In this manuscript, the author identified and characterized 10 UrSINAs by in-silico analysis and studied their spatial and temporal expression patterns using qRT-PCR. The main concern of mine is that root tissue was used to study the effects of ABA and MeJA treatment on the expression of 10 UrSINAs and 12 key enzyme genes for TIA biosynthesis when UrSINAs are relatively expressed at much lower levels in root compared to leaf and stem, and the stem of U. rhynchophylla is the main site for medicinal compounds. In addition, there are lots of inappropriate and wrong scientific statements in the text that needs to be fixed and refined.

1. Describe the full name of SINA.

2. Lines 115- 117 is an incorrect statement. “The number of aa varied from 206 aa (UrSINA6) to 388aa (UrSINA1)…..MW, which ranged from between 23.63 kDa (UrSIA6)……., with an average of 37.46 kDa” is incorrect. According to Table 1, it varied from 206 aa to 506 aa (UrSINA7)…..MW, which ranged between 23.63 kDa (UrSINA6) and 57.46 kDa (UrSINA7), with an average of 40.55 kDa.

3. Line 117-118. This is an incorrect statement. If a protein's pI value is higher than 7.0 (PI>7), it is considered a basic protein. Conversely, if a protein's pI value is lower than 7.0 (PI<7), it is considered an acidic protein.

4. Line 119. The statement that only UrSINA7 is stable is not correct because both UrSINA4 and UrSINA7 have essentially the same value (38.0) of instability index. Explain the criteria distinguishing between the stability and instability of proteins based on the instability index.

5. Line 121- “UrSINA1/4 were only located in the nucleus.” To support this claim, description of Table 1 needs to be clarified using demarcation lines between the rows to identify which ones are located in nucleus and/or mitochondrion.

6. Line 149-150. The present tense “have” should be used instead of past tense “had.”

7. Line 219. “UrSINA6 showing the lowest expression levels in materials.” This is an incorrect and unambiguous statement. According to Fig. 7, UrSIN4 exhibited the lowest expression in stem and leaf. All ten UrSINAs appear to be expressed at similar levels in root under normal conditions.

8. Line 219-220 statement is entirely incorrect. UrSINA4 showed the lowest expression in leaf; expression levels of UrSINA5/6/7 are indistinguishable between the root and leaf. The P value indication of leaf UrSINA4 is missing despite the apparently significant reduction in its expression in leaf.

9. Line 221-222 must be clarified. Provide the reference supporting this statement. Also, it is not clear what type of “most genes” show higher expression levels in other tissues. What do you mean by “other tissue”? If most genes refer to UrSINAs, then this statement does not make sense at all.

10. Line 233. Provide references supporting this statement.

11. In section 2.7. The qRT-PCR-based expression analysis of UrSINAs was implemented using root tissue. Authors must explain why root tissue was used instead of leaf or leaf when UrSINAs are relatively expressed at much lower levels in root compared to leaf and stem, as shown in Fig. 7, and the stem of U. rhynchophylla is the main site for medicinal compounds.

12. Fig.8b and Fig. 9b. In which tissue was used for analyzing expression patterns of the 12 key genes under ABA and MeJA treatment?

13. UrSINA4/7/10, which do not have ABA-responsive cis elements (Fig. 6), exhibited no differential temporal expression patterns (Fig. 8). In contrast, UrSINA2/6/9 showed differential temporal expression patterns, and UrSINA9 was first up-regulated at 1 h after ABA treatment despite the absence of ABA-responsive cis-acting elements. Explain this apparent discrepancy.

14. Line 241-244. This is an incorrect statement. According to Fig. 8a, not UrSINA5/6 but UrSINA9 was first up-regulated at 1 h and the highest expression levels at 12h after ABA treatment. UrSINA9 is the most sensitive gene in response to ABA.

15. Line 253-254. This is an incorrect statement. UrSINA1 showed significantly positive correlation with Ur7DLH and UrSTR, but not with UrLAMT.

16. Line 262-263. Authors ignored the following important point: Unlike the other UrSINAs, UrSINA4 showed a relatively stable (less variable) temporal expression pattern, and this pattern is consistent with the observation that only UrSINA4 lacks MeJA-responsive cis-acting element.

17. Description of reference 4 (Line 503) is incomplete, missing in the name, volume and page number of literature.

18. Line 309-311. Rewrite this inept and redundant sentence by simply stating – The number of UrSINA paralogous genes indicates a diversification of UrSINA functions in both spatial and temporal expression, as well as substrate specificity.

19. Line 313-315 needs to be clarified: “According to the predicted…….. different functions (Table 1).” Single or multiple UrSINA proteins might have different functions? The predicted cellular location of UrSINA suggests that it is involved in regulating the stability of nuclear and mitochondrial proteins.

20. Line 383. Provide the reason why the SINA ubiquitin E3 ligase targets crucial enzyme genes of TIA pathway rather than transcription factors controlling the expression of genes involved TIA biosynthesis pathway.

21. Line 353-355 statement is essentially the same as line 389-392. This redundancy must be removed.

22. Discussion is mostly repetition of the results. What are the potential substrates of the identified 10 UrSINAs? Authors needs to discuss this point. This could be inferred from the same clade group of phylogenetic tree and/or amino acid sequence similarities in SINA domain (responsible for the interaction with target proteins) between the UrSINAs and other species’ SINAs and the known substrates? For examples, SINAT5 (SINA of Arabidopsis thaliana 5) ubiquitinate the transcription factor NAC1. ATG6 is a target of the SINATs in Arabidopsis. OsDIS1 (Oryza sativa drought-induced SINA protein 1) is a negative regulator of drought response and directly interacts with and promotes the degradation of OsNek6. Conversely, SINA2 positively regulates drought tolerance in an ABA-dependent manner in Arabidopsis. A banana SINA E3 ligase MaSINA1 was recently found to ubiquinate and promote the degradation of MaICE1, which positively regulates cold tolerance i. These are a few examples, and there may be many others.

23. Discuss the significance of the presence of light-responsive cis-acting element in UrSINAs. It has been reported that SINAT protein levels are decreased in the dark and increased in the light, suggesting that darkness may promote autodegradation of SINATs.

24. Line 397 is an incorrect statement. SINA gene family was not identified from whole genome transcriptomic data and qRT-PCR expression analyses but by in-silico protein domain homology searches.

25. Line 436 is an incorrect statement. SINAs are not transcription factors but E3 ubiquitin ligase.

26. Line 400 is an inept sentence – “bioinformatics were analyzed” Bioinformatics is an analysis tool.