"Genome-wide Identification of Seven in absentia E3 ubiquitin ligase gene family and expression profiles in response to different hormones in *Uncaria rhynchophylla"*

Thank you for taking the time to review my manuscript and give me valuable feedback. In response to your suggestion, we have made the following modifications.

1. Line 37-41, information on the present pathway is needed. Where is the pathay coming from? Is there a reference reported to the figure? Information on each enzyme in the figure is necessary.

Response: Thank you for your advise. The full name of the key enzyme in TIAs biosynthesis pathway have added in Figure 1. The pathway comes from our previous research foundation. We have cited the reference 4 in manuscript.

2. Line 44-45, I think ABA is not completely an abiotic factor. Passage needs precision on that.

Response: Thank you for your suggestion. We have made the necessary modifications. "Plant growth and development is a highly complex process controlled by multiple hormones coordinatng effects, including abscisic acid (ABA) ."

3. Line 53-54, please specify how the influence of secondary metabolism on the regulation of stress-responsive genes could be.

Response: Thank you for your advice. What we want to demonstrate here is the influence of hormones on secondary metabolism and genes involved in secondary metabolism pathways, rather than stress response genes.

4. Line 112, precisions are required for the term 'the integrity of the conserved domain'. What information is incorporated into this activity?

Response: Thank you for your suggestion. We have supplemented it completely in manuscript. The conserved domain including RING box and SINA domian.

5. Reference 18, please provide a precise reference for the passage.

Response: Thank you for your suggestion. We made mistakes in reference. We have corrected it in our manuscript.

6. Line 86-87, reference is required

Response: Thank you for your suggestion. We have added reference in our manuscript. We would like to clarify that "Furthermore, SINA proteins also play an

important role in plant growth and development, defense response, and secondary metabolism [27-29]. A recent study demonstrated that the overexpression of SlSINA2 and SlSINA5 genes in tomatoes can result in light green leaves and alterations in flower structure [27]. Additionally, in bread wheat, the E3 ligase TaSINA has been shown to enhance biomass and crop yield in the presence of heat stress [28]. In Aquilaria sinensis, a C3HC4-type E3 ubiquitin ligase, RING3, enhanced the content of sesquiterpene by degrading AsWRKY44 through ubiquitination [29]. ” In fact, the following three examples correspond to plant growth and development, respectively, defense response, and secondary metabolism.

7. Does SlSINT5 or sisia5?

Response: Thank you for your suggestion. We made mistakes, and we have corrected in our manuscript.

8. Line 91-92, is a Sina ring

Response: Thank you for your suggestion. We have reviewed the literature again and provided a new description in manuscript. “In *Aquilaria sinensis*, a C3HC4-type E3 ubiquitin ligase, RING3, enhanced the content of sesquiterpene by degrading AsWRKY44 through ubiquitination. ”

9. 119-120, the stability adscription is not clear.

Response: Thank you for your suggestion. We have supplemented it in the manuscript. “Besides this, except for UrSINA4 and UrSINA7, which is a stable protein (Instability index < 40), other SINA proteins are unstable proteins (Instability index > 40). ”

10. Figure 1, I think the emphasis on the enantiomeric compound is not necessary since the paper does not generate data on it.

Response: Thank you for your suggestion. What we want to show in Figure 1 is the biosynthesis pathway of *U. rhynchophylla* alkaloids and the location of key enzyme genes along the pathway. In the following text, we analyzed the co-expression between key enzyme genes and *UrSINA* genes. In order to better show the location of key enzyme genes to readers, clarify the substrates and products catalyzed by the enzyme, there will definitely be the emergence of enantiomeric compounds. So we think it should be retained. Thank you for your understanding.

11. Figure 3, is not mentioned in the results section; Figure 4, lacks panels as figure legends

Response: Thank you for your kindness suggestions. These issues we have corrected in our manuscript.

12. Figure 5, panel a, does not make sense to include chromosomes without UrSINAs; Lines 166-167, do not make sense since only two genes map to the same chromosomes. It is evident that tandem duplication of results is not something that should be expected.

Response: Thank you for your reminder. The number of UrSINA members is not too many, while in order to display the UrSINA genes on 22 chromosomes and preserve the integrity of Figure 5, and we have consulted some articles on gene family based on genome-wide, they also retained chromosomes without distributed genes. As for tandem duplication, we have adopted your kind suggestion, and we have corrected in our manuscript.

13. Figure 6b is repetitive information, which is actually depicted in figure 6a. Also, the scale in figure 6b does not include the entire range of values.

Response: Thank you for your reminder. We think that Figure 6b can better demonstrate the number of *cis*-acting elements in each UrSINAs. The same display method also appeared in kiwifruit [1] and *Salvia miltiorrhiza* [2]. And the entire of values we have corrected in our manuscript.

[1] Tang, X., Hou, Y., Jiang, F., Lang, H., Li, J., Cheng, J., Wang, L., Liu, X., & Zhang, H. (2023). Genome-wide characterization of SINA E3 ubiquitin ligase family members and their expression profiles in response to various abiotic stresses and hormones in kiwifruit. Plant physiology and biochemistry : PPB, 201, 107891.

[2] Chen, C., Wang, C., Li, J., Gao, X., Huang, Q., Gong, Y., Hao, X., Maoz, I., Kai, G., & Zhou, W. (2022). Genome-Wide Analysis of U-box E3 Ubiquitin Ligase Family in Response to ABA Treatment in Salvia miltiorrhiza. Frontiers in plant science, 13, 829447.

14. Line 218-219, Double check the passage, it seems that UrSINA4 is the lowest expressed gene; Line 246, Double check the passage. It seems that it is related to enzymes that were analyzed.

Response: Thank you for your kindness suggestions. There were issues with our descriptions. We have corrected in our manuscript.

15. 325-327, I think the conclusion should be weakened as some SINA genes are specific to species rather than extensible across species.

Response: Thank you for your suggestion. We have made some modifications to the discussion.Modifications as follows: In this work, 10 *UrSINA* genes were identified from the genome, the number of UrSINA paralogous genes indicates a diversification of UrSINA functions in both spatial and temporal expression, as well as substrate specificity.

16. 329-332, I think the two passages contradict each other, and the conclusion should focus on the main findings.
Response: Thank you for your suggestion. We have corrected some details in our discussion part.
17. Lines 338-341 and 347-349. Where is the data coming from? What is the link between kiwifruit and the findings reported in this paper? Is it coffee instead of kiwifruit?

Thank you for your kindness suggestions. We have supplemented source of data in our manuscript, and we made some mistakes in description, so we have corrected in our manuscript.

18. Lines 397-398, It is not clear why the whole transcriptome data was mentioned; Figure 9 is not mentioned in the main text and its panel b is located on the left side.

Response: Thank you for your kindness reminder. It was an error in our description, we have carefully revised it in manuscript. As for the formatting issue, we have made adjustments.

19. Line 412, Does the seedling media fresmedia replace itself every 6 months? Line 416, Specified the solvent for the stock and spray solution of the material.

Response: Thank you for your suggestions. What we want to express here is that the plants we have selected are tissue cultured seedlings of *U. rhynchophylla* that have grown for six months. And MeJA and ABA were purchased from Beijing Colaber Company, and the reagents were directly prepared and can be used directly. We are also not sure about the formula inside. Thank you for your understanding and support!

20. Line 436, why transcription factors? Line 437, genome sources required

Response: Thank you for your kindness suggestions. It shouldn't be transcription factor, but rather candidate SINA members. And we have made some changes in the manuscript.

21. Line 430, The paper should benefit from adding the minimal requirements for qPCR repeatability required.

Response: Thank you for your kindness suggestions. We have supplemented some details in manuscript. “Three technical replications were used in each RT-qPCR reaction, and performed three biological replications.”

22. Table 1, GenBank ID should be useful. Units in the columns should be beneficial for the readability of the table.

Response: The genome of *U. rhynchophylla* has uploaded to NCBI (BioProject: PRJNA931770). At present, the data is still in the confidentiality stage. And the data is availbale from the corresponding author upon reasonable request. So we didn't upload the SINA sequence to NCBI. And we have supplemented the units in Table 1, thank you for your advise.

Finally, thank you again for your valuable feedback and understanding!