

Peer J Review

Morphological, histological, and transcriptomic mechanisms underlying different fruit shapes in Capsicum annuum (#84048)

Carvalho Jr's review:

Some QTLs related to fruit shape have been identified recently in the pepper genome, but they are not well characterized as in tomato. Thus, the authors propose identifying gene expression profiles from genes controlling the main types of fruit shapes that may be related to some of these QTLs. To do so, they propose morphological, histological, and transcriptional analyses of five types of fruit shape from 10 distinct accessions to establish correlations between gene expression and fruit morphology. Therefore, the appeal of this work is supposed to contribute to a better understanding of fruit morphogenesis and pepper breeding. Although this summary may be inferred from the well-written introduction session, it does not seem so appealing to mention that the helical pepper morphogenesis remains to be elucidated. Indeed, the authors sound contradictory in comparing the text segments from the introduction (lines 139-141) to the discussion session (lines 460-461).

The results session feels extensively descriptive, which is comprehensive based on the morphological analyses. However, the description might be more concise. First, the main result is presented by the fruit shape index (FSI) morphological parameter. This analysis shows that is a positive correlation (Pearson: 0.98) between the OSI and the FSI, which indicates that the elongated shape of the majority of the accessions ($FSI > 1$) is determined, in part, by the elongated ovary ($OSI > 1$). Since the ovaries were measured at anthesis, the authors assume that the pre-anthesis ovary development determines the final fruit shape. This is pretty logical and, it was already demonstrated in Tomato, as the authors mentioned in the discussion session. Although the demonstration that this same phenomenon also applies to the evaluated pepper accessions brings a new finding, it is far away to be a breakthrough. The same is true for the finding that the elongation of the ovary is due to the increase in cell number, whereas the fruit shape is determined by both cell number and size. These conclusions are based on both positive correlations of ovary height and cell number in the proximal-distal axis (OMH and OCN-PD), as well the ovary width and cell number in the medial-lateral axis (OMW and OCN-ML). The equivalent measures are valid to the fruit (FML-PCN-PD; FMW and PCN-ML; FMW and PCS-ML). It is already well established that in the growing stage after the anthesis of fruit set, there is an increase in cell number associated with auxin signaling followed by cell wall expansion induced by the synergic signaling of auxin and gibberellin. Therefore, the findings of Figures 1 to 3 are not surprising whatsoever. The data are well described and presented tough. The main conclusions are pertinent, except for this one from the discussion session (lines 389-390): "Fruit shape determination may be controlled by the same regulatory pathway before and after anthesis". It can not be assumed that a positive correlation between OSI and OSI indicates that the same pathway is controlling fruit shape before and after anthesis, respectively. Correlation is not causation. Taken together, the results presented from Figure 1 to Figure 3 are consistent and well evaluated, but Figure 2 can definitely be a supplementary figure. This figure is related to the supplementary tables.

Regarding the RNA seq analyses, the authors show that accession 19C1511 (the slender-chapped pepper) clearly presents a distinct transcriptome profile compared to the other accessions (Fig. 4A-D). The differential principal component distribution and the enrichment of the clustering of orthologous groups in this accession (Fig.4A-B) and the increase in the number of DEGs related

to this accession compared to the others (Fig. 4C) support this finding. Nevertheless, the authors propose changing the focus to accession 19C705 (the helical pepper). This just does not make sense based just on the regional interest for this variant of fruit in Asia or even though it is a “widespread developmental pattern in plant” (line 140). In the Venn Diagram (Fig. 4D), for example, considering the non-common genes in each of the 4 pair-based comparisons with the helical pepper, the “705 vs 1511” presents approximately an enrichment of 4-fold (1412) genes compared to the others (705 vs 961:393 > 705 vs 302: 350 > 705 vs 355: 244). This finding just reiterates that the elongated fruit (referred to as slender) should be the focus of these analyses. I do not agree with the interpretation that the data observed for this accession would be related to differential domestication processes from either the North or South regions of China (lines 422-429). The data simply indicates that elongated fruits have a distinct transcriptome than wide fruits, as it is shown in Figure 5. Indeed, the references mentioned in the discussion (lines 427-434) really support the statement that “the OFP-TRM pathway plays an important role in the regulation of pepper fruit shape” (lines 433-434), but for another reason. The reason why is in Figure S1, wherein it can be seen that genes from the OVATE pathway (OFP: light blue triangle) are clearly downregulated in the access 19C1511 (e.g. Chr 1: Capana 01g003670; Chr 6: Capana 06g00645; Chr10: Capana 10g0095,96,97 and 99). This is a clear correlation between the downregulation of OFP genes in the elongated pepper of the access 19C1511, which is the expected phenotype according to the classic loss-of-function ovate mutant of tomato. Thus, I disagree with the following conclusion in the discussion (lines 434-437): “...in this study, the expression patterns of the OFPs and TRMs were not associated with ovary or fruit shape variations (Fig. S1), indicating the mutations of those two gene families at the transcriptional level may not be involved in the ovary and fruit shape regulation of the studied accessions”.

Thereafter, the authors “back on track” to identify the clusters of genes that may be related to the differential OSI among the evaluated accessions. Consistently, the green module of the cluster dendrogram (Fig. 5A) presents a score of 0.85 related to the ovary width (Fig. 5B) with enrichment of 192 upregulated genes, especially in the accession 19C355 (Fig. 5C). Likewise, the black module (Fig. 5A) presents a score of 0.63 related to OSI (Fig. 5B) and the highest number of genes (435) of DEG genes, especially in the accession 19C1511 (the slender/elongated pepper). These findings from the black module should be first presented in the main Figure 5, followed by the data from the green module. Even so, the WGCNA analyses are strong and make sense with the previous data. In summary, the ovaries that became elongated fruit (e.g. accession 19C1511) present a cluster of differentially expressed genes that are distinct from the ovaries that originate wide fruits (e.g. accession 19C355). Accordingly, there are auxin genes present in both green and black modules (e.g. GH3.1; Fig. 5F, N), whereas the genes related to gibberellin (e.g. GA2ox1) are upregulated in the elongated ovaries of the accession 19C1511 (Fig. 5N). This is under the requirement of gibberellin to promote cell extension in this tissue, which contributes to the growth of the basal-distal axis.

Regardless of all these matters, I still consider that this study brings interesting findings, especially the global gene expression profile related to each of the 5 main evaluated accessions. Moreover, some of the gene expression signatures related to the elongated (19C1511; Fig. 5K-N) and blocky-shaped (19C355; Fig. 5C-F) may be helpful for future studies searching to unveil the cell and molecular mechanisms that these genes may regulate. Therefore, I strongly suggest the supplementary Figure 1 as the final main figure. It is an overall informative figure since it brings conclusive information regarding the identified DEGs and their location within or without the QTLs in each chromosome of the pepper genome. This suggestion is also in consonance with the workflow of the analysis presented in the text. Finally, I do not consider it necessary to

perform any additional experiments, although I strongly suggest reconsidering the highlighted misinterpretation regarding the downregulation of OFP genes in accession 19C1511. Hence, a proper explanation of this matter should be included in the “conclusions” session.

Minor suggestions:

- The description of the identified genes (lines 365-367) is not appropriate for the fluency of reading. Alternatively, the number of genes may be mentioned in parentheses following their respective families. I suggest adding the number of genes per family to Figure S1 (to be replaced as the main final figure).
- The following sentence (line 113) is not clear: “Morphological and histological analyses of developing fruits revealed that both fs10.1 and fs3.1 elongated fruit after anthesis...”. Consider rewriting it.
- Consider replacing the following expression (lines 109 and 487): “Besides what is mentioned above”.