Quantitative Resistance: More Than Just Perception of a Pathogen

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Review timeline:

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REPORT: (The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2016-00915-REV 1st Editorial decision – *accept with minor revision* Feb. 4, 2017

I agree with reviewer comments that the writing could be clearer and more concise in some places, but in general it seems an interesting and valuable contribution to the literature. I have made some comments and suggestions in the attached document. In addition, please address all reviewer comments to the best of your ability.

---------- Reviewer comments:

[Reviewer comments shown below along with author responses]

TPC2016-00915-REV1 1st Revision received Feb. 26, 2017

Reviewer comments and **author responses**:

Reviewer #1:

The basic content of this review is fine. Though it does not provide any major new insights, it is clear that a lot of work went into reviewing and synthesizing the literature in the field. The writing is not fine. Perhaps a little more leeway can be given for imprecise or bad writing in a research paper but in a review, clarity is paramount, and there are too many cases in this manuscript where clarity is lacking. There is a lot of overwriting also...the authors should try to be as concise as possible.

The review is rather wide ranging and perhaps it would be better to focus on one or two of the issues raised here.

> **RESPONSE: I have worked to change the entire manuscript to make the writing more concise. I would prefer to maintain the range of the review because I feel this is essential at this point in research on the topic of quantitative resistance. There are numerous concise reviews but from my reading, there is rarely an attempt, right or wrong, to span across the diverse areas to at least begin the discussion on a coherent thesis for quantitative resistance.**

L17. Abstract badly written. First sentence:...loci aren't the same as genes... what signal is being referred to..? 2nd sentence, explain what quantitative resistance is, what variation? 3rd sentence... I figure that polygenic means based on many genes, but it is hard for me to grasp what the phrase "highly polygenic suites of genes" conveys. 4th

sentence: just doesn't really make sense. Several other issues. This abstract needs to be completely rewritten...the phrase "These studies are developing an explosion in our understanding..." is dramatic but not good English...

RESPONSE: In the abstract, I have made it clear that the loci are caused by genes as I wish to keep loci (genetic regions found to affect the trait) and genes distinct as too often genes are used when loci is invariably meant. I have also worked to clarify that the community had been underestimating the number of genes possibly underlying the traits. Sentence four has been completely reworked.

L39- rather simplistic, arguably wrong, to say that "Plant hosts usually respond to biotrophic pathogens through local programmed cell death to inhibit pathogen proliferation". PCD is one aspect of a multi-faceted response. Sometimes PCD does not occur and in some cases where it does occur, its importance in resistance has been questioned.

RESPONSE: I have deleted this sentence as it is unnecessary.

L47: "strong resistance mechanisms to the defenses". I would say "robust means of overcoming the defenses...." Something like that....describing it as a resistance mechanism to a defence which is itself a resistance mechanism is a little odd. The last two sentences of this paragraph should be rewritten..."that can include the majority of tested plants" is an odd way of putting it...surely that depends on what plants you test... I'd say something more objective...methods enabling what?

RESPONSE: These sentences have been changed as suggested.

L52. Have to make clear what you're talking about here. In this case I think you're talking about a segregating population or possibly a collection of lines or ecotypes

RESPONSE: In this section I mean it in the context of any population.

L58 what does "determined by" mean here?

RESPONSE: This has been changed to the following "However, quantitative resistance governs the outcome of the vast majority of host-pathogen interactions."

L61...I think quantitative disease resistance loci have been being mapped for at least 25 years....

RESPONSE: I have added a statement to this effect. The goal of this was to say that in spite of the mapping, there is still much that is unknown. Including the simple question of how many genes underlie the trait.

L64 need to define RLK and NLR...

RESPONSE: This has been corrected.

L66" The diverse levels of complexity within quantitative resistance provides a ready barrier that has hindered detailed molecular analysis the causal genes." It's hard to know what to make of this sentence "diverse levels of complexity"?, "ready barrier"? why has it hindered the analysis? I think an "of" is missing

RESPONSE: I have changed this sentence to the following to clarify the meaning. "The multiple layers of biological complexity within quantitative resistance and technical complexity in identifying the underlying genes has hindered detailed molecular analysis of the causal genes."

L97. I disagree, to some extent. Quatitative resistance, to my mind, just means partial resistance. Quantitative resistance doesn't necessarily have to be distributed in a continuous way in a segregating population.... quant res could be based on the segregation of alleles at just one locus. I recognize that quantitative resistance can be defined in slightly different ways, phenotypically or genetically, and in this case the authors are assuming the genetic definition, which I think is the less common one.

The NIKs et al (2015) review that is referenced here talks about this very elegantly.

RESPONSE: There is some disagreement in the field as from my background with quantitative genetics, quantitative resistance is merely polygenic and quantitative. This definition is agnostic to the mechanisms by which quantitative resistance is achieved. I am hesitant to utilize the partial or durable resistance definitions as these imply that there is total resistance and stable resistance, which may not be true. In fact, I would argue that

the quantitative definition is both phenotypic and genetic while the partial resistance definition blends in mechanistic assumptions that may or may not be warranted.

L128. True, but a pretty large fraction...more than half I'd guess

RESPONSE: We think that we and the reviewer are disagreeing about the unit of fraction. For our sentence, we were focusing on the number of loci while we think the reviewer is focusing on fraction of variance. We have worked to clarify that these loci controlled more than half of the variation but that they are not a majority of the number of loci. We have also added the following conclusion "It remains to be seen if the identified loci represent the majority of causal loci or are a minority of the total number of causal loci."

L136… I'd like an example here… L139 an example would be good here too.

RESPONSE: I have added an example and expanded the grape example that is contained in the Fournier and Giraud 2008 and Johnston *et al.* 2014 citations.

L143. I think the point has to be made somewhere here that it is not that different genes are important in different populations- genes important for resistance in population A are very likely important for resistance in population B... it is just that alleles with contrasting effects need to be segregating in the population for the effect of a gene to be detected.

RESPONSE: We think that this request relates to the choice of terminologies for quantitative resistance. The genetic definition that we utilize requires *a priori* that a gene must have variation to control variation in quantitative resistance. While it is possible that there are absolutely conserved genes that have no variation and may be missed by this approach, there are key loci that show natural variation such as *COI1*, etc. We have worked to clarify that it is variation in quantitative resistance in this paragraph to minimize the confusion.

L222 "quantitative resistance loci are predominant in plant/pathogen interactions,..." Again, not sure what this means. I think that the authors' intended meaning is that " Quantitative resistance is the predominant form of resistance..."

RESPONSE: This has been corrected.

L244 This is arguably true but the conclusions in these papers are very tentative...

RESPONSE: We had caveated this paragraph at the end and feel that this is sufficient.

L249 Again the confusion of genes and loci. Also surely the smallest class would be the classes for which no genes were identified? Again, all the candidate genes identified in the papers mentioned here and in the following paragraph are *candidates*…it should be emphasized that these genes have not be definitively identified. So the authors should be careful to make too much of these connections.

RESPONSE: We worked to emphasize that a modest sample of candidate genes were tested using insertional mutants and that over 60% of these showed an effect on quantitative resistance. Thus, while the vast majority of the identified loci are candidates, the mutant sampling approach suggests that a large fraction of these candidates will affect the quantitative resistance phenotype. We have included this wording to clarify this aspect of the candidate genes to fully caveat them reflecting all the evidence "The candidate genes chosen for validation were randomly drawn from suggesting that there should be a similar rate of validation for the full candidate gene list. This same approach for calling candidate genes was previously shown to have a greater than 60% success rate while a corresponding analysis of genes with no GWA evidence showed almost no effect on the traits analyzed (Chan et al., 2011; Francisco et al., 2016). Thus, while the full gene list is not yet validated, it is likely that the experimental evidence supports the general observations from this list."

L277. Do the authors mean that it is not possible to identify every single gene with any effect at all on quantitative resistance? I suppose that will always be true. If it comes to that, I would argue that the main limitation may be the lack of segregation of functional alleles for certain genes...

RESPONSE: Actually, we do mean that it is formally possible that there are more causal genes within a population than it is possible to obtain sufficient recombination and line numbers to actually identify. Using natural populations, recombination and line numbers are more limiting than allelic variation based on our reading of the literature. We have included a direct citation to the work by Buckler and colleagues that show that NAM is still vastly underpowered to find all of the causal genes within that population.

L279 I think there is a misunderstanding here. First, what alleles are being referred to? Second, at any QTL that is associated with resistance, there will be one allele associated with increased resistance and another allele associated with increased susceptibility… that is true at every QTL.... not sure what point is being made here? Is it that resistant lines don't have resistance alleles at every QTL? That would be a point worth making and refers back to the classic studies on tomato fruit size (Large-fruit alleles from small-fruited lines)

RESPONSE: We have reworked this entire paragraph to make it clear that we are talking about transgressive segregation and how all alleles are not stacked as suggested by the reviewer. Then when you blend in multiple mechanisms, each with transgressive segregation, it might be beneficial to study individual mechanisms.

L307?? The paragraph is not well written in general. It starts talking about MAMPs and then goes to effectors, which are not MAMPs. It is not clear how these ideas are conntected (to me at least).

RESPONSE: This paragraph has been reworked to make it clear that the transition is from large effect MAMP/PAMP to the analysis of other effector derived signals to assess if these have a similar monogenic large effect behavior.

L312 and many places elsewhere elsewhere "polygenic response system". The *system* is not polygenic. The *inheritance* of the system is polygenic.

RESPONSE: Actually we had meant to blend the inheritance as well as the mechanism in the phrase to suggest that there may be multiple variable loci underlying the response in all plants and that this complexity was being overlooked.

The discussion of broad-spectrum and race-specific QTL could be shortened...basically, some QTL appear to be race-specific while others are associated with resistance to a whole species and others to several species....

RESPONSE: We have worked to improve the writing in this section as in the entire manuscript. We do however feel that while this is a relatively straightforward concept that we have seen confusion in the literature about this. With the term broad-spectrum being used to describe all of the above situations and we wanted to start a discussion about how broad spectrum is being misused. This is especially the case given the definition issue where quantitative resistance is sometimes being termed durable resistance, yet the loci being found do not support this claim.

Reviewer #2:

First, I welcome this review since there has not been, in my opinion, a good review that really tries to equate quantitative and qualitative resistance mechanisms. Irrespective of the enormous amount of effort and funding that has gone to investigation of qualitative resistance, I believe it is still an open question as to what relevance this has to quantitative resistance, which is what is seen predominantly, both in crops and wild plant species. Therefore, this review is important but needs to be complete.

In general, I believe the review does a nice job of identifying the various issues and supporting this discussion with pertinent papers. Hence, my criticisms below are brief but I also believe important.

1. First and foremost, I would ask that the authors consult the nice paper by Wang and Balint-Kurti that appeared in *Plant Physiology* in 2016. Similar to what the authors describe in this review, they found maize resistance QTL that mapped to genes apparently involved in lignin biosynthesis and, hence, at first glance, unrelated to known qualitative resistance pathways. However, upon further biochemical analysis, they showed that these lignin enzymes directly interacted with a resistance protein and, therefore, intersected with known ETI mechanisms. Besides being an interesting paper, this work raises the important caution that we cannot blindly formulate mechanistic hypotheses from gene annotations, an assumption that made throughout the current article. Genes annotated as being involved in vesicular trafficking, cell wall biosynthesis, etc., may indeed mediate their function in plant defense via this biochemistry or may actually be mediating their effects through unknown biochemistry that intersects known PTI and ETI pathways.

RESPONSE: These citations have been added and discussed. We did not use them in the section on polygenic nature of quantitative resistance but instead used them in the polygenic nature of pathogen detection as our reading of these papers suggests that their true importance is more to convince the community that pathogen detection will involve a larger than expected array of "sensors".

2. P. 13. I appreciate this discussion of the role of pathogen diversity but don't believe enough has been said about the role of plant host diversity. For example, it is well known that many QTLs are not reproducible when mapping studies are done with different parental populations. Indeed, some QTLs are not reproducible in different environments. Therefore, both host G and host GXE are important considerations. The authors need to stress this equally with their discussion of pathogen diversity.

RESPONSE: We have worked to improve this. We tend to disagree that lack of reproducibility is solely due to environmental effects as populations may simply differ in their genes with variable alleles. We have commented in defined areas that environment is part of the interaction but that we are not discussing that complication to maintain a focus on the complexity of simply the genetic component.

3. P14, one pet-peeve that I have is the often made claim that (previously well-known) phenomena are somehow new and can only be attributed to the recent advent of metagenomics studies of the microbiome. Microbial ecology was not invented with the advent of metagenomics. A case in point is the idea that members of the natural microbiome can affect the outcome of either symbiotic or pathogen infection. One paper that came rapidly to mind was the early work by Jo Handelsman (inventor of the term metagenomics) as part of her Ph.D. work in 1985. In this paper, she showed that Erwinia growing on alfalfa seeds could skew competition between different rhizobial strains. Hence, the presence of Erwinia was a major determinant of whether one strain was infective or not. There is no doubt that many other papers, perhaps before and after, have demonstrated similar findings. This is long before the advent of metagenomics.

RESPONSE: As per the suggestion of Reviewer 1, we have deleted this paragraph to help improve the focus within the manuscript. We agree with the reviewer that a single paragraph on this topic does not do it proper service.

Reviewer #3:

This is an interesting and insightful review of the current state of understanding of quantitative disease resistance in plants. The authors clearly have deep knowledge of the subject matter bring together a broad body of literature in their review, and make some intriguing points. The blending of insights from model plants and crops is particularly valuable. The figures do not reflect any new insight or addition to the literature, however.

RESPONSE: We were unsure as to what type of figure as we cannot quite yet figure out how to illustrate the concept of complexity in the 1000s of genes.

A missing citation is French *et al.* 2016, which gives a run-down on the implications of recent cloned loci implicated in quantitative disease resistance. [French, E., Kim, B.-S., & Iyer-Pascuzzi, A. S. (2016). Mechanisms of quantitative disease resistance in plants. *Seminars in Cell & Developmental Biology*, 56, 201-208. https://doi.org/10.1016/j.semcdb.2016.05.015]

RESPONSE: We apologize for having missed this citation. It is now included.

There seems to be a contradiction between the comments on lines 236-238 (saying that the alleles at genes downstream of NLR/RLK genes are likely diverse) and the comments in lines 288-291 (saying that they may be homogenized and irrelevant to quantitative resistance). Could both be true to whatever extent, but at least cross-ref/acknowledge the tension?

RESPONSE: We have worked better to make these not contradictory but complementary discussions. Basically response to a single signal is highly variable and polygenic and as such response to multiple signals is more polygenic and finally the outputs are equally polygenic. As such, the system is vastly more complex than current models account for. We hope that is now clearer throughout.

The review has a chatty tone, which has its charms but in some cases could be tightened up. Some sentences don't quite ake sense, implying a lack of editorial effort (e.g., Lines 190-191: "Mechanistic information also arose from a comparative transcriptomic study of how barley to study resists Stem Rust").

RESPONSE: We have worked to edit the entire manuscript to tighten it.

TPC2016-00915-REV1 2nd Editorial decision – *acceptance pending* **Mar. 2, 2017**

We are pleased to inform you that your paper entitled "Quantitative Resistance: More than just perception of a pathogen" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff.

Final acceptance from Science Editor **Mar. 16, 2017**