Author's Response to Peer Reviews

Authors' Response to Peer Reviews of "Relationship Between Seed Coat Color and Cytokinin Concentration in Efficiently Regenerating Leaf Lettuce Shoots: In Vitro Experimental Study"

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This is the authors' response to peer-review reports for "Relationship Between Seed Coat Color and Cytokinin Concentration in Efficiently Regenerating Leaf Lettuce Shoots: In Vitro Experimental Study."

Round 1 Review

Reviewer DQ [1]

The reviewer acknowledged the novelty and robustness of our study [2] but suggested improving the language, statistical reporting, and discussion depth.

Language and clarity: While the scientific content is strong, the manuscript would benefit from careful language editing for grammar and fluency.

Response: The manuscript was professionally proofread for grammar and clarity.

Statistical reporting: The statistical significance (eg, P values) is noted, but a more detailed description of the statistical models and effect sizes would enhance reproducibility.

Response: Statistical methods are now detailed in the Methods section (one-way ANOVA with Tukey test, $P<.05$).

Figures and tables: Ensure that all figures and tables referenced (eg, Figure 1, Table S1) are clearly labeled and formatted for clarity.

Response: All figures and tables have been relabeled and referenced in the correct order.

Discussion depth: The discussion of mechanisms linking seed coat pigmentation to shoot regeneration could be expanded, possibly integrating flavonoid biosynthesis and tissue culture responsiveness more.

Response: The Results and Discussion section now includes an expanded interpretation linking flavonoid metabolism to cytokinin responsiveness.

Conclusion: Consider sharpening the Conclusion to emphasize the practical applications of the findings, especially in the context of lettuce transformation systems.

Response: The Conclusion emphasizes the practical application of optimizing transformation efficiency in lettuce.

Including a visual summary (graphical abstract) could further enhance impact.

Response: A graphical abstract was considered but omitted because Figures 1-3 fully summarize the experimental results.

Reviewer FA [3]

At the beginning of your abstract, you should write a paragraph about the problem you want to solve.

Response: The Abstract begins with a clear problem statement: cultivar-dependent shoot regeneration efficiency.

The abstract mentions statistical significance but does not provide any details about how these were assessed or the significance level (eg, P value). Details on the statistical analysis methods used (eg, "significant at P<.05") should be added.

Response: Quantitative and statistical details ($P<.05$) have been added to the Abstract.

The Introduction contains well-documented data that are widely known. Hormonal information has been extensively reported and reviewed. Against this background, authors have to point out how this work is different from the earlier reported work; what are the innovative findings reported here? A strong and convincing justification is required.

Response: The Introduction has been rewritten to clarify the originality and novelty of our study.

The Methods section in its current form is not acceptable because it requires more details, such as the latitude and longitude of the culture area. Write a simple paragraph describing the climate of the area and date of study.

It is necessary to mention the active ingredient of commercial chlorine bleach.

KOH and HCl are used in the pH adjustment process.

Response: These Methods have been expanded to include climate information (humid subtropical, Cfa), bleach composition (6% NaOCl, final 1.2%), and pH adjustment (KOH/HCl).

Tween-20 is used with disinfectants to reduce surface tension, thus increasing the disinfectant's effectiveness.

Response: Tween-20 was mentioned by the reviewer but was not used in our sterilization protocol. Surface sterilization was performed using 70% ethanol and 20% bleach without surfactants.

It is necessary to mention the lighting intensity during the incubation period of the cultures.

Response: The light intensity during incubation was approximately 300 μmol m^{-2} s^{-1} under cool white fluorescent lamps.

The statistical analysis mentions EZR software, but there is no explanation of why this particular software was chosen.

Response: The rationale for using EZR software has been provided, noting that the software is a free R-based statistical platform suitable for general biological data analysis.

In the Discussion, authors have explained various biochemical interactions and mechanisms that are widely known and reported. Authors should give their own reflections of the work. It is essential to include the advantages and shortcomings of the work; what are the limitations of this technology and its shortfalls? Authors' own scrutiny of the data clarifications is decisive for the impending research on this subject. This work is field-oriented, the cost-benefit ratio is very significant, and micropropagation will increase the cost, but this has not been commented on in the text. Scale-up of the tissue culture plant is not an easy task and would be challenging work.

Response: The Discussion has been expanded with a new section, "Limitations and Future Applications," addressing the scalability, cost, and practical applicability of our method.

Conclusion: What does this infer for lettuce production? Need a little more work to show the significance of your work.

Response: The Conclusion has been revised to emphasize the implications of large-scale lettuce transformation.

References: It is advised to refer only to recent work and not old citations.

Response: The references have been updated to include recent literature (2022-2025).

References

1. Soufi H. Peer review of "Relationship Between Seed Coat Color and Cytokinin Concentration in Efficiently Regenerating Leaf Lettuce Shoots: In Vitro Experimental Study". JMIRx Bio. 2026;3:e89399. [doi: 10.2196/89399]
2. Kimura M, Yoshizumi T. Relationship between seed coat color and cytokinin concentration in efficiently regenerating leaf lettuce shoots: in vitro experimental study. JMIRx Bio. 2026;4:e70496. [doi: 10.2196/70496]
3. Al-Mayahi AMW. Peer review of "Relationship Between Seed Coat Color and Cytokinin Concentration in Efficiently Regenerating Leaf Lettuce Shoots: In Vitro Experimental Study". JMIRx Bio. 2026;4:e89401. [doi: 10.2196/89401]

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