

Dr. Van der Knaap
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RE: Manuscript 2019-00292

Please find attached a revised version of the above referenced manuscript. On behalf of the authors, I would like to express our sincere appreciation to you and the reviewers for the helpful comments regarding our work. Below, we have compiled a point-by point response to the comments and have highlighted sections of the manuscript that were revised. We are happy to provide additional clarification in case some of our responses are inadequate.

Sincerely

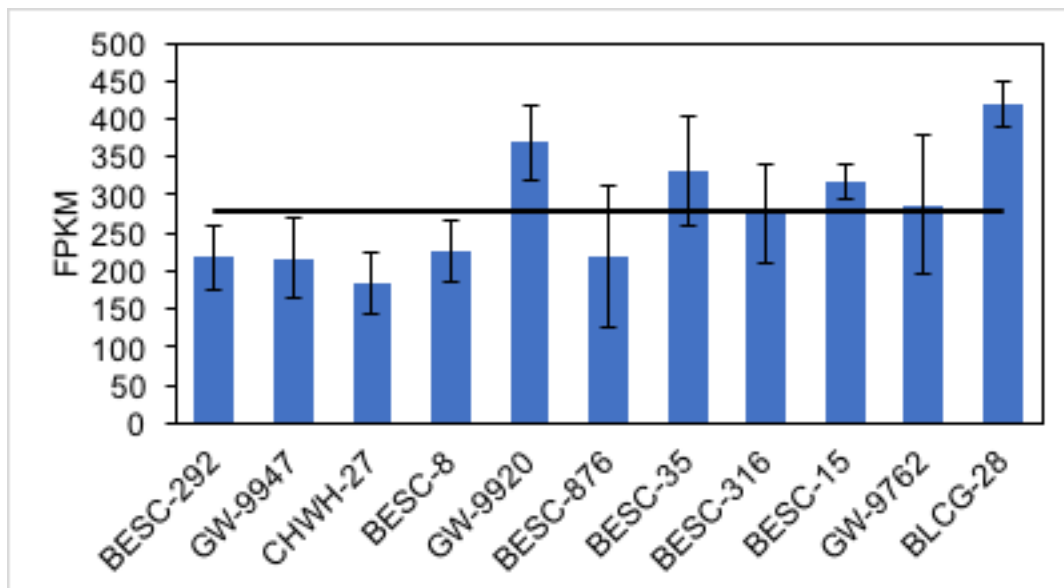


Wellington Muchero, PhD (on behalf of the authors)

Reviewer #1:

This nicely extends prior work that identified a novel transcription factor, where a DNA binding domain (HTH) was identified in an EPSP isoform in poplar. The HTH domain-EPSP is widespread in eudicots, but not in monocots. Thus, use of heterologous expression in rice was interesting as is not always the case for heterologous work. A rare allelic variant with a SNP near the HTH was associated with reduced lignin and this variant showed reduced DNA binding in vitro and in protoplast assays. Though the evidence is solid that the D to E substitution reduces binding/transcriptional activity, it would be good to include any information on expression of TF-EPSP in contrasting poplar genotypes (i.e., are regulatory differences also implicated?).

[Response:] Based on our unpublished RNA-seq data, the expression of *PtrEPSP-TF* in the two variants fell within the population range suggesting that mutations in BESC-35 and BESC-876 do not affect expression levels of these alleles. A statement has been added in the discussion section (Line 370 – 372) to clarify this point.



Reviewer #2:

In "Identification of functional single nucleotide polymorphism of *Populus trichocarpa* PtrEPSP-TF and determination of its transcriptional effect," the authors identify a SNP in PtrEPSP-TF, and demonstrate in rice the impact of this SNP on DNA binding, transcriptional activity, metabolite biosynthesis, and regulatory activity. Overall, the paper is well-written and easy to understand, with sufficient in-text and supplementary materials to evaluate results and conclusions. Furthermore, the study provides insights into the mechanisms controlling phenylpropanoid biosynthesis in rice. Below are specific comments:

Line 91: The period is missing after "etc."

[Response:] A period has been added after etc.

Line 113-114: I understand that this study provides a useful strategy for understanding phenylpropanoid regulation in rice, but what, if anything from this study can be useful for understanding phenylpropanoid biosynthesis in poplar, the species from which PtrEPSP-TF was identified?

[Response:] Yes, our study also revealed that *Populus* homologs of the five negative regulators found in rice (XND1, MYB48, MYB3R1, MYB3R3, and NAC047) had opposite expression pattern to PtrEPSP-TF in the woody-forming zone (Fig. 4B). Since PtrEPSP-TF has positive effect on lignin and phenylpropanoid biosynthesis, these *Populus* genes should negatively affect phenylpropanoid biosynthesis as their rice homologs do. Our studies provided evidence to link these *Populus* genes with the phenylpropanoid pathway. To clarify this, a description has been added in Line 113 - 115. Additionally, the last sentence of the introduction section (Line 115 - 117) has been modified to include the impact on understanding phenylpropanoid biosynthesis in poplar.

Line 158: At what age(s) was no apparent morphological change assessed, and what specific morphological attributes were examined?

[Response:] Since secondary cell wall modification affects leaf morphology and the elongation of internode, we examined morphological changes in leaf and height during the vegetable growth of our transgenic rice plants. A description has been added in Line 160.

Line 298: Can you clarify what you mean by background signals?

[Response:] The meaning of “background signals” is noises from RNA-seq. A description has been added in Line 301.

Line 304: I suggest revising the phrase "During the wood formation of Populus," to "During Populus wood formation."

[Response:] The phrase has been revised. (Line 307)

Line 369: In the discussion you mention that you found an increase in sugar release associated with variant PtrEPSP-TF, please clarify which results demonstrate this.

[Response:] The sugar release data is included in a published paper (Lu et al. 2019. Bioresource Technology). The reference has been added. (Line 593 - 595)

Line 380: You state that BESC-876 did not display additional reduction of DNA binding activity, compared to BESC-35. Are you drawing this conclusion strictly from the intensity of bands on the EMSA? Was a t-test or other statistical test performed to confirm this?

[Response:] Yes, it is based on the intensity of binding bands on EMSA. We used the same amount of BESC-35 and BESC-876 in EMSA assay. In our analysis, the two proteins had binding bands with similar intensity (Fig. 1B). If the band intensity of BESC-876 is set to 1, the relative band intensity of BESC-35 will be 1.263517 (quantified using Image Lab software (Bio-rad)). We repeated this assay for three times and got consistent results.

Line 460: Please specify the R package used for PCA. Were normalized genes used for PCA?

[Response:] Data of PCA and K-means clustering is not included in this manuscript. Their descriptions in the method section have been deleted.

Line 478: Please clarify "data were pooled across lines," when in figure 2 legend it specifies for metabolite data that "4 independent replicates were measured to calculate mean values and standard error."

[Response:] The description in the method section was modified to clarify that results of four replicates were pooled to calculate mean values and standard errors. (Line 483)

Line 494: I suggest adding "the" before "manufacturer."

[Response:] “the” has been added. (Line 499)

Line 506: I suggest adding "the" before "microscope."

[Response:] “the” has been added. (Line 511)

Line 508: Please include the URL accessed for the NCBI GEO database.

[Response:] The URL links have been added. (Line 514 and 516)

Line 513: The "PCC" acronym is not needed, since it is not used elsewhere in the text.

[Response:] “PCC” has been deleted. (Line 520)

Line 534: Please review the format of references. There is inconsistent capitalization and abbreviation of journal titles throughout the reference section.

[Response:] The inconsistent formats have been revised.

Line 727: Can you please indicate the range of strength of co-relationship associated with thickness of lines (e.g. upper and lower limits).

[Response:] Fig.5 shows correlation coefficient (r) >0.1 or <-0.1. This is also the range of thickness of lines. The Fig. 5 legends have been modified to clarify this. (Line 738 - 741) We also listed all the r values in supplemental dataset 5.

Figures 1 and 4 legends: Please specify what the error bars in Fig. 1C and Fig. 4A represent.

[Response:] A description of error bar has been added in Figure 1 and 4 legends. (Line 696 – 697 and Line 727 - 728)

Figure 1C legend: Please clarify t-test comparisons for relative GUS activity in figure legend. Were comparisons only between empty vector and PtrEPSP/BESC-35/BESC-876?

[Response:] The details of t-test comparisons have been added (Line 697 - 699). As shown in Fig. 3C, the comparisons are between empty vector and PtrEPSP-TF, as well as PtrEPSP-TF and BESC-35/BESC876.

Figure 2C: Did you evaluate whether there were any significant differences between PtrEPSP-TF and BESC-35/BESC-876, or were comparisons only made relative to the control?

[Response:] We did statistical analysis on differences between PtrEPSP-TF and BESC-35/BESC-876. BESC-35 and BESC-876 also have significant differences from PtrEPSP-TF. In Fig. 2C, we only show the statistical analysis between PtrEPSP-TF and the control in order to focus on these two samples in the following studies.