nature portfolio

# Peer Review File

## Conserved effector families render Phytophthora species vulnerable to recognition by NLR receptors in nonhost plants

**REVIEWER COMMENTS**

Reviewer #1 (Remarks to the Author):

Oh et al manuscript shows that multiple RxLR effectors are broadly conserved across Phytophthora species and most of them are recognized by corresponding Solanum NLRs. They also show that expression three of these NLRs conferred broad-spectrum resistance against multiple Phytophthora species. Based on these results, they suggest that nonhost resistance (NHR) can be mediated by NLRs. The manuscript is well written. The topic of this manuscript would be of broad interest and the results are potentially novel. Even though the experiments are well done, the data is not presented well and that made me less convincing as described below.

Major Comments:
Line 35: Author suggests that "they have developed a homology-based approach to identify functional NLR. However, it is not clear whether this method is used for the first time by the author or the method is adopted from previously published approaches. In addition, in the abstract it will be useful to state which scientific questions were addressed and how they were addressed. This could perhaps be done in a sentence or two.
Line 46-59: Although author has tried to explain NHR, however, it is not clear through this paragraph how the non-host resistance works. Author can briefly explain NHR mechanism before getting into unaddressed questions in NHR research domain.
Line 68-69: This is a convoluted sentence-authors are suggested to modify the sentence to convey the message-Functional homologs of solanum NLRs recognizing effectors of Potato late blight pathogen-Phytophthora infestans were found in non-host plant chilli pepper.
Line 76: Acronym RxLR appears without defining it for the first time. Although, this might be an obvious terminology for a specialist in the field perhaps non-specialist reader would be left puzzled.
Line 137: Author should clarify what is the rationale for cloning 69 out of 89 effectors chosen. Was the additional 20 effectors could not be cloned?
Line 138: Although, author mixed effectors and putative solanum NLR in a 1:1 fashion, however, simply mixing does not ensure that two Agrobacterium strains separately carrying an effector and NLR would deliver two of these molecules to a single plant cell, even though this approach is commonly used. The variation observed in cell death could be due to this issue. Perhaps this could have been avoided by cloning the effector and NLR into a single T-DNA vector for expression? I do acknowledge this increases the work significantly. Perhaps they can test few examples to see if they observe less variation in cell death.
Line 167-169: These sentences are miss leading. Author should amend the sentences to express clearly that Avr1, Avr8, or Avramr1 were transiently expressed in T0 plants expressing R1, R8, or Rpi-amr1. It is also not clear promoter and terminator used for the expression of R1, R8, or Rpi-amr1, and T-DNA backbone used for the cloning these genes. It will be useful if the author can list the sequences of the constructs used in supplementary info. In addition, it will be useful to provide representative map of construct used for the expression of R1, R8, or Rpi-amr1 and others in figure 3.
Line 229-335: Discussion section could be shortened a bit and include more references
Line 423: plasmid sequence of pICH31160 should be provided in the supplementary material. In addition, sequence of all 69 effectors cloned should be provided along with primers pairs used for their

amplification.
Fig. 2a is not well explained. What does the white blank space mean? No HR? but "-" also means no HR. The scale for the heat map doesn't make sense to me. There is no dark green in the scale. I presume dark green is strong HR.
Fig. 2b is also not well explained. There are single asterisks without connecting two box plots! What does this mean? It is statistically different than GFP control? The same applies to Supplemental figures 8, 11, and 12. There are single asterisks all over the place in some cases below the box plot! No explanation in the legend regarding what these asterisks are for! In Fig 2b, the lesion size of P. palmivora infection upon expression of R8 significantly increased instead of decreasing. The same is observed with P. capsici infection after expression of Rpi-amr1 and Rpi-blb2. The authors did not explain the reason behind this. How the R gene expression can make it more susceptible to disease?
There are serious issues with supplemental figures 7b, 9b, and 10b. I am not sure if this is data manipulation or inadvertent error. In 7b the images of PiAvr2::R2 and PiAvramr1::Rpi-amr1 interactions are identical! The same is observed with PiAvrblb1::Rpi-blb1 and PiAvrvnt1::Rpi-vnt1 interactions where the images are identical! In Fig 9b PiAvr8::R8 and PcacAvramr1::Rpi-amr1 are identical; PiAvrvnt1-Rpi-vnt1 and PcacAvrvnt1::Rpi-vnt1 are identical. In Fig 10b PiAvramr1::Rpi-amr1 is identical to Fig 9b. It is not clear if the experiment was repeated. I did not compare all the images across all the figures. There may be more like this.
Fig 3 is not well prepared and difficult to understand. In Fig 3 c-f is cited in the text before Fig 3a. In Fig 3c-f, I am not able to see red and green dotted lines in the graphs as mentioned in the legend! However, I do see a very light green or red shading. Maybe give different colors for R1, R8 and Rpi-amr1 since the shades given can be confusing especially between R8 and Rpi-amr1. The percentages shown in the graph is very tiny and hard to see unless zoomed. The reader will not be able to read this in a printed copy.
It is not clear what the difference between Supplemental figures 14 and 15 is.

Minor Comments:
Please expand gene names when appropriate during first mention. For example, Rpi-amr3.
Line 46: "now NHR" should be changed to "currently NHR" or "at present NHR".
Line 49: Need a reference.
Line 109, Avramr3 is missing in the Supplementary Fig 3 legend.
Line 165: agrobacterium is Agrobacterium
Line 198: Bit Score Score?
Line 221: In conclusion, among our homologous effector candidates-Authors are suggested to remove "our" from the sentence. Perhaps, this can be applied through the manuscript.
Line 237: needs a reference.
Line 241-251: Need a reference.
Line 337: Plant materials and growth conditions-Author should provide details about the nutrition of the plants or if not refer to an appropriate prior study.
Line 349: "Incubated" should be changed to "incubating".
Line 406, it should be P. infestans.
Line 451: Washed out thrice with what?
Line 452: growth for four weeks in MS media, whether it is light or dark?

Reviewer #2 (Remarks to the Author):

"I co-reviewed this manuscript with one of the reviewers who provided the listed reports". Please recognize my contributions accordingly.

Reviewer #3 (Remarks to the Author):

In the reviewed paper Oh et all. explored the hypothesis that NLRs conferring resistance to P. infestans can also recognise effectors and thus provide resistance against other Phytophthora species. The findings in this paper are overall interesting and provide new knowledge. I have some comments for the authors to consider:

Major points:
- The major hypothesis is that Rpi genes from Solanum species may confer non-host resistance to other Phytophthora species in addition to host resistance to P. infestans. However, care should be taken to provide evidence that the source of these genes (particularly S. americanum and S. demissum) actually are non-hosts to these other Phytophthora. Currently no references or experiments are provided to support this. Similarly, S. americanum is a reported non-host of P. infestans, so the suggestion that P. infestans is adapted to S. americanum are misleading.
- The finding that Rpi-amr1 confers recognition of P. cactorum and P. parasitica is already published (Witek et al 2021) while the study expands upon this and demonstrates additional recognition as well as resistance conferred by Rpi-amr1, care should be taken to make this clear.
- The quality of writing makes it difficult to understand at times

Minor points:

26 – The term ‘the corresponding Solanum NLR’ is misleading, there may be multiple NLRs capable of recognising these effectors (e.g. AVR2 is recognised by the unrelated NLRs Rpi-mcq1 and R2), not all may recognise the same orthologues from other Phytophthora species
- Similar in Line-80

57-59 – This statement directly contradicts the following paragraph and the general hypothesis of the paper.

121 – Functionally conserved is a big assumption, conserved predicted structure does not mean that function is conserved.

163/164 – ‘More natural condition’ means non-transient expression? Is transgenic N. benthamiana natural?

190-227 section and figure 4 – Isn't the finding obvious?
- 201/202 – "Overall, more closely related effectors were more possibly being recognized by corresponding Solanum NLRs."
- Similar effectors are more likely to be recognised than distantly related proteins

246-251 – What does this mean?

270-273 – This hypothesis is a repeat of the previous referenced statement

275-294
- Do NLRs that mediate indirect recognition have lower sequence conservation than NLRs which directly recognise effectors? Are there references for this?
- 280-282 – Both convergently and divergently evolved NLRs could mediate NHR? The implication is that any NLR could mediate NHR
- 293-294 - Is there any evidence that convergently evolved NLRs are more likely to have an indirect recognition mode? Or that NLRs cannot convergently evolve direct recognition of an effector?
- How is this section relevant to the findings in the paper? Direct and indirect recognition is not distinguished elsewhere in the manuscript.

304/305
- Do you mean suppression of the NRC helper NLR? Could you correlate NRC dependency of the tested sensors with resistance observed? E.g. does Rpi-amr1-mediated recognition of P. parasitica evade suppression due to signalling through additional NRCs compared to R8 or other tested NLRs?
- There may be other reasons that recognition does not translate into resistance like: the sensor NLRs could also be suppressed, there are differences in the expression levels of the effectors between pathogens or due to the difference in the interaction strength between effectors from each pathogen, the effectors are overexpressed in the HR assay.

338 – N. benthamiana is not tobacco

341-350 – Include details for P. capsici

406 – infestans rather than infestance

Figure 1
- Minor comment - It would be much easier to interpret if the species in (a) were not abbreviated, or if abbreviations were indicated in the legend
- "The presence or absence of parentheses indicates whether certain motif or domain is required or optional to be classified as each category" – It is not clear what is meant by this. Also, it appears that categories are distinguished by the presence/absence of Sig/WY, rather than the motif or domain in

parentheses.

Figure 2 (a): Green is not indicated on the scale, is this a pdf formatting issue? Positions without either -/HR show where there are no orthologues identified?
Was only one orthologue from each species cloned? There are 40 HR assay results shown, but 77 effectors are mentioned in the legend – in the text – "60.87% (42/69) of the tested effectors induced cell death upon co-expressed with their putative corresponding Solanum NLR". It is not clear how many effectors are represented in this figure, if multiple effectors fit into each category, it would be more informative to indicate how many induce HR with the tested NLR
(b) How many lesions were measured for each set? It would be helpful to represent this in a similar way to (c). Why were some combinations not tested – e.g. Rpi-vnt1 and P. cactorum which is shown as HR in panel (a)?

Figure 3 (a) Include representative images of all lines indicated in panel (b);
(b) The pathogen (P. capsici?) is not indicated in the figure or legend, the second R8 transgenic line is not resistant? Consider changing the colours or datapoint style as R8 #11 and Rpi-amr1#2 are indistinguishable. How many plants of each line were tested?
(c/d/e/f) WT lesion size plots are coloured the same as R1/R8/Rpi-amr1 lines, please use a different colour to indicate the WT control. It is strange that number of inoculation sites varies between the pictures, it should be consistent between WT and transgenic lines

Figure 4: Similar to line 121, functionally conserved is an assumption.

Figure 5d: Consider rephrasing 'number of functional NLRs' to something closer to 'compatible R-gene and avr-gene pairs'. Also evolutionary distance could be changed to 'coevolving to non-adapted"

Reviewer #4 (Remarks to the Author):

The manuscript by Oh et al investigates whether the conserved avirulence effectors across four Phytophthora spp. infecting different plant species were cross-recognised by immune receptors from different Solanaceous plants. They cloned 69 effectors closely related to 12 avirulence effectors into a binary vector and tested for their recognition by nine cognate immune receptors by agroinfiltration using both transient and stable transgenic Nicotiana benthamiana plants expressing these receptors. They found that some immune receptors recognised closely related effectors present in Phytophthora spp. that do not normally infect the plant species harboring those receptors. Additionally, they discovered that three of the immune receptors, tested using transgenic N. benthamiana plants, conferred broad resistance against "non-infecting" Phytophthora species. The authors concluded that these NLRs found in Solanaceous plants contribute to the non-host resistance (NHR) in this plant family.

The manuscript is mostly well written and logically laid out. Experiments were performed to excellent

standards and statistical analyses were included where applicable. The figures are of high quality (although there were some issues) with enough details for the readers to scrutinize. There are sufficient details in the materials and methods that other researchers can use to reproduce the results if need be. The authors are highly commendable for their thoroughness and robust experiments with meticulous presentation of the results.

I am convinced that the NLRs the authors have tested have the potential and capability of recognising the effectors from "non-infecting" pathogens and some do confer resistance against these pathogens when tested on a surrogate host such as N. benthamiana. However, my main concern is that the authors have not provided explicit enough answer to the question: Are these NLRs actually involved in NHR? In my opinion, the authors have not provided enough evidence to overcome the burden of proof that these NLRs are involved in NHR (e.g., Would NLR knock out in a host plant be successfully infected by a "non-infecting" pathogen in this experimental set up? Or would the "non-infecting" pathogen become infectious if the effectors that are recognised by non-host plant NLRs were knocked out? Are the NLRs and effectors expressed in the right places at the right time to the right amount? Would there be any NLRs that are involved in NHR against slightly more distant Albugo or Pythium spp?). The authors would be less controversial and more productive if they re-frame their findings along the evolutionary lines, such as evolutionary transitions, regressive evolution, diversification and differential loss of pathogenicity to host ranges etc, instead of tying them to NHR (although they can speculate about this phenomena). The fact that pathogen species as well as the host species used in this manuscript are "closely" related makes defining and resolving NHR difficult. As far as I am concerned, the infection assays conducted in N. benthamiana to show the context of NHR is confusing and not conclusive enough for authors' claims. N. benthamiana itself can be host or non-host to the pathogens they tested depending on circumstances as P. capsici and N. benthamiana might have never encountered each other in their evolutionary time until they were put together by humans. NHR could get more complicated if one considers P. capsici and P. palmivora to have wider host range than other Phytophthora species. Furthermore, the authors still cannot eliminate the possibility that other factors and genes, in addition to the NLRs, might be responsible for NHR in these interactions.

Some minor issues:

- The authors would benefit from reconciling some concepts with Panstruga and Moscou (2020) https://doi.org/10.1094/MPMI-06-20-0161-CR in their introduction and discussions.
- Perhaps discussions could be streamlined. It almost reads like a review article as it was written.

Some corrections:

Line 32: "Solanaceae palnts" should be either "Solanaceous plants" or "Solanaceae family of plants"
Line 325: "Similar with" should be "Similar to"
Line 333: "more identification of NLRs" should be "identification of more NLRs"
Line 338: Please delete "Tobacco" and parenthesis around N. benthamiana as it is not Tabacco plant
Line 342: "rye agar plate" should be "rye sucrose agar plate"

Line 346: Please define "TDW"
Line 349: "after incubated" should be "after being incubated"
Line 415: Please state the cutoff values for bit-score and pTM score
Line 429: GV3101- it should be precisely written as GV3101 (pMP90) if this is indeed used (refer to http://www.bio.net/bionet/mm/arab-gen/2016-January/013588.html). Otherwise, GV3101 alone cannot transfom plants as it is cured of Ti plasmid.
Line 432: "after adjusted" should be "after being adjusted"
Line 433: "were used for test" should be either "were used for testing" or "were used for agroinfiltration"
Line 435: "1~4 scale indexing method" - please provide a reference, or describe the scale in details
Line 456: "For the root infection assay" - please provide the soil type used, and whether it was sterilized
Line 459: "Phenotypes were scored" - please describe specific phenotypes being scored

Figure 2a - Color scale bar next to the heat map - gradient application is wrong, e.g. 300 and 0 will have the same color according to the scale.
Figure 2b - White bar on the leaves - what does this represent? A scale bar? This should be described in the legend.
Figure 5a - does not capture the concept described in the legend very well. Perhaps it needs to be re-considered, for example, effectors and NLRs are missing. Figure 1 in their reference #1 seems to better represent the concept.
Figure 5b - what does each tick on the X-axis represent? Number of effectors? Different effector groups? This should be included in the graph. "Effectors" is not sufficient to understand the graph.

## Reply to Reviewer's Commemts

## Reviewer #1 (Remarks to the Author):

Oh et al manuscript shows that multiple RxLR effectors are broadly conserved across *Phytophthora* species and most of them are recognized by corresponding *Solanum* NLRs. They also show that expression three of these NLRs conferred broad-spectrum resistance against multiple Phytophthora species. Based on these results, they suggest that nonhost resistance (NHR) can be mediated by NLRs.
The manuscript is well written. The topic of this manuscript would be of broad interest and the results are potentially novel. Even though the experiments are well done, the data is not presented well and that made me less convincing as described below.

**Major Comments:**

**Q1.** Line 35: Author suggests that "they have developed a homology-based approach to identify functional NLR. However, it is not clear whether this method is used for the first time by the author or the method is adopted from previously published approaches. In addition, in the abstract it will be useful to state which scientific questions were addressed and how they were addressed. This could perhaps be done in a sentence or two.

**Reply:** As the reviewer's concern, we revised the abstract as below:

'Moreover, considering that resistance genes against most *Phytophthora* species, except for *P. infestans,* have never been identified, a homology-based approach could provide an alternative strategy of genetic mapping for identifying functional NLRs against multiple pathogens threatening crop production.' In **Line 35-38**

Additionally, we referenced several papers performed similar approaches such as (Lin *et al., Mol. Plant* 2022 & Witek *et al., Nat. Plant* 2021; Laflamme *et al.,* Science 2020) and revised related parts (added statement about the previously reported result in **Line 150-151 / legend in Figure 2a**) to tone down previous statements. We also removed 'our' words from the most part of manuscript to emphasize the approach/methods used in the study is modified/adopted from the previous methods.

We added sentence and revised abstracts to clarify our question and how it was addressed as below.

**[**Question **in Line 21-22]** However, the evolutionary process of how plants develop receptors for recognizing wide range of non-adapted pathogens is still elusive.

**[**Finding and meaning **in Line 32-35]** Combined results suggest that conserved effector families of *Phytophthora* species allow Solanaceae family of plants to recognize a wide range of pathogens via NLRs that originally reported to recognize *P. infestans*. Thus, NLR-mediated recognition would contribute to NHR against pathogens that possess similar repertoires of effectors.

**Q2.** Line 46-59: Although author has tried to explain NHR, however, it is not clear through this paragraph how the non-host resistance works. Author can briefly explain NHR mechanism before getting into unaddressed questions in NHR research domain.

**Reply:** As the reviewer's concerns, we additionally describe about 'NHR' **in Line 47-50,** and

newly added reference (Panstruga and Moscou *MPMI.,* 2020) to link the what is NHR and receptor-mediated NHR (especially NLRs) in introduction part.

**Q3.** Line 68-69: This is a convoluted sentence-authors are suggested to modify the sentence to convey the message-Functional homologs of solanum NLRs recognizing effectors of Potato late blight pathogen-Phytophthora infestans were found in non-host plant chilli pepper.

**Reply:** As the reviewer's concerns, related part is removed from the revised manuscript.

**Q4.** Line 76: Acronym RxLR appears without defining it for the first time. Although, this might be an obvious terminology for a specialist in the field perhaps non-specialist reader would be left puzzled.

**Reply:** We added description as '(conserved N-terminal Arg-Xaa-Leu-Arg motif)' as suggested in **Line 23, and 82-83**.

**Q5.** Line 137: Author should clarify what is the rationale for cloning 69 out of 89 effectors chosen. Was the additional 20 effectors could not be cloned?

**Reply:** There were disparities between the genome information of reference strains (of *Phytophthora* species) and experimental (domestic/Korean) strains that we used in this study (as shown in Supplementary Table 4). We assume that several candidates were not existed (or possess SNPs in primer site) in our strain (were not amplified from PCR).

To clarify procedure and rationale, we revised method section (**Line 423 - 424**), and provided how we select (cut off, or detailed information about cloned effectors) in **Line 415-416**, and **Supplementary figure 3, 6,** and **supporting information** about initial cloning targets (with raw data, excel file named as 'initial_sets_effector_numbering').

**Q6.** Line 138: Although, author mixed effectors and putative solanum NLR in a 1:1 fashion, however, simply mixing does not ensure that two Agrobacterium strains separately carrying an effector and NLR would deliver two of these molecules to a single plant cell, even though this approach is commonly used. The variation observed in cell death could be due to this issue. Perhaps this could have been avoided by cloning the effector and NLR into a single T-DNA vector for expression? I do acknowledge this increases the work significantly. Perhaps they can test few examples to see if they observe less variation in cell death.

**Reply:** We agree with the reviewer's suggestion and we could identify additional combination by stabilizing co-expression of effector and NLRs by cloning them into single vector. However, we are concerned about the benefits that could be gained by further elaborating screening method would not be significant because we could already obtain a plenty of candidates which exhibited consistent (at least 3 replications) and intensive cell death phenotype with commonly used 1:1 co-expression screening. We beg reviewer's generous accept about this part.

**Q7.** Line 167-169: These sentences are miss leading. Author should amend the sentences to express clearly that Avr1, Avr8, or Avramr1 were transiently expressed in T0 plants expressing R1, R8, or Rpi-amr1. It is also not clear promoter and terminator used for the expression of R1, R8, or Rpi-amr1, and T-DNA backbone used for the cloning these genes. It will be useful if the author can list the sequences of the constructs used in supplementary info. In addition, it will be useful to provide representative map of construct used for the

expression of R1, R8, or Rpi-amr1 and others in figure 3.

**Reply:** As the reviewer's concerns, we revised sentences in **Line 171– 175**. And, we added whole plasmid sequencing data (in Supplementary table 7) of vectors used in this study, and we also submitted vector map files (as a **supporting information**).

**Q8.** Line 229-335: Discussion section could be shortened a bit and include more references.

**Reply:** We shortened the discussion section from 1,219 => 996 words by removing 'Convergent and divergent evolution of NLRs and NHR' section. We also revised first section 'NLRs recognizing effectors of broad-spectrum pathogens have a potential to be exploited to confer durable resistance in crops' in **Line 261-287** for the clarification, and added some references including (Schulze-Lefert *et al., Trends in Plant Sci.* 2011; Witek*et al., Nat. Plants* 2021).

**Q9.** Line 423: plasmid sequence of pICH31160 should be provided in the supplementary material. In addition, sequence of all 69 effectors cloned should be provided along with primers pairs used for their amplification.

**Reply:** As the reviewer's concern, sequences of all vectors used in this study is added in Supplementary table 7. And also, coding sequences of 69 cloned effectors are added with the primer information in Supplementary table 6.

**Q10.** Fig. 2a is not well explained. What does the white blank space mean? No HR? but "-" also means no HR. The scale for the heat map doesn't make sense to me. There is no dark green in the scale. I presume dark green is strong HR.

**Reply:** We corrected figure 2, absence of dark green part was a file converting error and we fixed it. We revised the figure legend and design of figures, described detailed information of scales, and added description about white blank spaces that mean no conserved homologs or not cloned, thus not tested.

**Q11.** Fig. 2b is also not well explained. There are single asterisks without connecting two box plots! What does this mean? It is statistically different than GFP control? The same applies to Supplemental figures 8, 11, and 12. There are single asterisks all over the place in some cases below the box plot! No explanation in the legend regarding what these asterisks are for! In Fig 2b, the lesion size of P. palmivora infection upon expression of R8 significantly increased instead of decreasing. The same is observed with P. capsici infection after expression of Rpi-amr1 and Rpi-blb2. The authors did not explain the reason behind this. How the R gene expression can make it more susceptible to disease?

**Reply:** It was not a single asterix without connecting boxes but data point which were excluded drawing boxes because they exceed threshold from average. However, we notice this type of presentation could cause misunderstanding, thus we replaced all the graph format in this manuscript (also in supplementary figures).

About the lesion size increasing: for now, we have no clear answer about this phenomenon, though it was consistently observed for several cases (Rpi-blb2 against *P. capsici* and R8 against *P. palmivora*) as reviewer's concern. At least, as shown in the Fig 2a, *Rpi-blb2* and *R8* cannot recognized effectors of *P. capsici* (Avrblb2 is not conserved) or Avr8 of *P. palmivora*. Thus, we could just assume both NLRs cannot function as resistance gene (because they are not activated) against each pathogen but the physiological changes occurred by over-expressing those NLRs make more infectious environment in *N. benthamiana* leaves to the each *Phytophthora* pathogen. But still, we have no clear answer about this phenomenon.

**Q12.** There are serious issues with supplemental figures 7b, 9b, and 10b. I am not sure if this is data manipulation or inadvertent error. In 7b the images of PiAvr2::R2 and PiAvramr1::Rpi-amr1 interactions are identical! The same is observed with PiAvrblb1::Rpi-blb1 and PiAvrvnt1::Rpi-vnt1 interactions where the images are identical! In Fig 9b PiAvr8::R8 and PcacAvramr1::Rpi-amr1 are identical; PiAvrvnt1-Rpi-vnt1 and PcacAvrvnt1::Rpi-vnt1 are identical. In Fig 10b PiAvramr1::Rpi-amr1 is identical to Fig 9b. It is not clear if the experiment was repeated. I did not compare all the images across all the figures. There may be more like this.

**Reply:** First of all, we thanks to the reviewer for pointing out this serious flaws and give us an opportunity for revising it. We thoroughly inspect our images (even for used in other figures) and replaced duplicated pictures with proper pictures, and also provided related raw data and information when each picture was taken with the picture of research note for each corresponding date.

As shown in the submitted raw data and research scheme (supporting information file named as '**HR_test_raw_data_scheme**'), although the experiments were performed properly, we regret that there was an image modification issue on editing Supplementary figures, and once again, thanks for the reviewer and editor for the concerns and devotion on revising our manuscript.

**Q13.** Fig 3 is not well prepared and difficult to understand. In Fig 3 c-f is cited in the text before Fig 3a. In Fig 3c-f, I am not able to see red and green dotted lines in the graphs as mentioned in the legend! However, I do see a very light green or red shading. Maybe give different colors for R1, R8 and Rpi-amr1 since the shades given can be confusing especially between R8 and Rpi-amr1. The percentages shown in the graph is very tiny and hard to see unless zoomed. The reader will not be able to read this in a printed copy.

**Reply:** Figure legend are revised [dotted lines => shades], and all the graph in the Figure 3 are re-designed and re-colored for the clarification as the reviewer's suggestion (font size of percentage are also increased).

**Q14.** It is not clear what the difference between Supplemental figures 14 and 15 is.

**Reply:** We presented combined data on the main Figure 3, and Supplementary figure 14 and 15 were the replication of same experiment (1st and 2nd trials). To clarifying it, we described as 1st / 2nd trials in figure legends and revised design of both Supplementary Figures, and we also marked as (3rd trial) for the newly added Supplementary figure 16.

**Minor Comments:**

**Q1.** Please expand gene names when appropriate during first mention. For example, Rpi-amr3.
**Reply:** we added 'resistance genes against *P. infestans'* in **Line 65-66**

**Q2.** Line 46: "now NHR" should be changed to "currently NHR" or "at present NHR".
**Reply:** revised as 'currently NHR' in **Line 45**

**Q3.** Line 49: Need a reference.
**Reply:** reference is added in **Line 51 (Oh *et al., EBC.* 2022).**

**Q4.** Line 109, Avramr3 is missing in the Supplementary Fig 3 legend.
**Reply:** We added Avramr3 in figure legend.

**Q5.** Line 165: agrobacterium is Agrobacterium
**Reply:** revised in the whole manuscript.

**Q6.** Line 198: Bit Score Score?
**Reply:** revised in **Line 205**

**Q7.** Line 221: In conclusion, among our homologous effector candidates-Authors are suggested to remove "our" from the sentence. Perhaps, this can be applied through the manuscript.
**Reply:** revised in the whole part of manuscript (removed 'our' word in related parts)

**Q8.** Line 237: needs a reference.
**Reply:** We added reference (Haverkort *et al., Potato Res.* 2016) in **Line 255.**

**Q9.** Line 241-251: Need a reference.
**Reply:** Added reference in **Line 266** (P. schulze-Lefert and R. panstruga et al., 2011), and revised related part.

**Q10.** Line 337: Plant materials and growth conditions-Author should provide details about the nutrition of the plants or if not refer to an appropriate prior study.
**Reply:** we added descriptions about detailed conditions for plant materials in **Line 335-338**.

**Q11.** Line 349: "Incubated" should be changed to "incubating".
**Reply:** revised as 'being incubated' in **Line 349**

**Q12.** Line 406, it should be *P. infestans*.
**Reply:** revised as concerned, in **Line 406**

**Q13.** Line 451: Washed out thrice with what?
**Reply:** we added detailed information about 'washing media' in **Line 453**.

**Q14.** Line 452: growth for four weeks in MS media, whether it is light or dark?
**Reply:** under the continuous light, revised in **Line 455.**

**Above all, we sincerely thanks to the Reviewer 1's devotion for polishing this manuscript.**

## Reviewer #2 (Remarks to the Author):

"I co-reviewed this manuscript with one of the reviewers who provided the listed reports". Please recognize my contributions accordingly.

**Reply:** We sincerely thanks to the Reviewer 2's contribution for revising this manuscript.

## Reviewer #3 (Remarks to the Author):

In the reviewed paper Oh et all. explored the hypothesis that NLRs conferring resistance to *P. infestans* can also recognise effectors and thus provide resistance against other *Phytophthora* species. The findings in this paper are overall interesting and provide new knowledge. I have some comments for the authors to consider:

**Major points:**

**Q1.** The major hypothesis is that *Rpi* genes from *Solanum* species may confer non-host resistance to other *Phytophthora* species in addition to host resistance to *P. infestans*. However, care should be taken to provide evidence that the source of these genes (particularly *S. americanum* and *S. demissum*) actually are non-hosts to these other *Phytophthora* species. Currently no references or experiments are provided to support this. Similarly, *S. americanum* is a reported non-host of *P. infestans*, so the suggestion that *P. infestans* is adapted to *S. americanum* are misleading.

**Reply:** We completely agree with the reviewer's concern that there is no actual evidence in this study whether the sources of the tested NLRs are nonhost or not against tested *Phytophthora* species (Though the pathogens used in this study could be regarded as 'normally not infecting the *Solanum* species harboring the tested NLRs' because it has never been reported). However, we are not able to obtain all the wild *Solanum* species described in this study.

Thus, we tried to tone down and reduced statements about nonhost resistance (started from the title of the manuscript, in total, the number of 'NHR' in the manuscript decreased from **22 => 11**; and 'non-adapted' decreased from **14 => 5**), and most of those parts remain only in discussion & very first of introduction section. As presented in the replaced title, we tried to state our result as the broad-spectrum resistance by recognizing conserved effectors but stated as these mechanisms 'would' contribute to the nonhost resistance.

We revised the related part for clarification, in **Line: 277-280** as below:
Considering that *S. americanum* is evolutionarily more distant from potato compared to the other wild *Solanum* species such as *S. demissum* or *S. bulbocastanum*, we could assume that *P. parasitica* is relatively well adapted to potato and its closely related species but not to *S. americanum*.

**Q2.** The finding that Rpi-amr1 confers recognition of *P. cactorum* and *P. parasitica* is already published (Witek et al 2021) while the study expands upon this and demonstrates additional recognition as well as resistance conferred by Rpi-amr1, care should be taken to make this clear.

**Reply:** As the reviewer's concern, we added statement about the previously reported result in **Line 151, 275** and referenced (Witek et al 2021; though we already cited this paper but we added statement about the result), and tried to emphasize the previous results in **Figure 2a** using asterix (*) mark with *Rpi-amr1/3*-related results and described it in figure legends.

**Q3.** The quality of writing makes it difficult to understand at times

**Reply:** We revised the whole manuscript according to the three reviewer's major/minor concerns. And corrected grammars. We sincerely thanks to the reviewers' contribution for revising the manuscript.

**Minor points:**

**Q1.** 26 – The term 'the corresponding Solanum NLR' is misleading, there may be multiple NLRs capable of recognising these effectors (e.g. AVR2 is recognised by the unrelated NLRs Rpi-mcq1 and R2), not all may recognise the same orthologues from other *Phytophthora* species. Similar in Line-80

**Reply:** we removed 'corresponding' from the **Line 26** (abstract) and **86** (introduction)

**Q2.** 57-59 – This statement directly contradicts the following paragraph and the general hypothesis of the paper.

**Reply:** Our intension was to state effectors are 'relatively variable' compared to PAMPs. Indeed, though PAMPs are conserved across the kingdom level, effectors are less broadly conserved (*Phytophthora* genus level in our results). We revised related part (**in Line 57-60**) to clarify our intension.

**Q3.** 121 – Functionally conserved is a big assumption, conserved predicted structure does not mean that function is conserved.

**Reply:** We removed 'functionally conserved' from the related part (also in the whole manuscript), and revised as 'Combined results suggest that multiple effector families are conserved among *Phytophthora* species.' In **Line 127-128**

**Q4.** 163/164 – 'More natural condition' means non-transient expression? Is transgenic *N. benthamiana* natural?

**Reply:** Revised as 'To validate *Solanum* NLR-mediated resistance against multiple *Phytophthora* species using transgenic plants' in **Line 169-170.**

**Q5.** 190-227 section and figure 4 – Isn't the finding obvious? 201/202 – "Overall, more closely related effectors were more possibly being recognized by corresponding *Solanum* NLRs." Similar effectors are more likely to be recognised than distantly related proteins

**Reply:** As a response to the reviewer's concern, we **removed** the concerned sentence and re-described the related section for the clarification (**Line 198-215 in revised manuscript**).

**Q6.** 246-251 – What does this mean?

**Reply:** We revised the related part for the clarification, in **Line 261-272**, and also revised related part of Figure 5 and its legend.

**Q7.** 270-273 – This hypothesis is a repeat of the previous referenced statement

**Reply:** We removed the repetitive statement and replace it with our own perspective about the durable resistance in **Line 283– 288.**

**Q8.** 275-294 - Do NLRs that mediate indirect recognition have lower sequence conservation than NLRs which directly recognise effectors? Are there references for this?

**Reply:** As the reviewer's concerns, we realized the part named 'Convergent and divergent evolution of NLRs and NHR' was not well matched with this study. Thus, the related part is removed from the revised manuscript (in the discussion section).

**Q9.** 280-282 – Both convergently and divergently evolved NLRs could mediate NHR? The implication is that any NLR could mediate NHR

**Reply:** As the reviewer's concerns, we realized the part named 'Convergent and divergent

evolution of NLRs and NHR' was not well matched with this study. Thus, the related part is removed from the revised manuscript (in the discussion section).

**Q10.** 293-294 - Is there any evidence that convergently evolved NLRs are more likely to have an indirect recognition mode? Or that NLRs cannot convergently evolve direct recognition of an effector? How is this section relevant to the findings in the paper? Direct and indirect recognition is not distinguished elsewhere in the manuscript.

**Reply:** As the reviewer's concerns, we realized the part named 'Convergent and divergent evolution of NLRs and NHR' was not well matched with this study. Thus, the related part is removed from the revised manuscript (in the discussion section) for the uniformity.

**Q11.** 304/305 - Do you mean suppression of the NRC helper NLR? Could you correlate NRC dependency of the tested sensors with resistance observed? E.g. does Rpi-amr1-mediated recognition of *P. parasitica* evade suppression due to signaling through additional NRCs compared to R8 or other tested NLRs?

**Reply:** As the reviewer's comment, NRC is one of the possible candidate to be suppressed by pathogen, and similar with the reviewer's concern, we also assumed that different NRC-dependency of tested sensor NLRs could be correlated with the disparities between HR cell death against effectors and resistance against *Phytophthora* pathogens that we observed in Figure 2. Indeed, *Rpi-amr1* (NRC2/3-dependent) cannot conferred resistance against *P. capsici* while *R1* (NRC4-dependent) and *R8* (NRC2/3/4-dependent) conferred significant resistance against *P. capsici*, even though all these three *Solanum* NLRs were able to recognize and induced cell death against effectors of *P. capsici*, Therefore, we hypothesized that *P. capsici* would suppress NRC2/3 but not NRC4 of *N. benthamiana*. To test this hypothesis, we used nrc4 knockout *N. benthamiana*. However, the result was negative (expression of *R8* were still able to confer resistance against *P. capsici* even in nrc4 knockout *N. benthamiana* as shown in the submitted **raw data of DLA experiment performed in '220415'using *P. capsici***). This result indirectly indicates that *NbNRC2/3* still properly work during the *P. capsici* infection. As suggested by the reviewer, if we conduct similar experiments with a more variety of NRC knockout plants, we may be able to find examples that explain the correlation of NRC-dependency and the discrepancies between resistance and HR cell death phenotypes, but unfortunately, with the materials we currently have, it was not achieved.

**Q12.** There may be other reasons that recognition does not translate into resistance like: the sensor NLRs could also be suppressed, there are differences in the expression levels of the effectors between pathogens or due to the difference in the interaction strength between effectors from each pathogen, the effectors are overexpressed in the HR assay.

**Reply:** Agree, we added the several more possible reasons in the discussion part including the reviewer3's concerns in **Line 300-303**.

**Q13.** 338 – *N. benthamiana* is not tobacco

**Reply:** revised in **Line 335**.

**Q14.** 341-350 – Include details for *P. capsici*

**Reply:** added details for *P. capsici* in **Line 343 and 346**.

**Q15.** 406 – *infestans* rather than *infestance*

**Reply:** revised in **Line 406**

**Q16.** Figure 1 (Minor comment) - It would be much easier to interpret if the species in (a) were not abbreviated, or if abbreviations were indicated in the legend
- "The presence or absence of parentheses indicates whether certain motif or domain is required or optional to be classified as each category" – It is not clear what is meant by this. Also, it appears that categories are distinguished by the presence/absence of Sig/WY, rather than the motif or domain in parentheses.

**Reply:** We have revised Figure 1 based on the reviewers' suggestions. Species names were assigned in Figure 1a rather than using abbreviations. The categories in Figure 1b were classified according to the presence or absence of a signal peptide (Sig) and WY domain (WY). Additionally, we have added a note to Figure 1b indicating that detailed domain and motif information regarding homologous effectors is provided in Supplementary Table 3.

**Q17.** Figure 2 (a): Green is not indicated on the scale, is this a pdf formatting issue? Positions without either -/HR show where there are no orthologues identified?

**Reply:** The absence of green part was a formatting issue, corrected.
Avr2 homologs of *P. capsici* were missed from dataset for drawing heat-map. It's also revised (added into the heatmap). Also, we revised the figure legend and design of figures, described detailed information of scales, and added description about white blank spaces that mean no conserved homologs or not cloned, thus not tested.

**Q18.** Was only one orthologue from each species cloned? There are 40 HR assay results shown, but 77 effectors are mentioned in the legend – in the text – "60.87% (42/69) of the tested effectors induced cell death upon co-expressed with their putative corresponding *Solanum* NLR".

**Reply:** We revised figure and its legend to clarify how to draw this figure, and 69 was correct number, we revised it.

**Q19.** It is not clear how many effectors are represented in this figure, if multiple effectors fit into each category, it would be more informative to indicate how many induce HR with the tested NLR

**Reply:** The number of HR positive / test effectors are presented as fraction for each cases in Figure 2a as the reviewer's concern.

**Q20.** (b) How many lesions were measured for each set? It would be helpful to represent this in a similar way to (c). Why were some combinations not tested – e.g. Rpi-vnt1 and *P. cactorum* which is shown as HR in panel (a)?

**Reply:** All dots presented as the reviewer's suggestion (similar with Figure 2c, for the all presented graph in main figures). However, the number of replications are different for each case and if it presented, the figures would become too complicated. **Thus, detailed information (numbers) are presented in Supplementary figures 7-12.**

**Not tested cases:** For the cases of *P. palmivora* and *P. cactorum*, we did not test for several case (NLRs) when the corresponding effectors are not conserved in each pathogen (only R3a was tested even though *P. cactorum* did not possess Avr3a homologs in our criteria).

However, as the reviewer's concern, for the case of *Rpi-vnt1*, We have auto-activated cell death issue with p35s:*Rpi-vnt1* construct. It induces moderate level of cell death on infiltrated region (weak cell death observed with naked eyes but merely detected using

FoBI machine – red leaves/black dead tissue images) at 2 dpi, but it kills most of infiltrated leaves at 2.5 dpi of agroinfiltration. While *P. parasitica* and *P. capsici* fully expand their lesion in *N. benthamiana* leaves within 2.0 days after inoculation, *P. palmivora* and *P. cactorum* take more than 3 days for the measureable lesion size. Thus, we could not test *Rpi-vnt1*-mediated resistance against *P. cactorum* in *N. benthamiana* (also, we could obtain other promising candidates *R1, R8,* and *Rpi-amr1,* it did not stimulate us to test *Rpi-vnt1* with different/optimized experimental conditions). For the clarification, we added auto-active phenotypes of *Rpi-vnt1* expressed leaves in Supplementary Figure 7a (weak cell death at 2 dpi) and 9a (severe cell death at 3 dpi), and described about these issues in legends. In addition, for the cell death test using *Rpi-vnt1,* we also provided the **raw data (file named as 'HR_test_raw_data_scheme')** with a proper control (photo taken before *Rpi-vnt1* induced cell death itself, as shown in experiments performed at 230214_*P. cactorum*, 230309_*P. parasitica*, and 230406_*P. sojae*, respectively), and presented control cases of *Rpi-vnt1* in Supplementary Figure 7a and 9a.

**Q21.** Figure 3 (a) Include representative images of all lines indicated in panel (b);

**Reply:** We could not take the pictures (as good as could be presented in the main figure) of representative images of R1 #3 and R8 #11, however we provided whole plant images in Supplementary figure 14 / 15, and newly added Supplementary figure 16, and all the pictures taken using transgenic plants (file named as **'transgenic_plant_raw_data'**)

**Q22.** Figure 3 (b) The pathogen (*P. capsici*?) is not indicated in the figure or legend, the second R8 transgenic line is not resistant? Consider changing the colours or datapoint style as R8 #11 and Rpi-amr1#2 are indistinguishable. How many plants of each line were tested?

**Reply:** It was *P. capsici*, the figure and legend are revised to include *P. capsici*.

For the concern about the second line (#11) expressing R8, we observed characteristics of 'heterozygous line' of R8-expressing plants (especially #11) because we used T1 plants which could exhibit genetic segregation. The similar patterns were also observed in R1-expressing lines (2~30% of plants were infected by *P. capsici* as wild type plants). And unfortunately, it was worse in the 1st trial (Supplementary figure 14). Thus we performed additional experiments (triplicate) as presented in Supplementary figure 15 (2nd trial) and Supplementary Figure 16 (3rd, newly performed) and observed that R8-expressing lines (#11) able to confer relatively weak resistance (compared to R1-expressing lines) against *P. capsici*. Indeed, this phenomenon was similarly observed in transient-expression-based experiments as shown in Figure 2c (R1 > R8, in terms of intensity of resistance).

In addition, we changed color of graph in Figure 3b, and the numbers of tested plants are presented in Figure 3a and Supplementary figure 14~16.

**Q23.** (c/d/e/f) WT lesion size plots are coloured the same as R1/R8/Rpi-amr1 lines, please use a different colour to indicate the WT control. It is strange that number of inoculation sites varies between the pictures, it should be consistent between WT and transgenic lines

**Reply:** We changed colors, re-design, and revised legend of graph for the clear distinguish between R1 / R8 / Rpi-amr1 lines as the reviewer's concern. We also replaced images and synchronized all the numbers of inoculation sites of photos in Figure 3c~f).

**Q24.** Figure 4: Similar to line 121, functionally conserved is an assumption.

**Reply:** We removed 'functionally conserved' as the reviewer's concern. In **Line 127-128,** and also from the whole manuscript.

**Q25.** Figure 5d: Consider rephrasing ‘number of functional NLRs’ to something closer to ‘compatible R-gene and avr-gene pairs’. Also evolutionary distance could be changed to ‘coevolving to non-adapted”

**Reply:** Revised as reviewer’s concerns. As [Number of functional NLRs => Compatible NLR/effector paris] [Evolutionary distance => Divergent time from adapted pathogen (A) of given plant (a)]

**Above all, we sincerely thanks to the Reviewer 3's suggestions and devotion for revising this manuscript**

## Reviewer #4 (Remarks to the Author):

The manuscript by Oh et al investigates whether the conserved avirulence effectors across four *Phytophthora* spp. infecting different plant species were cross-recognised by immune receptors from different Solanaceous plants. They cloned 69 effectors closely related to 12 avirulence effectors into a binary vector and tested for their recognition by nine cognate immune receptors by agroinfiltration using both transient and stable transgenic *Nicotiana benthamiana* plants expressing these receptors. They found that some immune receptors recognised closely related effectors present in *Phytophthora* spp. that do not normally infect the plant species harboring those receptors. Additionally, they discovered that three of the immune receptors, tested using transgenic *N. benthamiana* plants, conferred broad resistance against "non-infecting" *Phytophthora* species. The authors concluded that these NLRs found in Solanaceous plants contribute to the non-host resistance (NHR) in this plant family.
The manuscript is mostly well written and logically laid out. Experiments were performed to excellent standards and statistical analyses were included where applicable. The figures are of high quality (although there were some issues) with enough details for the readers to scrutinize. There are sufficient details in the materials and methods that other researchers can use to reproduce the results if need be. The authors are highly commendable for their thoroughness and robust experiments with meticulous presentation of the results.

**Major issues:**

**Q1.** I am convinced that the NLRs the authors have tested have the potential and capability of recognising the effectors from "non-infecting" pathogens and some do confer resistance against these pathogens when tested on a surrogate host such as *N. benthamiana*. However, my main concern is that the authors have not provided explicit enough answer to the question: Are these NLRs actually involved in NHR? In my opinion, the authors have not provided enough evidence to overcome the burden of proof that these NLRs are involved in NHR (e.g., Would NLR knock out in a host plant be successfully infected by a "non-infecting" pathogen in this experimental set up? Or would the "non-infecting" pathogen become infectious if the effectors that are recognised by non-host plant NLRs were knocked out? Are the NLRs and effectors expressed in the right places at the right time to the right amount? Would there be any NLRs that are involved in NHR against slightly more distant *Albugo* or *Pythium* spp?). The authors would be less controversial and more productive if they re-frame their findings along the evolutionary lines, such as evolutionary transitions, regressive evolution, diversification and differential loss of pathogenicity to host ranges etc, instead of tying them to NHR (although they can speculate about this phenomena). The fact that pathogen species as well as the host species used in this manuscript are "closely" related makes defining and resolving NHR difficult. As far as I am concerned, the infection assays conducted in *N. benthamiana* to show the context of NHR is confusing and not conclusive enough for authors' claims. *N. benthamiana* itself can be host or non-host to the pathogens they tested depending on circumstances as *P. capsici* and *N. benthamiana* might have never encountered each other in their evolutionary time until they were put together by humans. NHR could get more complicated if one considers *P. capsici* and *P. palmivora* to have wider host range than other *Phytophthora* species. Furthermore, the authors still cannot eliminate the possibility that other factors and genes, in addition to the NLRs, might be responsible for NHR in these interactions.

**Reply:** We sincerely thanks to the reviewer’s suggestion for the better presentation of our work

and pointing out the weak point of the manuscript. We completely agree with the reviewer's suggestion that 'to re-frame the findings along the evolutionary lines instead of tying them to NHR' would be less controversial and more productive.

Therefore, we tried to tone down and reduced statements about nonhost resistance (started from the title of the manuscript, in total, the number of 'NHR' in the manuscript decreased from **22 => 11**; and 'non-adapted' decreased from **14 => 5**), and those parts remain only in discussion & very first part of introduction section. As shown in the presented in the replaced title, we tried to state our result as the broad-spectrum resistance by recognizing conserved effectors of *Phytophthora* species but stated this kind of mechanism 'would' have potential to contribute to the nonhost resistance.

**Some minor issues:**

**Q1.** The authors would benefit from reconciling some concepts with Panstruga and Moscou (2020) https://doi.org/10.1094/MPMI-06-20-0161-CR in their introduction and discussions.

**Reply:** We newly cited (Panstruga and Moscou et al., MPMI. 2020) and tried to adopt their concept and description into this manuscript (in **Line 47-60** of introduction part), and we also revised related part in discussion section (in **Line 261-272** of discussion) for the clarification/reframing of our previous description.

**Q2.** Perhaps discussions could be streamlined. It almost reads like a review article as it was written.

**Reply:** We shortened the discussion section from 1,219 => 996 words by removing 'Convergent and divergent evolution of NLRs and NHR' part and revised most other parts to make it streamlined and reduce description about NHR or non-adapted pathogens.

**Some corrections:**

**Q1.** Line 32: "Solanaceae palnts" should be either "Solanaceous plants" or "Solanaceae family of plants"

**Reply:** revised in **Line 33**, and also in the whole manuscript.

**Q2.** Line 325: "Similar with" should be "Similar to"

**Reply:** revised **in Line 323**.

**Q3.** Line 333: "more identification of NLRs" should be "identification of more NLRs"

**Reply:** revised in **Line 330**.

**Q4.** Line 338: Please delete "Tobacco" and parenthesis around *N. benthamiana* as it is not Tabacco plant

**Reply:** revised in **Line 335.**

**Q5.** Line 342: "rye agar plate" should be "rye sucrose agar plate"

**Reply:** revised in **Line 341**.

**Q6.** Line 346: Please define "TDW"

**Reply:** revised in **Line 345.**

**Q7.** Line 349: "after incubated" should be "after being incubated"
**Reply:** revised in **Line 349.**

**Q8.** Line 415: Please state the cutoff values for bit-score and pTM score
**Reply:** We provided cut-off in **Line 415-416** as the reviewer's concerns and we also provided initially selected effectors for cloning in submitted raw data excel file named 'initial_sets_effector_numbering'

**Q9.** Line 429: GV3101- it should be precisely written as GV3101 (pMP90) if this is indeed used (refer to http://www.bio.net/bionet/mm/arab-gen/2016-January/013588.html). Otherwise, GV3101 alone cannot transfom plants as it is cured of Ti plasmid.
**Reply:** revised as commented, thanks for the notification.

**Q10.** Line 432: "after adjusted" should be "after being adjusted"
**Reply:** revised in **Line 433.**

**Q11.** Line 433: "were used for test" should be either "were used for testing" or "were used for agroinfiltration"
**Reply:** revised in **Line 435.**

**Q12.** Line 435: "1~4 scale indexing method" - please provide a reference, or describe the scale in details
**Reply:** it was our mistake, we only scored HR cell death phenotypes with positive/negative in this study, it revised as presented in Supplementary table 6, thus related part is removed.

**Q13.** Line 456: "For the root infection assay" - please provide the soil type used, and whether it was sterilized
**Reply:** details about soil is added in **Line 464-466.**

**Q14.** Line 459: "Phenotypes were scored" - please describe specific phenotypes being scored
**Reply:** we add descriptions about how we determined wilt/dead plants in material method in **Line 462-463.**

**Q15.** Figure 2a - Color scale bar next to the heat map - gradient application is wrong, e.g. 300 and 0 will have the same color according to the scale.
**Reply:** It was an error occurred during file converting in submission procedure, we corrected.

**Q16.** Figure 2b - White bar on the leaves - what does this represent? A scale bar? This should be described in the legend.
**Reply:** It's removed.

**Q17.** Figure 5a - does not capture the concept described in the legend very well. Perhaps it needs to be re-considered, for example, effectors and NLRs are missing. Figure 1 in their reference #1 seems to better represent the concept.
**Reply:** We added NLR and effectors in the picture, and revised figure legend and related part of the manuscript in **Line 261-272**.

**Q18.** Figure 5b - what does each tick on the X-axis represent? Number of effectors? Different effector groups? This should be included in the graph. "Effectors" is not sufficient to

understand the graph.

**Reply:** It was different group of effectors, we added detailed label in X-axis for each group.

**REVIEWERS' COMMENTS**

Reviewer #1 (Remarks to the Author):

In the revised manuscript, the authors have addressed most of my concerns but needs a bit more improvement as mentioned below.

Include NHR in keywords

In Fig 2a legend, please mention blank space means not tested.

In Fig 2b, regarding the author's response to my concern about the lesion size of P. palmivora infection upon expression of R8 significantly increased instead of decreasing, the authors need to discuss this in the discussion section.

Also in fig 2b, please explain in the legend that the dots are data points. The dots look more like asterisk. It will be good to distinguish this form the asterisk. Can actual dots be used instead of asterisk? Maybe even a different colored dots.

Regarding washing media in line 453, It will be useful to clarify what is BA in liquid MS media with cefotaxime.

Reviewer #4 (Remarks to the Author):

The manuscript by Oh et al is a resubmission by the authors with revisions. The authors have addressed majority of my concerns and I do not have any other issues except the title which could be re-worded so that it conveys the message more clearly, perhaps along the line of
"Conserved effector families renders Phytophthora species vulnerable to recognition by Nucleotide-binding leucine-rich repeat receptors in nonhost plants" or something similar.
I appreciate the authors' openness and willingness to converse with the reviewers.

REPLY to REVIEWERS' COMMENTS

Above all, thanks to the anonymous reviewers for their invaluable comments on our manuscript.

The new version of manuscript has been revised according to the reviewer's comments and replies to the reviewer's comments are followed.

Reviewer #1 (Remarks to the Author):

In the revised manuscript, the authors have addressed most of my concerns but needs a bit more improvement as mentioned below.

S1: Include NHR in keywords

Done

S2: In Fig 2a legend, please mention blank space means not tested.

Adjusted as suggested.

S3: In Fig 2b, regarding the author's response to my concern about the lesion size of P. palmivora infection upon expression of R8 significantly increased instead of decreasing, the authors need to discuss this in the discussion section.

Added description in Line 292-297 of discussion session.

S4: Also in fig 2b, please explain in the legend that the dots are data points. The dots look more like asterisk. It will be good to distinguish this form the asterisk. Can actual dots be used instead of asterisk? Maybe even a different colored dot.

We increased font size of asterisk for the clear distinguishment from data points.

S5: Regarding washing media in line 453, It will be useful to clarify what is BA in liquid MS media with cefotaxime.

We added full description as (BA => benzyladenine)

Reviewer #4 (Remarks to the Author):

The manuscript by Oh et al is a resubmission by the authors with revisions.

The authors have addressed majority of my concerns and I do not have any other issues except the title which could be re-worded so that it conveys the message more clearly, perhaps along the line of "Conserved effector families renders Phytophthora species vulnerable to recognition by Nucleotide-binding leucine-rich repeat receptors in nonhost plants" or something similar. I appreciate the authors' openness and willingness to converse with the reviewers.

We decided below sentence as a New Title according to the reviewer's suggestion and editorial guide lines (less than 16 words)

'Conserved effector families render Phytophthora species vulnerable to recognition by NLR receptors in nonhost plants