Genome-wide identification and characterization of caffeoyl-coenzyme A O-methyltransferase genes related to Fusarium head blight response in wheat

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Research Article

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Abstract

Background

Lignin is one of the main components of cell wall, which directly associates with the development and defense mechanisms in plants, especially in response to Fusarium head blight (FHB) tolerance. Caffeoyl-coenzyme A Omethyltransferase (CCoAOMT) is the main regulator determining the efficiency of lignin synthesis and composition. Although it has been widely characterized in many plants, the importance of CCoAOMT family in wheat is not well understood up to now.

Results

Here, a total 21 CCoAOMT genes were identified in wheat (TaCCoAOMT) through a in silico genome search method and they were classified into four groups based on phylogenetic analysis with the members in the same group sharing similar gene structures and conserved motif compositions. Furthermore, the expression patterns and co-expression network which these TaCCoAOMT involved in were comprehensively investigated using 48 RNA-seq samples from Fusarium graminearum-infected and control samples of 4 wheat genotypes. Combined with qRT-PCR validation of 11 Fg-responsive TaCCoAOMT genes, the potential candidates involving in FHB response and their regulation modules were preliminarily revealed. Additionally, we also investigated the genetic diversity and main haplotypes of these CCoAOMT genes in bread wheat and its relative populations based on resequencing data.

Conclusion

This study systematically identified and characterized the CCoAOMT gene family in wheat, which not only provided the targets for further functional analysis, but also contribute to the mechanism of lignin biosynthesis and its role in FHB tolerance in wheat and beyond.

Background

Wheat is considered as one of the most important staple crops all over the world, which accounts for approximately 30% of the global cultivated area, and provides 20% of the world's food consumption [1, 2]. Wheat is also an important source of human protein and mineral elements intake [3, 4]. Continuous increased and stable production of wheat holds the promise for ensuring global food security under the challenge of population booming and climate change as well as limited resource input in future [5]. Fusarium head blight (FHB), that is also called scab and caused mainly by Fusarium graminearum (Fg), is one of the most destructive diseases of wheat, resulting in huge loss of wheat yield and also imposing great health threats on both human beings and livestock due to the DON toxin [6, 7]. More seriously, FHB has gradually become the major hazard and limitation of wheat production in recent years because of the climate change and the expansion of conservation agriculture [8]. Thus, revealing the mechanism of FHB resistance and then breeding for FHB-tolerant wheat varieties are crucial to cope with these problems. Cell wall was mainly composed of polysaccharides, phenolic compounds and proteins. In plant cells, cell wall always acts mechanical and regulatory functions [9]. Extensive studies had reported that the components of cell wall endow plants with the ability to resist the invasion of pathogens [10, 11]. For example, Giancaspro et al reported the putative markers of FHB resistance through analyzing the expression pattern of pectin methyltransferase WheatPME-1 and β, 1–3 Glucanase (Glu-1), which involved in cell wall metabolism as well as regulated the non-specific lipid transfer protein (nsLTP-1) [10]. Lioi et al showed that PMEIs could dynamically regulate the PME activity and pectin methylsterification during Botrytis infection, and AtPME10, AtPME11 and AtPME12 were verified to play the significant roles in cell wall metabolism to enhance plant immunity [12].

Lignin is the second most abundant component of the plant cell wall. It has been demonstrated that the dehydrogenative polymerization of three hydroxycinnamyl alcohols, p-coumaryl, coniferyl, and sinapyl alcohols contributed to lignin biosynthesis, of which these three hydroxycinnamyl alcohols give rise to the p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units of the lignin polymer, respectively [13]. And O-Methyltransferases (OMTs) play an important role in regulating these secondary metabolic processes involving in lignin biosynthesis. OMTs are generally classified into two types, including caffeic acid O-methyltransferase (COMT) and caffeoyl-coenzyme A O-methyltransferase (CCoAOMT), of which COMT controls the pathway of S unit and CCoAOMT affects the pathway of S and G unit [14]. It is found that COMT could catalyze the O-methylation at the 5 position of the aromatic ring and CCoAOMT function to form the 3 position of the aromatic ring [15, 16]. The hydroxylation and methylation steps are crucial to determine the lignin composition and also the S/G ratio is a major determinant of lignin quality [14].

In light of its significance, CCoAOMT gene family has been systematically investigated and analyzed in many plants, such as Arabidopsis and rice [17], citrus [18], switchgrass [19], dove tree [20], tea plant [21] and sorghum [22]. In wheat, Nguyen et al analyzed the expression patterns and potential functions of some genes involving in lignin biosynthesis including several CCoAOMTs, and indicated that lodging resistance, tolerance against biotic and abiotic stresses and feedstock quality of wheat biomass were closely associated with its lignin content [23]. Ma and Luo verified that TaCCoAOMT1 was an important gene for regulating lignin biosynthesis, which is critical for stem development [24]. Soni et al silenced the function of TaNAC032 via VIGS method and found that the total lignin content in the rachis of TaNAC032 silenced wheat decreased significantly and the transgenic plant showed susceptible to Fg infection [25], which demonstrated the role of lignin in FHB tolerance.
Although some CCoAOMT gene has been functionally characterized in wheat, the genomic organization, evolutionary relationship and regulatory modules of wheat CCoAOMT gene family are not well understood up to now, especially those associated with FHB tolerance. In this study, we performed an in silico genome-wide search method to identify and characterize CCoAOMT family in wheat using the updated reference genome information. Furthermore, the phylogenetic relationship, conserved motif and cis-elements of them were systematically analyzed. Furthermore, the expression patterns and co-expression network of TaCCoAOMT genes under Fg treatment were studied and FHB-responsive TaCCoAOMTs as well as the regulation modules were obtained. Finally, the genetic diversity and genetic divergence of CCoAOMT genes in different Triticum species were also investigated based on resequencing data to reveal the evolutionary effect of this family during wheat formation.

Results

Genome-wide identification of CCoAOMT genes in wheat

Using the genome-wide search method described in Methods section, a total of 21 CCoAOMT genes were detected in the wheat genome. Since there is no standard nomenclature, these identified CCoAOMT genes were named as TaCCoAOMT1 to TaCCoAOMT21 based on their chromosome location (Table 1). It is found that these TaCCoAOMT genes were mainly located in chromosome group 7 (66.67%) while not found on chromosome group 2, 3 and 6. The gene size of TaCCoAOMT genes ranged from 447 (TaCCoAOMT17) to 4567 (TaCCoAOMT12) bp in length. The average lengths of CDS and amino acid sequences were 761 bp and 253 aa, respectively. Isoelectric point (pI) of them ranged from 4.89 (TaCCoAOMT15) to 11.11 (TaCCoAOMT17) and and molecular weight (Mw) ranged from 16284.2 (TaCCoAOMT17) and 34158.48 (TaCCoAOMT21), respectively. A search for orthologs of TaCCoAOMT genes revealed that 20 (95.24%) TaCCoAOMTs had the orthologs with Arabidopsis and rice, expect for TaCCoAOMT6. Subcellular localization prediction showed that most of them were located in the cytoplasm and chloroplast, and only two genes was located in the mitochondiral and nuclear, respectively. In these 21 TaCCoAOMT genes, we also found four homoeologous gene groups with each contained A, B and D homoeologous copies, and all of them were localized on chromosome group 7, resulting in chromosome group 7 having the most abundant TaCCoAOMT genes.

Phylogenetic relationship, exon-intron structure and conserved motifs analysis

Phylogenetic tree were constructed using the full-length protein sequences of the CCoAOMT genes in wheat, Arabidopsis and rice (Figure 1). Result showed they were classified into 4 groups based on phylogenetic relationship, with 4, 7, 3 and 7 TaCCoAOMT genes belonging to class I to IV, respectively. TaCCoAOMT and OsCCoAOMT genes were distributed in all of groups. However, AtCCoAOMT genes were mainly clustered in class III and just two genes (AtCCoAOMT5 and AtCCoAOMT6) in class I.

Then, the exon-intron and motif structures of TaCCoAOMTs were further analyzed (Figure 2). Exon number of TaCCoAOMTs ranged from 1 to 10, of which two genes contained only one exon, and 85.71% genes had 5 exon or less (Figure 2B). Furthermore, the conserved motif was identified and 10 high confidence motifs were predicted (Figure 2C and Figure S1). Compared to other classes, Motif 6 and 9 was the specific type in class I and III, respectively. Although few differences of motif were found between Class II and III, the exon number of TaCCoAOMT genes in class II was more than that of class III. Motif 2 was identified in 20 (95.24%) TaCCoAOMTs, with the exception of TaCCoAOMT2. Motif 3 was also identified in 20 TaCCoAOMTs with the exception of TaCCoAOMT17. In addition, with the exception of TaCCoAOMT5 and TaCCoAOMT17, Motif 1 was identified in 19 TaCCoAOMT genes, and Motif 4 was found in 19 TaCCoAOMT genes excepted for TaCCoAOMT2 and TaCCoAOMT17. This result showed that Motif 1, 2, 3, 4 was significantly abundant in TaCCoAOMTs, and all of them was found to be related to O-methyltransferase based on PFAM analysis, which further supported the prediction.Meanwhile, the members in the same groups based on phylogenetic relationship shared the similar exon-intron structure and motif compositions.

Cis-element analysis of TaCCoAOMTs

A total 44 types of cis-elements were identified in the 1.5-kb genomic sequences upstream from the transcription start sites (TSS) of TaCCoAOMT genes, with the functions primarily associating with stress response (Table S1 and Figure S2). CAAT-box was identified in all of TaCCoAOMTs (21), followed by CGTCA-motif (19) and TGACG-motif (19). Together with CGTCA motif and TGACG motif, ABRE related to the abscisic acid responsiveness was identified in 18 TaCCoAOMT genes. In addition, CCAAT-box (MYB Hv1 binding site), MBS (MYB binding site involved in drought-inducibility), TCA-element (cis-acting element involved in salicylic acid responsiveness), TC-rich repeats (cis-acting element involved in defense and stress responsiveness), MRE (MYB binding site involved in light responsiveness), SARE (cis-acting element involved in salicylic acid responsiveness) and WUN-motif (wound-responsive element) also were identified in 13, 7, 7, 2, 1, 1 and 1 TaCCoAOMT genes, respectively.

In order to get some clues about the biological function of TaCCoAOMTs, we performed GO (gene ontology) enrichment analysis of them as the all wheat protein as background. Results showed that they was significantly enriched into 10, 10 and 1 terms in biological process, molecular function and cell component classes, respectively (Figure S3 and Table S2). Among them, lignin biosynthetic process (GO:0009809), O-methyltransferase activity (GO:0008171) and caffeoyl-CoA O-methyltransferase activity (GO:0042409) were significant enriched, verifying the correlation between TaCCoAOMTs and lignin biosynthetic.

Expression patterns of TaCCoAOMT genes under different tissues and abiotic stress
The spatio-temporal expression patterns of TaCCoAOMTs were investigated using 58 RNA-seq samples of different tissues as well as under low temperature, salt, heat and drought stresses. A total of 20 TaCCoAOMTs were found to express in different tissues and some gene exhibited tissue-specific expression. Totally, 3 (TaCCoAOMT3, 14, 18) genes were highly expressed in all of the five differential tissues (Figure 3A). However, TaCCoAOMT1, 2, 3 were only expressed in leaf and TaCCoAOMT5 was only expressed in root. Most of the TaCCoAOMTs showed high expression in grain and leaf compared to root, spike and stem.

At low temperature treatments (Figure 3B), we found that the expression level of eight genes was higher at 23 °C than that of 4 °C, indicating these genes may be more responsive to low temperature stress. After salt stress treatments (Figure 3C), 13 out of 21 TaCCoAOMT genes were down-regulated in 6 h, 8 and 11 genes were down-regulated in 12 h and 24 h respectively, indicating their negative effects related to salt stress. Under drought stress (Figure 3D), 8 and 5 TaCCoAOMT genes were up-regulated in two time points respectively, four genes (TaCCoAOMT7, 8, 10, 11) were up-regulated at both time points. However, 9 genes were down-regulated at both time points, indicating the negative effects of TaCCoAOMTs exerted when in response to drought stress.

Comprehensive analysis of the expression profiles of TaCCoAOMT genes under Fg infection

According to previous study, lignin biosynthesis process related gene played the significant role in the response to F. graminearum (Fg) infection[26]. To identify the TaCCoAOMTs involving in Fg response, we further used 48 RNA-seq samples from Fg-infected and control samples of three FHB-tolerant genotypes and one FHB-sensitive genotype at 2 day and 4 days post inoculation (dpi) [27]. Expression pattern of TaCCoAOMT genes showed obvious differentiation between 2 and 4 dpi. In 2 dpi, 13, 12, 12 and 11 of TaCCoAOMTs expressed in genotype HC374, NyuBai, Wuhan 1 and Shaw, respectively (Figure 4), of which 10 (TaCCoAOMT3, 8, 9, 10, 11, 14, 15, 18, 19, 20), 10 (TaCCoAOMT3, 8, 9, 10, 11, 14, 15, 18, 19, 20), 6 (TaCCoAOMT8, 9, 10, 14, 19, 20) and 8 (TaCCoAOMT8, 9, 10, 11, 14, 15, 19, 20) were differentially expressed between Fg-infected and control treatments in HC374, NyuBai, Wuhan 1 and Shaw, respectively. Furthermore, the homologous group TaCCoAOMT 10, 14 and 19 showed differential expression among all of the four genotypes. In 4 dpi, there were 12, 11, 10 and 10 TaCCoAOMT genes expressed in HC374, NyuBai, Wuhan 1 and Shaw, respectively (Figure 4). Although there was no significant change in the number of TaCCoAOMTs expressed between 2dpi and 4dpi, the number of DEG varied. A total of 7 (TaCCoAOMT8, 10, 11, 14, 15, 19, 20), 9 (TaCCoAOMT3, 8, 10, 11, 14, 15, 18, 19, 20), 7 (TaCCoAOMT8, 9, 10, 11, 15, 19, 20) and 9 (TaCCoAOMT8, 9, 10, 11, 13, 14, 15, 19, 20) DEGs were found in HC374, NyuBai, Wuhan 1 and Shaw, respectively. Compared to 2 dpi, TaCCoAOMT11 displayed differential expression in Wuhan 1 and TaCCoAOMT13 showed differential expression in Shaw. It is worth noting that TaCCoAOMT 10, 14 and 19 were still significantly differentially expressed between Fg infection and control treatment in all of the four genotypes, suggesting these three genes played the vital role in response to Fg infection.

To further verify the expression of TaCCoAOMT genes under Fg treatment, 11 genes were randomly selected to validate their expression in the resistant and sensitive recombinant inbred lines (RIL) by qRT-PCR analysis (Figure 5). Results demonstrated the expression trend of these genes was consistent with that of RNA-seq analysis. Compared to control, 8 genes (TaCCoAOMT2, 9, 10, 12, 13, 16, 20) displayed the up-regulated expression after Fg treatment in both tolerant and sensitive lines, indicating that they might participate in the response process of Fg infection. Interestingly, the expression level of TaCCoAOMT8 and TaCCoAOMT15 displayed down-regulated expression in resistant line while up-regulated expression in sensitive line, suggesting their expression associated with the susceptibility to Fg infection. At the same time, TaCCoAOMT19 showed up-regulated expression in resistant line while down-regulated expression in sensitive line, indicating it might act the crucial role in FHB tolerance.

Co-expression network and regulation module of FHB-responsive TaCCoAOMTs

To better understand the function and regulation network of the identified FHB-responsive TaCCoAOMTs, we further constructed the WGCNA co-expression network based on these RNA-seq data. Through constructing weighted correlation network, 34 co-expression modules were obtained (Figure 6). Then, we linked the co-expression modules with the available phenotypic data of the Fg inoculation, including Percentage (Fusarium oxysporum inoculum), DON (Deoxynivalenol), GAPDH (Glyceraldehyde-3-phosphate dehydrogenase content) and inoculation time [27]. The module-trait association results showed that saddlebrown, blue, greenyellow, lightcyan and green module were highly correlated with Fg inoculation (Figure 6). Then, we linked the co-expression modules with the available phenotypic data of the Fg inoculation, including Percentage (Fusarium oxysporum inoculum), DON (Deoxynivalenol), GAPDH (Glyceraldehyde-3-phosphate dehydrogenase content) and inoculation time [27]. The module-trait association results showed that saddlebrown, blue, greenyellow, lightcyan and green module were highly correlated with Fg inoculation (Figure 6A). Meanwhile, tan, darkolivegreen, blue and greenyellow were highly correlated with Fg percentage, DON and GAPDH. These important modules also were correlated with the time of Fg inoculation. Interestingly, 2, 5, 1, 1 and 2 FHB-responsive TaCCoAOMTs were found in blue, green, lightyellow, turquoise and yellow module, respectively. Furthermore, there modules also showed the strong correlation and associated with Fg inoculation (Figure 6B). A total of 93 genes were associated by 25 miRNAs (Table S3) and 50 miRNA-TaCCoAOMT interactions were constructed, of which four different TaCCoAOMT genes (TaCCoAOMT8, 11, 15, 20) were targeted by miR1119, and miR9781 targeted three TaCCoAOMT genes, including TaCCoAOMT11, 15 and 20.
It is showed that TaCCoAOMT genes were mainly inhibited by miRNAs through transcript cleavage (86.27%). Combined with miRNA-TaCCoAOMT relationship and co-regulation modules TaCCoAOMT involved in, we further obtain the miRNA-mediated networks associated with FHB response and resistance that were mainly regulated by CCoAOMT genes in wheat (Figure 7), which provided the useful clues to regulate the expression of TaCCoAOMTs to control lignin biosynthesis and then enhance FHB tolerance through post-transcriptional approach.

2.7 Genetic diversity and haplotype analysis of CCoAOMT family in wheat and its relatives

Based on the resequencing data of Triticum species [28], the genetic variations of CCoAOMT genes in wheat and its diploid and tetraploid relatives were investigated, including the nucleotide diversity (\(\pi\)), population divergence (\(F_{st}\)) and Tajimas’D values. The average value of \(\pi\) in Triticum urartu, Aegilops tauschii, wild emmer, domesticated emmer, durum wheat and bread wheat was 0.000565, 0.00535, 0.00297, 0.00321, 0.00320 and 0.00233, respectively (Figure 8A), together with the average value of Tajimas’D of them was -0.834, 1.094, 0.145, 0.370, 0.292 and -0.137, respectively (Figure 8B). Due to too few SNPs identified from the re-sequencing data, Triticum urartu showed the abnormally lower value of nucleotide diversity. It is found that Aegilops tauschii displayed the highest nucleotide diversity in CCoAOMT genes, while bread wheat had the most lower nucleotide diversity except for T. urartu with the value decreased by 2 times, suggesting that significant genetic bottleneck occurred in CCoAOMT gene family during wheat evolution. Then, the gene flow and genetic divergence between bread wheat and its relatives were also detected. In A subgenome, the \(F_{st}\) value between bread wheat and T. urartu was 0.556, ranked the largest, followed by that of wild emmer and T. urartu with the value of 0.480 as well as bread wheat and wild emmer with the value of 0.248, indicating the high divergence of bread wheat with T. urartu compared to wild emmer at A subgenome level from the perspective of CCoAOMT gene family (Figure 8C). In B subgenome, the divergence between bread wheat and wild emmer was larger than that of durum and wild emmer, and bread wheat showed closer to durum wheat compared to wild emmer wheat (Figure 8D). In D subgenome, the \(F_{st}\) value of between bread wheat and Aegilops tauschii was 0.604 (Figure 8E). On the whole, the genetic divergence at D subgenome was highest, followed by A subgenome and B.

The samples of resequencing data contained 30 Aegilops tauschii samples, 29 Triticum urartu samples, 28 wild emmer (Triticum turgidum L. ssp. dicoccoides) samples, 29 domesticated emmer (Triticum turgidum L. ssp. dicoccon) samples, 13 durum (Triticum turgidum L. ssp. durum) samples and 163 bread wheat (Triticum aestivum L. ssp. aestivum) samples. Finally, we identified the haplotype organization and frequency of each TaCCoAOMTs in these populations based on the resequencing data (Table S4). A total 13 TaCCoAOMT genes were found to have the genetic variations among these populations, of which 4, 2, 7 were located on A, B, D subgenome, respectively. Then, the main haplotype and its frequencies were investigated (Figure S4, S5 and S6). It is obviously that the percentage of main haplotype in cultivated wheat was significantly larger than that of wild species at all subgenome levels, indicating the articial selection exerted on these CCoAOMT genes to result in the decline of genetic diversity and genetic bottleneck during wheat domestication and improvement processes.

Discussion

Lignin was the main component of cell wall and involved in response to the abiotic and biotic stresses [15]. The characteristic of CCoAOMT genes has been analyzed in Arabidopsis, sorghum, and other plants. However, its significance in wheat is not determined, especially its role in Fusarium head blight resistance is not well studied. In this study, we identified 21 CCoAOMT in wheat at the whole genome level. Based on the phylogenetic relationship, we divided these TaCCoAOMTs into four groups, and the TaCCoAOMTs belonging to the same group showed similar gene structure and motif organization. In sorghum, the CCoAOMT proteins were classified in class Ia, class Ib, class Ic and class II, of which class Ia was the orthologous genes with AtCCoAOMT1 and OsCCoAOMT1 and was considered as the true CCoAOMT gene, while the other classes were considered as CCoAOMT-like genes [22]. In our results, AtCCoAOMT1 and OsCCoAOMT1 also were cluster into same group (class III), which was consistent with that of sorghum. Meanwhile, CCoAOMT genes in rice and wheat can be found in each group, but AtCCoAOMTs just were found in class I and III, indicating that there was some divergence between the monocot and dicot CCoAOMT family. Additionally, class I and III has the specific motif 6 and 9, respectively. Although no specific motif found in class II and IV, their gene structure displayed the difference with the obvious difference of exon number, suggesting more type of splice variants or binding-sites may be existing in these two classes.

Based on RNA-seq samples, the expression level of these wheat CCoAOMT genes in different tissue, abiotic and biotic stresses were comprehensively analyzed. A total of 19 TaCCoAOMT genes were found to express in leaves, followed by 17 in roots and 16 in grains. Among them, TaCCoAOMT13, 14 and 18 expressed in five tissues, while TaCCoAOMT1, 2, 3 was only expressed in leaf and TaCCoAOMT5 only in root. These tissue-constitutive and specific TaCCoAOMT genes provided the useful targets for further functional study. Most of TaCCoAOMT genes were highly expressed in grains, indicating the lignin biosynthesis in grains were more active than other tissues. Simultaneously, 10 TaCCoAOMT showed significantly up-regulated expression after Fg treatment compared to the control, of which 3 (TaCCoAOMT10, 14 and 19) genes were shared in three resistant varieties and one susceptible variety, suggesting they played the important role in response to FHB. Interestingly, TaCCoAOMT10, 14 and 19 were the A, B and D homoeologues copies of the same homoeologues group respectively, of which the expression level of TaCCoAOMT19 was the highest, indicating the dominant role of them in response to FHB and asymmetry expression between homoeologues copies. It is well known that cis-regulatory elements can regulate gene expression level by binding to corresponding transcription factors, and then might determine the specific expression patterns in different tissue and stresses [29]. CGTCA-motif and TGACG-motif, which is related to MeJA-responsiveness, were identified in the promoter regions of all of the up-regulated TaCCoAOMTs. Previous study has proved that MeJA can not only
help the wheat to significantly delay the necrosis process of susceptible varieties, but also increase the activities of enzymes related to pathogen defense [30]. Therefore, these up-regulated TaCCoAOMTs containing CGTCA-motif and TGACG-motif might play the crucial role in regulating FHB tolerance through MeJA-mediated approach. We further re-constructed the co-expression by WGCNA method based on 48 RNA-seq samples of four wheat genotypes [27]. Results showed that there was a high correlation between seven modules and Fg infection. Meanwhile, TaCCoAOMT genes were found in five co-expression modules and acted as the hub factors, of which two (blue, green) modules were positively correlated with Fg infection and three (lightyellow, turquoise, yellow) modules were negative. These molecular modules not only provided the vital insight into the genetic basis and regulation network of TaCCoAOMT involved in, but also contributed to reveal the their roles in FHB resistance. Furthermore, we predicted the miRNAs which could target on the TaCCoAOMT genes. And eight microRNAs were found to target on the five TaCCoAOMT genes in the Fg infection related modules, including tae-miR167a and tae-miR1119. Previous studies had been reported that miR167a could mediate auxin signaling to respond to biotic stresses in tomato [31], and miR1119 was proven to regulate the expression of actin under stress conditions and activate plant defense signaling pathway in barley [32]. We postulated that these two miRNAs also play the regulatory role in the response to Fg infection through regulating on CCoAOMT genes in wheat. The miRNA-CCoAOMT mediated networks that associated with FHB response and resistance provided the insight to better understand the molecular mechanism underlying FHB resistance in wheat.

Finally, we used the resequencing data to investigate the genetic variations and divergence of CCoAOMT family in wheat and its relative populations. Results found that wild species showed high genetic diversity and rich haplotype composition in this family compared to cultivated species, suggesting selection effect was exerted on this family and obvious genetic bottleneck has occurred at it during wheat domestication and improvement processes[33]. Wild populations possessed the specific haplotypes on the CCoAOMT genes which have been lost in cultivated populations, which hold the promising for enriching the genetic diversity and also improving the traits that CCoAOMT genes controlling in cultivated wheat, such as FHB resistance.

Conclusion

It is the first study to identify the CCoAOMT family in wheat at the whole genome level. The genomic organization, phylogenetic relationship, exon-intron structure, conserved motif composition and cis-elements as well as expression profiles of this family were systematically investigated and characterized. Especially, the expression patterns and co-expression network of these TaCCoAOMTs involved in Fg infection were comprehensively identified and the FHB-responsive candidates and their regulation modules were obtained, which provided the insight on the roles of TaCCoAOMTs playing in the FHB response and tolerance. Additionally, we also detected the genetic diversity and haplotype frequencies of these CCoAOMT genes in wheat and its relative populations based on resequencing data. This study not only shed light on the potential function of CCoAOMT family in regulating wheat lignan biosynthesis and FHB tolerance, but also provided the vital clues for the evolution of this family in wheat and beyond.

Methods

Identification of CCoAOMT genes in wheat

For the identification the CCoAOMT family in wheat, the protein sequences of the wheat genome were retrieved from the Ensembl plant database to use as the local protein database (http://plants.ensembl.org/index.html). CCoAOMT gene of Arabidopsis and rice [17] were used to perform BLASTP search against the local protein database with the threshold of E-value < 1e-5 [34]. The domain (PF01596) was downloaded from PFAM database (https://pfam.xfam.org/) and used as the query to search against local protein database using the HMMER 3.0 with the threshold of E-value < 1e-5. The results of HMMER and BLASTP were integrated together, and the redundant were manually removed. Then, the putative wheat CCoAOMT genes were submitted to SMART (http://smart.embl-heidelberg.de/) and PFAM database(PF01596) (http://pfam.xfam.org/search) to predict the conserved protein domain, and then those having complete CCoAOMT domain were remained as candidates. The candidate TaCCoAOMT proteins were submitted to ExPAsy (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) database to compute theoretical isoelectric point (pl) and molecular weight (Mw). The cello tool (http://cello.life.nctu.edu.tw/) was used to predict the subcellular localization.

Phylogenetic, gene structure, conservative motif and cis-element analysis of TdPUB

Multiple sequence alignment was performed using ClustalX v2.0 [35]. Neighbor-joining method imbedded in MEGA-X program was used to construct the phylogenetic tree and bootstrap was set to 1000 [36]. Additionally, conserved motifs of TaCCoAOMT proteins were identified using MEME v5.2.0 with the default parameters. The gene and motif structures were displayed based on GTF annotation files using TBtools [37] The upstream 1500bp region of each TaCCoAOMT genes were extracted and submitted to the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) to predict cis-elements.

Expression analysis of TaCCoAOMT genes using RNA-seq

A total of 106 RNA-seq samples under different tissues (grain, leaf, root, spike, stem) and treatments (heat, salt, low temperature, drought, F. graminearum inoculation) were download from URGI (http://wheat-urgi.versailles.inra.fr/) and NCBI database (https://www.ncbi.nlm.nih.gov/) (accession no. SRP045409, SRP062745, SRP043554 and SRP045409). All of these RNA-seq data was mapped onto reference genome of wheat
reference genome IWSGCv1.1 by STAR v2.7.6a [38] and the fragments per kilobase per million (FPKM) were calculated by StringTie v2.1.2 [39]. The expression patterns were displayed using R package. Differential expression genes were determined by edgeR package [40]. The Gene ontology (GO) enrichment were analyzed using AgriGO v2 (http://systemsbiology.cau.edu.cn/agriGOv2/index.php) and Triticum aestivum was set as background. The GO enrichment results were plot by ggplot2 and divided into three classes.

**FHB related co-expression networks construction and miRNA analysis**

Co-expression network was constructed using the WGCNA tools based on the 48 RNA-seq samples of four genotype wheat under F. graminearum treatment. To obtain the correct module number and clarify gene interaction, we set the restricted minimum gene number to 30 for each module and used a threshold of 0.25 to merge the similar modules. Genes that have higher weight in important modules were chosen to constructed co-expression network. The traits data publically available, including Fg treatment, Fg time, Fg percent, Fg GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) and DON (Deoxynivalenol) were used for trait-module correlation analysis [27]. miRNA binding sites were predicted using psRNATarget (http://plantgrn.noble.org/psRNATarget/analysis) with default parameters and all of the wheat miRNAs were used. The regulatory network of TaCCoAOMT gene and miRNA were visualized using Cytoscape v3.8.0 [41].

**Haplotype and population genetics analysis of TdPUB**

VCF file of wheat resequencing were downloaded from Genome Variation Map (https://bigd.big.ac.cn/gvm) (accession no. GVM000082) [28], which contained the genome variations of a total of 163 bread wheat accessions, 13 durum wheat accessions, 29 domesticated emmer wheat accessions, 28 wild emmer wheat accessions, 29 T. urartu accessions and 30 Aegilops tauschii accessions. SNPs in the coding region of TaCCoAOMT genes were extracted based on the chromosome location using TBtool tool. Furthermore, the haplotype organization and frequency were investigated by an inhouse Python script.

**Validation of the expression of TaCCoAOMTs through qRT-PCR analysis**

For experiment verification, a FHB resistant RIL line (R75) and a FHB susceptible line (S98) from the wheat RIL population developed by single-seed descent from a cross between the susceptible US wheat variety Wheaton and the Chinese resistant wheat landrace HYZ, which were provided generously by Prof. Tao Li, Yangzhou University, China, are used. The plant material culture and Fg inoculation were performed following the previous described method by Li et al [42]. And the inoculated spikelet samples together with counterpart CK samples were collected from 4 to 5 spikes at 2 days post inoculation (dpi) and three biological replications were adopted. RNA Easy Fast Plant Tissue Kit (Tiangen, Beijing, China) was used to extract total RNA of all sample and RT Master Mix Perfect Real-Time kit (Takara, Dalian, China) was used to synthesized cDNAs according to the manufacturer's instruction. qRT-PCR reaction were performed on the QuantStudioTM 7 Flex System (Thermo Fisher Scientific, USA) using SYBR® Green Premix Pro Taq HS qPCR Kit (Accurate Biology, Hunan, China) with the thermal cycling condition was 95°C for 30 s followed by 40 cycles of 95°C for 3s, 60°C for 30 s. Three technological replications were applied. The expression levels of these 11 randomly selected TaCCoAOMTs were calculated using the 2−ΔΔCT method with TaActin2 as the internal reference gene. The primers used in this study were listed in additional file (Table S5).

**Abbreviations**

FHB: Fusarium head blight;

Fg: Fusarium graminearum;

CCoAOMT: caffeoyl-coenzyme A O-methyltransferase;

TaCCoAOMT: Wheat CCoAOMT;

HMM: Hidden Markov Model;

CDS: Coding sequence;

GO: Gene ontology;

qRT-PCR: Quantitative real-time polymerase chain reaction

**Declarations**

**Ethics approval and consent to participate**

Not applicable
Consent for publication

Not applicable

Availability of data and materials

All of the datasets supporting the results of this article are included within the article and its Additional files. And the datasets generated for phylogenetic tree analysis during the current study are available in the Treebase repository, http://purl.org/phylo/treebase/phylows/studyTB2:S27750?
x-access-code=7fc2b4648b76436128174b93773e0694&format=html.

Competing interests

The authors declare that they have no competing interests

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Author Contribution

YG performed analysis and drafted the manuscript. PWQ collected data and performed the RT-PCR experiments. ZRY and PY contributed to plant material collection. GQF completed the visualization of the data. SWN revised the manuscript. ZWJ and NXJ conceived this study and revised the manuscript. All authors read and approved the nal manuscript.

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Competent interest

All authors have declared that there no competent interest existed.

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Tables

Table 1. Characteristics of the CCoAOMT genes identified in wheat
| TaCCoAOMT   | Transcript       | Gene Length (bp) | Protein Length (aa) | Exon number | Splice variant | pl   | Mw (Da)     | Subcellular localization | Orthologs                  |
|------------|------------------|-----------------|---------------------|-------------|----------------|------|-------------|----------------------------|-----------------------------|
| TaCCoAOMT1 | TraesCS1B02G023200.2 | 2088            | 238                 | 5           | 2              | 5.03 | 26054.06   | Cytoplasmic                | AtCCoAMT, AtTSM1, OsOMT26   |
| TaCCoAOMT2 | TraesCS1B02G049800.1  | 1168            | 210                 | 2           | 1              | 5.55 | 23350.86   | Cytoplasmic                | AtCCoAMT, AtTSM1, OsOMT26   |
| TaCCoAOMT3 | TraesCS1D02G019000.1  | 1245            | 248                 | 2           | 1              | 5.02 | 27035.03   | Cytoplasmic                | AtCCoAMT, AtTSM1, OsOMT26   |
| TaCCoAOMT4 | TraesCS4A02G442400.1  | 1355            | 246                 | 2           | 1              | 4.98 | 27050.77   | Cytoplasmic                | AtCCoAMT, AtTSM1, OsOMT26   |
| TaCCoAOMT5 | TraesCS4D02G362500.1  | 942             | 190                 | 1           | 1              | 5.04 | 20387.36   | Chloroplast                 | AtCCoAMT, OsROMT27          |
| TaCCoAOMT6 | TraesCS5A02G257600.1  | 1136            | 252                 | 5           | 2              | 5.39 | 27304.38   | Chloroplast                 | -                           |
| TaCCoAOMT7 | TraesCS5D02G265900.1  | 1586            | 242                 | 5           | 1              | 5.15 | 26189.03   | Chloroplast                 | AtCCoAMT                   |
| TaCCoAOMT8 | TraesCS7A02G068600.1  | 1648            | 288                 | 4           | 2              | 7.01 | 31886.32   | Mitochondrial               | AtCCoAMT, AtTSM1, OsROMT27  |
| TaCCoAOMT9 | TraesCS7A02G127600.1  | 1093            | 284                 | 3           | 1              | 5.04 | 31491.93   | Cytoplasmic                 | AtCCoAOMT1, OsROMT16        |
| TaCCoAOMT10| TraesCS7A02G240300.1  | 1753            | 247                 | 2           | 1              | 4.99 | 27088.92   | Cytoplasmic                 | AtCCoAMT, AtTSM1, OsOMT26   |
| TaCCoAOMT11| TraesCS7A02G240400.1  | 1219            | 245                 | 3           | 1              | 4.95 | 27071.88   | Cytoplasmic                 | AtCCoAMT, AtTSM1, OsOMT26   |
| TaCCoAOMT12| TraesCS7A02G302500.1  | 4567            | 300                 | 10          | 2              | 8.59 | 32885.04   | Chloroplast                 | AtOMTF3                     |
| TaCCoAOMT13| TraesCS7B02G027200.1  | 1729            | 267                 | 4           | 1              | 5.11 | 29506.65   | Cytoplasmic                 | AtCCoAOMT1, OsROMT16        |
| TaCCoAOMT14| TraesCS7B02G135900.1  | 1854            | 302                 | 2           | 1              | 5.54 | 33139.88   | Chloroplast                 | AtCCoAMT, OsOMT26           |
| TaCCoAOMT15| TraesCS7B02G136000.1  | 1734            | 245                 | 3           | 1              | 4.89 | 27124.85   | Cytoplasmic                 | AtCCoAMT, AtTSM1, OsROMT17  |
| TaCCoAOMT16| TraesCS7B02G202600.1  | 4218            | 294                 | 9           | 1              | 7.57 | 32151.1    | Chloroplast                 | AtOMTF3                     |
| TaCCoAOMT17| TraesCS7D02G063100.1  | 447             | 148                 | 1           | 1              | 11.11 | 16284.2   | Nuclear                     | AtCCoAMT, AtTSM1             |
| TaCCoAOMT18| TraesCS7D02G126000.1  | 2465            | 260                 | 4           | 1              | 5.11 | 28864.96   | Cytoplasmic                 | AtCCoAOMT1, OsROMT16        |
| TaCCoAOMT19| TraesCS7D02G239200.1  | 1548            | 247                 | 2           | 1              | 4.98 | 27074.9    | Cytoplasmic                 | OsOMT26                     |
| TaCCoAOMT20| TraesCS7D02G239400.1  | 1185            | 245                 | 3           | 1              | 4.94 | 27121.94   | Cytoplasmic                 | OsOMT26                     |
| TaCCoAOMT21| TraesCS7D02G297800.1  | 3828            | 311                 | 9           | 2              | 8.38 | 34158.48   | Chloroplast                 | AtOMTF3                     |

**Additional Files**

**Supplementary Figures**

Figure S1. Motifs found in TaCCoAOMT genes.

Figure S2. Prediction of the cis-element in promoter regions of TaCCoAOMT genes.

Figure S3. GO enrichment for TaCCoAOMT proteins.
Figure S4. Main haplotype and frequency of TaCCoAOMT genes in A subgenome of Triticum.

Figure S5. Main haplotype and frequency of TaCCoAOMT genes in B subgenome of Triticum.

Figure S6. Main haplotype and frequency of TaCCoAOMT genes in D subgenome of Triticum.

Supplementary Tables

Table S1. Characteristics of cis-acting regulatory elements in the promoter region of CCoAOMT genes in wheat.

Table S2. GO enrichment analysis of the identified TaCCoAOMTs.

Table S3. Identification of miRNA bind sites in TaCCoAOMT genes.

Table S4. Haplotype of CCoAOMT genes in five Triticum species.

Table S5. The list of primers used for qRT-PCR in this study.