In the manuscript “Evolution and dynamic transcriptome of key genes of photoperiodic flowering pathway in water spinach (Ipomoea aquatica)”, the authors studied genes that were differentially expressed at different time points during two photoperiod treatment (short and long days) in water spinach to provide better understanding of the photoperiod-mediated molecular regulation mechanism of flowering time.

Experiments and data analysis were well done, and the study design was appropriate to answer their biological questions, but in the manuscript text some explanations are needed. Further clarification of how certain procedures were performed is necessary in the materials and methods section for the readers to validate their results. For example, authors are not providing important information on primers used to validate gene expression by qPCR, among other details). Furthermore, the cohesion and coherence of ideas in the results section needs to be improved so the reader understands the author analysis and conclusions. The points to be clarified and improved can be divided into major and minor issues as described below:

Response back: Dear reviewer, thank you for your time and important suggestion, your valuable suggestion; improved the manuscript quality. Thank you.

1. Title: Ipomoea aquatica in title is a scientific name and the convention for scientific name is that it needs to be italicized.

Response back: We have removed the scientific name from the title, upon suggestion of other reviewers.

2. Introduction (page 2, line 51): Arabidopsis thaliana is a scientific name and the convention for scientific name is that it needs to be italicized.

Response back: We have changed into italicized.

3. Materials and methods (page 3, line 146): How was water spinach propagated by liquid culture? At what age were the plants treated? Please provide details.

Response back: It was typo, we have corrected, propagated by hydroponic methods, experiment conducted under hydroponic conditions.

4. Materials and methods (page 4, line 158-162): How was the total RNA extracted? What kind of library was constructed? And what Illumina instrument was used for sequencing?

Please provide details. Also, software used for analysis (for example: Hisat2 v2.0.5) needs to have a citation. Which software was used to "removing reads containing adapter, reads containing ploy-N and low-quality reads from raw data"? What is ploy-N? Not sure what that term means, is this a typo?

Response back: We have mentioned all detailed in material and method section. We have provided the complete procedure of RNA extracted.

5. Materials and methods (page 4, line 165): Citation is needed for the package "DESeq2 R package (1.20.0)".

Response back: We have provided the suitable reference

6. Materials and methods (page 4, line 172): Citation is needed for the package "cluster Profiler R package".

Response back: We have provided the suitable reference,

7. Materials and methods (page 4, lines 176-179): Citations needed for the package "the WGCNA package in R" and the software "Cytoscape 179 (v.3.8.1)".

Response back: We have provided the suitable reference

8. Materials and methods (page 4, line 182-183): Citations are needed for "Bioedit software" and "MEGA 8.0".

Response back: We have provided the suitable reference

9. Materials and methods (page 4, line 186-191): What genes were selected when author indicate "10 flowering-related genes for RT-qPCR validation" Not clear. Which primers were used? Please include a table with gene, primer (F and R) name, primer (F and R) sequences, and corresponding melting temperature used during PCR. Please provide details on how the total RNA was retrotranscribed to cDNA and how the qPCR was performed? (SYBR Green kit? FAM kit? Instrument? Temperature conditions? Melting curve analysis? How many biological replicates per sample? among other important details for qPCR). The materials and methods section must be very specific so other people wanting to validate the results can do it exactly in the same way authors did.

Response back: We have provided the all details in material and methods section. Thank you for intension.

10. Results (page 4, line 199): Ipomoea aquatica is a scientific name and the convention for scientific name is that it needs to be italicized. What version of the reference genome was used for alignment? Also, where is the reference genome found (database)? Authors did not mention these details in the materials and methods section. Please specify it and provide more details in the materials and methods section.

Response back: We have changed italics, The reference genome was deposited at the BIGD under PRJCA002216.

11. Results (page 4, line 201): Table S1 is missing from the materials provided by the authors, so I have no way to check this table. Please, for future reviewers, provide the table, otherwise is like the table does not exist.

Response back: We have provided the table S1. Sorry for inconvenience.

12. Results (page 5, Figure 1): Font needs to be bigger in all three panels, so it can be readable in a printed version of the manuscript.

Response back: We have revised the figures 1.

13. Results (page 7, lines 253-256): Not clear if the paragraph refers to the results obtained in water spinach or Arabidopsis. Please clarify. Also, this paragraph need citations? It seems like is part of a discussion and not the results section. Please remove from results if it belongs to discussion. Not sure what the purpose of the paragraph is. The purpose is not clear.

Response back: We have revised the figure, also revised the results section and also moved to discussion section.

14. Results (page 7, Figure 3): Does S represents short days and L represent Long days? Please clarify in the figure description. And add it to all figures that have the S and L presented as well.

Response back: We have indicated in all the figures.

15. Results (page 8, Figure 4): Not clear where Figure 4 is referenced to in the results? What paragraph of the results described figure 4?

Response back: We have revised the figure resolution, and described the figures in result section.

16. Results (page 8, lines 270-272): Not clear if the paragraph refers to the results obtained in water spinach or Arabidopsis. Please clarify. Also, this paragraph need citations? It seems like is part of a discussion and not the results section. Please remove from results if it belongs to discussion. Not sure what the purpose of the paragraph is. The purpose is not clear.

Response back: We have moved the paragraph relavent materials to discussion section.

17. Results (page 8, line 299) Figure 4 was presented 2 figures ago and cited until now? Not clear what the purpose of Figure 4 really is. Please cite figure 4 in the results where it corresponds to cite it. It is confusing citing a figure later in the text when it was presented long ago.

Response back: We have revised the figures and updated the figure sequences. Also cited the figure 4.

18. Results (page 10, Figure 7): Font needs to be bigger in panel D, so it can be readable in a printed version of the manuscript.

Response back: We have revised the figure and increased figure resolution.

19. Results (page 10, no line number indicated here, please add the lines number to facilitate reviewer work): What do the authors mean by "meanwhile, SVP, AGL24, AP1, and FKF1 have no homologs in C. australis (Figure S1)." Where does this new species C. australis comes from? Results should refer to water spinach, not a new species. This is very confusing, please fix this.

Response back: The sentence was deleted.

20. Results (referring to all supplementary figures in the word document): No supplementary figure has a description under the figure name. Please add a description so readers can know why the figure is important to see.

Response back: We have described all figure in the results also added all supplementary files.

21. Results (page 10, no line number here, please add the lines number to facilitate reviewer work): Is FKFI the same as FPK1? Not clear where the results of this paragraph are found. All the paragraphs found in page 10 need to refer to where the results are found. Not just at the very end of the section in page 11. Cohesion and coherence of the section "3.4. Analysis of photoperiod-mediated flowering-related genes" needs to be improved, right now, as it is, it is not clear at all what the purpose of the author is here.

Response back: It was typo, I have corrected the gene name, we have revised the result section.

22. Results (page 11, section 3.5): Authors indicate here that "eight flowering-related genes for qRT-PCR analysis to verify the accuracy of RNA-seq results" but the materials and methods indicate ten (Materials and methods, page 4, line 186: "10 flowering-related genes for RT-qPCR validation"). What was it, ten or eight? Not clear.

Response back: It was typo, 10 flowering genes were selected for RNA-seq analysis. We have also provide supplementary file.

23. Results (page 11, section 3.6): Authors indicate "WGCNA analysis constructs a hierarchical clustering tree based on the correlation between the expression levels of genes and divided divides the different modules, the genes in the same module may have similar biological functions." Not clear what divided divides means. Is this a typo? Please fix to improve clarity of ideas.

Response back: It was typo, we have corrected the sentence and lines, thank you for intension.

24. Results (page 12, Figure 8): Font needs to be bigger in all three panels, so it can be readable in a printed version of the manuscript. What about correlations with light time? Not sure why correlations with long and short days are presented together in the same column in panel B. Shouldn't this be presented separately? What is the number in parenthesis? What does the colors in the scale are? Expression levels? Correlation level? Please clarify.

Response back: The colors represent different modules, and the numbers represent correlations.

25. Results (page 15, line 324): Authors indicate "A: yellow module; B: red module; C: pink module; D: darkmagenta module" What is this? Does this correspond to a supplementary figure description? Please see my previous comment on all supplementary figures, they all need a description with their names.

Response back: We have removed the unnecessary lines, it was typo, we have added the description in the supplementary files.

26. Results (page 16, Figure 10): Font needs to be bigger in all the four panels, so it can be readable in a printed version of the manuscript. Please indicate what the difference between

circles and triangles is in panel D. The quality needs to be improved, even zooming in in the figure, the letters disappear.

Response back: We have improved the figure quality and figure resolution, easy for readers.

27. Discussion (page 17, line 391): Arabidopsis thaliana is a scientific name and the convention for scientific name is that it needs to be italicized.

Response back: We have changed into italicized