# Round #1

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*by Wirulda Pootakham, 09 Sep 2022 07:05*
Manuscript: **https://doi.org/10.1101/2021.06.01.446506**

**Revision required**

Dear Authors,

The reviewers have looked at the manuscript, and two of them provided extensive and valuable feedbacks. Please take a look at their comments below and revise your manuscript accordingly. Please pay special attention to the comments on the enrichment test as I also think it is important to carry out such test. In addition, please make sure that the hypothesis or anything that is not supported by the evidence provided in this study is written in a way that conveys the "hypothetical" nature of it (the language used should not be too ascertain). Please let us know if you require an extension on the revision of the paper.

Regards,

Wirulda Pootakham

Dear recommender, dear referees, thanks a lot for your time and all your comments through which we improve the quality of our manuscript. We have tried to take all of them into account and we hope this reviewed version will suit you.

Tanks again for your careful reading,

Regards,

Emilie VERGNE

## Reviews

*Reviewed by anonymous reviewer, 06 Jul 2022 03:31*
I read the manuscript titled “Phenotypic and transcriptomic analyses reveal major differences between apple and pear scab nonhost resistance” by Vergne et al. which is an interesting paper. The authors present phenotypic and transcriptome data of apple and pear scab nonhost resistance to Venturia species infected during 24 and 72 hours post inoculation. Macro- and microscopic observations and gene expressions were different in the experiments. However, I have several concerns that I would like the authors to address my comments.

Comments
- Please correct typos and grammar errors in the manuscript.
I hope we have corrected the errors, let us know if some remains.

- I am a litter bit confused about your conclusion in the abstract. You concluded only the study

of pear (lines 38-40).
Lines 41-42. Abstract has been modified to add a conclusion on apple as well.

- The introduction did not provide references to previous related studies (lines 67-68). Please add them.
Lines 75-120. Introduction has been lengthened to add information on previous large scale works on apple (among them scab host resistance) and pear (among them scab host resistance).

- Need for writing clear well-organized results and discussion.
Lines 125-195. A short Results section has been created, with new results from enrichment test on DEGs.
- Figures should be numbers in order. Please check and edit them such as Fig. 1C (line 83), Fig. 1A (line 85), and Fig 1B (line 86).
Lines 130-133, 138-145, 1370-1376. Figure 1 and its caption have been modified to take this comment into account, along with comments from reviewer 3.

- Genus and species names should be italicized such as Arabidopsis and Pyrus communis.
Done in all the text.
- Topic of results should not be a sentence.
Lines 126, 147. Topics of the Results section have been modified.

- The conclusion should be concise and have no references.
Lines 746-777. References have been removed from the Conclusion section and it has been so slighty shortened. To shorten it more, we can move the summary about our findings in pear (757-767) at the end of the corresponding Discussion part (line 644). Let us know if you prefer this option.

- Some abbreviations had no full words.
I hope we have corrected these errors, let us know if some remains.

*Reviewed by anonymous reviewer, 27 Jun 2022 16:42*

Vergne and colleagues present a comprehensive investigation into the transcriptomic changes following pear scab nonhost resistance to a pathogen. The authors clearly have detailed knowledge of the expected (and possible) immune responses in pears and apples and I think the description of these pathways alone would be useful for researchers in this area.

However, this paper suffers from a severe flaw: it is largely qualitative after the initial differential expression testing. Specifically, the authors devote most of the manuscript to introducing various expected immune and physical pathways and phenotypes and then mention how many genes related to those traits were differentially expressed. They then dig into specifically what those genes are. The problem with this approach is that it is not at all convincing that genes overall related to the phenotypes of interest are differentially expressed. In other words, a few genes in each of these pathways/phenotypes of interest may be differentially expressed, but possibly a similar number would be found if genes were totally randomly sampled. Standard enrichment tests (e.g., Fisher's exact tests) are needed to support the authors' claims that these traits are specifically enriched or depleted for differentially expressed genes. Otherwise it is very possible that the authors could be interpreting noise in their data – the reader currently has little means to evaluate that.

As you suggested, thanks to the Functional Classification SuperViewer tool [Provart et al., 2003], the TAIR accessions have been used to class DEGs in functional categories according to MapMan software (https://mapman.gabipd.org/homemapman.gabipd.org; file Ath_AGI_LOCUS_TAIR10_Aug2012.txt; [Thimm et al., 2004]), and to highlight the enriched categories by calculating the frequency of DEGs per category, normalized to the numbers of Arabidopsis genes in each MapMan category, and bootstrapping the dataset to provide a confidence estimate for the accuracy of the result. The corresponding results gives a new figure 2 and a new Table 3 in the new Result section (lines 175-195). Material and Methods (lines 877-883) and Figure 2 caption (1379-1386) have also been amended accordingly. We hope these modifications correct the initial flaw.

This manuscript is also quite unique in that it has a very long results/discussion section, which tends to largely be a discussion of the predicted gene functions and other results in the literature. I think it would be much clearer if the authors elected instead to include a brief results section where they present the results of enrichment tests such as I alluded to above. They should also provide specific odd's ratios and p-values where relevant as well. A quick summary of the results in this way would make the paper much easier to interpret. The authors could then expand specific genes within the enriched pathways/phenotypes and make connections to other literature. As currently the paper is 90% discussion, I think keeping it in the current format would make it very difficult for the reader to disentangle exactly what the data presented here supports.

As you suggested, a brief Result section has been created (Lines 125-195) which present macroscopic and microscopic phenotypes, and enrichment tests. We hope the paper is easier to interpret in this new format.

Other major comments

In general, the authors are too strong in how they interpret the RNA-seq data. For instance, at L190-191 I disagree that the RNA-seq data is clear evidence that "some JA seems to be produced, but [is] rapidly converted [into] inactive compounds…" The data is consistent with this hypothesis, but I think the language should be toned down as you are not actually measuring JA levels here. Similar issues are at play in the conclusion paragraphs of each discussion section: the authors should make it clear that they are indirectly inferring the levels of calcium influx, HR, etc. Otherwise readers would get the wrong impression for how much confidence they should put in all of these very specific inferences. E..g, L263-266: the authors speak about calcium influx and development of specific stomatal closure pre-invasive defense as though it were experimentally shown, but there is no direct evidence for this. There are many similar examples as well, e.g., L325:327, L409:410, etc.

As you suggested, we have tried to tone down our interpretation of the results all along the Discussion section, as well as in the Discussion subsection titles. Let us know if these modifications are acceptable or insufficient.

The same problem is at play with the subsection titles. For instance, the authors do not have direct evidence that the cell wall carbohydrates content and cuticle content are altered", only that some genes likely involved in those processes are differentially expressed. This is a very important distinction.

As you suggested, we have tried to tone down our interpretation of the results all along the Discussion section, as well as in the Discussion subsection titles. Let us know if these modifications are acceptable or insufficient.

The introduction should be lengthened to discuss some more prior results. Currently it feels unbalanced as there is a very long results and discussion section, but concise introduction. I think this it would be especially important to introduce citation 11 more, which comes up numerous times. It would be helpful if the authors made the differences between this paper and their own clear from the offset (this can be inferred from the last sentence of the intro with a careful reading, but it would be better if this was explicit).
As you suggested, the introduction has been lengthened (lines 75-120) to discuss more prior results on large-scale analyses on resistance in apple and pear, but essentially on host resistance because we did not find such genome wide works on apple and pear nonhost resistance, especially against scab. We particularly explain Perchepied et al. (2021, now citation 13) findings on apple and pear scab host resistance, to better distinguish this work from ours.

I don't find Figure 2 very informative – the key question I have is what is the background proportion of all of those categories? I think rather than having the percent of total genes on the x-axis, it could just be the percent of all significant hits, and you could show the bars for the percents of background genes in all categories as well, to make it easier to evaluate whether any categories are particularly common (or depleted). This is of course related to my key critique of the manuscript as well.
Previous Figure 2 has been removed and replace by a new Figure 2 with results of the enrichment tests suggested earlier. The new Result section (lines 175-195), Material and Methods (lines 877-883) and Figure 2 caption (1379-1386) have been amended accordingly.

Exact sample sizes and replicate structure should be more clearly explained and brought up at the beginning of the results as well.
We have tried to better explain sample sizes and replicate structure at beginning of the results (lines 152-155). Let us know if these modifications are acceptable or insufficient.

Custom code used for project should be made available through an online repository, e.g., on GitHub. It is not sufficient to point to R and AnaDiff. Also, I don't think the authors explicitly mention AnaDiff in the manuscript itself – details on the actual statistical test for differential expression testing are needed.
(Lines 868-871) Briefs details on the statistical tests have been added in the Material and Methods section, and the online repository were the pipeline AnaDiff is available has been also added. The information about this availability of the pipeline has been added in the section Availability of data and materials as well (lines 938-939).

Minor

First line of each paragraph (except for the first paragraph of a section, which is optional) should be indented.
Done in all the text.

L19 (and elsewhere): is being "a nonhost" the correct term? Or should it be they "have nonhost resistance to …"
Lines 19-21. Replacement has been done and checked in all the text.

L49 – Add "." after "et al"
Done in all the text.

L80 – Make it clear that Gala and Conference are an apple and pear cultivar, respectively, upon first mention
Line 127. Done, and checked in all the text.

Table 1: Should add the description of each class as another column of table, so thjat the reader doesn't have to keep looking back and forth
Line 135 Table 1. Done in the first column of Table1, associated to the corresponding number.

Table 2: Perhaps change "without TAIR name" to "without Arabidopsis homolog"?
Line 163 Table 2. Done.

Should be space between number "hpi", e.g., 72hpi should be 72 hpi
Done in all the text.

L143 – "theses" should be "these"
Line 254. Done.

L159 – remove "basically"
Line 168. Done.

L159 – "DEGS" should be "DEGs" and "have been tested" should be "were tested"
Line 168-169. Done.

Figure 2: decimal places on x-axis should be period
Previous Figure 2 has been removed and replace by a new Figure 2 with results of the enrichment tests suggested by one of the reviewers. The new Result section (lines 175-195), Material and Methods (lines 877-883) and Figure 2 caption (1379-1386) have been amended accordingly.

L161: "weak" should be "low"
Line 170. Done.

L168 – Use active voice when describing results found in this work, or make it clear if you're referring to a different paper
Line 283. Done.

L191 – add "is" before "rapidly" and "into" instead of "in"
Lines 307-308. Done.

L270: "i. e." should be ", i.e.,"
Line 392. Done.
L316: add "the" before JA
Line 442. Done.

L333-L336 – Re-word, this sentence is very hard to follow.
Line 460-463. Done. Let us know if it is more understandable this way.

L606: Replace "As far as we know" with "To our knowledge"
Line 747. Done.

*Reviewed by anonymous reviewer, 07 Sep 2022 08:16*

Vergne and co-workers have here deciphered and compared the mechanisms underlying NHR of apple and pear using phenotypic and transcriptomic analyses. They have shown that the resistance differed in terms of phenotypic expression of the resistance, and that the difference was also mirrored by the gene expression underlying resistance, with DEGs being consistent with the phenotypic expression of the resistance in both plant species.

I particularly appreciated that the transcriptomic data were thoroughly explored and that authors have illustrated their findings through well-designed figures.

Nonhost resistance is increasingly considered as a promising field of research to identify sustainable disease-control methods with a low environmental impact, and the authors have provided a high-quality analysis of their data. Considering that, this paper brings valuable information for the community and I fully support the publication of this article in PCI Genomics once comments have been taken in consideration.

Major comments:

- even though very few genes are DEG in the apple / V. pyrina interaction, could you provide the list of these genes in suppl. data, with as much info regarding these genes as you have?
As requested, all our informations about the 60 DEG found in the apple / V. pyrina interaction have been added in the new Table S3 (in “Additional File 1” file).

- lines 82-90: could you indicate in the text the number of interactions tested for each pathosystem? Even though a few infections of pears with V. inequalis gave rise to HR or resistance symptoms, most interactions (90%) were symptomless. It was 100% for the apple X V. pyrina. Could the symptoms observed, as they are not on all interactions, be due to an environmental effect? Also, to be cautious, I would mitigate the statement that the interaction is NH type II, just by adding "seems" or "most interactions were asymptomatic except for X interactions, we are hypothesizing that it is type II". This is also confusing because a “small scale HR” is observed in both cases (lines 104-105). Moreover, in both cases, growing of the hyphae seems to be very limited (from figure 1), although the outcome of the interaction is not the same, could you comment on that? For the pear x V. inequalis interaction, it could be the same as for the “rare HR-like reactions” on apple x V. pyrina (lines 108-109). Please comment and clarify. I think it would be sound not to conclude here on the type of HR but to use the transcriptomic analysis to validate the hypothesis.
The number of interactions tested for each pathosystem has been added between brackets in Table 1 (Line 135).
Indeed, the difference between apple and pear does not seem to be at the level of the macroscopic symptoms (two symptomatic plants are indeed not sufficient to conclude), but rather at the level of the microscopic symptoms, which we had not explained /illustrated well. In pear at the microscopic level we observed more frequent HR than in apple, we have added two pictures (Fig. 1 J and H) in Figure 1 which illustrate this point and we have tried to better explain it in the results (lines 143-144) and the discussion (lines 227-228).
Throughout the text, we have also removed the argument on belonging to type I or II nonhost resistance, because you are right, make this judgment on very few plants with macroscopic symptoms is not legitimate.

We hope the paper is now clearer on these points, please let us know.

- Figure 1: could you add the same visualization of the infection of apple with V. inequalis and pear with V. pyrina as a control, for non-familiar people.
We have added two pictures (Fig. 1 A and B) in Figure 1 which illustrate V. pyrina VP102 strain / pear 'Conference' (A) and V. inaequalis EUB05 strain / apple 'Gala' (B) 21 days symptoms, as classical ones of susceptible host interactions. Results (line 130) and Figure 1 caption (1371-1373) have been amended accordingly.

- lines 117-119: justify the time points used for the transcriptomic analysis especially that later, 6 dpi are mentioned (line 146): do these timepoints correspond to a particular stage in the apple and / or pear infection by their respective adaptive pathogen? and are the inoculations made on detached leaf (cause possible leaf ageing is mentioned line 122)?
Lines 150-152. We have had this information in the text: 24 and 72 hours post inoculation time points were chosen in order to cover the period of establishment of the first intimate contacts between fungal and plant cells: conidia germination and appressoria formation.
Line 237. The inoculations are not made on detached leaves, they are made on actively growing shoots (lines 792-795 in Material and Methods section). Leaf ageing is mentioned because a previous reader of the paper advised us to verify the absence of DEG linked to ontogenetic resistance in our data. Our experimental design in "kinetic", with respect to time "0", could indeed give rise to results containing DEG related to this type of resistance.

- lines 120-122: this sentence is difficult to follow, could you rephrase to clarify, for instance, remove "not to the infection but" and "which" should be used instead of "whose".
Lines 233-237. We have rephrased to a better understanding. Let us know if it suits you like that.

- lines 149-150: have you compared these data to the infection of pear by V. pyrina or apple by V. inequalis (i.e type of gene deregulated due to infection by the adapted pathogen)? Authors may have done later in the paper.
Line 260-262. In the Discussion section (lines 677-744), we have compared and discuss the overall answers of pear host (against V. pyrina, from Perchepied et al., 2021, now reference 13) and nonhost (against V. inaequalis, present work) resistance, but we have not precisely compared the "type" of genes deregulated.

- part "calcium influx and ROS…": could you indicate which pathosystem this data corresponds to? And if it changes throughout this part? It was unclear at the first reading.
Lines 344-353. This part is all about pear / V. inaequalis interaction, we have added the information in the subsection title.

-line 476: "but further functional analyses…" could you indicate which analyses could be set up to conclude?
Lines 610-612. We have indicated that analyses such as histochemical staining, content measure by absorbance or Fourier-transform infrared (FTIR) spectroscopy analysis could help to conclude about the lignin status.

- lines 707-708: transposable elements were represented on the array, but no results were mentioned concerned these elements, could you provide the results?

Lines 707-709. We are sorry, we have not specifically analyzed the data about transposable element on the apple chip (present only in this chip). But the 60 apple DEG found in our work does not contain any of them.

-lines 798-710: were all genes of V. inaequalis represented on the microarray? Why were the data regarding these genes not presented?
Lines 709-710. The chip design was intended to effectively study the genome-wide transcriptome of V. inaequalis. Data regarding these genes are not presented because at the interaction times points chosen (24 and 72 hpi), no fungus RNA quantities were sufficient to allow to recover any signal.

Minor comments:

- in the text, sometimes “Malus x domestica”, sometimes “Malus domestica”, homogenize.
Done in all the text.

- line 80: add that leaf is inoculated even though it is mentioned in the methods.
Line 127. Done.

- Figure 1: images extend beyond the frame and are not aligned. “V. pyrina”: the "V" must be italicized
Lines 1370-1376 (caption). Figure 1 and its caption have been modified to take these comments into account and add pictures of infection of apple with *V. inequalis* and pear with *V. pyrina* as a control, and larger pictures of macroscopic views to show the greater number of microscopic HR observed in pear compared to apple.

- Table 1, line 97: a parenthesis is missing, add the number of interactions assessed in parenthesis.
Line 135 Table 1. Done.

- line 119-120: you can remove “kinetic”, “experimental design” is sufficient.
Line 233. Done.

- line 125: to be consistent, change “differently” to “differentially”.
Line 240. Done.
- table 2: to be consistent, add or remove “of” for the number of genes deregulated. Add the meaning of “TAIR”.
Line 163 Table 2. “of” has been added everywhere and “TAIR” has been replaced by “Arabidopsis homolog” to be clearer.

- table S1: header of columns G and H to be checked. Title should be “… BLAST analysis”.
Line 245 Table S5. Table S1 become Table S5. Header of columns G and H have been changed to be clearer and the title has been corrected.

- line 147: “a later” rather than “longer” should be written.
Line 259. Done.

- line 154: “at both time points of the experiment” rather than “of the kinetics”.
Line 160. Done.

- homegenise “up-regulated” or “upregulated”, same for down regulated throughout the paper.
Done in all the text.

- figure 2: replace “,” by “.”
Previous Figure 2 has been removed and replace by a new Figure 2 with results of the enrichment tests.

- line 159: remove “basically”
Line 168. Done.

- line 164: change “24phi” to “24hpi”
Line 173. Done.

- line 165: I would conclude this part with a biological conclusion by moving the validation of the microarray data earlier in the text and finishing on the functional categories identified.
Line 168-174. In the new Result section created, the paragraph about qPCR validation has been placed before the part on identified DEGs and functional categories.

-line 170: change “that is” to “corresponding to”
Line 285-286. Done.

-line 201: “…only two of the previously activated ones”?
Line 318. Done.

- line 209: what do you mean with “SA accumulation was also rather mixed”?
Line 325. We have rephrased to a better understanding. Let us know if it suits you like that.

- throughout the text, write “nonhost” rather than “non-host”, both are used and this needs to be consistent.
Done in all the text.

- line 312: change “hypersensitive reaction” to “HR”.
Line 438. Done.

- line 336: “plant cell wall interactions, xxx”. Remove the coma.
Line 463-467. We have rephrased to a better understanding. Let us know if it suits you like that.

- line 342: “xxx Table 3 xxx”
Line 470. Done (Table 3 become Table 4).

- Table 4: please, add the legend (for “*”) below the table
Done. (Table 4 become Table 5).

- line 433: change “hemi biotrophic” to “hemibiotrophic”
Line 564. Done.

- line 434: change “strong induction” to “Strong induction…”
Line 565. Done.

- lines 456-459: the “s” is missing for the verbs conjugated to the the 3rd person sing.
Line 588-591. Done.

- line 512: “no known function” to “unpredicted function”?
Line 649. Done.

-line 642: “in vitro” to be italicized
Line 784. Done.