Tangled in transcription

The web of transcription factors regulating tomato type VI glandular trichome development and specialized metabolsim

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Publication date
2026

Chapter 5

General discussion

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5.1 The interplay between SlHDZ38 and SlMYC1 in the trichome transcriptional network

In this thesis two transcription factors, SlMYC1 and SlHDZ38 have been investigated with regard to their role in the trichome transcriptional networks. Both in the regulation of the development and the specialized metabolism. Here the effect on the trichome transcriptome of mutating each of these genes will be discussed.

SlMYC1 is a tomato bHLH transcription factor that was initially identified as a regulator of the biosynthesis of volatile terpenes, capable of transactivating the promoter of several terpene synthase genes (TPS; Spyropoulou et al., 2014). When characterized by gene knockdown, knockout and overexpression in trichomes, the SlMYC1 role appeared more extensive than initially hypothesized (Hua et al., 2020; Xu et al., 2018). In fact, it has been demonstrated that in leaves of cultivated tomatoes, the expression of many *TPS*s is positively regulated by SlMYC1 and that in stem trichomes, SlMYC1 promotes the expression of monoterpenes synthases, but suppresses sesquiterpene synthase expression. Additionally, it has been discovered that SlMYC1 has a second role: it controls type VI glandular trichome formation, as its knockdown and knockout reduce their number on leaves or cause their almost total absence on leaves and stems, respectively (Hua et al., 2020; Xu et al., 2018). In chapter 4, by studying the tomato mutant *glandless*, we discovered that SlHDZ38, a tomato HD-ZIP-type I transcription factor, may have a similar double function as SlMYC1. Indeed, the “natural” mutation in *glandless*, for clarity from here on called “*SlHDZ38* mutant”, results in downregulation of many *TPS* genes in leaves and stem trichomes and overall lower volatile terpenes levels (Chapter 4). In addition, in this mutant we observed the differential expression of many known trichome regulatory genes and the disappearance of the glandular head from type VI trichomes (Chapter 4). The knockout of SlHDZ38 via CRISPR resulted in the complete disappearance of type VI glandular trichomes from the leaves, with no changes in the morphology of the other trichome types, and a dramatic reduction of volatile mono- and sesquiterpenes. Having seen the resemblances between SlHDZ38 and SlMYC1 roles, the question arises: what could be the connection and how much do their regulatory networks overlap? Comparing the trichome transcriptome data of SlMYC1 mutant lines (Chapter 3) and of the *SlHDZ38* mutant (Chapter 4) will shed light on the interplay between these two TFs.

Transcriptome analysis of stem trichomes

Interestingly, *SlMYC1* expression levels are strongly reduced (-3.66 $\log_2$ fold-change) in isolated stem-trichomes of the *SlHDZ38* mutant. This would mean that, in stem type VI trichomes, SlHDZ38 acts upstream of SlMYC1 or controls *SlMYC1* expression, although SlHDZ38 has not been found among the proteins binding *SlMYC1* promoter in the Y1H assay (Chapter 2). Additionally, it has been shown that *SlMYC1* is mostly expressed from the two-gland cells developmental stage of the glandular head of type VI trichomes (Hua et al., 2020). The development of type VI glandular trichomes in the *SlHDZ38* mutant seems to stop before this stage, this another possible reason why *SlMYC1* expression is lower when compared to WT. *SlHDZ38* has a slightly lower expression in isolated stem-trichomes of the *SlMYC1* knockout plants (-0.68 $\log_2$ fold-change). Instead, no significant effects were observed on the expression of SlMYC1 in leaves of the *SlHDZ38* mutant, nor on the expression of SlHDZ38 in leaves and stem trichomes of SlMYC1 knockdown and overexpression lines. Therefore, it seems unlikely that SlMYC1 could control *SlHDZ38* expression. To understand the nature of the interaction between *SlMYC1* and *SlHDZ38*, more research is necessary. A Y1H assay using *SlHDZ38* promoter to identify putative regulators, or a Y2H assay to find out if SlHDZ38 and SlMYC1 interact at the protein level could be very informative.

Comparing the transcriptome of trichomes from the *SlMYC1* and the *SlHDZ38* mutants revealed that more than two thousand genes were differentially regulated in both lines (26% of the total; Figure 1A). The genes affected in both lines were mostly downregulated, as could be expected when mutating a TF (Figure 1B). This points at a significant level of overlap of between SlMYC1 and SlHDZ38 function in stem-trichomes. It is probable that to a certain extent this overlap is determined by the type VI glandular trichome phenotype that is partially similar between *SlHDZ38* and *SlMYC1* mutants: in the *SlHDZ38* mutant the glandular cells of type VI trichomes are missing while in *SlMYC1* mutant lines this type of trichomes is almost absent. As discussed in Chapter 4, it cannot be excluded that, especially for genes exclusively or highly expressed in type VI glandular trichomes, the lower expression levels were caused by the reduced number or absence of type VI trichomes from the stems or the lack of the glandular cells.

Transcriptome analysis of leaf trichomes

Both the *SlHDZ38* and *SlMYC1* mutants, the impact of the mutation in terms of number of differentially expressed genes is bigger in stem-trichomes than in leaves. In fact, in leaves samples not only the total number of differentially expressed genes is much lower, but also the genes affected in both *SlHDZ38* and *SlMYC1* mutants represent only the 6% of the total (Figure 1C). In the *SlHDZ38* mutant, the majority of affected genes were down-regulated, while in the *SlMYC1* mutants these were up-regulated (Figure 1D). Altogether these results could indicate that SlMYC1 and SlHDZ38 have a different role in leaves than in stem-trichomes, but they still control the expression of the same few regulatory genes that are crucial for trichome development and metabolism. This difference in regulation of terpene metabolism in leaves and stem-trichomes has been reported before (Z. Gong et al., 2021; Schilmiller et al., 2010b; Xu et al., 2018).

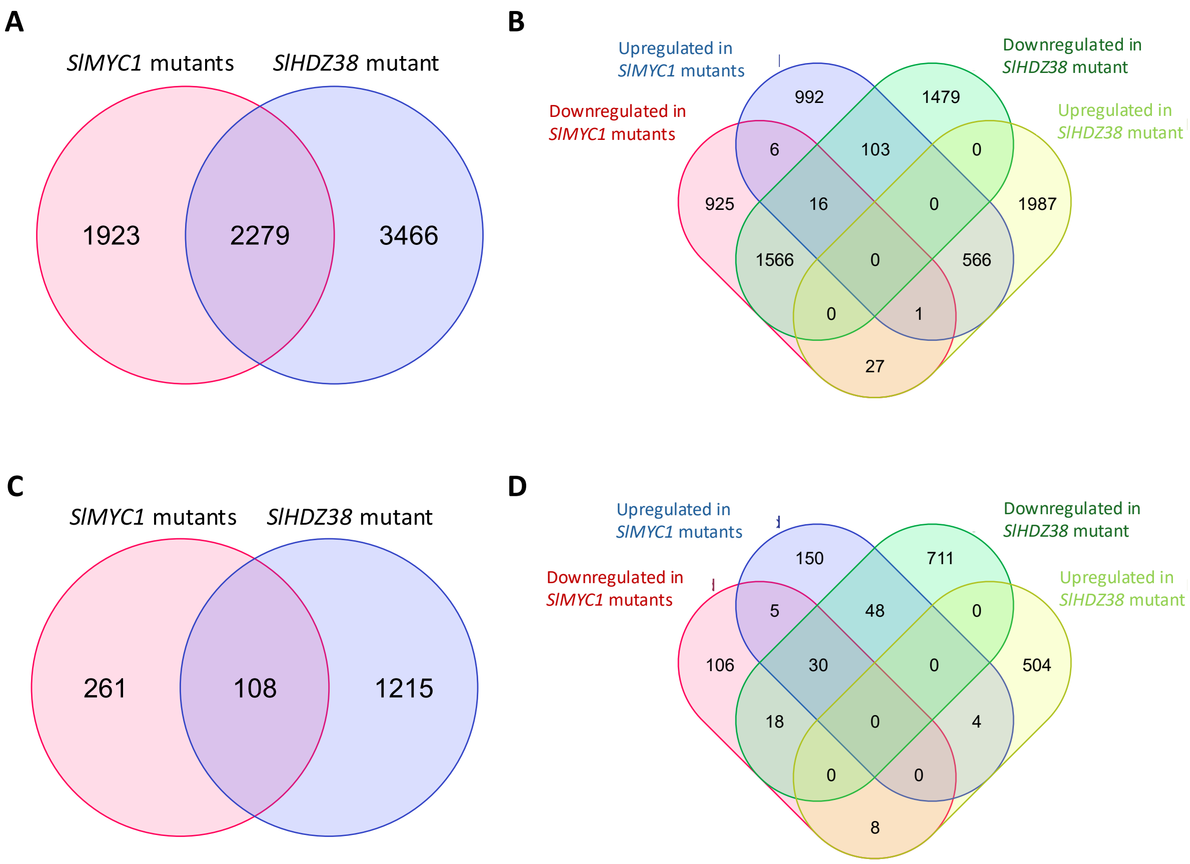

Figure 1 DEG overlap in SlHDZ38 and SlMYC1 mutants. *Venn diagram representing the number of differentially expressed genes (p-value < 0.05) in: (A) isolated stem-trichomes and (C) leaves of SlHDZ38 and SlMYC1 mutants, or both. The number of down- and up-regulated genes is specified for (B) isolated stem-trichomes and (D) leaves.*

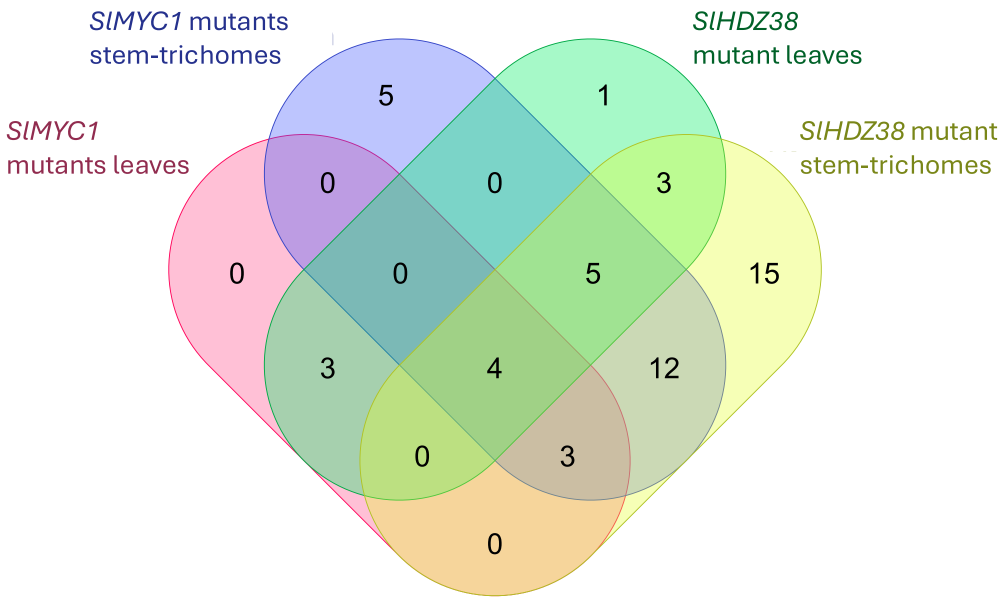

Figure 2 Differentially expressed trichome-related genes overlap in SlHDZ38 and SlMYC1 mutants. *Venn diagrams representing the number genes with a trichome-related function that are differentially expressed (p-value < 0.05) in isolated stem-trichomes and leaves of SlHDZ38 and SlMYC1 mutants, or both. See supplemental table 4 for details.*

Further insight in the interplay between SlMYC1 and SlHDZ38 regulatory networks can be obtained also by focusing on the expression levels of genes coding for TFs previously characterized as regulators of trichome initiation, development or specialized metabolism including TPSs (Supplemental table 2 and 3). Comparing the transcriptome changes in *SlHDZ38* and *SlMYC1* mutants (Figure 2, Supplemental table 4), it appears that more than half of trichome-related genes (27) are regulated by both SlMYC1 and SlHDZ38, the majority of which in stem-trichomes.

Regulation of terpene synthases

In stem trichomes, only few terpene synthases (*SlTPS16, SlTPS27, SlTPS43*) are regulated exclusively by SlMYC1, while SlHDZ38 controls three other terpene synthases (*SlTPS21, SlTPS35, SlTPS7*). Four monoterpene synthases (*SlTPS1, SlTPS5, SlTPS20, SlTPS39*) and many sesquiterpene synthases (*SlTPS12, SlTPS16, SlTPS17, SlTPS18, SlTPS31, SlTPS41*) are positively regulated by both SlHDZ38 and SlMYC1. In leaves, both SlHDZ38 and SlMYC1 are involved in regulating the same four terpene synthases: SlTPS1, SlTPS12, SlTPS20 and SlTPS41. Also SlTPS46 is regulated by both SlHDZ38 and SlMYC1, but only in leaves and not in stem-trichomes. It remains to be tested, for example via a transactivation assay in *N. benthamiana*, whether the regulation of these genes by SlHDZ38 occurs directly by binding *TPS*s promoters, as it happens for SlMYC1 in a module with SlWO, or indirectly via the mediation of some other TFs, or in both ways.

Transcription factors affected by SlHDZ38 and SlMYC1

The involvement of other TFs that mediate the regulation downstream seems more probable in stem-trichomes, where both the knockout of SlHDZ38 and of SlMYC1 causes dramatic downregulation of *SlEOT1* and *SlEOT2*, two known regulators of TPS genes expression (Spyropoulou et al., 2014a; Xu, 2023). Also *SlSCL3*, coding for a TF regulating terpenes metabolism in type VI glandular trichomes (Yang et al., 2021), is downregulated in stem-trichomes of the *SlHDZ38* mutant and *SlMYC1* knockdown and knockout lines. Having seen the similar expression pattern of *SlEOT1*, *SlEOT2* and *SlSCL3* in *SlMYC1* and *SlHDZ38* mutants, and considering the putative hierarchy in the regulatory pathway, it could be that SlHDZ38 control of terpene metabolism in stem-trichomes works via regulation of SlMYC1, which then downstream regulates TPSs, both directly binding their promoter and via SlEOT1, SlEOT2 and SlSCL3. The alternative hypothesis would be that SlHDZ38 controls directly *SlEOT1*, *SlEOT2* and *SlSCL3* expression without SlMYC1 mediation. This could be verified via a transactivation assay in *N. benthamiana*. At this stage our data are not sufficient to exclude completely the hypothesis that TPS genes as well as SlEOT1, SlEOT2 and SlSCL3, are downregulated in SlHDZ38 and SlMYC1 mutants because of the absence of type VI glandular trichomes or of their gland cells. However, this seems less likely because almost all the common differentially expressed *TPS*s were found not only in the *SlHDZ38* mutant, that has type VI trichomes with no glandular head, but also in *SlMYC1* knockout plants, where type VI trichomes are absent. In addition, in *SlMYC1* knockdown plants, where type VI trichomes still have the glandular head, although reduced in diameter, the expression of these *TPSs* is also affected. Altogether these results support the conclusion that SlMYC1 and SlHDZ38 functions overlap in controlling terpene biosynthesis via positive regulation of different subsets of TPS in leaves and stem trichomes.

More limited seems to be the overlap between SlMYC1 and SlHDZ38 regarding their role in trichome development: only two TFs are found in common and solely in stem-trichomes. SlSH, known to involved in the regulation of non-glandular trichomes development (R. Li et al., 2021), is found upregulated in both SlHDZ38 and SlMYC1 knockout. SlMTR1, previously known as SlCycB2, implicated in a negative feedback mechanism with SlWO during trichomes fate determination and development (M. Wu et al., 2023), is downregulated in both SlMYC1 knockdown plants and in the *SlHDZ38* mutant. SlHDZ38, seems to control the expression of more genes (14 in total) in stem trichomes, which are not regulated by SlMYC1 (Figure 2, Supplemental table 4). Some of the known trichome development regulators were found upregulated, including *SlWO* and its downstream targets *SlMX1* and *SlWOX3b*, which together regulate the initiation and morphogenesis of long hairy trichomes (Wu et al., 2023, 2024). Interestingly, in leaves, SlLN, which positively regulates hairy trichomes formation (Xie et al., 2022) is exclusively regulated by SlHDZ38. Finally, SlHDZ38 regulates SlTOEb1 and SlGCR1, both recently characterized as involved in regulation of trichome gland cells formation via controlling downstream SlLFS (Chang et al., 2024). Taken together these observations could mean that SlHDZ38 is high up in the hierarchy of TFs in the trichome development regulatory pathway, especially in the stem trichomes.

JAZ proteins affected by SlHDZ38 and SlMYC1

In leaves of the *SlHDZ38* mutant, *SlJAZ2* and *SlJAZ4* expression is lower than in the wild type (Chapter 4). *SlJAZ2* and *SlJAZ4* are both upregulated in leaves where *SlMYC1* is overexpressed, but no effect on their expression levels was observed in SlMYC1 knockout and knockdown mutants (Chapter 3). SlJAZ4 has been previously found to interact with SlMYC1, SlHD8 (Hua et al., 2020, 2021) and with SlMYC2 (S. Wu et al., 2024) indicating a role in modulating JA-induced responses, among which also the enhancement of trichome formation and stem cell elongation. SlJAZ2 interacts with both SlMYC1 and SlWO, hindering the formation of the Myc1-Wo regulatory module that, in presence of JA, promotes TPSs expression (Hua et al., 2020). In summary, not only SlMYC1 is regulated by SlJAZ2 and SlJAZ4, dependent on JA levels, but probably, via positive feedback mechanism, higher levels of SlMYC1 determine higher expression of these JAZ proteins. Differently than SlMYC1, SlHDZ38 expression remains unaltered both in leaves after herbivore feeding and in stem-trichomes following JA treatment (E. A. Spyropoulou et al., 2014b). This indicates that SlHDZ38 function is independent from JA signalling. Instead, we hypothesize SlHDZ38 to be responsible, independently from JA signalling, for maintaining the levels of *SlJAZ2* and *SlJAZ4* and hence stabilize their inhibitory function in absence of JA (Chapter 4). Overall, in leaf trichomes there is probably a great difference between how SlMYC1 and SlHDZ38 are regulated. In stem trichomes, instead, we found that neither SlHDZ38 nor SlMYC1 regulate SlJAZ2 and SlJAZ4. The expression of SlJAZ7, which normally is highly expressed in trichomes (Hua et al., 2020), seems to be negatively regulated by both SlHDZ38 and SlMYC1. Unexpectedly, its expression is found to be higher when SlMYC1 is overexpressed. Our results suggest that in leaves SlHDZ38 is positioned higher in the hierarchy of the regulatory pathway responsible of regulating terpenes biosynthesis in absence of JA. It cannot be excluded though that also in stem-trichomes the two TFs may share some downstream targets. The difference in expression of TPS genes in leaves and stem trichomes indicates that the terpene metabolic pathway is differently controlled in these two tissues, maybe by involving different mediators. Our results are still not sufficient to fully clarify this issue. We would have to gather more insight by generating SlHDZ38 overexpression lines and analyse the levels of SlJAZ2 and SlJAZ4. As it has been done for SlMYC1, we should investigate whether SlHDZ38 interacts with JAZs proteins in a Y2H assay, or if it binds JAZ gene promoters with a transactivation assay. This will be crucial to understand the interplay between JA signalling and SlHDZ38 regulatory function.

5.2 Other transcription factors under the control of SlHDZ38

The results in this thesis have implicated several transcription factors in metabolic regulation and development of tomato glandular trichomes. In Chapter 2, we found that SlZHD18 (Solyc05g007580) binds the *SlMYC1* promoter, thus representing a putative upstream regulator of this gene. The knock-down (VIGS) and knock-out (CRISPR) of *SlZHD18* both resulted in a reduction of the density of leaf type VI trichomes. In Chapter 3, among differentially expressed genes in *SlMYC1* mutants, we identified three candidate TFs, putatively playing a role in the regulatory pathway of SlMYC1, namely SlbHLH114 (Solyc01g096370), SlWRKY1 (Solyc07g047960) and SlMYB14 (Solyc06g053610). The CRISPR knock-out of either *SlbHLH114* or *SlWRKY1*, resulted in both cases in a significant reduction in the number of type VI glandular trichomes on leaves. Instead, *SlMYB14* knockout did not change type VI trichomes density or metabolic content on leaves and stems. These observations led us to hypothesize for SlbHLH114, SlWRKY1 and SlZHD18 to be involved in promoting type VI trichomes development in leaves. Whether this could be valid also for stem-trichomes has to be further investigated by checking their density on the CRISPR knockout plants. However, not only the density, but also the metabolic activity of type VI trichomes will contribute to the levels of volatile terpenes produced. We discovered that, despite the lower density of type VI glandular trichomes, the levels of mono- and sesquiterpenes in leaves of *SlbHLH114* and *SlWRKY1* knockout lines and of sesquiterpenes in *SlZHD18* knockout lines were unchanged. This suggest that more terpenes are produced per type VI trichome, although this hypothesis has to be verified by analyzing the volatile terpene levels of individually picked trichomes. Nonetheless, the level of volatile terpenes is also dependent on the metabolic flux of precursors via the plastidial MEP pathway. This could be regulated at different stages, for instance the carbon/sugar influx, the abundance or allelic variants of synthetic enzymes, or the transport and accumulation end products in a specialised cavity (R. W. J. Kortbeek, 2022). Therefore, it could be that SlbHLH114, SlWRKY1 and SlZHD18 function as negative regulators in any of these levels of control. To gain a better insight it would be important to analyze the expression levels of TPS genes or even better the whole transcriptome, of the glandular trichomes of *SlbHLH114*, *SlWRKY1* and *SlZHD18* CRISPR-knockout lines. This will help to entangle the intertwined regulatory pathways controlling type VI specialized metabolism.

Interestingly, when analysing the transcriptome of the *SlHDZ38* mutant (Chapter 4), *SlbHLH114*, *SlWRKY1*, *SlMYB14* and *SlZHD18* transcript levels were all influenced by the mutation in isolated stem trichomes (Table 1).

Table 1 Differential expression of selected candidate TFs in SlHDZ38 mutant.

Solyc ID	Gene	Tissue	log2FC	p-value	Class	Family
Solyc07g047960	WRKY1	Stem trichomes	-1.92	2.31E-19	TF	WRKY
Solyc01g096370	bHLH114	Stem trichomes	-1.15	6.88E-08	TF	bHLH
Solyc05g007580	ZHD18	Stem trichomes	0.82	2.60E-04	TF	Zinc Finger HD
Solyc06g053610	MYB14	Stem trichomes	-2.42	1.83E-17	TF	MYB
Solyc06g053610	MYB14	Leaves	-1.16	1.02E-2	TF	MYB

Specifically, *SlbHLH114*, *SlWRKY1* and *SlMYB14* are downregulated in the *SlHDZ38* mutant, similarly to what we observed in *SlMYC1* knockdown and knockout lines (Chapter 3). This result could be explained by the hypothetical scenario where SlMYC1 and SlHDZ38 work redundantly on the same regulatory pathway, controlling a similar set of downstream genes regulating type VI development and their specialized metabolism, among which *SlbHLH114* and *SlWRKY1*. The role of *SlMYB14* remains unclear, since it is the only candidate gene of the four that does not show a trichome or terpene phenotype when knocked down via CRISPR and to be found regulated by SlHDZ38 also in leaves (Table1). A possibility to gain more insight on the role of *SlMYB14* could be to run an untargeted metabolomic analysis on the glands of the SlMYB14 mutants to determine if other specialized metabolites are affected.

Finally, *SlZHD18*, whose expression is not influenced by the knockdown and knockout of SlMYC1 (Chapter 2), is found to be upregulated (1.6-fold) in stem trichomes of the *SlHDZ38* mutant. Taken together, these results are in line with the hypothesis that SlZHD18 is higher in hierarchy than SlMYC1 (Chapter 2) and further suggest that, beside being involved in promoting type VI trichome development, SlZHD18, under the negative control of SlHDZ38, could positively control *SlMYC1* expression in stem-trichomes. However, although SlZHD18 is upregulated in *SlHDZ38* mutant stem trichomes, the expression of *SlMYC1* is still downregulated. This could mean that SlZHD18 is not the only TF necessary to control *SlMYC1* expression. This specific regulatory interplay could be better understood by analyzing the transcriptome of stem-trichomes of the SlZHD18 mutant lines. If our hypothesis is true, we expect to observe that when SlZHD18 is knocked down, the levels of SlMYC1 in stem trichomes would be lower. This would be in line with what previously observed by in stem-trichomes of the SlMYC1 mutants: higher transcript levels of *SlTPS12* and consequent higher production of sesquiterpenes (Xu et al., 2018), which are normally found in leaf trichomes rather than in stem trichomes (Schilmiller et al., 2010b). Furthermore, the analysis of the transcriptome of also the leaves of the SlZHD18 mutant will help to understand whether SlMYC1 is regulated by SlZHD18 in leaves in this same as in stem-trichomes. If so, this would explain why, when SlZHD18 is knocked down, the fewer type VI trichomes that are left on the leaves can produce sesquiterpene at the same levels as the wild type (Chapter 2).
Another open point remains the timing of the hypothesized regulatory interplay between SlHDZ38, SlZHD18 and SlMYC1 during type VI trichome development. To follow in time their activation, it could be useful to fuse these three TF with fluorescent proteins. With our current knowledge we can only speculate that *SlHDZ38* expression is activated to initiate type VI trichomes gland cells formation. At this stage SlHDZ38 inhibit *SlZHD18* expression that consequently does not promote *SlMYC1* expression. At a later developmental stage, when the gland cells are formed, SlZHD18 gets switched on and thus SlMYC1, starting up the biosynthesis of volatile terpenes.

5.3 An updated regulatory network for type VI trichome terpene metabolism

In the introduction, the state of the art of the regulatory networks controlling terpene metabolism and development of tomato type VI glandular trichomes has been summarized. The findings of the three experimental chapters and the results of the RNA-sequencing of leaf with trichomes and isolated stem-trichomes samples from SlMYC1 and SlHDZ38 mutants (Supplemental table 2 A-B and 3 A-B), make it possible to further add to the complexity of these networks (Figure 3 and 4).

Looking at the differentially expressed genes in SlMYC1 mutants, this TF positively regulates the expression of the monoterpene synthases SlTPS20 and of the sesquiterpene synthase SlTPS12 in both leaf and stem type VI trichomes. Also the diterpene synthase SlTPS41 seems to be under the positive control of SlMYC1 in both leaves and trichomes. Moreover, in stem trichomes, SlMYC1 could positively control also SlTPS18, SlTPS27, SlTPS31, and SlTPS43. Additionally, we found that SlMYC1 could be in stem trichomes a positive regulator of the expression of four known TFs involved in controlling terpene metabolism: SlEOT1, SlEOT2, SlWRKY73 and SlSCL3. This finding indicates that, while SlMYC1 is probably capable of binding directly to the promoter of some TPSs, the regulation of terpene metabolism by SlMYC1 is also achieved by the mediation of these four TFs. This is valid also for two of the TFs characterized in Chapter 3, SlbHLH114 and SlWRKY1, that can regulate terpenes biosynthesis:

their expression appears to be promoted by SlMYC1 in stem trichomes. Instead, SlMYB14, although its expression is induced by SlMYC1 in stem trichomes, seems not to be involved in type VI trichome specialized metabolism (Chapter 3).

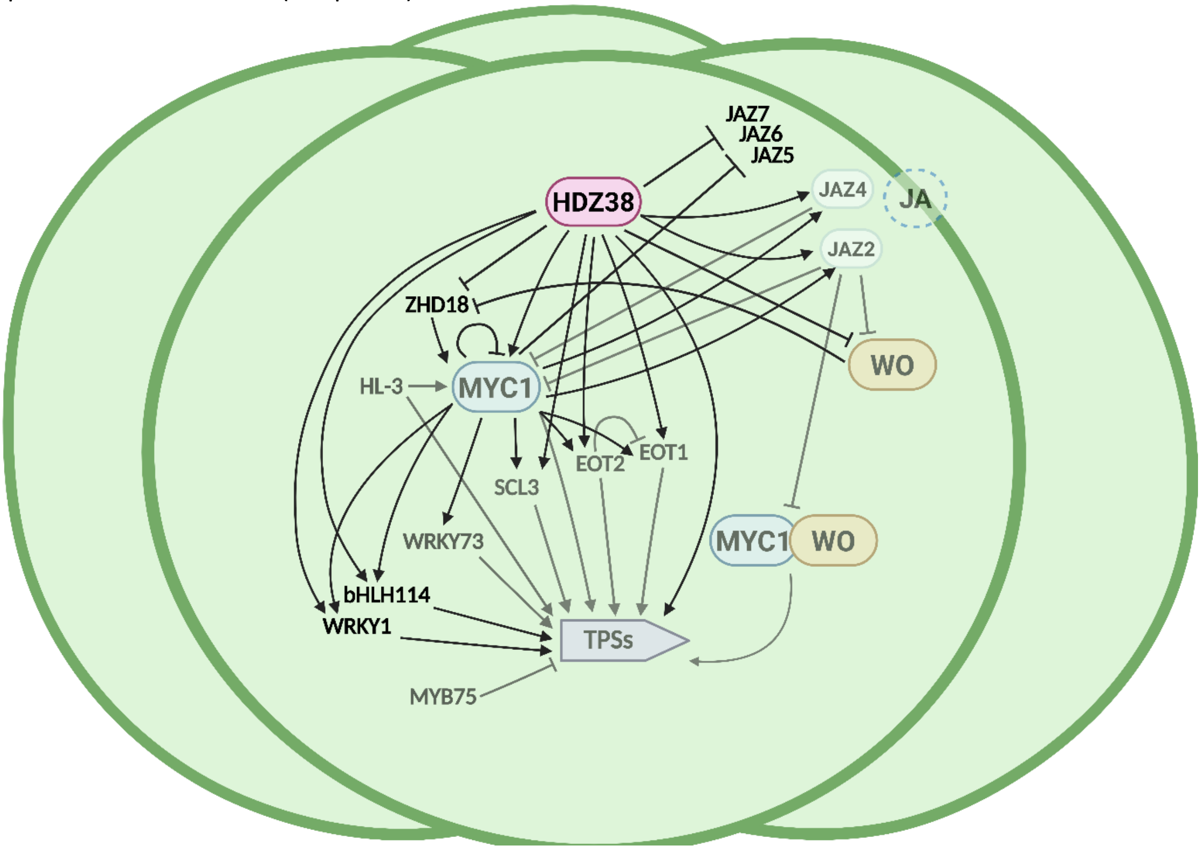

Figure 3 Regulation of type VI trichome terpene metabolism. *Updated network of transcription factors that are known to control the specialized metabolism in the four-celled head of tomato type VI glandular trichomes, focussing on the transcriptional regulation of terpene synthases (TPSs, in grey). Existing knowledge (depicted also in figure 2 of chapter 1) is marked with transparency for clarity. The results of the experimental studies of chapter 2, 3 and 4 are added in black. The newly identified master regulator, HDZ38, is highlighted in a pink box. The master regulators MYC1 and WO are highlighted, including their protein-protein interaction. Circled are also JAZ repressors whose action is dependent from the hormone Jasmonic Acid (JA, in dotted circle). Created in https://BioRender.com*

In Chapter 2, studying *SlMYC1* promoter, we identified an uncharacterized TF, SlZHD18, that shows strong interaction with *SlMYC1* promoter in yeast, therefore suggesting it could be an upstream regulator of *SlMYC1*. Moreover, knocking out SlZHD18 resulted in the reduction of type VI trichome density, but not of monoterpenes, suggesting SlZHD18 could be also a negative regulator of monoterpenes metabolism. More studies will be needed to verify these two hypotheses but, as it has been shown that *SlZHD18* expression is regulated by SlWO (Wu et al., 2023), an involvement of SlZHD18 in the control of terpene metabolism seems likely. In Chapter 2, also SlMYC1 itself was found to interact with *SlMYC1* promoter, suggesting a self-regulatory feedback mechanism, as it has been found in the case of SlWO (Hua et al., 2020).

Finally, in Chapter 4, the characterization of SlHDZ38 pointed out a major role for this TF in the regulation of terpene biosynthesis. In both leaves and isolated stem-trichomes SlHDZ38 promotes the expression of the genes coding for the monoterpene synthases SlTPS20 and SlTPS39, for the sesquiterpene synthases SlTPS12, SlTPS17, SlTPS18 and for the diterpene synthase SlTPS41. Moreover, in leaf it positively regulates the diterpene synthase SlTPS46, and in stem-trichomes the monoterpene synthases SlTPS5 and SlTPS7, and the sesquiterpene synthases SlTPS16, SlTPS21, SlTPS31 and SlTPS35. Whether this regulation is direct or indirect is yet clear. However, as previously discussed, it is probable that this regulation goes via other intermediary TFs downstream. In fact, in isolated stem trichomes of the SlHDZ38 mutant, SlMYC1 is downregulated and SlWO is upregulated. This suggest SlHDZ38 could be working upstream these two master regulators of type VI trichomes specialized metabolism and controlling their expression. Finally, this TF also appears to be involved in controlling many other type VI trichomes terpene metabolism regulators in isolated stem trichomes, such as the well known SlEOT1, SlEOT2, and SlSCL3 but also the recently characterized SlZHD18, SlbHLH114 and SlWRKY1 (Chapter 2 and 3). This supports once more the hypothesis of SlHDZ38 being a TF high up in the hierarchy of the regulatory network.

It is well known that JA can induce terpene biosynthesis by disrupting JAZ proteins that repress key regulators of TPSs such as SlMYC1 and SlWO. Looking at the transcriptome of SlHDZ38 and SlMYC1 mutants, we discovered that SlMYC1 and SlHDZ38 could in turn change JAZ protein expression. In fact, SlMYC1 seems to positively regulate the JA-signalling repressors SlJAZ2 and SlJAZ4 in leaves and to negatively control SlJAZ7 expression in both leaves and stem trichomes. This could be caused by the presence of a feedback mechanism between SlMYC1 and some of the JAZ repressors, who participate in a complex with SlWO in the JA-dependent induction of terpenes metabolism. SlHDZ38 instead, appears to positively regulate both SlJAZ2 and SlJAZ4 in leaves with trichomes. Moreover, SlJAZ5, SlJAZ6 and SlJAZ7 seems to be downregulated by SlHDZ38 in stem-trichomes. The precise role of SlJAZ5, SlJAZ6 and SlJAZ7 is not well known yet, making it difficult to make any hypothesis. However, our findings about SlMYC1 and SlHDZ38 being capable of regulating JAZ2 and JAZ4 expression, could possibly mean a new layer of regulation in connection with JA-dependent induction of type VI trichomes terpenes metabolism.

5.4 An updated regulatory network for type VI glandular trichome formation

With regard to the development of type VI trichomes, the results of Chapter 2, 3 and 4 allow us to add new players to the network of TFs involved and to build an updated model to better understand how this complex process could be regulated (Figure 4). As discussed in Chapter 4, SlHDZ38 seems to be located high up in the hierarchy in the network of TFs regulating type VI trichome development, as many of them are differentially expressed in the SlHDZ38 mutant, especially in isolated stem trichomes. One of the most important is SlWO, which, together with its downstream targets SlMX1 and SlWOX3b, are found upregulated in isolated stem-trichomes of the SlHDZ38 mutant. This suggest SlHDZ38 negatively regulates them, directly or indirectly by the mediation of other TFs. For example, also *SlMTR1* and *SlMTR2,* whose expression is activated by SlWO, are both involved in the feedback mechanism controlling the concentration of SlWO. *SlMTR1* appears to be positively regulated *by* SlHDZ38 in both leaves and isolated stem trichomes and *SlMTR2* negatively regulated in isolated stem trichomes. Moreover, SlHDZ38 seems to be a positive regulator of the leaf expression of *SlLN*, which is known to positively regulate *SlMTR1* and *SlMTR2*. Although the levels of SlLFS, that promotes glandular trichome development under the control SlWO and SlMX1, are unchanged in the SlHDZ38 mutant, SlHDZ38 influences the expression levels in leaves and isolated stem trichomes of both SlGCR1, which directly inhibits SlLFS, and of SlTOEb1, part of SlLFS negative feedback mechanism. Also SlH2 is found upregulated in SlHDZ38 mutant stem trichomes, suggesting SlHDZ38 positive regulation of *SlH2* expression. However, this was not observed for its homolog SlH.
The other master regulator of type VI glandular trichomes development, SlMYC1, appears to be positively controlled by SlHDZ38, as it is found strongly downregulated in isolated stem trichomes of the *glandless* mutant. SlZHD18, identified in Chapter 2 as a putative upstream regulator of SlMYC1 and as a positive regulator of type VI trichome development, is downregulated in SlHDZ38 mutant stem trichomes. This, and the fact that SlHDZ38 has not been found among TFs binding *SlMYC1* promoter (Chapter 2), suggest that SlZHD18 could be downstream of SlHDZ38 and mediate the regulation of SlMYC1. In Chapter 3, three previously uncharacterized TFs, namely SlbHLH114, SlWRKY1 and SlMYB14, all three positively regulated by SlMYC1, were selected as putative co-players in SlMYC1 regulatory network. When knocked-out by CRISPR-Cas9, SlbHLH114 and SlWRKY1 gave a trichome

phenotype that suggest their involvement in type VI glandular trichome development. Instead, although regulated by SlMYC1, SlMYB14 appears not to be directly involved in type VI trichomes development. Interestingly, SlHDZ38 looks like to be able to positively regulate the expression of all three of them, SlMYB14 in both leaves and stem trichomes, SlbHLH114 and SlWRKY1 only in stem trichomes. Also SlHD8, who controls *SlHL* and *SlHL2* expression, seems to be positively regulated by SlHDZ38. Finally, both SlCD2 and SlMIXTA-like were found upregulated stem trichomes of the SlHDZ38 mutant, suggesting the possibly undergo a negative regulation by SlHDZ38.

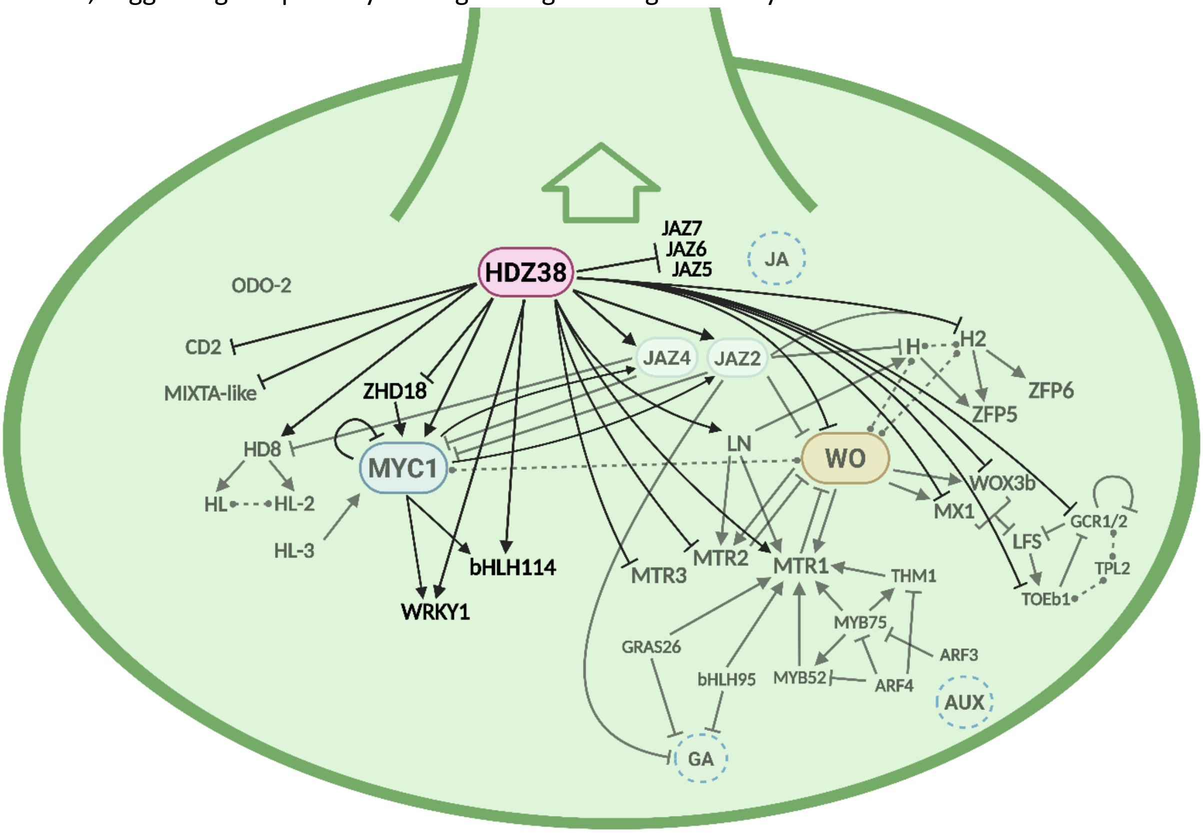

Figure 4 Regulation of type VI trichomes development. *Updated network of transcription factors that are known to control the development of type VI glandular trichomes (illustrated by the large arrow pointing upward). Existing knowledge, (depicted also in figure 3 of chapter 1) is marked with transparency for clarity. The results of the experimental studies of chapter 2, 3 and 4 are added in black. The new master regulator, HDZ38, is highlighted in a pink box. The master regulators MYC1 and WO are highlighted with coloured boxes. Circled are also JAZ2 and 4 repressors, whose action is dependent from the hormone Jasmonic Acid (JA). Highlighted in dotted circles are the two hormones which influence the regulatory network, Gibberellic Acid (GA) and Auxin (AUX). Dotted lines represent known protein-protein interactions. Created in https://BioRender.com*

Jasmonic acid (JA) can affect type VI trichome development (L. Li et al., 2004; Xu et al., 2018; Yan et al., 2013). This probably happens thanks to the mediation of JAZ-repressors, as a reduced type VI trichome density on new leaves were observed after overexpressing JAZ2 and shorter type VI trichome stalks were measured when JAZ4 was overexpressed (Hua et al., 2020; Yu et al., 2018). As described for the regulation of trichome type VI terpene metabolism, SlWO, SlMYC1, SlH and SlH2 have all been found to be targets of SlJAZ2 repression in absence of JA (Hua et al., 2020, 2022). Additionally, SlJAZ4 targets SlMYC1 and SlHD8 (Hua et al., 2020, 2021). We observed that both SlMYC1 and SlHDZ38 can positively regulate the expression of several JAZ genes, including SlJAZ2 and SlJAZ4. Taken together these observations lead to a hypothetical model where, with basal level of JA, known type VI trichome regulators, such as SlMYC1 and SlHDZ38, are maintained in a homeostasis state via the repression of JAZs at the protein level. This results in the production of basic levels of volatile terpenes and the development of a basal number of type VI trichomes. When JA concentration increases, the repression of JAZs is ceased, the homeostasis state of trichome regulators like SlMYC1 and SlHDZ38 changes and as a result terpenes biosynthesis is induced and more type VI trichomes develop on newly forming leaves. These same regulatory TFs are then involved in terminating JA signalling and re-establish the homeostasis by downregulating JAZ genes expression. Studying if JA can influence the activity of SlHDZ38 and if other of these trichome-regulator TFs, like SlWO, SlH and SlH2, can regulate JAZ genes expression are crucial to validate this hypothesis.

In conclusion, our experimental results and characterizing more TFs taking part in the control of the development of type VI glandular trichomes, combined with previous knowledge, reveal an even more intricate regulatory network at the base of this process.

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5.6 Supplemental data

Supplemental Table 1: Type VI trichomes-related genes

Solyc ID	Gene	Family	Name	Function	References
Solyc02g077560	**ARF3**	ARF		Positive regulation of trichome type I, V and VI density; Auxin-based regulation	Zhang *et al.*, 2015; Gong *et al.*, 2021
Solyc11g069190	**ARF4**	ARF		Positive regulation of trichome type II, V and VI density; Auxin-based regulation; regulates SlTHM1 and SlMYB52	Yuan *et al.*, 2021; Gong *et al.*, 2021
Solyc01g096370	**bHLH114**	bHLH		New candidate: positive regulator of type VI trichomes development, negative regulation of terpene metabolism	Chapter 3
Solyc10g079050	**bHLH95**	bHLH		Negative regulation of type I and V initiation; regulates MTR1	Chen *et al.*, 2020
Solyc01g091630	**CD2**	HD-ZIP	Cutin deficient 2	Positive regulation of trichome type VI density	Nadakuduti *et al.*, 2012
Solyc02g067870	**CHI1**		Chalcone isomerase	Positive regulation of trichome density and gland cell formation	Kang *et al.*, 2010b
Solyc08g066780 -840	**DL**		Dialytic	Trichome morphogenesis; not cloned	Chang *et al.*, 2016
Solyc09g014980	**dt-1**	ARP2/3	Distorted trichomes 1	Trichomes morphogenesis	Chang *et al.*, 2019
Solyc12g098430	**dt-2**	SCAR-WAVE	Distorted trichomes 2	Trichomes morphogenesis	Chang *et al.*, 2019
Solyc02g094320	**dt-5/dt-6**	ARP2/3	Distorted trichomes 5/6	Trichomes morphogenesis	Chang *et al.*, 2019
Solyc02g062400	**EOT1**	SHI/STY	Expression of Terpenoids 1	Trichome-specific expression, regulation of terpenes metabolism	Spyropoulou *et al.*, 2014b
Solyc03g033680	**EOT2**	SHI/STY	Expression of Terpenoids 2	Trichome-specific expression, regulation of terpenes metabolism	Xu J., 2023
Solyc02g076670	**GCR1**	GARP-G2-like	Gland cell repressor	Negative regulation of trichomes gland cells formation	Chang *et al.*, 2024
Solyc02g092570	**GRAS26**	GRAS		Trichomes initiation; regulates MTR1	Zhou *et al.*, 2018
Solyc10g078970	**H**	C2H2	Hair	Positive regulator of trichome type I, III, V, VI, VII density, and type I, III, VI elongation	Chang *et al.*, 2018; Zheng *et al.*, 2022
Solyc10g078990	**H2**	C2H2	Hair 2, Sparce Hair (SH), Hair-like, SlZFP8L	Positive regulator of trichome type I, III, V, VI, VII density, and type I, III, VI elongation	Li *et al.*, 2021; Chun *et al.*, 2021; Hua *et al.*, 2022b; Zheng *et al.*, 2022
Solyc03g098200	**HD8**	HD-ZIP	Homeodomain 8, HDZIV8, HDG12, HDZ15	Positive regulation of type I, II, III, IV, V, VI initiation and morphogenesis	Gao *et al.*, 2015; Xie *et al.*, 2020; Hua *et al.*, 2021a;
Solyc09g008810	**HDZ38**	HD-ZIP	Glandless	Trichomes density, trichome type VI development	Chapter 4
Solyc11g013290	**HL**	SCAR-WAVE	Hairless	or dt-4, Trichome type I, IV, VI, VII density and morphogenesis	Kang *et al.*, 2010a; Tian *et al.*, 2012; Kang *et al.*, 2016
Solyc02g068720	**HL-2**	SCAR-WAVE	Hairless 2	Trichome type I density and trichomes morphogenesis	Xie *et al.*, 2020
Solyc05g006470	**HL-3**	ARP2/3	Hairless 3, dt-3, ARPC1	Positive regulation of trichome type I and VI density and trichomes morphogenesis	Chun *et al.*, 2022
Solyc12g009220	**JAZ2**	Tify	Jasmonate ZIM-domain protein 2	Repressor of JA induced trichome initiation, interacts with MYC1 and WO	*Yu et al., 2018; Hua et al., 2020*
Solyc12g049400	**JAZ4**	Tify	Jasmonate ZIM-domain protein 4	Repressor of JA induced trichome initiation, interacts with MYC1 and HD8	Hua et al., 2020; Hua *et al.*, 2021
Solyc03g118540	**JAZ5**	Tify	Jasmonate ZIM-domain protein		Chapter 4
Solyc01g005440	**JAZ6**	Tify	Jasmonate ZIM-domain protein		Chapter 4
Solyc11g011030	**JAZ7**	Tify	Jasmonate ZIM-domain protein		Chapter 4

Solyc05g013540	**LFS**	AP2/ERF	Leafless	Positive regulator of glandular trichome differentiation	Wu, Chang et al.,2023
Solyc03g031760	**LN**	HD-ZIP	Lanata	Trichomes initiation; Interacts with WO, regulates H, MTR1 and MTR2	Xie *et al.*, 2022
Solyc02g088190	**MIXTA-like**	MYB		Trichome type I, IV, V, VI, VII, initiation	Galdon-Armero *et al.*, 2020
Solyc10g083140	**MTR1**		Multicellular trichome repressor 1, CyclinB2 (CycB2)	Negative regulation of type I, III, IV and VI initiation	Yang *et al.*, 2011, Gao *et al.*, 2017, Xie *et al.*, 2022; Wu *et al.*, 2023;
Solyc06g073990	**MTR2**		Multicellular trichome repressor 2, CyclinB3 (CycB3),	Negative regulation of type I, III, IV and VI initiation	Gao *et al.*, 2017; Xie *et al.*, 2022; Wu *et al.*, 2023
Solyc01g007870	**MTR3**		Multicellular trichome repressor 3	Putatively redundant function to MTR1 and MTR2	Wu *et al.*, 2023
Solyc01g010910	**MX1**	MYB	Mixta	Trichome type I, III, IV, V, VI, VII, VIII initiation; non-glandular trichome development	Ewas *et al.*, 2016; Ewas *et al.*, 2017; Wu *et al.*, 2023
Solyc06g053610	**MYB14**	MYB		New candidate	Chapter 3
Solyc01g079620	**MYB52**	MYB		Negative regulation of type V; Regulates MTR1	Yuan *et al.*,2021
Solyc10g086250	**MYB75**	MYB		Negative regulation of type II, V and VI initiation; Regulates MTR1	Gong *et al.*, 2021
Solyc08g005050	**MYC1**	bHLH		Regulates trichome type VI initiation and morphogenesis and terpene synthases	Xu *et al.*, 2020; Hua *et al.*, 2022
Solyc11g019950	**ODO2**		Odorless-2	Positive regulation of terpenoids biosynthesis, trichome type I and VI development	Kang, Liu, *et al.*, 2010
Solyc12g099900	**SCL3**	GRAS	Scarecrow Like 3	Trichome type VI metabolism and gland cell formation	Yang *et al.*, 2021
Solyc08g081500	**THM1**	MYB	Tomato hypocotyl MYB	Trichome type II abdVI initiation; regulates MTR1	Yuan *et al.*,2021
Solyc04g049800	**TOE1b**	AP2/ERF		Trichomes gland cells formation	Chang *et al.*, 2024
Solyc08g076030	**TPL2**		Topless 2	Trichome gland cell formation; Binds GCR1	Chang *et al.*, 2024
Solyc01g105850	**TPS1**			Mutated terpene synthase, non functional	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc06g059930	**TPS12 (CAHS)**			Sesquiterpene synthase, β-Caryophyllene/α-humulene, cytosol	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc07g008690	**TPS16**			Sesquiterpene synthase, δ-Cadinene, cytosol	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc12g006570	**TPS17**			Sesquiterpene synthase, Valencene, cytosol	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc08g005720	**TPS18**			Sesquiterpene synthase, not determined, mitochondria	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc08g005670	**TPS19**			Sesquiterpene synthase, β-Myrcene, β-ocimene, plastid	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc08g005665	**TPS20 (PHS1)**			Monoterpene synthase, Phellandrene, plastid	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc08g005640	**TPS21**			Sesquiterpene synthase, Lycosantalene, plastid	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc02g079910	**TPS27**			Sesquiterpene synthase, α-farnesene, cytosol	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc01g101170	**TPS31**			Sesquiterpene synthase, Viridiflorene, cytosol	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc01g101210	**TPS35**			Sesquiterpene synthase, (Z,Z)-Farnesol, cytosol	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc10g005390	**TPS39**			Terpene synthase, Linalool/ E-nerolidol, cytosol	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc08g005710	**TPS41**			Diterpene synthase, Copalyl diphosphate, mitochondria	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc05g026590	**TPS43**			Mutated terpene synthase, non functional	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc03g006550	**TPS46**			Diterpene synthase, Geranyllinalool, cytosol	Falara *et al.*, 2011; Zhou *et al., 2020*
Solyc01g105890	**TPS5 (MTS1)**		Monoterpene synthase 1	Monoterpene synthase, Linalool, plastidial	Falara *et al.*, 2011; Zhou *et al., 2020*

Solyc01g105920	**TPS7**			Monoterpene synthase, β-Myrcene/limonene, plastid	Falara *et al.,* 2011; Zhou *et al., 2020*
Solyc06g059885	**TPS9 (SST1)**		Sesquiterpene synthase 1	Sesquiterpene synthase, Germacrene, α-humulene	Falara *et al.,* 2011; Zhou *et al., 2020*
Solyc02g080260	**WO**	HD-ZIP	Woolly	Regulation of trichomes initiation, development and morphogenesis	Hua *et al.,* 2021; Tian *et al.*, 2012; Yang *et al.*, 2011b; Wu *et al.*,2023; Wu, Bian, Hu *et al.*, 2024;
Solyc11g072790	**WOX3b**	WUSCHEL Homeobox		Non-glandular trichome development	Wu *et al.,* 2023
Solyc07g047960	**WRKY1**	WRKY		New candidate: positive regulator of type VI trichomes development, negative regulation of terpene metabolism	Chapter 3
Solyc03g113120	**WRKY73**	WRKY		Putative regulator terpenoids biosynthesis	Spyropoulou *et al.*, 2014
Solyc05g006310	**ZFP5**	C2H2		Positive regulation of long hairy trichomes initiation	Li *et al.*, 2021
Solyc05g009170	**ZFP6**	C2H2		Positive regulation of type I, III, V, VI, VII initiation and type I, III, VI elongation	Zheng *et al.*, 2022
Solyc05g007580	**ZHD18**	Zinc finger HD		New candidate: positive regulator of type VI trichomes development, negative regulation of sesquiterpene metabolism; binds MYC1 promoter	Chapter 2

Supplemental table 2A: Differentially expressed trichome genes in leaves of glandless mutant: ***downregulated and upregulated genes are indicated with a negative and positive log2FC respectively***

Solyc ID	**Gene**	**log2FC**	**p-value**	**Class**	**Family**	**Annotation**
Solyc06g059930	TPS12	-10.3	2.92E-15	NA	NA	Sesquiterpene synthase, β-Caryophyllene/α-humulene, cytosol
Solyc08g005710	TPS41	-7.91	7.90E-12	NA	NA	Diterpene synthase, Copalyl diphosphate, mitochondria
Solyc01g105850	TPS1	-6.51	2.59E-05	NA	NA	Mutated terpene synthase, non functional
Solyc10g005390	TPS39	-5.19	3.38E-02	NA	NA	Terpene synthase, Linalool/ E-nerolidol, cytosol
Solyc12g006570	TPS17	-4.65	3.18E-06	NA	NA	Sesquiterpene synthase, Valencene, cytosol
Solyc08g005670	TPS20	-2.94	3.74E-02	NA	NA	Monoterpene synthase, Phellandrene, plastid
Solyc12g049400	JAZ4	-2.55	6.96E-13	TF	Tify	Jasmonate ZIM-domain protein, Tify
Solyc03g006550	TPS46	-2.4	2.19E-09	NA	NA	Diterpene synthase, Geranyllinalool, cytosol
Solyc08g005720	TPS18	-2.27	3.17E-03	NA	NA	Sesquiterpene synthase, not determined, mitochondria
Solyc12g009220	JAZ2	-1.2	1.31E-06	TF	Tify	Jasmonate ZIM-domain protein 1
Solyc06g053610	MYB14	-1.16	1.02E-02	TF	MYB	R2R3MYB transcription factor 14, THM18 protein
Solyc02g076670	GCP1 HD8	-1.03	3.17E-04	TF	GARP-G2-like	MYB-like domain, Homeodomain-like protein
Solyc10g083140	MTR1	-0.77	1.45E-03	NA	NA	Hypothetical protein
Solyc03g031760	LN	-0.64	3.60E-05	TF	HB-HD-ZIP	Homeobox-leucine zipper protein ROC2
Solyc03g098200	HDZ8	-0.56	2.70E-02	TF	HB-HD-ZIP	Homeobox-leucine zipper protein HDG12
Solyc04g049800	TOE1b	0.79	1.40E-02	TF	AP2/ERF	AP2/ERF transcription factor

Supplemental table 2B: Differentially expressed trichome genes in isolated stem-trichomes of glandless mutant

Solyc ID	Gene	log2FC	p-value	Class	Family	Annotation
Solyc10g005390	TPS39	-8.66	2.90E-14	NA	NA	Terpene synthase, Linalool/ E-nerolidol, cytosol
Solyc08g005720	TPS18	-8.64	1.72E-17	NA	NA	Sesquiterpene synthase, not determined, mitochondria
Solyc01g105850	TPS1	-8.41	5.65E-13	NA	NA	Mutated terpene synthase, non functional
Solyc08g005670	TPS20	-8.41	7.85E-10	NA	NA	Monoterpene synthase, Phellandrene, plastid
Solyc06g059930	TPS12	-8.38	6.90E-08	NA	NA	Sesquiterpene synthase, β-Caryophyllene/α-humulene, cytosol
Solyc01g101170	TPS31	-8.38	4.20E-12	NA	NA	Sesquiterpene synthase, Viridiflorene, cytosol
Solyc07g008690	TPS16	-8.28	3.93E-20	NA	NA	Sesquiterpene synthase, δ-Cadinene, cytosol
Solyc08g005710	TPS41	-8.02	1.85E-13	NA	NA	Diterpene synthase, Copalyl diphosphate, mitochondria
Solyc08g005640	TPS21	-5.6	5.40E-65	NA	NA	Sesquiterpene synthase, Lycosantalene, plastid
Solyc01g105890	TPS5	-5.52	7.16E-17	NA	NA	Monoterpene synthase, Linalool, plastidial
Solyc01g101210	TPS35	-5.35	8.40E-03	NA	NA	Sesquiterpene synthase, (Z,Z)-Farnesol, cytosol
Solyc02g062400	EOT1	-3.96	2.92E-79	TF	SRS	Expression of terpenoids 1
Solyc08g005050	MYC1	-3.66	8.94E-55	TF	bHLH	Transcription factor MYC1
Solyc06g009710	MYB111	-3.31	1.36E-30	TF	MYB	MYB-like transcription factor, candidate 7, not selected
Solyc01g105920	TPS7	-2.9	1.49E-04	NA	NA	Monoterpene synthase, β-Myrcene/limonene, plastid
Solyc03g033680	EOT2	-2.45	1.60E-21	TF	SRS	Expression of terpenoids 2
Solyc06g053610	MYB14	-2.42	1.83E-17	TF	MYB	R2R3MYB transcription factor 14, THM18 protein
Solyc12g099900	SCL3	-2.08	5.06E-17	TF	GRAS	SCL3 (scarecrow-like 3)
Solyc07g047960	WRKY1	-1.92	2.31E-19	TF	WRKY	WRKY transcription factor 1
Solyc12g006570	TPS17	-1.71	2.67E-10	NA	NA	Sesquiterpene synthase, Valencene, cytosol
Solyc10g005330	HDZ40	-1.58	3.72E-11	TF	HB-HD-ZIP	Homeobox-leucine zipper protein MERISTEM, close homolog to WO
Solyc09g008810	HDZ38	-1.56	5.36E-04	TF	HB-HD-ZIP	Homeobox-leucine zipper protein ATHB-22
Solyc01g096370	bHLH114	-1.15	6.88E-08	TF	bHLH	Transcription factor MYC2
Solyc01g079620	MYB12	-1.1	2.10E-08	TF	MYB	Trichome specific expression, regulates flavonoid pathway in fruit
Solyc10g083140	MTR1	-0.56	8.48E-03	NA	NA	hypothetical protein
Solyc09g014980	DT1	0.34	4.72E-03	NA	NA	Protein SCAR4
Solyc03g098200	HD8	0.41	1.15E-03	TF	HB-HD-ZIP	Homeobox-leucine zipper protein HDG12
Solyc04g049800	TOE1b	0.48	3.76E-02	TF	ARF2/ERF	AP2-like ethylene-responsive transcription factor
Solyc01g091630	CD2	0.52	8.96E-07	TF	HB-HD-ZIP	Cutin deficient 2
Solyc03g118540	JAZ5	0.66	4.43E-02	TF	Tify	Jasmonate ZIM domain protein
Solyc02g088190	MIXTA-like	0.71	4.88E-07	TF	MYB	MYB transcription factor
Solyc02g076670	GCR1	0.72	7.84E-04	TF	GARP G2-like	MYB-like domain, Homeodomain-like protein
Solyc05g007580	ZHD18	0.82	2.60E-04	TF	zf-HD	Zinc-finger homeodomain protein
Solyc01g005440	JAZ6	0.99	3.38E-07	TF	Tify	Jasmonate ZIM-domain protein
Solyc06g073990	MTR2	0.99	7.18E-06	NA	NA	Hypothetical protein
Solyc02g080260	WO	1.01	2.49E-11	TF	HB-HD-ZIP	Woolly
Solyc01g007870	MTR3	1.09	1.88E-07	NA	NA	hypothetical protein
Solyc11g011030	JAZ7	1.11	5.60E-03	TF	Tify	Pto-responsive gene 1
Solyc01g010910	MX1	1.21	3.45E-03	TF	MYB	MYB transcription factor subfamily 9
Solyc11g072790	WOX3b	1.90	1.67E-04	TF	HB-WOX	WOX3b
Solyc02g092570	GRAS26	3.99	2.22E-02	TF	GRAS	Transcription factor GRAS26
Solyc10g078990	H2	4.17	4.23E-04	TF	C2H2	Zinc finger protein 6

Supplemental table 3A: Differentially expressed trichome genes in leaves of MYC1 mutants

Genotype	**Solyc ID**	**Gene**	**log2FC**	**p-value**	**Class**	**Family**	**Annotation**
OE-1	Solyc12g009220	JAZ2	2.09	2.59E-02	TF	Tify	JAZ jasmonate ZIM-domain protein 1
OE-1	Solyc12g049400	JAZ4	4.59	6.33E-05	TF	Tify	JAZ jasmonate ZIM-domain protein
OE-1	Solyc11g011030	JAZ7	4.19	3.73E-05	TF	Tify	JAZ jasmonate ZIM-domain protein, Pto-responsive gene 1
KD-1	Solyc08g005050	MYC1	-2.63	1.91E-33	TF	bHLH	Transcription factor MYC1
OE-1	Solyc08g005050	MYC1	1.16	5.09E-03	TF	bHLH	Transcription factor MYC1
OE-1	Solyc01g105850	TPS1	3.61	1.20E-02	NA	NA	Mutated terpene synthase, non functional
KD-1	Solyc08g005670	TPS20	-5.18	2.18E-02	NA	NA	Monoterpene synthase, Phellandrene, plastid
OE-1	Solyc07g008690	TPS16	3.25	4.93E-02	NA	NA	Sesquiterpene synthase, δ-Cadinene, cytosol
OE-1	Solyc08g005710	TPS41	2.26	2.15E-03	NA	NA	Diterpene synthase, Copalyl diphosphate, mitochondria
OE-1	Solyc03g006550	TPS46	-1.64	3.78E-02	NA	NA	Diterpene synthase, Geranyllinalool, cytosol
KD-1	Solyc06g059930	TPS12	-5.99	1.45E-05	NA	NA	Sesquiterpene synthase, β-Caryophyllene/α-humulene, cytosol
OE-1	Solyc06g059930	TPS12	1.98	1.47E-03	NA	NA	Sesquiterpene synthase, β-Caryophyllene/α-humulene, cytosol

Supplemental table 3B: Differentially expressed trichome genes in isolated stem-trichomes of MYC1 mutants

Genotype	Solyc ID	Gene	log2FC	p-value	Class	Family	Annotation
KO-1	Solyc01g096370	bHLH114	-2.24	5.78E-30	TF	bHLH	MYC transcription factor MYC
KD-1	Solyc01g096370	bHLH114	-1.58	5.57E-14	TF	bHLH	MYC transcription factor MYC
KO-1	Solyc02g062400	EOT1	-1.98	2.05E-16	TF	SRS	Expression of terpenoids 1
KD-1	Solyc02g062400	EOT1	-1.51	1.12E-08	TF	SRS	Expression of terpenoids 1
KO-1	Solyc03g033680	EOT2	-1.72	3.56E-06	TF	SRS	Expression of terpenoids 2
KD-1	Solyc03g033680	EOT2	-1.12	2.87E-03	TF	SRS	Expression of terpenoids 2
KO-1	Solyc09g008810	HDZ38	-0.68	4.53E-02	TF	HB-HD-ZIP	Homeobox-leucine zipper protein ATHB-22
KO-1	Solyc10g005330	HDZ40	-1.59	4.64E-19	TF	HB-HD-ZIP	Homeobox-leucine zipper protein MERISTEM, close homolog to WO
KD-1	Solyc10g005330	HDZ40	-1.26	1.37E-17	TF	HB-HD-ZIP	Homeobox-leucine zipper protein MERISTEM, close homolog to WO
KD-1	Solyc11g011030	JAZ7	1.03	1.37E-04	TF	Tify	Pto-responsive gene 1
KO-1	Solyc11g011030	JAZ7	1.19	4.45E-05	TF	Tify	Pto-responsive gene 1
OE-1	Solyc11g011030	JAZ7	2.13	1.66E-04	TF	Tify	Pto-responsive gene 1
KD-1	Solyc10g083140	MTR1	-0.56	7.63E-05	NA	NA	
KO-1	Solyc06g009710	MYB111	-4.15	7.65E-33	TF	MYB	MYB-like transcription factor, candidate 7, not
KD-1	Solyc06g009710	MYB111	-3.97	8.83E-66	TF	MYB	MYB-like transcription factor, candidate 7, not
KO-1	Solyc01g079620	MYB12	-1.15	6.90E-05	TF	MYB	Trichome specific expression, regulates flavonoids pathway in fruit
KD-1	Solyc01g079620	MYB12	-0.60	5.72E-03	TF	MYB	Trichome specific expression, regulates flavonoids pathway in fruit
KO-1	Solyc06g053610	MYB14	-4.79	3.96E-83	TF	MYB	R2R3MYB transcription factor 14, THM18 protein [Source:UniProtKB/TrEMBL;Acc:Q40174]
KD-1	Solyc06g053610	MYB14	-3.11	1.26E-25	TF	MYB	R2R3MYB transcription factor 14, THM18 protein [Source:UniProtKB/TrEMBL;Acc:Q40174]
KO-1	Solyc08g005050	MYC1	-5.10	4.46E-142	TF	bHLH	Transcription factor MYC1
KD-1	Solyc08g005050	MYC1	-4.56	7.98E-151	TF	bHLH	Transcription factor MYC1
KO-1	Solyc12g099900	SCL3	-2.73	9.45E-48	TF	GRAS	SCL3 (scarecrow-like 3)
KD-1	Solyc12g099900	SCL3	-2.62	4.64E-52	TF	GRAS	SCL3 (scarecrow-like 3)
KO-1	Solyc10g078990	SH	1.19	1.71E-02	TF	C2H2 ZFP	Zinc finger protein 6
KO-1	Solyc01g105850	TPS1	-10.59	4.13E-36	NA	NA	Mutated terpene synthase, non functional
KD-1	Solyc01g105850	TPS1	-4.59	1.40E-129	NA	NA	Mutated terpene synthase, non functional
KO-1	Solyc06g059930	TPS12	-6.87	3.77E-40	NA	NA	Sesquiterpene synthase, β-Caryophyllene/α-humulene, cytosol
KD-1	Solyc06g059930	TPS12	0.68	2.79E-02	NA	NA	Sesquiterpene synthase, β-Caryophyllene/α-humulene, cytosol
OE-1	Solyc06g059930	TPS12	0.95	1.65E-02	NA	NA	Sesquiterpene synthase, β-Caryophyllene/α-humulene, cytosol
KO-1	Solyc07g008690	TPS16	-8.38	4.36E-17	NA	NA	Sesquiterpene synthase, δ-Cadinene, cytosol
KO-1	Solyc07g008680	TPS16	-5.40	1.25E-04	NA	NA	Sesquiterpene synthase, δ-Cadinene, cytosol
KD-1	Solyc07g008690	TPS16	-2.53	5.84E-13	NA	NA	Sesquiterpene synthase, δ-Cadinene, cytosol
KO-1	Solyc12g006570	TPS17	-10.16	8.81E-170	NA	NA	Sesquiterpene synthase, Valencene, cytosol
KD-1	Solyc12g006570	TPS17	-5.48	7.67E-136	NA	NA	Sesquiterpene synthase, Valencene, cytosol
KD-1	Solyc08g005720	TPS18	-6.12	1.07E-19	NA	NA	Sesquiterpene synthase, not determined,
KO-1	Solyc08g005720	TPS18	-5.81	9.03E-23	NA	NA	Sesquiterpene synthase, not determined,
KO-1	Solyc08g005670	TPS20	-12.54	1.12E-89	NA	NA	Monoterpene synthase, Phellandrene, plastid
KD-1	Solyc08g005670	TPS20	-7.84	8.15E-195	NA	NA	Monoterpene synthase, Phellandrene, plastid
OE-1	Solyc02g079910	TPS27	4.97	2.55E-02	NA	NA	Sesquiterpene synthase, α-farnesene, cytosol
KO-1	Solyc01g101170	TPS31	-6.79	2.78E-10	NA	NA	Sesquiterpene synthase, Viridiflorene, cytosol
KD-1	Solyc01g101170	TPS31	-6.70	6.59E-10	NA	NA	Sesquiterpene synthase, Viridiflorene, cytosol
KO-1	Solyc10g005390	TPS39	-9.45	5.76E-29	NA	NA	Terpene synthase, Linalool/ E-nerolidol, cytosol
KD-1	Solyc10g005390	TPS39	-8.95	2.69E-32	NA	NA	Terpene synthase, Linalool/ E-nerolidol, cytosol
KO-1	Solyc08g005710	TPS41	-8.88	3.99E-68	NA	NA	Diterpene synthase, Copalyl diphosphate,
KD-1	Solyc08g005710	TPS41	-3.37	5.74E-83	NA	NA	Diterpene synthase, Copalyl diphosphate,
KO-1	Solyc05g026590	TPS43	-7.96	1.71E-15	NA	NA	Mutated terpene synthase, non functional
KO-1	Solyc05g026600	TPS43	-5.78	4.07E-07	NA	NA	Mutated terpene synthase, non functional
KD-1	Solyc05g026590	TPS43	-4.09	1.33E-15	NA	NA	Mutated terpene synthase, non functional
KD-1	Solyc05g026600	TPS43	-3.27	9.94E-05	NA	NA	Mutated terpene synthase, non functional
KO-1	Solyc01g105890	TPS5	-5.86	1.61E-78	NA	NA	Monoterpene synthase, Linalool, plastidial
KD-1	Solyc01g105890	TPS5	-4.46	7.46E-68	NA	NA	Monoterpene synthase, Linalool, plastidial
KO-1	Solyc07g047960	WRKY1	-2.29	5.12E-28	TF	WRKY	WRKY transcription factor 1,
KD-1	Solyc07g047960	WRKY1	-2.09	8.54E-25	TF	WRKY	WRKY transcription factor 1,
KD-1	Solyc03g113120	WRKY73	-1.22	1.64E-04	TF	WRKY	WRKY transcription factor 73

Supplemental table 4: genes in Venn diagram from Figure 3

***SlHDZ38* leaves**		**Expression in SlMYC1 mutants**	**Expression in SlHDZ38 mutant**
Solyc03g031760	*SlLN*	-	Down

***SlHDZ38* stem-trichomes**		**Expression in SlMYC1 mutants**	**Expression in SlHDZ38 mutant**
Solyc01g005440	*SlJAZ6*	-	Up
Solyc01g007870	*SlMTR3*	-	Up
Solyc01g010910	*SlMX1*	-	Up
Solyc01g091630	*SlCD2*	-	Up
Solyc01g101210	*SlTPS35*	-	Down
Solyc01g105920	*SlTPS7*	-	Down
Solyc02g080260	*SlWO*	-	Up
Solyc02g088190	*SlMIXTA-like*	-	Up
Solyc02g092570	*SlGRAS26*	-	Up
Solyc03g118540	*SlJAZ5*	-	Up
Solyc05g007580	*SlZHD18*	-	Up
Solyc06g073990	*SlMTR2*	-	Up
Solyc08g005640	*SlTPS21*	-	Down
Solyc09g014980	*SlDT1*	-	Up
Solyc11g072790	*SlWOX3b*	-	Up

***SlMYC1* stem-trichomes**		**Expression in SlMYC1 mutants**	**Expression in SlHDZ38 mutant**
Solyc02g079910	SlTPS27	Up (OE)	-
Solyc03g113120	SlWRKY73	Down	-
Solyc05g026590	SlTPS43	Down	-
Solyc05g026600		Down	-
Solyc07g008680	*SlTPS16*	Down	-

***SlHDZ38* leaves ∩ *SlHDZ38* stem-trichomes**		**Expression in SlMYC1 mutants**	**Expression in SlHDZ38 mutant**
Solyc02g076670	*SlGCR1*	-	Up
Solyc03g098200	*SlHDZ8*	-	Up
Solyc04g049800	*SlTOE1b*	-	Up

***SlHDZ38* leaves ∩ *SlMYC1* leaves**		**Expression in SlMYC1 mutants**	**Expression in SlHDZ38 mutant**
Solyc03g006550	*SlTPS46*	Up (OE)	Down
Solyc12g009220	*SlJAZ2*	Up (OE)	Down
Solyc12g049400	*SlJAZ4*	Up (OE)	Down

***SlHDZ38* stem-trichomes ∩ *SlMYC1* stem-trichomes**		**Expression in SlMYC1 mutants**	**Expression in SlHDZ38 mutant**
Solyc01g079620	*SlMYB12*	Down	Down
Solyc01g096370	*SlbHLH114*	Down	Down
Solyc01g101170	*SlTPS31*	Down	Down
Solyc01g105890	*SlTPS5*	Down	Down
Solyc02g062400	*SlEOT1*	Down	Down
Solyc03g033680	*SlEOT2*	Down	Down
Solyc06g009710	*SlMYB111*	Down	Down
Solyc07g047960	*SlWRKY1*	Down	Down
Solyc09g008810	*SlHDZ38*	Down	Down
Solyc10g005330	*SlHDZ40*	Down	Down
Solyc10g078990	*SlSH*	Up	Up
Solyc12g099900	*SlSCL3*	Down	Down

SlHDZ38* leaves ∩ *SlHDZ38* stem-trichomes ∩ *SlMYC1* leaves ∩ *SlMYC1		**Expression in SlMYC1 mutants**	**Expression in SlHDZ38 mutant**
Solyc01g105850	*SlTPS1*	Up (OE leaf), Down (KD and KO stem trichomes)	Down
Solyc06g059930	*SlTPS12*	Up (OE leaf), Down (KD leaf, KD and KO stem trichomes)	Down
Solyc08g005670	*SlTPS20*	Down (KD leaf, KD and KO stem trichomes)	Down
Solyc08g005710	*SlTPS41*	Up (OE leaf), Down (KD and KO stem trichomes)	Down

***SlHDZ38* stem-trichomes ∩ *SlMYC1* leaves ∩ *SlMYC1* stem-trichomes**		**Expression in SlMYC1 mutants**	**Expression in SlHDZ38 mutant**
Solyc07g008690	*SlTPS16*	Up (OE leaf), Down (KD, KO stem trichomes)	Down
Solyc08g005050	*SlMYC1*	Up (OE leaf), Down (KD leaf and stem trichomes, KO stem	Down
Solyc11g011030	*SlJAZ7*	Up	Up

***SlHDZ38* leaves ∩ *SlHDZ38* stem-trichomes ∩ *SlMYC1* stem-trichomes**		**Expression in SlMYC1 mutants**	**Expression in SlHDZ38 mutant**
Solyc06g053610	*SlMYB14*	Down	Down
Solyc08g005720	*SlTPS18*	Down	Down
Solyc10g005390	*SlTPS39*	Down	Down
Solyc10g083140	*SlMTR1*	Down	Down
Solyc12g006570	*SlTPS17*	Down	Down