Molecular Profiles of Contrasting Shade Response Strategies in Wild Plants: Differential Control of Immunity and Shoot Elongation

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Review timeline:

TPC2016-00790-RA	Submission received:	Oct. 14, 2016
	1st Decision:	Nov. 21, 2016 *accept with minor revision*
TPC2016-00790-RAR1	1st Revision received:	Dec. 20, 2016
	2nd Decision:	Jan. 6, 2017 *accept with minor revision*
TPC2016-00790-RAR2	2nd Revision received:	Jan. 10, 2017
	3rd Decision:	Jan. 10, 2017 *acceptance pending, sent to science editor*
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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.)

TPC2016-00790-RA 1st Editorial decision – *accept with minor revision* Nov. 21, 2016

We have received reviews of your manuscript entitled "Molecular profiles of contrasting shade response strategies in wild plants: differential control of immunity and shoot elongation." 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:

Although the goal of "finding mechanisms" has not been accomplished and the specific genes studied are not shown to be causal for the difference in shade avoidance in these species, the reviewers and editors support communication of this well performed study but ask the authors to downplay the hyperbole and address several issues. The authors are urged to modify the text to align objectives, data and conclusions, particularly in line with the criticisms of Reviewer 3. The authors should be careful not to extrapolate too far from a single shade tolerant and shade avoiding species comparison, and to acknowledge the earlier work by Harry Smith and colleagues on this topic, i.e. Morgan, D.C., and Smith, H. (1979). A systematic relationship between phytochrome-controlled development and species habitat, for plants grown in simulated natural radiation. Planta 145, 253-258; Gilbert, I.R., Jarvis, P.G., and Smith, H. (2001). Proximity signal and shade avoidance differences between early and late successional trees. Nature 411, 792-795). Other considerations of the three reviewers should be acknowledged in the revised manuscript, as these should improve the manuscript by providing suitable caveats for what conclusions are solid, and what questions remain to be addressed. In addition, the authors need to clarify the description of replicates and statistical analysis of RT-PCR presented in Figure 4C, e.g., how many biological replicates were used and to clarify the precise meaning of n=5. The authors need to make this more explicit in this figure and also check the other legends to ensure that the information on biological replicates is provided.

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

[Reviewer comments shown below along with author responses]

TPC2016-00790-RAR1 1st Revision received Dec. 20, 2016

Editor comments [shown in decision letter above] and **author responses**:

RESPONSE: We have referenced these studies on shade tolerance in the introduction by modifying the text (lines 89-97): "Indeed, variation in low R:FR-induced stem elongation rates have been documented between (Morgan and Smith, 1978; Gilbert et al., 2001) and within species (Sasidharan et al., 2008; Filiault et al., 2008). Species from forest understories, in addition to suppressing low R:FR-induced stem elongation have evolved shade tolerance strategies, such as optimal leaf morphology for low light photosynthesis (Morgan and Smith, 1978) and tolerance to biotic and abiotic stresses (reviewed in Valladares and Niinemets, 2008)."

Reviewer comments and **author responses**:

Reviewer #1:

One very interesting aspect of shade avoidance is the fact that some plants are shade tolerant where others are shade avoiders. The mechanistic bases for these differences are mostly unknown. This paper represents a first step in trying to address the basis of these differences. The authors study two species of geranium, one is a shade avoider and the other is shade tolerant. Interestingly they show that both have an initial growth response to shade but the shade tolerant one then slows its growth so that there is no net change between shaded and control after a day. This is an interesting finding because it shows that the shade tolerant species can sense shade but that it responds differently. The authors then investigate transcriptional responses to shade in both species at an early and late time point. Consistent with the elongation result, the shade avoiding species shows a much larger transcriptional response to shade, especially at the later time point. Shade avoiding plants often dampen their immune responses, perhaps due to a trade off between growth and defense. The transcriptional profiles lead to a hypothesis that the shade tolerant species will not dampen its immune response in shade; this is tested and verified. For the final experiments, the authors examine transcriptional profiles to find candidate upstream genes that are differentially regulated between the two species. They then test mutants in the Arabidopsis homologs of these genes for shade avoidance defects. All three have phenotypes, meaning that three new shade avoidance genes have been identified. Interestingly, mutants in one of these genes do not affect induction of the downstream marker genes IAA19 and ATHB-2, suggesting that it is working independently of these core genes. Importantly they show reduced elongation in these mutants in specific to low R:FR; the mutants elongate at least as much as wild type in response to IAA and low blue. The manuscript is very clearly and logically written. The experiments are sound and for the most part interpreted appropriately.

Point 1. The abstract and discussion exaggerate the conclusions that can be drawn. In both sections the authors imply that the candidate genes validated in Arabidopsis could be responsible for the differences in shade avoidance responses between the two geranium species. There is no evidence to support this claim. The authors show that these genes are regulated differently in the two species, not that they have different intrinsic activity levels or functions. The presented evidence suggests that these genes are downstream of the causative difference. Finding the genes(s) that are actually causative for the difference in shade avoidance would be outside the scope of this study and I am NOT requesting that this be done. Instead reword the manuscript to be consistent with what can actually be concluded.

RESPONSE: Abstract: we have rephrased the final line (lines 38–39) to tone down the claim and it reads now: "We propose that these components may be associated with either or not showing shade avoidance responses".

Point 2. No mention is made of data availability. The raw reads, assembled transcripts, and gene expression levels for each sample must be deposited in a public repository such as NCBI or EMBL.

RESPONSE: We have submitted the Geranium raw sequencing files and assembled transcriptomes (TSA's) to the European Nucleotide Archive (ENA, EMBL), and expression data are available at Array Express (EMBL). The accession numbers have been added to the manuscript. In case these numbers are still modified at EMBL, this should happen within the next week and we will still correct that.

Reviewer #2:

It was a pleasure to read through this latest submission by the Pierik lab. By comparing different Geranium species adapted to different habitats this well executed study elucidated two contrasting shade response strategies. *G. pyrenaicum*, an "open habitat" plant responded to simulated vegetation shade (low R:FR) in a similar manner to Arabidopsis- with marked (petiole) extension growth at the expense of immunity. *G. robertianum*, an understory plant,

lacked the elongation response, but instead exhibited heightened defence responses. The manuscript is well written, it proceeds in a logical order and the main scientific claims are well supported by data.

Point 1. Figure 1D shows that FR is effective in eliciting a period of petiole elongation in *G. pyrenaicum* when provided at any time during the day. This is not the case for *G. robertianum*, where timing matters. To me the *G. robertianum* response has the hallmarks of a circadian-gated response, while *G. pyrenaicum* does not (although the second peak may be clock dependent... its hard to see?). The authors do not mention this even though the data are clear. Given that the *G. robertianum* response could be circadian-gated, does the transcriptome data support this notion? e.g., are there more clock-regulated genes in the 2-h *G. robertianum* samples?

RESPONSE: We are aware of the fact that this response seems clock-dependent. However, we did not find strong evidence for clock components in the transcriptomes, and therefore we did not follow up in detail. Nevertheless, because of this apparent role for time of day, we did implement the 'clock' in the summarizing Figure 10.

Point 2. On P9 line 187 I was confused by the term "positive species*treatment (thus higher fold changes for *G. robertianum* than *G. pyrenaicum*). Could this please be simplified?

RESPONSE: We have rephrased the sentence, which makes this comparison clearer.

Point 3. In Figure 4 the authors show that in *G. pyrenaicum* low R:FR suppresses the expression of meJA-induced genes, and compromises plant defence response to *B. cinerea*. In *G. robertianum* low R:FR is less effective in repressing meJA-induced genes, and these plants are more resistant to *B. cinerea*. The authors however, do not comment on the greater susceptibility of *G. robertianum* to *B. cinerea* in control conditions, even though expression of meJA-induced genes is higher. Thus the correlation between gene expression and infection is not true for all conditions tested. [Also, *G. robertianum* has a lower me-JA response.] As the genome-wide data broadly support the assertions made- perhaps a more solid case could be made by plotting the low R:FR induced expression patterns of defence pathway clusters at 2 h and 11.5 h in the two species?

RESPONSE: We are aware of the fact that susceptibility in control situations is higher in *G. robertianum* compared to *G. pyrenaicum*. This may not necessarily have to do with gene expression differences, but is likely associated with different leaf morphologies between the species: *G. robertianum* has much thinner cotyledons than *G. pyrenaicum*, which makes the infection by the pathogen more effective. Direct comparisons of absolute lesion sizes between species is, in general, very difficult. Therefore, we prefer to compare only between treatments within species. As suggested by the reviewer, and in order to support the assertions made, we have plotted the expression patterns of a number of OMCL groups from these defence clusters presented in Fig. 4A. These graphs are shown in Fig. S5A.

Point 4. KDR, THE1 and FER1 emerged from the transcriptomic data as candidate regulators of low R:FR induced elongation in *G. pyrenaicum*. The authors provided genetic data to support this claim, identifying these genes as novel SAR components in Arabidopsis, that potentially operate independently of auxin. Although expression data are shown it would have been useful to see a timecourse — to establish whether the dynamic regulation of these genes is conserved in Arabidopsis and *G. pyrenaicum*.

RESPONSE: The time points for expression data in Arabidopsis have been chosen based on earlier studies on Arabidopsis in the host laboratory where several SAS marker genes were tested, and peaked after 4 hours of FR-enriched light (De Wit et al. 2016, Curr. Biol.).

Point 5. Genetic data suggested that PIFs (especially PIF7) are implicated in shade-induced KDR, THE1 and FER expression (especially in hypocotyls). The authors did not test if this was direct, but given the novel insights this does not detract from the study.

RESPONSE: Indeed, we consider this as one of several interesting leads for future work.

Reviewer #3:

Shade-tolerant plants show poor shade-avoidance responses when exposed to low R:FR, typical of shade. As argued by Gommers et al., identifying the molecular mechanisms underpinning the suppression of these responses in shade-tolerant species has great potential to understand different plant strategies and to inform crop-breeding programs.

To address this interesting issue, Gommers et al. compare growth, immunity and transcriptome responses to low R:FR in two species from contrasting habitats: *Geranium robertianum* (forest understory) and *Geranium pyrenaicum* (open habitats). Then, Gommers et al. select three genes that had not been previously included among those related to shade-avoidance responses and characterize Arabidopsis loss-of-function mutants or overexpressors of these genes. The experiments were carefully conducted and the results provide useful information. However, in its current status the work shows some limitations.

Point 1. To the best of my knowledge the lack of immunity responses to R:FR in shade-tolerant responses has not been reported before, which makes the results presented here potentially very interesting. However, we cannot make a general statement by comparing only two species. These two species may have differences not necessarily linked to their condition of shade tolerant or shade intolerant plants. As a matter of fact, the basal levels of immunity under high R:FR appear much lower (larger lesion diameter) in *G. robertianum* than in *G. pyrenaicum* (Fig. 4D). Furthermore, the induction of the expression of immunity-related genes by MeJA is reduced also much weaker in *G. robertianum* than in *G. pyrenaicum* (Fig. 4C). It would be necessary to conduct one of these tests in several species of each class to provide a more general picture. The present result is only descriptive of the response pattern in two different species.

RESPONSE: Indeed, a two-species comparison does not cover all species. Nevertheless, the results in the shade avoider *G. pyrenaicum* are consistent with previous results in other shade avoiding species such as Arabidopsis and tomato. A direct connection between the lack of the shade avoidance response and lack of defence suppression, like we show for *G. robertianum*, has never been shown before, but ecological studies have in the past found a correlation between seedling shade tolerance and disease resistance (Auspurger 1984). As for the constitutive differences between the two species, see reply to point 5 by reviewer 2. In these experiments we were mostly interested in the effect of the low R:FR light treatment, which is clearly opposite for the two species. Additional studies on a wider variety of species would give more knowledge on the generality of the contrasts identified here, but lie beyond the scope and feasibility of this manuscript. We have carefully gone through the manuscript and revised such generalisations.

Point 2. While in the Introduction Gommers et al. convincingly argue in favor of the convenience of identifying the molecular mechanisms underpinning the suppression of shade-avoidance responses in shade-tolerant species, the results partially deviate from this aim. Actually, the work identifies genes necessary for shade avoidance, rather than genes that suppress shade avoidance. These genes were selected by the correlation of their expression with the occurrence of growth responses to low R:FR in both species. Therefore, we could conclude that the shade-tolerant species likely fails to show a promotion of petiole growth by low R:FR in part because this treatment fails to promote the expression of growth-related genes, including FERONIA, THESEUS1 and KIDARI. While this information is important, it would be interesting to know why these genes fail to respond in *G. robertianum*, i.e., the mechanisms of suppression. Furthermore, *G. robertianum* actually shows a negative growth response to low R:FR at 11.5 h, which is necessary to compensate for the normal promotion observed earlier during the day. This compensatory response remains unaccounted. Perhaps the authors could explore among the genes expressed in *G. robertianum* and not in *G. pyrenaicum*. At the very least, the function and relative position of the selected genes in the context of other genes involved in shade avoidance should be defined with more precision.

RESPONSE: The reviewer provides excellent ideas for further studying the mechanisms of regulation of KDR, THE1 and FER in *G. robertianum*. Unfortunately, until now, we have not been able to successfully create transgenic lines of these two species. We used the model Arabidopsis to prove that proper regulation of these candidate genes is necessary to induce a full shade avoidance response in different plant organs. Nevertheless, future studies will search for SAS-inhibitory regulators that may or may not differentially regulate the genes mentioned here.

Exploration of the genes up-regulated at the late time point in *G. robertianum*, but not *G. pyrenaicum,* did not provide any clear expression patterns to be correlated with growth, but did provide the list of JA- and SA-associated gene ontology terms, which is presented in Fig. S4 and 4. Nevertheless, this is a topic that we hope to include in follow-up projects.

In the Discussion, and using Figures 8 and 9, we discuss the relative position of the selected genes in the context of other shade avoidance genes. We discuss that they are specific to low R:FR and not controlling low blue responses,

and that they are not downstream of the major shade avoidance hormone auxin. We discuss that THE1 expression is PIF7-dependent and that it likely represents a branch of regulation that is independent of classic SAS genes, such as IAA19 and ATHB2. THE1 also does not seem to affect expression of KDR. We also discuss that FER, the other RLK identified here for SAS, does seem to be involved in ATHB2 upregulation during low R:FR conditions, indicating an (upstream) interaction with at least part of the canonical SAS pathway.

TPC2016-00790-RAR1 2nd Editorial decision – *accept with minor revision* Jan. 6, 2017

We apologize for our delay due to holidays. The reviewing editor had initiated a brief consultation on your revised manuscript with the three reviewers, but unfortunately one of the reviewers is unavailable until Jan 9th. We therefore have decided to proceed with an editorial decision. Two reviewers and the editors agree that the revised manuscript is of the quality and significance commensurate with publication in The Plant Cell. Reviewer 3 raised a couple of issues that follow up on their earlier review (see below). We would like to give you the opportunity to address their concerns prior to formal acceptance of your manuscript. This acceptance will be determined by the editors without the need for additional reviewer input.

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

[Reviewer comments shown below along with author responses]

TPC2016-000790-RAR2 2nd Revision received Jan. 10, 2017

Reviewer comments and **author responses**:

Reviewer #1:

My concerns have been appropriately addressed by this revision.

Reviewer #3:

Following the comments on the previous version of the manuscript, the authors have introduced some modifications in the text. Although in the revised version of the paper the objectives, data and conclusions look more consistent, I think that some specific issues require further attention.

Point 1. The authors argue that "high biotic stress resistance is considered an important aspect of shade tolerance, protecting fixed carbon which is scarce in low light (Augspurger, 1984; Valladares and Niinemets, 2008)". However, the latter statement is contradicted by the observation that the shade tolerant species that they use here is actually more susceptible and not more resistant (Figure 4D). I would simply avoid the above comment. In order to provide a clearer picture to the reader, I would also note in Results that *G. robertianum* shows higher background susceptibility to *B. cinerea* than *G. pyrenaicum* (that might be related to differences in leaf structure).

RESPONSE: We removed this statement (line 335).

Point 2. Also, the authors argue that "JA-mediated defences are repressed by low R:FR in *G. pyrenaicum*, but enhanced in *G. robertianum*". What are the genes that follow this pattern in *G. robertianum*? In Figure 4C, PA1 tends to go in the proposed direction but, according to the letters indicated in the figure, the differences between meJa and meJA + low R:FR are not significant. It would be more adequate to state simply that *G. robertianum* lacks the reduction in defenses observed in *G. pyrenaicum*.

RESPONSE: To prevent this from becoming a very wordy piece, we have followed the suggestion to simply state that "*G. robertianum* lacks the reduction in defenses observed in *G. pyrenaicum*" (Lines 214-215).

Point 3. Regarding my request to define with more precision the function and relative position of the selected genes in the context of other genes involved in shade avoidance, I do not find any highlighted modification in the text to address this issue. I am not saying that that information provided by the authors is incorrect. The problem is that the message is not clear. After reading the paper I had to go back to Results and draw my own scheme, where I place KDR and THE1 under the positive regulation of PIFs (based on Figure 9) and KDR, THE1 and FER as positive

regulators of growth (based on Figure 7). If this is correct, it might be useful to show it in Figure 10 (model). However, I have doubts because based on previously published data (Figure S9), the authors argue that these genes do not respond strongly to shade signals.

RESPONSE: Indeed, the original text already held this information, so we had not changed this. We have considered adding PIFs (and auxin) to the scheme, but this makes it very difficult to comprehend. The scheme now nicely shows the differential regulations between the two species. Including PIFs, although interesting, would also complicate things since we do not know whether PIFs are differentially regulated between the two species. All we know is that *KDR* and *THE* expression in Arabidopsis is PIF-dependent. We have added this remark to the legend of the summarizing Fig. 10. We have also implemented two additional (recent) references (Das et al., 2016; Kohnen et al., 2016) that support our findings of *THE1* and *FER* induction in shade (line 316–320).

Point 4. At several places the authors refer to "phenotypes" without indicating the actual effect on growth, which would be more informative.

RESPONSE: We have searched through the entire document and specified where appropriate as per the reviewer's suggestion: lines 171, 270, 303–304).

Point 5. I do not think that the authors need to conclude that the effects are specific of R:FR treatments because the blue-light response also shows some effect of the mutations.

RESPONSE: Even though variation exists among the genotypes when exposed to blue-depleted light, and not all respond similar to WT, there is no consistent effect of different mutations of the same genes or complexes (*the1-1* vs *the1-4*, *fer5* vs *llg1-2*). This led us to conclude that growth in this treatment, unlike low R:FR, is not clearly affected by the RLK mutations, and the effect is thus rather R:FR-specific. We have rephrased the sentence in the results to make this more clear and the statement more modest (line 285–290).

TPC2016-00790-RAR2 3rd Editorial decision – *acceptance pending* Jan. 10, 2017

We are pleased to inform you that your paper entitled "Molecular profiles of contrasting shade response strategies in wild plants: differential control of immunity and shoot elongation" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff.

Final acceptance from Science Editor Jan. 30, 2017