Transcriptional regulation of KCS gene by bZIP29 and MYB70 transcription factors during ABA-stimulated wound suberization of kiwifruit (Actinidia deliciosa)

Xueyuan Han1,2, Xiaopeng Wei2,3, Wenjing Lu2,4, Qiong Wu5, Linchun Mao2,6* and Zisheng Luo2,6

Abstract
Background: Our previous study has demonstrated that the transcription of AchnKCS involved in suberin biosynthesis was up-regulated by exogenous abscisic acid (ABA) during the wound suberization of kiwifruit, but the regulatory mechanism has not been fully elucidated.

Results: Through subcellular localization analysis in this work, AchnbZIP29 and AchnMYB70 transcription factors were observed to be localized in the nucleus. Yeast one-hybrid and dual-luciferase assay proved the transcriptional activation of AchnMYB70 and transcriptional suppression of AchnbZIP29 on AchnKCS promoter. Furthermore, the transcription level of AchnMYB70 was enhanced by ABA during wound suberization of kiwifruit, but AchnbZIP29 transcription was reduced by ABA.

Conclusions: Therefore, it was believed that ABA enhanced the transcriptional activation of AchnMYB70 on AchnKCS by increasing AchnMYB70 expression. On the contrary, ABA relieved the inhibitory effect of AchnbZIP29 on transcription of AchnKCS by inhibiting AchnbZIP29 expression. These results gave further insight into the molecular regulatory network of ABA in wound suberization of kiwifruit.

Keywords: Suberization, KCS, Transcriptional regulation, Abscisic acid

Background
Fruits are often bruised or mechanically wounded during the harvesting, transportation and storage processes, which leads to the susceptibility to microbial infection and quality degradation. However, the damaged surface of the postharvest kiwifruit would suberize to accumulate suberin and further form a healing layer, which can reduce the outflow of cell water and nutrients and limit the invasion of pathogens [1–3]. Suberin layer was observed after wounding by means of fluorescence and staining microscopy and component analysis in kiwifruit [1]. Wounding-induced suberization also commonly occurs in potato tuber [4], Arabidopsis root [5] and postharvest tomato [6]. Suberin is a plant cell-wall biopolymer composed of glycerol-based aliphatic polyester and the associated polymeric aromatics [7, 8]. It is biosynthesized initially from the acylation of fatty acids by long chain acyl-CoA synthetase (LACS), following fatty acyl elongation controlled by fatty acid elongation enzyme complex (FAE), acyl reduction by fatty acyl reductase (FAR), fatty acyl oxidation by cytochrome P450 enzyme (CYP) and esterification of ω-hydroxy fatty acids and α, ω-dicarboxylic acids by glycerol 3-phosphate

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
acyltransferase (GPAT) [9]. The polymeric aromatics are biosynthesized from phenylpropanoid pathway [7].

Exogenous abscisic acid (ABA) could stimulate the accumulation of suberin with induced expression of genes encoding β-ketoacyl-coenzyme A synthases (KCSs) related to suberin synthesis [1, 10]. It was suggested that ABA signaling stimulated the formation of a periderm including suberin in the apple and tomato fruit with defective cuticle formation [11, 12]. KCSs, as the components of FAE, catalyze the condensation of long-chain fatty acyl CoA and malonyl CoA to produce β-ketoacyl CoA with a carbon chain extension of two-carbon unit (Fig. 1), participating in the synthesis of very long chain fatty acids (VLCFAs) that are the precursors of suberin biosynthesis. Recent research also reported that KCSs were associated with peridermal skin formation in kiwifruit [14].

The coding sequence (CDS) of AchnKCS (Achn030011) of 1512 bp was cloned from Actinidia deliciosa ‘Xuxiang’ in our previous work [15]. The homology analysis of amino acid sequence displayed that the KCSs in plant were highly conserved, and AchnKCS had a high homology with AtKCS20 in Arabidopsis [16] and SlKCS11 in tomato [17]. In addition, the endoplasmic reticulum (ER) localization of AchnKCS protein was confirmed [15].

QsMYB1 (Quercus suber) was reported to target two QsKCS involved in suberin biosynthesis by Chip-seq assay [18]. Recently, it was revealed that AchnbZIP12 responding to ABA signaling positively regulated the transcription of AchnKCS during wound suberization of kiwifruit [15]. AtMYB41 [19], AtMYB9 [12], AtMYB107 [20] and AtMYB93 [21, 22] were demonstrated to be associated with the regulation of suberin biosynthesis. The over expression of MYB92 in leaves of Nicotiana benthamiana significantly increased the transcript level of KCS1 and the deposition of corresponding suberin monomers with carbon chain length of > 20 [23]. Similarly, the transcript levels of KCS2 and KCS20 were elevated in MYB39 overexpression leaves of N. benthamiana, and KCS1 and KCS2 in MYB39 overexpression root of Arabidopsis [24]. Moreover, some of these transcription factors involved in suberization regulation have been shown to be ABA-responsive, such as AtMYB41 [19], AchnbZIP12 [15] and AchnMYB107 [25]. Besides, ABA signaling cascades was suggested to play a mediating role in suberin biosynthesis regulated by MYB39 in the Arabidopsis root endodermis [24].

Therefore, based on our previous report and related literatures, the present study was to explore the regulatory mechanism of ABA in inducing AchnKCS (Achn030011) expression during suberin deposition by investigating the transcriptional control of transcription factors on AchnKCS. AchnbZIP29 and AchnMYB70 transcription factors were speculated and verified to regulate the transcription of AchnKCS in response to ABA-stimulated wound suberization. It was expected to give further insight into the molecular regulatory network of ABA in promoting wound suberization of kiwifruit.

Methods

Fruit treatment

Kiwifruit (Actinidia deliciosa ‘Xuxiang’) were harvested at commercial maturity with the uniformity of shape and size from a commercial orchard in Fuyang District, Hangzhou, China. Treatment was based on Han et al. [15]. The surface was sterilized with 0.5% (v/v) NaClO solution for 3 min, washed with sterile water and air-dried naturally. Artificial wound was made by cutting the fruit into halves lengthwise. Nighty halves were treated with 0.5 mmol L⁻¹ ABA (≥ 90%, Aladdin Industrial Inc., China) and another 90 halves were treated with sterile water (control) by vacuum infiltration. Afterwards, fruit halves were stored in a sterile incubator at 20 °C and 85% relative humidity for wound healing under darkness. Suberized tissue was separated from the scarred outmost layer of the wound surface after incubating for 2, 3 and 4 days and stored at −80 °C until further analysis.

RNA extraction

The cetyltrimethylammonium bromide (CTAB) method was carried out to extract the total RNA

---

Fig. 1 Catalysis and substrate specificity of KCSs in the elongation steps of carbon chains involved in the synthesis of VLCFAs in Arabidopsis [13]. Numbers represent the number of carbon units of VLCFAs.
DNA extraction
The total DNA was extracted by implementing the CTAB method [27]. The implementation details referred to Han et al. [15]. Briefly, 2% CTAB extraction buffer and LiCl solution (12 mol L$^{-1}$) were applied to extract and denature the RNA on the first day. On the second day, the SSTE buffer (containing 1.0 mM EDTA, 10 mM Tris-HCl pH 8.0, 0.5% (m/v) SDS and 1.0 M NaCl), chloroform and ethanol were added to dissolve, purify and precipitate the RNA, respectively. Finally, wash the RNA pellet with pre-chilled 75% ethanol for twice and dissolve the RNA pellets again using RNase-free water. The quality of the RNA samples was measured using a NanoDrop 2000 (Thermo Fisher Scientific, USA).

Molecular cloning and amino acid sequence homology
The gene sequence of transcription factor AchnbZIP29 (Achn340751) and AchnMYB70 (Achn117821) were determined based on the Cornell University kiwifruit database (http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/home.cgi). The cloning conditions were according to Han et al. [15]. Based on the primers in Supplementary Table 1 (AchnbZIP29-Full and AchnMYB70-Full), both genes of AchnbZIP29 and AchnMYB70 were cloned from reverse transcribed cDNA. And the promoter of AchnKCS was cloned from the extracted total DNA using the corresponding AchnKCS-Pro primers. After linking the amplified product with pEASY-T1 simple vector and transferring it into Escherichia coli, the test of white colony on target AchnKCS-Pro-pABAi and empty pGADT7 were cloned into pGADT7 vector (AD) via restriction enzyme cutting sites (EcoRI and XhoI sites, SmaI and SacI sites, respectively). Transformed Y1H Gold harboring both AchnKCS-Pro-pABAi and AchnbZIP29-pGADT7 or AchnMYB70-pGADT7 were cultured to test the interaction on SD/-Leu with AbA at 30 °C for 3 days. Y1H Gold co-transformed with p53-promoter and pGADT7-Rec I and I and XhoI cutting sites (SacI sites, SmaI and SacI sites, respectively) were used as positive control. Y1H Gold co-transformed with AchnKCS-Pro-pABAi and empty pGADT7 were used as negative control.

Yeast one-hybrid assay (Y1H)
In order to test the protein-DNA interaction of AchnbZIP29, AchnMYB70 and AchnKCS promoter, Y1H assay was carried out according to the Matchmaker® Gold Yeast One-Hybrid Library Screening System (Cat. No. 630491, TaKaRa, Dalian, China). Auto-activation analysis of AchnKCS promoter was conducted at first and the minimum inhibitory concentration of aureobasidin A (AbA, a yeast toxin) was determined. The recombinant plasmid of AchnKCS-Pro-pABAi was transferred into Y1H Gold through PEG/LiAc after linearizing. The full-length regions of AchnbZIP29 and AchnMYB70 were cloned into pGADT7 vector (AD) via restriction enzyme cutting sites (EcoRI and XhoI sites, SmaI and Sacl sites, respectively). Transformed Y1H Gold harboring both AchnKCS-Pro-pABAi and AchnbZIP29-pGADT7 or AchnMYB70-pGADT7 were cultured to test the interaction on SD/-Leu with AbA at 30 °C for 3 days. Y1H Gold co-transformed with p53-promoter and pGADT7-Rec I and I and XhoI cutting sites (SacI sites, SmaI and SacI sites, respectively) were used as positive control. Y1H Gold co-transformed with AchnKCS-Pro-pABAi and empty pGADT7 were used as negative control.

Dual luciferase assay
Dual-luciferase assay was carried out to determine the trans-activation role of AchnbZIP29 and AchnMYB70 on target AchnKCS promoter. The implementation details referred to Tao, et al. [28]. The promoter sequence of AchnKCS was inserted into LUC vector (pGreen II 0800-LUC, cut by HindIII and BamHI). The CDSs of AchnbZIP29 and AchnMYB70 were amplified and inserted into pGreen II 0029 62-SK vector (SK) (cut by HindIII and BamHI), respectively. The ClonExpress II One Step Cloning Kit (C112-01, Vazyme, China) was applied to drive the connection reactions. The procedures of Agrobacterium tumefaciens transformation and the preparation of the infection buffer of Agrobacteria were according to Han et al. [15]. Afterwards, the Agrobacteria culture mixtures of respectively empty pSK, AchnbZIP29-pSK and AchnMYB70-pSK were added to Agrobacteria to Han et al. [15]. The obtained Agrobacteria were amplified and inserted into the 1300-35S-eGFP vector. The obtained AchnbZIP29-GFP and AchnMYB70-GFP fusion expression vectors were respectively transferred into Agrobacterium strain. The preparation of the infection buffer of Agrobacteria and the inoculation of tobacco (Nicotiana benthamiana) leaves were according to Han et al. [15]. After inoculation for 48 h, a confocal microscope (Leica SP8, Leica Microsystems Co., Germany) was used to observe the GFP fluorescence of the leaf discs at 488 nm excitation.

Subcellular localization of AchnbZIP29 and AchnMYB70
After cloning the coding sequence (CDS) of AchnbZIP29 and AchnMYB70, the sequence with no stop codon was amplified and inserted into the 1300-35S-eGFP vector. The obtained AchnbZIP29-GFP and AchnMYB70-GFP fusion expression vectors were respectively transferred into Agrobacterium strain. The preparation of the infection buffer of Agrobacteria and the inoculation of tobacco (Nicotiana benthamiana) leaves were according to Han et al. [15]. After inoculation for 48 h, a confocal microscope (Leica SP8, Leica Microsystems Co., Germany) was used to observe the GFP fluorescence of the leaf discs at 488 nm excitation.

The total DNA was extracted by implementing the CTAB method [27]. The implementation details referred to Han et al. [15]. Briefly, 2% CTAB extraction buffer and LiCl solution (12 mol L$^{-1}$) were applied to extract and denature the RNA on the first day. On the second day, the SSTE buffer (containing 1.0 mM EDTA, 10 mM Tris-HCl pH 8.0, 0.5% (m/v) SDS and 1.0 M NaCl), chloroform and ethanol were added to dissolve, purify and precipitate the RNA, respectively. Finally, wash the RNA pellet with pre-chilled 75% ethanol for twice and dissolve the RNA pellets again using RNase-free water. The quality of the RNA samples was measured using a NanoDrop 2000 (Thermo Fisher Scientific, USA).
or AchnMYB70-pSK and AchnKCS promoter-pLUC (v/v 10:1) were prepared to infect tobacco (Nicotiana benthamiana) leaves with needleless syringes. A total of three tobacco plants were used and two leaves of each plant were selected for infection. That was six biological replicates were considered to determine the results. After 72 h for infiltration, the Dual-Luciferase Reporter Assay System (E1910, Promega, USA) with Modulus Luminometers (Promega, USA) was employed to detect the activities of firefly luciferase (LUC) and renilla luciferase (REN).

Real-time quantitative reverse transcription PCR analysis (qRT-PCR)
The first-strand cDNA was obtained by RNA reverse transcription according to the manufacturer’s instructions of PrimeScript™ RT reagent Kit (Perfect Real Time, TaKaRa Bio Inc., China). The CFX96-Touch™ Deep Well Sequence Detection system (Bio-Rad Laboratories, Inc. CA, USA) was applied to detect gene transcription levels with SYBR® Premix Ex Taq™ II (TliRNaseH Plus, TaKaRa Bio Inc., China). Each gene was analyzed in triplicate and Actin was used as reference gene. The relative expression levels of genes were calculated by the 2^△△CT method [29] and presented in multiples relative to the initial value without any treatment (normalized to 1).

Statistical analysis
Each experiment included at least three biological replicates. Data represented the mean value minus or plus standard deviation (± SD). SPSS software (version 20.0, IBM Corporation, New York, America) was used to analyze the difference significance by Least significant difference (LSD) test and Origin 9.0 software (OriginLab Corporation, Massachusetts, America) for mapping. The difference was considered to be statistical significance when p ≤ 0.05 or 0.01, and expressed with different letters or “*” in figures.

Results
Analysis of AchnKCS promoter sequence
Based on the total DNA template of kiwifruit, a 709 bp sequence of AchnKCS promoter was successfully amplified by the primer of AchnKCS-Pro-F/R in Supplementary Table 1. The sequence analysis through PlantCARE software (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) showed that cis-acting elements of ABRE (ABA responsive element), G-box, MBS and MRE were contained (Table 1). ABRE was considered to be specifically recognized by bZIP transcription factors and involved in ABA response, while G-box was supposed as coupling of ABRE [30, 31]. MBS and MRE were the binding sites of MYB transcription factors [32].

Amino acid sequence homology
Through the promoter sequence analysis by PlantCARE and bioinformatics searching by NCBI BLAST software, a bZIP (Achn340751) and an MYB (Achn117821) transcription factor were inferred to be downstream responses of ABA signaling and be associated with suberin biosynthesis based on the involvement of their close homologs in ABA responding and mechanical stress [33–38]. Using cDNA as template, the CDS of Achn340751 and Achn117821 were cloned. Furthermore, the BLAST online software was used to analyze the sequence homology from the NCBI database. Based on its homology with Arabidopsis transcription factors presented as phylogenetic tree by means of DNAMAN8 and MEGA7 software in Fig. 2, they were temporarily designated as AchnbZIP29 and AchnMYB70. And it showed that AchnbZIP29 and AchnMYB70 respectively belonged to Group I of bZIP transcription factors and R2R3-MYB 22 subgroup, which involved in the regulation of fatty acid biosynthesis [39–41].

Subcellular localization
In order to speculate the functional mechanism, the subcellular localization of both transcription factors was determined by observing the fluorescence signal of GFP based on the fusion expression vectors of the reporter gene GFP with AchnbZIP29 or AchnMYB70. The result displayed that compared with the green fluorescence appearing in the whole cell of the hollow vector, the GFP green fluorescence signal of the

| Element | Description                                             | Sequence (5′-3′) | Position |
|---------|---------------------------------------------------------|-----------------|----------|
| ABRE    | cis-acting element involved in the abscisic acid responsiveness | TACGTT          | −1025(+) |
| ABRE    | cis-acting element involved in the abscisic acid responsiveness | TACGTT          | −1498(−) |
| G-Box   | cis-acting regulatory element involved in light responsiveness | CAGTGT          | −1587(−) |
| G-Box   | cis-acting regulatory element involved in light responsiveness | CAGGTC          | −961(−)  |
| MBS     | MYB binding site involved in drought-inducibility       | CAACTG          | −1203(−) |
| MRE     | MYB binding site involved in light responsiveness        | AACCTAA         | −999(+)  |

Note: Position represents the cis-acting element is counted from the position of ATG
fusion expression vector with the AchnbZIP29 or AchnMYB70 appeared specifically in the nucleus (Fig. 3). It indicated that AchnbZIP29 and AchnMYB70 were located in the nucleus, conforming their functional characteristics of regulating gene transcription.

**Interaction between AchnbZIP29, AchnMYB70 and AchnKCS promoter**

Y1H was carried out to investigate whether AchnbZIP29 and AchnMYB70 can interact with AchnKCS promoter. Firstly, the self-activation test showed that
the yeast transformed with AchnKCS-Pro-pABAi cannot grow on the medium containing 100 ng\,mL\(^{-1}\) AbA (Fig. 4). Subsequently, Y1H displayed that the positive control strain (AD-Rec-p53+p53 promoter, not shown) and Y1HGold transformed with AchnbZIP29+AchnKCS Pro, and AchnMYB70+AchnKCS Pro can grow in the medium with 100 ng\,mL\(^{-1}\) AbA and no leucine (–Leu) (Fig. 4), which verified the interaction of individually AchnbZIP29 and AchnMYB70 with AchnKCS promoter. Besides, in order to further clarify the regulatory effect of AchnbZIP29 and AchnMYB70 on AchnKCS, a dual luciferase assay was applied. It presented that AchnMYB70 can significantly enhance the transcriptional activity of AchnKCS promoter, and the ratio of LUC/REN was 2.32 times that of the control (SK) (Fig. 5). In contrast, AchnbZIP29 negatively regulated the transcriptional activity of AchnKCS promoter, and its LUC/REN ratio was only 0.44 that of SK (Fig. 5).

**Effect of exogenous ABA on the transcription levels of AchnbZIP29 and AchnMYB70**

The relative transcription levels of AchnbZIP29 and AchnMYB70 in ABA-stimulated suberizing tissue of kiwifruit were analyzed by qRT-PCR. As shown in Fig. 6, the transcription level of AchnbZIP29 was
reduced by exogenous ABA and decreased to 0.45 of the initial value (normalized to 1) on the third day after treatment. On the contrary, the transcription level of AchnMYB70 was significantly up-regulated by ABA. From the second day after treatment, the transcription level of AchnMYB70 in the suberizing tissue increased significantly and reached the maximum abundance on the third day, which was 2.1 times of the initial control value. The difference in relative transcript abundance induced by ABA further illustrated that AchnbZIP29 and AchnMYB70 were ABA signal-responsive transcription factors.
**Discussion**

Abscisic acid (ABA) is a stress resistance hormone in plant, which is involved in a variety of biotic and abiotic stresses [42, 43]. Relevant studies in recent years have shown that ABA promoted suberin accumulation in Arabidopsis root [5, 43], potato tuber [4, 44], tomato fruit [6, 45] and kiwifruit [1]. Wounding also induced the increase of ABA level in potato tuber [4]. The increased expression of genes in suberin pathway with an ABA-dependent manner in russet apple further suggested the important role of ABA signaling in suberin development [11]. Moreover, the inhibition of ABA biosynthesis by fluridone was reported to block the wound suberization in potato tuber [4] and tomato fruit [6]. ABA has been verified to be a positive regulator in suberin deposition and confirmed the role in wound suberization of kiwifruit [1, 6, 46]. In detail, ABA treatment could induce suberin precursor VLCFAs accumulation during wound suberization [4, 47, 48]. In VLCFAs biosynthesis, KCSs are the rate-limiting enzymes in the chain elongation of fatty acids [49]. It was further found that the KCS gene was significantly induced in response to ABA-stimulated suberization of kiwifruit [15].

The promoter sequence of ABA-responsive genes generally has a conserved cis-acting element, namely ABA-responsive element (ABRE; PyACGTGG/TC) [50, 51]. Transcription factors of bZIP family in plant could interact with cis-acting elements containing ACGT sequence to participate in ABA signaling [52–54]. In Arabidopsis, it has identified eighty bZIP transcription factors, which are divided into 13 groups based on the similarity of their basic regions and other conserved motifs [55]. It was reported that AchnABF2 and AchnbZIP12 in Group A responding to ABA activated the transcription of AchnFHT and AchnKCS involved in suberin biosynthesis, respectively [15, 25]. In this work, AchnbZIP29 was cloned from kiwifruit and the analysis of amino acid sequence showed that it was classified into Group I. The bZIPS of Group I in Arabidopsis were likely to be involved in the development of vascular tissue and cell wall [56]. AchnbZIP29 presented high homology with AtbZIP29. Related research revealed that ABA decreased the expression of AtbZIP29 in guard cells [57]. Similarly, the transcription level of AchnbZIP29 was down-regulated by ABA during wound suberization in this work.
was also speculated that AtbZIP29 regulated the expression of CYP707A3 and CYP707A1 which were two key enzymes involved in ABA catabolism [37]. Accordingly, it was inferred that AchnbZIP29 negatively correlated with the expression of ABA-responsive genes and it was likely to participate in the regulation of wound suberization on the cell wall, but its target gene was possible not only AchnKCS.

However, cis-acting element alone was not sufficient for regulating the transcription of ABA-responsive genes. The interaction between AREB (ABRE binding proteins) and ABRE required the participation of coupling elements [58]. Considered as a coupling element of ABRE motif, the G-box element was reported to play roles in regulating gene expression under various environmental stresses [59]. Certain bZIP transcription factors contained motifs that recognized and bound to G-box element [30, 59]. In this work, the cloned AchnKCS promoter region contained not only two ABRE elements, but also two G-box elements. It allowed us to further determine that bZIP transcription factors played an important regulatory role in the ABA-promoted suberization.

MYB transcription factor family has a wide range of function diversity, including the regulation of suberin biosynthesis [12, 19, 21]. In this work, AchnMYB70 was found to activate the AchnKCS promoter and positively regulate the AchnKCS transcription. Most MYB proteins bound to one or more cis-acting elements (MBS/MRE) with the conserved sequence of CNGTT(A/G) or C(G/T)T(A/T) GTT(A/G) [32]. It showed that AchnMYB70 had high homology with AtMYB70, AtMYB73 and AtMYB44, which were involved in secondary metabolism and resisting biotic and abiotic stress in Arabidopsis [33, 60, 61]. The lipid content in seeds and leaves of transgenic Arabidopsis overexpressing the GmMYB73 (Glycine max) gene was significantly increased [40]. It was also reported that osmotic stress induced the transcription of AtMYB30 and AtMYB44, which was associated with the FAE complex and contributed to the synthesis of VLCFAs [62]. In addition, AchnMYB107 and AchnMYB41 were induced by exogenous ABA during wound suberization of kiwifruit and were demonstrated to activate the transcription of AchnFHT, AchnFAR and AchnCYP86A1 that were involved in suberin biosynthesis [25, 46, 63]. In this study, the transcription level of AchnMYB70 was also up-regulated by exogenous ABA treatment and was proved to possibly have an activating effect on AchnKCS transcription during wound suberization of kiwifruit.

The transcription of a gene may be comprehensively regulated by multiple transcription factors, and the interaction between transcription factors may jointly play a role in the transcriptional regulation of the target genes. In this work, any interaction or other cooperative regulation between the transcription factors that can interact with the AchnKCS promoter, including AchnbZIP29, AchnMYB70 and AchnbZIP12 we reported previously, still needed to be further studied.

Conclusions

In conclusion, the present work explored a potential regulatory pathway of ABA on AchnKCS involved in suberin biosynthesis (Fig. 7). AchnKCS promoter was activated by the interaction with AchnMYB70 but suppressed by the interaction with AchnbZIP29. The transcription level of AchnMYB70 was induced by ABA, but AchnbZIP29 expression was reduced by ABA. Therefore, ABA played a key role in the transcriptional activation of AchnKCS possibly by up-regulating AchnMYB70 expression and down-regulating AchnbZIP29 expression.

Abbreviations

ABA: Abscisic acid; AbA: Aureobasidin A; ABRE: ABA responsive element; AREB: ABRE binding proteins; CDS: Coding sequence; CTAB: Cetyltrimethylammonium bromide; FAE: Fatty acid elongation enzyme complex; GFP: Green fluorescent protein; KCS: β-ketoacyl-coenzyme A synthase; LUC: Firefly luciferase; NCBI: National Center of Biotechnology Information; qRT-PCR: Real-time quantitative reverse transcription PCR; REN: Renilla luciferase; VLCFAs: Very long chain fatty acids; Y1H: Yeast one-hybrid.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-021-03407-6.

Acknowledgments

Not applicable.

Authors’ contributions

Conception and design: LC Mao and XY Han; Analysis and interpretation of the data: XY Han, XP Wei and WJ Lu; Drafting of the article: XY Han and XP Wei; Critical revision of the article: Q Wu, ZS Luo and LC Mao. Final approval of the article: LC Mao, XY Han, XP Wei, WJ Lu, Q Wu and ZS Luo. All authors read and approved the final manuscript.

Funding

This research was supported by the National Natural Science Foundation of China (32172637) and the General program of the Education Department of Zhejiang Province (Y20204419).

Availability of data and materials

All data generated or analyzed during this study are included in this article (and its supplementary information files) or are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.
Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 School of Life Science, Shaoshan University, Shaoshan 312000, Zhejiang Province, China. 2 College of Biosystems Engineering and Food Science, Zhejiang Key Laboratory of Agro-Food Processing, Key Laboratory of Agro-Products Postharvest Handling of Ministry of Agriculture and Rural Affairs, Zhejiang University, Hangzhou 310058, China. 3 College of Food and Bioengineering, Zhengzhou University of Light Industry, Zhengzhou 450002, Henan, China. 4 Institute of Food Science, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China. 5 College of Food Science and Engineering, Henan University of Technology, Zhengzhou 450001, China. 6 Ningbo Research Institute, Zhejiang University, Ningbo 315100, China.

Received: 13 July 2021 Accepted: 9 December 2021
Published online: 08 January 2022

References
1. Han XY, Mao LC, Wei XP, Lu WJ. Stimulatory involvement of abscisic acid in wound suberization of postharvest kiwifruit. Sci Hortic. 2017;224:444–50.
2. Fransen RB, Domblik I, Schreiber L. Suberin goes genomics: use of a short living plant to investigate a long lasting polymer. Front Plant Sci. 2012;3:1–8.
3. Pollard M, Beisson F, Li Y, Hohlgrohe JB. Building lipid barriers: biosynthesis of cutin and suberin. Trends Plant Sci. 2008;13:236–46.
4. Lulai EC, Suttle JC, Pederson SM. Regulatory involvement of abscisic acid in potato tuber wound-healing. J Exp Bot. 2008;59:1175–86.
5. Barberon M, Vermeer JM, De Bells D, Wang D, Naseer S, Andersen TG, et al. Adaptation of root function by nutrient-induced plasticity of endo- dermal differentiation. Cell. 2016;164:447–59.
6. Tao X, Mao L, Li J, Chen J, Lu W, Huang S. Abscisic acid mediates wound-healing in harvested tomato fruit. Postharvest Biol Technol. 2016;118:28–33.
7. Bernards MA. Demystifying suberin. Can J Bot. 2002;80:227–40.
8. Graça J. Hydroxycinnamates in suberine formation. Phytochem Rev. 2010;9:85–91.
9. Pollard M, Beisson F, Li Y, Hohlgrohe JB. Building lipid barriers: biosynthesis of cutin and suberin. Trends Plant Sci. 2008;13:236–46.
10. Han X-Y, Mao L-C, Lu W-J, Tao X-Y, Wei X-P, Luo Z-S. Abscisic acid induces differential expression of genes involved in wound suberization in post- harvest tomato fruit. J Integr Agric. 2020;20:57–67.
11. Falgellina L, Andre C, Legay S, Lin-Wang K, Dare AP, Deng C, et al. Differential regulation of triterpene biosynthesis induced by an early failure in cuticle formation in apple. Horticulture Res. 2021;8:1–15.
12. Lashbrooke JS, Chen H, Levy-Samocha D, Pederson SM. Regulatory involvement of abscisic acid in potato tuber wound-healing. J Exp Bot. 2008;59:1175–86.
13. Barberon M, Vermeer JM, De Bells D, Wang D, Naseer S, Andersen TG, et al. Adaptation of root function by nutrient-induced plasticity of endo- dermal differentiation. Cell. 2016;164:447–59.
14. Tao X, Mao L, Li J, Chen J, Lu W, Huang S. Abscisic acid mediates wound-healing in harvested tomato fruit. Postharvest Biol Technol. 2016;118:28–33.
15. Bernards MA. Demystifying suberin. Can J Bot. 2002;80:227–40.
16. Graça J. Hydroxycinnamates in suberine formation. Phytochem Rev. 2010;9:85–91.
17. Legay S, Andre C, Guerriero G, Deleruelle A, Lateur M, Evers D, Andre CR, et al. Functional characterization of the Arabidopsis transcription factor MdMyb93 is a regulator of suberin deposition in russeted apple fruit skins. New Phytol. 2016;212:977–91.
18. Legay S, Guerrier S, Lolutou E, Merchand G, Cocco C, Charton S, et al. MdMyb93 is a regulator of suberin deposition in russeted apple fruit skins. New Phytol. 2016;212:977–91.
19. Kosma DK, Murmu J, Razaq FM, Santos P, Bourgault R, Molina I, et al. AtMYB81 activates ectopic suberin synthesis and assembly in multiple plant species and cell types. Plant J. 2014;80:216–29.
20. Gou M, Hou G, Yang H, Zhang X, Cai Y, Kai G, et al. The MYB107 transcription factor positively regulates suberin biosynthesis. Plant Physiol. 2017;173:1045–58.
21. Legay S, Guerrier S, André C, Guinand G, Cocco C, Charton S, et al. MdMyb93 is a regulator of suberin deposition in russeted apple fruit skins. New Phytol. 2016;212:977–91.
22. Legay S, Guerrier S, Lolutou E, Merchand G, Cocco C, Charton S, et al. MdMyb93 is a regulator of suberin deposition in russeted apple fruit skins. New Phytol. 2016;212:977–91.
23. To A, Joubès J, Thueux J, Kazi Z, Lepinecin L, Baud S. AtMYB92 enhances fatty acid synthesis and suberin deposition in leaves of Nicotiana benthamiana. Plant J. 2020;103:660–76.
24. Cohen H, Fedyuk V, Wang C, Wu S, Aharoni A. SUBERMAN regulates developmental suberization of the Arabidopsis root endodermis. Plant J. 2020;102:431–47.
25. Wei X, Lu W, Mao L, Han X, Wei X, Zhao X, et al. ABF2 and MYB transcription factors regulate feruloyl transferase FHT involved in ABA-mediated wounded suberization of kiwifruit. J Exp Bot. 2020;71:305–17.
26. Jaakola L, Pirttilä AM, Halonen M, Hohotl A. Isolation of high-quality RNA from bilberry (Vaccinium myrtillus L.) fruit. Mol Biotechnol. 2001;19:201–3.
27. Persobski S, Bailey LG, Baum BR. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rep. 1997;15:8–15.
28. Tao RY, Bai SL, Ni JB, Yang QS, Zou Y, Teng YW. The blue light signal transduction pathway is involved in anthocyanin accumulation in ‘Red Zoas’ pear. Planta. 2012;248:37–48.
29. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCt method. Methods. 2001;25:402–8.
30. Baena-González E, Rolland F, Thevelein JM, Sheen J. A central integrator of transcription networks in plant stress and energy signalling. Nature. 2007;448:938–U10.
31. Iglesia-Fernandez R, Barnerro-Sicilia C, Carrillo-Barral N, Onate-Sanchez L, Carbonero P. Arabidopsis thalianna bi2ZP44: a transcription factor affecting seed germination and expression of the mannanase-encoding gene AtMAN7. Plant J. 2013;74:767–80.
32. Romero I, Fuertes A, Benito MJ, Malpica JP, Levy A, Paz-Ares J. More than 80% of MYB regulatory genes in the genome of Arabidopsis thalianna. Plant J. 1998;14:273–84.
33. Jung C, Seo JS, Han SW, Koo YI, Kim CH, Song SJ, et al. Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis. Plant Physiol. 2008;146:623–35.
34. Persak H, Pitzschke A, Tight interconnection and multi-level control of Arabidopsis MYB44 in MAPK Cascade Signalling. PLoS One. 2013;8:e57547.
35. Tsugami D, Liu S, Fujino K, Takano T. Possible inhibition of Arabidopsis VIP1-mediated mechanosensory signaling by streptomycin. Plant Signal Behav. 2018;13:e121236.
36. Tu M, Wang X, Zhu Y, Wang D, Zhang X, Cui Y, et al. YbZiP30 of grapevine functions in dehydration tolerance via the abscisic acid core signaling pathway. Horticulture Research. 2018;5:1–15.
37. Van Leene J, Blomme J, Kulkarni SR, Cannoot B, De Winne N, Eeckhout D, et al. Functional characterization of the Arabidopsis transcription factor bZIP29 reveals its role in leaf and root development. J Exp Bot. 2016;67:5825–40.
38. Zhao Y, Xing L, Wang XG, Hou YJ, Gao JH, Wang PC, et al. The ABA receptor PYL8 promotes lateral root growth by enhancing MYB77-dependent transcription of auxin-responsive genes. Sci Signal. 2014;7:11.
39. Gibalová A, Steinbachová L, Hafidh S, Bláhová V, Gadiou Z, Michailidis C, et al. Characterization of pollen-expressed bZIP protein interactions and the role of AtbZIP18 in the male gametophyte. Plant Reproduction. 2017;30:1–17.
40. Liu YF, Li QT, Lu X, Song QX, Lam SM, Zhang WK, et al. Soybean GmMYB73 promotes lipid accumulation in transgenic plants. BMC Plant Biol. 2014;14:16.
41. Yu Y, Qian Y, Jiang M, Xu J, Yang J, Zhang T, et al. Regulation mechanisms of plant basic leucine zippers to various abiotic stresses. Front Plant Sci. 2020;11:1258.
42. Leng P, Yuan B, Guo YD. The role of abscisic acid in fruit ripening and responses to abiotic stress. J Exp Bot. 2014;65:4577–88.

43. Tao Q, Jia P, Liu Y, Luo J, Li J, Kováč J, et al. Abscisic acid-mediated modifications of radial apoplastic transport pathway play a key role in cadmium uptake in hyperaccumulator Sedum alfredii. Plant Cell Environ. 2019;42:1425–40.

44. Kumar GM, Lulai EC, Suttle JC, Knowles NR. Age-induced loss of wound-healing ability in potato tubers is partly regulated by ABA. Planta. 2010;232:1433–45.

45. Leide J, Hildebrandt U, Hartung W, Riederer M, Vogg G. Abscisic acid mediates the formation of a suberized stem scar tissue in tomato fruits. New Phytol. 2012;194:402–15.

46. Wei X, Mao L, Wei X, Xia M, Xu C. MYB41, MYB107, and MYC2 promote ABA-mediated primary fatty alcohol accumulation via activation of AChnFAR in wound suberization in kiwifruit. Horticulture Res. 2020;7:1–10.

47. Han XY, Lu WJ, Wei XP, Li L, Mao LC, Zhao YY. Proteomics analysis to understand the ABA stimulation of wound suberization in kiwifruit. J Proteome. 2018;173:42–51.

48. Lulai EC, Neubauer JD. Wound-induced suberization genes are differentially expressed, spatially and temporally, during closing layer and wound periderm formation. Postharvest Biol Technol. 2014;90:24–33.

49. Paul S, Gable K, Beaudoin F, Cahoon E, Jaworski J, Najer JA, et al. Members of the Arabidopsis FAE1-like 3-ketoacyl-CoA synthase gene family substitute for the Elop proteins of Saccharomyces cerevisiae. J Biol Chem. 2006;281:9018–29.

50. Hattori T, Totsuka M, Hobe T, Kagaya Y, Yamamoto-Toyoda A. Experimentally determined sequence requirement of ACGT-containing abscisic acid response element. Plant Cell Physiol. 2002;43:136–40.

51. Zhang WX, Ruan JH, Ho TD, You Y, Yu TT, Quatrano RS. Cis-regulatory element based targeted gene finding: genome-wide identification of abscisic acid- and abiotic stress-responsive genes in Arabidopsis thaliana. Bioinformatics. 2005;21:3074–81.

52. Choi HI, Hong JH, Ha JO, Kang JY, Kim SY. ABFs, a family of ABA-responsive element binding factors. J Biol Chem. 2000;275:1723–30.

53. Uno Y, Furuhata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K. Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proceed Natl Acad Sci USA. 2000;97:1632–7.

54. Yoshida T, Fujita Y, Sayama H, Kodokoro S, Maruyama K, Mizoi J, et al. AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. Plant J. 2010;61:672–85.

55. Jakoby M, Weisshaar B, Droege-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, et al. bZIP transcription factors in Arabidopsis. Trends Plant Sci. 2002;7:106–11.

56. Fukazawa J, Sakai T, Ishida S, Yamaguchi I, Kamiya Y, Takahashi Y. Repression of shoot growth, a bZIP transcriptional activator, regulates cell elongation by controlling the level of gibberellins. Plant Cell. 2000;12:901–15.

57. Pandey S, Wang RS, Wilson L, Li S, Zhao ZX, Gookin TE, et al. Boolean modeling of transcriptome data reveals novel modes of heterotrimeric G-protein action. Mol Syst Biol. 2010;6:17.

58. Agarwal PK, Jha B. Transcription factors in plants and ABA dependent and independent abiotic stress signalling. Bioplant. 2010;54:201–12.

59. Menkens AE, Schindler U, Cashmore AR. The G-box: a ubiquitous regulatory DNA element in plants bound by the GBF family of bZIP proteins. Trends Biochem Sci. 1995;20:506–10.

60. Shin R, Burch-AY, Huppert KA, Tiwari SB, Murphy AS, Guilfoyle TJ, et al. The Arabidopsis transcription factor MYB77 modulates auxin signal transduction. Plant Cell. 2007;19:2440–53.

61. Stracke R, Werber M, Weisshaar B. The R2R3-MYB gene family in Arabidopsis thaliana. Curr Opin Plant Biol. 2001;4:447–56.

62. Raffaele S, Vailleau F, Léger A, Joublé A, Miersch O, Huard C, et al. A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in Arabidopsis. Plant Cell. 2008;20:752–67.

63. Wei X, Mao L, Lu W, Wei X, Han X, Guan W, et al. Three transcription activators of ABA signaling positively regulate suberin monomer synthesis by activating cytochrome P450 CYP86A1 in kiwifruit. Front Plant Sci. 2020;10:1650.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.