# REVIEWERS’ COMMENTS

## Reviewer 1:

Comments to the Author

The present manuscript entitled “A high-throughput phenotyping pipeline for quinoa (Chenopodium quinoa) panicles using image analysis with convolutional neural networks” is a relevant article for the scientific community and therefore falls within the scope of the Plant Breeding Journal. The authors designed a panicle phenotyping pipeline for accurate and cost-effective phenotyping of quinoa panicles. The data analysis presented in the manuscript is accurate and sound. However, the following comments should be considered for the improvement of the manuscript:

**Response.:** Dear Reviewer, many thanks for your comments and positive feedback. We have provided detailed responses to each of your comments below, including the corresponding line numbers where the information has been incorporated. All changes in the manuscript have been highlighted in red for your convenience.

**Main issues:**

1. **The developed pipeline for image analysis can distinguish between glomerulate or amarantiform panicles. However, many quinoa accessions have an intermediate panicle type, which has been excluded from this study due to its complication. This is also the reason behind the relatively high Ve and the consequent low heritability for panicle shape. Authors should however discuss the possible optimization of their method to be able to phenotype those panicles as well, given the high frequency of accessions with such intermediate panicles in quinoa germplasm.**

**Response.:** Thank you for your observation. We recognize the significance of including the intermediate panicle shape in quinoa phenotyping. This study represents the first attempt to analyze quinoa panicles using a high-throughput pipeline, focusing on a recombinant inbred line (RIL) population from the F6 to F10 generations. At these generations, the trait exhibits high homozygosity, and diversity in panicle shape is largely fixed. Initially, our analysis considered all three panicle shapes—glomerulate, amarantiform, and intermediate. However, due to the low frequency of intermediate panicles and the challenges in accurately distinguishing this class from the two primary shapes, we decided to exclude it from the analysis. This decision aimed to ensure the robustness and accuracy of the pipeline in identifying the two predominant panicle shapes.

To address this concern, we have extended the explanation, and the additional information has been included in Lines 399–405

1. **It is not really clear why using the deep-learning pipeline developed in this study is easier and faster than using a conventional image analysis tool (e.g. ImageJ). Both methods rely on taking high quality pictures using certain backgrounds, which can be complicated due to background color, image position, shadow, and image rotation. For the developed pipeline high number of figures are required to improve the efficiency of the pipeline. Authors should discuss more extensively in the Discussion, why their deep learning pipeline should be preferred over the available image analysis software.**

**Response.:** The pipeline developed in this study provides significant advancements in efficiency, scalability, and robustness, making it a superior option for large-scale phenotyping in quinoa breeding programs compared to manual image analysis tools such as ImageJ.

We have included a detailed explanation of the advantages of implementing a deep learning approach over conventional tools in Lines 410–422.

1. **Phenotyping the panicle form (glomerulate or amarantiform) is certainly much easier and faster by manual phenotyping rather than taking pictures and running the deep learning platform to identify the panicle shape! Authors should discuss why their pipeline is particularly of interest for phenotyping panicle type.**

**Response.:** Thank you for your comment. Manual phenotyping is inherently subjective, relying heavily on the evaluator's expertise to distinguish panicle shapes, which can result in inconsistencies across different operators or phenotyping sessions, particularly when analyzing large populations. In contrast, our pipeline offers objective and consistent classification by leveraging learned patterns from the training dataset. This ensures reproducibility across diverse environments and datasets, making it a valuable tool for standardized evaluations in breeding programs.

We have included this information in the Discussion section, Lines 348–350.

1. **Previous studies show correlations between panicle density and seed yield from repeated field trials. The authors shortly mentioned that the panicle density can also be phenotyped with their pipeline but no further description of the possible required modifications to the pipeline is provided. This point should be discussed in the Discussion.**

**Response.:** Currently, our pipeline is optimized for classifying panicle shapes (glomerulate and amarantiform) but not the panicle density; however, extending its functionality to include panicle density would require dedicated annotations. These annotations would need to capture the variation between lax and compact regions within the panicle, specifically focusing on areas with inflorescences in relation to the overall panicle structure and mask.

This limitation has been acknowledged and included in Lines 454–457.

1. **The regression analysis between the manual measurement of the panicle traits (in cm) and the deep learning image analysis pipeline (in pixel), did not show any correlation of the results generated by the pipeline with the actual panicle length. The correlations of the pixel values generated by the pipeline and the width/length and length/width measurements were also low. Only for panicle width high correlations between the manual measurement and the deep learning image analysis pipeline was recorded. Authors quickly mentioned two possible reasons for this, due to lower number of pictures and non-availability of a scale in the picture frames taken in the field. The question is, if this pipeline really accelerates quantifying panicle traits in quinoa plants, when 100s of pictures should be taken in a more time and labor-consuming way (holding a scale in the frame of every picture!) to be able to accurately phenotype the traits. Authors should discuss their strategies to address these points for accurate phenotyping of panicle traits in quinoa.**

**Response.:** The application of deep learning for quinoa panicle phenotyping represented a valuable learning experience under field condition that laid the foundation for future model improvements. Based on the insights gained, we proposed a redesigned board for future image acquisition, incorporating a perimeter color scale. This enhancement will enable color adjustments for images taken under field conditions, improving the quality and consistency of data for subsequent analyses. To address this point, we have included the proposed board design for future panicle image acquisition in Supplementary File 3.

Furthermore, deep learning-based image analysis offers significant advantages by enabling the precise extraction of traits such as RGB color values and panicle area, which are challenging to measure rapidly in field conditions. These variables are crucial for selecting genotypes with desirable agronomic traits and can also support advanced genetic studies, including GWAS and QTL mapping.

This information is already detailed in the manuscript in Lines 450–454.

1. **The authors used images from multi-environmental trials from 2016 to 2019, where seed yield and other yield components were certainly also recorded to select the best lines for registration. It would be interesting to see the correlations between the panicle data generated by the pipeline and the actual yield of the lines.**

**Response.:** Thank you for the comment. We appreciate the interest in exploring the correlations between the panicle data generated by the pipeline and the seed yields. However, we would like to clarify that this information has already been published in a separate study, where the relationship between panicle traits and yield components was analyzed. To avoid redundancy and to maintain the focus of the present study on the development and application of the deep-learning-based pipeline, this information was not included here.

Lozano-Isla, F., Apaza, JD., Mujica Sanchez, A. et al. Enhancing quinoa cultivation in the Andean highlands of Peru: a breeding strategy for improved yield and early maturity adaptation to climate change using traditional cultivars. Euphytica 219, 26 (2023). <https://doi.org/10.1007/s10681-023-03155-8>

1. **I strongly recommend improving the English language of the manuscript, specifically in the Discussion section.**

Thank you for the suggestion. To enhance the quality of the writing, we initially utilized the Curie AI tool (<https://www.aje.com/curie/>) to generate suggestions for improvement. Following this, the text was reviewed and refined by a native speaker to ensure the highest standard for the final revision.