Dear editor and reviewers:

Thank you for your email of December 14, 2023. We highly appreciate the editor and reviewers for their helpful and constructive comments to our manuscript entitled "Enhancing Salt Tolerance in Poplar Seedlings through Arbuscular Mycorrhizal Fungal (AMF) Symbiosis" (Manuscript ID: plants-2774587). According to the reviewers' detailed suggestions, we have made a careful revision of the original manuscript. All revised portions are marked in red font in the revised manuscript which we would like to submit for your kind consideration. A point-to-point response to the editor and reviewers' comments is provided as follows. We sincerely hope to meet the publication requirements of the journal.

We look forward to hearing from you soon.

With best wishes,

Yours sincerely,

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Point to point response to the editor and reviewers' comments:

1. The impacts of AM symbiosis on salt tolerance have been reported in many plant species. Many reports demonstrated the detailed mechanisms affected. I suggest to provide the information in the introduction.

Answer: Thank you for your suggestion. We have supplemented the detailed mechanism of AMF mediating plant salt tolerance in lines 59-62 and 65-68 in the introduction.

2. The authors used 84K poplar as material. Please explain the reason to choose this material in the introduction.

Answer: In the introduction, we supplement the reasons for choosing 84K and the sources of 84K in lines 86-90.

3. Based on the experimental design shown in Figure 8, salt treatment was performed at 90 d after transplanting and after 10-d treatment, plants were

recovered for 30 d before harvesting.

(1) Is there any reason to treat the plants with 100 mM and 200 mM NaCl solution but not solution with higher concentration?

(2) Is the level of salt stress increased gradually?

(3) How about fertilization? The authors did not mention in the methods.

(4) Please explain why not harvest plants right after salt treatment?

Answer:(1) The selection of 100 mM and 200 mM NaCl concentrations for the salt treatment was based on a careful consideration of the experimental objectives and the need to induce a moderate to high level of salinity stress while ensuring the feasibility of plant recovery. Previous studies and pilot experiments indicated that these concentrations are effective in eliciting a significant salt stress response without causing severe plant damage. Higher concentrations were not used to avoid potential negative effects that could compromise the recovery period or lead to excessive plant mortality, which might hinder the assessment of subsequent physiological and molecular responses. The chosen concentrations align with the research goals and provide a balance between inducing a meaningful stress response and maintaining experimental viability.

(2) The level of salt stress was not increased gradually in our experimental design. A fixed concentration of NaCl was applied directly to the plants at two specific concentrations, namely 100 mM and 200 mM. They were selected to induce different levels of salinity stress. The plants were exposed to these concentrations for a duration of 10 days, after which they were allowed to recover for 30 days. This approach aimed to assess the immediate and delayed responses to distinct salt stress levels rather than a gradual increase in stress over time.

(3) Regarding the fertilization treatment, no additional fertilization was carried out in this study, and supplementary comments were made in line 445.

(4) Allowing a recovery period of 30 days after the 10-day salt treatment was intended to observe the delayed effects and potential restoration of plant physiological and molecular responses following the cessation of salt stress. Harvesting immediately after salt treatment might capture only the immediate stress-induced changes, while the

extended recovery period provided insights into the more sustained impacts and adaptive mechanisms.

4.Strongly suggest to reorganize the figures in the text, especially Figures 3 and 5. The authors combined the data derived from shoots and roots in the same bar that make it hard to read. And in Figure 4 Why the authors used line chart instead of bar chart? The color codes in Figure 4 and Figure 7 is confusing at first glance. For Figure 2, I suggest the authors to present the data in bar chart and evaluate the difference by ANOVA.

Answer: According to your suggestion, we have redrawn Figure 3 and Figure 5 in the form of upper and lower bar charts.

In Figure 4, for the analysis of elements and ions, we aim to explain the continuous change of parameters at different concentrations. This is beneficial when the data points have logical order or series, and the lines connecting the data points help readers to perceive the overall trends and changes more easily. Although only two concentrations were used in this study, we hope to provide reference for the study of using other concentrations in more research on salt stress in the future.

For Figure 4, Figure 7 and the pictures with the same color, we have adjusted the color of the pictures to make them great.

For Figure 2, we chose to present the data using a lollipop plot primarily due to considerations of the data characteristics. Lollipop plots are beneficial for illustrating the distribution within each treatment group and clearly depicting individual variations. We want to emphasize the obvious difference of glomus mycin protein between NM and AM groups through this graph. The ANOVA results of GRSP differences in Figure 2 are in Table S1.

5. Inoculation of AMF alters plant physiology. The authors described the percentage of increase or decrease of certain parameters they measured. However, they use two different salt treatments simultaneously. Which treatment caused the alteration they described in the text?

Answer: For an index, at an equivalent concentration, we calculated the difference between the inoculated and non-inoculated groups, normalized by the data from the

non-inoculated group, to obtain the changes described in the manuscript.

6. The AM symbiosis-mediated salt tolerance has been reported in many species. I would expect the authors to discuss the novelty of this study.

Answer: Thank you for pointing it out, we added lines 105-107 of the novelty of this study in the Introduction.

7. The abbreviation must be spelled out on initial appearance in the text.

Answer: We have checked the full text and supplemented the definitions of abbreviations such as "GRSP", "EEG", "TG" and "MDA".

8. It looks like line no. in the upper left in Fig 1 and 7. Please remove it.

Answer: The line numbers on the upper left of Fig 1 and Fig 7 have been deleted.

9. The authors used MI method to evaluate colonization intensity. What does MI stand for? Based on which reference?

Answer: In line 459, we changed the abbreviation to the full name "magnified intersections method" and added the reference [69].

10. In Table S1, some of the indices, such as CR, cannot be found in the main text.

Answer: “CR” in Table S1 has been changed to "MCR" (Mycorrhizal colonization rate), and all indices are defined in the footnote.

11. I suggest the authors to make the manuscript concise and precise and let the text edited by English editor before resubmit. For example, in line 82 "inoculating with Rhizophagus irregularis after colonization" and the subtitle "2.9. PLS-PM of AMF improving salt tolerance of poplar". The structure of sentences is not easy to understand.

Answer: We changed " inoculating with Rhizophagus irregularis after colonization" to " inoculated 84K poplar with AMF (Rhizophagus irregularis)" in line 93. Replace " PLS-PM of AMF improving salt tolerance of poplar" with " Partial Least Squares Path Modeling (PLS-PM) of AMF enhancing poplar's salt tolerance" in line 254. The expression structure has been modified, for example, in lines 22-23, 114-117, etc. The expression of the full-text sentence was checked, and some contents were modified to make the expression more reasonable.