EjMYB8 Transcriptionally Regulates Flesh Lignification in Loquat Fruit

Wen-qiu Wang[1,2☯], **Jing Zhang**[1,2☯], **Hang Ge**[1], **Shao-jia Li**[1], **Xian Li**[1,2], **Xue-ren Yin**[1,2]*, **Donald Grierson**[1,3], **Kun-song Chen**[1,2]

1 Zhejiang Provincial Key Laboratory of Horticultural Plant Integrative Biology, Zhejiang University, Zijingang Campus, Hangzhou, P.R. China, 2 The State Agriculture Ministry Laboratory of Horticultural Plant Growth, Development and Quality Improvement, Zhejiang University, Zijingang Campus, Hangzhou, P.R. China, 3 Plant & Crop Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdom

☯ These authors contributed equally to this work.
* xuerenyin@zju.edu.cn

Abstract

Transcriptional regulatory mechanisms underlying lignin metabolism have been widely studied in model plants and woody trees, but seldom in fruits such as loquat, which undergo lignification. Here, twelve *EjMYB* genes, designed as *EjMYB3-14*, were isolated based on RNA-seq. Gene expression indicated that *EjMYB8* and *EjMYB9* were significantly induced in fruit with higher lignin content resulting from storage at low temperature (0°C), while two treatments (low temperature conditioning, LTC; heat treatment, HT) both alleviated fruit lignification and inhibited *EjMYB8* and *EjMYB9* expression. Dual-luciferase assays indicated that EjMYB8, but not EjMYB9, could trans-activate promoters of lignin-related genes *EjPAL1*, *Ej4CL1* and *Ej4CL5*. Yeast one-hybrid assay indicated that EjMYB8 physically bind to *Ej4CL1* promoter. Furthermore, the putative functions of *EjMYB8* were verified using transient over-expression in both *N. tabacum* and loquat leaves, which increased lignin content. Moreover, combination of EjMYB8 and previously isolated EjMYB1 generated strong trans-activation effects on the *Ej4CL1* promoter, indicating that *EjMYB8* is a novel regulator of loquat fruit lignification.

Introduction

Lignin is one of the main components required for plant secondary cell wall formation and its biosynthesis and transcriptional regulatory networks have been studied in various plants, particularly in *Arabidopsis* [1,2]. The synthesis of lignin monomers involve the phenylpropanoid pathway, initiated by L-phenylalanine ammonia-lyase (PAL), followed by Cinnamate 4-hydroxylase (C4H), 4-coumarate:coenzyme A ligase (4CL), Hydroxycinnamoyl CoA shikimate hydroxycinnamoyl transferase (HCT), Caffeoyl CoA 3-O-methyltransferase (CCoAOMT), Cinnamoyl CoA reductase (CCR), Caffeic acid 3-O-methyltransferase (COMT) and Cinnamyl alcohol dehydrogenase (CAD) [3].

Genes encoding enzymes of the phenylpropanoid pathway have been widely reported to be involved in controlling lignin biosynthesis, regulated by transcription factors, including MYB

and NAC. For instance, at least 11 *AtMYB* genes were identified as regulators for lignin biosynthesis, including *AtMYB4*, *AtMYB26*, *AtMYB32*, *AtMYB41*, *AtMYB46*, *AtMYB58*, *AtMYB61*, *AtMYB63*, *AtMYB83*, *AtMYB85* and *AtMYB103* [2,4,5]. However, among these *AtMYB* genes, only *AtMYB58*, *AtMYB63* and *AtMYB85*, were identified as lignin-specific [6,7], while others were considered mainly to regulate secondary cell wall production. Similar transcriptional regulatory mechanisms have been uncovered in various woody trees, such as *Eucalyptus* and *Populus trichocarpa*, and *EgMYB2*, *PtrMYB3* and *PtrMYB20* are transcriptional activators [8], while *EgMYB1* represses lignin biosynthesis [9]. More lignin-related MYB transcription factors have recently been characterized from other plants, such as *PpMYB8* from pine tree [10] and *PdMYB221* from poplar [11].

MYB transcription factors could also interact with other transcription factors, such as members of the NAC transcription factor group. For instance, Arabidopsis *AtMYB83* is directly regulated by *SND1*, a NAC transcription factor [12], while *AtMYB26* up-regulated lignin related *NAC* genes, *NST1* and *NST2* [13]. There is some evidence that different MYB transcription factors participate in a transcriptional regulatory cascade, by regulating promoters of other MYBs, for instance *AtMYB46* binds to the secondary wall MYB-responsive element in the *AtMYB63* promoter [14]. Furthermore, MYBs could also form protein-protein complexes with other transcription factors and in *Arabidopsis*, MYB75 and KNAT7 interact to regulate secondary cell wall development in stems and seed coats [15].

Lignin accumulation occurs not only in model plants, woody trees and field crops, but is also important in some fleshy fruit, such as loquat (*Eriobotrya japonica* Lindl.) [16] and mangosteen (*Garcinia mangostana* L.) [17]. In different fruit, lignification occurred in different layers, in the flesh in loquat fruit and the pericarp in mangosteen. In loquat fruit, two MYB transcription factors were reported as regulators of loquat flesh lignification, with an activator type *EjMYB1* and repressor type *EjMYB2* [18]. Both *EjMYB1* and *EjMYB2* directly interacted with the promoter of the lignin-related biosynthetic gene *Ej4CL1*, and *EjMYB1* could trigger lignin accumulation in *N. tabacum* leaves [18]. In addition to MYB transcription factors, *EjNAC1* was characterized from loquat fruit, and shown to transcriptionally activate lignin biosynthetic genes. As with *EjMYB1*, *EjNAC1* over-expression enhanced lignin biosynthesis, however, the regulatory mechanisms involving *EjNAC1* remain unclear [19]. Most recently, an *AP2/ERF* gene, *EjAP2-1*, was also reported as an indirect regulator for loquat fruit lignification, via protein-protein interaction with EjMYB1 and EjMYB2 [20]. These transcription factors expanded the mechanistic understanding of the regulation of fruit lignification, but understanding is still limited compared to model or woody plants.

In the present study, twelve *EjMYB* genes were isolated from loquat fruit using RNA-Seq. Using the materials described in Xu *et al.* (2014), correlations between *EjMYB* and loquat fruit lignification were investigated and the transcriptional regulatory roles of *EjMYB* in controlling lignin biosynthetic genes were studied by dual-luciferase assay and yeast one-hybrid assay. Functional characterization of the key candidate, *EjMYB8*, was performed by transient overexpression in leaves of both *N. tabacum* and loquat seedlings. Furthermore, a significant additive transactivation of the *Ej4CL1* promoter was observed after *EjMYB1* and *EjMYB8* in combination.

Materials and Methods

Plant materials and treatments

Loquat fruit (*Eriobotrya japonica* Lindl. cv. Luoyangqing, 'LYQ') were harvested and bought from an orchard (name Butoutang) at Luqiao, Zhejiang province, China, at 2011. We confirm that the field studies did not involve endangered or protected species. The fruit were

transported to the lab at Zhejiang University (Hangzhou, Zhejiang) at same day, for posthar-vest treatments. Three different treatments were set: the fruit were treated at 40°C (Hot air, 90–95% RH) for 4 h then transferred to 0°C storage (termed as HT); or pre-stored at 5°C for 6 d then transferred to 0°C storage (termed as LTC); and stored directly at 0°C (control). Details of treatments, sampling and physiological data (eg. Lignin content, firmness) were as previously described [18].

Gene/promoter isolation and analysis

Differentially expressed *MYB*-related unigenes were selected, based on the annotations RNA-seq of 'LYQ' loquat fruit. The UTR and full ORF regions were amplified using a SMART™ RACE cDNA amplification Kit (Clontech) and the primers are listed in S1 and S2 Tables. A phylogenetic tree for R2R3 type *MYB* was constructed by Fig Tree (version 1.4.2).

Regulatory roles of newly isolated *EjMYB* genes were investigated with both lignin biosyn-thetic genes and previous isolated transcription factors related to lignification. Promoters of loquat lignin biosynthesis related structural genes *EjMYB1* and *EjMYB2* were isolated in our previous reports [18,20]. Putative promoters of *EjAP2-1*, were isolated with a GenomeWalker kit (Clontech), using the primers described in S3 Table. The sequence of the putative promoter region is given in S4 Table.

Real-time PCR analysis

For real-time PCR, gene specific oligonucleotide primers were designed and are described in S5 Table. The quality and specificity of each pair of primers was checked with melting curves and sequencing analysis [21]. *EjACT* (Genbank no. JN004223) was chosen as house keeping gene. Gene expression levels were expressed as a ratio relative to the fruit harvest time point (0 d, for fruit experiments) or empty vector (SK, for transient overexpression experiments), which was set to 1.

Total RNA extraction from loquat flesh and leaves used the published protocol [19]. cDNAs used for realtime PCR were synthesized using iScript™ cDNA Synthesis Kit (Biorad).

PCR reactions were performed on a LightCycler 480 instrument (Roche). PCR reaction mixtures comprised 10 µl of LightCycler 480 SYBR Green I Master mix (Roche), 1 µl of each primer (10 µM), 2 µl diluted cDNA and 6 µl PCR grade water [22]. The PCR program was initi-ated with a preliminary step of 5 min at 95°C, followed by 50 cycles at 95°C for 10 s, 60°C for 10 s and 72°C for 15 s. Melting curve analysis were performed for each gene, at the end of each run.

Dual-luciferase assay and yeast one-hybrid

Transcription factors recognize and regulate target promoters, which can be measured by dual-luciferase assays and yeast one-hybrid (Y1H) interactions [18,23]. Full-length transcription factors were cloned into pGreen II 0029 62-SK vector (SK), while the target promoter was inserted into pGreen II 0800-LUC vector [24]. Thus, full-length *EjMYB8* and *EjMYB9*, and the promoter of *EjAP2-1* were newly constructed, using the primers described in S3 and S6 Tables; while the other constructs were previously reported [18,20]. The dual-luciferase assays were performed in *N. benthamiana* leaves, according to protocols described in Zeng *et al.* (2015). Dual-luciferase assays were performed with at least three independent experiments (five bio-logical replicates in each experiment).

According to the results of dual-luciferase assay, Y1H was further performed to verify physical binding of transcription factor and target promoter. Y1H was performed, using the Matchma-ker™ Gold Yeast One-Hybrid Library Screening System (Clontech, USA). Promoters of *Ej4CL1*

was constructed into pAbAi vector by Xu *et al.* (2014), while the full-length *EjMYB8* sequence was subcloned into pGADT7 AD vector (primers are listed in S6 Table). TF-promoter interactions were tested on SD/-Leu containing 0-200ng/ml aureobasidin A (-Leu+AbA^{200}) at 30°C for 3 d.

Transient over-expression analysis of *EjMYB* in *N. tabacum* and loquat leaves

As loquat is a perennial woody fruit tree, it is difficult to perform stable transformation. In order to determine the roles of *EjMYB* genes, an unstable transient over-expression system, was adapted. Firstly, the transient expression analyses were performed in *N. tabacum*, using the same batch of *Agrobacterium* stock and infiltration protocol of the dual-luciferase assay. Target genes (MYB) and empty vector controls (SK) were infiltrated into two sides of the same leaves. Five days after infiltration, the infiltrated leaves (permeation ranges were recorded at infiltration) were sampled and used for lignin analysis. The transient expression analyses were repeated in at least three independent experiments (three biological replicates for each experiment). Similar experiments were also performed on young leaves of loquat seedlings. Transient over-expression experiments were repeated twice (three biological replicates in each). Five days after infiltration, the infiltrated leaves were sampled and used for lignin analysis

Transient over-expression experiments in both *N. tabacum* and loquat were conducted in a growth chamber (light: dark = 16 h: 8 h). Lignin content measurements were conducted according to the protocol described by Xu *et al.* (2014).

Statistical analysis

The statistical significance of differences was calculated using Student's *t*-test. Least significant differences (LSD$_{0.05}$) were calculated using DPS7.05 (Zhejiang University, Hangzhou, China).

Results

Gene isolation and analysis

Twelve *EjMYB* genes were isolated and designated as *EjMYB3-14* (Genbank no. KU534356-KU534367), in addition to the previously characterized *EjMYB1* and *EjMYB2* [18]. These *EjMYB* genes were of several different types with different MYB domain (S1 Fig), *EjMYB1-8* were R2R3 MYB, as indicated by possession of the conserved R2R3 domain (S2 Fig). Phylogenetic analysis that *EjMYB3* and *EjMYB8* were clustered with previously characterized *EjMYB2* and *EjMYB1* (Fig 1), while other *EjMYB* genes were in different sub-group, with *EjMYB6* in the same sub-group as the lignin-related *AtMYB46* and *AtMYB83* (Fig 1).

Association between *EjMYB* expression and loquat fruit lignification

Using the loquat fruit materials described in Xu *et al.* (2014), the expression patterns of 12 *EjMYB* genes were analyzed. Most of *EjMYB* genes showed increasing expression during low temperature (0°C) treatment, either during the whole storage period (eg. *EjMYB8*, *EjMYB9* and *EjMYB14*) or at specific sampling points (eg. *EjMYB5*, *EjMYB11* and *EjMYB13* at 8 d; *EjMYB12* at 4 d), However, only *EjMYB8* and *EjMYB9* were substantially repressed by HT and LTC treatments, which also alleviated, in which loquat fruit lignification, while the other low temperature-inducible *EjMYB* genes were much less responsive to HT and LTC treatments (Fig 2). *EjMYB8* and *EjMYB9* were also highly responsive to low temperature, and their mRNAs increased in abundance 111- and 36-fold, respectively. In contrast, transcript abundance of *EjMYB7* decreased during low temperature storage, and expression in HT and LTC treated fruit showed little change compared with low temperature stored fruit (Fig 2).
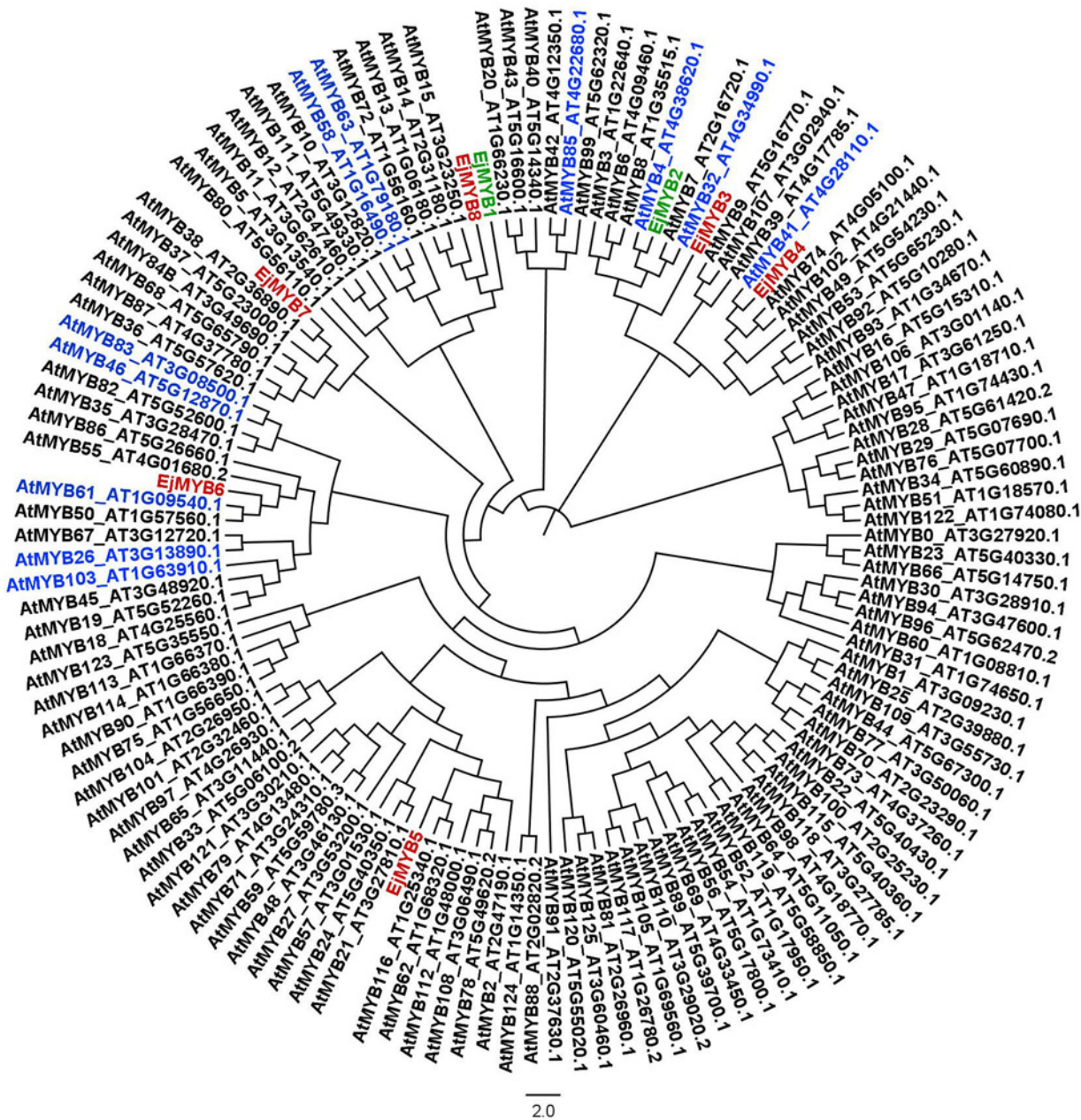
Fig 1. Phylogenetic analysis of R2R3 MYB transcription factors from loquat and Arabidopsis. Lignin-related AtMYBs are highlighted in blue and previously reported loquat EjMYB in green. Deduced amino acid sequences of *Arabidopsis* AtMYB were obtained from The Arabidopsis Information Resource (TAIR). Alignment was performed using the neighbor-joining (NJ) method in ClustalX (v. 1.81) and a phylogenetic tree was constructed with Fig Tree (v. 1.4.2).

doi:10.1371/journal.pone.0154399.g001

Regulatory roles of EjMYB8 and EjMYB9 on lignin biosynthesis genes and related transcription factors

EjMYB8 and EjMYB9 had particularly interesting expression patterns and were selected for further analysis. Dual-luciferase assays indicated that EjMYB8 significantly activated activities of *EjPAL1*, *Ej4CL1* and *Ej4CL5* promoters, with the *Ej4CL1* promoter being up-regulated about 6-fold, while *EjMYB9* had smaller effects ([Fig 3](#)). Y1H analysis indicated EjMYB8 could bind directly to the *Ej4CL1* promoter ([Fig 4](#)). These results indicated EjMYB8 could interact
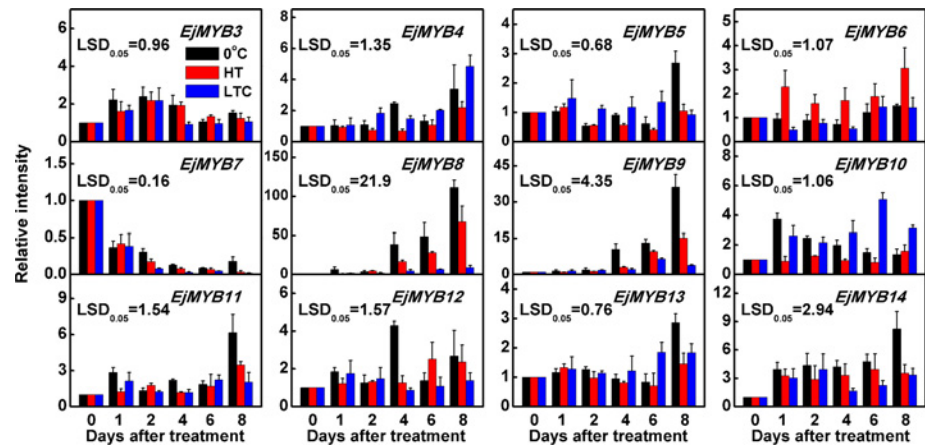
Fig 2. Expression of *EjMYB* genes in response to 0°C (Control), HT and LTC treatments. HT, heat treatment; LTC, low temperature conditioning. All of the materials were obtained from Xu *et al*., (2014). mRNA levels were expressed as a ratio relative to the harvest time point (0 d), which was set at 1. Error bars indicate S.E.s from three replicates.

doi:10.1371/journal.pone.0154399.g002

with and activate lignin biosynthetic genes. Possible regulatory roles of EjMYB8 on lignin related transcription factors were also investigated. However, dual-luciferase assay indicated that EjMYB8 was not able to influence promoters activities of the transcription factor genes *EjMYB1*, *EjMYB2* and *EjAP2-1* (S3 Fig), suggesting that EjMYB8 does not regulate these known lignin-related transcription factors in loquat.

Functional characterization of *EjMYB8* in controlling lignin biosynthesis in both *N. tabacum* and loquat

Due to the lack of a stable transformation system for loquat, transient over-expression approaches were adopted. *EjMYB8* transiently over-expressed in *N. tabacum* leaves
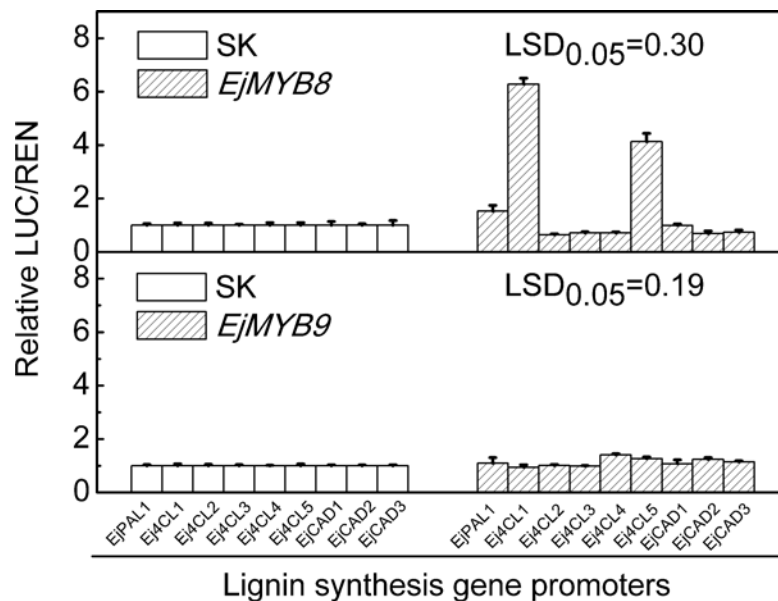

Fig 3. Regulatory effects of EjMYB8 and EjMYB9 on the promoters of lignin biosynthesis genes, using the dual luciferase assay. The ratio of LUC/REN of the empty vector (SK) plus promoter was used as calibrator (set as 1).

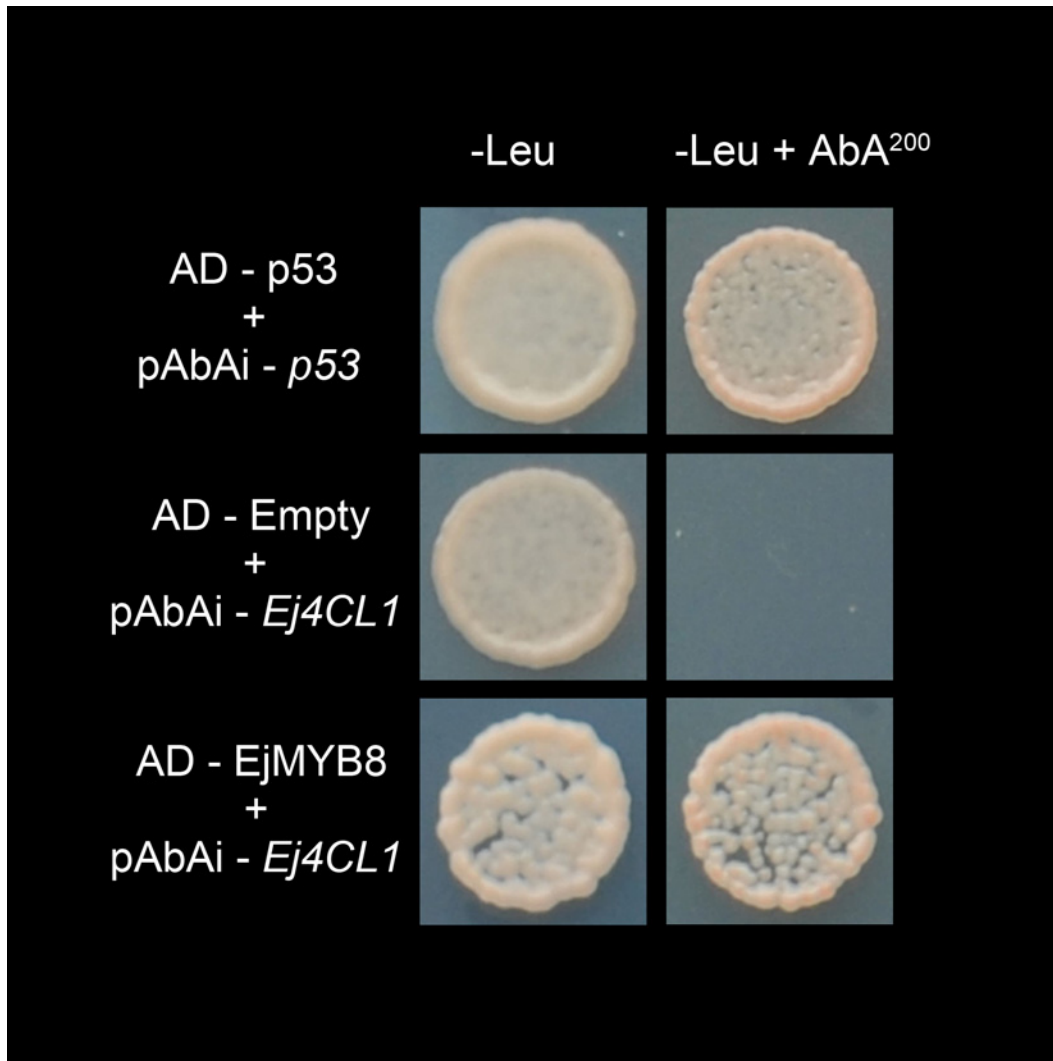doi:10.1371/journal.pone.0154399.g003

Fig 4. Yeast one-hybrid analysis of EjMYB8 binding to *Ej4CL1* promoter. Interaction was determined on SD medium lacking Leu in the presence of aureobasidin A (-Leu+AbA^{200}). AD-p53 and pAbAi-*p53* were used as positive control; AD-empty and pAbAi-*Ej4CL1* were used as negative control.

significantly ($P<0.01$) promoted lignin accumulation, reaching 1.81×10^3 A$_{280}$kg^{-1} FW^{-1} compared with 1.26×10^3 A$_{280}$kg^{-1} FW^{-1} for the empty vector (Fig 5). Similar results were found in loquat leaves, where the half blades over-expressing *EjMYB8* had a higher lignin content of 19.8×10^3 A$_{280}$kg^{-1} FW^{-1}, compared with the empty vector control half blades of 16.2×10^3 A$_{280}$kg^{-1} FW^{-1} (Fig 5).

Combination effects of EjMYB8 and EjMYB1

The results of gene expression, dual-luciferase assay, Y1H and transient over-expression indicate that EjMYB8 is an activator of loquat fruit lignification. It promotes transcription from the promoters of lignin biosynthesis genes, as found for the previously reported transcriptional activator, EjMYB1 using dual-luciferase assay [18]. EjMYB1 and EjMYB8 together caused a substantially higher induction (approximate 21 folds) of expression from the *Ej4CL1* promoter (Fig 6). The results of yeast two hybrid assays, however, indicated that EjMYB8 could not directly interact with EjMYB1 (S4 Fig).
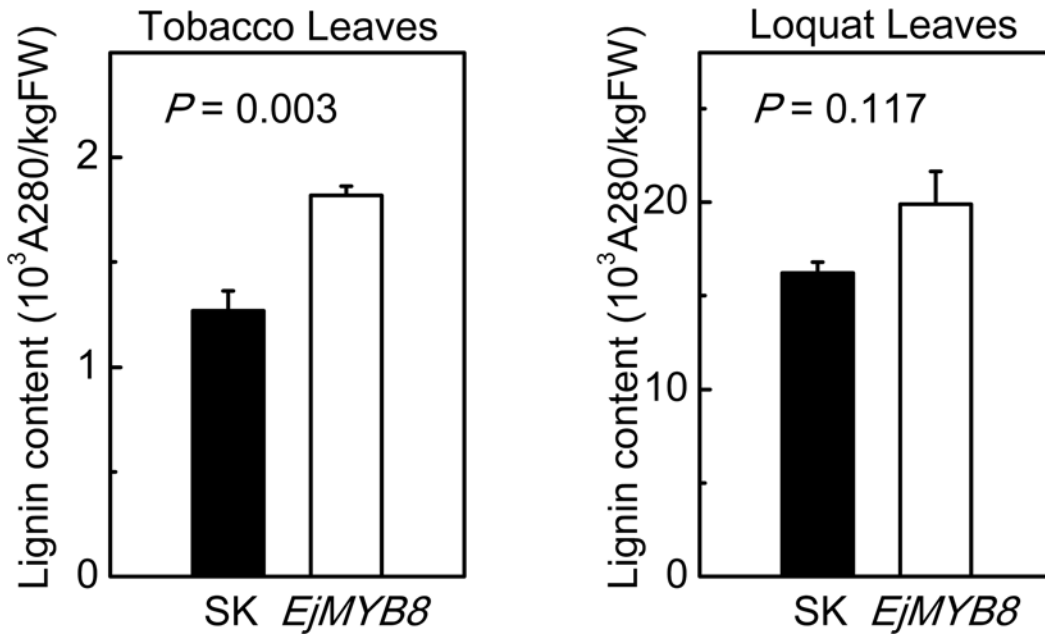
Fig 5. Transient over-expression of *EjMYB8* in *N. tabacum* and loquat leaves. The transient over-expression experiments were conducted with empty vector and *EjMYB8* on opposite sides of the same leaf. Error bars indicate S.E.s from three biological replicates. Student's t-test was applied for the lignin content analysis and the significance levels indicated.
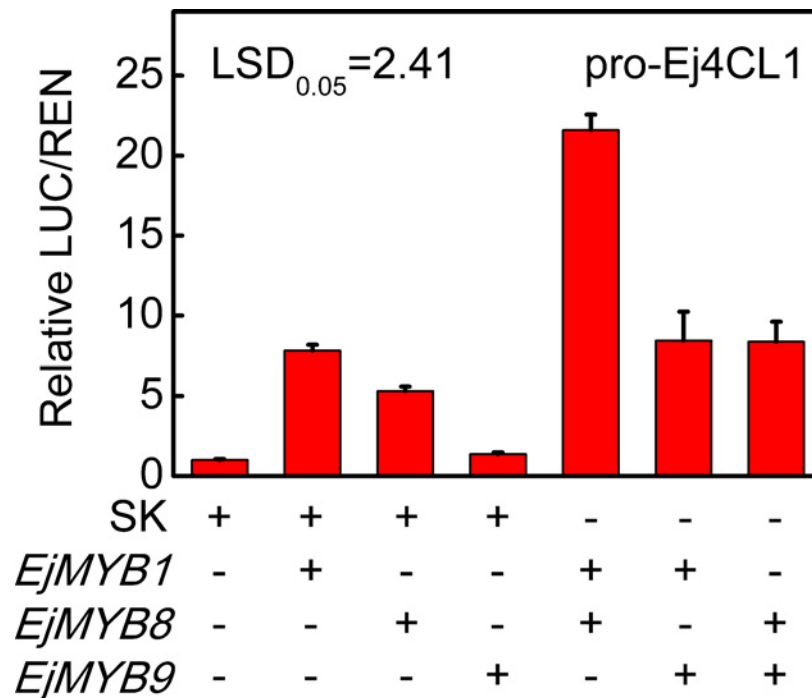
Fig 6. Synergistic trans-activation of the *Ej4CL1* promoter by combination of EjMYBs. The ratio of LUC/REN of the empty vector (SK) plus promoter was used as calibrator (set as 1). + and—means presence and absence of indicated constructs. Error bars indicate S.E.s from five replicates. LSDs represent least significant difference at $p = 0.05$.

Discussion

Fleshy fruit lignification is important both commercially and scientifically. For industry, lignification adversely influences fruit storability and quality, for instance, loquat fruit lignification is usually accompanied by flesh browning reduced juice extractability. Thus, many artificial technologies have been developed to alleviate lignification, such as LTC [16], 1-MCP [25], HT [18], Methyl Jasmonate (MeJA) [26]. Mechanisms controlling fruit lignification are particularly interesting, as it occurs in productive edible organs, not vegetative organs of model plants. The underlying regulatory mechanisms of low temperature induced lignification and the technologies developed to alleviate it remain generally unknown.

Previous research has indicated that *EjMYB1* and *EjMYB2* transcriptionally regulate loquat fruit lignification, and both *EjMYB* genes were cloned based on their similarity to *Arabidopsis* lignin regulators *AtMYB58* and *AtMYB4* [18]. We investigated whether there could be additional *EjMYB* genes involved in loquat lignification. Twelve *EjMYB* were isolated based on RNA-seq and RACE. Phylogenetic analysis suggested, that other R2R3 type *EjMYB* genes could be involved in loquat fruit lignification, as they were clustered with known lignin-related MYB, such as *AtMYB4*, *AtMYB46* and *AtMYB58* [7,27,28]. However, gene expression indicated that only *EjMYB8* and *EjMYB9* (not R2R3 types) were significantly correlated with loquat fruit lignification, as their transcripts abundance were significantly prohibited by LTC and HT treatment, which reduced lignification.

A role for *EjMYB8* was confirmed by the demonstration that it had the ability to trans-activate promoters of lignin biosynthesis related genes (*EjPAL1*, *Ej4CL1* and *Ej4CL5*) from loquat fruit and Y1H assay indicated a physical interaction between *EjMYB8* and the *Ej4CL1* promoter. On contrast, *EjMYB9* could not regulate these promoters. Significantly, all results for *EjMYB8*, including gene expression, dual-luciferase assay and Y1H, mimicked those for *EjMYB1* [18] and *EjMYB8* was clustered with *EjMYB1*. At least two *AtMYB* genes, *AtMYB58* an *AtMYB63*, which fall in the same sub-group as *EjMYB1* and *EjMYB8*, have also been characterized as regulators of lignin biosynthesis [7], suggesting multiple MYB family members may have similar function. However, *EjMYB8* encoded a novel protein, with only 52% amino acid sequence identity to *EjMYB1* (data not shown), indicating that, *EjMYB8* is a novel *MYB* transcription factor that participates in loquat fruit lignification, via transcriptional regulated of structural genes. The function and regulatory mechanisms of *EjMYB9* on loquat fruit lignification remain unknown, which might not a direct regulator on lignin biosynthesis genes and, require further investigation.

The function of *EjMYB8* was verified in *N. tabacum* and loquat leaves. For perennial fruit, functional verifications are generally a bottleneck, due to the lack of stable transformation systems and transient expression systems have been widely adopted for functional analysis of fruit genes, such as *MdMYB10* for apple anthocyanin regulation [29]; *PpMYB10.4* for peach anthocyanin biosynthesis [30]; *AdGT4* for kiwifruit aroma [31]. Here, transient over-expression of *EjMYB8* in loquat and *N. tabacum* supported the role of *EjMYB8* as a regulator of loquat fruit lignification.

MYB transcription factors have been widely reported to be involved in many aspects of plant metabolisms, via interaction with other transcription factors, such as MYB-bHLH-WD40 for anthocyanin regulation [32,33] and also MYB-ZML for lignin biosynthesis [34]. In loquat, protein-protein interactions have also been observed between lignification related transcription factors EjMYB1/2 and EjAP2-1 [20]. However, both dual-luciferase and yeast two hybrid assays indicated that EjMYB8 could not interact with the EjMYB1 protein or promoter (S3 and S4 Figs). Thus, the underlying mechanisms of the addictive effects of *EjMYB1* and *EjMYB8* require further investigation.

Conclusions

Twelve *EjMYB* genes were isolated from loquat fruit and *EjMYB8* was characterized as a novel activator for loquat fruit lignification, according to the results of gene expression and dual-luciferase assay. Y1H indicated EjMYB8 could directly interact with *Ej4CL1* promoter. Furthermore, *EjMYB8* and *EjMYB1* acted synergistically to enhance expression of *Ej4CL1*. The present study has thus identified a novel MYB transcription factor (*EjMYB8*) and possible MYB-MYB linkage for loquat fruit lignification.

Supporting Information

S1 Fig. Distribution of conserved domains in EjMYB protein sequences.
(TIF)

S2 Fig. Alignment of R2R3 domains from EjMYB transcription factors.
(TIF)

S3 Fig. Regulatory effects of EjMYB8/9 on the promoters of lignification-related transcription factors, using the dual luciferase assay.
(TIF)

S4 Fig. Yeast two-hybrid assay for EjMYB1 and EjMYB8.
(TIF)

S1 Table. Primes sequences for 5'-RACE analysis.
(DOCX)

S2 Table. Primer sequences for 3'-RACE analysis.
(DOCX)

S3 Table. Primers for *EjAP2-1* promoter isolation and vector construction.
(DOCX)

S4 Table. Sequence of *EjAP2-1* promoter.
(DOCX)

S5 Table. Primers for Real-time PCR.
(DOCX)

S6 Table. Primers for EjMYB full-length sequences clone.
(DOCX)

Author Contributions

Conceived and designed the experiments: WqW XrY. Performed the experiments: WqW JZ HG SjL. Analyzed the data: WqW XrY XL DG KsC. Contributed reagents/materials/analysis tools: WqW JZ HG XL. Wrote the paper: WqW XrY DG KsC.

References

1. Zhong RQ, Ye ZH. Transcriptional regulation of lignin biosynthesis. Plant Signal Behavior 2009; 4: 1028–1034.

2. Zhao Q, Dixon RA. Transcriptional networks for lignin biosynthesis: more complex than we thought? Trends Plant Sci. 2011; 16: 227–233. doi: 10.1016/j.tplants.2010.12.005 PMID: 21227733

3. Vermerris W, Abril A. Enhancing cellulose utilization for fuels and chemicals by genetic modification of plant cell wall architecture. Curr Opin Biotechnol. 2015; 32: 104–112. doi: 10.1016/j.copbio.2014.11.024 PMID: 25531269

4. Öhman D, Demedts B, Kumar M, Gerber L, Gorzsás A, Goeminne G, et al. MYB103 is required for FERULATE-5-HYDROXYLASE expression and syringyl lignin biosynthesis in Arabidopsis stems. Plant J. 2013; 73: 63–76. doi: 10.1111/tpj.12018 PMID: 22967312

5. Kosma DK, Murmu J, Razeq FM, Santos P, Bourgault R, Molina I, et al. AtMYB41 activates ectopic suberin synthesis and assembly in multiple plant species and cell types. Plant J. 2014; 80: 216–229. doi: 10.1111/tpj.12624 PMID: 25060192

6. Zhong RQ, Lee C, Zhou JL, McCarthy RL, Ye ZH. A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in *Arabidopsis*. Plant Cell 2008; 20: 2763–2782. doi: 10.1105/tpc.108.061325 PMID: 18952777

7. Zhou JL, Lee C, Zhong RQ, Ye ZH. MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in *Arabidopsis*. Plant Cell 2009; 21, 248–266. doi: 10.1105/tpc.108.063321 PMID: 19122102

8. McCarthy RL, Zhong R, Fowler S, Lyskowski D, Piyasena H, Carleton K, et al. The poplar MYB transcription factors, PtrMYB3 and PtrMYB20, are involved in the regulation of secondary wall biosynthesis. Plant Cell Physiol. 2010; 51: 1084–1090. doi: 10.1093/pcp/pcq064 PMID: 20427511

9. Legay S, Lacombe E, Goicoechea M, Brière C, Séguin A, Mackay J, et al. Molecular characterization of EgMYB1, a putative transcriptional repressor of the lignin biosynthetic pathway. Plant Sci. 2007; 173: 542–549.

10. Craven-Bartle B, Pascual MB, Cánovas FM, Ávila C. A Myb transcription factor regulates genes of the phenylalanine pathway in maritime pine. Plant J. 2013; 74: 755–766. doi: 10.1111/tpj.12158 PMID: 23451763

11. Tang XF, Zhuang YM, Qi G, Wang D, Liu HH, Wang KR, et al. Poplar PdMYB221 is involved in the direct and indirect regulation of secondary wall biosynthesis during wood formation. Sci Rep. 2015; 5: 12240. doi: 10.1038/srep12240 PMID: 26179205

12. McCarthy RL, Zhong R, Ye ZH. MYB83 is a direct target of SND1 and acts redundantly with MYB46 in the regulation of secondary cell wall biosynthesis in Arabidopsis. Plant Cell Physiol. 2009; 50: 1950–1964. doi: 10.1093/pcp/pcp139 PMID: 19808805

13. Yang C, Xu Z, Song J, Conner K, Barrena GV, Wilson ZA. Arabidopsis *MYB26/MALE STERILE35* regulates secondary thickening in the endothecium and is essential for anther dehiscence. Plant Cell 2007; 19: 534–548. PMID: 17329564

14. Zhong RQ, Ye ZH. MYB46 and MYB83 bind to the SMRE sites and directly activated a suite of transcription factors and secondary wall biosynthetic genes. Plant Cell Physiol. 2012; 53: 368–380. doi: 10.1093/pcp/pcr185 PMID: 22197883

15. Bhargava A, Ahad A, Wang S, Mansfield SD, Haughn GW, Douglas CJ, et al. 2013. The interacting MYB75 and KNAT7 transcription factors modulate secondary cell wall deposition both in stems and seed coat in Arabidopsis. Planta 2013; 237: 1199–1211. doi: 10.1007/s00425-012-1821-9 PMID: 23328896

16. Cai C, Xu CJ, Shan LL, Li X, Zhou CH, Zhang WS, et al. Low temperature conditioning reduces postharvest chilling injury in loquat fruit. Postharvest Biol Technol. 2006a; 41: 252–259.

17. Kamdee C, Imsabai W, Kirk R, Allen AC, Ferguson IB, Ketsa S. Regulation of lignin biosynthesis in fruit pericarp hardening of mangosteen (*Garcinia mangostana* L.) after impact. Postharvest Biol Technol. 2014; 97: 68–76.

18. Xu Q, Yin XR, Zeng JK, Ge H, Song M, Xu CJ, et al. Activator- and repressor-type MYB transcription factors are involved in chilling injury induced flesh lignification in loquat via their interactions with the phenylpropanoid pathway. J Exp Bot. 2014; 65: 4349–4359. doi: 10.1093/jxb/eru208 PMID: 24860186

19. Xu Q, Wang WQ, Zeng JK, Zhang J, Donald G, Li X, et al. A NAC transcription factor, *EjNAC1*, affects lignification of loquat fruit by regulating lignin. Postharvest Biol Technol. 2015; 102: 25–31.

20. Zeng JK, Li X, Xu Q, Chen JY, Yin XR, Ferguson IB, et al. *EjAP2-1*, an *AP2/ERF* gene, is a novel regulator of fruit lignification induced by chilling injury, via interaction with *EjMYB* transcription factors. Plant Biotechnol J. 2015; 13: 1325–1334. doi: 10.1111/pbi.12351 PMID: 25778106

21. Yin XR, Shi YN, Min T, Luo ZR, Yao YC, Xu Q, et al. Expression of ethylene response genes during persimmon fruit astringency removal. Planta 2012a; 235: 895–906.

22. Yin XR, Zhang Y, Zhang B, Yang SL, Shi YN, Ferguson IB. Differential expression of kiwifruit *ERF* genes in response to postharvest abiotic stress. Postharvest Biol Technol. 2012b; 66: 1–7.

23. Min T, Fang F, Ge H, Shi YN, Luo ZR, Yao YC, et al. Two novel anoxia-induced ethylene response factors that interact with promoters of deastringency-related genes from persimmon. PLoS ONE 2014; 9: e97043. doi: 10.1371/journal.pone.0097043 PMID: 24805136

24. Hellens RP, Allan AC, Friel EN, Bolitho K, Grafton K, Templeton MD, et al. Transient expression vectors for functional genomics, quantification of promoter activity and RNA silencing in plants. Plant Methods 2005; 1: 13. PMID: 16359558

25. Cai C, Chen KS, Xu WP, Zhang WS, Li X, Ferguson IB. 2006b. Effect of 1-MCP on postharvest quality of loquat fruit. Postharvest Biol Technol. 2006b; 40: 155–162.

26. Jin P, Duan Y, Wang L, Wang J, Zheng Y. Reducing Chilling Injury of Loquat Fruit by Combined Treatment with Hot Air and Methyl Jasmonate. Food Biopro Technol. 2014; 7: 2259–2266.

27. Jin HL, Cominelli E, Bailey P, Parr A, Mehrtens F, Jones J, et al. 2000. Transcriptional repression by AtMYB4 controls production of UV-protecting sunscreens in *Arabidopsis*. EMBO J. 2000; 19: 6150–6161. PMID: 11080161

28. Zhong RQ, Richardson EA, Ye ZH. The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in *Arabidopsis*. Plant Cell 2007; 19: 2776–2792. PMID: 17890373

29. Espley RV, Hellens RP, Putterill J, Stevenson DE, Kutty-Amma S, Allan AC. Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. Plant J. 2007; 49: 414–427. PMID: 17181777

30. Zhou Y, Zhou H, Lin-Wang K, Vimolmangkang S, Espley RV, Wang L, et al. Transcriptome analysis and transient transformation suggest an ancient duplicated MYB transcription factor as a candidate gene for leaf red coloration in peach. BMC Plant Biol. 2014; 14: 388. doi: 10.1186/s12870-014-0388-y PMID: 25551393

31. Yauk YK, Ged C, Wang MY, Matich AJ, Tessarotto L, Cooney JM, et al. Manipulation of flavour and aroma compound sequestration and release using a glycosyltransferase with specificity for terpene alcohols. Plant J. 2014; 80: 317–330. doi: 10.1111/tpj.12634 PMID: 25088478

32. Liu XF, Yin XR, Allan AC, Lin-Wang K, Shi YN, Huang YJ, et al. The role of *MrbHLH1* and *MrMYB1* in regulating anthocyanin biosynthetic genes in tobacco and Chinese bayberry (*Myrica rubra*) during anthocyanin biosynthesis. Plant Cell Tiss Organ Cult. 2013; 115: 285–298.

33. Tian J, Peng Z, Zhang J, Song TT, Wan HH, Zhang ML, et al. McMYB10 regulates coloration via activating *McF3'H* and later structural genes in ever-red leaf crabapple. Plant Biotechnol J. 2015; 13: 948–961. doi: 10.1111/pbi.12331 PMID: 25641214

34. Vélez-Bermúdez IC, Salazar-Henao JE, Fornalé S, López-Vidriero I, Franco-Zorrilla JM, Franco-Zorrilla E, et al. A MYB/ZML Complex Regulates Wound-Induced Lignin Genes in Maize. Plant Cell 2015; 27: 3245–3259. doi: 10.1105/tpc.15.00545 PMID: 26566917