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Population studies of the wild tomato species *Solanum chilense* reveal geographically structured major gene-mediated pathogen resistance

Parvinderdeep S. Kahlon, Shallet Mindih Seta, Gesche Zander, Daniela Scheikl, Ralph Hückelhoven, Matthieu H. A. J. Joosten and Remco Stam

Article citation details
Proc. R. Soc. B **287**: 20202723.
http://dx.doi.org/10.1098/rspb.2020.2723

Review timeline

Original submission:	31 August 2020
1st revised submission:	30 October 2020
2nd revised submission:	29 November 2020
Final acceptance:	30 November 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2020-2148.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
In this work, the authors work to show that there is geographic variation in S. chilense to C. fulvum. This work develops on the large body of literature investigating the molecular mechanism of C. fulvum recognition within the solanum. The show a distinct north/south cline in sensitivity that has been reported for other pathogens in this system. This is an interesting beginning of studying how the plant may be responding to shifts in either the presence of the pathogen or the presence of specific Avrs within the pathogen.

I had seen this manuscript previously and the authors focused their work around the strengths of the data with a focus on the populations and the interaction. The first two paragraphs of the discussion took a particular beating in this editing to refocus. I might encourage blending them a bit to help the reader.

In Table 1, are there no indels as the headers only discuss SNPs? It might help to give an idea of frequency and independence. For example, are the 4 non-syn SNPs in SOBIR1 in LA2932 all in one individual or separate SNPs in four individuals, i.e one or four haplotypes?

I have a question about line 379-382, the work by Joy Bergelson on gene-for-gene systems has indicated that R gene selective pressures are greatest when there is genetic variation in the population. So if a population has bottlenecked to the sensitive allele, would there be detriment in the absence of migration?

Similarly, is it safe to argue that everything is about the loss of resistance and that the authors are sure that similar to the LOV story that there may not be a pathogen able to use the resistance alleles for C. fulvum as susceptibility alleles?

It would help to say if there are any quantitative modifiers of Hcr9 known in any of the published tomato systems. I know that a number of other tomato loci like PTO have quantitative modifiers found in the original mapping studies but never followed-up upon.

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

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Is it accessible?
No

Is it clear?
No

Is it adequate?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
This manuscript describes the diversity in recognition ability of Cladosporium fulvum avirulence genes and thus, resistance, in plants of different populations of Solanum chilense. The results reveal a striking diversity that is geografically structure, with southern populations being completelly susceptible to the pathogen. These results fit into a gene-for-gene evolutionary model and suggest the existance of costs of unnecessary resistance that the authors hypothesis to be related with trade offs with abiotic tolerance. Another stricking result of this work is the absence of correlation between the presence of homologs to Cf-9 and Cf-4 genes nd the recognition ability of the plant. This point to different recognition abilities of these genes in the species S. chilense comparade to other wild tomato species or with domesticated tomato. The authors speculate that the lack of recognition of Avr9 by plants expressing Cf-9 may be due to mutetions in the Cf-correceptors in southern populations. However, the authors disregard the possibility that mutations in S. chilense homologs also affect Avr9 recognition. The authors should sequence the amplified putative Cf-9 orthologs in responsive and non responsive plants in order to be able to assverate that the lack of response is due to co-receptors and not to the putative resistance gene.

In fact, the authors should also sequence Cf-4 homologs in plants representing the different response possibilities (gene and response present; gene present but no response). This is a factible experiment since they can amplify those regions, and would give more light to the complex interactions that the observations suggest.
Besides, there are minor questions to revise:
Line 57. Change "(effectors)" by "(avirulence factors, Avrs)". Join this pragraph with the following one.
Line 60. "the seventeen wild tomato species known"
Line 61. Eliminate "to"
Line 74. Fusarium spp. (not spec)
Line 82. Eliminate "also called avirulence factors (Avrs)". Add "as avirulence factors, following the gene-for-gene model of interaction" to the end of the sentence. This sentence, should be moved after the sentence finishing in "secreted by C. fulvum".
Line 85. Eiminate "During evolution", subsituting the sentence "This recognition facilitates host resistance following the gene-for-gene model"
Line 94. Subsititute "Both genes" by Cf-9 and Cf-4
Line 95. Write "Homologues of Cladosporium resistance 9" before Hcr9
Line 98. This sentence is too taxative. Authors should nuance it
Line 109. Authors should mention here that regions of accessions and number of plants tested are described in Table S1.
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Lines 349-351. The authors mix the presence of Ct-9 and Ct-4 homlogs and the response to plants to Avr9 and Avr4 to aseverate that they are not allelic in S. chilense, but in fact they have shown that the presence of the homolog does not correlate with the resistance reaction. Athors should correct the writing of this reasoning.
When the authors sequence the homologs of Cf-9 and Cf-4, there would be more arguments to be discussed about the conformation of these loci.
In general, the authors should revise the whole text for verb tenses. They use either the present

and the past. Normally, results should be written in past tense.

Decision letter (RSPB-2020-2148.R0)

20-Oct-2020

Dear Dr Stam:

I am writing to inform you that your manuscript RSPB-2020-2148 entitled "Population studies of the wild tomato species Solanum chilense reveal geographically structured major gene-mediated pathogen resistance" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.
4) Data - please see our policies on data sharing to ensure that you are complying (https://royalsociety.org/journals/authors/author-guidelines/#data).

To upload a resubmitted manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Sincerely,
Professor Hans Heesterbeek
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Two experts have now reviewed your work "Population studies of the wild tomato species Solanum chilense reveal geographically structured major gene-mediated pathogen resistance" (RSPB-2020-2148). Both reviewers agree on the interest of the study.
Reviewer 1 recommends only modifications in the text and changes in the focus of some parts of the Discussion. However, Reviewer 2 points that the possibility that lack of recognition of Avr9 is

due to mutations in S. chilense Cf9 homologs is not considered, and recommends sequencing the amplified putative Cf-9 orthologs in responsive and non-responsive plants in order to be able to ascertain that the lack of response is due to co-receptors and not to the putative resistance gene. Same exploration is recommended for Cf-4. In my opinion these additional data would much add to the relevance of the study.

Reviewer(s)' Comments to Author:
Referee: 1

Comments to the Author(s)
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Referee: 2

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In general, the authors should revise the whole text for verb tenses. They use either the present and the past. Normally, results should be written in past tense.

Author's Response to Decision Letter for (RSPB-2020-2148.R0)

See Appendix A.

RSPB-2020-2723.R0

Review form: Reviewer 2

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors have addressed most of my comments and have given a convincing explanation to the comment that they have not been able to address.

Decision letter (RSPB-2020-2723.R0)

24-Nov-2020

Dear Dr Stam

I am pleased to inform you that your Review manuscript RSPB-2020-2723 entitled "Population studies of the wild tomato species Solanum chilense reveal geographically structured major gene-mediated pathogen resistance" has been accepted for publication in Proceedings B.

The referee does not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

To upload your manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, upload a new version through your Author Centre.

Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: https://royalsociety.org/journals/authors/author-guidelines/

4) Data-Sharing and data citation
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=RSPB-2020-2723 which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

5) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Professor Hans Heesterbeek
mailto:proceedingsb@royalsociety.org

Associate Editor
Board Member
Comments to Author:
Thank you for the revised version of your manuscript. In my opinion, all concerns and suggestions of both referees have been fully addressed, including the detailed explanation of why the experiments suggested by Referee 2 cannot/need not be performed. Referee 2 is also fully satisfied with your response to his/her comments. I think this is a valuable contribution to the field of plant-pathogen interaction and coevolution.

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s).
The authors have addressed most of my comments and have given a convincing explanation to the comment that they have not been able to address.

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Decision letter (RSPB-2020-2723.R1)

30-Nov-2020

Dear Dr Stam

I am pleased to inform you that your manuscript entitled "Population studies of the wild tomato species Solanum chilense reveal geographically structured major gene-mediated pathogen resistance" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page

length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

Your article has been estimated as being 10 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Dear Professor Heesterbeek,

We are pleased to submit this revision of our manuscript called "Population studies of the wild tomato species *Solanum chilense* reveal geographically structured major gene-mediated pathogen resistance" to Proceedings B.
We greatly appreciate the comments of both reviewers. They have helped us reshape the manuscript and we think that the legibility and descriptions of the results and the discussion have improved over our last submission.

All comments by reviewer 1 relate to minor text changes, as do most of the comments by reviewer 2. All of these are now addressed.

The only comment that we did not and cannot address is the one related to sequencing some of the *Cf* alleles. As mentioned in our previous email exchange, my co-authors and I are of the opinion that using Sanger sequencing this is not possible or would not provide new or relevant insights. One could potentially address the issue using a long read target capture sequencing, however, we think that such an approach goes beyond the scope of the current manuscript.

I have provided a point-by-point reply to all reviewers comments below, including a more elaborate explanation as on why we think that sequencing *Cf* genes is at the moment not feasible. All line numbers mentioned in this response refer to the track changes version of the resubmission.

As you already indicated that the previous version of the paper was interesting and the experiments suggested by reviewer 2 are not essential we think we now fulfill the criteria for publication in Proceedings B. If there is anything we overlooked or if you have any additional questions, please don't hesitate to contact me directly.

Best regards,

Remco Stam

REPLIES TO THE REVIEWER COMMENTS

Associate Editor
Board Member: 1
Comments to Author:
Two experts have now reviewed your work "Population studies of the wild tomato species Solanum chilense reveal geographically structured major gene-mediated pathogen resistance" (RSPB-2020-2148). Both reviewers agree on the interest of the study.
Reviewer 1 recommends only modifications in the text and changes in the focus of some parts of the Discussion. However, Reviewer 2 points that the possibility that lack of recognition of Avr9 is due to mutations in S. chilense Cf9 homologs is not considered, and recommends sequencing the amplified putative Cf-9 orthologs in responsive and non-responsive plants in order to be able to ascertain that the lack of response is due to co-receptors and not to the putative resistance gene. Same exploration is recommended for Cf-4. In my opinion these additional data would much add to the relevance of the study.

REPLY: We provide a detailed reply to this comment below, under the comments of Reviewer 2

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
In this work, the authors work to show that there is geographic variation in S. chilense to C. fulvum. This work develops on the large body of literature investigating the molecular mechanism of C. fulvum recognition within the solanum. The show a distinct north/south cline in sensitivity that has been reported for other pathogens in this system. This is an interesting beginning of studying how the plant may be responding to shifts in either the presence of the pathogen or the presence of specific Avrs within the pathogen.

I had seen this manuscript previously and the authors focused their work around the strengths of the data with a focus on the populations and the interaction. The first two paragraphs of the discussion took a particular beating in this editing to refocus. I might encourage blending them a bit to help the reader.

REPLY: We have added a sentence to link the two paragraphs (LINES 319-320).

In Table 1, are there no indels as the headers only discuss SNPs? It might help to give an idea of frequency and independence. For example, are the 4 non-syn SNPs in SOBIR1 in LA2932 all in one individual or separate SNPs in four individuals, i.e one or four haplotypes?

REPLY: Thanks for pointing out the discrepancy between header and table. We have updated the table to point out the indels separately.
We should point out that these data come from three individuals only. As described in the methods, we used the data from one single plant from LA3111, one single plant from LA2932 and one single plant from LA4330. Other data are not fully available yet. Thus we cannot provide information on the number of haplotypes.
That said, our collaborators are analyzing additional *S. chilense* genomes and could confirm that these and additional mutations do occur also in other plants from these populations. In a follow-up study we aim to look deeper into these mutations and want to see whether some correlate with the HR phenotypes that we observed.

I have a question about line 379-382, the work by Joy Bergelson on gene-for-gene systems has indicated that R gene selective pressures are greatest when there is genetic variation in the population. So if a population has bottlenecked to the sensitive allele, would there be detriment in the absence of migration?

REPLY: We are not entirely sure how to interpret this comment. We think that the reviewer wonders what the effect of the presence of sensitive alleles would be if a population experiences a bottleneck (e.g. population size reduction) without migration (e.g. remains at the same location).
We show that there are individuals in the central population which do not recognize the AF. This would mean that if in that area there is a *C. fulvum* isolate that possesses the same or a very similar effector mix as present in our AF (which we think is a reasonable assumption), then it would be detrimental for these populations. However, as we discuss further on in the manuscript (LINES 392-44) and also in response to the next question, there are several possibilities why such a situation might occur. Further on in the discussion we highlight that several Cf-gene co-receptors are also involved in responses to environmental stresses. Populations of the central genotype group experience much more diverse environmental stresses than the southern populations. They experience larger temperature fluctuations, larger variation in precepitation events and more irregular flooding. The mutations in some of the coreceptors could have evolved in response to such events. At the same time, we know that *C. fulvum* can be found in these regions, thus there might be a need for balancing selecting on these extreme stresses.
In fact our collaborators at the Chair of Population Genetics have indications that these populations have larger seed banks than the populations in the south coast and highlands, which would confirm this hypothesis. We expect these findings to be published in the next couple of months.
A trade off similar to the one described for LOV is another possibility. It might be that there are two different pathogens present in the central regions, one or which the Cf genes are resistance genes, another for which the Cf genes are an susceptibility factor.
We have made a few minor changes to the discussion to make both hypotheses clearer (LINES 410-414), but due to length limitations and for the ease of reading we refrain from overly long discussions and philosophizing.

Similarly, is it safe to argue that everything is about the loss of resistance and that the authors are sure that similar to the LOV story that there may not be a pathogen able to use the resistance alleles for C. fulvum as susceptibility alleles?

REPLY: This is a very interesting thought that we did overlook and have now briefly addressed at the end of the paragraph and added a citation to the LOV paper (LINES 410-414).
The reviewer might find it interesting to hear that in general we still know very little about the pathogens that are present in the different geographical locations. From our own field work (yet unpublished, but manuscript in preparation), we know that *C. fulvum* is present in the central regions and in those regions several *Alternaria* species are also present. So far we did not find *C. fulvum* in the southern regions, but we did find *Alternaria* spp. there. *C. fulvum* is seen as a biotrophic pathogen, that can be stopped by an hypersensitive response as the one caused by the product of Cf genes. *Alternaria* species are considered to be necrotrophic pathogens. This kind of pathogens directly kills the hosts cells and as such a hypersensitive response is counter probable. It would thus be feasible that *Alternaria* fungi try to "hi-jack" the Cf receptors and turn them into susceptibility factors. This is definitely we will keep in mind for further studies as well.

It would help to say if there are any quantitative modifiers of Hcr9 known in any of the published tomato systems. I know that a number of other tomato loci like PTO have quantitative modifiers found in the original mapping studies but never followed-up upon.

REPLY: There are indeed several papers that indeed observe minor differences in quantitative responses of the different Cf allles (see for example Kruijt et al, [20] in our manuscript). Similarly, for NLR resistance genes, different recombinant alleles show small differenes in the intensity of the HR that they produce (see Witek et al, [38] in our

manuscript). However we have not been able to assess the quantitative differences in our assay in detail. The authors of the manuscripts above used transient expression systems in *N benthamiana*, under growth chamber conditions. We performed infiltrations in whole plants, in a semi-controlled glass house. In our plants, quantitative differences could also seen between leaves (e.g. dependent on the age as shown in figure S Figure 5) and in a minor way, between experiments (e.g. dependent on the conditions in the glass house; on warmer days the response is stronger). Performing all our assays with adult (1.5-2m high) plants in growth chambers is not feasible.
Whereas we do think that this is a interesting phenomenon we decided not to address it in the manuscript, due to the length limitations.

Referee: 2

Comments to the Author(s)
This manuscript describes the diversity in recognition ability of Cladosporium fulvum avirulence genes and thus, resistance, in plants of different populations of Solanum chilense. The results reveal a striking diversity that is geografically structure, with southern populations being completelly susceptible to the pathogen. These results fit into a gene-for-gene evolutionary model and suggest the existance of costs of unnecessary resistance that the authors hypothesis to be related with trade offs with abiotic tolerance. Another stricking result of this work is the absence of correlation between the presence of homologs to Cf-9 and Cf-4 genes nd the recognition ability of the plant. This point to different recognition abilities of these genes in the species S. chilense comparade to other wild tomato species or with domesticated tomato. The authors speculate that the lack of recognition of Avr9 by plants expressing Cf-9 may be due to mutetions in the Cf-correceptors in southern populations. However, the authors disregard the possibility that mutations in S. chilense homologs also affect Avr9 recognition. The authors should sequence the amplified putative Cf-9 orthologs in responsive and non responsive plants in order to be able to assverate that the lack of response is due to co-receptors and not to the putative resistance gene. In fact, the authors should also sequence Cf-4 homologs in plants representing the different response possibilities (gene and response present; gene present but no response). This is a factible experiment since they can amplify those regions, and would give more light to the complex interactions that the observations suggest.

REPLY:
We thank the reviewer for this valuable suggestion and we agree that sequence data of *Cf* alleles would be very valuable, however we think the reviewer might have overlooked some important aspects and that in fact, it does not make much sense to sequence *Cf-9* and *Cf-4* in the non responding populations in regard to this study. There are several reasons for this.

1) We show in Figure 1 and Table S1 that these non responding southern populations not just lose the response to Avr9 and Avr4, but to the complete mix of avirulence factors secreted by *C. fulvum* (AF mix). As we mention in the introduction and the discussion, this mixture
contains likely over 70 potential avirulence proteins and we do identify several plants that show a response to this AF mix, but not to individual Avr4 or Avr9. If the response to all these Avrs is lost, it is very unlikely that this is the result of mutations in all individual receptors, like *Cf-4* and *Cf-9*, and more likely to be the result of mutations in coreceptors or other regulatory elements. We also wrote this in our result and discussion (now LINES: 290-291, 376-380).
Additionally, we have updated table S1 and created table S2, to illustrate these phenomena more clearly and added appropriate references to these tables in the main text (LINES 229, 235, 244, 250)

2) Sequencing of the receptors is not always feasible in our case. As we show in figure 4 that the presence of the *Cf*-4 gene region doesn't correspond to Avr recognition. Some populations respond to Avr4, but the canonical gene region for *Cf-4* is actually not present. If this is the case: how are we to sequence *Cf-4* if it might not even be present? And how informative would sequence information really be if we sequence one of the cases where it is present?

3) We in fact did try to sequence the full *Cf-9* gene from various populations. For this we used previously described primers (Hoorn et al 2001 and Kruijt et al 2005) and their recommended PCR settings. Note that the lead author of the Kruijt et al. paper is co-author in this study. When trying, we often got PCR products of with a size different from the expectations. When we got products with the right size, sequencing yielded very confusing results. In some cases sequencing results yielded genes with premature DNA stop codons and in some cases, it turned out that we sequenced a different gene or close paralog, more similar to a gene annotated as *Cf-19* in *S. lycopersicum*.
These combined results actually lead us to the hypothesis that the *Cf* gene family is subject to tremendous variation as the result of either historical or ongoing recombination events. I have recently observed this phenomenon in a related plant defence gene family (NLRs) in a wild potato species (see Witek et al 2020 BioRxiv, resubmitted to Nature Plants).

We chose not to present these data in this manuscript, first of all, because it is implicit to the data for *Cf-4* and explained under 2) and also, because it makes the manuscript even more complex without adding any real information on the effect of sequence diversity. The only way to resolve this issue completely is by targeted resequencing all *Cf* gene

orthologs in all plants with long reads. This is something that would be way beyond the scope of this submission and is in fact the topic of my latest submitted grant.

Besides, there are minor questions to revise:
Line 57. Change "(effectors)" by "(avirulence factors, Avrs)". Join this pragraph with the following one.
Line 60. "the seventeen wild tomato species known"
Line 61. Eliminate "to"
Line 74. Fusarium spp. (not spec)
REPLY: All done

Line 82. Eliminate "also called avirulence factors (Avrs)". Add "as avirulence factors, following the gene-for-gene model of interaction" to the end of the sentence. This sentence, should be moved after the sentence finishing in "secreted by C. fulvum".
Line 85. Eiminate "During evolution", subsituting the sentence "This recognition facilitates host resistance following the gene-for-gene model"
REPLY: We have rewritten the whole paragraph taking these two comments into account (LINES 79-91).

Line 94. Subsititute "Both genes" by Cf-9 and Cf-4
Line 95. Write "Homologues of Cladosporium resistance 9" before Hcr9
Line 98. This sentence is too taxative. Authors should nuance it
Line 109. Authors should mention here that regions of accessions and number of plants tested are described in Table S1.
REPLY: All done

Lines 112-114. This sentence and the following look contradictory. Authors should explain this better.
REPLY: We thank the reviewer for making us aware of this and now we have made changes in the text to make it clear (LINES 114-115)

Line 122. "A strain of C. fulvum race 5". Also, the authors should explain here the virulence attribute of this strain.
REPLY: We have added the information on virulence (LINES 125-126)

In general, it is not clear in material and methods how are the experiments done. Are all of them done in detached leaves? Only the infiltraton experiments are in detached leaves? And the spray experiments? They should also specify in mterial and methods the number of leaves or plants analyzed in ech experiment
REPLY: In the materials and methods section we now clearly state that we used adult plants for all experiments (LINES 131-133, 163-164). We also discussed that this gives our study an advantage and more reliable outcomes over previously published studies (LINES 337-345).

Line 135. Eliminate "(five plants per popultion, three per control", this is better explained below in the same paragraph. Ad "at" before 14 dpi
REPLY: done

Line 156. Are these detached leaves?
REPLY: No, we have done all assays on plants. We have stressed the used of plants in the methods (LINES 132-134, 164-165) .We also discussed that this gives our study an advantage and more reliable outcomes over previously published studies (LINES 337-345).

Line 166 "DNA samples were obtained" (not "was used")
Line 168. "analyzed in" not "analyzed by". Eliminated the "a" before PCR control
Line 189. I suppose it should be "in one responsive plant from LA3111" and "one each from non responsive southern populations..."
Line 202 TO 204. Insert "Plants from" before LA3111 and bEfore LA4330. Change "shows" by "showed" and "as" by "to"
Line 206. Change "a" by "the"
Line 211. Add "of this host" after "the populations"
Line 217. Move "For the inoculation" after "was used". Eliminate "to" after AF.
Line 288 as wel as BIR2
Line 317. Eliminate "probably due to loss of pathogen pressure in these locations". This is highly speculative and it is discussed further below
Line 322. by the small number of plants
REPLY: All done

Line 323. I do not understand this sentence. Explain better.
REPLY: When reading again, we understand why confusion may have arisen. In the second part of the sentence we meant to say that the proportions of Avr4 and Avr9 recognizing plants in each population were not documented in previous studies. That said, even when these fractions would have been reported, these might not have been very relevant or comparable to our outcomes. As we discuss in LINES 337-345, in previous studies the authors used young seedlings, which we show in our S Figure 5 with possible explanation of age effect in this kind of infiltration assays. We have now edited this paragraph to clarify these issues (LINES 331-345).

Line 324. Insert “a previous work,” before S. chilense
Line 326 insert “previous” before “study”
Line 349. We show the presence
REPLY: All done

Lines 349-351. The authors mix the presence of Ct-9 and Ct-4 homlogs and the response to plants to Avr9 and Avr4 to aseverate that they are not allelic in S. chilense, but in fact they have shown that the presence of the homolog does not correlate with the resistance reaction. Athors should correct the writing of this reasoning.
REPLY: We have now made our statement clearer (LINES 361-363) and we have added a S Table 3 to make the message clearer.

When the authors sequence the homologs of Cf-9 and Cf-4, there would be more arguments to be discussed about the conformation of these loci.
REPLY: As mentioned above, we are waiting to hear whether our proposal to do such sequencing using long read sequencing technology get approved.

In general, the authors should revise the whole text for verb tenses. They use either the present and the past. Normally, results should be written in past tense.
REPLY: We have corrected the tense used in the results.