SlMYC1 Regulates Type VI Glandular Trichome Formation and Terpene Biosynthesis in Tomato Glandular Cells

Jiesen Xu, Zeger O. van Herwijnen, Dörthe B. Dräger, Chun Sui, Michel A. Haring, and Robert C. Schuurink

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Corresponding author: Robert C. Schuurink r.c.schuurink@uva.nl.

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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.)

TPC2018-00057-RA 1st Editorial decision – *declined* Feb. 23, 2018

As you will see when you review their comments, the two reviewers felt that the work you report is important and will constitute a significant contribution to the field. That said, there were a few recurring themes in the reviewers' comments that if addressed, will improve the manuscript.

1. One recurring theme among the reviewers involved the clarity of writing and the overall length of the manuscript. Some passages are very clear while others are much more challenging to follow, and would be particularly so for the broad readership of The Plant Cell. The number of mutant and transgenic lines involved, and the varying effects on gene expression and metabolite accumulation, make the passages on these topics somewhat difficult to follow in places. We would ask you to do your best to simplify the presentation of these data where possible and avoid the use of abbreviations that need to be defined. Similarly, one of the reviewers commented that there is significant reiteration of results in the Discussion, and that an overall tightening up of the manuscript would be desirable. We suggest that you reduce the Discussion by 33% in word count and 10% in the Introduction and Results.

2. Both of the reviewers also raised concerns or had suggestions about improving quantification of the trichome phenotypes of your various lines.

3. Reviewer 2 has concerns about the spider mite experiments and suggests that they should be expanded somewhat. The reviewer also points out that these experiments are not mentioned in the Discussion. You might want to consider whether these experiments should be expanded or deleted entirely if they were not important enough to discuss.

4. Both reviewers have provided suggestions for missing controls that would allow your results to be interpreted with more confidence.

5. We ask you to remove the additional results presented within the Discussion. These could either be deleted entirely or moved to the Results if you believe them to be essential to your story.

6. Please see the attached file for suggestions on improving the figures, if you choose to resubmit. Apply these suggestions also to the supplemental figures. The supplemental table must be visible at 100% view and the font must be Arial or Helvetica. It may be possible to submit it as an Excel file.

7. There are too many supplemental files. Please read the instructions for authors for criteria for supplemental figures. If your figures do not meet these criteria, please move them to the main paper.

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

Reviewer comments and **author responses:**

Reviewer #1:

The manuscript by Xu et al. describes research to explore the role of SlMYC1 in the regulation of tomato trichome development and volatile terpene biosynthesis in these organelles. They convincingly show the relevance of this bHLH TF through transcript and metabolite profiling of knockdown, knockout and overexpression tomato lines.
I have two major considerations and some minor comments.

Point 1. I was wondering how the authors would envisage the exact role of SlMYC1 in type VI trichome development considering that reduced SlMYC1 expression (as in the RNAi lines) leads to shorter trichomes but no real effects on trichome numbers or densities, thus suggesting that SlMYC1 is not involved in trichome initiation per se, whereas the homozygous SlMYC1 mutant has no type VI trichomes at all, suggesting that SlMYC1 would be crucial for initiation as well?

RESPONSE: We envision that SlMYC1 is essential for initiation of type VI glandular trichomes, as a knock-out leads to their absence, and that it also plays a role in their development, as knock-downs have smaller type VI glandular trichomes and have fewer type VI trichomes on the abaxial leaves, just like the heterozygous MYC1/*myc1*. In addition, SlMYC1 plays a role in regulating terpene synthases. We have made this clearer in the manuscript.

Point 2. Linked with the above, it could be mentioned on p 14 after line 302 already (now only in the Discussion) that JA elicitation cannot overrule total loss of type VI trichomes in the MYC1 mutant. In this regard, it is needed to confirm this observation by analyzing the effect of JA on the MYC1 CRISPR KO line.

RESPONSE: We did mention that JA application cannot overrule the loss of type VI trichomes in the homozygous *myc1* mutant already on p14 line 287-289 but have now made this even clearer for the reader. This is very similar to the fact that JA application cannot restore the phenotype of the *myc2* mutant in Arabidopsis (Plant Cell. 2004 Jul;16(7):1938-50) nor that of the *myc3* or *myc4* mutant (Plant Cell. 2011 Feb;23(2):701-15). Still, we tested whether JA application of the SlMYC1 knockdown lines could rescue the smaller phenotype of the type VI glandular trichomes, which was indeed not the case. Thus, since both the knockdown and knockout lines cannot be rescued by application of JA, we do not see the need to perform the same experiment with the CRISPR-Cas9 line as well.

Point 3. Results, p 5, lines 129-130. The authors note a positive correlation between the extent of *SlMYC1* gene silencing and the levels of all of the monoterpenes (as well as of all of the monoterpene genes). It is therefore striking that exactly the same trends (thus now anti-correlated with the extent of *SlMYC1* gene silencing) are observed for *SlTPS12* expression and sesquiterpene levels.

RESPONSE: This is precisely what one would expect if SlMYC1 is a positive regulator of monoterpene synthases and a negative regulator of sesquiterpene synthases in stem trichomes. We have tried to explain this better in the text, as this is indeed a very interesting and exciting finding that also shows that these smaller type VI glandular trichomes are still very metabolically active with regard to terpene biosynthesis and that the reduction of terpene biosynthesis is not due to the smaller size of the glandular head of the trichomes.

Point 4. Results, p 9, lines 193-207. There does seem to be a statistically significant effect on type VII trichomes in the RNAi lines (Fig S6A), contrary to what the authors claim in the text. Hence, this paragraph needs rewriting, as in

its current state it is confusing and does not represent the experimental observations correctly. E.g. Figure 4A neither shows a significant reduction in type VI trichome density on the stem surface nor does it show significant reduction in type VI trichome density on the adaxial leaf surface for line 25. Supplemental Figure 6A does show a significant increase in type VI trichome density on the adaxial leaf surface for line 8. The sentence that spans from line 200 to 202 is very confusing.

RESPONSE: Indeed, there seems to be only a significant effect on type VII/VII-like trichomes for two of the RNAi lines, and only a significant reduction of type VI trichomes on the abaxial leaves. We have made the text more accurate to reflect the data.

Point 5. Results, p 17, lines 368-376. The evidence that SlMYC2 is not involved in type VI trichome development or terpene biosynthesis is now only conclusively shown for stems, not for leaves. I suggest that the authors either attenuate their statement accordingly, or alternatively, carry out some analysis on leaves of SlMYC2 silenced plants.

RESPONSE: We have performed qRT-PCR analysis to determine the transcript levels of several terpene synthases in the leaves that show that these are not affected (New Supplemental Figure 9). We have changed the text accordingly.

Point 6. Discussion, p 23, lines 509-511. In my view, reduced terpene levels look like a combined effect of reduced trichome numbers and sizes and reduced terpene biosynthesis within them, rather than an exclusive effect of reduced levels within the trichomes, no?

RESPONSE: On stems, the ir-MYC1 lines have similar numbers of type VI trichomes as wt (Figure 4), but the reduction in transcript levels of terpene synthases is mostly more than 90%, whereas the reduction in the size of the glandular heads of the trichomes is only between 20 and 40% (Figure 3). These trichomes still produce more β-caryophyllene and α-humulene, correlating with higher *TPS12* expression, as also shown by collecting individual trichomes (Figure 6). Also, the experiments with the heterozygous *MYC1/myc1* mutant in which the size of the type VI trichome is not altered (Figure 3) support the conclusion that SLMYC1 controls the expression of terpene synthases: on stems, on which the trichome density is not altered, the trichomes still produce more sesquiterpenes; on leaves, which have a reduced number of trichomes on the abaxial site, the trichomes still produce normal amounts of sesquiterpenes, with monoterpenes slightly reduced (Figures 4,5). Analysis of individual trichomes confirmed this (Figure 7). Of course we cannot exclude that there might be an additional effect of a reduced cavity size, but this would require further investigations.

Reviewer #2:

The manuscript describes allelic tomato mutants defective in MYC1, a bHLH transcription factor. Mutants were generated by RNAi knockdown, forward mutant screening to obtain a C-terminal truncated mutant, and CRISPR editing. The strong mutants showed complete loss of type VI trichome and RNAi lines had more mild phenotypes. Plants defective in MYC1 showed reduced terpene volatile accumulation in type VI trichomes, and this correlated in some cases with reduced expression of some terpene synthase genes but not others. RNAi lines also became susceptible to herbivorous spider mites. The major finding is the identification of MYC1 as being specifically required for the development of type VI trichomes, which are a major model system for studying glandular trichomes. This is of general interest to plant biologists. The following suggestions are provided for how to improve the manuscript.

Point 1. There are several significant concerns with the interpretation of the E161K mutant data. First, because there is no control in which MYC1 WT protein expressed from the *TPS5* promoter (or I missed it), its unclear if the observed phenotypes are caused by mis/overexpression from the *TPS5* promoter or if these phenotype indeed are caused by E161K mutation. It seems likely that the *TPS5* promoter is much stronger than the native *SlMYC1* promoter, which in turn could lead to protein dosage effects, especially when multiple TFs may interact in a complex. A related point is that the authors have not shown that E161K mutant is constitutively activated due to loss of JAZ repressor binding or other reasons. Re text lines 266, 517 and elsewhere: the mutant protein should not be referred to as a "stabilized" MYC -- no evidence is presented to back this up or explain what is meant by stabilized.

RESPONSE: The aim of this experiment was to use a trichome-specific promoter to determine whether MYC1 could activate the terpene synthases in the trichomes. We did not want to use an ectopic promoter or the *SlMYC1* promoter itself, as it would not address this question directly. The overexpression by the *TPS5* promoter is modest,

as depicted in Figure 8.

We first tried expressing MYC1 from the stronger TPS9 promoter but did not get any overexpression, only one co-suppression line. Next, we tried expressing a MYC mutant shown to be released from JAZ repression in Arabidopsis, D94N (New Phytol. 2015 Jun;206(4):1229-37). We obtained one tomato line expressing this SlMYC1^{D94N} driven by the *TPS5* promoter showing slightly higher transcript levels of TPS3 and lower levels of TPS12 than wild type. Since we were still not entirely convinced, we used the SlTPS5:MYC1^{E161K} and SlTPS9:MYC1^{E161K} constructs to transform tomato. Also, the one line obtained with SlTPS9:MYC1^{E161K}, which expressed low levels of MYC1^{E161K} without having any effects on total MYC1 transcripts, expressed TPS3 and TPS12 at slightly higher and lower levels, respectively. Thus we don't think that our phenotype is due to a dosage effect.

Point 2. Spider mite experiments. Although it is shown that mites produce more eggs when grown on the SlMYC1 RNAi line, this could be backed up by data about whether mites did more damage to the mutant compared to wt. In general, after reading the manuscript, I did not come away with a good sense of how/if MYC1 (as opposed to MYC2) contributes to herbivore defense. Based on the differential accumulation of phytohormones and herbivore-induced genes between WT and RNAi lines, authors conclude that MYC1 is essential for JA responses, but the RNAi lines are more susceptible to spider mites, which potentially contribute to higher herbivore damage and therefore amplified plant responses to herbivory. So, to analyze the herbivore responses, the authors should control the damage levels by herbivore or analyze short-term responses.

RESPONSE: As suggested by the editor, we have now deleted the spider mite experiments.

Point 3. I can appreciate experiments to understand how the expression of various TPS genes in wt and mutants correlates with terpene profiles. For example, one interesting result reported is that some sesquiterpene synthesis genes are upregulated rather than downregulated on stems but not leaves of *myc1* mutants, leading the authors to speculate about how this could happen through repressors, TF complexes, etc. I find several weaknesses in these experiments. Analysis of terpene levels/TPS expression was presented for *myc1/+* heterozygotes, but I find this data difficult to interpret without knowing more about the mechanism and MYC1 targets. There also appear to be some inconsistent results between the RNAi lines and E161K-OE lines in terms of *TPS17* and *TPS31* expression.

As a general observation, it seems difficult to determine if reduced terpene levels are due to misregulation of TPS genes or simply developmental problems in *myc1* mutants, or both. The outcome to the reader is a fairly complex if not confusing picture of what MYC1 regulates and where (leaves and stems). Although it would require some additional work, directed ChIP experiments testing if MYC1 binds to the promoters of select TPS genes would greatly strengthen the conclusions, similar to what was reported by Du et al. TPC in their characterization of SlMYC2.

RESPONSE: We have no good antibodies against SlMYC1 (a paper using AtMYC2 antibodies was just retracted, Plant Physiol Vol. 170 : 2432–2443, 2016). We do not know if SlMYC1-GFP is fully functional, and no one has even done Chip experiments with glandular trichomes. We believe this is outside the scope of this paper, as well that it would not be so informative, since SlMYC2 has been shown to regulate 74% of the genes indirectly (Du et al, TPC, who used in their very nice paper the 35S promoter for ectopic expression). Thus, if SlMYC1 regulates terpene synthases indirectly, we might not even detect them.

It has been known for a long time that the sesquiterpene synthase TPS12, β-caryophylene/α-humulene synthase, is expressed in leaf trichomes and not in stem trichomes (Plant Physiol. 2010 Jul;153(3):1212-23). Our data shed new light on how this differential regulation might occur and the potential role that SlMYC1 plays in it. In planta, although ATTAs showed the transactivation of *TPS12* promoters by MYC1, we found a differential regulation of *TPS12* by MYC1 in tomato leaf and stem trichomes. In this case, directed ChIP experiments may not be of great help to strengthen the conclusions we obtained in planta.

Indeed, besides consistent results between the RNAi lines, such as the effect on *TPS12* and *TPS39* expression, there are some seemingly inconsistent results for *TPS17* and *TPS31*. Thus, we have only made a start with solving parts of the transcriptional network regulating the many terpene synthases in tomato. The community can now pick this up to continue this.

As described above, we strongly believe that the reduced terpene levels are not due to developmental problems, as downregulation of *SlMYC1* led to a higher production of the sesquiterpenes β-caryophylene and α-humulene. Also, expression of MYC1^{E161K} in the trichomes led to higher expression of some monoterpene synthases while

repressing *SlTPS12* expression in stem trichomes, indicating that SlMYC1 regulates terpene synthases.

TPC2018-00179-RA 1st Editorial decision – *declined* **July 27, 2018**

Our post-review discussion of your manuscript has been extensive and intense in order to arrive at a fair decision and one that recognizes the value of your work to the scientific community. We recognize the improvements made to this version of the manuscript but as the reviewers point out, there are still significant issues with interpreting the trichome results. As you will see, the reviewers and editors are not fully convinced that the sole interpretation of the results are that SlMYC1 is required for Type VI trichome initiation rather than maturation. Instead, the reviewers put forward alternative interpretations. The reviewers are requesting more experiments to test the sole interpretation. We are not fully convinced that these experiments are going to provide as clear-cut an answer as the reviewers. If you have any experiments on these lines, they would be greatly helpful to include. Alternatively, you may want to consider broadening the scope of your interpretation.

In our reading of the manuscript, the key novelty is that a MYC2 paralogue/orthologue is responsible for trichome specification, which is unknown in other systems. Thus, an alternative is to optimize the MYC/bHLH phylogenetics and trichome quantification and then to provide other mechanistic/developmental interpretations that could equally explain the data. The singular mechanistic interpretation is in our view not the key novelty of the manuscript. We also agree that at least some of the JA-related data could be removed without significantly impacting the message of the manuscript.

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

[Reviewer comments shown below along with author responses]

TPC2018-00571-RA Submission received **Sept. 27, 2018**

Reviewer comments on previously declined manuscript and **author responses:**

Reviewer #3:

Point 1. This manuscript is concerned with the activity of SlMYC1 in development of type VI trichomes of tomato. There is a lot of other analysis included in the manuscript based on data (some already published) that SlMYC1 is a regulator of terpenoid biosynthesis, or at least of expression of genes encoding some terpene synthases in tomato. My view is that the terpene synthase work serves only to complicate and obscure the most important points being made in this manuscript. As some of the work on SlMYC1 regulating TPS expression has already been published and some of it is flawed, I suggest that this entire section of the manuscript be published separately.

RESPONSE: The first paragraph of the comments of reviewers 3 contains remarks that are very suggestive (I have underlined them). It is insinuating that we publish the same data twice and that we include data to complicate and obscure points. The statement that our data (which?) are flawed is not substantiated at any point.

Point 2. However, that then leaves the new observation that SlMYC1 regulates type VI trichome initiation and development rather under supported in terms of appropriate analysis to establish this claim convincingly. I will outline what I think is necessary to substantiate the claim that, 'SlMYC1 is essential for the initiation and formation of Type VI trichomes' in tomato (lines 183-184) as well as comment on the data concerning the role of SlMYC1 in regulating terpene biosynthesis.

The authors have a non-functional knockout mutation (EMS) as well as a homozygous CRISPR deletion of SlMYC1. It would be much easier to follow the arguments about the role of SlMYC1 if these mutants were examined first, and fully characterised. Presumably these data were added later to the paper, which may consequently have suffered from preconceived ideas built on the less reliable or less easily interpretable RNAi lines. The data from these RNAi lines could then be discussed in terms of weak alleles compared to the KO mutants.

RESPONSE: The reviewer presumes that we added data later and that we have preconceived ideas. Based on what?

Why our RNAi lines are less reliable is not substantiated anywhere.

Point 3. Trichomes develop relatively early in the development of the leaf epidermis, but work from Arabidopsis has shown that trichome density differs with the particular leaf on the plant and whether they are on the adaxial or abaxial surface. It is not clear whether or not similar changes occur on the stem of tomatoes, but it is important to know whether or not the tissues were fully mature and had stopped expanding before estimating trichome densities. Very helpful, also, is to express trichome densities per total number of epidermal cells rather than per unit area. The Methods section gives no indication of whether the leaves or stems sampled for each experiment were fully expanded - taking the youngest leaflet from the 5th leaf or the leaflet from the growing tip seems to indicate that the material was still expanding, which would give rise to unacceptable errors in recording trichome densities. (There is evidence for this in Supplementary Figure 1 - WT-RZ). Similarly, reports of trichome density recorded per cm^2 may be unreliable if the tissue is still expanding.

RESPONSE: We took fully mature stems and also looked at fully mature leaflets of plants at different developmental stages. We have now indicated this better in the Methods section. Counting per cm^2 of fully developed stems and leaves is thus reliable. Supplemental Figure 1 showed a young developing leaf for illustration, not for counting. We have replaced this figure with SEMs of type VII-like trichomes (SFig.1A).

Point 4. There is no indication of how many electron micrographs were used for each biological replicate (one?).

RESPONSE: The suggestion that we would have taken only one micrograph is an unfair comment of this reviewer. We have made 80 SEMs, and we have clearly indicated in Figure 3C that 3 or 4 SEMs per line were used to determine the sizes of the gland and stalk.

Point 5. If only one picture was taken per biological replicate, this could be heavily biased by counting trichomes close to vascular tissue. Similarly, no indication is given of whether the leaf material sampled was imaged on the abaxial or adaxial surface in most instances. The light micrographs of stem trichomes are completely uninterpretable.

RESPONSE: We have clearly indicated in the Methods section that we used a Leica MZFLIII microscope to count the trichomes. We have also plainly indicated whether the leaf counts were ab- or adaxial. The light micrographs clearly illustrate the absence or presence of the type VI glandular trichomes.

Point 6. The derivation of the CRISPR/Cas9 knockout mutation of SlMYC1 needs to be properly described and the mutant phenotype fully described in terms of the criteria listed above.

RESPONSE: We clearly described the making of the CC mutant and included the mutated sequences. We have characterized the phenotype with regard to several parameters.

Point 7. One of the most important comments in the excellent paper by Bergau et al. is that it is impossible to distinguish which type of trichome a tomato trichome is going to be at the initiation stage. Although subcellularly Type VI trichomes can be distinguished at later stages, this would not be obvious at stages up to ~50 um long. So it seems quite possible that the Type VII-like trichomes newly observed in the homozygous mutant and some RNAi lines could actually be modified Type VI trichomes resulting from a lack of SlMYC1 activity. Similarly, in the single micrograph of the stems of WT and homozygous *slmyc1* mutants, there are a number of short stumpy trichomes that are not as long as Type IV trichomes. No comment is made about these, but they are not visible in the micrographs of the other lines. Were these trichomes counted? What were they classified as? Failure to include these in the analyses would have biased the data and their interpretation.

RESPONSE: We clearly state in the manuscript: "these have a single stalk cell, with a wide intermediate cell and a berry-shaped glandular head composed of multiple cells similar to type VII glandular trichomes". The wide intermediate cell is also present in type VII trichomes, and not in type VI trichomes. Therefore, these were categorized as type VII-like and counted as such. We have added two new figures (SFig. 1A) to illustrate more clearly the nature of these aberrant trichomes.

Point 8. I suggest that these could be interrupted type VI trichomes that fail to develop a proper glandular head and so were therefore not classified as Type VI trichomes. This possibility (that the aberrant non-glandular and Type VII-like trichomes are modified Type VI trichomes) needs to be investigated properly. If true, then the authors would need

to retract the idea that SlMYC1 is involved in Type VI trichome initiation, which, in the absence of reliable trichome density measurements, is not supported by the data available.

RESPONSE: As stated above, our trichome density counts are reliable. The lower numbers of the type VI trichomes are not complemented by the type VII-like trichomes. We have now broadened the scope of our interpretation in the Discussion along the lines suggested by this reviewer – i.e. modified type VI trichomes - and by the editor.

Point 9. Such an interpretation might also address the issue of SlMYC1 being a direct regulator of terpenoid biosynthesis as well as Type VI trichome formation. If SlMYC1 were required for the formation of the gland cells of Type VI trichomes, it could have a pleiotropic effect on the production of terpenoids, because the glandular cells are the site of synthesis of specific terpenoids. Then the effect of *slmyc1* on *TPS* expression could be indirect through the fact that *TPS5* is trichome-specific in its expression. The induction of the *TPS5* promoter by SlMYC1 in *N. benthamiana* is quite modest (maximum about 4-fold, suggesting that it might be an indirect effect). Is the *TPS5* promoter expressed in *N. benthamiana* specifically in trichomes?

RESPONSE: The reviewer missed a point: sesquiterpene biosynthesis is higher when SlMYC1 is downregulated (and fewer and smaller trichomes are present), so a pleiotropic effect leading to less terpene biosynthesis is not what we observed. The *Agrobacterium*-mediated transient expression assay was performed in *Nicotiana benthamiana* leaves: expression did not take place in glandular trichomes but in mesophyll cells.

Point 10. The data on the over-expression of the mutated E16K mutant are flawed. As reviewer #2 originally pointed out, there is no proper control (pTPS5:SlMYC1) to compare to the effects of expression of pTPS5:SlMYC1(E16K). No evidence is given that that SlMYC1(E16K) is, in fact, a stabilised version of SlMYC1. Indeed, the mutation was made by homology to a mutation in AtMYC2, which clearly has functions that are distinct from SlMYC1 despite them being structurally related in the same clade of bHLH proteins. Clarification of the relatedness of these different proteins through provision of a phylogenetic tree would also be most helpful. Although the authors addressed Reviewer 2's concerns about the use of the *TPS5* promoter, the responses to the points relating to the interpretation of the SlMYC1(E16K) mutation were conspicuous by their absence.

RESPONSE: I do not understand why the data are "flawed". Why does reviewer 3 state that points are conspicuous by their absence? We have provided data of other transgenic lines to accommodate the request of the previous reviewer. The choice of the E16K mutation is based on solid science on AtMYC2.

Point 11. As for the JA experiments, they seem to have little relevance to this work. After wading through a lot of histograms, the only conclusion one can draw is that JA has no effect on the functioning of SlMYC1 which is, perhaps, not unsurprising, since the maximum effect, reported by the authors, of JA on *SlMYC1* expression was 1.4 fold, so it was hardly likely that SlMYC1 activity could be responsible for the big effect of JA on Type VI trichome numbers.

RESPONSE: Because MYC proteins are known to interact with JAZ proteins and thus orchestrate the JA response, we investigated the effect of JA treatment. It is not the induction that is important but the interaction of the MYC protein with JAZ proteins and a predicted effect of MYC1 on the JA-pathway in light of MYC2. We have deleted Figure 6 and S. Fig. 8 to make the manuscript more compact without losing its message.

Point 12. In terms of relating the activity of SlMYC1 to terpene biosynthesis, the authors need to consider its effect on other structural genes required for biosynthesis. Transcription factors tend to regulate secondary metabolic pathways as regulons and if the second or third steps in the pathway are not also regulated by SlMYC1, then the correlation between specific terpenoids and SlMYC1 and TPS activity will be lost.

RESPONSE: We are aware of this, but do not see the necessity to talk about textbook hierarchical TFs, as we discussed sesquiterpene biosynthesis and MYC complexes in our paper.

Point 13. The authors have omitted to cite some important papers on the regulation of trichome formation in tomato:

Ewas, M et al., 2017. RNA-seq reveals mechanisms of SlMX1 for enhanced carotenoids and terpenoids accumulation along with stress resistance in tomato. Science Bulletin, 62(7), pp.476-485.

Ewas, M., et al 2016. Manipulation of SlMXl for enhanced carotenoids accumulation and drought resistance in tomato. Science bulletin, 61(18), pp.1413-1418.

They need to also revise their discussion about the factors controlling trichome initiation and development in the light of data from species other than Arabidopsis (Serna and Martin, 2006) and most importantly data from cotton: Wu, H., et al., 2018. Genetics and evolution of MIXTA genes regulating cotton lint fiber development. New Phytologist, 217(2), pp.883-895

RESPONSE: We have added the (first two) references and included some text about them. As we were instructed to reduce the size of our paper, we don't have enough space to discuss the other references.

Reviewer #4:

Xu et al. examine the role of the basic helix-loop-helix transcription factor SlMYC1 in the formation and density of type VI glandular trichomes as well as terpene synthase transcript and terpene metabolite levels in leaf and stem trichomes of *Solanum lycopersicum*. The major finding of the study is that SlMYC1 is required for type VI glandular trichome formation since these trichomes are absent in a homozygous *myc1* mutant and CRISPR lines and their morphology is affected in *SlMYC1* knockdown lines. Furthermore, the results show that SlMYC1 regulates monoterpene synthase and sesquiterpene synthase gene expression and terpene levels. SlMYC1 differentially controls the transcript abundance of a caryophyllene/humulene synthase in leaf and stem trichomes, which correlates with different levels of these terpenes in these trichomes. The authors further investigated the effects of reduced *SlMYC1* expression on the induction of terpene synthase transcription and terpene levels by jasmonic acid.

Overall, the authors present a comprehensive study of the role of a MYC transcription factor that was identified previously by the same group in a *S. lycopersicum* trichome transcriptome. The work appears quite robust, since it is based on results obtained from a combination of knockdown lines, EMS mutants, CRISPR and overexpression lines. The authors have improved the manuscript by including several revisions on data interpretation and style based on comments by previous reviewers.

Point 1. While this is not the first study of a transcription factor with a function in trichome development, this study shows that SlMYC1 is a major regulator in the formation of type VI trichomes and, therefore, terpene metabolism in tomato. The work also points toward a complex regulation of the development of different trichomes by different transcription factors, since the absence of type VI trichomes gives rise to type VII-like trichomes.

What remains unclear from this work is (this has been addressed by one of the previous reviewers) whether SlMYC1 affects terpene synthase gene transcription directly by binding to the respective promoters or indirectly, as hinted by the authors, by downstream transcription factors or in complex with other factors. The manuscript should provide more clarification and data about this point. What is the effect of knocking down *SlMYC1* on trichome-specific transcription factors that were previously identified by the group? In particular, previous studies showed that SlEOT1, identified by a yeast-one-hybrid screen, transactivates the *SlTPS5* promoter. How is *SlEOT1* expression affected in *SlMYC1* knockdown lines?

RESPONSE: Thank you for your comments! We have indeed looked at *EOT1* expression in three independent T1 lines in which *SlMYC1* is silenced and did not observe an effect on *EOT1* transcript levels. Since we were told to reduce the numbers of (supplemental) figures and text, we hesitate to include (a figure of) this in the manuscript. Indeed, we do not know whether SlMYC1 binds directly to the respective promoters or acts indirectly. As we tried to indicate to the previous reviewer, this would be a fair amount of challenging work to find out.

Point 2. While I understand that this study is focused on type VI trichomes, it would be interesting to obtain more information about the compound content of the VII like trichomes that appear in the *myc1* mutants.

RESPONSE: We fully agree, it would be very interesting to know the primary and specialized metabolites present in the type VII-like trichomes. This would require quite some work, as we need to find a way to collect them without the real type VII like trichomes (of which the compound content is still unknown).

Point 3. Can the results on terpene levels in the individual type VI trichomes (Fig. 7) be complemented by terpene synthase transcript data?

RESPONSE: That is an interesting question. We have not tried qRT-PCRs on individual trichomes yet and do not know how many we should collect these for proper RNA isolation. One hundred heads relates to 400 cells and although transcript levels have been determined in fewer – but other types of - cells, the terpenes in the trichomes

make the RNA isolation very difficult. We would have to test if existing kits would work or design our own.

TPC2018-00571-RA 1st Editorial decision – *accept with minor revisions* Oct. 12, 2018

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 your previous e-mail correspondence with Dr. Merchant in which you agreed to shorten portions of the manuscript.

[Email from Sabeeha Merchant: I see that your manuscript has been re-submitted but without many of the revisions suggested by the editors. Our editors take their jobs seriously and they would really like to see these changes. Therefore, I would typically return the manuscript to the authors in such a situation, but in this case, I thought I would open the door to some communication so that the work can eventually be published in The Plant Cell.

The editors had requested that you shorten the JA aspects of the Discussion to focus on what are new insights rather than summarize all the data. I think that this can be accomplished readily. May I suggest that you shorten the discussion by 25-30% in word count? Our suggestion is designed to help you to increase the impact of your work. As you know, we do not have a page limit for papers, and this has resulted in uncontrolled increase in the length of our papers. Readers often get lost in the weeds and the key take-home points are missed. Tightening up a paper can only benefit you. Yes, it is work for you, but it will be well worth it. You may even wish to hire a professional editor to help you with this. We use planteditors.com but you may have preference for others that you wish to use.

In addition, I understand your argument that you do not want to include your responses to reviewer 2 in the manuscript because it would add to the length. In this case, we can deal with it by publishing the peer review process file, so perhaps you can let us know whether this is acceptable to you. The option to include your response to the reviewer within the text of your paper is of course still open.

I hope you are willing to make these changes to avoid unnecessary delay in publishing your work. If you are willing to do these things, I will communicate with the editors so that we can move forward to accept your paper. Then, you can make these changes at the science editor stage. I will communicate with them so that you can work with them to make it happen. I hope this compromise is acceptable.]

TPC2018-00571-RAR1 1st Revision received Nov. 7, 2018

AUTHOR RESPONSE: Once again thank you for handling our manuscript and the constructive email conversation. We have worked with the professional Plant Editors (planteditors.com) to improve the scientific writing and to reduce the Discussion by a little over 30% as requested. I have added a manuscript version with track changes for your perusal. The Discussion has been reduced from 1802 to 1125 words to focus on new insights. I hope that this much-improved version of our manuscript can be published in the Plant Cell.

TPC2018-00571-RAR1 2nd Editorial decision – *acceptance pending* Nov. 12, 2018

We are pleased to inform you that your paper entitled "SlMYC1 is involved in tomato type VI glandular trichome formation and in regulating terpene biosynthesis in glandular cells" 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 Nov. 21, 2018