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Peer Review File

Sugar transporters PpSWEET9a and PpSWEET14 synergistically mediate peach sucrose allocation from source leaves to fruit

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Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript by Luo et al. describes the characterization of the sucrose transporters PpSWEET9a and PpSWEET14. The authors confirmed sucrose transport and oligomerization in yeast and showed the effect of over-expressing these genes in an Arabidopsis sugar-loading deficient mutant and peach leaves. Overall, the results are confirmatory with minimal experimental novelty; similar results have been shown for a multitude of other SWEETS using the same techniques implemented in the manuscript. Furthermore, two significant points dampened my enthusiasm.

Major points:
1) There is no experimental data showing that PpSWEET9a and PpSWEET14 loss of function in peach decreases sugar loading. I understand it's challenging to create crop mutants (or at least find a polymorphism on these genes), but this would be necessary to support a functional role in sugar loading.
2) The controls to support the claim that PpSWEET9a and PpSWEET14 act synergistically are not appropriate. The co-overexpression of these two genes results in a total over-expression of sucrose exporters larger than the single overexpression of PpSWEET9a or PpSWEET14. A better comparison for Figure 4 would have been PpSWEET9a+PpSWEET14-OE versus PpSWEET9a+PpSWEET9a-OE and PpSWEET14+PpSWEET14-OE to account for total gene copy number. Alternatively, the authors could have quantified total gene expression (as done in Arabidopsis) to rule out my concern.

Minor points:
Line 234: The lower end of the range of predicted amino acids in the 19 SWEETs in the peach genome seems low. The authors should include the number of predicted transmembrane domains for each SWEET in Figure 1B to confirm that the 19 SWEETs could indeed form the seven transmembrane domains characteristic of this family of proteins.
Line 257: The claim that genomic loci of PpSWEET9a and PpSWEET14 are enriched putative light responsiveness elements should be verified by quantifying gene expression of the genes in the presence and absence of light stimulation.
Line 269: The authors should specify that membrane localization was tested in Nicotiana benthamiana. Furthermore, higher magnification of plasmolyzed epidermal cells would corroborate plasma membrane localization, as sometimes cytosolic proteins can appear membrane localized when using low-magnification imaging. The nuclear localization of the negative control is not sufficient to conclude that PpSWEET9a and PpSWEET14 are not localized in the cytosol, as the fusion proteins could simply be excluded from the nucleus.
Figure 3: The serial dilution for PpSWEET14 does not seem consistent between the sucrose and glucose control images. There is a higher concentration of cells in the next-to-last dilution for the sucrose sample.
Line 595: It's unclear what the authors mean by "Values followed by different letters are significantly different between transgenic samples (B) or in the same tissue." What statistical test was used here? If these are pairwise comparisons, which pairs are being compared?
Line 306: Reference to figure 4D missing.
Line 338: apple.
Line 347: inClade

Reviewer #2

(Remarks to the Author)
Comments

Overall, the manuscript entitled “Sugar transporters PpSWEET9a and PpSWEET14 synergistically mediate peach sucrose allocation from source leaves to fruit” presents a comprehensive investigation into the role of SWEET transporters in sucrose allocation in peach fruit. Here are some comments and suggestions for improvement:

1. The introduction effectively outlines the importance of sugar transporters in fruit quality and briefly mentions the role of SWEET proteins. However, it could benefit from a more detailed explanation of why sucrose allocation in peach fruit is specifically important, perhaps by highlighting its impact on flavor, texture, or shelf life. This would provide a clearer context for readers regarding the significance of the study.

2. Authors mentioned that 'Pink fruit' peach grown at Chongqing Yunduo Agricultural Development Co., Ltd. were used in this study. However, they should provide the variety name or line name of the peach for clarity and reproducibility.

3. More details and specific data on the expression of the selected genes "PpSWEET9a and PpSWEET14" should be provided. The authors only state, "Moreover, PpSWEET9a and PpSWEET14 were preferentially expressed at the highest levels in the mature leaves and branches..." (Figure 2A). Including additional information such as fold-change expression values or statistical significance would enhance the rigor of their analysis.

4. The protein interaction assays for PpSWEET9a and PpSWEET14 should be validated using one of the following assays: co-immunoprecipitation (co-IP), pull-down assay, or far-western blot analysis. Validating these interactions would provide a solid foundation for the results and strengthen the conclusions drawn from the study.

5. In lines 222 to 230, too many gene names are listed. It would be clearer and more organized to present this information in a table format to improve readability and comprehension for the readers.

Reviewer #3

(Remarks to the Author)
I found the current manuscript interesting. The authors identified two Clade III SWEET genes, PpSWEET9a and PpSWEET14, mediating sucrose translocation from source leaves to fruits. The findings can provide a scientific basis for the quality improvement of peach. However, there are some problems need to be concerned and corrected:
1.The subcellular localization study should be performed with 1-2 control proteins with known localization patterns, for instance in plasma membrane or cell wall. And the legends of Figure 2D is absent.
2.Only through the yeast two-hybrid system is not sufficient to illustrate the protein interaction and should be supplemented with experiments such as BiFC and Co-IP to further verify the interaction of PpSWEET9a and PpSWEET14.
3.There are some grammar and format errors in the article. I hope the authors can carefully polish it again.

Author Rebuttal letter:

Reviewer 1
Dear reviewer,
We appreciate for your hard work on our manuscript entitled "Sugar transporters PpSWEET9a and PpSWEET14 synergistically mediate peach sucrose allocation from source leaves to fruit” (COMMSBIO-24-1144). As you are concerned, many efforts have been made in the revision. We hope that these revisions will meet with approval.
Major points:
1) There is no experimental data showing that PpSWEET9a and PpSWEET14 loss of function in peach decreases sugar loading. I understand it’s challenging to create crop mutants (or at least find a polymorphism on these genes), but this would be necessary to support a functional role in sugar loading.
Response: Thank you for your nice suggestion. Creating mutants is very challenging because of the lack of a stable transgenic system and the long juvenile phase in peach. Besides, we did not ensure that there is polymorphism in these genes in peach germplasm. Even so, we conducted a loss-of-function experiment for PpSWEET9a and PpSWEET14 in peach using TRV mediated VIGS system. This transient transgenic system has been widely applied for gene functional analysis in fruit crops including peach, apple, pear, and etc. Moreover, our data verified that the loss-of-function for one or both genes could decrease sugar loading. These results are included in the revision.

2) The controls to support the claim that PpSWEET9a andPpSWEET14 act synergistically are not appropriate. The co-overexpression of these two genes results in a total over-expression of sucrose exporters larger than the single overexpression of PpSWEET9a or PpSWEET14. A better comparison for Figure 4 would have been PpSWEET9a+PpSWEET14-OE versus PpSWEET9a+PpSWEET9a-OE and PpSWEET14+PpSWEET14-OE to account for total gene copy number. Alternatively, the authors could have quantified total gene expression (as done in Arabidopsis) to rule out my concern.
Response: We agreed with your concern regarding the effect of expression level on the analysis of

synergistic action of PpSWEET9a and PpSWEET14 in the co-expression experiment. In fact, in this experiment, to ensure the equal copy number of the transgene, the total volume of injected solution was controlled at 1 mL per mature leaf. Besides, the total gene expression was quantified with the value sum of PpSWEET9a and PpSWEET14 absolute expression. The total gene expression for PpSWEET9a and PpSWEET14 was comparable between PpSWEET14-OE with PpSWEET9a+ PpSWEET14-OE groups, but different with PpSWEET9a-OE, which indicated a limited effect for individual PpSWEET9a or PpSWEET14, but a synergistic effect for these two proteins on sucrose allocation.
In addition, the synergistic action of PpSWEET9a and PpSWEET14 was also supported by their similar spatial expression pattern and their mutual expression stimulation in individual transient overexpression assay. Moreover, transgenic and complement assays indicated both PpSWEET9a and PpSWEET14 act as sucrose exporters to mediate the sucrose allocation from leaves to fruit. Thus, we believe that these data still support our claim.

Minor points:
Line 234: The lower end of the range of predicted amino acids in the 19 SWEETs in the peach genome seems low. The authors should include the number of predicted transmembrane domains for each SWEET in Figure 1B to confirm that the 19 SWEETs could indeed form the seven transmembrane domains characteristic of this family of proteins.
Response: Thank you for your nice suggestion. In the revision, the number of transmembrane domains for all 19 SWEETs was included in the Figure 1B. Four proteins could not form standard even transmembrane domains. Moreover, this result was added in the sections of the result and discussion.

Line 257: The claim that genomic loci of PpSWEET9a and PpSWEET14 are enriched putative light responsiveness elements should be verified by quantifying gene expression of the genes in the presence and absence of light stimulation.
Response: Thank you for your suggestion. In the revision, we analyzed the expression level of PpSWEET9a and PpSWEET14 in response to light stimulation. Results showed that both genes were up-regulated by light exposure.

Line 269: The authors should specify that membrane localization was tested in Nicotiana benthamiana. Furthermore, higher magnification of plasmolyzed epidermal cells would corroborate plasma membrane localization, as sometimes cytosolic proteins can appear membrane localized when using low-magnification imaging. The nuclear localization of the negative control is not sufficient to conclude that PpSWEET9a and PpSWEET14 are not localized in the cytosol, as the fusion proteins could simply be excluded from the nucleus.
Response: In the revision, the subcellular localization was performed again and the plasm membrane-localized marker PM-RK-mCherry (Xiang et al., 2022, Mol Plant Pathol. 23: 1792-1806) was used to specify the plasm membrane localization. Results showed that both PpSWEET9a and PpSWEET14 were co-located with the plasm membrane marker.

Figure 3: The serial dilution for PpSWEET14 does not seem consistent between the sucrose and glucose control images. There is a higher concentration of cells in the next-to-last dilution for the sucrose sample.
Response: This inconsistency may be a consequence of mis-operation in serial dilution or dropping. Thus, we performed this experiment for PpSWEET14 again and confirmed its activity for sucrose transportation. Meanwhile, the serial dilution for PpSWEET14 was replaced with a new result in the revision.

Line 595: It's unclear what the authors mean by "Values followed by different letters are significantly different between transgenic samples (B) or in the same tissue." What statistical test was used here? If these are pairwise comparisons, which pairs are being compared?
Response: The one-way ANOVA was used for statistical analysis. We want to claim that different letters were used to compare one parameter from the same tissue, such as starch from mature leaves between mature leaves, branches, and fruit. To avoid misunderstanding, this sentence was corrected with "Values followed by different letters are significantly different ($P < 0.05$)." in the revision.

Line 306: Reference to figure 4D missing.
Line 338: apple.
Line 347: inClade
Response: We are sorry for these mistakes and these issues were corrected in the revision.
Reviewer 2

Dear reviewer,
We appreciate your earnest work on our manuscript. These suggestions are very helpful for improving our paper. Many efforts have been made in the revision.

1. The introduction effectively outlines the importance of sugar transporters in fruit quality and briefly mentions the role of SWEET proteins. However, it could benefit from a more detailed explanation of why sucrose allocation in peach fruit is specifically important, perhaps by highlighting its impact on flavor, texture, or shelf life. This would provide a clearer context for readers regarding the significance of the study.
Response: Thank you for your nice suggestion. In the revision, we provided some information about the impact of sucrose allocation on flavor, texture, and shelf life in peach fruit.

2. Authors mentioned that 'Pink fruit' peach grown at Chongqing Yunduo Agricultural Development Co., Ltd. Were used in this study. However, they should provide the variety name or line name of the peach for clarity and reproducibility.
Response: In the revision, this name was corrected with a variety name.

3. More details and specific data on the expression of the selected genes "PpSWEET9a and PpSWEET14" should be provided. The authors only state, "Moreover, PpSWEET9a and PpSWEET14 were preferentially expressed at the highest levels in the mature leaves and branches..." (Figure 2A). Including additional information such as fold-change expression values or statistical significance would enhance the rigor of their analysis.
Response: In the revision, we provided a detailed description of the spatial expression pattern of PpSWEET9a and PpSWEET14, including the fold-change of expression and comparison with other genes.

4. The protein interaction assays for PpSWEET9a and PpSWEET14 should be validated using one of the following assays: co-immunoprecipitation (co-IP), pull-down assay, or far-western blot analysis. Validating these interactions would provide a solid foundation for the results and strengthen the conclusions drawn from the study.
Response: Thank you for the suggestion. In the revision, the protein interaction for PpSWEET9a and PpSWEET14 has been verified using a split-luciferase complementation method. Results showed that both PpSWEET9a and PpSWEET14 could form homodimers and heterodimers in vivo.

5. In lines 222 to 230, too many gene names are listed. It would be clearer and more organized to present this information in a table format to improve readability and comprehension for the readers.
Response: In fact, these genes were well organized in Figure 1A. We considered that this information could be easily followed by readers.
Reviewer 3
Dear reviewer,
We appreciate your earnest work on our manuscript. According to your suggestions, efforts have been made in the revision.

1. The subcellular localization study should be performed with 1-2 control proteins with known localization patterns, for instance in plasma membrane or cell wall. And the legends of Figure 2D is absent.
Response: In the revision, we performed the subcellular localization again. Moreover, the plasm membrane-localized marker PM-RK-mCherry (Xiang et al., 2022, Mol Plant Pathol. 23: 1792-1806) was used to specify the plasm membrane localization. Results showed that both PpSWEET9a and PpSWEET14 were co-located with the plasm membrane marker. Besides, the figure legend was added.

2.Only through the yeast two-hybrid system is not sufficient to illustrate the protein interaction and should be supplemented with experiments such as BiFC and Co-IP to further verify the interaction of PpSWEET9a and PpSWEET14.
Response: Thank you for your nice suggestion. In the revision, the protein interaction of PpSWEET9a and PpSWEET14 has been further verified by a split-luciferase complementation method. Resulted showed that both PpSWEET9a and PpSWEET14 could form homodimers and heterodimers in vivo.

3.There are some grammar and format errors in the article. I hope the authors can carefully polish it again.
Response: We carefully checked the grammar and format and made the necessary corrections in the revision.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed my previous concerns, and I find the manuscript a lot improved by the additional controls and experiments.

Major concern:

1) I am worried by the predicted SWEETs with less than 7 TMs, since these “shorter” SWEETs seem to lack UTR’s (if I am understanding Fig.1b correctly) and potentially some exons. This indicates that these genes are misannotated and thus it is unfair to name them until their full protein sequences are determined. It is possible that upon discovering the full sequence of these proteins, their alignment with the Arabidopsis SWEETs may change and they may need to be renamed; thus, causing a problem. I would prefer it if the authors did not name these particular SWEETs yet and just used their peach gene name in the manuscript.

Minor concerns:

1) There are published crystal structures of SWEETs suggesting that proteins with less than 7 TM would not make a functional pore. The authors should state this in a manuscript after listing the number of TMs for each SWEET, as many readers would not understand the implications of the predicted number of membranes if they were unfamiliar with SWEETs.
2) The scale bars in Fig. 3 are too small. It would not be visible when imbedded into the PDF text and printed on standard A4 paper.
3) Similarly, the font of figure 1 is too small. I would suggest moving this figure to a supplemental file where it can take a whole A4 page instead of including it in the main text.
4) There were a few typos, specially commas. Please double check.

Reviewer #2

(Remarks to the Author)
The authors have clarified most of the questions I raised in my previous review. Now I feel the manuscript could be accepted for publication.

Reviewer #3

(Remarks to the Author)
This manuscript is greatly improved and I have no other suggestions. I think it can be accepted for publication in Communications Biology.

Author Rebuttal letter:

Reviewer 1
Dear reviewer,
We appreciate your hard work on our manuscript again. Efforts have been made in the revision regarding your concerns. We hope that these revisions will meet with approval.
Major concern:
1) I am worried by the predicted SWEETs with less than 7 TMs, since these “shorter” SWEETs seem to lack UTR’s (if I am understanding Fig.1b correctly) and potentially some exons. This indicates that these genes are misannotated and thus it is unfair to name them until their full protein sequences are determined. It is possible that upon discovering the full sequence of these proteins, their alignment with the Arabidopsis SWEETs may change and they may need to be renamed; thus, causing a problem. I would prefer it if the authors did not name these particular SWEETs yet and just used their peach gene name in the manuscript.
Response: Thank you for your nice suggestions. We agreed with your concern and renamed these four “shorter” SWEETs as SemiSWEETs according to the rule suggested by Jia et al (2017, Front Plant Sci. 8, 2178).

Minor concerns:
1) There are published crystal structures of SWEETs suggesting that proteins with less than 7 TM would not make a functional pore. The authors should state this in a manuscript after listing the number of TMs for each SWEET, as many readers would not understand the implications of the predicted number of membranes if they were unfamiliar with SWEETs.
Response: This statement was provided in the revision.

2) The scale bars in Fig. 3 are too small. It would not be visible when imbedded into the PDF text and printed on standard A4 paper.
Response: The scale bars were revised with a clear visualization.

3) Similarly, the font of figure 1 is too small. I would suggest moving this figure to a supplemental file where it can take a whole A4 page instead of including it in the main text.
Response: We considered that the Figure 1 inserted in the main text would be easy follow by readers. Thus, we re-arranged Figure 1 and enlarged the font in the revision, making a clear visualization.
4) There were a few typos, specially commas. Please double check.
Response: We double-checked the manuscript and corrected the mistakes.
Reviewer 2 and Reviewer 3
Dear reviewer,
We appreciate your earnest work again and the approval of our manuscript.