The AP2/ERF Transcription Factor TINY Modulates Brassinosteroid-Regulated Plant Growth and Drought Responses in Arabidopsis

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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.)

TPC2018-00066-RA 1st Editorial decision – *declined* Mar. 4, 2018

Thank you for choosing to send your manuscript entitled "The AP2/ERF Transcription Factor TINY Modulates Brassinosteroid-Regulated Plant Growth and Drought Response in Arabidopsis" for consideration at *The Plant Cell*. Your submission has been evaluated by members of the editorial board as well as expert reviewers in your field, and we regret to inform you that we are not able to recommend publication of this manuscript. We have not made this decision lightly. We have had input from multiple scientists, and have solicited post-review comments as well. Our present policy is to offer streamlined decisions and to not advise on the direction of the work by requesting extensive modifications or substantial additional experiments.

Based on the reviewers' comments, this study represents a substantial work with promising general interest in plant hormone biology. Nevertheless, the reviewers also raised numerous concerns and suggestions that include the lack of adequate controls in some cases, statistical analysis, including proper use of biological controls, the need for additional experimental verification and clear description. The unstated mechanism on how TINY antagonize with BES1 is a key question requiring further exploration, and the rationale linking TINY to drought responses is another issue that should be further addressed. Based on these comments, I must decline the current version of the manuscript but encourage you to resubmit a new manuscript that fully addresses the concerns raised during this review process.

TPC2018-00918-RA Received Dec. 1, 2018

Reviewer comments on declined paper and **author responses**:

In this revised manuscript, we have addressed most of the reviewer's comments. We have also made new findings that further strengthened the manuscript. First, we demonstrated that TINY inhibited plant growth by inhibiting BR pathway and antagonizing BES1's transcriptional activity on plant growth-related genes. TINY and BES1 oppositely regulated these genes though inhibiting each other's transcriptional activity. Second, we illustrated that TINY reduced plant water loss during water deficient conditions by promoting ABA-induced stomatal closure, and directly activated drought responsive gene expression to positively regulate drought response.

The detailed responses to reviewer's comments are as follows:

Reviewer #1:

This manuscript mainly reported the crosstalk between drought stress and BR signaling. The authors found that overexpression of TINY inhibits plant growth, probably through reducing BES1 level and inhibiting BR signaling. TINY can interact with BES1, and they antagonize each other's transcriptional activity. The authors also find that BIN2 can phosphorylate and stabilize TINY. Although the authors proposed several aspects of mechanism about the interaction between TINY and BR signaling pathway, many key points were not fully supported by the results. In addition, this study touched several aspects, but lacks very logic experimental design. My concerns are as follows.

1, There are a number of the studies on the molecular mechanism of the interaction between BR signaling and stresses responses, but the authors did not mention and discuss these studies. The whole study lacks the basic understanding on the field.

Responses: We have included recent studies on BR and stress responses (especially BR d androught) in the Introduction.

2, In figure 1, where are the marker gene expression to evaluate the BR signaling. The author proposed that TINY inhibits the stability of BES1 and even bes1-D protein. What are the possible mechanisms? This should be a major question to ask and address in this study.

Responses: BR-responsive marker genes, especially those involved in cell elongation, were tested in TINY overexpression plants and *tiny tiny2 tiny3* triple mutants (Figure 1E and 1F). These results indicated that TINY inhibited BR signaling. In the revised manuscript, we focused on studying TINY-BES1 interactions in the regulation of growth and stress-related genes and the regulation of TINY by BR signaling through the BIN2 kinase. The regulation of BES1-D by TINY overexpression is not a focus in current manuscript as wild-type (WT) *BES1* transcript is not repressed by TINY overexpression. Our new results demonstrated that TINY inhibited the BR pathway mainly by oppositely regulating BR-responsive genes and antagonizing BES1's transcriptional regulation of these genes.

3, In figure 2B, after BL treatment of #6 line, the TINY-FLAG was completely gone, but the BES1 level was much higher than that in #2 line, which has more TINY-FLAG protein after BL treatment.

Responses: We redid the experiment with different concentrations of BR and showed that BR treatment clearly reduced TINY protein levels (Figure 2C).

4, The author claimed that BIN2 stabilizes TINY. In figure 2I, J, K, L, where is the wild type control.

Responses: We redid the experiment with the WT control and showed that BIN2 stabilized TINY (Figure 2I-2L).

5, For figure 3, what's the physiological reason of TINY on drought responses. By enhancing water uptake and/or by reducing water loss? The drought responses phenotype for other BR-related mutants is needed.

Responses: We found that TINY overexpression reduced plant water loss using detached leaf assays and that TINY promoted ABA-induced stomatal closure to positively contribute to the drought response. The phenotype of BR-related mutants was reported by several studies where BR loss-of-function mutant *bri1-301* and BR gain-of-function mutant *bes1-D* are tolerant or sensitive to drought, respectively (Nolan et al., 2017; Ye et al., 2017; Fàbregas et al., 2018). We also confirmed that *bes1-D* is more sensitive to drought (Supplemental Figure 4).

6, In the Figure 4, since TINY can significant regulate the stability of BES1, I am surprised that it only regulates a very low percentage (19.4%) of BES1-target genes.

Responses: As discussed above (point 2), TINY overexpression reduced BES1-D in the *bes1-D* mutant but not BES1 in WT (Supplemental Figure 5B and 5C), which should explain the low percentage of overlap.

7, In figure 5F and G, I would like to see whether the DNA binding domain and partial Phospho-domain of BES1 together is sufficient for its interaction with TINY.

Responses: We re-generated several BES1 fragments including the BES1 DNA binding domain, and two truncated phosphorylation fragments for GST pull-down assay. The results indicated that the DNA binding and phosphorylation domains are responsible for TINY interaction.

8, For the model in Figure 7, the author did not answer the key finding at beginning how TINY regulate the stability of BES1, which is independent on 26S proteasome, because the amount of BES1-D was reduced in the TINY-OE line. For the model, the expression level of TINY is induced by drought, but the drought signal was apparently put on a wrong position.

Responses: We re-modified the model. For TINY regulation of BES1-D stability, please see our responses to point 2.

Reviewer #2:

In this manuscript, the authors describe research about the function of the AP2/ERF-like transcription factor TINY and attempt to understand how it interacts with the brassinosteroid signaling pathway. Their results indicate that TINY promotes stress responses and inhibit growth by interfering with BR signaling, which promotes growth and inhibits stress responses. Though the presentation of the results is somewhat confusing, they appear to support this concept. Though some of their conclusions seem to me to be specious, overall, it is an interesting study that adds to our understanding of the feedback regulation that controls the balance between growth and stress response. However, I have several concerns about the reliability and interpretation of the data as outlined below.

1. At least five different gene constructs with four different promoters are used to create plants that, apparently, over-express TINY. A construct in which flag-tagged TINY is under control of its native promoter (TINYpro:TINY-FLAG), one in which it's under control of a estradiol inducible promoter (XVE:TINY-FLAG), one in which the *BES1* promoter was used (BES1pro:TINY-FLAG, abbreviated, for some reason, as TINYOE) and one in which GFP-tagged and myc-tagged TINY is under control of the 35S promoter (35S:TINY-GFP and 35S:TINY-MYC). Testing of these lines is not adequately described. How many lines were produced and tested? How were the lines that were ultimately used in experiments selected?

There is no mention of expression analysis of any of these lines. Since the TINYpro:TINY-FLAG lines use the native promoter, it seems likely that many of the them will express TINY-FLAG at levels no higher than the native gene. I need to see expression analysis at both the mRNA and protein levels to have confidence in this data. Why was the *BES1* promoter chosen? Since *BES1* is apparently negatively regulated by TINY, it seems like there could be feedback regulation between the promoter and the gene product in these lines.

Responses: In the revised manuscript, we used BES1:TINY-FLAG transgenic line 8 and line 3 to perform the majority of experiments, (and line 3 for gene expression and RNA-seq analysis). The protein and transcript levels are shown in Supplemental Figure 1C. The *BES1* promoter is ubiquitous, allowing stable and higher expression of transgene. In contrast, the 35S promoter-driven transgene is not stable as the TINY overexpression phenotype in T3 generation is reduced by certain extent.

We also used TINY:TINY-FLAG and XVE:TINY-FLAG transgenic lines. The protein and transcript levels are shown in Supplemental Figure 3G-3I. TINY native promoter also caused TINY overexpressed phenotype which might due to positional effect of trans-gene insertions. TINY:TINY-FLAG line 2 was used to cross into bin2-1 and bin2 bil1 bil2 mutants and genetic complementation experiments. Using the *TINY* native promoter allowed us to study TINY function in its "normal" expression domain.

The XVE:TINY-FLAG lines with inducer promoter can avoid the effect of BR on *BES1* and *TINY* promoters. We used these lines to study how BR regulates TINY protein level, without the need to consider *TINY* transcript changes. All overexpression lines displayed similar stunted growth phenotypes.

Below is the summary about lines produced and used:

Trangenic plants	Lines generated and homozygous (HM) lines	Lines for use
BES1:TINY-FLAG	Generate 33 lines and 4 HM lines	#3, #8
TINY:TINY-FLAG	Generate 19 lines and 3 HM lines	#2, #5
XVE:TINY-FLAG	Generate 25 lines and 6 HM lines	#2, #6

2. In general, the reproducibility of the results is questionable. Though, in some places, the authors state that experiments were repeated three times, this is inadequate and more details are needed. How many lines were used? How many plants from each line were assayed? For example, in Figure 2B TINY protein was not detectable in WT, and the two XVE:TINY-FLAG lines showed very different results, with only minor effects of BL on TINY-FLAG accumulation in line #2 and nearly complete loss in line #6. Which pattern represents reality in WT plants?

Responses: The information on numbers of repeats and plant lines are indicated in the figure legends. Please also refer to Reviewer #1 question 2 and 3. We redid the experiment with different concentrations of BR and showed that BR treatment clearly reduced TINY protein levels (Figure 2C).

3. Line 105-106. The authors state that TINY3 and TINY4 were repressed by BL in WT and *bes1-D*. This is not apparent in the figure (Supplemental Figure 1A). TINY3 is shown to be slightly higher in BL-treated WT plants, not lower and TINY4 is the same. Levels of TINY3 do appear to be repressed in *bes1-D* plants somewhat more so in bes1D plants treated with BL.

Responses: In the new manuscript, we renamed TINY homologs with TINY2 (At5G11590) and TINY3 (AT4G32800) and re-performed qRT-PCR analysis again with two technical replicates and three biological replicates. The results are shown in Supplemental Figure 1B that TINY and TINY2 were induced by BL, whereas TINY3 was repressed by BL.

4. Line 132-133. The authors state that TINYOE suppressed the growth phenotype of *bes1-D* in both seedlings and adult plants (Figure 1E). This is not clear. The double mutant plants look much more like WT and *bes1-D* plants than TINYOE plants. Would be more accurate to say that *bes1-D* suppresses TINYOE than to say TINYOE suppresses *bes1-D*, in my opinion.

Responses: We found that overexpression of TINY in *bes1-D* background repressed BES1 transcript by 80%, which resulted in decreased BES1 protein and suppressed *bes1-D* phenotype. However, since overexpression TINY in WT background didn't reduce *BES1* transcript, we reasoned that TINY didn't affect BES1 protein stability under normal condition. Since TINY has this specific effect on BES1-D expression, we decided not to use the double mutant to explain their relationship. As shown in the new manuscript, BES1 and TINY antagonize each other's activities on target gene promoters upon binding to their corresponding DNA binding sites.

5. Phosphorylated and non-phosphorylated forms of TINY-FLAG are not readily apparent in Figure 2C and are not clear at all in the CIP experiment (Figure 2D). The two bands migrate closely together and the bands are not well resolved. Are there supposed to be two bands in the -CIP lane? Looks like figure was cut from the edge of a larger gel blot image in which the gel was "smiling".

Though the kinase activity leaves little doubt that TINY can be phosphorylated by BIN2 in vitro, better images should be used. Also, it should be noted that a different construct (35S:TINY-FLAG) was used for the MG132 treatment assays (Supplemental Figures 3C and 3D).

5. Line 196 to Line 199. The authors state that two classes of phenotypes were observed in the F1 generation of a cross to produce TINYOE2 *bin2-1* plants. Were these plants actually genotyped directly or are the genotypes mentioned based on speculation?

Responses: We redid CIP treatment using TINY protein precipitated from TINYOE #3 transgenic line and loaded two repeats (Figure 2E). After compared with background bands, we concluded that TINY was phosphorylated in plants as CIP treatment led the shift of non-phosphorylated of TINY.

6. As stated in line 202, phosphorylation of BES1 by BIN2 apparently destablizes but phosphorylation of TINY by BIN2 apparently stabilizes it. This could happen if BES1 is susceptible to degradation when phosphorylated and TINY is susceptible when unphosphorylated. However, if this is he model that authors are proposing, it does not agree with that depicted in Figure 7D. On the normal conditions side, the line with the bar showing repression of BES1 should be thin rather than tick, showing little repression while the corresponding line on the drought side should be thick rather than thin. Also, this concept is not clearly stated in the abstract.

Responses: The genotyping results are shown in Figure 2I, bottom panel. We corrected the line widths in the model.

7. In Figure 5G, why does the apparent pull-down efficiency decrease gradually with shorter probes? Are there multiple sites of interaction? Was this interaction mapping also tested using Y2H?

Responses: Please see response to Reviewer #1 question 7. In our GST pull-down assay, BES1 DNA binding domain and phosphorylation domains are responsible for TINY interaction.

8. The experiment shown in Figure 7 is not fully described. Obviously, both reporter and effector constructs have to be introduced. This is not clearly stated. Also, not clear how expression was measured. Luminescence or RT-qPCR?

Responses: Luciferase (LUC) reporter enzyme activity was measured with a luminometer after adding luciferin substrate. We changed the label "Relative LUC expression" into "LUC relative activity" to make the statement more clear (Figure 6 and Figure 7).

9. Given that they have epitope tagged TINY expressing Arabidopsis plants and specific antibodies, why not confirm the enrichment of TINY at the RD29A locus in vivo using ChIP? The co-enrichment of BES1 could also possibly be assayed.

Responses: We performed ChIP assay to confirm TINY and BES1 association with several growth-related genes and drought responsive genes (Figure 6C and Figure 7D and 7E).

All the minor problems are addressed in the main manuscript.

Reviewer #3:

In the present paper, Xie et al. report that the transcription factor TINY negatively regulates plant growth but positively drought responses by interacting with BES1 and the BR signaling/response pathway.

Although the paper sounds very interesting and innovative, I have some problems with the adequacy of some results and the applied statistics.

1) Upon my request, the authors stated that they performed the physiological tests three times independently with similar results. I don´t like this kind of statement. Either the authors calculate the mean {plus minus} SE of the mean of the values of the three experiments or they provide the independent data sets in the supplements.

Responses: In the manuscript, we used SD to measure the variability within samples, and we presented data with mean and SD instead of mean {plus minus}. Since we included *tiny tiny2 tiny3* triple mutants, we re-preformed most physiological tests with individual plate or tray. The data collected from the individual plate or tray was treated as biological replicate. All statistical analysis used at least three biological replicates.

2) Regarding the gene expression experiments, the authors wrote that they display the mean {plus minus}

SD from three technical (!) replicates and that the additional independent biological replicates showed similar results. Technical replicates are meaningless but only biological replicates actually "count". How similar (or different) are the biological replicates?

Responses: We re-preformed gene expression experiments with two technical repeats and three biological replicates. We used the average of two technical replicates to calculate relative gene expression and used the three biological results to do statistical analysis.

3) Most of the results were obtained by using TINYpro:TINY-Flag overexpression lines or estradiol-inducible TINY lines. Why are the TINYpro:TINY-Flag overexpressors and to which extend? Were the constructs transformed into the wild type and/or the tiny triple mutant background? Do the constructs complement the mutant phenotype of the tiny triple mutant? Why was the tiny triple mutant not included in most of the physiological experiments (e.g. gene expression experiments, hypocotyl length measurements)?

Responses: We re-preformed all physiological tests and gene expression studies including tiny tiny2 tiny3 triple mutant, as shown in the manuscript.

For the usage of transgenic lines, please refer to response to reviewer #2 question 1. Briefly, we used BES1:TINY-FLAG transgenic lines for most experiments. XVE:TINY-FLAG lines with inducer promoter was used to examine BR

regulation on TINY protein level, as these lines help eliminate BR regulation on transgene. TINY:TINY-FLAG line 2 in WT Arabidopsis was used to cross into bin2-1 and bin2 bil1 bil2 mutants. By using this line, we can study BIN2 regulation of TINY with its native promoter. Overexpressing TINY:TINY-FLAG into *tiny tiny2 tiny3* triple mutants was used for genetic complementation experiments. Our complementation tests showed that *tiny tiny2 tiny3* triple mutant phenotype was caused by loss-of-function of *TINY* and its close homologs. All the transcript and protein levels were shown in Supplemental Figure 1C and Supplemental Figure 3G-3I. All overexpression lines displayed similar stunted growth phenotype.

4) I see the BiFC experiments very critically. The cYFP/nYFP fragments are bad controls because they are usually unstable in tobacco. The authors need to test an unrelated control BiFC protein, which localizes to the same compartment as TINY and BES do (e.g. an unrelated transcription factor). Furthermore, they need to prove by western blot that this control protein is actually co-expressed with TINY or BES in one and the same tobacco cell. I would like to highly recommend the ratiometric BiFC approach, which reduces the probability of BiFC artefacts significantly.

Responses: We performed Co-IP experiments to further support TINY-BES1and TINY-BIN2 interactions, as shown in Figure 2H and Figure 2E.

5) The transcriptome analysis focuses on the data sets achieved with the TINYOE compared to wild type. However, the overexpression of any transcription factor can lead to transcriptional artefacts. The authors neglect the DE genes which are differently expressed between the tiny triple mutant and wild type. These genes are much more reliable for the interpretation of in vivo TINY function.

Responses: TINY is a stress induced transcription factor, whose expression level is low under normal growth condition, but highly induced by stresses (Sun et al., 2008). TINY homologs function redundantly to regulate growth and drought (Figure 1B and 3A). This can explain why the growth and drought phenotypes of tiny triple mutant are not strong and a smaller number of genes are differential expressed in *tiny tiny2 tiny3* triple mutants. So we focused on DE genes from TINYOE, but also presented data on *tiny tiny2 tiny3* triple mutants.

6) The BL and BZR concentrations appear non-physiologically high. Concentrations above 10 to 20 nM BL usually induce an inhibition of growth - at least in the root. Please comment on this.

Responses: The root system is more sensitive to BL and BRZ treatments: even 1 nM BL treatment causes the inhibition on root growth. 10 nM BL and 250 nM BRZ are widely accepted concentrations to quantify the hypocotyl elongation and inhibition respectively in the BR field. To accurately quantify TINYOE and *tiny tiny2 tiny3* BL and BRZ response, we added more BL and BRZ concentrations, as shown in Figure 1D and Figure 2B.

7) The EMSAs look experimentally well done but the authors should avoid the one-to-one transfer of these data to the in vivo situation. The in vivo situation can only be revealed by (reciprocal) X-ChIP experiments.

Responses: We conducted ChIP assay using TINYOE plants with anti-BES1, anti-FLAG, and anti-IgG antibodies. The results showed TINY and BES1 were associated with CESA5, IAA19, RD29A and COR15A promoters (Figure 6C and Figure 7B-7C).

TPC2018-00918-RA 1st Editorial decision – *accept with minor revisions* **Feb. 1, 2019**

We have received reviews of your manuscript entitled "The AP2/ERF Transcription Factor TINY Modulates Brassinosteroid-Regulated Plant Growth and Drought Response in Arabidopsis." 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:

As mentioned in reviewers' comments, the editors agree that the TINY protein stability assay, commercial antibodies to detect phosphorylation, negative control of BiFC assay and other comments must be addressed or well discussed in your next revision. Please see editorial in *The Plant Cell* on BiFC and controls.

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

[Reviewer comments shown below along with author responses]

TPC2018-00918-RAR1 1st Revision received Date 3/29/2019

Reviewer comments and **author responses**:

We want to thank the reviewers for the constructive comments. In this revised manuscript, we have addressed the reviewer's remaining comments. We have determined the half-life of TINY protein as suggested by Reviewer #2 and showed that TINY protein is indeed less stable in the presence of BR. Moreover, we verified the protein levels in BiFC experiments. We also addressed other minor comments made by the reviewers. Together, the detailed responses to reviewer's comments are as detailed below:

Reviewer #2:

In this revised manuscript the authors have addressed most of the concerns raised in the previous review. Several experiments are repeated and the results, in general are more convincing. The presentation has also been improved. However, I still have reservations about some of the results and their interpretations and feel the need to point out additional technical weaknesses and over- interpretations.

Primary concerns:

Line 155-158. Data for the change in stability of TINY protein in response to BL remains weak. In order to accurately assay protein stability, new protein synthesis in the tissues should be inhibited with cyclohexamide and the protein half-life calculated.

As suggested, we used cyclohexamide (CHX) to block new protein synthesis and checked TINY protein degradation rate (TINY protein half-life) with or without BL treatment. We found that TINY protein had a shorter half-life (about 25 min) after BL treatment compared to a mock control without BL (about 50 min) (Figure 2D and 2E). This result clarifies our conclusion in the manuscript that BR promotes TINY protein degradation.

Line 159-164. Use of CIP to dephosphorylate TINY and detection of a shift in electrophoretic mobility is a relatively crude method to assay protein phosphorylation. Commercial antibodies able to detect phosphorylated Ser or Thr (or both) are available and could provide a more direct method of detection.

We tried to detect phosphorylated form of TINY using commercial Monoclonal Anti- Phosphoserine antibody (Sigma, P3430-2ML). Unfortunately, this commercial antibody did not work well due to poor specificity (recognizing multiple protein ladder bands) and high background. As a control, we used a well-established phosphorylated protein (BES1), which shows a clear shift after CIP treatment (Yin et al., 2002, Cell). The phosphoserine antibody also did not work well for BES1. Thus, the CIP treatment appears to be the most appropriate assay for detection of phosphorylated proteins under our experimental conditions.

Line 209 and Lines 478-479. Use of the term "drought" in the context of a stress treatment. Drought is a complex stress that also includes high temperatures and high irrradiance. Should use "water deficit stress" for the simple water withholding treatments used here. Using the term "drought" in the context of stereotypical stress responses is acceptable.

We carefully went through the whole manuscript, especially water deficit stress test part, to make sure all descriptions are accurate.

Line 373 and Line 396-397. The mechanism for TINY conferring drought tolerance is not fully revealed. I agree that the results increase our understanding of the mechanism by which TINY confers drought tolerance. This mechanism has not been fully established. The importance of the protein-protein interaction between TINY and BES1 remains unknown. It is clear that these factors bind to distinct upstream elements upstream of the promoters of various BR-responsive and stress responsive genes but the mechanisms of their action are not clarified by the results presented here. The results suggest that TINY and BES1 do not act cooperatively. Rather, their actions seem to be additive (subtractive?). Again, what is the significance of their physical interaction? Is it necessary for their function?

Our results revealed that TINY confers drought tolerance though two mechanisms: 1. TINY positively regulates ABA-mediated stomatal closure (Figure 3); 2. TINY promotes drought responsive gene expression (Figure 3-4 and Supplementary Figure 5). While the focus of our study is to reveal how TINY interacts with BES1 to regulate BR-

mediated plant growth and drought response, these two mechanisms can at least partially explain TINY's function in drought response.

We further discussed the importance of TINY and BES1 interactions in discussion part (lines 408-416).

Line 393-395. It is not clear what this statement means. Perhaps something like: The negative effects of TINY on BR-regulated growth support the notion that inhibition of plant growth is dependent on the repression of genes that are required for cell elongation and proliferation. Is cell division different from proliferation? Also, it is not clear that inhibition of growth is sufficient to achieve stress tolerance.

After carefully reading of the references cited (Claeys and Inzé, 2013; Kudo et al., 2018), we changed the sentence as reviewer suggested that "The negative effects of TINY on BR-regulated growth support the notion that inhibition of plant growth is dependent on the repression of genes that are required for cell elongation and division." Also at least, in our case, we observed that inhibition of plant growth by TINY was coincided with increased drought stress tolerance.

Line 400-401. As clarified below (Line 407-409) TINY and BES1 bind to different DNA elements. This should be clarified here. The use of the word "corresponding" in this context is confusing.

We clarified as follows: TINY and BES1 bind to different DNA elements and inhibit each other's activities.

Line 415. In my opinion, the use of the term "major finding" is hyperbolic as it suggests that the finding is extraordinary. It is an interesting finding, though, in my opinion, not unambiguously supported by the evidence.

We changed the sentence as follows: "In this study, we showed that the function of TINY is negatively regulated by BR signaling through BIN2."

Minor editorial comments:

Line 40-41. Though understandable, the phrasing in the leading sentence in the introduction is cumbersome. I recommend something like: Environmental challenges such as water deficit and extreme temperatures are associated with decreased plant growth and can cause severe crop losses.

We changed as the reviewer suggested.

Line 118. "....transgenic lines overexpressing TINY...." Should be: ....transgenic lines that overexpress TINY....

We changed as the reviewer suggested.

Line 137. It does not look to me that the triple mutant plants are "resistant to Pcz treatment" because some effects are still evident. I recommend changing this to less sensitive to Pcz treatment.

We changed as the reviewer suggested.

Line 192. This sentence is cumbersome: All genotype were confirmed with genotyping before testing. First, genotype should be plural, second, what is "genotyping"? If you don't want to define what is meant by genotyping here, just say that: All genotypes were confirmed before testing.

We changed as the reviewer suggested.

Line 195. What does HT stand for?

We are not using "HT" anymore. We have clarified that HZ is an abbreviation for heterozygous.

Line 363. "BIN2 kinase" This term suggests that you are referring to an enzyme that phosphorylates BIN2, a BIN2 kinase. Something like: "The GSK protein kinase BIN2" or just "BIN2" would be more clear.

We have clarified the wording throughout the manuscript.

Line 383. "*TINY* transcript" should be plural.

We changed "*TINY* transcript" into "*TINY* transcripts"

Reviewer #3:

The authors responded well to most of my criticisms and comments and improved the text.

TPC2018-00918-RAR1 2nd Editorial decision – *acceptance pending* **April 22, 2019**

We are pleased to inform you that your paper entitled "The AP2/ERF Transcription Factor TINY Modulates Brassinosteroid-Regulated Plant Growth and Drought Response in Arabidopsis" has been accepted for publication in *The Plant Cell*, pending a final minor editorial review by journal staff. At this stage, your manuscript will be evaluated by a Science Editor with respect to scientific content presentation, compliance with journal policies, and presentation for a broad readership.

Final acceptance from Science Editor **May 20, 2019**