Short title: Epigenetic Control Regulates Wheat Wax Synthesis

Epigenetic Activation of Enoyl-CoA Reductase By An Acetyltransferase Complex Triggers Wheat Wax Biosynthesis

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One-sentence summary:
A transcriptional activator recruits a histone acetyltransferase complex that epigenetically regulates the biosynthesis of wheat cuticular wax, which is essential for triggering the germination of the powdery mildew pathogen.
ABSTRACT

The epidermal surface of bread wheat (*Triticum aestivum*) is coated with a hydrophobic cuticular wax layer that protects plant tissues against environmental stresses. However, the regulatory mechanism of cuticular wax biosynthesis remains to be uncovered in bread wheat. Here, we identified wheat Enoyl-CoA Reductase (TaECR) as a core component responsible for biosynthesis of wheat cuticular wax. Silencing of *TaECR* in bread wheat resulted in a reduced cuticular wax load and attenuated conidia germination of the adapted fungal pathogen powdery mildew (*Blumeria graminis* f.sp. *tritici*; *Bgt*). Furthermore, we established that *TaECR* genes are direct targets of *TaECR* promoter-binding MYB transcription factor 1 (*TaEPBM1*), which could interact with the adapter protein Alteration/Deficiency in Activation 2 (*TaADA2*) and recruit the histone acetyltransferase General Control Non-derepressible 5 (*TaGCN5*) to *TaECR* promoters. Most importantly, we demonstrated that the *TaEPBM1-TaADA2-TaGCN5* ternary protein complex activates *TaECR* transcription by potentiating histone acetylation and enhancing RNA polymerase II enrichment at *TaECR* genes, thereby contributing to the wheat cuticular wax biosynthesis. Finally, we identified very-long-chain aldehydes as the wax signals provided by the *TaECR-TaEPBM1-TaADA2-TaGCN5* circuit for triggering *Bgt* conidia germination. These results demonstrate that specific transcription factors recruit the *TaADA2-TaGCN5* histone acetyltransferase complex to epigenetically regulate biosynthesis of wheat cuticular wax, which is required for triggering germination of the adapted powdery mildew pathogen.

Keywords: ECR, ADA2, GCN5, cuticular wax biosynthesis, wheat, *Blumeria graminis* f.sp. *tritici*

INTRODUCTION

The epidermal surfaces of aerial plant organs are coated with a hydrophobic layer, the cuticle, to protect plant tissues against enormous environmental stresses such as desiccation, ultraviolet radiation, excessive light, extreme temperatures, and even pathogen infections (Nawrath 2006; Samuels et al., 2008; Domínguez et al. 2017). Although the composition and structure of the cuticle vary among plant species, organs, developmental stages, and even environmental conditions, plant cuticle generally consists of a macromolecular scaffold of cutin impregnated by and covered with the cuticular wax mixture (Nawrath et al., 2006; Fernández et al., 2016; Domínguez et al., 2017). Increasing evidence reveals that many microbial pathogens have acquired the capacity to utilize the plant cuticular wax components to initiate their pre-invasion and infection processes (Serrano et al., 2014; Aragón et al., 2017; Ziv et al., 2018). For instance, *Medicago truncatula* mutants with loss of abaxial epicuticular wax exhibit retarded infection of two rust pathogens, *Puccinia emaculata* and *Phakopsora pachyrhizi*, during pre-invasion processes (Uppalapati et al. 2012).

Cuticular wax is a mixture of very-long-chain (VLC, > C<sub>20</sub>) fatty acids and their derivatives, such as aldehydes, alcohols, alkanes, ketones, and esters (Nawrath, 2006; Lee and Suh 2013; Yeats and Rose, 2013; Martin and Rose, 2014). It is well established in model plant *Arabidopsis thaliana* that cuticular wax biosynthesis begins with the esterification of CoA to the plastid-derived C<sub>16</sub> and C<sub>18</sub> fatty acids by long-chain acyl-CoA synthetase (AtLACS) proteins in the endoplasmic reticulum, and the generated C<sub>16</sub> and C<sub>18</sub> acyl-CoAs are elongated to VLC acyl-CoAs under the action of the fatty acid elongase (FAE) complex and ECERIFERUM2 (AtCER2) proteins (Xia et al., 1996; Todd et al., 1999; Fiebig et al., 2000;
Hooker et al., 2002; Schnurr et al., 2004; Zheng et al., 2005; Bach et al., 2008; Beaudoin et al., 2009; Lee et al., 2009; Lu et al., 2009; Weng et al., 2010; Haslam et al., 2012; Haslam and Kunst, 2013; Kim et al., 2013; Haslam et al., 2015). The elongated VLC acyl-CoAs are then modified into aldehydes, alkanes, secondary alcohols, and ketones by an alkanone-forming pathway, or into primary alcohols and wax esters by an alcohol-forming pathway (Aarts et al., 1995; Millar et al., 1999; Chen et al., 2003; Rowland et al., 2006; Greer et al., 2007; Rowland et al., 2007; Bourdenx et al., 2011; Bernard et al., 2012; Yang et al., 2017; Pascal et al., 2019). As a core component of fatty acid elongase complex, enoyl-CoA reductase (ECR) catalyzes the final step in the biosynthesis of VLC acyl-CoAs (Zheng et al., 2005). In Arabidopsis thaliana, silencing of AtECR results in a reduction of all cuticular wax compositions such as VLC fatty acids, alcohols, aldehydes, alkanes, and ketones, suggesting that Arabidopsis AtECR gets involved in the VLC acyl-CoAs biosynthesis (Zheng et al., 2005). Increasing research in Arabidopsis reveals that AtECR expression is governed by multiple transcriptional regulators. For instance, the AtECR transcription is up-regulated by the MYB type transcription factors such as AtMYB30 and AtMYB94, but negatively regulated by the AP2/ERF-type transcription factor DECREASE WAX BIOSYNTHESIS (AtDEWAX) in Arabidopsis (Raffaele et al., 2008; Go et al., 2014; Lee and Suh 2014). However, the biological function and transcriptional regulation of ECR remain to be uncovered in important cereal crops such as bread wheat (Triticum aestivum).

Chromatin modifications such as acetylation, methylation, and ubiquitination play important roles in the regulation of transcriptional reprogramming associated with plant development and stress responses (Jenuwein and Allis 2001; Strahl and Allis 2000). As important epigenetic modifications, trimethylation of histone H3 lysine 4 and deubiquitination of histone H2B could induce a permissive chromatin structure for gene activation (Kurdistani and Grunstein, 2003; Daniel et al. 2004; Schmitz et al. 2009). Similarly, acetylation of histone lysine residues catalyzed by histone acetyltransferases (HAT) also promotes gene transcription (Kurdistani and Grunstein, 2003). As the first HAT linked to gene transcriptional activation, General Control Non-derepressible 5 (GCN5) interacts with the adaptor protein Alteration/Deficiency in Activation 2 (ADA2) in the HAT module of the transcriptional co-activator Spt-Ada-Gcn5-acyetyltransferase (SAGA) complex, which is engaged in histone acetylation, histone deubiquitination, and even recruitment of the RNA polymerase II (RNA Pol II) (Grant et al., 1997; Weake and Workman, 2012; Wang and Dent, 2014; Moraga and Aquea, 2015). The Alteration/Deficiency in Activation 2-General Control Non-derepressible 5 (ADA2-GCN5) complex is reported to function in concert with specific transcription factors (TFs) to regulate gene transcription associated with plant development and response to environmental stresses in Arabidopsis, rice (Oryza sativa), and even Populus trichocarpa. For instance, the rice WUSCHEL-RELATED HOMEobox11 (OsWOX11) could interact with the ADA2-GCN5 complex to establish gene expression programs of crown root meristem in rice (Zhou et al., 2017). Similarly, the Populus transcription factor Abscisic Acid-Responsive Element (PtAREB1) could recruit the ADA2-GCN5 complex to induce expression of drought-responsive PtrNAC gene expression during drought stress (Li et al., 2018; Castroverde 2019). However, to date, whether and how the TF-ADA2-GCN5 complex regulates the gene transcription involved in the plant cuticular wax biosynthesis remains unknown.

As the causal agent of wheat powdery mildew disease, Blumeria graminis f.sp. tritici (Bgt) is the airborne biotrophic fungal pathogen that is capable of infecting the important crop bread wheat, leading to the wheat yield losses of 10% to 40% (Zhu et al., 2015; Zhang et al., 2016; He et al., 2018; Koller et al., 2019).
2018; Xing et al., 2018; Zou et al., 2018; Zheng et al., 2020). On the aerial surface of wheat, the first contact between Bgt and wheat takes place at the cuticle, and the Bgt conidia germination is induced to initiate the infection processes (Nielsen et al., 2000; Wright et al., 2002). In bread wheat, silencing of 3-KETOACYL-CoA SYNTHASE (TaKCS6) and WAX INDUCER 1 (TaWIN1), two positive regulators in wheat cuticular wax biosynthesis, results in a reduction of Bgt conidia germination, suggesting that the cuticular wax biosynthesis is essential to stimulate the Bgt conidia germination in bread wheat (Kong and Chang, 2018; Wang et al., 2019). However, the function of other components responsible for the wheat cuticular wax biosynthesis in modulating Bgt conidia germination needs to be characterized.

In this study, we showed that wheat enoyl-CoA reductase (TaECR) is a core component responsible for the cuticular wax biosynthesis in bread wheat. TaECR promoter-binding MYB transcription factor 1 (TaEPBM1) recruits the TaADA2-TaGCN5 histone acetyltransferase complex to activate TaECR transcription by potentiating histone acetylation and enhancing RNA Pol II enrichment at TaECR genes and thus stimulate the cuticular wax biosynthesis required for stimulating Bgt conidia germination. Besides, VLC aldehydes were identified as the wax signals provided by the TaECR-TaEPBM1-TaADA2-TaGCN5 circuit for Bgt germination in bread wheat. Thus, we revealed that the TaECR-TaEPBM1-TaADA2-TaGCN5 circuit regulates the wheat cuticular wax biosynthesis essential for the germination of powdery mildew fungus.

RESULTS

Characterization of the Enoyl-CoA Reductase (ECR) Gene in Bread Wheat

In this study, we are interested in exploring the function of the wheat Enoyl-CoA Reductase (ECR) gene in regulating the cuticular wax biosynthesis required for stimulating Bgt conidia germination. To this end, we first identified the wheat TaECR genes based on the sequence of the Arabidopsis AtECR gene (AT3G55360) and the reference genome of the hexaploid bread wheat (International Wheat Genome Sequencing Consortium 2018). Three highly conserved homologous sequences of TaECR genes separately located on chromosomes 3AS, 3BS and 3DS were isolated from the hexaploid bread wheat cultivar Jing411, and were designated as TaECR-A, TaECR-B, and TaECR-D (Supplemental Fig. S1). The open reading frames (ORFs) of these TaECR genomic sequences all contained four exons and three introns, encoding proteins with over 99% amino acid sequence identity (Supplemental Figs. S1). To analyze the evolution of ECR in land plants, we employed protein sequences of TaECR as query sequences to search the genomes of representative land plant species from the Joint Genome Institute (JGI) Phytozome v12.1 database. As shown in Supplemental Figure S2, highly homologous ECR proteins were obtained from all test plant species including the hornwort Marchantia polymorpha and moss Physcomitrella patens, suggesting that ECR proteins might be evolutionarily conserved among land plants.

Since the biosynthesis of cuticular wax mainly occurs in the endoplasmic reticulum (ER) in plant epidermal cells, we first analyzed the localization of TaECR in ER. TaECR-YFP fusion proteins were transiently co-expressed with mCherry-HDEL, an ER indicator, in Nicotiana benthamiana leaves. Confocal microscopic images showed that the fluorescence signal of TaECR-YFP was co-localized with that of mCherry-HDEL at ER, suggesting that TaECR proteins localize to the ER in N. benthamiana cells.
In addition, we expressed TaECR-HA in the wheat protoplast and performed a sucrose density-gradient fractionation to validate the ER-localization of TaECR in wheat cells. Since TaECR-A, TaECR-B, and TaECR-D share more than 99% amino acid sequence identity, TaECR-A was selected as a representative TaECR in this experiment. As shown in Supplemental Figure S3, TaECR-HA cofractionated with the ER marker BiP and exhibited the same Mg$^{2+}$-dependent density shift as the ER marker BiP, further confirming the ER-localization of TaECR in bread wheat. Thereafter, the expression profiles of TaECR were analyzed in different tissues of wheat cultivar Jing411 using reverse transcription quantitative PCR (RT-qPCR). As shown in Figure 1B, TaECR exhibits the lowest expression level in roots and the highest expression levels in epidermis of leaves and stems. Its expression levels were much higher in leaves and stems than in roots (Fig. 1B).

To explore whether TaECR involved in the wheat cuticle biosynthesis is required for stimulating Bgt conidia germination, we first conducted the barley stripe mosaic virus (BSMV) induced gene silencing (BSMV-VIGS) to silence all endogenous TaECR genes, including TaECR-A, TaECR-B, and TaECR-D, in wheat cultivar Jing411, and characterized the chemical composition of two major cuticle components, cutin and cuticular wax, in wheat leaves about 15 days post-BSMV-infection through using gas chromatography-mass spectrometry (GC-MS) (Supplemental Fig. S4). Compared with the mock control, inoculation with BSMV-γ has no significant effect on the deposition of cutin and cuticular wax in bread wheat (Supplemental Fig. S5). As shown in Figure 1C, the total cutin load was not significantly changed by the silencing of TaECR, but the wax load decreased from 12.1 μg cm$^{-2}$ on wild-type Jing 411 leaves to a significant level of 2.6 μg cm$^{-2}$ on TaECR-silenced wheat leaves. Further quantitative analysis of wax constituents revealed that VLC fatty acids and their derivatives such as aldehydes, alcohols, alkanes, ketone, and even C$_{46}$-C$_{50}$ esters showed a remarkable decrease in the TaECR-silenced plants (Fig. 1D). Thereafter, the conidia germination of Bgt strain E09 was examined on leaves of wild-type and TaECR-silenced plants using light microscopy. Compared with the mock control, inoculation with BSMV-γ has no significant effect on Bgt conidia germination in bread wheat (Supplemental Fig. S5). As shown in Figures 1E and 1F, the Bgt conidia germination was affected on TaECR-silenced leaves, with 26% more conidia failing to germinate. Taken together, these results suggest that wheat TaECR, resembling its homolog in Arabidopsis, acts as a core component responsible for the cuticular wax biosynthesis and positively regulates the Bgt conidia germination in bread wheat.

**TaEPBM1 Functions as a Transcriptional Activator of TaECR**

To identify the transcriptional regulator that directly binds to TaECR promoters, we performed yeast one-hybrid screening against a wheat leaf cDNA library using TaECR promoter regions as baits. One R2R3 type MYB transcription factor was independently and repeatedly isolated as the binding protein of the TaECR promoter, and designated as TaEPBM1 (for TaECR Promoter-Binding MYB transcription factor 1). Three highly conserved homologous sequences of TaEPBM1 genes located on chromosomes 2AS, 2BS and 2DS were obtained from Jing411 and encode TaEPBM1-A, TaEPBM1-B, and TaEPBM1-D with more than 96% amino acid sequence identity, among which, TaEPBM1-A was selected as a representative TaEPBM1 in the following experiments (Supplemental Fig. S6). Previous studies revealed that the R2R3 type MYB transcription factor could recognize multiple cis-elements including MBS (CACCAT), which was present in the TaECR promoters. Electrophoretic mobility shift
assay (EMSA) showed that TaEPBM1 exclusively associated with the wild-type MBS but not mutant MBS fragments, suggesting that TaEPBM1 has the MBS-binding activity (Supplemental Fig. S7). Yeast one-hybrid assays revealed that TaEPBM1 could bind to the wild-type TaECR promoters, but not the MBS cis-element mutated TaECR promoters, suggesting that TaEPBM1 could recognize the MBS cis-element and directly bind to TaECR promoters in yeast cells (Fig. 2A).

To examine whether TaEPBM1 could bind to TaECR promoters in plant cells, we employed the wheat protoplast transfection system, in which luciferase (LUC) reporters containing wild-type or mutant TaECR promoter regions were cotransfected with effector constructs over-expressing TaEPBM1 (Fig. 2B). As shown in Figure 2C, the LucA ratio obtained from LUC reporters containing wild-type TaECR promoters increased to a significant level of above 4.8 in the presence of TaEPBM1, compared with the basal LUC activity of the Gal4 DNA binding domain (DBD). In contrast, the LucA ratio obtained from LUC reporters containing the MBS-mutated TaECR promoters was not significantly changed by the addition of TaEPBM1 (Fig. 2C). This result suggests that TaEPBM1 could bind to TaECR promoters and activate their expression in wheat cells.

To determine the association of TaEPBM1 with TaECR promoters in bread wheat, we generated an antibody specifically against TaEPBM1 and performed chromatin immunoprecipitation (ChIP) assay in Jing 411 leaves (Supplemental Fig. S8A). The wheat elongation factor 1 (TaEF1) gene was employed as control. As shown in Figure 2D and E, two genomic regions (represented by 1 and 2) containing the MBS cis-element in TaECR promoters were subjected to the ChIP assay and found to be enriched in DNA samples precipitated with the α-TaEPBM1 antibody, suggesting that TaEPBM1 associated with TaECR promoters in bread wheat. At the same time, nuclear run-on assays revealed that TaECR was transcribed at much lower rates in the BSMV-TaEPBM1as-infected wheat plants compared with BSMV-γ plants (Fig. 2F). Consistent with this, TaECR mRNA levels significantly decreased by 75% in the TaEPBM1-silenced wheat leaves compared with controls, suggesting that TaEPBM1 activates the TaECR transcription (Fig. 2G). In contrast, the transcription rate and expression level of TaEF1 were not affected by silencing of TaEPBM1 (Supplemental Fig. S9). Together, these results indicate that TaEPBM1 is a bona fide transcriptional activator of TaECR in bread wheat.

TaEPBM1 Interacts with Transcriptional Adapter TaADA2 and Forms the TaEPBM1-TaADA2-TaGCN5 Ternary Protein Complex at TaECR Promoters

To explore the molecular mechanism by which TaEPBM1 regulates TaECR transcription, we performed a yeast two-hybrid screening against a wheat leaf cDNA library to identify TaEPBM1-interacting proteins. One of the isolated interacting proteins was homologous to the rice ADA2 (LOC_Os03g53960) and was designated as TaADA2. Three highly-homologous sequences of TaADA2 genes located on chromosomes 5AL, 5BL and 5DL were obtained from Jing411 and encode TaADA2-A, TaADA2-B, and TaADA2-D with more than 99% amino acid sequence identity, among which, TaADA2-A was selected as a representative TaADA2 in the following experiments (Supplemental Fig. S10). As shown in Figure 3A, the interaction between TaEPBM1 and TaADA2 was detected in the EGY48 yeast cells. Further yeast two-hybrid analysis with truncated TaEPBM1 and TaADA2 revealed that the C-terminal region of TaEPBM1 and the N-terminal region of TaADA2 were responsible for their interaction (Fig. 3A).
To validate the TaEPBM1-TaADA2 interaction, we performed both in vitro and in vivo protein interaction assays. As shown in Figure 3B, GST-TaEPBM1, but not GST alone, could retain TaADA2-His instead of TaGCN5-His in the GST pull-down assay, suggesting that TaEPBM1 directly interacts with TaADA2 but not TaGCN5 in vitro. In the bimolecular fluorescence complementation (BiFC) assay, YFP was reconstituted in the nucleus only in the co-expression pair of nYFP-TaEPBM1 and cYFP-TaADA2, but not in control pairs (Fig. 3C). In addition, we generated an antibody specifically against TaADA2 and performed co-immunoprecipitation (co-IP) assay to analyze the TaEPBM1-TaADA2 association in Jing 411 leaves (Supplemental Fig. S8B). As shown in Figure 3D, TaEPBM1 was found co-immunoprecipitated with TaADA2, which was not detected in the TaEPBM1- or TaADA2-silenced plants, suggesting that TaEPBM1 interacts with TaADA2 in bread wheat.

In yeast and plants, ADA2 interacts with GCN5 to constitute the ADA2-GCN5 complex (Grant et al., 1997; Weake and Workman, 2012; Wang and Dent, 2014; Moraga and Aquea, 2015; Zhou et al., 2017; Li et al., 2018; Castroverde 2019). The finding that TaEPBM1 interacts with TaADA2 prompted us to ask whether TaEPBM1, TaADA2, and TaGCN5 could form a complex. To this end, we first identified the wheat TaGCN5 genes using the sequence of rice OsGCN5 gene (LOC_Os10g28040) as a query to search the chromosome-based reference genome of the hexaploid bread wheat (International Wheat Genome Sequencing Consortium 2018). Three highly conserved homologous sequences of TaGCN5 genes separately located on chromosomes 1AS, 1BS and 1DS were isolated from the hexaploid bread wheat cultivar Jing411 and encode TaGCN5-A, TaGCN5-B, and TaGCN5-D with more than 99% amino acid sequence identity, among which, TaGCN5-A was selected as a representative TaGCN5 in the following protein interaction assays (Supplemental Fig. S11). To determine the association among TaEPBM1, TaADA2, and TaGCN5, we conducted yeast two-hybrid, luciferase complementation imaging (LCI) and BiFC assays. As shown in Figures 4, A, B, and C, TaADA2 interacts with TaGCN5 in yeast and plant cells. Notably, the association of TaEPBM1 with TaGCN5 was observed in the presence but not the absence of TaADA2, suggesting that TaADA2 could function as an adapter to bridge the TaEPBM1-TaGCN5 interaction in plant cells (Fig. 4, B and C). Thereafter, we performed the co-IP assay to determine whether TaEPBM1, TaADA2, and TaGCN5 could form a complex in vivo. As shown in Figure 4D, TaEPBM1 and TaADA2 were coimmunoprecipitated with TaGCN5-HA, but not with GFP-HA. Besides, the coimmunoprecipitation of TaEPBM1 with TaGCN5-HA was abrogated by the silencing of TaADA2, indicating that TaEPBM1, TaADA2, and TaGCN5 could form a ternary complex, in which TaADA2 functions as an adapter to bridge the interaction between TaEPBM1 and TaGCN5 (Fig. 4D).

Having already demonstrated that TaEPBM1 directly binds to TaECR promoters and TaEPBM1, TaADA2 and TaGCN5 could form a complex, we next ask whether the TaEPBM1-TaADA2-TaGCN5 ternary protein complex associates with TaECR promoters in bread wheat. To test this hypothesis, we co-transfected the wheat protoplast with TaGCN5-HA and RNAi constructs, and performed a ChIP assay to characterize the distribution of TaGCN5-HA, TaADA2, and TaEPBM1 at TaECR promoters (Fig. 5). TaGCN5-HA and TaADA2 were found enriched at the promoter regions of TaECR-A, TaECR-B, and TaECR-D (Fig. 5, top and middle rows), in a pattern similar with that of TaEPBM1 (Fig. 5, bottom row), suggesting that they associate with TaECR promoters as a ternary complex. Notably, the levels of TaADA2 and TaGCN5-HA at TaECR promoter regions were significantly reduced by the silencing of TaEPBM1 (Fig. 5, Supplemental Fig. S12). Also, the silencing
of TaADA2 decreased the accumulation of TaGCN5-HA but not TaEPBM1 at TaECR promoters (Fig. 5, Supplemental Fig. S12). Together, these results indicate that TaEPBM1 interacts with the adapter protein TaADA2 and recruited TaADA2-TaGCN5 complex to TaECR promoters.

TaEPBM1-TaADA2-TaGCN5 Protein Complex Activates TaECR Transcription by Potentiating Histone Acetylation and Enhancing RNA Pol II Enrichment at TaECR Genes

In yeast and other plants, the ADA2-GCN5 complex was reported to activate gene transcription through enhancing histone acetylation and recruitment of the RNA polymerase II (Grant et al., 1997; Weake and Workman, 2012; Wang and Dent, 2014; Moraga and Aquea, 2015; Zhou et al., 2017; Li et al., 2018; Castroverde 2019). The finding that TaEPBM1 recruits the TaADA2-TaGCN5 complex to TaECR promoter regions led us to ask whether the complex regulates histone acetylation at TaECR promoters. To this end, we separately silenced all endogenous TaEPBM1, TaADA2 and TaGCN5 genes using BSMV-VIGS, and performed a ChIP assay to analyze histone acetylation such as H3K4ac, H3K9ac, H3K14ac, H3K27ac, and H4K5ac at TaECR promoters (Supplemental Fig. S12). As shown in Figure 6A, the levels of H3K4ac, H3K9ac, H3K14ac, H3K27ac, and H4K5ac at TaECR promoters were remarkably reduced by silencing of TaEPBM1, TaADA2 or TaGCN5, indicating that TaEPBM1, TaADA2, and TaGCN5 mediate histone acetylation at TaECR promoters. Notably, simultaneous silencing of TaEPBM1, TaADA2, and TaGCN5 failed to cause a further change in histone acetylation at TaECR promoters, compared with single-silencing of TaEPBM1, TaADA2 or TaGCN5, which is consistent with the fact that TaEPBM1, TaADA2, and TaGCN5 function in a ternary protein complex (Fig. 6A).

Recent studies in P. trichocarpa revealed that the recruitment of ADA2-GCN5 complex enables the enhancement of histone acetylation and enrichment of RNA polymerase II at PtrNAC genes for the development of plant drought tolerance (Li et al., 2018). To examine the potential regulation of RNA Pol II recruitment at TaECR genes by TaEPBM1-TaADA2-TaGCN5 protein complex, we separately silenced all endogenous TaEPBM1, TaADA2 and TaGCN5 genes using BSMV-VIGS, and analyzed the occupancy of total RNA Pol II at promoters and coding regions of TaECR genes using ChIP-qPCR assay (Supplemental Fig. S12). Since allelic TaECR-A, TaECR-B, and TaECR-D shared over 95% nucleotide sequence identity at coding regions, we chose TaECR-A as a representative TaECR gene in these ChIP-qPCR assays (Fig. 6B, Supplemental Fig. S1). As shown in Figure 6C, the levels of total RNA Pol II decreased by over 25% at promoters and coding regions of TaECR genes with the silencing of TaEPBM1, TaADA2 or TaGCN5, suggesting that TaEPBM1, TaADA2, and TaGCN5 stimulate the RNA Pol II recruitment at promoters and coding regions of TaECR genes. At the same time, nuclear run-on assays revealed that TaECR was transcribed at much lower rates in the TaEPBM1, TaADA2 or TaGCN5-silenced wheat leaves compared with controls (Fig. 6D; Supplemental Fig. S12). Consistent with this, TaECR transcript levels decreased by over 70% in the wheat leaves infected with BSMV-TaEPBM1as, BSMV-TaADA2as or BSMV-TaGCN5as compared with BSMV-γ plants (Fig. 6E; Supplemental Fig. S12). Additionally, simultaneous silencing of TaEPBM1, TaADA2, and TaGCN5 failed to cause a further change in RNA Pol II recruitment and transcription at TaECR genes, compared with single-silencing of TaEPBM1, TaADA2 or TaGCN5, suggesting that TaEPBM1, TaADA2 and TaGCN5 function in a protein complex to facilitate the RNA Pol II recruitment and activate the TaECR transcription (Fig. 6, C,
D, and E; Supplemental Fig. S12). In contrast, the transcription rate and expression level of TaEF1 were not affected by the silencing of TaEPBM1, TaADA2 or TaGCN5 (Supplemental Fig. S9). Taken together, these results indicate that the TaEPBM1-TaADA2-TaGCN5 protein complex activates TaECR transcription by potentiating histone acetylation such as H3K4ac, H3K9ac, H3K14ac, H3K27ac, and H4K5ac, as well as enhancing RNA Pol II occupancy at TaECR genes.

Reduced Expression of TaEPBM1, TaADA2 or TaGCN5 in Bread Wheat Decreased the Cuticular Wax Biosynthesis and Attenuated Bgt Germination

The findings that TaECR acts as a core component responsible for the cuticular wax biosynthesis and that the TaEPBM1-TaADA2-TaGCN5 protein complex directly activates the TaECR transcription led us to ask whether the TaEPBM1-TaADA2-TaGCN5 protein complex regulates the cuticular wax biosynthesis in bread wheat. To examine this hypothesis, we separately silenced all endogenous TaEPBM1, TaADA2 and TaGCN5 genes using BSMV-VIGS, and determined leaf cuticular wax load in wheat leaves about 15 days post-BSMV-infection by GC-MS (Supplemental Fig. S12). As shown in Figure 7A, the total cuticular wax load decreased by over 50% in TaEPBM1, TaADA2 or TaGCN5-silenced wheat leaves compared with controls. Further quantitative analysis of wax components revealed that VLC wax components including fatty acids, aldehydes, alcohols, alkanes, ketones, and even C_{46}-C_{50} esters all showed a significant decrease in the TaEPBM1, TaADA2 or TaGCN5-silenced plants (Fig. 7B). Thereafter, the conidia germination of Bgt strain E09 was examined on leaves of wild-type Jing411 and TaEPBM1, TaADA2 or TaGCN5-silenced plants. As shown in Figure 7C and 7D, Bgt conidia on TaEPBM1, TaADA2 or TaGCN5-silenced wheat leaves exhibit remarkably decreased germination rates than did Bgt conidia on Jing411 wild-type wheat leaves. Notably, simultaneous silencing of TaEPBM1, TaADA2, and TaGCN5 failed to cause a further decrease in cuticular wax biosynthesis and Bgt conidia germination compared with single-silencing of TaEPBM1, TaADA2 or TaGCN5. Taken together, these results support that TaEPBM1, TaADA2 and TaGCN5 function in a protein complex to activate the TaECR transcription and thus contribute to the cuticular wax biosynthesis and Bgt germination.

VLC Aldehydes are the Wax Signals Provided by TaECR-TaEPBM1-TaADA2-TaGCN5 Circuit for Stimulating Bgt Germination in Bread Wheat

Increasing evidence revealed that the cuticular wax provides signals for stimulating conidia germination of powdery mildew fungus Blumeria graminis (Nielsen et al., 2000; Wright et al., 2002; Weidenbach et al., 2014; Kong and Chang, 2018; Li et al., 2018; Wang et al., 2019). The finding that TaECR-TaEPBM1-TaADA2-TaGCN5 circuit regulates both the cuticular wax biosynthesis and Bgt conidia germination led us to ask whether the cuticular wax biosynthesis regulated by TaECR-TaEPBM1-TaADA2-TaGCN5 circuit is essential to Bgt conidia germination. To this end, we manipulated the cuticular wax on TaECR, TaEPBM1, TaADA2 or TaGCN5-silenced wheat leaves by spraying cuticular wax isolated from BSMV-γ control plants and then analyzed the Bgt conidia germination. As shown in Figure 8A, the application of cuticular wax isolated from BSMV-γ plants enabled the Bgt germination rates on the TaECR, TaEPBM1, TaADA2 or TaGCN5-silenced wheat leaves.
to increase by about 30%, a value nearly identical to that on BSMV-γ leaves. Similarly, Bgt germination rates on the BSMV-γ leaves remarkably decreased in the presence of cuticular wax isolated from TaECR, TaEPBM1, TaADA2 or TaGCN5-silenced wheat leaves (Fig. 8A). These results indicated that the cuticular wax biosynthesis regulated by the TaECR-TaEPBM1-TaADA2-TaGCN5 circuit is required for stimulating Bgt conidia germination in bread wheat.

Previous studies revealed that the physical properties, namely hydrophobicity, and chemical composition of the cuticle determine the beginning of plant-fungi interaction (Aragón et al., 2017). To analyze the hydrophobicity of leaf cuticle, we measured the contact angle of water droplets on the leaf surface in wheat leaves separately infected with BSMV-TaECRas, BSMV-TaEPBM1as, BSMV-TaADA2as, BSMV-TaGCN5as or BSMV-γ. As shown in Figure 8A, the leaf surfaces of BSMV-γ plants showed contact angles about 153°, whereas the leaf surfaces of TaECR, TaEPBM1, TaADA2 or TaGCN5-silenced plants exhibited contact angles less than 131°, indicating that silencing of TaECR, TaEPBM1, TaADA2 or TaGCN5 led to reduced hydrophobicity in wheat leaf cuticle. Thereafter, we employed a Formvar resin-based in vitro system, which could minimize the hydrophobicity difference through providing highly homogeneous surfaces, to examine the Bgt conidia germination. As shown in Figure 8B, glass slides covered with Formvar/cuticular wax isolated from wheat leaves infected with BSMV-γ, BSMV-TaECRas, BSMV-TaEPBM1as, BSMV-TaADA2as, or BSMV-TaGCN5as exhibit nearly identical contact angles. The germination rate of Bgt conidia on glass slides covered with Formvar/BSMV-γ cuticular wax is about 77%, but less than 59% Bgt conidia could germinate on glass slides coated with the Formvar/ cuticular wax isolated from wheat leaves infected with BSMV-TaECRas, BSMV-TaEPBM1as, BSMV-TaADA2as, or BSMV-TaGCN5as (Supplemental Fig. S13), suggesting that the chemical composition of cuticular wax was responsible for the difference in Bgt germination rate between wild-type and TaECR, TaEPBM1, TaADA2 or TaGCN5-silenced plants.

To characterize the role of single wax components including aldehydes, fatty acids, alcohols, alkanes, and even esters (which are reduced by silencing of TaECR, TaEPBM1, TaADA2, and TaGCN5), in stimulating Bgt germination, we employed glass slides covered with Formvar/wheat cuticular wax supplemented with corresponding synthetic chemicals and examined the Bgt conidia germination. As shown in Figure 8B, a supplement of C26, C28 or C30-aldehyde could restore the Bgt germination penalty on the glass slides coated with Formvar/cuticular wax isolated from the TaECR, TaEPBM1, TaADA2 or TaGCN5-silenced plants, whereas addition of other synthetic wax components such as VLC fatty acid, alkane, alcohol or alkyl ester failed to promote Bgt germination. Together, these results support that the VLC aldehydes are the wax signals provided by the TaECR-TaEPBM1-TaADA2-TaGCN5 circuit for stimulating Bgt germination.

DISCUSSION

Powdery mildew caused by the biotrophic fungal pathogen Bgt seriously threatens wheat production. Therefore, characterizing the molecular mechanism by which wheat genes regulate Bgt infection is vital to breeding Bgt-resistant wheat. In this study, we explored the transcriptional regulation of wheat cuticular wax biosynthesis required for stimulating Bgt conidia germination, and revealed that the
transcription factor TaEPBM1 recruits TaADA2-TaGCN5 complex to activate transcription of TaECR, a core gene controlling wheat cuticular wax biosynthesis, by enhancing histone acetylation and RNA Pol II occupancy at TaECR gene, and thereby stimulating the cuticular wax biosynthesis essential for Bgt conidia germination.

TaECR is a Core Component Responsible for the Wheat Cuticular Wax Biosynthesis

In previous studies, Enoyl-CoA Reductase (ECR) was revealed to function in the endoplasmic reticulum (ER) to catalyze the reduction of the enoyl-CoA, the final step of VLC acyl-CoA elongation (Kohlwein et al., 2001). Arabidopsis cer10 mutant disrupted in the AtECR gene exhibited reduced cuticular wax load, indicating that ECR participates in VLC acyl-CoA’s elongation reactions in Arabidopsis (Zheng et al., 2005). However, the biological function of ECR in other plants, especially the important crops, is still unknown. In this study, we characterized the function of wheat TaECR, which had more than 88% amino acid identities with Arabidopsis AtECR. Both confocal microscopy imaging and sucrose density-gradient fractionation assay showed that TaECR was localized to the ER, the site of cuticular wax biosynthesis. Tissue-specific analysis of TaECR transcription demonstrated that TaECR is highly expressed in the epidermis of wheat leaves and stems, where the cuticular wax is accumulated. Consistently, the silencing of TaECR in bread wheat led to a reduction in deposition of all cuticular wax components such as VLC fatty acids, alcohols, aldehydes, alkanes, and ketones, suggesting that wheat TaECR, resembling Arabidopsis AtECR, acts a core component responsible for the wheat cuticular wax biosynthesis.

Increasing evidence revealed that the function of core components mediating the cuticular wax biosynthesis is largely conserved among dicots and monocots. For instance, constitutive overexpression of SHINE1 (SHN1) in bread wheat increased the content of cuticular wax constituents such as VLC alkanes, aldehydes and primary alcohols, which is consistent with the results of overexpression of SHN1 in rice, Arabidopsis and even mulberry trees (Aharoni et al., 2004; Wang et al., 2012; Sajeevan et al., 2017; Bi et al., 2018). Similarly, disruption of 3-KETOACYL-CoA SYNTHASE 6 (KCS6) in Arabidopsis, barley and wheat all led to the attenuated deposition of cuticular wax (Fiebig et al., 2000; Hooker et al., 2002; Weidenbach et al., 2014; Wang et al., 2019). However, in contrast to disruption of β-ketoacyl-CoA Synthase (HvKCS1)-attenuated wax synthesis in barley, AtKCS1 has little affect on wax biosynthesis in Arabidopsis, suggesting that some cuticle biosynthetic components have acquired divergent functions in the evolution of dicots and monocots (Todd et al., 1999; Li et al., 2018).

TaEPBM1 Recruits the TaADA2-TaGCN5 Histone Acetyltransferase Complex to Activate TaECR Transcription

In Arabidopsis, AtECR transcription is activated by the MYB type transcription factors such as MYB30 and MYB94, and suppressed by the AP2/ERF-type transcription factor DEWAX (Raffaele et al., 2008; Go et al., 2014; Lee and Suh 2014). Through employing multiple approaches such as EMSA, yeast one-hybrid, and ChIP-qPCR assay, we demonstrated that the MYB type transcription factor TaEPBM1 could recognize the MBS cis-element and directly bind to the promoters of TaECR genes. Wheat protoplast transactivation assay and RT-qPCR revealed that TaEPBM1 acts as a transcriptional...
activator of TaECR. The closest homolog of TaEPBM1 in Arabidopsis was AtMYB96 with 41.3% identity, suggesting potential functional conservation of the transcription factor in monocots and dicots. In previous studies, multiple MYB type transcription factors, including the Arabidopsis MYB16, MYB30, MYB94, MYB96, MYB106, maize GL3, and saltwater cress EsWAX1, were reported to regulate cuticular wax biosynthesis (Avato et al., 1987; Raffaele et al., 2008; Seo et al., 2011; Liu et al., 2012; Oshima et al., 2013; Zhu et al., 2014; Lee and Suh, 2015b; Lee et al., 2016). Here, we found that TaEPBM1 activates TaECR transcription and positively regulates wheat cuticular wax biosynthesis, further implicating the essential role of MYB type transcription factors in the transcriptional regulation of plant cuticular wax biosynthesis.

In yeast and plants, ADA2 and GCN5 are integrated into the histone acetylation (HAT) module of the transcriptional activator complex SAGA, which comprises more than 20 subunits grouped into 4 modules, including the HAT module, histone deubiquitination module, coactivator architecture module, and recruiting module (Grant et al., 1997; Weake and Workman, 2012; Wang and Dent, 2014; Moraga and Aquea, 2015). Recent studies revealed that the ADA2-GCN5 complex activates gene transcription through enhancing histone acetylation and recruitment of the RNA polymerase II in rice and P. trichocarpa (Zhou et al., 2017; Li et al., 2018). Here, we showed that TaADA2 and TaGCN5 were enriched at TaECR promoters in a similar pattern, and silencing of TaADA2 and TaGCN5 led to the attenuated histone acetylations such as H3K4ac, H3K9ac, H3K14ac, H3K27ac, and H4K5ac, as well as reduced RNA Pol II recruitment at TaECR genes, suggesting that the TaADA2-TaGCN5 HAT module might directly regulate the TaECR transcription by potentiating histone acetylation and enhancing RNA Pol II enrichment. Therefore, it will be intriguing to test whether other subunits or modules of SAGA complex are involved in the epigenetic control of TaECR transcription and cuticle wax biosynthesis in the future.

Increasing evidence revealed that the ADA2-GCN5 complex complex is recruited to the target promoters through interaction with specific transcription factors, in which ADA2 proteins serve as an adapter to bridge the association between ADA2-GCN5 complex and transcription factors. For instance, the rice homeodomain transcription factor WOX11 directly interacts with rice ADA2 and recruits the ADA2-GCN5 complex to target root-specific genes involved in cell proliferation of crown root meristem (Zhou et al., 2017). Similarly, the Arabidopsis AP2 transcription factor CBF directly interacts with ADA2 to activate transcription of cold-responsive genes (Mao et al., 2006). Recent research in P. trichocarpa also revealed that the AREB1 transcription factor interacts with the ADA2-GCN5 complex to regulate gene expression during drought stress (Li et al., 2018; Castroverde 2019). Through employing multiple approaches such as yeast two-hybrid, GST pull-down, BiFC, co-IP, and ChIP-qPCR assay, we demonstrated that the TaEPBM1 directly interacts with TaADA2 and recruits the TaADA2-TaGCN5 complex to TaECR promoters to activate TaECR transcription by enhancing histone acetylation and RNA Pol II occupancy at TaECR genes. Silencing of TaEPBM1, TaADA2 or TaGCN5 resulted in the attenuation of cuticular wax biosynthesis required for stimulating Bgt germination, suggesting that TaEPBM1 recruits TaADA2-TaGCN5 complex to establish the epigenetic regulation of cuticular wax biosynthesis required for triggering Bgt conidia germination in bread wheat.
TaECR-TaEPBM1-TaADA2-TaGCN5 Circuit Regulates the Cuticular Wax Biosynthesis Exploited by Bgt for Triggering Conidia Germination

It has been demonstrated that the fungal pathogen Blumeria graminis could utilize the plant cuticular wax components to initiate its pre-penetration processes (Nielsen et al., 2000; Wright et al., 2002; Weidenbach et al., 2014; Kong and Chang, 2018; Li et al., 2018; Wang et al., 2019). Additionally, VLC aldehydes promote the in vitro conidia germination and appressorial development of B. graminis in a dose-dependent manner (Hansjakob et al., 2010; Hansjakob et al., 2011; Kong and Chang, 2018; Wang et al., 2019). Here, we showed that silencing of TaECR, TaEPBM1, TaADA2 or TaGCN5 in bread wheat attenuated Bgt conidia germination, which was fully restored by application of cuticular wax isolated from wild-type wheat leaves. Consistently, spraying of cuticular wax isolated from TaECR, TaEPBM1, TaADA2 or TaGCN5-silenced leaves led to the reduced germination of Bgt conidia on wild-type wheat leaves, indicating that the TaECR-TaEPBM1-TaADA2-TaGCN5 circuit involved in the cuticular wax biosynthesis has been exploited by Bgt for triggering conidia germination. Through using an in vitro system based on Formvar-coated glass slides, we showed that application of synthetic VLC aldehydes, but not other synthetic wax components such as VLC fatty acids, alkanes, alcohols or alkyl esters, could overcome the Bgt germination penalty on cuticular wax isolated from TaECR, TaEPBM1, TaADA2 or TaGCN5-silenced wheat leaves, suggesting that VLC aldehydes are the wax signals provided by the TaECR-TaEPBM1-TaADA2-TaGCN5 circuit for triggering Bgt conidia germination in bread wheat.

These results allowed us to establish a model about how the TaECR-TaEPBM1-TaADA2-TaGCN5 circuit regulates the cuticular wax biosynthesis required for stimulating Bgt conidia germination in bread wheat. As shown in Figure 9, transcription factor TaEPBM1 recognizes the MBS cis-element and directly targets TaECR, an essential gene in cuticular wax biosynthesis. Through directly interacting with the adapter protein TaADA2, TaEPBM1 recruits the TaADA2-TaGCN5 histone acetyltransferase complex to TaECR promoters, which lead to activation of TaECR transcription by potentiating histone acetylation and enhancing RNA Pol II enrichment at TaECR genes. Consequently, the cuticular wax biosynthesis is stimulated, leading to the accumulation of VLC aldehyde wax constituents and thereby triggering the germination of Bgt conidia. Our findings support that the recruitment of the TaADA2-TaGCN5 histone acetyltransferase complex by specific transcription factor plays an important role in the epigenetic control of cuticular wax biosynthesis essential for stimulating Bgt conidia germination, and provide insight for the improvement of wheat powdery mildew resistance in the future.

MATERIALS AND METHODS

Plant Materials and Fungal Inoculation

The cultivar Jing411 of common wheat (Triticum aestivum L.) and the isolate E09 of wheat powdery mildew fungus (Blumeria graminis f. sp. tritici) were used for the wheat-powdery mildew interaction and maintained under conditions as previously reported (Liu et al., 2019). The Bgt inoculation was performed in the same condition. At 12 hours post-Bgt inoculation, wheat leaves were subjected to the microscopic analysis of Bgt conidia germination as described by Kong and Chang (2018).
**TaECR** coding regions were amplified using primers listed in Supplemental Table S1 and cloned into vector pCAMBIA1300-YFP via the pENTRY-TaECR construct using the GATEWAY cloning technology and then transformed into the *Agrobacterium tumefaciens* strain GV3101. The pCAMBIA1300-derivatives expressing the endoplasmic reticulum (ER) marker mCherry-HDEL were also transformed into the *A. tumefaciens* strain GV3101. *Nicotiana benthamiana* plants used in this study were grown in a growth chamber at 22°C with a 14/10 h light/dark photoperiod. *N. benthamiana* leaves co-infiltrated with *A. tumefaciens* strain GV3101 expressing TaECR-YFP and mCherry-HDEL were imaged using the confocal microscope (Leica TCS SP5) at 48 hours post-Agro-infiltration.

For the subcellular localization analysis in wheat protoplasts, the TaECR coding region was amplified using primers listed in Supplemental Table S1 and cloned into the pCAMBIA1300-HA vector via the pENTRY-TaGCN5 construct using GATEWAY cloning technology to generate the fusion protein TaECR-HA. The wheat protoplasts were prepared as previously described by Liu *et al.* (2019). 10 µg plasmid of pCAMBIA1300-TaECR-HA construct was mixed with 200 µl wheat protoplasts in 250 µl PEG solution containing 40% (w/v) PEG4000, 0.2M mannitol, and 0.1M CaCl₂ and kept 30 minutes in the dark for transfection. After being washed three times with W5 solution (pH 5.7, 2 mM MES, 5 mM KCl, 120 mM CaCl₂ and 150 mM NaCl), the transformed protoplasts were cultured in W5 solution for at least 48 hours for the sucrose density-gradient fractionation assay. The sucrose density-gradient fractionation assay was performed as described previously (Chen *et al.*, 2002). The antibodies α-HA (Millipore, 05-904), α-BiP (Stressgen, SPA-818), and α-H⁺ ATPase (Agrisera, AS07260) were employed for immunoblotting. For confocal microscopy imaging and sucrose density-gradient fractionation assays, three independent biological replicates were performed with consistent results.

**Barley Stripe Mosaic Virus (BSMV)-mediated Silencing of TaECR, TaEPBM1, TaADA2, and TaGCN5**

For the BSMV-mediated gene silencing, fragments of TaECR, TaEPBM1, TaADA2, and TaGCN5 (approximately 200-bp) were amplified using primers listed in Supplemental Table S1 and cloned in the antisense orientation into the pCa-γbLIC vector through the ligation independent cloning technique to create the BSMV-TaECRas, BSMV-TaEPBM1as, BSMV-TaADA2as, and BSMV-TaGCN5as constructs, respectively. The BSMV-mediated gene silencing was performed as described (Yuan *et al.*, 2011). About 3 weeks post-inoculation with BSMV virus, nascent wheat leaves with visible BSMV symptoms were subjected to cuticle chemical analysis and powdery mildew infection.

**Cuticle Chemical Analysis**

As described in previous studies, wheat leaves with virus symptoms about 15 d post-BSMV-infection were subjected to wax analysis (Kong and Chang, 2018; Wang *et al.*, 2019). For the cutin composition analysis, lyophilized wheat leaves from at least 5 BSMV-VIGS wheat plants were delipidated in an isopropanol-chloroform-methanol solution containing 0.01% (v/v) butylated hydroxytoluene. After being dried under an N₂ stream and weighed, the extracts were depolymerized into methyl esters in a
reaction mixture containing methanol and methyl nonadecanoate. After extraction with dichloromethane and dried under an N\textsubscript{2} stream, the samples were derivatized with pyridine and bis-N,O-trimethylsilyl trifluoroacetamide, and then subjected to GC-MS analysis as described previously (Kong and Chang, 2018). The oven temperature program was set at an initial temperature 75°C, increased to 200°C at 15°C min\textsuperscript{-1}, then increased to 280°C at 1.5°C min\textsuperscript{-1}. Methyl nonadecanoate was added as the internal standard for the FID peak-based quantification.

The cuticular wax composition analysis was performed as described by Hansjakob et al. (2010). Briefly, wheat leaves from at least 5 BSMV-VIGS wheat plants were dipped into chloroform (Merck). The extracts dried under N\textsubscript{2} were derivatized at 70°C for 30 mins through reaction with bis-N,O-trimethylsilyl trifluoroacetamide and analyzed by the same GC-MS column in cutin analysis, in which H\textsubscript{2} was used as the carrier gas. The oven temperature was programmed at an initial 50°C for 2min, increased to 200°C at 40°C min\textsuperscript{-1}, kept at 200°C for 2min, and then increased to 320°C at 3°C min\textsuperscript{-1}, and finally kept at 320°C for 30min. n-Tetracosane was added as an internal standard for the FID peak-based quantification and Agilent/HP chemstation software (Agilent Technologies) were employed for the cutin and wax compound identification. For cuticle chemical analysis, three biological replicates were statistically analyzed.

**Wheat Protoplast Transient Gene Silencing and Expression Assay**

For the gene silencing in wheat protoplasts, 198-, 209-, 212-bp fragments of *TaEPBM1*, *TaADA2* and *TaGCN5* were amplified using primers listed in Supplemental Table S1 and cloned in the antisense orientation into the pIPKb007 vector via the pENTRY-RNAi-*TaEPBM1*as, pENTRY-RNAi-*TaADA2*as, and pENTRY-RNAi-*TaGCN5*as constructs using GATEWAY cloning technology to create the RNAi-*TaEPBM1*as, RNAi-*TaADA2*as, and RNAi-*TaGCN5*as constructs, respectively. For gene expression in wheat protoplasts, the *TaGCN5* coding region was amplified using primers listed in Supplemental Table S1 and cloned into the pCAMBIA1300-HA vector via the pENTRY-*TaGCN5* construct using GATEWAY cloning technology to generate the fusion protein *TaGCN5*-HA. 10 µg plasmids for RNAi and pCAMBIA1300-*TaGCN5*-HA constructs were co-transfected into wheat protoplasts as previously described by Liu et al (2019). The transformed protoplasts were cultured in W5 solution for at least 48 hours for the next gene expression analysis or ChIP-qPCR assays.

For the wheat protoplast transactivation assay, the *TaEPBM1* coding region was amplified using the primers listed in Supplemental Table S1 and cloned into the vector pIPKb004 vector via the pENTRY-*TaEPBM1* construct using the GATEWAY cloning technology. Similarly, *TaECR* promoters were amplified using the primers listed in Supplemental Table S1 and ligated into the vector 5XGAL4-LUC, which was then co-transfected with pIPKb004 derivatives and internal control pPTRL into the wheat protoplast as previously described by Liu et al (2019). LUC activity was measured at 48 hours post-transfection using a Promega dual-luciferase reporter assay system (Promega, E1910)
according to the manual. In wheat protoplast transactivation assay, three biological replicates were
statistically analyzed.

**Gene Expression Analysis**

For gene expression analysis, wheat leaves with visible BSMV symptoms were collected at 3 weeks
post-inoculation with indicated BSMV virus, and wheat protoplasts were harvested at 48 hours
post-transfection with indicated RNAi constructs. In the nuclear run-on assay for measuring gene
transcription rate, wheat cell nuclei were isolated and mixed with reaction buffer (25 mM biotin-16-UTP
and 0.75 mM of ATP, CTP, and GTP) for the transcription reaction as described by Ding et al (2012).
After RNA extraction using Trizol, the nascent RNA was enriched by streptavidin magnetic beads
(Invitrogen) and subjected to the RT-qPCR assay using the primers listed in Supplemental Table S1. For
the RT-qPCR assay, total RNA was extracted using Trizol reagent and treated with Dnase I for the
gDNA removal. 2μg RNA was then employed to synthesize the first-strand cDNA template using the
cDNA synthesis supermix (Transgen) according to the manual. RT-qPCR was performed using the
qPCR Master Mix (Invitrogen) under the following programs: 95°C for 3 min, 40 cycles at 95°C for 20 s,
55°C for 20 s, and 72°C for 15 s, followed by 72°C for 1 min. The expression levels of *TaGADPH*,
*TaECR*, *TaEPBM1*, *TaADA2*, and *TaCHR729* were analyzed using the primers listed in Supplemental
Table S1 and the *TaGADPH*, whose expression is stable among various treatments, was used as the
internal control for reference. For the nuclear run-on and RT-qPCR assays, three biological replicates
were statistically analyzed.

**Yeast One- and Two-Hybrid Experiments**

In yeast one-hybrid analysis, the *TaEPBM1* coding region was amplified using the primers listed in
Supplemental Table S1 and cloned into the vector pGADT7 via the pENTRY-TaEPBM1 construct using
GATEWAY cloning technology to generate protein fusions to the GAL4 transcription-activating domain
(AD). Similarly, *TaECR* promoters were amplified using the primers listed in Supplemental Table S1
and cloned into the vectors pHIS2, which were then co-transformed with pGADT7 derivatives into
competent cells of yeast strain Y187 according to the manual. Yeast transformants were then grown on
the SD/-Trp-Leu-His plate with 15% (w/v) 3-amino-1,2,4-triazole (3-AT) to test for HIS2 expression. In
yeast two-hybrid analysis, the coding fragments of *TaEPBM1*, *TaADA2*, and *TaGCN5* were amplified
using primers listed in Supplemental Table S1 and separately cloned into the vectors pLexA and
pB42AD via the pENTRY-TaEPBM1, pENTRY-TaADA2, and pENTRY-TaGCN5 constructs using
GATEWAY cloning technology; these were co-transformed into competent cells of yeast strain EGY48
according to the Clontech Yeast Protocols Handbook. For the truncated *TaEPBM1* and *TaADA2* used in
the yeast two-hybrid assay, the *TaEPBM1-NT*(1-120), *TaEPBM1-CT*(121-314), *TaADA2-NT*(1-244),
and *TaEPBM1-NT*(245-568) were amplified using primers listed in Supplemental Table S1 and cloned
into the vectors pLexA and pB42AD. The pB42AD-derived prey wheat cDNA library was constructed
and screened as previously described by Liu et al (2019). Yeast transformants were grown on the
SD/-Ura-Trp-Leu-His plate with X-gal to test for expression of LEU2 and LacZ. The yeast strains Y187 and EGY48 were maintained on YPAD and SD/-Ura medium, respectively. For the yeast one- and two-hybrid experiments, at least three independent biological replicates were performed with consistent results.

Electrophoretic Mobility Shift Assay (EMSA)

The TaEPBM1 coding region was amplified using the primers listed in Supplemental Table S1 and cloned into the vector pET32, and TaEPBM1-His recombinant protein was expressed and purified from E. coli using Ni-NTA resin according to the manual. The Pchn0 probe (GGTCCATAACCATCTCTGTGGGTCCATACACCATCCTCTGTGTCT) contains two copies of wild type MBS cis-elements and flanking sequences from TaECR promoters. In the mutated Probe mPchn0 (GGTCCATAACCATCTCTGTGGGTCCATACACCATCCTCTGTGTCT), the MBS cis-element CACCAT was replaced by CACCAT. Pchn0 and the mPchn0 probe were generated by annealing oligonucleotides (see Supplemental Table S1 for sequence information) as described previously (Su et al. 2015). The EMSA was performed as described by Kong and Chang (2018). The DNA-protein complexes were visualized by exposing the resultant gel on the Phosphor screen for 8 hours. For the EMSA, at least three independent biological replicates were performed with similar results.

Pull-Down Assay

For pull-down assays, the TaEPBM1 coding region was amplified using the primers listed in Supplemental Table S1 and cloned into the vector pGEX4T-1 to generate the fusion protein GST-TaEPBM1, while the coding regions of TaADA2 and TaGCN5 were amplified using the primers listed in Supplemental Table S1 and cloned into the vector pET32 to create proteins fusions to the His-tag. The pull-down assay was performed as described by Wang et al (2019). Briefly, the recombinant proteins with GST and His tags were expressed and purified from E.coli using glutathione sepharose and Ni-NTA resin according to the manual. Recombinant proteins with GST and His tags were mixed as pairs indicated and incubated with glutathione sepharose, and then subjected to the centrifugation-assisted precipitation. After being washed five times with PBS buffer, the precipitates were subjected to SDS-PAGE separation, and the co-precipitation of TaADA2-His or TaGCN5-His with GST or GST-TaEPBM1 was resolved by immunoblotting with α-His antibody (CWBIO, CW0286). For the pull-down assays, at least three independent biological replicates were performed with similar results.

Bimolecular Fluorescence Complementation (BiFC) Assay

For BiFC assays, coding regions of TaEPBM1, TaADA2, and TaGCN5 were amplified using primers listed in Supplemental Table S1 and cloned into vectors pCAMBIA1300-YN and pCAMBIA1300-YC via the pENTRY-TaEPBM1, pENTRY-TaADA2, and pENTRY-TaGCN5 constructs using GATEWAY cloning technology to express protein fusions to the N-terminal or C-terminal domain of YFP.
respectively. Similarly, the *TaADA2* coding region was amplified using primers listed in Supplemental Table S1 and cloned into vectors pCAMBIA1300 to express TaADA2 alone. The BiFC assay was performed as described by Liu et al (2019). The interaction was imaged using a confocal microscope (Leica TCS SP5) at 48 hours post-Agro-infiltration. All BiFC images were collected on a Leica TCS SP5 confocal laser scanning system (Leica, Mannheim, Germany) connected to an inverted motorized microscope with the following settings: pinhole 1 airy unit, scan speed 400 Hz bidirectional. DAPI and YFP were excited with a 405 nm diode laser and a 514 nm argon laser, respectively. Fluorescence emissions were collected using the following wavelengths: 420–480nm (for DAPI) and 529–540nm (for YFP). Digital confocal images were analyzed using Adobe Photoshop (Version CS5) and adjusted with ImageJ (Version 1.38) for the optimized intensity projection. At least three independent biological replicates were performed for this BiFC assay.

**Co-immunoprecipitation (Co-IP) Assay**

For the Co-IP assay, wheat leaves with visible BSMV symptoms were collected at 3 weeks post-inoculation with indicated BSMV virus, and wheat protoplasts were harvested at 48 hours post-transfection with indicated constructs. The Co-IP assay was performed as described by Wang et al. (2019). The nuclear extracts were treated with DNase I to remove the potential DNA-protein interaction during the Co-IP. The epitope sequences SAFEYDRKPAVLAPD and SGHKTNRPMKLETGDS were chosen and synthesized, coupled to keyhole limpet hemocyanin, and immunized mice to generate antibodies against TaEPBM1 and TaADA2, respectively. The antibody specificities were analyzed by Western-blot assay against total leaf protein isolated from indicated BSMV-VIGS plants (shown in Supplemental Fig. S8). The antibodies α-HA (Millipore, 05-904), α-TaEPBM1, and α-TaADA2 were employed for immunoprecipitation. The co-immunoprecipitation of TaEPBM1, TaADA2, and TaGCN5 was analyzed by immunoblot with antibodies α-HA (Millipore, 05-904), α-TaEPBM1, and α-TaADA2. The ECL chemiluminescence kit (Pierce Biotechnology) was employed for the immunoblot visualization. For the Co-IP assay, at least three independent biological replicates were performed with similar results.

**Luciferase Complementation Imaging (LCI) Assay**

For LCI assay, coding regions of *TaEPBM1*, *TaADA2*, and *TaGCN5* were amplified using primers listed in Supplemental Table S1 and cloned into vectors pCAMBIA-nLUC and pCAMBIA-cLUC via the pENTRY-TaEPBM1, pENTRY-TaADA2, pENTRY-TaGCN5 constructs using GATEWAY cloning technology to express protein fusions to the N-terminal or C-terminal domain of firefly LUCIFERASE, respectively. The LCI assay was performed as described by Kong and Chang (2018). The luminescent signal was collected at 60 hours post-Agro-infiltration trough using a cooled CCD camera (iXon, Andor Technology). At least three independent biological replicates were performed for this LCI assay.

**Chromatin Immunoprecipitation (ChIP) Assay**
For the ChIP assay, wheat leaves and protoplasts were harvested at the same time as for the Co-IP assay. The ChIP assay was performed as described by Wang et al (2019). The antibodies α-HA (Millipore, 05-904), α-TaEPBM1, α-TaADA2, α-histone H3 (Abcam, ab1791), α-H3K4ac (Millipore, 07-539), α-H3K9ac (Abcam, ab10812), α-H3K14ac (Abcam, ab46984), α-H3K27ac (Abcam, ab4729), α-H4K5ac (Millipore, 07-327), and α-Pol II (Abcam, ab817) were employed for the immunoprecipitation. DNA recovery after chromatin immunoprecipitation was quantified as the percentage of input. For H3K4ac, H3K9ac, H3K14ac, and H3K27ac analysis, relative enrichments were calculated by normalizing the histone H3 acetylation ChIP with histone H3 ChIP. For H4K5ac analysis, relative enrichments were calculated by normalizing the histone H4 acetylation ChIP with histone H4 ChIP. The ChIP-qPCR was performed using qPCR Master Mix (Invitrogen) under the following programs: 95°C for 3 min, 40 cycles at 95°C for 20 s, 55°C for 20 s, and 72°C for 1 min. For the ChIP assay, three biological replicates were statistically analyzed.

Characterization and Manipulation of Leaf Surface and Glass Slide

The cuticle hydrophobicity on the wheat leaf surface and glass slide was analyzed by measuring the contact angle of 1 μL water droplets on the indicated surface. Angles from at least 50 water droplets were separately measured for 5 s using the contact angle system (SDP-300, Sindin) and five independent surface samples were statistically analyzed using Student’s t-test. The cuticle wax on the wheat leaf and glass slide were manipulated as described previously (Hansjakob et al., 2011, Wang et al., 2019). Briefly, wheat leaves were sprayed with cuticular wax extracts in chloroform at 480 μg mL⁻¹ or chloroform only (control). Histobond glass slides (Marienfeld) were coated with chloroform solution containing 0.5% (w/v) Formvar resin (Applichem) and leaf cuticular wax extract (480 μg ml⁻¹) or wax chemical components (7x10⁻⁵ mol l⁻¹). For this study, VLC aldehydes were synthesized from VLC alcohols as previously described, and other wax chemical components were purchased from Sigma-Aldrich. At least three independent biological replicates were performed with similar results.

Accession Numbers

Sequence data in this study can be found in the GenBank database using the following accession numbers: AcECR, MT181952; A/JECR, MT181953; AtECR, At3g55360; BdECR, MT181954; MpECR, MT181955; OsECR, MT181956; PpECR, MT181957; PsECR, MT181958; ScECR, MT181959; SjECR, MT181960; SIECR, MT181961; SmECR, MT181962; TaECR-A, MT180310; TaECR-B, MT180311; TaECR-D, MT180312; TaEPBM1-A, MT211783; TaEPBM1-B, MT211784; TaEPBM1-D, MT211785; TaADA2-A, MT180316; TaADA2-B, MT180317; TaADA2-D, MT180318; TaGCN5-A, MT180319; TaGCN5-B, MT180320; and TaGCN5-D, MT180321.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1 The nucleotide sequences, structures and encoding proteins of TaECR genes.

Supplemental Figure S2 Protein sequence alignment of TaECR with putative orthologs from representative land plant lineages.
**Supplemental Figure S3** Subcellular localization analysis of TaECR-HA in wheat cells by sucrose density-gradient fractionation.

**Supplemental Figure S4** RT-qPCR analysis of TaECR expression in the wheat leaves separately infected with BSMV-γ and BSMV-TaECRas.

**Supplemental Figure S5** Analysis of cuticle chemical composition and Bgt conidia germination on the wheat leaves treated with mock control and BSMV-VIGS.

**Supplemental Figure S6** The nucleotide sequences and encoding proteins of TaEPBM1 genes.

**Supplemental Figure S7** EMSA analysis of TaEPBM1 binding to the MBS cis-element.

**Supplemental Figure S8** Immunoblot analysis of wheat leaf protein using antibodies α-TaEPBM1 and α-TaADA2.

**Supplemental Figure S9** Nuclear run-on analysis of TaEF1 transcription and RT-qPCR analysis of TaEF1 expression in the wheat leaves infected with BSMV-γ, BSMV-TaEPBM1as, BSMV-TaADA2as, or BSMV-TaGCN5as.

**Supplemental Figure S10** The nucleotide sequences and encoding proteins of TaADA2 genes.

**Supplemental Figure S11** The nucleotide sequences and encoding proteins of TaGCN5 genes.

**Supplemental Figure S12** RT-qPCR analysis of expression of TaGCN5, TaADA2, and TaEPBM1 in wheat protoplasts and leaves.

**Supplemental Figure S13** Statistical analysis of Bgt conidia germination on glass slides coated with Formvar/cuticular wax (480 μg ml⁻¹) with indicated contact angles.

**Supplemental Table S1** Primers used in this study.

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**FIGURE LEGENDS**

**Figure 1.** Characterization of TaECR in bread wheat. A, Subcellular co-localization of TaECR-YFP with Endoplasmic reticulum (ER) marker mCherry-HDEL in *Nicotiana benthamiana* leaves. YFP fluorescence signal of TaECR-YFP merged with the mCherry signal of the ER marker. Bars= 10 μm. B, Transcriptional profiles of TaECR in different wheat tissues. The relative transcript abundance of TaECR was compared with that in roots. C, Quantification of total cutin monomer and wax per unit surface area (μg cm⁻²) in the BSMV-γ and BSMV-TaECRas wheat leaves. D, Amount of major wax constituents per unit surface area (μg cm⁻²) in the wheat leaves infected with BSMV-γ and BSMV-TaECRas. N. I., not identified compound. E, Bgt germination on the BSMV-γ and BSMV-TaECRas wheat leaves. Black arrows indicate successfully germinated conidia with a germ tube, and red arrow indicates the non-germinated conidia without germ tube. Bars= 30 μm. F, Statistical analysis of Bgt conidia
germination on the wheat leaves infected with BSMV-γ or BSMV-TaECRas. More than 10
BSMV-VIGS plants were employed for inoculation of Bgt conidia and at least 500 Bgt conidia were
analyzed for each experiment. For B, C, D, and F, three biological replicates were statistically analyzed
for each treatment, and data are presented as the mean±SE (Student’s t-test; ** P<0.01).

**Figure 2.** Transcription factor TaEPBM1 binds to TaECR promoters. A, TaEPBM1 binds to TaECR
promoters in yeast. Yeast cells were co-transformed with a bait vector, harboring a wild type or mutated
TaECR promoter fragment (labeled as pro and mpro) fused to the pHIS2 reporter, and a prey vector,
containing TaEPBM1 fused to a GAL4 activation domain, and were then grown on SD-Trp-Leu-His
plate with 15% (w/v) 3-AT to test for HIS2 expression. Optical density (OD) of diluted culture for each
spot is shown at the top of the panel. B, Schematic diagram of the reporter constructs harboring a
wild-type or mutated TaECR promoter fragment (labeled as pro and mpro) fused to the 5xGAL4
upstream activating sequences (UAS) and the reporter gene firefly LUCIFERASE (LUC). C,
Transactivation of TaECR by TaEPBM1 in wheat protoplast. Indicated pairs of effectors and LUC
reporters were transfected into the wheat protoplasts, and the LUC activity was measured and expressed
as a ration of LUC activity normalized to that obtained from protoplasts expressing DBD effectors. D,
Schematic diagram of TaECR promoter structures. MBS cis-element is shown as a blue box, and
promoter regions subjected to ChIP-qPCR analysis are labeled with numbers. Black triangle indicates
the start point of coding region of TaECR gene. E, ChIP-qPCR analysis of TaEPBM1 enrichment at
TaECR promoters in the wheat leaves infected with BSMV-γ and BSMV-TaEPBM1as. Antibody
α-TaEPBM1 was used for immunoprecipitation. Nuclear run-on analysis of TaECR transcription (F) and
RT-qPCR analysis of TaECR expression (G) in the wheat leaves infected with BSMV-γ and
BSMV-TaEPBM1as. For C, E, F, and G, three biological replicates were statistically analyzed for each
treatment, and data are presented as the mean±SE (Student’s t-test; ** P<0.01). (t-test; ** P<0.01).

**Figure 3.** TaEPBM1 directly interacts with TaADA2 in vitro and in vivo. A, Yeast two-hybrid analysis
of the interaction between TaEPBM1 and TaADA2. Different fragments of TaEPBM1 and TaADA2
were fused with AD and LexA vectors and cotransformed into the yeast cells. TaEPBM1 NT and CT
represent the N-terminal region (1-120) and C-terminal region (121-314) of TaEPBM1, whereas
TaADA2 NT and CT indicate the N-terminal region (1-244) and C-terminal region (245-568) of
TaADA2. The transformants were grown on the SD-Ura-Trp-Leu-His plate with X-gal to test for
expression of LEU2 and LacZ. B, GST pull-down analysis of the interaction of TaEPBM1 and TaADA2.
GST-TaEPBM1 or GST protein bound on GST affinity resins was incubated with TaADA2-His and
TaGCN5-His, and the bound protein was then detected by immunoblotting using an anti-His antibody.
C, BiFC analysis of the interaction between TaEPBM1 and TaADA2 in N. benthamiana leaves. The nuclei
were revealed by DAPI staining. The YFP fluorescence signals were detected 40 hours
post-Agro-infiltration. Bars= 100 μm. D, Co-IP analysis of the interaction of TaEPBM1 and TaADA2.
Nuclear protein was extracted from wheat leaves infected with indicated BSMV-γ, BSMV-TaEPBM1as,
BSMV-TaADA2as, or BSMV-TaEPBM1/TaADA2as. Antibodies α-TaEPBM1 and α-TaADA2 were
used for immunoprecipitation and Western-blot assays.

**Figure 4.** TaGCN5-TaADA2 complex interacts with TaEPBM1. A, Yeast two-hybrid analysis of the
interaction among TaGCN5, TaADA2, and TaEPBM1. The yeast transformants were grown on the
SD-Ura-Trp-Leu-His medium with X-gal to test for interaction analysis. B, LCI analysis of the interaction among TaGCN5, TaADA2, and TaEPBM1 in *N. benthamiana* leaves. Bars= 1 cm. C, BiFC analysis of the interaction among TaGCN5, TaADA2, and TaEPBM1 in *N. benthamiana* leaves. The nuclei were revealed by DAPI staining. The YFP fluorescence signals were detected 40 hours post-Agro-infiltration. Bars= 100 μm.

**Figure 5.** Distribution of TaGCN5-HA, TaADA2, and TaEPBM1 on TaECR promoters in wild-type and TaEPBM1-, TaADA2- or TaGCN5-silenced wheat leaves. The distribution of TaGCN5-HA (upper panel), TaADA2 (middle panel) and TaEPBM1 (lower panel) on TaECR promoters in the wheat leaves infected with BSMV-γ (dark), BSMV-TaEPBM1as (dark grey), BSMV-TaADA2as (grey), BSMV-TaGCN5as (light grey) or BSMV-TaEPBM1as + BSMV-TaADA2as + BSMV-TaGCN5as + BSMV-TaECRas (white). Antibodies α-HA, α-TaADA2, and α-TaEPBM1 were used for immunoprecipitation and Western-blot assays.

**Figure 6.** Characterization of histone acetylation, RNA polymerase II occupancy and gene transcription at TaECR loci in wild-type and TaEPBM1-, TaADA2- or TaGCN5-silenced wheat leaves. A, ChIP-qPCR analysis of abundance of H3K4ac, H3K9ac, H3K14ac, H3K27ac, and H4K5ac on TaECR promoters in the wheat leaves infected with BSMV-γ (dark), BSMV-TaEPBM1as (dark grey), BSMV-TaADA2as (grey), BSMV-TaGCN5as (light grey) or BSMV-TaEPBM1as + BSMV-TaADA2as + BSMV-TaECRas (white). Antibodies α-H3K4ac, α-H3K9ac, α-H3K14ac, α-H3K27ac, α-H4K5ac were used for immunoprecipitation. Fragments for ChIP-qPCR analysis were shown in Figure 2D. B, Schematic diagram of the TaECR gene structure. Fragments subjected to ChIP-qPCR analysis are labeled with numbers. Black triangle indicates the start point of coding region of TaECR gene. C, ChIP-qPCR analysis of RNA Pol II abundance on promoters and coding regions of TaECR genes in the BSMV-γ (grey), BSMV-TaEPBM1as (blue), BSMV-TaADA2as (green), BSMV-TaGCN5as (orange) or BSMV-TaEPBM1as + BSMV-TaADA2as + BSMV-TaGCN5as + BSMV-TaECRas (white) wheat leaves. Nuclear run-on analysis of TaECR transcription rates (D) and RT-qPCR analysis of TaECR expression levels (E) in the wheat leaves infected with BSMV-γ, BSMV-TaEPBM1as, BSMV-TaADA2as, BSMV-TaGCN5as or BSMV-TaEPBM1as + BSMV-TaADA2as + BSMV-TaGCN5as + BSMV-TaECRas. For A, C, D, and E, three independent biological replicates per treatment were statistically analyzed, and data are presented as the mean ± SE (Student’s t-test; ** P<0.01).

**Figure 7.** Characterization of cuticle chemical composition and *Bgt* conidia germination on wild-type and TaEPBM1-, TaADA2- or TaGCN5-silenced wheat leaves. A, Quantification of total cuticular wax per unit surface area (μg cm⁻²) in the wheat leaves infected with BSMV-γ (grey), BSMV-TaEPBM1as (blue), BSMV-TaADA2as (green), BSMV-TaGCN5as (orange) or BSMV-TaEPBM1as + BSMV-TaADA2as + BSMV-TaGCN5as (yellow). B, Amount of major wax constituents per unit surface area (μg cm⁻²) in the BSMV-γ (grey), BSMV-TaEPBM1as (blue), BSMV-TaADA2as (green),
BSMV-TaGCN5as (orange) or BSMV-TaEPBM1as + BSMV-TaADA2as + BSMV-TaGCN5as (yellow) infected wheat leaves. N. I., not identified compound. C, Bgt germination on the wheat leaves infected with BSMV-γ, BSMV-TaEPBM1as, BSMV-TaADA2as, BSMV-TaGCN5as or BSMV-TaEPBM1as + BSMV-TaADA2as + BSMV-TaGCN5as. Black arrows indicate successfully germinated conidia with a germ tube, and red arrows indicate the non-germinated conidia without germ tube. Bars= 30 μm.

Statistical analysis of Bgt conidia germination on the BSMV-γ (dark grey), BSMV-TaEPBM1as (dark blue), BSMV-TaADA2as (dark green), BSMV-TaGCN5as (dark orange) or BSMV-TaEPBM1as + BSMV-TaADA2as + BSMV-TaGCN5as (dark yellow) infected wheat leaves. More than 10 BSMV-VIGS plants were employed for inoculation of Bgt conidia and at least 500 Bgt conidia were analyzed for each experiment. For A, B, and D, three biological replicates were statistically analyzed for each treatment, and data are presented as the mean ± SE (Student’s t-test; ** P<0.01).

Figure 8. Characterization of Bgt conidia germination on TaECR-, TaEPBM1-, TaADA2 or TaGCN5-silenced wheat leaves or glass slides coated with Formvar/wheat leaf wax. A, Statistical analysis of Bgt conidia germination on wheat leaves infected with BSMV-γ (dark grey), BSMV-TaECRas (dark blue), BSMV-TaEPBM1as (dark green), BSMV-TaADA2 (dark orange) or BSMV-TaGCN5as (dark yellow). Wheat leaves with indicated contact angles were sprayed with cuticular wax extracted in chloroform from indicated wheat leaves, or with chloroform only (control treatment). B, Statistical analysis of Bgt conidia germination on glass slides coated with Formvar/cuticular wax (480 μg ml⁻¹) and pure waxes (7×10⁻⁵ mol l⁻¹), or with Formvar/cuticular wax (480 μg ml⁻¹) only (control treatment). For A and B, more than 500 Bgt conidia were analyzed in one experiment, and three biological replicates were statistically analyzed for each treatment, and data are presented as the mean ± SE (Student’s t-test; ** P<0.01).

Figure 9. Proposed model for the regulation of wheat cuticular wax biosynthesis and Bgt conidia germination by the TaECR-TaEPBM1-TaADA2-TaGCN5 circuit. Transcription factor TaEPBM1 recognizes the MBS cis-element and directly targets TaECR, an essential gene in cuticular wax biosynthesis, and then recruits the TaADA2-TaGCN5 transcriptional activator complex to TaECR promoters, which potentiates histone acetylation such as H3K4ac, H3K9ac, H3K14ac, H3K27ac, and H4K5ac, as well as enhances occupancy of RNA polymerase II at TaECR genes. Consequently, the cuticular wax biosynthesis is stimulated, leading to the accumulation of VLC aldehydes and thereby triggering the germination of Bgt conidia.

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