Final Author Comments (ACs)

RC1: 'Comment on egusphere-2024-2628', Imre Cseresnyes, 25 Oct 2024

General comments: High-quality paper with actual choice of topic and important novelties. Congratulations for the Authors!

Reply: Thanks for your careful and detailed review, and for providing interesting comments and suggestions that would greatly improve the manuscript.

Specific comments:

1) L36: Consider to add "electrical impedance" and/or "phase angle" to the keywords.

Reply: "electrical impedance" and "phase angle" will be added to the keywords in the revised version.

2) L42–44: Besides low water potential and ion toxicity, salinity provokes an oxidative stress as well by excessive ROS generation. Consider to mention it as a third effect.

Reply: Thanks for pointing this out, it would be included in the revised version

3) L55: Among plant physiological processes, diurnal cycles in root uptake activity has recently been monitored by impedance measurement, see: <u>https://doi.org/10.1186/s13007-023-01133-8</u>

Reply: The authors have read this paper and agree that it is worth mentioning here, it will be included in the revised version

4) L59: Basically, there is a composite water pathway inside the root cylinder, including three routes temporally variable: 1) apoplastic pathway (cell walls and extracellulars), 2) symplastic pathway (plasmodesmata), 3) transcellular pathway through (aquaporin channels). The last two are often named "cell-to-cell pathway".

Reply: As you rightly noted, "cell to cell pathway" or "intercellular pathway" is often used to refer to the last two above. This was the intention of the authors. L59 would be revised to read "intercellular (plasmodesmata and aquaporins)..."

5) L68–70: It is worth mentioning that living tissues are equivalent to parallel RC circuits, which has a characteristic phase angle depending on AC frequency. It could be important later.

Reply: The authors agree and would add this in the revised version

6) L71: There is another work to evaluate salinity effect on impedance phase angle at a single frequency: <u>https://doi.org/10.1016/j.biosystemseng.2018.03.004</u>

Reply: Having read the suggested article, the authors agree that it is very relevant to the topic and would cite it here too.

7) L74 (caption of Fig. 1): "Low" and "high" frequency is rather relative. I think that a specification of frequency ranges (according to alpha and beta dispersion regions) would give a help for the readers.

Reply: Thanks for your suggestion. While it appears that specifying frequency ranges would be helpful here, there is no "specific frequency threshold" that controls the current pathway in all

plants. The frequency at which the current pathway changes is expected to vary between one plant and another depending on the properties of their cell membranes.

We agree that the alpha, beta and gamma dispersion ranges are used to differentiate the three polarization mechanisms in biomaterials such as plant roots, but the dispersion ranges overlap, which explains why different polarization processes are often found within a dispersion range that could not be explained using one time constant model.

8) L87–104: Consider to shorten the description of the previous work by Ben Hamed et al. (2016), focusing the main finding only. I think this long description is not necessary.

Reply: The authors will shorten this paragraph where necessary in the revised version

9) L113–114: "More studies are still needed to better understand how roots respond to salt stress." I fully agree with it, as root cells are the first target of soil salinity. I may be emphasized here.

Reply: Yes, we will emphasize this as suggested.

10) L123–125: As maize tolerance to salinity depends on genotype (as you write), specify the cultivar of maize applied in the experiment, and add some information (if available) of its salinity tolerance level.

Reply: Thank you for this suggestion, we agree that it would be nice to specify the cultivar and it's salinity threshold (if available). We tried to retrieve this information but unfortunately we were not successful, mainly because this study was conducted back in 2019 and we lost track of the exact source of the maize seeds.

11) L146: Add terminal (input) voltage of the AC signal used for measurement.

Reply: The input voltage used is 5V, this will be added to the text here during revision

12) L147 and thereafter: In my opinion, it would be better to always use the conventional symbol ϕ (phí) for phase angle both in the text and in the figures. Likewise, symbol "R" is worth using for the magnitude of resistance.

Reply: Thanks for your suggestion. The authors think that the use of "phase" or the symbol " ϕ " is mainly a matter of style as both means exactly same.

Also, in the context of SIP, we measure complex impedance (see L149) and use the geometric factor to compute the complex resistivity magnitude which we showed, the use of the symbol "R" does not apply in this case.

13) L208–209: "Polarization (phase peak) of Brachypodium showed a decrease and a shift towards lower frequencies while that of Maize first showed an increase followed by a stabilization." The sentence is difficult to follow. Make clear that changes occurred over root exposition time, and consider to give frequencies at which the phase shift reach a peak.

Reply: We agree, this will be clarified during revision

14) L222: I think "larger canopy transpiration" could be written instead of "larger transpiration pull". The latter characterize the negative xylem pressure, which was not obviously higher in maize.

Reply: Thanks for the suggestion, L222 will be revised to read "larger canopy transpiration"

15) L230–231: "Polarization (phase peak) of Brachypodium showed no clear trend while that of Maize remained mostly constant after an initial increase for a broad range of frequencies" For clarity,

supplement the sentence that there was a temporal trend, according to the absorption time of DM water.

Reply: The authors agree with your suggestion and will revise the paragraph accordingly

16) L258–259: "Drought is also known to cause wilting of leaves (e.g. UCANR, 2021; Ji et al. 2022; PlantDitech 2023; Bayer 2024)..." This is evident, references are not necessary, and should be deleted from here.

Reply: We agree that references are not necessary here, and will delete them during the revision

17) L266–268: "The consistent decrease in resistivity magnitude and phase for both species suggests excessive accumulation of ions in the cytoplasm and apoplast, which makes the roots more conductive" Additionally, salinity can lead to membrane damage with increased permeability (https://doi.org/10.1016/j.biosystemseng.2018.03.004). I think this also contributed to the changes observed in the present study.

Reply: Membrane damage was mentioned earlier (L193), linked with root desiccation. It makes sense that salinity could lead to membrane damage due to increased permeability

18) L300–302: Add literature, if available, to show the salinity thresholds tolerated by some maize genotypes.

Reply: Some Maize genotypes tolerate up to 6 mS/cm (e.g. Islam et al. 2024). This will be included in the revision

19) Fig. 9: For maize, one data seems to be an outlier. Have you tested the correlation without it? Perhaps it would be improved.

Reply: Thanks for your observation, we will do that during the revision.

Technical corrections:

1) Begin a new paragraph from L55.

Reply: OK

2) Write Brachypodium in italics.

Reply: OK

3) Write "maize" with lowercase letter, not capital.

Reply: OK

4) Fig 2–7: Using more contrasting colors for the curves may improve the visibility of the results.

Reply: We will edit figures 2-7 with more contrasting colors

5) Consider to merge Table 2 and 3.

Reply: Table 2 and 3 will be merged in the revised version

6) Fig. 7–9: It is confusing that the ranking of the two species (maize a-b, Brachypodium c-d) is the opposite to those of the previous figures. It is worth changing them.

Reply: Thanks for the observation, we will change the order as noted.