Genome-wide identification and expression analysis of the coronatine-insensitive 1 (COI1) gene family in response to biotic and abiotic stresses in Saccharum

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Abstract

Background: The coronatine insensitive 1 (COI1) gene is the core member of jasmonate signaling pathway, which is closely related to plant biotic and abiotic resistance. However, there have been no reports on COI1 in sugarcane (Saccharum spp.). Hence, systematically investigating the characteristics of the COI1 multigene family in sugarcane can provide a means to study and manipulate the jasmonic acid signaling pathway.

Results: A total of 156 COI1 proteins were obtained from the genomes of 19 land plants, while none were obtained from five algae species. A phylogenetic tree demonstrated that these COI1 proteins were classified into four groups, while 31 proteins of SsCOI1 from Saccharum spontaneum, SbCOI1 from Sorghum bicolor, and ShCOI1 from Saccharum spp. hybrid cultivar R570 clustered into three groups. Synteny analysis and duplication patterns revealed that COI1 genes expanded through various genome replication events and could have experienced strong purifying selective pressure during evolution in S. spontaneum, S. bicolor, and R570. An investigation of cis-acting elements suggests that COI1 genes may be involved in plant growth and development and response to various stresses. Expression analysis implied that 21 SsCOI1 genes were constitutively expressed, and had positive responses to drought, cold, and Sporisorium scitamineum stresses with different expression patterns. Among them, seven SsCOI1 haplotype genes may play different roles in response to methyl jasmonate. Furthermore, the ShCOI1–4, ShCOI1–5, and ShCOI1–6 genes were cloned from Saccharum spp. hybrid cultivar ROC22. Real-time quantitative PCR (RT-qPCR) analysis demonstrated that these three ShCOI1 genes had divergent expression profiles in response to salicylic acid, abscisic acid, polyethylene glycol, cold, and S. scitamineum.

Conclusions: These results suggest that COI1 genes may act in sugarcane growth, development, and response to various stresses via different regulatory mechanisms, which laying a foundation for the functional identification of the sugarcane COI1 gene.

Keywords: Sugarcane, COI1 gene family, Whole genome analysis, Biotic and abiotic stresses, Expression analysis

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the core members of the JA-signaling pathway, including SClF^COI1 (SCF, SKP1 + Cdc53/cullin+Rbx1 + F-box; COI1, coronatine insensitive 1) E3 ubiquitin ligase, jasmonate ZIM-domain (JAZ) repressor proteins, and myelocytomatosis2 (MYC2) transcription factor, have been defined as the COI1/ JAZs/MYC2 module [5, 6].

In recent years, the mechanistic details of the JA signal transduction pathway and its regulatory network have gradually been revealed [7–9]. Research on jasmonics mainly focuses on their interaction with other hormones [10–12], and the responses of JA signals to pathogenic bacteria or pests [10, 13, 14]. JA content is low in plants under normal growth conditions, and JAZ inhibits the expression of JA-response genes through direct interaction with transcription factors such as MYC2. JA-mediated responses are therefore repressed by JAZ proteins [6, 15]. In response to stresses, such as that caused by insect feeding or pathogen infection, JA is accumulated rapidly, and jasmonyl-l-isoleucine (JA-Ile) is formed under the action of jasmonic acid-amido synthetase (JAR1) [6, 16]. The increase of JA-Ile levels promotes the interaction between the JAZ repressor protein and the F-box protein encoded by COI1, making JAZs ubiquitinate and degrade via the 26S proteasome pathway, releasing DNA-binding transcription factors (such as MYC2) and inducing the expression of JA response genes [6, 15]. In addition, the deletion of the COI1 locus or its functionally deficient mutations can lead to the elimination or weakness of plant responses to JA [13, 17]. These results illustrate the importance of COI1 in JA signal transduction.

It has been reported that COI1 is not only involved in plant developmental processes, such as leaf senescence [18, 19], seed maturation [13], flowering [20], male fertility [21], anthocyanin formation [22], and root growth [22, 23], but also plays a part during various physiological processes in the plant defense response to insect attack and pathogen infection [13, 24, 25]. COI1 belongs to a multigene family. There are one F-box domain and 16 leucine-rich repeats (LRR) in COI1 protein [26, 27]. The F-box protein participates in the formation of the SCF^COI1 E3 ubiquitin ligase complex involved in the ubiquitin-dependent proteolytic pathway [28], and regulates the expression of JA-responsive genes [28, 29]. As a JA receptor, COI1 protein is maintained at a protein level essential for proper biological functions during plant development and defense, which is strictly regulated by the dynamic balance of SCF^COI1-mediated stabilization and 26S proteasome pathway-mediated degradation [30, 31]. To date, 35 COI1-dependent JA-regulated proteins have been identified in Arabidopsis thaliana [19]. Among them, rubisco activase (RCA), which is correlated with JA-induced leaf senescence, can be down-regulated by JA in a COI1-dependent manner [19]. In addition, Arabidopsis coi1 mutants were observed to be male-sterile [17], apical dominance defective (coi1–37) [32], susceptible to pests and bacterial pathogens [17], insensitive to JA [17], and lacking the expression of JA-induced proteins [33]. Arabi-
dopsis coi1–1 mutant plants were infertile and showed a stay-green phenotype under dark-induced senescence conditions, but those phenotypes could be rescued in mutants overexpressing 35S:OsCOI1a or 35S:OsCOI1b due to the fact that the JA signaling insensitivity of coi1–1 mutants was complemented. This finding suggests that coi1 plays a key role in leaf senescence and fertility [18, 34]. In a study by Huang et al. [22], amino acid changes in COI1 could significantly attenuate its function, not only in regulating JA-inhibited root growth and JA-induced anthocyanin accumulation, but also in JA-mediated plant response to inoculation with the pathogen Pst DC3000. However, different mutations in the COI1 gene have distinct effects on COI1 function in regulating male fertility. GhCOI1 silencing in Gladiolus hybridus impaired inducible defense and increased susceptibility to the necro-trophic pathogenic fungus Alternaria brassicicola [24]. The above findings have provided evidence of the importance of the COI1 gene during the processes of plant growth and development, as well as defense responses. However, there have been no reports on the COI1 gene in sugarcane (Sacharum spp.). Hence, a systematic investigation of the characteristics of the COI1 multigene family in sugarcane should provide an efficient basis for the study and manipulation of the JA signaling pathway.

Sugarcane is an important sugar and biofuel crop in the world [35, 36]. However, various stresses, such as pathogens, low temperatures, and drought, seriously restrict the healthy development of the sugarcane industry [37]. Due to the complex genetic background and long growth period of sugarcane, genetic engineering has great advantages in the cultivation of resistant sugarcane varieties compared to traditional cross breeding [38]. Therefore, the discovery of resistance candidate genes is of great significance. In the present study, first, 21 SsCOI1, three ShCOI1, and seven SbCOI1 genes were identified from the genomes of the sugarcane-related wild species Saccharum spontaneum [36], the sugarcane-related cultivated species Saccharum spp. hybrid cultivar R570 [38], and the sugarcane proximal species Sorghum bicolor [39], respectively. Second, the protein physicochemical properties, chromosome location, evolutionary relationship, protein motif, gene structure, cis-acting elements, tissue-specific expression, and expression profiles of the COI1 gene family under methyl jasmonate (MeJA), cold, drought, and Sporisorium scitamineum stresses were analyzed [40–42].
Third, the full-length sequences of three ShCOI1 genes (ShCOI1–4, ShCOI1–5, and ShCOI1–6) were isolated from Saccharum spp. hybrid cultivar ROC22 using a homologous cloning method. In addition, the real-time quantitative PCR (RT-qPCR) technique was used to analyze the gene expression patterns of ShCOI1–4, ShCOI1–5, and ShCOI1–6 under cold, drought, salicylic acid (SA), abscisic acid (ABA), and S. scitamineum stresses [42–44]. This study aims to uncover and identify the COI1 gene family in sugarcane, understand their sequence characteristics and gene expression patterns, and thus provide candidate gene resources for sugarcane resistance molecular breeding.

**Results**

**Identification, classification, and phylogenetic analysis of COI1 gene family**

A total of 156 COI1 proteins were obtained from 19 sequenced plant species among five lineages, including 33 COI1s in five eudicots (eight in Medicago truncatula; seven in A. thaliana, Capsella rubella, and Vitis vinifera; and four in Fragaria vesca), 87 COI1s in 10 monocots (21 in S. spontaneum; 13 in Zea mays; eight in Brachypodium distachyon and Triticum aestivum; seven in Oryza sativa, Panicum hallii, Setaria italica, and S. bicolor; six in Ananas comosus; and three in R570), four COI1s in one basal angiosperm (Amborella trichopoda), 28 COI1s in two mosses (15 in Sphagnum fallax and 13 in Physcomitrella patens), and four COI1s in one lycophyte (Selaginella moellendorffii). However, no COI1 was identified in five algae plants that belonged to Rhodophyta (Chondrus crispus, Cyanidoschyzon merolae, and Galdieria sulphuraria) and Chlorophyta (Coccomyxa subellipsoidea C169 and Micromonas pusilla CCMP1545) (Fig. 1 and Supplemental Table S1).

On the basis of the topology of phylogenetic trees and the conserved amino acid sites of F-box and JAZ-binding sites on COI1 proteins (Supplemental Fig. S1 and Supplemental Table S2) [45], 156 COI1 proteins...
were classified into four groups (group A, group B, group C, and group D) (Fig. 2). Among them, COI1 genes from the same lineage, such as mosses, monocots, and eudicots, tended to be clustered to the same branch in group A, group B, and group D, and only COI1 proteins from mosses were clustered in group C. In detail, group A contained nine SsCOI1s (SsCOI1–4a, −4b, −4c, −4e, −5, −6a, −6b, −6c, and −6d) and two SbCOI1s (SbCOI1–5 and SbCOI1–6). Group B included five SsCOI1s (SsCOI1–1a, −1b, −3a, −3b, and −3c), two SbCOI1s (SbCOI1–2 and SbCOI1–4), and ShCOI1–2. There were seven SsCOI1s (SsCOI1–2a, −2b, −7a, −7b, −8a, −8b, and −8c), three SbCOI1s (SbCOI1–1, SbCOI1–3, and SbCOI1–7), and two ShCOI1s (ShCOI1–1 and ShCOI1–3) in group D.

Characteristics of the COI1 gene family in S. bicolor, R570, and S. spontaneum

As shown in Supplemental Table S3, the number of amino acids of 31 COI1s (including 21 SsCOI1s, seven SbCOI1s, and three ShCOI1s) was 434–665, and their corresponding molecular weights (MWs) ranged from 47.94 to 73.14 kDa. The predicted isoelectric point (pI) values of 31 COI1s varied from 5.25 to 8.40. The results of the instability index suggested that 30 of 31 COI1s were unstable proteins (instability index > 40), and the grand average of hydropathicity (GRAVY) showed that 22 of 31 COI1s were hydrophilic proteins (GRAVY < 0). There were no signal peptides or transmembrane structures in these COI1 proteins, suggesting that they were all non-secreted proteins (Supplemental Table S3). Moreover, among 31 COI1 proteins, nine were predicted to be located in the cytoplasm and nucleus, four in the cytoplasm, and 18 in the nucleus (Supplemental Table S3). For the secondary structures of these proteins encoded by the SsCOI1s, ShCOI1s, and SbCOI1s, alpha helices (45.41–53.46%) and random coils (28.80–37.89%) were the main components, the extended chain (10.90–14.60%) was secondary, and beta turns (3.20–5.84%) accounted for the smallest proportion (Supplemental Table S4).

Conserved motifs and gene structures of the COI1 gene family

Homology analysis showed that the amino acid sequence similarity among 31 COI1 proteins ranged from 27.00 to 100.00% (Supplemental Table S5). A phylogenetic tree (Fig. 3) demonstrated that the protein sequences of 21 SsCOI1s, seven SbCOI1s, and three ShCOI1s were divided into three groups (groups A, B, and D), which was consistent with the above classification in Fig. 2. The number of conserved motifs in 31 COI1 proteins varied from eight to 12, and motifs 1–7 and motif 10 were included in all of these COI1 protein sequences (Fig. 3). Motif 3, motif 2, and motif 1 represented an F-box_5 domain (pfam18791), a typical LRR sequence domain, and a transport inhibitor response 1 protein domain (cl40087), respectively (Supplemental Table S6). However, several motifs were specific in subgroup members. For example, motif 9 was present in all members of group B and group D, but only in three members of group A. Group D members had two motif 8 and one motif 4, except for SsCOI1–2b. In contrast, motifs in the members of group A were relatively irregular. Likewise, among 11 members of group A, four had two motif 4, five had two motif 7, three had motif 5, and three had two motif 8. These results indicate that motif 1, motif 3, motif 6, and motif 10 are relatively conserved in the evolution of the COI1 gene family. The number of introns contained in the COI1 gene family of S. bicolor, S. spontaneum and R570 ranged from two to six (Fig. 3). The numbers of group B had two introns. In group D, except for SsCOI1–2b, SsCOI1–2a, and SsCOI1–8b, all the other nine members had two introns. However, the gene structures of group A members were irregular, with intron numbers ranging from two to six (Fig. 3).

Chromosomal location, duplication events, and synteny analysis of COI1 gene family

Chromosomal mapping showed that 21 SsCOI1 genes were unevenly distributed on 18 of 32 S. spontaneum chromosomes (Supplemental Fig. S2 and Supplemental Table S1). Among them, chromosomes Ss1A, Ss4B and Ss5C had two SsCOI1 genes, and each of the remaining 15 chromosomes had one SsCOI1 gene. Three ShCOI1 genes were evenly distributed on chromosomes Sh01, Sh04, and Sh09 among 10 R570 chromosomes (Supplemental Fig. S2). However, SbCOI1–1 and SbCOI1–2 were distributed on Sb01, and the other five SbCOI1 genes were evenly distributed on chromosomes Sb03, Sb05, Sb06, and Sb09 of S. bicolor (Supplemental Fig. S2).

To explore the expansion mechanisms, the gene types of COI1 in S. spontaneum, R570 and S. bicolor, including singleton, dispersed, proximal, tandem, and whole-genome duplication (WGD)/segmental duplications, were analyzed (Fig. 4a and Supplemental Table S7). In 21 SsCOI1 genes, 14 WGD/segmental (66.67%), three dispersed (14.29%), two proximal repeat genes (9.52%), one tandem (4.76%) duplication, and one singleton gene (4.76%) were found. Interestingly, all three ShCOI1 genes in R570 had dispersed duplications. Among seven SbCOI1 genes, five were detected as dispersed genes (71.43%), and two were WGD/segmental duplications (28.57%) (Fig. 4a and Supplemental Table S7). Therefore, it can be speculated that the SsCOI1 gene family mainly expanded through WGD or segmental duplication events, and the dispersed duplications appear to be the main expansion mechanisms for the SbCOI1 gene family and ShCOI1 gene family.
Fig. 2 Phylogenetic analysis and classification of 156 COI1 proteins in 19 plant species. The phylogenetic tree was constructed using the maximum likelihood method (JTT + G model, complete deletion, and 1000 bootstrap replicates) using MEGA 6.60 based on the full-length sequences of COI1 proteins. Four colored arcs indicated four groups of COI1 proteins. The different colored shapes corresponded to various species were shown in labels. All the accession numbers of COI1 proteins were listed in Supplemental Table S1.
The gene collinearity among *S. spontaneum*, *R570*, and *S. bicolor* was analyzed to investigate the evolutionary mechanism of the COI1 gene family (Fig. 4b, c and Supplemental Table S8). In *S. spontaneum*, 12 pairs (14 genes) of collinearity relationships of 21 SsCOI1s were observed (Fig. 4b and Supplemental Table S8). Furthermore, all of these 14 genes had WGD/segmental duplications, including 11 pairs (13 SsCOI1 genes) of homoeologous genes that were distributed in different chromosomes. In *R570*, there was no collinear relationship among three ShCOI1 genes in *R570* (Fig. 4b and Supplemental Table S8). As shown in Fig. 4c, there were 12 orthologous pairs between *S. spontaneum* and *S. bicolor*, four between *S. spontaneum* and *R570*, and four between *S. bicolor* and *R570*. The nonsynonymous (Ka)/synonymous (Ks) ratios of all duplicated COI1 genes in *S. bicolor*, R570, and *S. spontaneum* were <1, indicating that the COI1 gene family might have experienced strong purifying selective pressure during evolution (Supplemental Table S8).

**Cis-acting elements in the promoter regions of the COI1 gene family**

The cis-acting regulatory elements in the promoters of COI1 genes were predicted to assist the gene function elaboration. There were many core elements in the promoter sequences of 31 COI1s, which were involved in stress responsiveness, hormone responsiveness, light responsiveness, and growth and development (Fig. 5 and Supplemental Table S9). The light, ABA and MeJA response elements were the most numerous in the COI1 gene promoter regions, followed by anaerobic induction, drought-inducibility, and low-temperature responsiveness. Among them, light response elements were observed in all COI1 promoter regions. In 31 COI1 promoter regions, 30 COI1s contained MeJA response elements (CGTCA-motif and TGACG-motif), except for ShCOI1–5, 26 (83.87%) contained abscisic acid response elements (ABRE), and 24 (77.42%) contained anaerobic induction elements (ARE), while 22 (70.97%) contained drought-inducibility (MBS) and low-temperature response elements (LTR). In addition, the numbers of COI1 promoter regions that contained auxin (IAA), gibberellin (GA), and SA response elements, were 16, 18, and 12, respectively. The existence of these functional elements indicates that COI1 genes may participate in the induction of multiple stress responses and thus play a role in sugarcane defense against various environmental stresses.
Fig. 4 Expansion mechanisms and synteny analysis of COI1 gene family. a Expansion mechanisms of COI1 gene family in Sorghum bicolor, Saccharum spp. hybrid cultivar R570, and S. spontaneum. The numbers in the pie chart represented the effects of WGD or segmental (whole-genome duplication/segmental duplication), Dispersed (dispersed duplication), Proximal (proximal duplication), Tandem (tandem duplication), and Singleton (single copy). b Synteny analysis of COI1 genes in S. spontaneum and R570. c Synteny analysis of COI1 genes in S. bicolor, R570, and S. spontaneum. All replicated genes in the genome were represented by gray lines in figures b and c; the red lines in figure b represented the replicated ScCOI1 genes; the red lines in figure c represented the homologous COI1 gene pairs in S. spontaneum and R570; the green lines represented the homologous COI1 gene pairs in S. bicolor and R570, and the blue lines represented the homologous COI1 gene pairs in S. spontaneum and S. bicolor. "Ss," "Sh," and "Sb" represented the name of each chromosome in S. bicolor, R570, and S. spontaneum, respectively. The detailed information was shown in Supplemental Table S7 and Table S8.
The results of transcriptome sequencing (RNA-seq) showed that 21 SsCOI1 genes were constitutively expressed in the root, bud, leaf, stem pith, and epidermal tissues of sugarcane cultivar ROC22 (Saccharum spp. hybrid), but with various expression levels (Fig. 6). Among them, SsCOI1–6d had low expression in all tissues, and the expression levels of SsCOI1–1a, −1b, −3a, −3b, and −3c (clustered into group B) were the highest in the bud and the lowest in the leaf. SsCOI1–2a and SsCOI1–2b had the lowest expression levels in the bud and the highest in the stem pith. The expression levels of SsCOI1–6a, −6b, −6c, −7a, −7b, −8a, −8b, and −8c were the highest in the stem pith and the lowest in the root. The highest expression levels of SsCOI1–4a, −4b, −4c, and −5 were observed in bud tissues, followed by those in the epidermis, stem pith, root, and leaf. There were different expression patterns between SsCOI1–4e and its duplicated gene, SsCOI1–4c. It was noteworthy that the SsCOI1 genes that clustered to group D showed a higher expression level compared with the other group in general. These results suggest that SsCOI1 genes may play a role in sugarcane growth and development, but with different function modes.

Expression profiles of COI1 genes under cold and drought treatments

Due to the fact that the drought-inducibility (MBS) and low-temperature response elements (LTR) were observed in most of the promoter sequences of SsCOI1s, their expression profiles under drought and cold stresses were analyzed to further investigate the function of SsCOI1 genes. As shown in Fig. 7, SsCOI1 responded to both drought and cold stresses, but with different expression patterns.

Under drought stress, SsCOI1–1a, −1b, −3a, −3b, −3c, −8a, −8b, and −8c had the highest transcription levels at 10 d and the lowest expression levels after water recovery. The expression levels of SsCOI1–7a and SsCOI1–7b were down-regulated at 6 d, but up-regulated at 10 d and after water recovery. Compared with the control, the expression levels of SsCOI1–4a, −4b, −4c, −4e, −5, −7a, and −7b were increased and reached a single peak at 2 d, but decreased to the lowest after 10 d water recovery. The transcription levels of SsCOI1–6a, −6b, −6c, and −6d were increased and had the highest levels at 10 d after drought treatment, but all of them were decreased after water recovery. Under cold stress, the expression levels of all SsCOI1s were elevated.
For instance, \textit{SsCOI1–4b} and \textit{SsCOI1–4e} showed the highest expression levels at 0.5 h and the lowest expression levels at 1 h. The expression levels of \textit{SsCOI1–1a}, \textit{−1b}, \textit{−4c}, \textit{−5}, \textit{−8a}, \textit{−8b}, and \textit{−8c} were all enhanced to a peak at 0.5 h and then decreased. The transcription levels of \textit{SsCOI1–2b}, \textit{−3a}, \textit{−3c}, \textit{−4a}, \textit{−6b}, \textit{−6c}, and \textit{−6d} were continuously up-regulated from 0 to 6 h after cold treatment, while \textit{SsCOI1–2a}, \textit{−3b}, and \textit{−6a} decreased first and then increased and showed the highest levels at 6 h. In addition, \textit{SsCOI1–7a} and \textit{SsCOI1–7b} were up-regulated at 0.5 h and remained stable at 1 h after cold treatment.

Expression profiles of \textit{COI1} genes in response to sugarcane smut pathogen infection

To study the function of \textit{SsCOI1}s in response to smut pathogen infection, the gene expression patterns during the interaction between two different sugarcane genotypes and \textit{S. scitamineum} were analyzed. As shown in Fig. 8, in sugarcane smut-resistant \textit{Saccharum} spp. hybrid cultivar YC05–179, \textit{SsCOI1–3a} had no significant expression change while \textit{SsCOI1–1a} and \textit{SsCOI1–1b} were down-regulated. \textit{SsCOI1–4a} and \textit{SsCOI1–6b} were up-regulated and reached the highest expression levels at 1 day post-inoculation (dpi),
while SsCOI1–2a, −2b, −3b, −3c, −4b, −4c, and −4e were up-regulated and reached a single peak at 2 dpi. The transcription levels of SsCOI1–5, −6b, −6c, and −6d were increased to a peak at 5 dpi. In addition, the expression levels of SsCOI1–7a, −7b, −8a, −8b, and −8c were decreased at 1–2 dpi and then increased at 5 dpi. In sugarcane smut-susceptible Saccharum spp. hybrid cultivar ROC22, the transcription levels of SsCOI1–1a, −1b, −2a, −2b, −3a, −3c, −4a, −4b, and −4e were inhibited after smut pathogen inoculation. The expression levels of SsCOI1–3b, −4c, and −5 were increased at 1 dpi, and then decreased at 2 dpi to 5 dpi. The expression levels of SsCOI1–6a, −6b, −6c, −6d, −8a, −8b, and −8c were decreased and reached the lowest point at 2 dpi, and then increased at 5 dpi. The expression levels of SsCOI1–7a and SsCOI1–7b remained stable from 1 dpi to 2 dpi, and then were up-regulated at 5 dpi.

These results indicated that all 21 members of the SsCOI1 gene family could be induced during the interaction between sugarcane and smut pathogen, and these allele genes showed similar expression patterns.

Expression profiles of COI1 genes under MeJA treatment via RT-qPCR

The COI1 gene is the core member of the JA signaling pathway. To understand the expression pattern of the COI1 gene in response to JA, the expression levels of seven SsCOI1 haplotype genes under MeJA stress were evaluated via RT-qPCR (Fig. 9). After MeJA treatment, the expression levels of SsCOI1–4 and SsCOI1–6 (the members of group A) remained unchanged from 0 h to 24 h. The expression levels of the SsCOI1–1 gene (a member of group B) decreased significantly at 3 h, but increased significantly at 12 h and remained at a high level at 24 h, at levels 1.62- and 1.92-fold that of the control, respectively. The expression levels of SsCOI1–2 (a member of group B) were increased remarkably at 3 h and 12 h, at levels 1.54- and 1.40-fold higher than the control, and returned to the control level at 24 h. The transcription levels of SsCOI1–2 and SsCOI1–7 (members of group D) were up-regulated at 3 h and then stayed at a relatively stable level. For the SsCOI1–8 gene (a member of group D), its expression levels increased with a single peak at 12 h, which was 1.67-fold higher...
than the control. The above results show that various SsCOI1 genes might play different roles in response to MeJA.

Cloning and sequence analysis of three COI1 genes in the sugarcane cultivar ROC22

To further understand the functions of COI1 genes in a sugarcane cultivar, three candidate COI1 genes, SsCOI1–4b (clustered into subgroup A), SsCOI1–1b (clustered into group B), and SsCOI1–3b (clustered into group B), were cloned from ROC22 and termed as ShCOI1–4, ShCOI1–5, and ShCOI1–6, respectively. The full lengths of the cDNA of the ShCOI1–4, ShCOI1–5, and ShCOI1–6 genes were 1953 bp, 2152 bp, and 2322 bp, respectively, with 592, 600, and 661 encoding amino acids, respectively. The amino acid sequence similarities of ShCOI1–4, ShCOI1–5, and ShCOI1–6 to SsCOI1–4b, SsCOI1–1b, and SsCOI1–3b, respectively, were 99.50, 99.70, and 99.2%, respectively (Supplemental Table S5). All three ShCOI1 proteins contained relatively conservative F-box domains, Transp_inhibit domains, and AMN1 domains (leucine-rich repeat protein) (Supplemental Fig. S1). Furthermore, compared with ZmCOI1a (GRMZM2G125411), ZmCOI1b (GRMZM2G151536), ZmCOI1c (GRMZM2G353209), and ZmCOI2 (GRMZM2G079112), three ShCOI1 proteins possessed only 4–5 of 16 key amino acid residues (asterisks and sites 1–4 in Supplemental Fig. S1) that are supposed to be the binding sites of JA-Ile or JAZ proteins, suggesting that there may be functional differentiation among these three ShCOI1s and ZmCOIs.

Gene expression patterns of ShCOI1–4, ShCOI1–5, and ShCOI1–6 in response to different abiotic stresses via RT-qPCR

According to previous reports, JA and SA are mostly related to plant resistance to pathogen infection [11, 46], and ABA is mostly associated with abiotic stress...
In this study, RT-qPCR was used to analyze the expression levels of \( ShCOI1-4 \), \( ShCOI1-5 \), and \( ShCOI1-6 \) in ROC22 under SA, ABA, cold (4 °C), and drought (polyethylene glycol, PEG) treatments (Fig. 10). Under ABA stress, the transcripts of \( ShCOI1-4 \) were up-regulated by 2.86- and 2.99-fold at 3 h and 6 h, respectively, and returned to the control levels at 12 h. After SA treatment, the expression levels of \( ShCOI1-4 \) increased with a single peak at 12 h that was 1.56-fold higher than the control. Under cold and drought stresses, compared to the control, the transcripts of \( ShCOI1-4 \) were decreased. Under SA treatment, the transcripts of the \( ShCOI1-5 \) gene increased with a single peak at 12 h that was 2.17-fold higher than the control. Based on these findings, it is speculated that \( ShCOI1-4 \), \( ShCOI1-5 \), and \( ShCOI1-6 \) actively respond to biotic and abiotic stresses in plants via different signal pathways.

Gene expression patterns of \( ShCOI1-4 \), \( ShCOI1-5 \), and \( ShCOI1-6 \) in response to smut pathogen infection via RT-qPCR

In the sugarcane-smut pathogen biosystem, the expression patterns of \( ShCOI1-4 \), \( ShCOI1-5 \), and \( ShCOI1-6 \) in six Saccharum spp. hybrid cultivars (including smut-resistant cultivars YZ03–258, LC05–136, and YT96–86, and smut-susceptible cultivars GT02–467, FN40, and YZ03–103) were evaluated using RT-qPCR (Fig. 11). Compared with the control, the expression levels of \( ShCOI1-5 \) were all significantly increased in three smut-resistant sugarcane cultivars and two smut-susceptible cultivars (GT02–467 and YZ03–103), but were significantly decreased in FN40 at 7 dpi. Except for the increased expression levels of \( ShCOI1-4 \) in LC05–136 at 7 dpi and in GT02–467 at 3 h.
dpi, the expression levels of the *ShCOI1–4* and *ShCOI1–6* genes were both significantly increased in YZ03–258 and YT96–86 and decreased or remained stable in the other four sugarcane cultivars. In summary, *ShCOI1–4*, *ShCOI1–5*, and *ShCOI1–6* can be induced by smut pathogen attack, but their expression patterns vary during the interaction between different sugarcane cultivars and the smut pathogen.

**Discussion**

According to previous reports, jasmonic signal molecules can induce plants to activate resistance-related genes and systematically accumulate defense-related proteins to resist biotic and abiotic stresses [7, 8, 49]. As the essential member in the JA signaling pathway, *COI1* has received increasing attention in recent years. It has been reported that the expression levels of *AsCOI1* in *Aquilaria sinensis* can be significantly induced by MeJA, mechanical wounding, and heat [26]. The transcription levels of *HbcO11* in latex can be induced by JA and tapping [50]. In *O. sativa*, the mutation of *OsCOI1b* can delay leaf senescence, down-regulate several senescence-associated genes (including homologs of *A. thaliana* ETHYLENE INSENSITIVE 3 and ORESARA 1), and result in significant decreases in spikelet fertility and grain filling [18]. When *OsCOI1* was inhibited by RNA interference...
(RNAi), *O. sativa* plants showed a decrease in resistance to *Cnaphalocrocis medinalis* and activity declines of trypsin protease inhibitor (TrypPI), polyphenol oxidase (PPO), and peroxidase (POD) [25].

Currently, as a polygenic family, the COI1 gene has been identified and reported in many plants, such as *A. thaliana* [31], *O. sativa* [18], *Artemisia annua* [51], *T. aestivum* [27], and *Hevea brasiliensis* [50]. In this study, a total of 156 COI1 proteins were identified from 19 land plant genomes, including 21 SsCOI1s from *S. spontaneum*, three ShCOI1s from *S. bicolor*, but no COI1 was obtained from five algae plants. It is speculated that COI1 may only exist in terrestrial plants because the COI1 gene family originated after the divergence of the algae and the ancestor of terrestrial plants. Of four groups, only group D in the phylogenetic tree was present in all terrestrial plants, indicating that a common ancestor was shared among the COI1 gene family from terrestrial plants after the divergence from algae (Fig. 2). In the present study, the numbers of COI1 observed in five eudicots were eight in *M. truncatula* (2n = 4x = 32, autotetraploid) [52]; seven in *A. thaliana* (2n = 2x = 10, diploid) [53], *C. rubella* (2n = 2x = 16, diploid) [54], and *V. vinifera* (2n = 2x = 38, diploid) [55]; and four in *F. vesca* (2n = 2x = 14, diploid) [56] (Fig. 1 and Supplemental Table S1). This result is in conflict with previous reports that the diploid dicots have a single copy of the COI1 gene, while the polyploid or paleopolyploid dicots may possess a number of COI1 orthologues in their genomes [21]. In addition, 31 COI1 proteins (three ShCOI1s, seven SbCOI1s, and 21 SsCOI1s) consisted of 434–665 amino acids with MWs ranging from 47.94 to 73.14 kDa, and most of them were predicted as unstable hydrophilic non-secreted proteins (Supplemental Table S3). These sequence characteristics of ShCOI1s, SbCOI1s, and SsCOI1s are common with those in other plants, such as the *TaCOI* gene family [27, 57], *AsCOI1* [26], and *HbCOI1* [50]. Furthermore, ShCOI1, SbCOI1, and SsCOI1 proteins were predicted to be located in the cytoplasm or nucleus (Supplemental Table S3), which was consistent with the subcellular localization of the *AsCOI1* [26], *SmCOI1* [58], and *TaCOI* proteins [57]. However, more empirical evidence needed to be provided.

As it shown in phylogenetic tree (Fig. 2), the COI1 genes from the same lineage, such as mosses, monocots, and eudicots, tended to be clustered to the same clade in group A, group B, and group D, and only COI1 proteins from mosses were clustered in group C. It is speculated that lineage-specific expansion and divergence events have occurred. Interestingly, COI1 proteins from the same species were clustered into different clades. For example, seven SbCOI1 and 21 SsCOI1 proteins were clustered into three groups (A, B, and D) and three ShCOI1s from R570 were clustered into two groups (B and D), revealing that the COI1 gene family exhibited differences in evolution among species.

According to the phylogenetic tree, protein motifs, and gene structure analysis, those COI1 proteins in the same group showed a similar motif composition and exon/intron structure, but varied among different groups (Fig. 3). For example, most members of COI1 clustered into group B and group D had two introns, while there were more intron numbers in group A. Furthermore, the number, length, and positions of introns exhibited diversity. Thus, we speculated that the intron loss or gain events occurred during the process of structural evolution of the COI1 gene family [59, 60]. Motif analysis results revealed the diversity of protein-conserved motifs among the COI1 gene family, such as the different numbers of motifs 4, 5, 7, 8, and 9 among various COI1
proteins that clustered into different clades. Therefore, the classification and evolution of COI1 genes might be related to their structural divergence and diversification.

As reported, gene duplication events can provide a primary source of material for the origin of evolutionary novelties, including new gene functions and expression patterns [61]. Chromosomal location, gene type, and gene collinearity analysis are often used to investigate the expansion and evolutionary mechanism of gene families [61, 62]. In this study, 21 SsCOI1 genes were unevenly distributed among 18 of 32 chromosomes of S. spontaneum, and not every COI1 had homoeologous genes on the homologous chromosomes A, B, C, and D, suggesting that some homologous COI1 genes may have been lost during the polyploidization of the genome [63]. There was a wide homologous relationship among S. spontaneum, R570, and S. bicolor, and the Ka/Ks ratios of all duplicated COI1 genes were <1, indicating that the COI1 gene family might have experienced strong purifying selective pressure during evolution (Fig. 4b, c, and Supplemental Table S8). In addition, it was observed that the COI1 gene family was expanded by various genome duplication events in S. spontaneum, S. bicolor, and R570 (Fig. 4a and Supplemental Table S7). The different expansion mechanisms demonstrated that the driving forces in the evolution of each COI1 gene or the COI1 gene family among different species were diverse, and there may have been functional differentiation among various COI1 gene family members.

As a key component in the regulation of gene expression, the analysis of cis-acting regulatory elements in gene promoters can assist to elaborate the regulation and function of individual genes and their interaction with other genes [64, 65]. In the present study, a large number of promoter core elements were identified in the promoter sequences of ShCOI1s, SbCOI1s, and SsCOI1s that were involved in stress responsiveness (such as drought, low-temperature, and wound stress), hormone responsiveness (like SA, ABA, and MeJA), light responsiveness, and growth and development (Fig. 5 and Supplemental Table S9). This finding was similar to that observed in the promoter regions of HbCOI1 [50] and TaCOI genes [57]. The existence of these functional elements indicates that COI1 genes may play a role in sugarcane development and defense against various environmental stresses via participating in different regulatory mechanisms.

Gene expression patterns are usually related to their function [66]. As reported, the expression levels of COI1 genes in different plants are spatiotemporal [26, 50, 57, 58]. For instance, in H. brasiliensis, HbCOI1 has high transcription levels in laticifers, but low levels in bark and leaf tissues [50]. In Solanum melongena, the transcription levels of SmCOI1 are significantly down-regulated in anther indehiscence, which is related to the normal development of anthers [58]. In A. sinensis, the AsCOI1 gene was highly expressed in roots and stems, the two major organs of agarwood formation [26]. In addition, the members of the TaCOI gene family are expressed differently in various tissues, with the higher expression levels in stem, leaf, petal, pistil, stamen, and glume tissues than in roots [57]. Analogously, COI1 genes were constitutively expressed in sugarcane cultivar ROC22, but their expression patterns were diverse in different tissues (Fig. 6). For example, the expression levels of the group B genes (SsCOI1–1a, −1b, −3a, −3b, and −3c) were the highest in the bud and the lowest in the leaf. Compared with the other group genes, SsCOI1 genes, which clustered into group D, showed abundant transcripts and had the highest expression levels in stem pith. These results indicate that COI1 genes exhibit a tissue-specific pattern, and the same expression pattern suggests a similar function in the growth and development process.

RNA-seq data revealed that the COI1 gene family played a positive role in sugarcane response to drought and cold stresses with different expression patterns (Fig. 7). Interestingly, this finding is consistent with the prediction that the COI1 promoter sequence contains a large number of drought and low temperature response elements. To validate the function diversities of sugarcane COI1 genes, the expression levels of SsCOI1s under MeJA treatment were assessed by RT-qPCR (Fig. 9). The results show that various SsCOI1 genes may play different roles in response to MeJA. To further elucidate the functions of the COI1 gene family in a sugarcane cultivar, three ShCOI1 genes homologous with SsCOI1–4b, SsCOI1–1b, and SsCOI1–3b were cloned from ROC22. RT-qPCR results revealed that the transcription of ShCOI1–4 was decreased under cold (4 °C) and drought (PEG) stresses, while it was up-regulated significantly after SA and ABA treatments. The expression levels of ShCOI1–5 were up-regulated remarkably under SA and drought stresses, and down-regulated under ABA stress, and remained unchanged under cold stress. The transcriptions of the ShCOI1–6 gene were increased under SA, ABA, and drought stresses, but remained unchanged under cold stress (Fig. 10). These results were consistent with previous reports that COI1 was involved in the response of plants to various abiotic stresses and exhibited functional divergence [21, 57]. Likewise, in the TaCOI gene family, TaCOI2 (TaCOI2-A and TaCOI2-B) and TaCOI6 (TaCOI6-A, TaCOI6-B, and TaCOI6-D) were clustered into group A, while TaCOI5 (TaCOI5-A, TaCOI5-B, and TaCOI5-D) was clustered into group D [57]. The expression levels of TaCOI2 were up-regulated under the ABA, GA, and low-temperature treatments, but down-regulated under the IAA and MeJA treatments.
The transcripts of TaCOI6 could be induced by ABA and MeJA, but were suppressed by IAA and PEG [57]. The transcription levels of TaCOI5 were increased in the GA, low temperature, and PEG treatments, while they were decreased after the ABA, IAA, MeJA, and salinity treatments [57]. In Z. mays, the expression levels of four ZmCOIs (clustered into group A) responded to plant hormones were detected, and the results showed that ZmCOI1a and ZmCOI1b were strongly induced by JA and ABA, while ZmCOI1c and ZmCOI2 were less-expressed in maize tissues and slightly induced by JA and ABA, but there was no significant induction for ZmCOI1a, ZmCOI1b, ZmCOI1c and ZmCOI2 by 1-Aminocyclopropane-1-carboxylic acid, GA, 1-Naphthylacetic acid, and SA [21]. In addition, the restoration of male fertility in Arabidopsis mutant coi1–1 could result in plants that overexpressed ZmCOI1a, ZmCOI1b, or ZmCOI1c, but not ZmCOI2, indicating the successful complementation of coi1–1 sterility by ZmCOI1a, ZmCOI1b, and ZmCOI1c and the functional divergence of ZmCOIs [21]. It should be stressed that COI1 genes may exhibit inconsistent expression patterns under certain environmental stresses even if they are clustered into the same group.

It has been reported that JA and SA are mostly related to plant resistance to pathogen infection [11, 46]. Therefore, the COI1 gene family may be involved in sugarcane response to pathogen infection. Transcriptome analysis showed that SsCOI1 genes could be induced during the interaction between sugarcane and the smut pathogen, and the alleles showed similar expression patterns. Among them, the expression levels of SsCOI1–4b and SsCOI1–3b were up-regulated in smut-resistant cultivar YC05–179, but down-regulated in smut-susceptible cultivar ROC22, while SsCOI1–1b was down-regulated in both YC05–179 and ROC22. Furthermore, the expression patterns of ShCOI1–4, ShCOI1–5, and ShCOI1–6, the homologous genes of SsCOI1–4b, SsCOI1–1b, and SsCOI1–3b, respectively, were different in the interaction between the six different sugarcane cultivars and the smut pathogen (Fig. 11). Similarly, TaCOI1 took part in the early defense of compatible and incompatible wheat responses to Blumeria graminis (Bgt), and the response time was earlier in the resistant cultivars than in the susceptible ones [27]. Using virus-induced gene silencing, the expression of TaCOI1 decreased significantly, and the rate of successful penetration by Bgt was higher than that of the control. This indicates that TaCOI1 may play a key role in wheat-Bgt interactions [27]. In A. thaliana, two mutant alleles of coil conferred hypersusceptibility to the necrotrophic pathogen Sclerotinia sclerotiorum than wild-type or heterozygous plants [67]. Furthermore, overexpressed ZmCOIs in the Arabidopsis coi1–1 mutant plants can cause the restoration of resistance to the leaf pathogen Botrytis cinerea and the soil-borne pathogen Pythium arsisto{porum [21]. Taking the above findings into consideration, we conclude that COI1 genes have multiple functions and participate in sugarcane defense against various environmental stresses via different regulatory mechanisms.

**Conclusion**

A total of 156 COI1s, including 21 SsCOI1s, seven SbCOI1s, and three ShCOI1s, were identified from 19 species and could be clustered into four groups. The analysis of cis-acting elements, tissue-specific expression, and expression profiles under various stresses suggests that COI1 genes participate in growth, development, and response to various stresses in sugarcane. Furthermore, three COI1 genes, ShCOI1–4, ShCOI1–5, and ShCOI1–6, were obtained by homologous cloning in the sugarcane cultivar ROC22 and could be induced by the stresses of drought, cold, ABA, SA, and S. scitamineum with divergent expression profiles. The results illustrate the fact that sugarcane COI1 genes may actively respond to biotic and abiotic stresses via different regulatory mechanisms. The present study laid a foundation for the functional identification of sugarcane COI1 genes and provided a theoretical basis for molecular breeding of sugarcane resistance.

**Materials and methods**

**Plant materials**

Eight Saccharum spp. hybrid cultivars (including YT96–86, LC05–136, YZ03–258, ROC22, GT02–467, YZ03–103, FN40, and YC05–179) and smut whip were obtained from the Key Laboratory of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture and Rural Affairs (Fuzhou, China).

The root, stem pith, leaf +1, bud, and epidermis tissues of nine consistent 10-month-old ROC22 plants (the prevalent sugarcane cultivar in mainland China) were collected [42]. Four-month-old hydroponic ROC22 tissue-cultured plantlets were sprayed with 100 mM ABA, 5 mM SA (containing 0.01% Tween-20, v/v), 100 μM MeJA (containing 0.1% ethanol and 0.05% Tween-20, v/v), and 25% PEG 8000 at 28°C with 16 h light and 8 h darkness [42, 68]. The leaves under SA and MeJA treatments were harvested at 0, 3, 12, and 24 h, the leaves under ABA treatment were collected at 0, 3, 6, and 12 h, and the leaves under PEG stress were harvested at 0, 0.5, 3, 6, and 24 h [42, 68]. For cold stress, the whole ROC22 plantlets were kept at a low temperature of 4°C with 16 h light and 8 h darkness for 0, 6, 12, 24, and 48 h [42, 68].

The stems of eight 10-month-old sugarcane cultivars, including smut-resistant cultivars YC05–179, YZ03–258, LC05–136, and YT96–86; and smut-susceptible cultivars GT02–467, ROC22, FN40, and YZ03–103, were cut into
two-bud sets, immersed for 1 day in flowing water, and cultivated under a light–dark regime (16h of light and 8h of darkness) at 32°C until the germinating seedlings with a bud height of about 2 cm [44, 69]. Then, the bud was inoculated with 5 x 10² spores/ml -1 S. scitamineum (0.01% Tween-20, v/v), and the control group was inoculated with sterile water (0.01% Tween-20, v/v) [43, 44]. All the materials were cultivated at 28°C with a photoperiod of 16h light and 8h darkness [43, 44]. Five buds of YC05–179 and ROC22 were collected at 0, 1, 3, and 7 dpi were harvested and immediately frozen in liquid nitrogen for gene expression analysis [44, 103]. At 0, 1, 3, and 7 dpi, samples were immediately frozen in liquid nitrogen and stored at −80°C.

RNA extraction and first-strand cDNA synthesis
Total RNA was extracted from the collected samples using TRIzol™ (Invitrogen, Carlsbad, USA). RNA (1.0μg) was reverse transcribed to the first-strand cDNA using a Prime-Script™ RT Reagent Kit (TaKaRa, Dalian, China) for RT-qPCR analysis. The cDNA used as cloning templates was synthesized from the RNA of ROC22 buds using a HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China).

Identification of the COI1 gene family
To identify the COI1 gene family, the genomic data of a total of 24 species were collected (Supplemental Table S10). The genomic data of 19 plants (A. thaliana, A. trichopoda, A. comosus, B. distachyon, C. rubella, F. vesca, M. truncatula, O. sativa, P. hallii, P. patens, R570, S. italicca, S. bicolor, S. spontaneum, S. fallax, S. moellendorffii, T. aestivum, V. vinifera, Z. mays, C. subellipsosidea C169, and M. pusilla CCMP1545) were downloaded from Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html). The genomic data of C. crispus, C. merolae, and G. sulphuraria were downloaded from Ensembl (http://plants.ensembl.org/index.html). The genomic data of S. spontaneum was downloaded from the link of http://www.life.illinois.edu/ming/ downloads/Spontaneum_genome/ [36]. The monoploid reference genome of R570 was obtained from the Sugarcane Genome Hub (http://sugarcane-genome.cirad.fr/) [38]. Two Hidden Markov Model (HMM) profiles (PF18511.1 and PF18791.1), which were predicted by Pfam (http://pfam.xfam.org/search#tabview=tab1), were download from HMMER (https://www.ebi.ac.uk/Tools/hmmer) and used for the HMMER search [71]. Hmmssearch (HMMER package version 3.1b2) was used to search candidate COI1s from the genomic data of 24 species [71]. All obtained sequences were input into the Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/cdd) to search the protein domain [72]. The COI1 gene family members were confirmed after removing incomplete sequences. Allele genes were designated as the same name followed by the letters “a,” “b,” “c,” and “d,” and duplicated genes were designated as the same name followed by the letter “e” in S. spontaneum.

The COI1 genes in T. aestivum and Z. mays were named according the research of Bai et al. [57] and An et al. [21], respectively.

Sequence characteristics of the COI1 gene family
All the identified COI1 genes in S. bicolor, R570, and S. spontaneum were submitted to ExPASy (http://web.expasy.org/protparam/) to analyze their amino acid numbers, MW, theoretical pI, instability index, and GRAVY. All full-length proteins were submitted to SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) for secondary structure analysis. The predictions of signal peptides, transmembrane structures, and subcellular localizations were conducted by SignalP-5.0 (http://www.cbs.dtu.dk/services/SignalP/), TMHMM (http://www.cbs.dtu.dk/services/TMHMM/), and Plant-mPloc (http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/), respectively. In addition, the percent identity matrixes between COI1 proteins in S. bicolor, R570, S. spontaneum, and sugarcane hybrid cultivar ROC22 were calculated using DNAMAN.

Multiple sequence alignment and phylogenetic analysis
Multiple sequence alignment (MSA) of COI1 proteins was conducted by ClustalW in MEGA 6.60 with default parameters. Three phylogenetic trees in this study, including one phylogenetic tree of the 156 COI1 proteins from 19 plant species, one phylogenetic tree of 21 SsCOI1s, seven SbCOI1s, and three ShCOI1s proteins, and one phylogenetic tree of 21 SsCOI1s, were constructed using the maximum likelihood method (JTT + G model, complete deletion, and 1000 bootstrap replicates) based on the above alignments [73]. Evolview (https://evolview.info/evolview-v2/#mytrees/SHOWCASES/showcase%202002) [74] was used to display and edit the phylogenetic tree.

Motif and gene structure analysis of SbCOI1s, ShCOI1s, and SsCOI1s
Amino acid sequences of SbCOI1s, ShCOI1s, and SsCOI1s were submitted to the Multiple Em for Motif Elicitation online program (http://meme-suite.org/tools/meme) to identify the conserved motifs [75]. The parameters were as follows: maximum motif number, 10; maximum motif width, 50; minimum motif width,
6; and distribution of motif occurrences with zero or one per sequence. Diagrams of exon-intron structures were drawn using the Gene Structure Display Server 2.0 (http://gsds.gao-lab.org/). TBTools (Toolbox for Biologists) v1.0.9832 and Adobe Illustrator CS6 were used to display and edit the phylogenetic tree, conserved motifs, and gene structures [76].

Chromosomal locations and collinearity analysis of SbCOI1s, ShCOI1s, and SsCOI1s

The physical locations of COI1s on the chromosomes of *S. bicolor*, R570, and *S. spontaneum* were analyzed using MapGene2Chrom (MG2C) software (http://mg2c.iask.in/mg2c_v2.1/). Multiple Collinearity Scan toolkit (MCScanX) and TBTools were used with the default parameters to analyze the synteny block and gene duplication pattern [76, 77]. The values of Ka/Ks between orthologous gene pairs were calculated by TBTools to study the selection pressure acting on the evolution of the COI1 gene family [76].

Cis-acting regulatory element analysis in the promoter regions of COI1 genes

A 2000 bp sequence upstream of the start site of gene translation of *SbCOI1s*, *ShCOI1s*, and *SsCOI1s* was retrieved from genomic data as the promoter sequence, and its cis-regulatory elements were predicted using the PlantCARE online program (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [78]. The results of the prediction were visualized using TBTools [76].

Expression profiles of SsCOI1s in sugarcane based on RNA-seq

The RNA of the roots, stem piths, leaves, buds, and epidermis in ROC22 and the buds of YC05–179 and ROC22 inoculated with *S. scitamineum* for 0, 1, 2, and 5 d [70] were sequenced and assembled by the Biomarker Technologies Company limited (Beijing, China). The original data were obtained by Illumina technology, and after passing quality control, the data were analyzed using the *S. spontaneum* genome as the reference annotation library. The fragments per kilobase of transcript per million mapped (FPKM) was used as an indicator to measure the expression levels of transcripts. For drought and cold treatments, the original data (PRJNA590595 and PRJNA636260) were downloaded from the Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/sra/). The leaves of *Saccharum* hybrid cultivar Co 8021 under drought treatment were harvested at 0, 2, 6, and 10 d, and water-recovered at 10 d. The leaves of *S. spontaneum* under cold treatment were collected at 0, 0.5, 1, and 6h. The sequence quality of these data was improved by Fastp [79]. The Hisat2 program was used to map sequence data to the *S. spontaneum* genome [80]. The count read and normalization of the data were conducted by the featureCounts in the Subread package and the trimmed mean of M-values (TMM) [81, 82]. The gene IDs of these transcriptomes followed the format of the original gene ID of *S. spontaneum*, which was relevant to the related search of their homologous genes. The expression levels of *SsCOI1s* in different sugarcane tissues and in response to drought, cold, and *S. scitamineum* stresses were mined from these RNA-seq data. The heat map showing the log2 (FPKM or TMM) expression profiles was generated by TBTools [76].

Cloning and sequences analysis of candidate *S. spontaneum* SsCOI1 genes in sugarcane cultivar

According to the sequences of *SsCOI1s*, the specific primers for *ShCOI1–4*, *ShCOI1–5*, and *ShCOI1–6* (Supplemental Table S11) which were clustered into two different groups of COI1 gene family and with different expression patterns in response to MeJA were designed using Primer premier 5.0 software. The cDNA of ROC22 bud was used as a template for gene cloning. The transcription-polymerase chain reaction (RT-PCR) system contained 1.0 μL cDNA template, 1.0 μL each of the forward and reverse primers (10μM), 12.5 μL 2× Phanta Max buffer, 0.5 μL dNTPs (2.5 mM), and 0.5 μL Phanta Max Super-Fidelity DNA Polymerase (Vazyme, Nanjing, China), and 8.5 μL ddH2O. The PCR reaction conditions were as follows: 95 °C for 3 min; 35 cycles of 95 °C for 15 s, 56 °C for 15 s, and 72 °C for 2 min 30 s; and 72 °C for 5 min. PCR products were gel-purified, cloned into pMD19-T vector (TaKaRa, Dalian, China), and sequenced [42]. Amino acid sequence alignment among *ShCOI1–4*, *ShCOI1–5*, *ShCOI1–6*, and ZmCOIs (ZmCOI1a, GRMZM2G125411; ZmCOI1b, GRMZM2G151536; ZmCOI1c, GRMZM2G533209; ZmCOI2, GRMZM2G079112) was performed by NTI software [21, 31, 45].

RT-qPCR analysis

Seven primer pairs of non-allelic *SsCOI1* genes were designed by Beacon Designer 8.0 (Supplemental Table S11). Due to the high amino acid sequences similarity (Supplemental Table S5), the primer pairs of *SsCOI1–4*, *SsCOI1–1*, and *SsCOI1–3* used in RT-qPCR analysis were the same as those of *ShCOI1–4*, *ShCOI1–5*, and *ShCOI1–6*, respectively (Supplemental Table S5 and Supplemental Table S11). The expression levels of the seven *SsCOI1* genes under MeJA stress and those of *ShCOI1–4*, *ShCOI1–5*, and *ShCOI1–6* in the six sugarcane cultivars (YZ03–258, LC05–136, YT96–86, GT02–467, FN40, and YZ03–103) infected by smut pathogen and under hormones and abiotic stresses (ABA, SA, and PRJNA636260) were downloaded from the Sequence...
cold, and PEG) were detected using RT-qPCR. The RT-qPCR was performed on Applied biosystems Q3 (ThermoFisher, Waltham, USA) system using the SYBR-green dye method with the conditions of 50 °C for 2 min; 95 °C for 10 min; 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The total volume of the RT-qPCR reaction system was 20 μL, which included 10 μL of the 2 × ChamQ Universal SYBR qPCR Master Mix, 0.4 μL of the primer (10 μM), 20 μL, which included 10 μL of the template (10 μg/mL diluted liquid), and 8.2 μL ddH₂O. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Supplemental Table S11) was used as a reference gene [83]. Each sample was assessed using three replicates. Expression levels of COI1 genes were calculated using the 2^−ΔΔCT algorithm [84]. Significant differences (p < 0.05) and standard error (SE) were determined by the Duncan’s new multiple range test by using Data Processing System v9.50 software, and the histogram was graphed by Origin 9.0.

**Abbreviations**

COI1: Coronatine insensitive 1; JA: Jasmonic acid; SCF: SKP1+ Cdc53/ Cullin4-Rbx1 + F-box; JAZ: Jasmonate ZIM-domain; MYC2: Myeloid differentiation 2; JA-ile: Jasmonoyl-L-ileucine; JAR1: Jasmonic acid-amido synthetase; LRR: Leucine rich repeats; RCA: Rubisco activase; RT-qPCR: Real-time quantitative PCR; MeJA: Methyl jasmonate; ABA: Abscisic acid; MWS: Molecular weights; pl: pI; point; GR: Grand average of hydrophaticity; UTR: Untranslated regions; WGD: Whole-genome duplication; K/Ks: Nonsynonymous/synonymous; IAA: Auxin; GA: Gibberellin; RNA-seq: Transcriptome sequencing; FPKM: Fragments per kilobase of transcript per million mapped; TMM: Trimmed mean of M-values; dpi: day(s) post-inoculation; RT-PCR: Transcription-polymerase chain reaction; PEG: Polyethylene glycol; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; SE: Standard error; RNAi: RNA interference; Trypi: Trypsin protease inhibitor; PPO: Polyphenol oxidase; POD: Peroxidase; Bl: Blumenia graminis; HHM: Hidden Markov Model; CDD: Conserved Domain Database; MSA: Multiple sequence alignment; TBtools: Toolbox for Biologists; MG2C: MapGene2Chrom; MCScanX: Multiple Collinearity Scan toolkit.

**Supplementary Information**

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**Authors’ contributions**

Y.Q. and Y.S. conceived and supervised the study, Y.M. and T.S. performed the experiments and analyzed the data, G.C., A.F., W.S., Y.C., and C.Y. assisted with bioinformatic analysis and aided in performing the experiments; T.S. drafted the manuscript; Y.Q. and Y.S. revised the final version of the paper. All of the authors carefully checked and approved the final manuscript.

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**Availability of data and materials**

The data supporting the conclusions of this article are within the paper.

**Declarations**

**Ethics approval and consent to participate**

The sugarcane materials used in this study were supplied by the Key Laboratory of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture and Rural Affairs/Fujian Agriculture and Forestry University. They are widely planted in China, and no permits are required to collect plant samples. This article did not contain any studies with human participants or animals and did not involve any endangered or protected species.

**Consent for publication**

Not applicable.

**Competing interests**

The authors have no conflict of interest to declare.

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