in biological system, but we performed several trial measurements with replicates of maize and Brachypodium plants before the actual experiments reported here. In all the replicates, we observed similar response for drying, water and saline water uptake, and we have attached figures here for some of the replicate measurements (**see attached additional figures 1-4**), thus we argue that the issue of variability and reproducibility were taken into account in the experiment. These additional figures could be added to the appendix to help clarify the question of replicates.

- **Fig. 3:** If I understand correctly, you suggest that the initial increase in resistivity (from the baseline to 5 minutes) is attributed to water loss through evaporation from the root. However, the changes in resistivity between 5 and 20 minutes are much smaller. Could you elaborate on the factors contributing to the decrease in the evaporation rate during this period?

Reply: Before measurement, the plant was removed from the soil and placed on the sample holder, at this time the root is moist on the surface. The water loss to evaporation in the first 5 minutes is due to evaporation of the water film on the root surface. What we observe from 5-20 minutes is a response from within the root.

In addition, I assume that the SIP measurement duration is on the same order of magnitude as the times reported here. Can you clarify?

Reply: Each SIP measurement takes about 1.5-2 minutes to complete. After the baseline measurement, subsequent measurements were started exactly at 5 minutes intervals.

The Mettler PM 2000 balance has a weighing capacity of 2,100 grams with a readability of 10 mg, a reproducibility of 5 mg, and a linearity of ±20 mg. Given these specifications, measuring changes as small as 20 mg approaches the balance's linearity limit, potentially compromising the accuracy of such measurements. Therefore, the accuracy of the 20 mg changes reported in Tables 2 and 3 is questionable when using this balance.

Reply: Thank you for your suggestion. We agree that 20 mg is close to the linearity limit of the balance. However, we conducted several trial measurements with this PM 2000 balance prior to the reported results (see attached additional figures 1-4), and we are confident that our results are valid and reproducible (please see the attached additional figures).

However, in subsequent experiments, we will use a more robust balance to avoid similar challenges.

According to your data, the water uptake by the plant appears to be constant over time. How do you explain the nonconstant change in resistivity over time in light of this observation?

Reply: Thanks for your observation. This is due to a combination of two factors:

1. The drying out of the exposed root surface as in the case of desiccation test

2. The uptake of water by the root tip

The baseline measurement was performed with root exposed, then the root tip is placed in water, and measurements are taken at 5 minutes interval. We expect that the surface of the root will dry out first and increase the resistivity, then as the root takes up more water the resistivity becomes more stabilized.

It is also challenging to understand how water uptake by the plant is equal or higher than evaporation. Typically, transpiration represents a fraction of the evaporation from a free surface. In this case, the free surface is the water in the tube, and the plant extracts water only from its root tip. Given the relatively small contact area between the root and the water, could you clarify

Reply: Thanks for your comment, this experiment was performed in a controlled environment where the temperature and humidity were kept relatively constant, with a focus to reducing evaporation in order to observe and quantify the uptake of water from a single root segment (see L179-185). We argue that this outcome is actually expected.

Also, we measured evaporation from the tube without the root in it for same duration of the experiment (see Table B1 in appendix), this helped us to properly separate water uptake by the roots from the water loss due to evaporation.

I am concerned about interpreting physiological mechanisms based solely on SIP measurements without supporting data, such as direct measurements of root salt levels. Without corroborating evidence, these claims remain speculative and weaken the study's impact.

Reply: Thanks for your comment. While we did not directly measure salt levels in the root, we have evidence that wilting of leaves occurred during the salinity tests in 20 minutes but not during desiccation tests of the same 20 minutes duration (see appendix)

I recommend reporting such an experiment with repetitions, which is far more important than studying various salt levels at this stage.

Reply: For the saline water uptake, we sampled a total of 14 plants (L177-179), thus we argue that the issue of repetitions was accounted for here.

Also, we mentioned earlier that prior to the reported results, we repeated these measurements several times with different replicates of maize and Brachypodium roots and the response to desiccation, water and saline water uptake were all the same. We attached some of those results here and will include them in the appendix in the revised version.

L203: Could you clarify what you mean by "Maize roots were observed to be more saturated than Brachypodium"? How was the saturation level of the different plants assessed, and what criteria or methods were used to draw this comparison?

Reply: We used the wrong choice of words here, what we mean is that the sampled maize roots were observed to be succulent and white in color, while Brachypodium roots were dry and brownish in color.

We will update L203 in the revised version to read “the sampled maize roots were observed to be succulent and whitish in color, while Brachypodium roots were dry and brownish in color..”

L212-214: The statements made here are quite strong. Can you provide supporting evidence or references from the literature to substantiate these claims?

Reply: The statements on L212-214 will be toned down during the revision to read “Maize roots were probably not plasmolyzed but rather experienced osmotic adjustments”.

The following literatures which described “osmotic adjustments” in Maize roots under stress will be cited here in the revised manuscript:

1. Sharp et al., 1990: https://doi.org/10.1104/pp.93.4.1337

2. Voetberg and Sharp, 1991: https://doi.org/10.1104/pp.96.4.1125

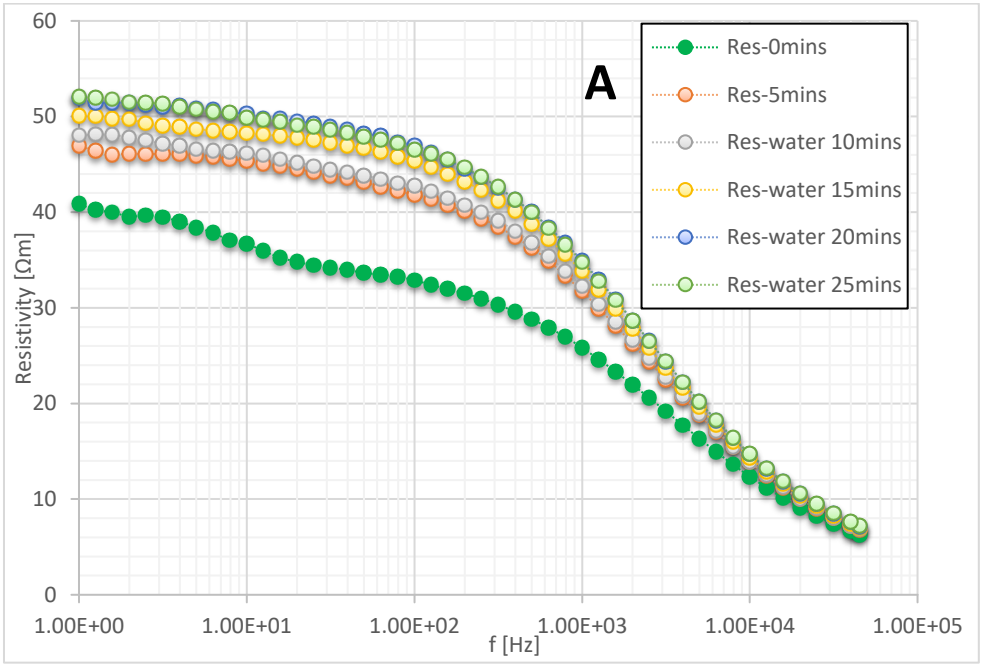
3. Ogawa and Yamauchi, 2006: https://doi.org/10.1626/pps.9.27

4. Hajlaoui et al., 2010: https://doi.org/10.1016/j.indcrop.2009.09.007

Additional Figures

1. Water uptake

Maize root



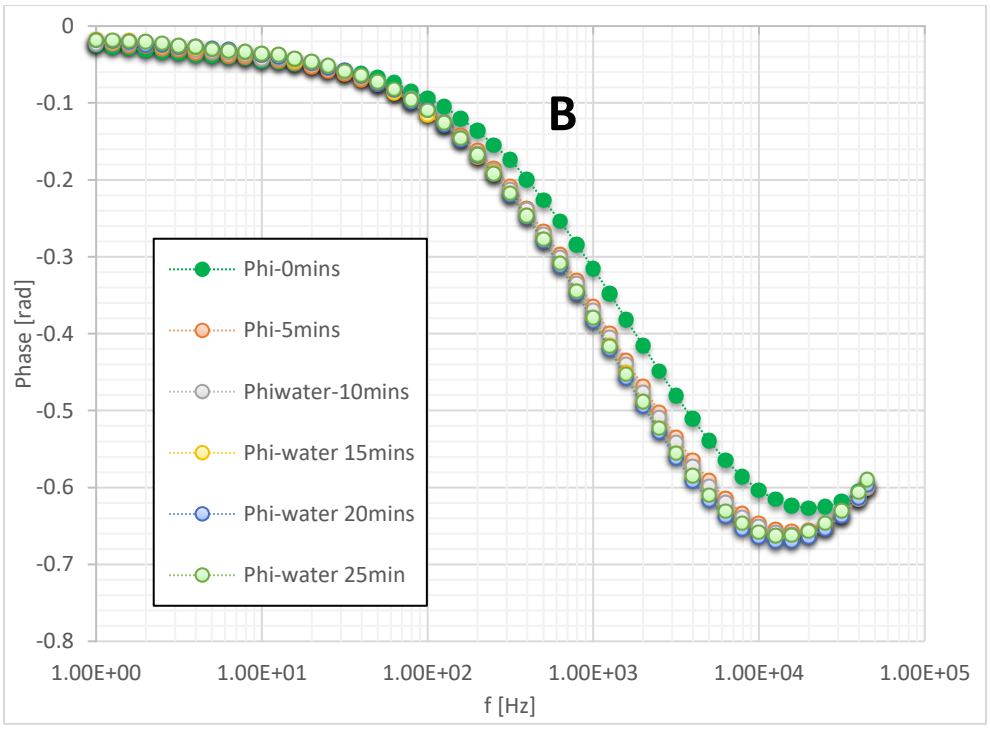
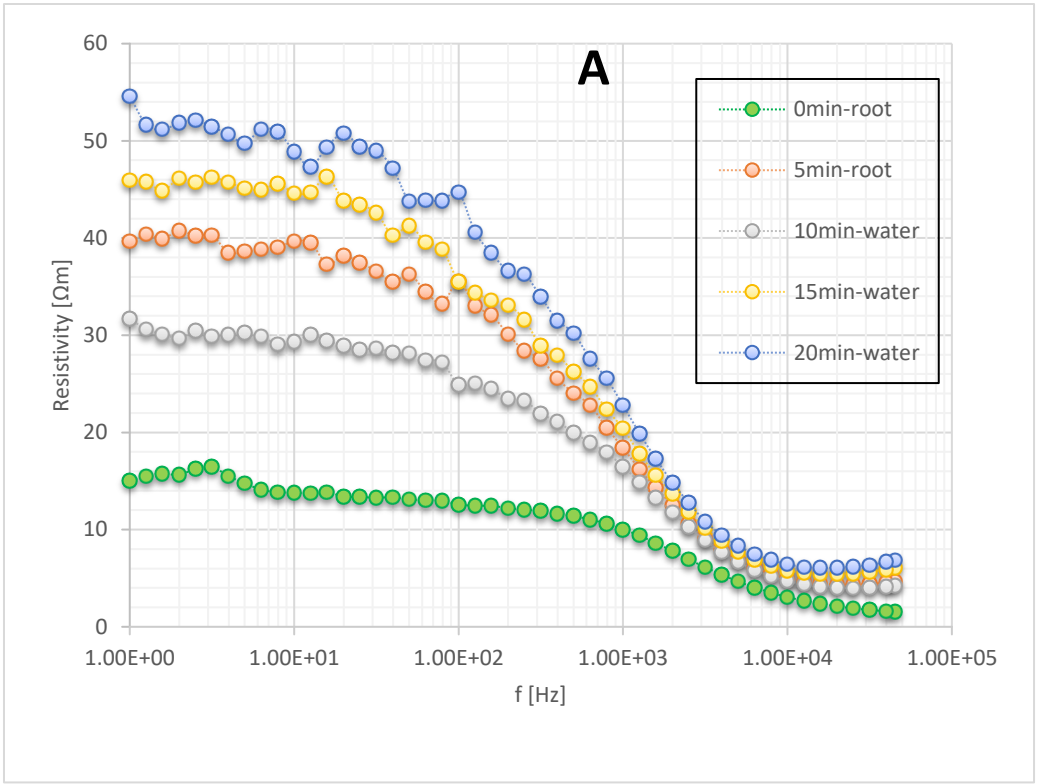


Figure 1. Resistivity and Phase response (a-b) of Maize during water uptake for 25 minutes. Measurement at 0 minute represents the baseline, measurement was repeated after 5 minutes (to observe drying effect) before putting the root tip in water at 10, 15, 20 and 25 minutes.

Brachypodium root



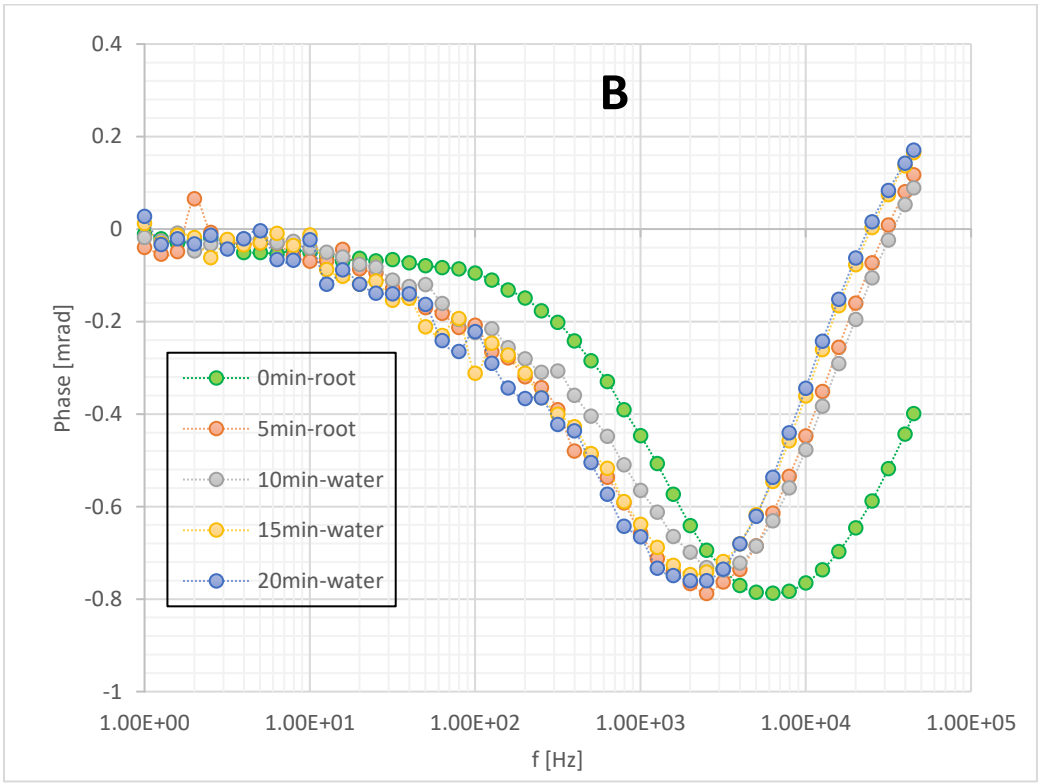


Figure 2. Resistivity and Phase response (a-b) of Brachypodium root during water uptake for 25 minutes. Measurement at 0 minute represents the baseline, measurement was repeated after 5 minutes (to observe drying effect) before putting the root tip in water at 10, 15, 20 and 25 minutes.

Saline water uptake replicates

Maize Root (salt-H)

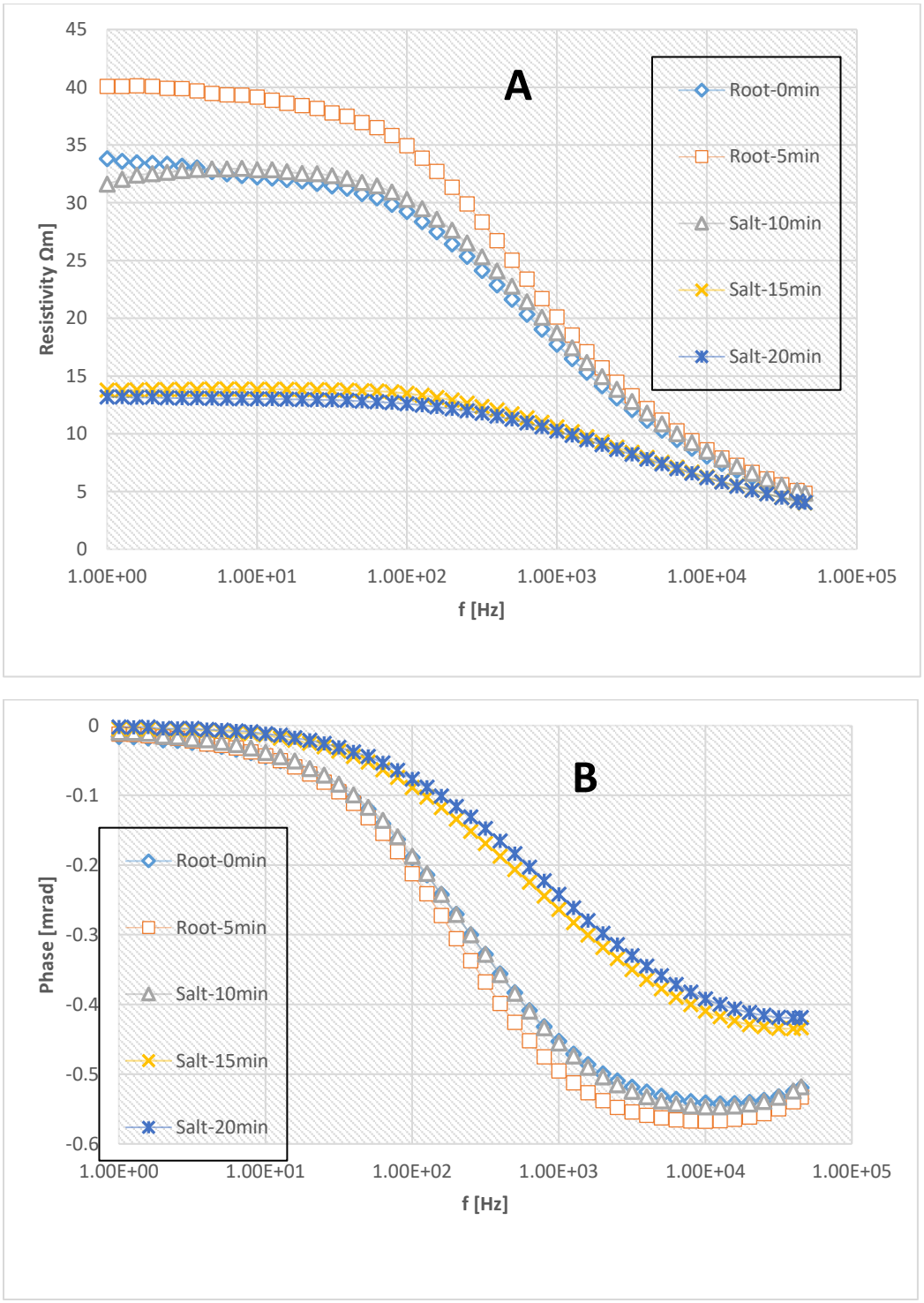


Figure 3. Resistivity and Phase response (a-b) of Maize during saline water uptake (Salt-H) for 20 minutes. Measurement at 0 minute represents the baseline, measurement was repeated after 5 minutes to observe drying effect, before putting the root tip in water at 10, 15 and 20 minutes.

Brachypodium Root (Salt-L)

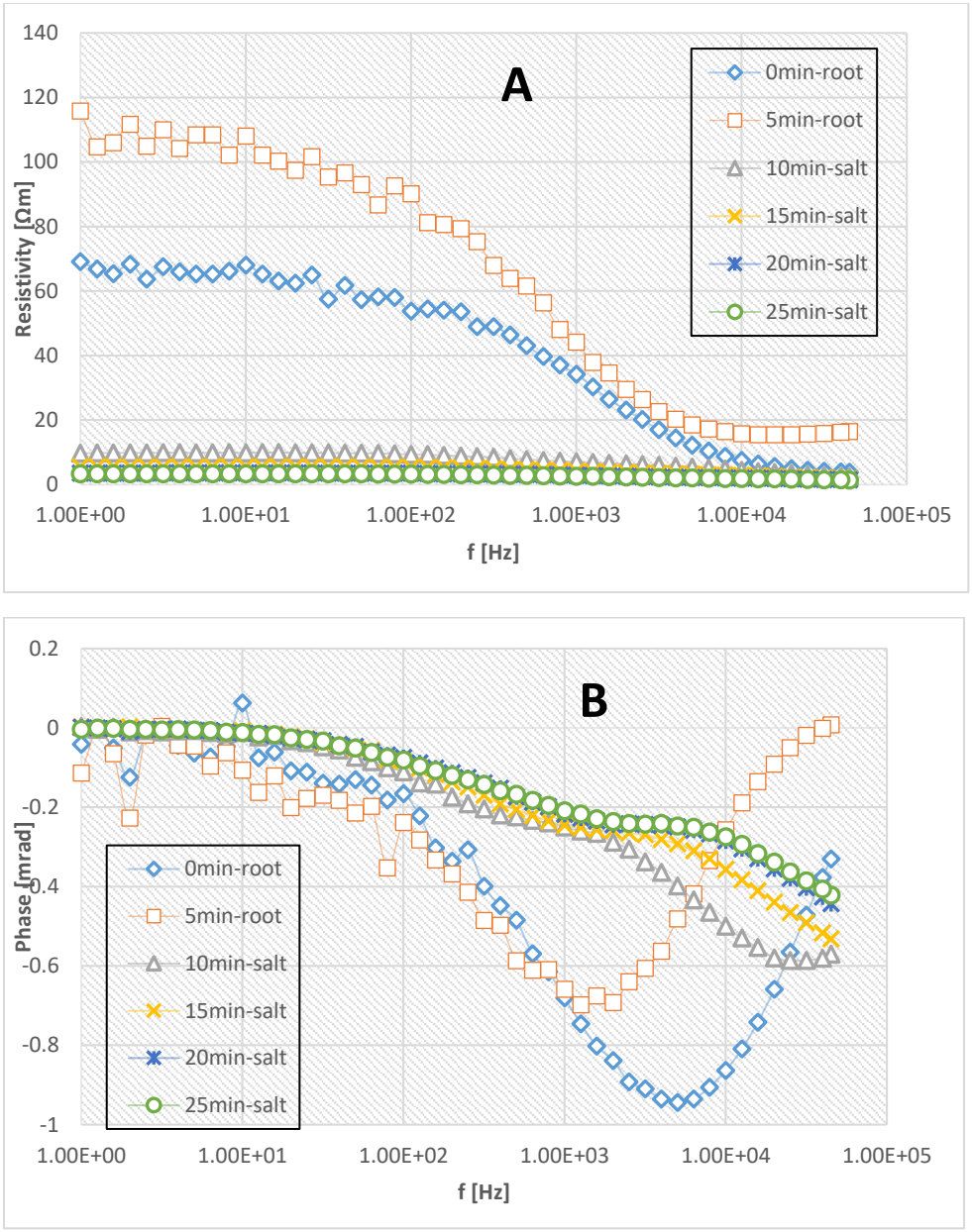


Figure 4. Resistivity and Phase response (a-b) of Brachypodium root during saline water uptake (Salt-L) for 20 minutes. Measurement at 0 minute represents the baseline, measurement was repeated after 5 minutes, before putting the root tip in water at 10, 15 and 20 minutes.