

**Mechanisms to Mitigate the Tradeoff between Growth and Defense**

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*Plant Cell. Advance Publication March 20, 2017; doi: 10.1105/tpc.16.00931*Corresponding author: Joy Bergelson [jbergels@uchicago.edu](mailto:jbergels@uchicago.edu)**Review timeline:**

<b>TPC2016-00931-REV</b>	Submission received:	December 14, 2016
	1 <sup>st</sup> Decision:	February 2, 2017 <i>revision requested</i>
<b>TPC2016-00931-REV1</b>	1 <sup>st</sup> Revision received:	February 23, 2017
	2 <sup>nd</sup> Decision:	March 2, 2017 <i>acceptance pending, sent to science editor</i>
	Final acceptance:	March 16, 2017
	Advance publication:	March 20, 2017

**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.)

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**TPC2016-00931-REV 1<sup>st</sup> Editorial decision – revision requested** **February 2, 2017**


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On the basis of the advice received, the board of reviewing editors would like to accept your manuscript for publication in The Plant Cell. This acceptance is contingent on revision based on the comments of our reviewers. In particular, please consider the following:

Thanks for this well-written article. Reviewers make fairly minor comments that could improve the manuscript. Both reviewers thought Figure 3 could be omitted - probably a good idea, unless it could be extended to add more of a synthesis of major events occurring. Please contact us if there are ambiguous comments or if you wish to discuss the revision.

----- Reviewer comments:

[Reviewer comments shown below along with author responses]

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**TPC2016-00931-REV1 1<sup>st</sup> Revision received** **February 23, 2017**


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Reviewer comments and **author responses:**

**RESPONSE TO EDITOR:** We have attempted to address the reviewer comments below (and in the manuscript).

Regarding the title of the manuscript, we respectfully wish to use a more general title that excludes the mention of *R*-genes. Two of the three major sections of the manuscript do not involve *R*-genes, hence we feel the broader focus of the current title is more appropriate.

Reviewer #1:

This review is timely in that it draws together latest information and ideas on the costs and benefits of harboring large panels of Resistance genes in plants. It highlights the need to maintain immunity/growth homeostasis and how R gene/protein mechanistic and interaction data can now be rationalized at the level of R locus composition and behaviors of plant genomes and populations in nature. At several points in the review the authors identify the importance in future work of investigating the adaptive significance of R gene architectures, co-regulation and functions in the field. Their mention of new directions in plant-microbiota research to address the extent to which host genotypes (together with environmental factors) shape resident microbe communities as part of an adaptive process is helpful. Overall, the analysis is balanced and well-structured. It is likely to be informative to a broad set of

researchers in immunity, population genetics, ecology and genome biology. Several suggestions below to improve the text/clarify points:

Point 1. In Section III (R-genes) it would be good to see mention of R-gene mutations in eg. SNC1, CHS3 and RPP4, that confer environment-specific growth defense trade-offs (Plant J. 2010 Jul;63(2):283-96. doi: 10.1111/j.1365-313X.2010.04241.x.Epub 2010 Apr 28., PLoS Pathog. 2010 Apr 1;6(4):e1000844. doi: 10.1371/journal.ppat.1000844., Plant Physiol. 2010 Oct;154(2):796-809. doi: 10.1104/pp.110.157610. Epub 2010 Aug 10.) suggesting that trade-offs observed under laboratory conditions might have a different outcome in field conditions. This is in line with what the authors propose in the conclusion. An important addition would be New Phytol. 2017 Jan;213(2):900-915. doi: 10.1111/nph.14155. Epub 2016 Sep 2, showing that Arabidopsis lines harboring necrosis-inducing alleles of ACD6 impaired in lab conditions did as well as reference lines in the field.

**RESPONSE: References added.**

Point 2. Fig 1 helps to make the point that R-proteins seem to be highly connected and consistent with the few molecular studies of plant NLR R-protein functional interactions showing they often monitor/guard other modified host proteins. However, it is important to state that the interactome network analysis is inferred mainly from yeast 2-hybrid associations and most have so far not been functionally validated.

**RESPONSE: Text added.**

Point 3. Fig 2 makes a speculative point about the benefit of studying R-gene linkage using a hypothetical locus. It would make more sense to show linkage with a known example. This would allow the authors to suggest more strongly that this type of analysis provides a good basis for identify co-evolving, potentially co-functioning pairs.

**RESPONSE: Figure with known example now included (Figure 2).**

Point 4. Fig 3 is not informative. It doesn't show potential outcomes of the relationship between classical immunity responses to external pathogens and the role/recruitment of other microorganisms. Given the number of recent in-depth reviews on plant microbiota and multitrophic interactions, this doesn't add anything.

**RESPONSE: Figure removed.**

Point 5. In 110 'optical allocation of defense is not static...'. This is an interesting point but is there published evidence for it which could be cited?

**RESPONSE: References added.**

Point 6. In 370 - 374. Is it really clear that de-methylation/methylation status 'drives' changes in gene expression of R and immunity genes? Drives implies this is causal rather than consequence/associated with other important triggers? Some clarity needed here to support claim.

**RESPONSE: Sentence changed to "DNA (de)methylation can regulate gene expression in response to both pathogen infection (Downen et al., 2012; Yu et al., 2013; reviewed by Deleris et al. 2016)".**

Point 7. In 434 and paragraph. Authors have described beforehand evidence from many studies for the importance of transcriptional control of R-genes in mitigating defense-growth tradeoffs. This doesn't sit easily with the statement that the deleterious effect is not due to R-gene mis-expression but rather R-protein properties. Some rewording needed to clarify the authors meaning and would be helpful to give the examples where this is shown to be the case and its molecular basis.

**RESPONSE: Sentence deleted.**

Reviewer #2:

The authors review some of the mechanisms that can contribute to mitigate the growth-defense tradeoff, with special emphasis in the R-genes. In general, I found the manuscript hard to read and follow. The manuscript overall can be shortened and tightened. There are multiple areas in which appropriate references are lacking. These should be

added. Also, in many places, substituting lengthy wording for information would make this a much more interesting paper to read. I point out some such instances below.

My main concern is the repeated use of teleological phrasing throughout the manuscript. This should be removed. The title of the manuscript should reflect the focus on R-genes. As it stands, I find it misleadingly general. Maybe something along the lines of "The Role of R genes in mitigating..." Or "Mechanisms to mitigate the growth-defense tradeoff: the R-genes as a study case". The subtitles in general are too long and wordy. The authors use immunity and defense interchangeably. This might very well be the common use in the plant pathology community but it should be clarified somewhere at the beginning of the text (and possibly be justified/explained). In my opinion, Figure 3 is rather simplistic and not needed. However, there are two sections in the manuscript that could benefit from a figure. These are in lines 153-159 and 321-330.

Point 1. L 11. Teleological phrasing implying there is a purpose in evolution should be removed.

**RESPONSE: Sentence removed.**

Point 2. L 20. Teleological phrasing. L 23. Teleological phrasing.

**RESPONSE: Sentence changed to: "The fine-scale regulation of R-genes is critical to alleviate the burden of their expression, and furthermore the genomic organization of R-genes into co-regulatory modules reduces costs. Plants can also recruit protection from other species."**

Point 3. Introduction, L 29-29. "It is hardly surprising..." → I find hard it to understand this sentence. Could just remove.

**RESPONSE: Sentence changed to: "That this metabolic expenditure entails a cost with a concomitant decrease in growth and/or reproductive output is hardly surprising."**

Point 4. L 51-54. Move section of ecological costs down to next paragraph (L55 on), which needs to be tightened.

**RESPONSE: Section rearranged and rewritten.**

Point 5. L 75. "it is reasonable to imagine" → I find this quite speculative.

**RESPONSE: Reworded. "Both growth and immunity involve the expression of thousands of genes (Thilmony et al., 2006) and the synthesis of myriad compounds (Bennett and Wallsgrove, 1994). If nutrients are limiting, then allocation to defense will come with a reduction in allocation to growth."**

Point 6. IB. Ecology/life history and the optimal growth-defense relationship → It is made clear in the review that optimality is hard to achieve in complex responses and their correlates. I would remove references to optimality from the heading. Suggestion: Ecology, life history, and the growth-defense relationship

**RESPONSE: Done.**

Point 7. IC. Co-regulation of growth and immunity → The paragraph starts with "Evolutionary reasons..." and it is unclear which evolutionary scenarios are discussed, if any.

**RESPONSE: The preceding paragraph discusses possible reasons for the coordination.**

Point 8. L 153-159. A figure to illustrate the relationships among these different hormones would be helpful. L 161. I find this sentence hard to understand. Remove "it comes at no surprise".

**RESPONSE: We respectfully disagree.**

Point 9. L 168. Add explanation of why the statement would be 'questionable and highly unlikely', or remove this clause.

**RESPONSE: We argue that it is unlikely given the extensive conservation of this co-regulation. "the tight co-regulation of phytohormones, at least in the case of SA and JA, is largely conserved across land plant species, suggesting that their co-regulation is important for fitness (Thaler et al., 2012)".**

Point 10. L 169-170. Please be more specific on the degree of conservation of SA and JA. Across Angiosperms? Land plants? All green plants?

**RESPONSE: Done (Land Plants)**

Point 11. L 173. What exactly is a 'pervasive mechanism'? It would be better if more specific information is provided and leave to the reader decide how pervasive it is.

**RESPONSE: In the previous paragraphs, we detail how many hormones are co-regulated, and how the co-regulation of SA and JA is preserved across land plants. These details warrant the description 'pervasive'.**

Point 12. L 180. This line has many issues. Perhaps the most important is that the use of 'to' has teleological implications. Suggestion: Plants employ many strategies that reduce allocation towards defense. One such strategy is controlling the timing and duration in which the immune response is active.

**RESPONSE: Done.**

Point 13. IIA. The use of induced (downstream) responses upon attack. L 187-197. I like this paragraph. It is informative and concise. L 200. "Plants have therefore evolved additional adaptive strategies to fine tune...!!" This sentence is as teleological as it can get. Plants did not evolve anything with any purpose. This is not how evolution works.

**RESPONSE: We have reworded to: "Plants have evolved additional mechanisms that fine tune metabolic expenditures on defense."**

Point 14. L 300. "... exposed to a broad diversity of microbiota and changing environmental conditions."

**RESPONSE: Section reworded.**

Point 15. L 302-304. "Hence...seedlings and in the laboratory." I do not understand this sentence.

**RESPONSE: Reworded: "Hence, it is possible that some components of priming machinery are triggered in all field settings. An unprimed state could be limited to recently germinated seedlings or to the laboratory."**

Point 16. It should be acknowledged that micro-RNAs targeting certain genomic areas did not necessarily evolve de novo in all cases, but rather this kind of regulation is likely to have preceded at least some speciation events (i.e., is the ancestral state).

**RESPONSE: It is unclear how to incorporate the described information.**

Point 17. The argument for the regulation being adaptive should to be provided explicitly.

**RESPONSE: Changed to: "More broadly, R-gene expression has been found to be induced by several abiotic stresses which perhaps indicates adaptive regulation."**

Point 18. IIIC. When regulation of expression is not enough: the genetic load of the R-gene surveillance system 1. The cost of the system: L 385-L389. References should be added. Without these, this paragraph is quite speculative.

**RESPONSE: Several references are provided.**

Point 19. L 437-440. I do not understand. Findings of genetic load in *A. thaliana* might indicate that this possible in other systems but there is no information that I know of that allows to state that findings in one species are 'generalizable'. The rest, I do not understand.

**RESPONSE: We reference several other studies demonstrating autoimmune genetic interactions similar to those we reference for *A. thaliana*. These results are consistent with the genetic load in *A. thaliana*.**

Point 20: IIID. Insight on function from evolutionary history; L 471. Either here or above it would help to specify how many R-genes have been described. Why or how are these among the most diverse plant genes?

**RESPONSE: The origin and maintenance of this diversity is discussed in detail in several of the listed references.**

Point 21. L 472. Selection also could generate the opposite effect (lower diversity). Could drift contribute to this process?

**RESPONSE: The origin and maintenance of this diversity is discussed in detail in several of the listed references.**

Point 22. L 475. Other processes also leave signatures in the genome.

**RESPONSE: The origin and maintenance of this diversity is discussed in detail in several of the listed references.**

Point 23. Section IV. Getting something for nothing → I am not sure I understand what the authors try to communicate with this heading.

**RESPONSE: We are trying to convey the idea that tritrophic interactions can reduce the physiological cost of prevent pathogen attack.**

Point 24. L 572. 'pathogens'. This is the first mention of soils in the manuscript. It sure feels like this is coming out of nowhere.

**RESPONSE: Changed to: "Studying the immune responses of plants exposed to pathogen in the presence or absence of protective microbes or commensal invertebrates could provide evidence for how plants use their immune systems in the presence of other beneficial organisms."**

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**TPC2016-00931-REV1 2<sup>nd</sup> Editorial decision – *acceptance pending***

**March 2, 2017**

We are pleased to inform you that your paper entitled "Mechanisms to Mitigate the Tradeoff between Growth and Defense" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff.

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**Final acceptance from Science Editor**

**March 16, 2017**