For editor:

In the manuscript entitled “Genome-wide analysis of the lignin toolbox for morus and the roles of lignin related genes in response to zinc stress”, by Chao et al., conducted the genome-wide identification of lignin toolbox genes in mulberry, de novo transcriptome assembly of *Morus alba*, phylogenetic analysis, and RT-qPCR analysis. This set of genes is very important to understand to be identified and characterized for future biotechnological applications. The work is well conducted, but need to check minor and major consideration to improve the paper.

Minor revision:

Line 21, word "denovo" should be modify for de novo

Line 24, word "bona fide" is italic. Please, check it along the manuscript.

Line 28, word "morus should be change by Morus

Line 29, word "morus" should be substituted by this species or genus, which could valorize the work.

Line 72 and 73, authors use the word morus in italic. I didn`t understand such use. The word in this case shouldn`t be without italic or a general word for the meaning. For example: Zea mays is in italic, but you can also talk about this species in a general sense such as maize (non-italic). Don`t you have a word like that for Morus?

Line 80, bona fide word should be in italic. Please, check it along the manuscript.

Line 81, in the sentence 19 genes in *Morus notabilis* or 17 genes in *Morus alba*, the word or should be changed by "and".

Line 103, in the word "Sm" from SmF5H, authors should mean that in parenthesis

Figure 3, authors should state what is the meaning of empty red circle in the legend.

Line 325, in the sentence "with high protein sequence identity", authors should add that information.

Major:

Line 62, authors explore information about Morus species. As authors conducted a genome-wide gene families studies, authors should state more about the genetics and ploidy information of this genus and species. Authors, also should explore the evolutionary relationship of Morus and other eudicots and rosids species that already contain genome available, such as Arabidopsis, Fragaria, Malus, Populus, and so on, indicating for example, their divergence in mya. It could help authors to explore and compare their data with other species, including a comparative table in results or as supplementary information.

Line 70, authors cite plant species that have been the focus of lignin biosynthesis, like poplar, eucalyptus, and medicago. Authors should be carefull with references. There is a paper from Carocha and collaborators (2016) from New Phytologist 206:1297-1313 that must be cited there about Eucalyptus; Lee and collaborators (2011) from PLoS Comput Biol 7(5):e1002047 about lignin in alfalfa; Hamberger et al., (2007) from Canadian Journal of Botany 85(12):1182-1201 and Shi et al., 2010 from Plant Cell Physiology about lignin in populus. The papers that author used are reviews and they should cite full paper that have been worked with those species and genome-wide analysis of lignin toolbox. Of course, authors can find better worked paper about lignin in those species to cite there.

Line 92, authors should add one reference for the MS medium used including information about quantity of nutrients.

Line 93 to 94, authors wrote "On the 15th day, samples were collected". Authors should describe better why they use 15th day. Why they shouldn`t use more or less day to collect samples? What is the biological meaning in 15th day? Is there any other experiment in the literature showing that time used is a better one method? Is there any markers to show that plants absorbed zinc?

Line 122 to 123, authors mention different tissues and organs. I suggest authors to write those tissues and organs, instead to use general words. For example, why they studied winter bud? They could explain this physiological aspect. They should also explain it from male-flower, which period of year those samples were collected, period of day and light. Some lignin pathway genes are light and stress responsive. Also, it would be interesting to include picture as supplementary information from those organs and tissues studied for both species.

Line 129, authors should describe better if all 23 identified genes are complete ORFs, including their UTRs regions. Authors also should describe the region of gene used to designed primers. Authors also should state if Actin is a good reference gene for their experiment and why they didn`t used more than one reference gene for normalization of RT-qPCR. Authors also should show as supplemental information the melt curve for all RT-qPCR experiments showing only one pick of amplification.

Figure 1 to 6. Legends should state if they use DNA or protein for phylogenetic analysis. This affirmative in important because in the manuscript line 152 to 154, authors didn`t use italic, which means that they worked with protein sequence. Authors also compared their data with Eucalyptus, however they didn`t showed it in their phylogeny. I guess, they missed an reference in the manuscript. It is very important to authors read again each line of manuscript to correct if they want to mean gene (italic) or protein (non-italic).

Figure 1 to 6. Legends should include all species used to construct each phylogeny, as we can see a distinct set of species for each figure. They also should describe how they got those sequences into material and methods to develop this part of work and cite papers that have already conduct similar work into genome-wide identification and annotation.

Figure 1 to 6. Legends should state the meaning of L0, S0, and R0. I see that in M&M L, S and R can be leaf, stem, and root. But, it would be interesting to add that in the legends and also to explain why use 0 from L0, and so on, in the figure?

Line 217, in the sentence "HCT belongs to the BAHD acyltransferase" authors cite an incorrect paper. There is a paper that describe this information, such as D'Auria JC. Acyltransferases in plants: a good time to be BAHD. Curr Opin Plant Biol. 2006;9(3):331–340.

Line 341 to 344, authors cite Figure S4 and wrote "indicated as a blue star and a red star". However, it was not clear which one is cluster I and cluster II. Also, if you looked at legends of figure S4, there is no information about Figure A and B. This figure is very nice and I`m wondering why authors don`t explored it in the main text? Besides, the suggest authors to explore the meaning of Bark in the text, as it seems that authors want to compare with stem. A little explanation could help readers, mainly those that are not familiar with those species and the anatomy of species.

Line 364 to 365, authors discuss about tandem duplication. However this kind of data was not explored in the results. Authors should explore it in the results adding information from other rosids plant closely related species, such as, Arabidopsis, grapevine, and so on. It could help them into discussion for each gene family or superfamily.

Line 386 to 389, authors describe the importance of zinc to human and emphasize that mulberry is rich in zinc. All of that information without any paper cited. I suggest authors also to explore that subject concerning the nutrition of man using mulberry and their benefits. I mean, what the concentration of zinc in mulberry play into human that eats that fruit or even drink as a tea?

Lines 390 to 392, in the sentence "core genes involved in lignin biosynthesis showed obvious expression changes". It is not clear what lignin genes should change under zinc treatment. Authors should describe it better in Morus and closely related species, similar kind of experiments showing that.