HY5 and phytochrome activity modulate shoot-to-root coordination during thermomorphogenesis in *Arabidopsis*

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MS TITLE: Shoot and root thermomorphogenesis are linked by a developmental trade-off

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I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

This paper studies thermomorphogenesis in roots. The latter term was coined by analogy to photomorphogenesis and reflects the existence of a developmental program carrying out responses to temperature. The process has been reasonably well characterized for shoots but far less so for roots. What is known so far suggests that the shoots and roots run at least partially distinct programs. Given that temperature along with light and water is a major driver of plant development, learning more about the process in roots is worthwhile and likely to interest readers of Development.

The authors focus on the transcription factor HY5, which is one of the players previously implicated in shoot thermomorphogenesis. They show that roots in various hy5 backgrounds have a diminished response. They show that a diminished response also occurs in a phyab double mutant. Importantly, while these mutants have a diminished root growth rate response, the change in meristem length is unaffected or even exaggerated, potentially separating these two aspects of thermomorphogenesis. The authors confirm that a loss of function mutant for pif4 has a wild type response (as previously reported by Martins et al.) but interestingly show that a PIF4 over-expressor has a diminished growth response, in keeping with the elucidated pathway in shoots. The authors then show that there is a nice, negative correlation between root and hypocotyl growth at 27C. This leads the authors to invoke the notion of a trade-off between shoot and root growth, so when the shoot responds a lot, the roots respond only a little.

That the effect of HY5 is exerted at least in part in the shoot is shown by transforming hy5 with the wild type copy driven by a shoot-specific promoter and finding partial rescue of the root phenotype.

The authors next look at the transcriptome at 4 and 18 h after a temperature shift. They find a core pathway of genes that change regardless of genotype, but they also find a large number of genes that are mis-regulated in the same with in both hy5 and phyab, supporting the idea that these both work in the same pathway. Finally the authors implicate auxin by finding that root thermomorphogenesis is also aberrant in several auxin mutants.

Altogether I found this paper fascinating, particularly as it brings up the integrated function of the plant. But in some cases I found the interpretations confusing or incomplete. I think all of these problems are readily addressed but some modest revision, tho in one case a simple experiment would be useful.

Comments for the author

In thinking about this paper, there seem to be two strands that are tangled up. The first is whether HY5 is active in the root. This gets tangled because a phrase such as “a pathway regulating thermomorphogenesis in the root” has two meanings: one is pathways that act in the root and another is pathways acting anywhere, in the shoot for example, that determine the behavior of the root. I am not clear from the author’s language whether they are suggesting that HY5 is acting in the root. Or is affecting the root based on manipulating the shoot. Some clear statement of this would be helpful.

But it would be even more helpful to know whether HY5 acts in the root. The authors attempt to answer this question by expressing HY5 in only the shoots of a hy5 mutant. But the results are ambiguous the rescue is partial. Does this mean that HY5 acts partly in the root? According to the images provided CAB3 is off in the hypocotyl, and so the partial rescue could mean that HY5 needs to be on in the hypocotyl in addition to the leaves/cotyledons. As for real experiments, it might be more convincing to express HY5 in the hy5 mutant root specifically, but that of course is a lot of work. A simpler experiment would be to follow the protocol of Bellstaedt et al. (2019, cited by the authors) and quantify the response of isolated roots. If the severed root of the hy5 mutant responds weakly to the raised temperature-change then that would be good evidence for a role for HY5 in the root. Conversely if the severed hy5 root responded like wild type then that would put the action of HY5 fully in the shoot.

The second tangled-up strand is the nature of the developmental trade-off. The authors talk about HY5 and PHYAB as regulating root thermomorphogenesis, presumably by repressing the massive response to temperature in the shoot and promoting the modest growth response of the root. But what the authors don't discuss is the role of the massive growth response itself. At 27C, the hypocotyl growth rates of hy5 or phyab are ten to 20 times faster than that of the wild type, a spectacular increase, and one likely to be consequential. To what extent can the root behavior be explained by the massive growth response itself? Thus, were one to express expansin in the hypocotyl say and stimulate elongation that way, then perhaps root growth would be diminished too? This is a thought experiment, used to illustrate this ambiguity that I think merits discussion.

Indeed, the authors show there is a negative correlation between hypocotyl growth rate and root growth rate at 27C (Figure 3 G). Does this indicate direct regulation or physical limitation (e.g., limited sucrose or water)? I don't think this question is easy to answer. But I do think it needs to be accepted as a possibility.

The other general comment I want to make concerns auxin. A previous paper (Martins et al. 2017 cited by the authors) has results that conflict with those of the authors. Martins et al. rule out a role for auxin in root thermomorphogenesis by finding no difference in taa1, yuc8 (two auxin synthesis genes), tir1 afb2, or tir1/afb2 mutants, and no difference in DR5-GFP expression. A key difference between the current paper and Martins et al. is that the latter grew plants continuously at the test temperature (21 and 26C in the case of Martins et al.); whereas here, plants are shifted. Likewise, other papers reporting a role for auxin in thermomorphogenesis all use a temperature-shift. Where kinetics have been examined, the response to the shift is transient (e.g., Hanzawa et al. 2013, cited by the authors). It is possible that the role of auxin concerns the response to a shift, possibly between any strongly different conditions, and not only to temperature itself. I think the authors should be open to this interpretation.

The relevance of the shift is also underlined by thinking about the fact that when hy5 or phyab are shifted from 21 to 27C the hypocotyl growth rate is increased by 10 to 20 fold. Insofar as kT is concerned we might expect rates to double or at most triple if some natural repression were being relieved. But at the same time, hypocotyl growth at 21 is not affected in the mutants. So either HY5 and PHYAB action in thermomorphogenesis cuts on abruptly above 21C or the shift to the sharply elevated temperature initiates strong responses that the transcription factors are needed to repress. This feature of the authors' experimental system should be discussed.

The other comment that needs to be made about auxin is this. In themselves, lowered auxin levels in the root would be associated with *greater* root growth rate. In the root, auxin levels are inhibitory. Thus in gravitropism, auxin is transferred from the top to the bottom of the root, stimulating the growth of the former and inhibiting the latter. The lowered auxin levels reported here are not associated with increased growth rate in the root and for that reason are difficult to interpret. Greater care is warranted.

Minor comments

Title: I cannot tell what the paper is about by reading the title. It is not obvious what a 'developmental trade-off' means. I think a more accurate title would be to say that the thermomorphogenesis pathways in shoots and roots are linked or coordinated. Perhaps even say something about the role of HY5 in the linkage.

Line 9: Change adaptation to acclimation.

Lines 49 - 57. This discussion here of the role of auxin is one-sided. First, both Feraru et al. and Wang et al. transfer plants to 29C. Insofar as strong responses to stress are clearly induced at 30C, the results of those two papers at 29C might relate to some difficult-to-interpret mixture of thermomorphogenesis and response to stress. Auxin could be important for the latter rather than the former. The authors are welcome to discount such a possibility but they need to acknowledge that it exists. Furthermore, as mentioned above Martins et al. present several lines of evidence excluding auxin from thermomorphogenesis and this should be acknowledged too.

Line 93. In view of the developmental root-shoot trade off, it would be interesting to show data for dark-grown hypocotyls.

Line 96. Spell out root apical meristem. No reason for the acronym.

Line 121. The authors describe the meristem response in hy5 and phyab as being hypersensitive. It may be so. But it is also true that measuring meristem length based on cell length is not particularly accurate. I would say that the shortening of the meristem with temperature might be enhanced in the mutants but is certainly not altered (in comparison to root elongation response, which clearly is altered). By the way, the authors don't discuss meristem shortening in the context of the developmental trade-off but I wonder why if the trade-off suppresses the growth response it does not suppress (and might even enhance) the division response.

Line 190. I would appreciate a statement of what "DOF" stands for.

Line 195. CAB3 and CER6 are described as 'shoot' specific but the images show no staining in hypocotyls.
This caveat should be mentioned.

Lines 259 - 261. The author's write: "Taken together, the analysis of transcriptional responses suggests that HY5 and phytochrome activity regulate root growth at higher temperature by modulating energy metabolism." At the risk of repeating myself, this sentence encapsulates what I see as a problem with the language here. At the general level, the statement is true. But it might not be true that in the root HY5 and PHYAB are in charge of energy metabolism; instead, the growth burst of the hypocotyl could be driving the changes in the root metabolism.

Figure 2D. The y-axis legend should say 'normalized meristem length'.

Figure 3G. I would like to see the correlation between the ratio of root growth at the two temperatures plotted vs hypocotyl growth rate at 27C. The authors plot the ratio of both organ response rates in Sup Fig. 2F but I think the trade off will be easier to see plotted as I just mentioned. I predict that the negative correlation between the magnitude of the root response and hypocotyl growth rate at 27C will be striking.

[As an aside, these plots might be clearer if the authors plotted the average + both x and y ranges, rather than showing all of the individual data points.] Along with this, it might in worthwhile to add dark grown hypocotyls.

Reviewer 2

Advance summary and potential significance to field

In Arabidopsis, developmental responses triggered by warm ambient temperatures (thermomorphogenic responses) include the elongation of shoots as well as roots. How temperature triggers elongation of the embryonic stem (hypocotyl) is relatively well understood and a genetic framework controlling this response has been revealed over the last decade. In contrast, factors promoting root elongation at warm temperatures have only begun to be identified. In their manuscript, Gaillochet et al. show that phytochromes as well as HY5 and PIF transcription factors, which are known to control thermomorphogenesis of the shoot, also regulate root growth in a temperature-dependent manner, upstream of auxin biosynthesis and signalling genes. They also demonstrate that the phytochrome/HY5 module can act in the shoot to affect root growth, and that this module appears to balance root versus shoot elongation.

With the threat of climate change becoming more apparent, plant temperature responses have attracted a strong interest over the last decade. But while a plethora of publications has addressed the control of hypocotyl elongation in response to temperature, very few reports focus on temperature-induced root growth. In particular, this is the first report revealing a trade-off between elongation of shoot and root under high temperature. Thus, the manuscript by Gaillochet

et al. is timely, of general interest to the plant science community and advances our understanding of the impact temperature exerts on plant development.

Comments for the author

There are a few aspects of the current manuscript that need to be addressed:

1) I find the use of the term “growth rate” in the manuscript quite confusing – usually this refers to growth over time (e.g. mm/h), but here, it is used to describe a dimensionless ratio of growth at different temperatures. Using something like “ratio” or “% elongation” might be more suitable.

2) The authors did not observe an altered root growth phenotype in higher order pif mutants, but a PIF4 overexpressor was able to affect root elongation. Recent publications indicated a prominent role of PIF7 in thermomorphogenesis alongside PIF4 (Fiorucci et al., 2020, New Phytol.; Chung et al., 2020, Nature Plants), but the contribution of PIF7 has not been tested. It might be worth to investigate the phenotype in a pif4 pif5 pif7 (de Wit et al., 2015, New Phytol.) or a pifQ pif7 (Leivar et al., 2020, Physiol. Plant.) mutant.

3) In Figure 3 B, C, the authors conclude from the analysis of double mutants that cop1 and det1 mutations suppress the hy5 long hypocotyl and short root phenotype – however, since the respective cop1 and det1 single mutants aren’t included in that particular experiment, it is not really possible to judge whether they fully or only partially suppress the hy5 phenotypes. The experiment should be repeated with all single mutants included.

4) The warm temperature-induced hypocotyl elongation observed in wild type is rather weak (Supplementary data to Figure 3B, E). This is probably due to a relatively high light intensity used in the experiments, as the hy5 mutant also does not show a strong elongation phenotype at 21°C. This does not impact on the genetic data obtained for root growth, but may affect the trade-off observed between root and shoot growth. Repeating the experiment e.g. with wild-type and hy5 mutants at lower light intensities could further strengthen the conclusions drawn regarding the balance between root and shoot growth.

Another conclusion that could be strengthened by additional experiments is the activity of HY5 in the shoot rather than the root. The authors use a HA-YFP-HA-(“DOF”-)tagged HY5 fusion protein expressed from a shoot-specific promoter to demonstrate that shoot-derived HY5 regulates root growth. Since they do not observe a YFP signal in roots, they conclude that HY5 acts in the shoot and does not travel to the root to regulate elongation. However, while it seems that the DOF tag restricts protein movement, a fusion protein of 51 kDa is still within the size range of proteins observed in the phloem sap (Paultre et al., 2016, Plant Cell). A larger tag such as 3xYFP would be an even more convincing tool to investigate HY5’s function with restricted mobility.
However, given the current restrictions for experimental work at many institutions, I would not ask for this to be a mandatory revision.

Reviewer 3

Advance summary and potential significance to field

This paper describes root elongation responses to elevated temperature. It tries to establish a role for HY5 and auxin in this response.

Comments for the author

Gaillochet et al argue they have identified a developmental trade-off for shoot and root thermomorphogenesis. I was very excited to read your paper, given the claims made in the title and abstract. Unfortunately, I was rather disappointed to see that these grand claims are not supported by rigorous studies and a trade-off was not even studied. So despite my initial enthusiasm about the topic and the collection of interesting data, I am rather skeptical about your manuscript.

I am quite uncomfortable about your use of sucrose in the plates. Plants are photoautotrophic organisms and sugars are the one thing they do not need from their environment. Feedings them sugars has a huge risk of studying the developmental artifacts arising from these conditions and interpreting them as genuine developmental pathways. Now, in this particular case it is even worse: one very likely explanation for the interaction between shoot and root (adequately addressed in Kircher and Schopfer's 2012 PNAS paper on the matter) is possible interactions with sugars, as you also discuss. Unfortunately, this choice of growth conditions dramatically impacts the quality of your study and leaves this entirely out in the open.

In figure 5 the results of an RNAseq experiment are shown. What I find problematic here is that entire plants are temperature-treated, whilst only the transcriptomes of the roots are shown. There really is no meaningful way to temp-treat a whole organism and just consider the root transcriptome in isolation, especially if one is interested in a "shoot-root trade-off". So, I strongly urge you to give much better consideration to the shoot tissues.

A similar issue relates to IAA quantifications in Fig 6: you show root IAA levels and these are essentially not affected by temperature. However, you do not show shoot IAA levels, even knowing that phytochromes control IAA in the shoot, and the shoot is one major source of IAA for the root. In your discussion you clearly struggle with this in lines 397-406 where you are trying to interpret about shoot-to-root IAA transport.

I find it very difficult to understand why this is not considered and see this as a major shortcoming of the manuscript: it makes no sense to consider the roots so entirely independent from the shoot, whilst looking at regulators such as auxin and HY5 that are so well known for their mobility between the shoot and the root.

I struggle with your interpretations around auxin involvement. Global knockouts for auxin receptors have very marginal phenotypes (after upping the sample size drastically compared to other assays…), but the biological significance of such marginal differences should probably not even be considered. The yucQ mutant gives a slightly larger effect (again increasing the sample size even further to get this to be significant) but also here the variation is too large to consider these differences as convincing. IAA levels in the roots are not affected at all by temperature (Fig. 6E), suggesting that auxin concentration variations are NOT part of the mechanism under study. The DR5v2 auxin reporter data in supplemental figure 5A-B indicate a small increase of signal in the root tip. It is possible that this small increase, even if it is through elevated IAA, is too small to contribute significantly to the whole-root IAA quantifications. To be sure, you should use the DII reporter, and ideally also cross these lines to the different mutant backgrounds of interest, such as hy5. Also, studies on pin mutants (certainly pin1) are needed to understand any role for auxin at all.
In conclusion: IAA levels in the root at the sampled time point are not affected by temperature and auxin receptor mutants have nearly identical root growth responses to temperature as wildtype. From the very limited data, the only reasonable conclusion is that auxin is not an important regulator of the enhanced root elongation response to elevated temperature. You, however, propose the exact opposite and I think this is misleading.

As a follow-up: you conclude that the [temperature] dynamics of auxin accumulation is disrupted in hy5 and phyAB mutant. Your data show the opposite: there is no significant temperature effect in WT, phyAB and hy5 (Fig. 6E). I am surprised to see such incorrect statements, that now look like working towards a favorite hypothesis that is not really supported by the data. All you could conclude from this is that these two mutants have reduced IAA levels in the root, regardless of temperature. Any interpretation about temperature effects has to be: no effect of temp, no effect of the genotype.

Root growth must be expressed differently: please show the actual growth rates (mm / h) at the two different temperatures for each of your genetic lines. It is obvious that many of your phenotypes are very marginal, and many important insights can go missing in the conversion process to relative growth rates expressions.
Presenting the real growth rates is also crucial to be able to evaluate if growth rates are consistent between independent experiments that are displayed in different figures and panels. Right now,

these data are available in the supplemental source files, but they should be the only data presented in the main figures.
I also have major objections to the way relative root growth is calculated: “Normalized growth rates were calculated by dividing root growth rate at 27 degrees C by the average growth rate at 21 degrees C” (lines 467-468). This way, all statistics and even biological variation become uninterpretable and essentially meaningless because you use the average of plants at 21 degrees C. In other words, you pretend there is no variation at 21 degrees C.
This shows all the more why the real data, separately for 21 and 27 degrees, must be displayed and included in the statistical design. A simple 2-way ANOVA will then be the appropriate design to analyze your data (not the 1-way ANOVA that is now used in the supplementary files; there are two fixed factors: genotype and temperature, so a 2-way ANOVA).

In your approaches you do not seem to consider a rather obvious explanation for the hy5 mutant phenotypes:
their shoots are hugely elongated and overall large. Would there be any opportunity left for these plants to elongate their roots at all? Judging from the images (but again, the real data must be presented for this) in Figure 1A, at 21 degrees C this issue would not exist in the hy5 mutant since its shoots are still reasonable but at elevated temperature there may be no further opportunity for root investments, given the incredibly elongated shoot. In fact, this is true for all of your genetic lines where the root elongation response to elevated temperature is reduced: phyAB, hy5 alleles, PIF4-OX all have an exaggareated shoot response and a reduced root response (and keep in mind also that the root response is anyway already of a different magnitude of resource investment compared to the shoot).
This is a rather obvious explanation that is consistent with what has been known for a very long time of course: resources can be invested only in one place at a time and investments into shoot can go at the expense of investments in the roots. You acknowledge this in your discussion, but do not really consider this in your approach. I see no reason, based on literature or on your data, to consider the focus on auxin be more relevant than the lack of focus on sugars and/or other resources. So, yes there is a trade-off (long-
established), and this is also seen in your data, but you are not resolving the molecular mechanism underlying this trade-off.

Figure 3G shows that hypocotyl elongation at elevated temperature is negatively correlated to root elongation at elevated temperature. I find the data presentation a bit misleading here: The graph first of all is biased by deliberately enhancing sample size for hy5 knockouts (three independent lines, versus a single one for all others). In addition, it only shows the elevated temperature data, whereas in order to make any interpretation we should also have the graph for 21 degrees C. And finally, this is perhaps the one case where the ratio of averaged 27 / averaged 21 degrees root elongation would be appropriate. Figure 3H is a copy of Figure 3G but with regression added, this would have been better superimposed on 3G.

Minor comments:
Fig 2D has a wrong y-axis label (shoot be meristem size, not growth rate).

First revision

Author response to reviewers' comments

Reviewer Point-by-Point Response

We are thankful for the reviewers’ efforts and their constructive suggestions. We were able to address almost all of the concerns and suggested experiments.

Reviewer 1 Comments for the author

In thinking about this paper, there seem to be two strands that are tangled up. The first is whether

HY5 is active in the root. This gets tangled because a phrase such as “a pathway regulating thermomorphogenesis in the root” has two meanings: one is pathways that act in the root and another is pathways acting anywhere, in the shoot for example, that determine the behavior of the root. I am not clear from the author’s language whether they are suggesting that HY5 is acting in the root. Or is affecting the root based on manipulating the shoot. Some clear statement of this would be helpful.

But it would be even more helpful to know whether HY5 acts in the root. The authors attempt to answer this question by expressing HY5 in only the shoots of a hy5 mutant. But the results are ambiguous, the rescue is partial. Does this mean that HY5 acts partly in the root? According to the images provided, CAB3 is off in the hypocotyl, and so the partial rescue could mean that HY5 needs to be on in the hypocotyl in addition to the leaves/cotyledons. As for real experiments, it might be more convincing to express HY5 in the hy5 mutant root specifically, but that of course is a lot of work. A simpler experiment would be to follow the protocol of Bellstaedt et al. (2019, cited by the authors) and quantify the response of isolated roots. If the severed root of the hy5 mutant responds weakly to the raised temperature-change then that would be good evidence for a role for HY5 in the root. Conversely if the severed hy5 root responded like wild type then that would put the action of HY5 fully in the shoot.

A: We thank the reviewer for this thoughtful comment. We have tested whether HY5 function is locally required in the root during thermomorphogenesis by removing the shoots of wild type plants, hy5-221 and phyAB and analyzed the root growth response to temperature (Fig. S3K-M). We have conducted the sections on the upper part of the hypocotyl to minimize damages of the root.

We found that removal of the shoot of hy5 did not rescue root growth response to wild type levels, suggesting that in addition to its role in the shoot, HY5 function is required in the root to mediate the growth response to temperature. In contrast, sectioning shoots in phyAB was able to rescue the root growth response to levels close to wild type. Together these assays suggest that phytochromes and HY5 regulatory function might differ during root thermo-response. We have discussed these results in the main text (line 222-233) and in the discussion section (line 448-451)

The second tangled-up strand is the nature of the developmental trade-off. The authors talk about HY5 and PHYAB as regulating root thermomorphogenesis, presumably by repressing the massive response to temperature in the shoot and promoting the modest growth response of the root. But what the authors don’t discuss is the role of the massive growth response itself. At 27C, the hypocotyl growth rates of hy5 or phyab are ten to 20 times faster than that of the wild type, a spectacular increase, and one likely to be consequential. To what extent can the root behavior be explained by the massive growth response itself? Thus, were one to express expansin in the hypocotyl say and stimulate elongation that way, then perhaps root growth would be diminished too? This is a thought experiment, used to illustrate this ambiguity that I think merits discussion. Indeed, the authors show there is a negative correlation between hypocotyl growth rate and root growth rate at 27C (Figure 3 G). Does this indicate direct regulation or physical limitation (e.g., limited
sucrose or water)? I don’t think this question is easy to answer. But I do think it needs to be accepted as a possibility.

A: We have further discussed the potential influence of strong hypocotyl growth rate on root growth in the discussion section. We have also suggested experiments to modulate the expression of expansin genes to investigate the role of hypocotyl growth on root thermomorphogenesis (line 403-405).

The other general comment I want to make concerns auxin. A previous paper (Martins et al. 2017, cited by the authors) has results that conflict with those of the authors. Martins et al. rule out a role for
auxin in root thermomorphogenesis by finding no difference in taa1, yuc8 (two auxin synthesis genes), tir1, afb2, or tir1/afb2 mutants, and no difference in DR5-GFP expression. A key difference between the current paper and Martins et al. is that the latter grew plants continuously at the test temperature (21 and 26C in the case of Martins et al.); whereas here, plants are shifted. Likewise, other papers reporting a role for auxin in thermomorphogenesis all use a temperature-shift. Where kinetics have been examined, the response to the shift is transient (e.g., Hanzawa et al. 2013,

cited by the authors). It is possible that the role of auxin concerns the response to a shift, possibly between any strongly different conditions, and not only to temperature itself. I think the authors should be open to this interpretation.

A: We have now added further discussion on the role of auxin in temperature shifts vs long term temperature treatment conducted by Martins et al (line 406-409). We have also mentioned the broad role of auxin for growth response to environmental cues (line 412-413).

The relevance of the shift is also underlined by thinking about the fact that when hy5 or phyab are shifted from 21 to 27C the hypocotyl growth rate is increased by 10 to 20 fold. Insofar as kT is concerned we might expect rates to double or at most triple if some natural repression were being relieved. But at the same time, hypocotyl growth at 21 is not affected in the mutants. So either HY5 and PHYAB action in thermomorphogenesis cuts on abruptly above 21C or the shift to the sharply elevated temperature initiates strong responses that the transcription factors are needed to repress. This feature of the authors' experimental system should be discussed.

A: We have now added that important hypocotyl growth from shifting could have an impact on metabolism and thus an indirect impact on root growth (line 380-382).

Furthermore, the light condition used in the main experiments might have an impact on the important growth response that we observed in hy5 and phyAB mutant as light and temperature signals are integrated to control hypocotyl growth (Legris et al 2016). Thus, we have also measured hypocotyl growth rate and root growth rate on seedlings grown in a lower light intensity condition in which hy5 mutant display longer hypocotyl elongation at 21°C (mean of 1,35mm vs 0,5mm in main growth condition)(source data, Fig. S1D, Fig. S2D-E). Although the strength of the phenotype is influenced by the basal growth of the hypocotyl at 21°C, we have observed that hy5 shows lower root growth response than wild type, further supporting that HY5 is required for root thermomorphogenesis (Fig. S2D-E).

The other comment that needs to be made about auxin is this. In themselves, lowered auxin levels in
the root would be associated with *greater* root growth rate. In the root, auxin levels are inhibitory. Thus in gravitropism, auxin is transferred from the top to the bottom of the root, stimulating the growth of the former and inhibiting the latter. The lowered auxin levels reported here are not associated with increased growth rate in the root and for that reason are difficult to interpret. Greater care is warranted.

A: We thank the reviewer for this important suggestion. We have adjusted our wording regarding the auxin levels and focus on change rather than on increase. Based on our genetic evidences and IAA measurements (Fig. 6), we state that auxin signaling can have a permissive role on root thermomorphogenesis (line 422-424)

Minor comments

Title: I cannot tell what the paper is about by reading the title. It is not obvious what a 'developmental
trade-off' means. I think a more accurate title would be to say that the thermomorphogenesis pathways in shoots and roots are linked or coordinated. Perhaps even say something about the role of HY5 in the linkage.

A: We have changed the title to "HY5 and phytochrome activity modulate shoot to root coordination during thermomorphogenesis" to closely describe the findings of our study.

Line 9: Change adaptation to acclimation.

A: We have edited the text.

Lines 49 - 57. This discussion here of the role of auxin is one-sided. First, both Feraru et al. and Wang et al. transfer plants to 29C. Insofar as strong responses to stress are clearly induced at 30C, the results of those two papers at 29C might relate to some difficult-to-interpret mixture of

thermomorphogenesis and response to stress. Auxin could be important for the latter rather than the former. The authors are welcome to discount such a possibility but they need to acknowledge that it exists. Furthermore, as mentioned above Martins et al. present several lines of evidence excluding auxin from thermomorphogenesis and this should be acknowledged too.

A: We have now commented on the possible confounding role of auxin for response to higher ambient temperature and temperature stress response in the previously published reports (Feraru et al and Wang et al) (line 410-412). We have also acknowledged the findings from Martins et al on the function of brassinosteroid signaling for long term response to higher ambient temperature (line 406-409).

Line 93. In view of the developmental root-shoot trade off, it would be interesting to show data for dark-grown hypocotyls.

A: We have measured dark grown wild type (Fig. S2B,C). Dark grown seedlings display a dramatic increase in hypocotyl length, while root elongation in response to temperature is reduced to level similar than at 21°C (line 169-170). These results are consistent with our observation that a developmental trade-off modulates shoot and root growth response to temperature.

Line 96. Spell out root apical meristem. No reason for the acronym.

A: We have edited the text.

Line 121. The authors describe the meristem response in hy5 and phyab as being hypersensitive. It may be so. But it is also true that measuring meristem length based on cell length is not particularly accurate. I would say that the shortening of the meristem with temperature might be enhanced in the mutants but is certainly not altered (in comparison to root elongation response, which clearly is altered). By the way, the authors don't discuss meristem shortening in the context of the developmental trade-off but I wonder why if the trade-off suppresses the growth response it does not suppress (and might even enhance) the division response.

A: Although we have not measured directly division responses, we have now repeated root meristem measurements of wild type, hy5-221 and phyAB at 72 hours after temperature shift and have calculated the cumulative cell length to display the number of cells in root meristem upon cellular differentiation (Fig. S1F). The onset of cell elongation occurs earlier at 27°C in wild type, and this difference is enhanced in both hy5 and phyAB. These results are in line with the reduced levels of auxin that we have measured in hy5 and phyAB at 21°C and 27°C and the current model of hormonal regulation of root meristem differentiation (Delle-Ioio et al 2008, Mahonen et al 2014)

Line 190. I would appreciate a statement of what "DOF" stands for.

A: We have clarified that DOF denominates a HA:YFP:HA tag as defined in (Burger et al, 2017) (line 473-474). An explanation for the abbreviation has not been provided in the original article.

Line 195. CAB3 and CER6 are described as 'shoot' specific but the images show no staining in hypocotyls.This caveat should be mentioned.

A: All pictures have been acquired with similar laser settings and were processed in the same way to give an estimate of the HY5-YFP expression strength. Although the signal in the hypocotyl is lower than in the leaves, we do observe nuclear fluorescence for CAB3-DOF and CER6-DOF lines (Fig. S3B-F). We have now clarified this observation in the main text (line 197-198).

Lines 259 - 261. The author's write: "Taken together, the analysis of transcriptional responses suggests that HY5 and phytochrome activity regulate root growth at higher temperature by modulating energy metabolism." At the risk of repeating myself, this sentence encapsulates what I see as a problem with the language here. At the general level, the statement is true. But it might not be true that in the root HY5 and PHYAB are in charge of energy metabolism; instead, the growth burst of the hypocotyl could be driving the changes in the root metabolism.

A: We have mentioned the possibility of indirect effect of hypocotyl growth on root metabolism in the discussion section (line 381-382)

Figure 2D. The y-axis legend should say ‘normalized meristem length’.

A: We have edited the figure

Figure 3G. I would like to see the correlation between the ratio of root growth at the two temperatures
plotted vs hypocotyl growth rate at 27C. The authors plot the ratio of both organ response rates in Sup
Fig. 2F but I think the trade off will be easier to see plotted as I just mentioned. I predict that the negative correlation between the magnitude of the root response and hypocotyl growth rate at 27C will be striking. [As an aside, these plots might be clearer if the authors plotted the average + both x and y ranges, rather than showing all of the individual data points.] Along with this, it might in worthwhile to add dark grown hypocotyls.

A: We have generated the correlation plot and (attached figure). The correlation observed in the suggested plot and in the one presented in Fig. 3H are similar and therefore we decided to keep the former.
Regarding the plot in Fig. 3G, we like to show individual data points to directly represent variability of the measures and sample size. To increase clarity, we have separated the plots in 2 panels (Fig. 3G and Fig. 3H).
As the experimental setup for measuring dark grown seedlings was different than for the genotypes analyzed in Fig. 3G, we could not calculate growth rates but only organ length. Thus we have decided to present the data separately (Fig. S2B,C)

Reviewer 2 Comments for the author
There are a few aspects of the current manuscript that need to be addressed:

1) I find the use of the term “growth rate” in the manuscript quite confusing – usually this refers to growth over time (e.g. mm/h), but here, it is used to describe a dimensionless ratio of growth at different temperatures. Using something like “ratio” or “% elongation” might be more suitable.

A: We thank the reviewer for pointing this out this inaccuracy. We have edited the text and figures. We now mention growth rate only when referring to increase in organ length size over time (mm/day).

2) The authors did not observe an altered root growth phenotype in higher order pif mutants, but a PIF4 overexpressor was able to affect root elongation. Recent publications indicated a prominent role of PIF7 in thermomorphogenesis alongside PIF4 (Fiorucci et al., 2020, New Phytol.; Chung et al., 2020, Nature Plants), but the contribution of PIF7 has not been tested. It might be worth to investigate the phenotype in a pif4 pif5 pif7 (de Wit et al., 2015, New Phytol.) or a pifQ pif7 (Leivar et al., 2020, Physiol. Plant.) mutant.

A: We have now compared root growth response to temperature in wild type, pif7, pifq and pifq,7. Our data show that pif7 single mutant or in a higher order mutant in pif7 pifq does not display impaired root thermomorphogenesis (Fig. S1E).

3) In Figure 3 B, C, the authors conclude from the analysis of double mutants that cop1 and det1 mutations suppress the hy5 long hypocotyl and short root phenotype – however, since the respective cop1 and det1 single mutants aren’t included in that particular experiment, it is not really possible to judge whether they fully or only partially suppress the hy5 phenotypes. The experiment should be repeated with all single mutants included.

A: We thank the reviewer for this comment. We had previously included these genotypes during the root growth assays and have now included these measurements in Fig. 3B,C,E,F.

4) The warm temperature-induced hypocotyl elongation observed in wild type is rather weak (Supplementary data to Figure 3B, E). This is probably due to a relatively high light intensity used in the experiments, as the hy5 mutant also does not show a strong elongation phenotype at 21°C. This does not impact on the genetic data obtained for root growth, but may affect the trade-off observed between root and shoot growth. Repeating the experiment e.g. with wild-type and hy5 mutants at lower light intensities could further strengthen the conclusions drawn regarding the balance between root and shoot growth.

A: We have measured hypocotyl and root elongation in wild type and hy5 under a lower light intensity condition (Growth condition 2). Under these conditions, we observe that hy5-221 plants show higher hypocotyl elongation than in the previous conditions (mean of 1,35mm vs 0,5mm in main growth condition; attached figure).
We have plotted the relation between root growth rate and hypocotyl growth rate under this condition and observed a negative correlation between these two parameters (R2 = 0.67) at 27°C (Fig. S2D,E). These results are in line with our data presented in Fig. 3G and support our conclusions that shoot and root thermomorphogenesis are coordinated (line 181-183).

Another conclusion that could be strengthened by additional experiments is the activity of HY5 in the shoot rather than the root. The authors use a HA-YFP-HA-("DOF"-)tagged HY5 fusion protein expressed from a shoot-specific promoter to demonstrate that shoot-derived HY5 regulates root growth. Since they do not observe a YFP signal in roots, they conclude that HY5 acts in the shoot and does not travel to the root to regulate elongation. However, while it seems that the DOF tag restricts protein movement, a fusion protein of 51 kDa is still within the size range of proteins observed in the phloem sap (Paultre et al., 2016, Plant Cell). A larger tag such as 3xYFP would be an even more convincing tool to investigate HY5's function with restricted mobility. However, given the current restrictions for experimental work at many institutions, I would not ask for this to be a mandatory revision.

A: We have now suggested in the discussion that fusing HY5 to a large tags such as 3xYFP could immobilize the protein and could be driven by organ specific promoter to complement and strengthen our HY5/hy5 chimera approach (line 446-448).

Reviewer 3 Comments for the author
Gaillochet et al argue they have identified a developmental trade-off for shoot and root thermomorphogenesis. I was very excited to read your paper, given the claims made in the title and abstract. Unfortunately, I was rather disappointed to see that these grand claims are not supported by rigorous studies and a trade-off was not even studied. So despite my initial enthusiasm about the topic and the collection of interesting data, I am rather skeptical about your manuscript.

I am quite uncomfortable about your use of sucrose in the plates. Plants are photoautotrophic organisms and sugars are the one thing they do not need from their environment. Feedings them sugars has a huge risk of studying the developmental artifacts arising from these conditions and interpreting them as genuine developmental pathways. Now, in this particular case it is even worse: one very likely explanation for the interaction between shoot and root (adequately addressed in Kircher and Schopfer's 2012 PNAS paper on the matter) is possible interactions with sugars, as you also discuss. Unfortunately, this choice of growth conditions dramatically impacts the quality of your study and leaves this entirely out in the open.

A: The use of sucrose provides homogeneity in the germination and allows more reliable measurements when studying root development at early seedling stage. Other studies on root thermomorphogenesis have also used sucrose (Hanzawa 2013, Feraru et al 2019).
To make sure that our genetic analysis is relevant under multiple growth conditions, we have tested hy5 growth response to temperature on medium without sucrose (Fig. S1C), as well as in two different light conditions (Fig. S1A,B). Although the root length absolute values and the responses varied between experimental conditions, we have consistently observed that hy5 root was less responsive to higher ambient temperature than wild type, supporting our conclusions (line 92-96).

In figure 5 the results of an RNAseq experiment are shown. What I find problematic here is that entire plants are temperature-treated, whilst only the transcriptomes of the roots are shown. There really is no meaningful way to temp-treat a whole organism and just consider the root

transcriptome in isolation, especially if one is interested in a “shoot-root trade-off”. So, I strongly urge you to give much better consideration to the shoot tissues.

A: During our experiments for the RNAseq, we had harvested and profiled shoots and roots of wild type, hy5 and phyAB 4 hours and 18 hours after increase in ambient temperature. We have added the corresponding shoot transcriptome analysis in Fig. S4A,B,D.
We have observed for all genotypes an enrichment of genes involved in response to sucrose in the shoot upon increased in higher temperature (Fig. S4A,B). This result is consistent with our observation that sucrose transport gene are enriched in the root in response to higher temperature and further suggest that increase in temperature modulates the energy metabolism in both the shoot and the root (line 253-255). These datasets also confirm the quality of the temperature transfer as well as the convergence of HY5 and phytochrome regulatory function at common target genes, and are consistent with what we have observed in the root (Fig. S4C).

A similar issue relates to IAA quantifications in Fig 6: you show root IAA levels and these are essentially not affected by temperature. However, you do not show shoot IAA levels, even knowing that phytochromes control IAA in the shoot, and the shoot is one major source of IAA for the root. In your discussion you clearly struggle with this in lines 397-406 where you are trying to interpret about shoot-to-root IAA transport. I find it very difficult to understand why this is not considered and see this as a major shortcoming of the manuscript: it makes no sense to consider the roots so entirely independent from the shoot, whilst looking at regulators such as auxin and HY5 that are so well known for their mobility between the shoot and the root.

A: We agree that it is an important point to further refine the molecular mechanisms coordinating shoot and root thermomorphogenesis. We have clearly stated this point in the discussion (line 430-433).
However, we feel that understanding the mechanism of auxin movement between the shoot and the root goes beyond the scope of this paper.

I struggle with your interpretations around auxin involvement. Global knockouts for auxin receptors have very marginal phenotypes (after upping the sample size drastically compared to other assays…), but the biological significance of such marginal differences should probably not even be considered.

A: One of the reasons for this mild phenotype might be the functional redundancy among TIR/AFB receptors, we have therefore aimed at measuring the tir1, afb2, afb3 triple mutant. The triple mutants unfortunately display strongly impaired roots that could not be measured as they were too small to be visualized on our scanning system. We have repeated the measurement of tir1 afb2 (see attached figure panel A). Although the decrease in the root response is modest, we show that this reduction is significant ($n<24$; Student t-test; $p = 0.0082$) and can be consistently observed. To test whether the effect size is significant (and not just if the means are statistically different), we used Cohen D-test (which is not affected by larger sample sizes). We find that the difference between the mean value of the root response show a large effect size according to its Cohen’s d (Cohen D-test; $d= 0.817$, $r=0.378$), further supporting our results that tir afb2 display a lower response to temperature than wild type plants. Nevertheless, because this was one of the few experiments, in which a two-way ANOVA yielded only into a marginal P-value (line 297-298), we reworded our conclusions regarding these tir1 afb1.
Furthermore, we have shown that tmk1,4 mutants that have impaired auxin perception/signaling show significant reduction in root growth response to temperature ($n<28$, Student t test: $p= 5,42$ e-15. Cohen D-test; $d= 3.431$, $r= 0.864$)

The yucQ mutant gives a slightly larger effect (again increasing the sample size even further to get this to be significant) but also here the variation is too large to consider these differences as convincing.

We have attached results from an independent assay comparing the wild type and yucQ root growth response to temperature and have again assessed the effect size. Using a sample size of $n<25$ we observe that the relative root growth in yucQ is significantly lower than wild type and the effect size is very large according to Cohen’s d (Student t-test; $p=1.1$ e-05. Cohen D-test; $d=1.482$, $r=0.595$). Furthermore, we have grown seedling on medium supplemented with yucasin, which

inhibits YUCCA function (Nishimura 2013) and have similarly observed reduced root growth response to higher ambient temperature (n<23, Student t test: p=2,86 e-09).

IAA levels in the roots are not affected at all by temperature (Fig. 6E), suggesting that auxin concentration variations are NOT part of the mechanism under study. The DR5v2 auxin reporter data in supplemental figure 5A-B indicate a small increase of signal in the root tip. It is possible that this small increase, even if it is through elevated IAA, is too small to contribute significantly to the whole-root IAA quantifications. To be sure, you should use the DII reporter, and ideally also cross these lines to the different mutant backgrounds of interest, such as hy5.

A: Given the complexity of auxin homeostasis, we agree that it would be good to analyze the local dynamics of auxin concentration in the root. To tackle this point, we have first tried to use the R2D2 reporter but have observed that the R2 component of the reporter (which is used to normalize the DII signal) is responsive to higher ambient temperature. This prevents from uncoupling the change in signal resulting from the transcriptional change of R2 and protein degradation of the DII component, which might have different sensitivities to temperature change. Importantly, this was also observed in Feraru et al 2019, who came to similar conclusions. As a consequence, we have focused on direct IAA and auxin precursor measurements (Fig. 6E,F, Fig. S5G,H).

We also agree that further genetic analyses on the role of auxin in coordinating shoot and root thermomorphogenesis is of great interest, nevertheless we feel that it is out of scope of our current study. We have discussed this point (line 430-433)

Also, studies on pin mutants (certainly pin1) are needed to understand any role for auxin at all.

A: Hanzawa et al 2013 and Feraru et al 2018 have previously shown evidence of the involvement of the auxin transporters AUX1, PIN2 and PILS6 during root thermomorphogenesis (line 51-53). We have now further clarified this point also in the discussion (line 406-409).

In conclusion: IAA levels in the root at the sampled time point are not affected by temperature and auxin receptor mutants have nearly identical root growth responses to temperature as wildtype. From the very limited data, the only reasonable conclusion is that auxin is not an important regulator of the enhanced root elongation response to elevated temperature. You, however, propose the exact opposite and I think this is misleading.

A: We agree with the reviewer that we did not observe an increase in root auxin levels upon increased temperature and we have clearly stated this in the text (line 328-330). We have revised the text further to accurately reflect the lack of strong evidence for a temperature dependent role of HY5 and phyAB on root auxin level (line 338-341). However, we think that there is still several lines of evidence suggesting a role of auxin for root thermomorphogenesis. These include:

1) Pharmacological and genetic perturbation of YUC function impairs the root growth response to higher temperature (Fig. 6B, attached figure panel B,C)
2) tmk1,4 mutants show reduced root growth response to temperature (Fig. 6C)
3) From our RNA-seq, we have found a significant overlap between genes that are transcriptionally responding to IAA treatment in the root and that are misregulated in hy5 and phyAB roots (both at early and late time points) (Fig. 6D, Fig. S5E)

We therefore propose that auxin signaling has a permissive rather than an inductive role on growth during root thermomorphogenesis (line 422-424). We have further clarified this idea in the discussion section and have toned down some of our conclusions by suggesting a molecular mechanism but by clearly stating that the process remains to be understood (line 430-433).

As a follow-up: you conclude that the [temperature] dynamics of auxin accumulation is disrupted in hy5 and phyAB mutant. Your data show the opposite: there is no significant temperature effect in WT, phyAB and hy5 (Fig. 6E). I am surprised to see such incorrect statements, that now look like working towards a favorite hypothesis that is not really supported by the data. All you could conclude from this is that these two mutants have reduced IAA levels in the root, regardless of temperature. Any interpretation about temperature effects has to be: no effect of temp, no effect of the genotype.

A: While the comparison on auxin accumulation between 27°C and 21°C refers to the analysis in Fig. 6F, where we found that the relative accumulation of auxin level in hy5 and phyAB compared to wild type was mildly reduced in hy5 and phyAB compared to wild type. However, the two-way ANOVA suggested by the reviewer didn't support this. We now state that taken together these results show that HY5 and phytochrome are required to maintain auxin levels, but lack strong evidence that this is temperature dependent. Further data will be required to thoroughly test the role of hy5 and phyAB dependent auxin levels in root thermomorphogenesis (line 338-341).

Root growth must be expressed differently: please show the actual growth rates (mm / h) at the two different temperatures for each of your genetic lines. It is obvious that many of your phenotypes are very marginal, and many important insights can go missing in the conversion process to relative growth rates expressions.
Presenting the real growth rates is also crucial to be able to evaluate if growth rates are consistent between independent experiments that are displayed in different figures and panels. Right now, these data are available in the supplemental source files, but they should be the only data presented in the main figures. I also have major objections to the way relative root growth is calculated: "Normalized growth rates were calculated by dividing root growth rate at 27 degrees C by the average growth rate at 21 degrees C" (lines467-468). This way, all statistics and even biological variation become uninterpretable and essentially meaningless because you use the average of plants at 21 degrees C.
In other words, you pretend there is no variation at 21 degrees C.

A: We agree with the reviewer that presenting measurements at individual temperature is important and we have included these data as supplemental figures as well all individual measurements as source data. To address the variation issues, we have conducted Two-way ANOVA analyses when data were analyzed separately at 21°C and 27°C (All panels in Supplementary data root measurements).
As we focus on the root response to temperature, we think that showing the growth ratio (27/21°C) is the most consistent trait to analyze and interpret for assessing the root growth response to temperature.

This shows all the more why the real data, separately for 21 and 27 degrees, must be displayed and included in the statistical design. A simple 2-way ANOVA will then be the appropriate design to analyze your data (not the 1-way ANOVA that is now used in the supplementary files; there are two fixed factors: genotype and temperature, so a 2-way ANOVA).

A: We thank the reviewer for this comment. We have conducted Two-way ANOVA as well as Three-way ANOVA analyses when data were analyzed separately at 21°C and 27°C. We have now displayed the results in the supplementary data root measurements and have also compiled the results in the supplementary source data. We have checked all conclusions that we had reached via our analysis of the relative growth data with the results of the ANOVAs and have reported the three instances that these didn't agree. In particular, the ANOVA results of the auxin levels prompted us to revise our conclusions from the auxin levels (line 338-341).

In your approaches you do not seem to consider a rather obvious explanation for the hy5 mutant phenotypes:their shoots are hugely elongated and overall large. Would there be any opportunity left for these plants to elongate their roots at all? Judging from the images (but again, the real data must be presented for this) in Figure 1A, at 21 degrees C this issue would not exist in the hy5 mutant since its shoots are still reasonable, but at elevated temperature there may be no further opportunity for root investments, given the incredibly elongated shoot. In fact, this is true for all of your genetic lines where the root elongation response toelevated temperature is reduced: phyAB, hy5 alleles, PIF4-OX all have an exaggareated shoot response and a reduced root response (and keep in mind also that the root response is anyway already of a different magnitude of resource investment compared to the shoot).
This is a rather obvious explanation that is consistent with what has been known for a very long time of course: resources can be invested only in one place at a time and investments into shoot can go at the expense of investments in the roots. You acknowledge this in your discussion, but do not really consider this in your approach. I see no reason, based on literature or on your data, to

consider the focus on auxin be more relevant than the lack of focus on sugars and/or other resources. So, yes there is a trade-off (long-established), and this is also seen in your data, but you are not resolving the molecular mechanism underlying this trade-off.

A: We have provided genetic evidences for a shoot to root growth trade-off during thermomorphogenesis by measuring hypocotyl and root growth of individual plants for nine different genotypes (wild type, hy5-221, hy5, hy5-215, hy5 pifQ, hy5 cop1, hy5 det1, phyAB and PIF4OX) and showing a quantitative growth trade-off (Fig. 3G). Furthermore, we have rescued hy5 root phenotypes by driving HY5 specifically in the shoot (Fig. 4).
We agree that we do not provide a clear molecular mechanism underlying this trade-off, but rather suggest that a complex interaction between light signaling, energy metabolism and auxin homeostasis might regulate this process. We have further discussed this point in the discussion (line 376-379)
We also feel that deciphering the fine mechanisms would require to establish a set of genetic tools and conduct a set of experiments that would go beyond the scope of this study. We agree that further research on these questions will be required to solve this.

Figure 3G shows that hypocotyl elongation at elevated temperature is negatively correlated to root elongation at elevated temperature. I find the data presentation a bit misleading here: The graph first of all is biased by deliberately enhancing sample size for hy5 knockouts (three independent lines, versus a single one for all others). In addition, it only shows the elevated temperature data, whereas in order to make any interpretation we should also have the graph for 21 degrees C. And finally, this is perhaps the one case where the ratio of averaged 27 / averaged 21 degrees root elongation would be appropriate. Figure 3H is a copy of Figure 3G, but with regression added, this would have been better superimposed on 3G.

A: We have used different genotypes of hy5 as multiple alleles were available to us and have been used as background in higher order mutants that we have used in this study (line 466-471). We think that they should be analyzed and represented separately. The plot with root growth rate ratios are shown in Fig. S2F-G. For clarity in the presentation, we decided to keep Fig. 3G and Fig. 3H separated.

Minor comments:
Fig 2D has a wrong y-axis label (shoot be meristem size, not growth rate).

A: We have edited the figure

Second decision letter

MS ID#: DEVELOP/2020/192625

MS TITLE: HY5 and phytochrome activity modulate shoot to root coordination during thermomorphogenesis

AUTHORS: Christophe Gaillochet, Yogev Burko, Matthieu Pierre Platre, Ling Zhang, Jan Simura, Bjoern Willige, Vinod Kumar, Karin Ljung, Joanne Chory, and Wolfgang Busch
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 2

Advance summary and potential significance to field

Please see my review of the original submission for appropriateness/timeliness of the topic. The authors have substantially toned down their claims with regard to the molecular mechanism (i.e. that the phytochrome/HY5-auxin module underlies the trade-off between root and shoot growth). I think this more careful interpretation reflects their data more appropriately, but it does reduce the impact of the story to some extent.

Comments for the author

The authors have added additional experiments to the manuscript and substantially revised the text. Specific comments are given below.

1) The authors have satisfactorily addressed my suggestions for including det1/cop1 single mutant phenotypes and analysing a possible involvement of PIF7.

2) The authors have changed the wording from “normalised root/hypocotyl growth rate” to “normalised root/hypocotyl growth” in the supplement, but not in the main figures. This should be amended.

3) The authors refer to “growth condition 2” as low light, although a light intensity of 122 (I presume umol m-2 s-1? This should be clarified in the methods) compared to 146 is not a very strong difference and could have been performed at light intensities <100 umol m-2 s-1. Analysing root/shoot elongation at lower light intensities would also represent an opportunity to further test the hypothesis that phytochromes/HY5 specifically coordinate root versus shoot elongation at warm temperature (also further investigating the concerns raised by reviewer 3).

4) Nevertheless, in their response the authors mention that “growth condition 2” did result in a more prominent long hypocotyl phenotype of hy5 at 21 degrees – this is however not evident from the data included in the figures. The elongation data at the different temperatures (plus relevant statistics) should be included in the supplemental figures, and a regression analysis similar to Figure S2D should also be performed for growth rates at 21 degrees; it also appears that the raw data (i.e. raw hypocotyl/root lengths) are not given in the source data, but rather the elongation data from day 4 to day 6 (given that there are a lot of 0/very low values).

Reviewer 3

Advance summary and potential significance to field

This paper reports that Phy's and HY5 contribute to thermomorphogenesis in Arabidopsis. This topic is of great interest. Many of the morphogenic responses to elevated temperature share overlap with photomorphogenic responses that also involve Phy's and HY5. The interesting distinction is that root elongation is promoted in elevated temp, whereas this the opposite for shade responses. Although interesting, this is not novel.

Comments for the author

This manuscript would be potentially interesting because of the claims it makes, both in the abstract and summarising figure. Despite you efforts since the former submission, I find it still falls short. In fact, by re-analysing your data and adding additional data, you have shown that several concerns raised by the reviewers upon the original submission were correct. It is quite clear that any role for auxin actively regulating the root responses to elevated temperature under your conditions and treatments is uncertain. Therefore, I believe that this part of the story is important for the manuscript but does not constitute part of the overall mechanisms as depicted in the summary and final figure. The Discussion should also conclude that auxin plays only a minor role under your experimental conditions, as far as this can be concluded from your data:

minor phenotypes in mutants, no effect of temperature on root IAA levels, and too little resolution (only DII in root tip) for IAA spatial distribution to draw conclusions about local changes.
Related to this: I find it really very problematic that in Fig 6E you show that temperature does NOT affect root [IAA] and then in Fig 6H you still plot the 27/21 degrees ratio (Knowing there is NO effect) to make a diagram to promote the suggestions there IS an effect.
The contrasting observations published previously on long-terms versus short-term temperature responses and auxin indicate that there is probably a very subtle complexity here.

The second major concern is that your new data on cut seedlings show that HY5 is needed in the roots for a full response to elevated temp. This means that in your response system, HY5 is NOT a shoot module, but rather a WHOLE-PLANT module. This has considerable implications for everything you write about a HY5-based mechanism.

Thirdly, rather than talking about the example of inducing expansins (cell wall loosening proteins) in the shoot to boost growth locally, such experiments (probably different ones with the same principal behind it) should be done and included.
This links to the fundamental question of shoot and root plasticity being linked. Of course they are, but I do not think you provided compelling evidence to show that this happens through the HY5/Phy/Auxin module. In other words, I don't think you have satisfactorily resolved the mechanism of such interaction in temperature response.

I find it problematic to see that you stick with your original concepts, logic and mechanisms, despite the proof you provide against it. At some point, one has to decide that things are more complicated than anticipated and require new ideas. You acknowledge this complexity in the discussion now, but you have not majorly changed your conclusions, or in fact the manuscript altogether.