GmCOI1*, a Soybean F-Box Protein Gene, Shows Ability to Mediate Jasmonate-Regulated Plant Defense and Fertility in *Arabidopsis

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The F-box protein gene *COI1* from *Arabidopsis* plays a fundamental role in response to jasmonates, which regulate plant root growth, pollen fertility, wounding and healing, and defense against pathogens and insects. Null mutations in *COI1* were previously found to abolish all the jasmonate responses, and the *Arabidopsis coi1-1* mutant is male sterile and susceptible to pathogen infection. In this study, we isolated an F-box protein gene from soybean, which shares significant homology with the *Arabidopsis COI1* and similarly contains an F-box motif and leucine rich repeats (LRR), here designated *GmCOI1* (*Glycine max* L. (Merr.) *COI1*). To test whether the sequence homology and structural similarity are indicative of functional conservation, we expressed *GmCOI1* in the *Arabidopsis coi1-1* mutant. The transgenic *coi1-1* plants with expression of the *GmCOI1* gene were found to exhibit normal jasmonate responses, including jasmonate-regulated plant defense and fertility. In addition, the chimerical proteins with swapped domain of the F-box motif or LRR between *GmCOI1* and *COI1* were shown to functionally complement the *coi1-1* mutation. Furthermore, *GmCOI1* was found to assemble into the Skp1-Cullin-F-box (SCF) complexes, similar to the formation of the *Arabidopsis* SCF^{COI1}. These data demonstrate the soybean F-box protein gene *GmCOI1* is able to mediate jasmonate-regulated plant defense and fertility in *Arabidopsis*, which implies a generic jasmonate pathway with conserved signal components in different plant species.

Plant hormones influence diverse developmental processes, ranging from seed germination to root, leaf, shoot, and flower formation. Jasmonates (JA), including jasmonic acid and its cyclopentanone derivatives, are a newly discovered class of plant hormones that are widely distributed in the plant kingdom. JA are synthesized from linolenic acid in a lipoxygenase-dependent biosynthetic pathway, in which linolenic acid is oxygenated by lipoxygenase (LOX) and then is converted to 12-oxo-phytodienoic acid by allene oxide synthase (AOS) and

allene oxide cyclase (AOC), and is finally converted to jasmonic acid through reduction and three steps of β -oxidation (Creelman and Mullet 1997; Staswick 1992). MeJA (methyl JA) was first discovered in the floral scent of jasmine; subsequent studies have demonstrated the presence of JA in all higher plants tested, including *Arabidopsis*, maize, potato, rice, soybean, tobacco, and tomato (Bell et al. 1995; Creelman et al. 1992; Engelberth et al. 2003; Schmelz et al. 2003; Seto et al. 1996; Weber et al. 1997). Many JA biosynthetic genes isolated from different plant species have been characterized and have been found to be functionally conserved, such as *LOX* from *Arabidopsis* (Bell et al. 1995), barley (Voros et al. 1998), potato (Royo et al. 1999), soybean (Bell and Mullet 1991), tomato (Heitz et al. 1997), and wheat (Bohland et al. 1997), *AOS* from *Arabidopsis*, barley, flax, guayule, *Nicotiana attenuata*, rice, tomato (Ha et al. 2002; Harms et al. 1995; Itoh et al. 2002; Kubigsteltig et al. 1999; Sivasankar et al. 2000), and *AOC* from *Arabidopsis* (Stenzel et al. 2003b), barley (Maucher et al. 2004), and tomato (Stenzel et al. 2003a).

JA are well-known to act as a regulator in plant growth and development as well as a signal in plant defense and wound responses. They are involved in regulating the expression of numerous genes and influencing diverse processes including pollen development, fruit ripening, root growth, tendril coiling, wound responses, and resistance against insects and pathogens (Creelman and Mullet 1997; Liechti and Farmer 2002; Mandaokar et al. 2003; Reymond and Farmer 1998; Staswick 1992; Vijayan et al. 1998; Wasternack and Parthier 1997). The JA responses were found in a variety of plant species, such as *Arabidopsis*, barley, maize, soybean, tomato, and wheat (Cordero et al. 1994; Creelman and Mullet 1997; Ma and Wang 2003; Rakwal et al. 2001; Reinbothe et al. 1993). A wide range of genes are regulated by JA, including vegetative storage proteins, thionin, defensin, and pathogenesis-related proteins from various plants, such as *Arabidopsis*, maize, rice, and soybean (Berger et al. 1995; Chen et al. 2002; Cordero et al. 1994; He et al. 2004; Jwa et al. 2001; Manners et al. 1998; Mason and Mullet 1990; Rakwal and Komatsu 2000; Santamaria et al. 2001; Vignutelli et al. 1998).

Various JA responses have been defined through analysis of genetically identified mutants, including *jar1* (Staswick 1992; Staswick et al. 1998), *coi1* (Feys et al. 1994), *jin* (Berger et al. 1996), *fad3-2 fad7-2 fad8* (McConn and Browse 1996), *opr3* (Sanders et al. 2000; Stintzi and Browse 2000; Stintzi et al.

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2001), *cev1* (Ellis et al. 2002), *mpk4* (Petersen et al. 2000), *cet1-9* (Hilpert et al. 2001), *cex1* (Xu et al. 2001), *jue1*, *jue2*, and *jue3* (Jensen et al. 2002), *iop1* (Penninckx et al. 2003) and others (Lee and Howe 2003; Li et al. 2001, 2002). The *jar1* and *jim* mutants exhibit decreased sensitivity to JA; JAR1 encodes an enzyme with JA adenylation activity (Staswick et al. 2002), while JIN1 encodes a MYC transcription factor functional in JA and abscisic acid signaling and is essential to JA and ethylene-regulated defense responses (Abe et al. 2003; Anderson et al. 2004; Boter et al. 2004; Lorenzo et al. 2004). The *coil-1* mutant is male sterile, insensitive to JA, and lacks the expression of JA-induced genes (Benedetti et al. 1995; Feng et al. 2003; Feys et al. 1994; Penninckx et al. 1998; Xie et al. 1998). The identification of COI1 as an F-box protein pointed to the involvement of the ubiquitination pathway in JA signaling (Xie et al. 1998). The F-box protein COI1 is able to associate with *Arabidopsis* SKP1-like proteins ASK1 and ASK2, AtCUL1, and RBX1 in planta (Xu et al. 2002).

In this study, we isolated *GmCOI1* from soybean (*Glycine max* L. (Merr.)), which shares significant homology with the *Arabidopsis* COI1 sequence and also similarly contains an F-box motif and leucine rich repeats (LRR). To test whether the sequence homology and structural similarity are indicative of functional conservation, we expressed *GmCOI1* in the *Arabidopsis* *coil-1* mutant, and found that *GmCOI1* is capable of substituting COI1 and assembling into the Skp1-Cullin-F-box (SCF) complexes to restore normal JA responses, including JA-regulated plant defense and fertility in the *coil-1* mutant of *Arabidopsis*, suggesting a functional conservation of COI1 in soybean and *Arabidopsis* and implying a generic JA pathway with conserved signal components in different plant species.

RESULTS

GmCOI1 is an F-box protein gene and highly related to COI1.

To investigate the potential conservation of the COI1-mediated JA signal transduction in the plant kingdom, we performed the BLAST search to identify the sequences highly homologous to COI1 in GenBank or the expressed sequence tags (EST) databases. As a result, we identified soybean EST fragments that are highly identical to COI1. We then obtained the full coding sequence by screening the soybean cDNA library with the EST fragment as a probe and by sequencing these isolated cDNA clones. Sequence analysis indicated that the identified gene is structurally similar to COI1, having both the F-box motif and the LRR domain; it shares significant identity, 68.5% with COI1 (Xie et al. 1998), 70% with LeCOI11 (the tomato homolog of COI1) (Li et al. 2004), and 57.3% with OsCOI1 (*Oryza sativa* COI1) (the rice homolog of COI1) (Figs. 1A and 2A); this gene is here referred to as *GmCOI1* (*Glycine max* L. (Merr.) COI1). In summary, *GmCOI1* exhibits significant structural similarity and strong sequence homology with the *Arabidopsis* COI1, suggesting that *GmCOI1* may be a COI1-like gene and bear COI1 function as an F-box protein assembled into SCF complexes essential for JA signaling.

Transient expression of *GmCOI1* in *coil-1* restores COI1-dependent activation of the JA-responsive reporter *Thi2.1:GUS*.

In *Arabidopsis*, transient expression of COI1 is able to complement the *coil-1* mutation (Xie et al. 1998) in activation of expression of many COI1-dependent genes such as *Thi2.1*, a gene that encodes an antimicrobial protein. To efficiently test whether the *GmCOI1* is functionally similar to *Arabidopsis* COI1, we introduced *GmCOI1*, which was driven by the cauliflower mosaic virus (CaMV) 35S constitutive promoter (Fig.

2A), into the *coil-1* plants transgenic for the *Thi2.1* promoter-driven β -glucuronidase gene (*GUS*) reporter (referred to as *coil-1;Thi2.1:GUS*) by particle bombardment (Xie et al. 1998). Histochemical staining revealed sites of transformation as blue spots of GUS activity in leaves of the *coil-1;Thi2.1:GUS* plants, indicating transient complementation of *coil-1* by the introduced genes (Xie et al. 1998). The results shown in Figure 2B clearly demonstrated that *GmCOI1*, like COI1, was able to activate the *Thi2.1* promoter to drive GUS expression in the *coil-1;Thi2.1:GUS* plants. As expected, the control vector or the cloned *coil-1* mutant fragment failed to complement *coil-1* under the identical condition (Fig. 2) (Xie et al. 1998). These results suggest that *GmCOI1* is functionally similar to COI1 in activation of the COI1-dependent gene expression.

Transgenic expression of *GmCOI1* in *coil-1* restores normal JA sensitivity and male fertility.

To further investigate whether *GmCOI1* is functionally similar to COI1 in all JA responses, including JA sensitivity and male fertility, we created stable transgenic *coil-1* plants expressing the Myc-tagged *GmCOI1* (referred to as *coil-1;GmCOI1*). As *coil-1* homozygous mutant plants are not suitable for plant transformation (Xie et al. 1998), we first introduced the Myc-tagged *GmCOI1* driven by the CaMV 35S constitutive promoter and other control constructs, including empty vector and the *Arabidopsis* COI1 gene driven by the CaMV 35S promoter into *coil-1* heterozygous plants. The *coil-1* homozygous mutant plants expressing the transgenes, including *GmCOI1* (*coil-1;GmCOI1*), vector (*coil-1;vector*), and COI1 (*coil-1;COI1*) were verified using the *coil-1*-specific cleaved amplified polymorphic sequence (CAPS) marker (Xie et al. 1998) (Fig. 3A) and then were examined by Western blot analysis (Fig. 3B). Three independent *coil-1;GmCOI1* lines (GMC1 through GMC3) were used for further analysis.

To verify the *Thi2.1:GUS* expression patterns from transient complementation shown in Figure 2B, we introduced the *Thi2.1* promoter-driven *GUS* reporter (*Thi2.1:GUS*) from the *Thi2.1:GUS* transgenic plant (Xie et al. 1998) into the GMC1 line (*coil-1;GmCOI1*) as well as the control lines VEC1 (*coil-1;vector*) and the ARC1 line (*coil-1;COI1*) via genetic crossing. As shown in Figure 3C, the *Thi2.1:GUS* activity was clearly detected in the *coil-1;GmCOI1;Thi2.1:GUS* plants (GMC1) and the *coil-1;COI1;Thi2.1:GUS* plants (ARC1) but not in the *coil-1;vector;Thi2.1:GUS* plants (VEC1), consistent with the data shown in Figure 2B.

To further examine whether the *Thi2.1:GUS* expression patterns are indicative of expression of endogenous *Thi2.1* gene in planta, we analyzed the *Thi2.1* transcript level, using Northern hybridization. As shown in Figure 3D, the *Thi2.1* transcripts were accumulated upon JA induction in the GMC1 line (*coil-1;GmCOI1*) but not in the control line VEC1 (*coil-1;vector*), consistent with the *Thi2.1:GUS* expression patterns in the ARC1 line (*coil-1;COI1*). Moreover, Northern blot analysis revealed that *GmCOI1* could functionally substitute COI1 to mediate the expression of other JA-responsive genes, including *PDF1.2* and *AtVSP* (Fig. 3D). Similar results were obtained from other independent transgenic GMC2 and GMC3 lines (Fig. 3D and data not shown). These results indicated that *GmCOI1* is able to functionally substitute COI1 and mediate the expression of JA-responsive genes in *Arabidopsis*.

As assayed for JA-inhibitory root growth, three GMC lines (*coil-1;GmCOI1*) all exhibited JA sensitivity at a level similar to the ARC1 line (*coil-1;COI1*), whereas the VEC1 line (*coil-1;vector*) was resistant to JA (Fig. 3E and data not shown), demonstrating that the *GmCOI1* is able to fully substitute *Arabidopsis* COI1 to mediate JA sensitivity.

The *coil-1* mutant is deficient in the production of viable pollen grains causing male sterility (Feys et al. 1994; Xie et al. 1998); therefore, we examined whether *GmCOI1* is able to functionally complement the defects of pollen development in *coil-1*. Three GMC lines (*coil-1*;*GmCOI1*), similar to the ARC1 line (*coil-1*;*Myc-COI1*), were able to produce viable pollen grains and generated fertile siliques (Fig. 3F). These re-

sults demonstrate functional conservation of *COI1* and *GmCOI1* in modulation of pollen development.

Transgenic expression of *GmCOI1* in *coil-1* restores plant defense.

The *coil-1* mutations were previously found to cause loss of plant defense responses (McConn et al. 1997; Norman-Setterblad

Fig. 1. Sequence comparison between AtCOI1 from *Arabidopsis* (AAB95279.1), GmCOI1 from soybean, LeCOI1 from tomato (AAR82925.1), and OsCOI1 from rice (BAD81943.1). Black boxes represent identical residues. The F-box domain is marked with one single solid line, while the leucine-rich repeats domain is located between the two inverted arrows.

et al. 2000; Stintzi et al. 2001; Thomma et al. 1998; Vijayan et al. 1998). To test whether *GmCOI1* is able to restore JA-regulated defense responses in the *Arabidopsis coi1-1* mutant plants, we inoculated *Botrytis cinerea* onto the *coi1-1* plants transgenic for *GmCOI1* (*coi1-1;GmCOI1*), the Myc-tagged *Arabidopsis COI1* (*coi1-1;Myc-COI1*), and vector (*coi1-1;vector*). As expected, all the seedlings from VEC1 (*coi1-1;vector*) were dead (100%) 14 days after pathogen inoculation, whereas those from the ARC1 line (*coi1-1;Myc-COI1*) were resistant to these pathogens, exhibiting only approximately 5% death rate under identical treatment (Fig. 4A). The *coi1-1;GmCOI1* transgenic lines, including GMC1, all displayed disease resistance by showing only a 4.5% death rate, similar to that of the ARC1 line (Fig. 4A and data not shown). Consistent with the phenotypes of disease resistance, expression of the defense-related genes, such as the plant defensin *PDF1.2*, upon pathogen inoculation was observed in the *coi1-1;GmCOI1* and *coi1-1;Myc-COI1* transgenic plants but not in the *coi1-1;vector* plants (Fig. 4C). These data suggest that the *GmCOI1* restores the plant defense in the *Arabidopsis coi1-1* mutant plants. A similar conclusion was reached when we tested the GMC1 line (*coi1-1;GmCOI1*) for the plant resistance against the bacterium *Erwinia carotovora* (Fig. 4B).

Domain swapping of the F-box motif and the LRR domain between COI1 and GmCOI1.

GmCOI1 is speculated to encode F-box proteins that are structurally similar to COI1, containing an F-box motif and 16

Fig. 2. Plasmid constructs and transient complementation. **A**, Plasmid constructs. *AtCOI1*, *GmCOI1*, *SF-CL*, and *CF-SL* genes were 5' myc tagged (m) under control of the cauliflower mosaic virus 35S promoter. *SF-CL* consists of the F-box domain (F) from *GmCOI1* and the leucine-rich repeat (LRR) domain from *COI1*, whereas *CF-SL* contains the F-box domain from *COI1* and the LRR domain from *GmCOI1*. The numbers indicate the locations of the amino acid residues. The schematic diagram is not on scale. **B**, Particle bombardment in the *coi1-1;Thi2.1:GUS* plants with the plasmid constructs *AtCOI1* and *GmCOI1* described in A. The vector *pMYC2* was used as a control. The blue spots indicated positive β -glucuronidase activity (Xie et al. 1998).

LRR (Fig. 1A). To further analyze the functional conservation of their F-box and LRR domains, we in-frame fused the F-box motif of soybean *COI1* to the LRR domain of *Arabidopsis COI1*, resulting in a chimerical gene designated *SF-CL* (soybean *COI1* F-box–*COI1* LRR). We also swapped with the *Arabidopsis COI1* F-box motif in the soybean *COI1* to generate another chimerical gene designated as *CF-SL* (*COI1* F-box–soybean *COI1* LRR) (Fig. 2A). Similar to generation and characterization of *coi1-1;GmCOI1* transgenic plants, we created the *coi1-1* transgenic plants expressing the *SF-CL* and *CF-SL* chimerical genes, which were designated as *coi1-1;SF-CL* and *coi1-1;CF-SL*, respectively. Three independent lines for each transgenes (SFCL1 to SFCL3 for *coi1-1;SF-CL* and CFSL1 to CFSL3 for *coi1-1;CF-SL*) were used for further analysis and were found to display similar results. The data from the transgenic lines SFCL1 and CFSL1 are shown in Figure 3.

As assayed for JA-inducible gene expression revealed by Northern blot analysis (Fig. 3D) and for JA inhibitory effect on root elongation (Fig. 3E), pollen development, and plant fertility (Fig. 3F), we found that *SF-CL* and *CF-SL* complemented the *coi1-1* mutation. When assayed for resistance against the fungus *Botrytis cinerea* (Fig. 4A) and against the bacterium *Erwinia carotovora* (Fig. 4B), the *coi1-1;SF-CL* and *coi1-1;CF-SL* transgenic plants exhibited resistance, though resistance level is lower than that conferred by *Myc-COI1* or *GmCOI1*. Consistently, the plant defensin *PDF1.2* transcripts upon pathogen infection were accumulated in the *coi1-1;SF-CL* and *coi1-1;CF-SL* transgenic plants (Fig. 4C and D), though at a lower level than that in the *coi1-1;Myc-COI1* or *coi1-1;GmCOI1* plants (Fig. 4). These results further demonstrate the functional conservation of the F-box and LRR domains in the *GmCOI1* and *Arabidopsis COI1*.

Formation of GmCOI1-containing SCF complexes.

The F-box protein COI1 was found to interact with the *Arabidopsis* SKP1-like proteins ASK1 and ASK2 in the yeast two-hybrid system and to associate with AtCUL1 and ASK1 or ASK2, assembling the SCF^{COI1} complexes that mediate JA signal transduction in *Arabidopsis* (Xu et al. 2002). To test whether *GmCOI1* in *Arabidopsis* is able to form SCF complexes similar to SCF^{COI1}, we used the yeast two-hybrid system and coimmunoprecipitation assay to examine the interaction of *GmCOI1* with SCF components. As shown in Figure 5A, the soybean COI1 was also able to interact with *Arabidopsis* ASK1 and ASK2 in the yeast two-hybrid system, which is similar to the interaction of COI1 with ASK1 and ASK2. As revealed by coimmunoprecipitation assay, the *GmCOI1* was found to associate with AtCUL1, RBX1, ASK1, or ASK2 and form SCF complexes in the *coi1-1;GmCOI1* transgenic plants (Fig. 5B and data not shown). These data demonstrate that *GmCOI1* is capable of substituting COI1 and assembling into the SCF complexes in *Arabidopsis*, suggesting that the COI1 orthologs may mediate JA responses in different plant species via SCF formation similar to SCF^{COI1}.

DISCUSSION

The model organism *Arabidopsis* has been used as a powerful system to study *Arabidopsis* homologous genes from other species through ectopic expression, particularly the availability of many well-characterized *Arabidopsis* mutants offered a unique opportunity to investigate the predicted function of the homologous genes from other species via heterologous expression. This approach has been successfully used for functional analysis of *LeCTR1*, a tomato gene with strong homology to the *Arabidopsis CTR1* essential for ethylene signal transduction, which was expressed in the ethylene response-deficient

Fig. 3. *GmCOI1*, *SF-CL*, and *CF-SL* restore jasmonate (JA) sensitivity and pollen fertility in the *Arabidopsis coi1-1* mutant. **A**, The transgenic lines transformed with the constructs containing *AtCOI1*, *GmCOI1*, *SF-CL*, or *CF-SL* genes were homozygous for *coi1-1*, which was revealed by the *coi1-1*-specific cleaved amplified polymorphic sequence markers. WT = wild type (*Arabidopsis Columbia*). The VEC1 line = the *coi1-1* mutant transgenic for the *pMYC2* vector. The transgenic lines ARC1, GMC1, SFCL1, or CFSL1 represent the *coi1-1* plants transgenic for the constructs *AtCOI1*, *GmCOI1*, *SF-CL*, and *CF-SL*, respectively. **B**, Western analysis of the transgenic lines mentioned in A. Top panel: the Myc-fused proteins were detected with Myc antibody (α -myc). Bottom panel: the ASK1 protein detected by the α -ASK1 antibody was used as loading control. **C**, The *coi1-1;Thi2.1:GUS* plants containing the constructs *AtCOI1* (the ARC1 line), *GmCOI1* (the GMC1 line), *SF-CL* (the SFCL1 line), or *CF-SL* (the CFSL1 line) were examined for β -glucuronidase activity upon JA induction. **D**, Northern analysis of the JA-treated (+) or untreated (-) transgenic lines probed with *PDF1.2*, *Thi2.1*, and *AtVSP*. 18S rRNA or 28S rRNA was provided as the loading control. **E**, Phenotypes of nine-day-old seedlings grown on the plant growth (MS) medium or the medium containing 25 μ M methyl (Me)JA (top panel). The bottom panel represents the MeJA dose-response curve of root growth. Root length of the indicated seedlings grown on medium containing various MeJA (5, 10, 25, 50, or 80 μ M) for 9 days was expressed as a percentage of root length grown on MS. Each datapoint is the mean of more than 30 samples. The experiment was repeated three times. Error bars represent standard deviation ($n > 30$). **F**, The *GmCOI1*, *SF-CL*, and *CF-SL* transgenes are able to restore fertility in *coi1-1*. The arrows in the top panel indicate the viable pollen grains in anthers from the transgenic plants indicated, which were stained with 1% fluorescein diacetate and were visualized under fluorescence microscopy. The arrows in the bottom panel indicate fertile siliques from the plants described.

Fig. 4. *GmCOI1*, *SF-CL*, and *CF-SL* are able to restore jasmonate-regulated defense responses in the *Arabidopsis coi1-1* mutant. **A**, Two-week-old seedlings were inoculated with *Botrytis cinerea* (*B. c*) (bottom panel) or 1% D-Glucose (CK) (top panel) and were photographed on the 14th day after inoculation. The death rate and standard deviation ($n > 40$) for each line are indicated, except when standard deviation is 0. The experiment was repeated seven times. **B**, Three-week-old seedlings were inoculated with *Erwinia carotovora* (*E. c*) (bottom panel) or Luria-Bertani (LB) medium (CK) (top panel) and were photographed on the 14th day after inoculation. The death rate and standard deviation ($n > 40$) for each line are indicated, except when standard deviation is 0. The experiment was repeated seven times. **C**, Northern blot analysis of the expression of plant defense gene *PDF1.2* in the indicated transgenic lines inoculated without (–) or with (+) *Botrytis cinerea* (*B. c*). **D**, Northern blot analysis of the expression of plant defense gene *PDF1.2* in the indicated transgenic lines inoculated without (–) or with (+) *Erwinia carotovora* (*E. c*).

mutant *ctr1-1* of *Arabidopsis* and was found to be capable of restoring normal ethylene signaling (Leclercq et al. 2002). Here, we used a similar approach to carry out functional analysis of *GmCOI1*, which is isolated from soybean and highly homologous to the *Arabidopsis COI1*. We demonstrate that *GmCOI1* from soybean is capable of substituting *AtCOI1* and mediating JA-regulated plant defense and fertility in *Arabidopsis*.

F-box proteins are generally composed of a conserved F-box motif at N-termini and a protein-protein interaction motif such as LRR, WD40, or Kelch repeats at C-termini and have been widely identified in mammals, plants, and yeast (Deshaies 1999; Hershko and Ciechanover 1998). In *Arabidopsis*, nearly 700 potential F-box genes have been identified (Gagne et al. 2002). Several F-box genes have also been characterized in other plant species, including rice (Sasaki et al. 2003), tomato (Li et al. 2004), *Antirrhinum* (Lai et al. 2002; Simon et al. 1994), pea (Taylor et al. 2001), Lotus (Zhang et al. 2003), almond, and cherry (Entani et al. 2003; Ushijima et al. 2003; Yamane et al. 2003). However, there is no information regarding molecular cloning of F-box genes in soybean. In this work, for the first time, we isolated the F-box gene *GmCOI1* from soybean and established the biological functions for the homolog of *COI1* from other plant species in JA signaling in *Arabidopsis*. Through transient or transgenic expression, or both, of *GmCOI1*, the soybean homolog of *COI1*, in the JA-response-deficient mutant *coi1-1* of *Arabidopsis*, we found that *GmCOI1* is capable of complementing the *coi1-1* mutation and restoring normal JA responses, including plant defense and fertility. These results suggest that *GmCOI1* is functionally conserved with the *COI1*, implying that *COI1*-mediated JA signaling pathways may be conserved in different plant species. Our finding is in good agreement with the previous studies that the JA biosynthesis and inducible genes are quite conserved in different plant species.

Despite the functional conservation, developmental and evolutionary modification among different plant species may influence JA responses and alter biological functions for the homologous genes of *COI1* in the corresponding host plant. It is noticeable to point out a discrepancy in JA responses between *Arabidopsis* and tomato. The *Arabidopsis* mutants defective in JA biosynthesis (*fad3-2 fad7-2 fad8*, *dad1*, *dde1/opr3*) or perception (*coi1-1*) are male sterile (Feys et al. 1994; Ishiguro et al. 2001; McConn and Browse 1996; Sanders et al. 2000; Stintzi et al. 2001), whereas tomato mutants apparently defective in JA biosynthesis (*def-1*, *spr-1*, *spr-2*) or perception (*jai1*) are male fertile (Lee and Howe 2003; Li et al. 2001, 2002), suggesting that the *Arabidopsis* *COI1* partner, which is essential for pollen development, may be altered in tomato and other specific components or signal pathways have been evolutionarily developed to mediate pollen development in tomato. Indeed, the recently identified tomato homolog of *COI1* (*LeCOI1*) from the mutant *jai1* is essential for JA-regulated gene expression and defense response but is not necessary for pollen development (Li et al. 2004). It was reported that the *jai1* mutant exhibited reduced pollen viability and the sterility was caused by a defect in the maternal control of seed maturation, which was associated with the loss of accumulation of JA-regulated proteinase inhibitor proteins in reproductive tissues (Li et al. 2001, 2004). Recently, a rice mutant, *hebiba*, was found to display impaired induction of the JA pathway and exhibit male sterility (Riemann et al. 2003), suggesting that the *COI1*-regulated pollen fertility might be conserved in rice and *Arabidopsis*. Identification and characterization of rice mutant with mutation in *OsCOI1* would further test the possible conservation in JA responses between rice and *Arabidopsis*.

The SCF complex is composed of four subunits, Cullin/Cdc53, SKP1, RBX1/ROC1/HRT1, and a variable component F-box protein (Deshaies 1999; Hershko and Ciechanover 1998). Except for this structural conservation, the homologs of the SCF core components from different species are likely conserved in the basic function. For example, disruption of Cullin caused similar developmental defects in embryogenesis in mice (Dealy et al. 1999; Wang et al. 1999) and *Arabidopsis* (Shen et al. 2002; Ren et al. 2005), yet there were some differences due to evolutionary modification. It is interesting to know whether the homologs of F-box genes from different species share similar functions. Several homologs of F-box genes among the plant species have been characterized. The *Arabidopsis* unusual floral organs (UFO) (Samach et al. 1999) shares conserved function with the UFO orthologs from pea (Taylor et al. 2001), *Antirrhinum* (Ingram et al. 1997; Simon et al. 1994), and Lotus (Zhang et al. 2003). The F-box gene *GID2* from rice and its homologous gene from *Arabidopsis* (*SLY1*) exhibited similar function in gibberellin signaling (McGinnis et al. 2003; Sasaki et al. 2003). OsRALyase1, a putative F-box protein from rice, displayed identical enzyme activity as wheat RALyase did (Ito et al. 2002; Ogasawara et al.

Fig. 5. Formation of GmCOI1-containing Skp1-Cullin-F-box complexes. **A**, GmCOI1 interacts with ASK1 and ASK2 in the yeast two-hybrid system. 1, pLexA-COI1/pB42AD-ASK1; 2, pLexA-GmCOI1/pB42AD-ASK1; 3, pLexA-COI1/pB42AD-ASK2; 4, pLexA-GmCOI1/pB42AD-ASK2; 5, pLexA-COI1/pB42AD; and 6, pLexA-GmCOI1/pB42AD. Positive interaction of the indicated proteins was revealed by blue color of the colonies showing positive β -galactosidase activity. **B**, Coimmunoprecipitation assays. Protein extracts from the indicated transgenic lines ARC1 (*coi1-1* transgenic plants expressing Myc-tagged COI1) and GMC1 (*coi1-1* transgenic plants expressing Myc-tagged GmCOI1) were immunoprecipitated (IP) with α -Myc antibody. The resulting immunoprecipitates were analyzed by Western blotting probed with the antibodies α -Myc, α -AtCUL1, α -ASK1, and α -RBX1 (indicated on the left side). The VEC1 line (the *coi1-1* transgenic for vector) was used as the control to demonstrate the specificity of coimmunoprecipitation assays. Hc = immunoglobulin heavy chain.

1999). The homologous F-box genes from the rosaceous species almond and *Antirrhinum*, and three species of cherry, *Prunus cerasus*, *P. avium*, and *P. mume*, all defined the self-incompatibility locus (Entani et al. 2003; Lai et al. 2002; Ushijima et al. 2003; Yamane et al. 2003). Our results, restoration of normal JA-regulated plant defense and fertility by the soybean F-box protein GmCOI1 in *Arabidopsis coi1-1* mutant plants, provide a clue that COI1 may be functionally conserved in *Arabidopsis* and soybean. The results that GmCOI1 interacted with the *Arabidopsis* COI1-associated proteins and assembled into the SCF complex in *Arabidopsis* indicate that the COI1 homologs mediate JA responses, probably via SCF complexes in various plant species; yet, the potential differences are not excluded. Combined with the data from other plant F-box homologous genes characterized, these results indicate a possibility that homologous F-box genes from different plant species might also share similar basic functions. This notion will be determined via further characterization of more F-box homologous genes from various plant species.

MATERIALS AND METHODS

Identification of GmCOI1 and domain swapping between GmCOI1 and AtCOI1.

The *Arabidopsis* COI1 sequence was used for the BLAST search in the EST databases. Following routine methods, the partial fragments highly identical to COI1 were identified from soybean EST databases, were subsequently polymerase chain reaction (PCR)-amplified and labeled with [α - 32 P] dCTP as probes, to screen the soybean (*Glycine max* L. (Merr.) cDNA library (Stratagene, La Jolla, CA, U.S.A.) for the full coding region; the identified cDNA was finally sequenced.

The full-length coding region of the *GmCOI1* gene was PCR-amplified from the soybean cDNA clone with primers GmCOI1-ATG (5'-ATGACGGAGGATCGGAACGT-3') and GmCOI1-END (5'-CTAGGTGTCAACGCATGTG-3') and were subsequently cloned into the pMYC2 vector with 5'-tagged Myc in sense orientation under control of the CaMV 35S promoter (Xu et al. 2002). The *Arabidopsis* COI1 was previously Myc-tagged in pMYC2 (Xu et al. 2002).

Domain swapping between GmCOI1 and COI1 was performed by PCR. To fuse the F-box motif of *Arabidopsis* COI1 to the LRR domain of soybean *GmCOI1*, we used primers COI1-ATG (5'-ATGGAGGATCCTGATATC-3') and CS-R (5'-GTAGCAGAGCGCGATGGTCACATGCTCTCTCGTCTC-3') to PCR-amplify, from COI1, a 183-bp fragment A containing the COI1 F-box motif tagged with an 18-bp sequence (underlined in the CS-R primer sequence) identical to the start region of the *GmCOI1* LRR. Also, primers CS-F (5'-GAGACGAGA-GAGCATGTGACCATCGCGCTCTGCTAC-3') and GmCOI1-END were used to PCR-amplify, from *GmCOI1*, fragment B containing the *GmCOI1* LRR tagged with an 18-bp sequence (underlined in the CS-F primer sequence) identical to the end region of the *Arabidopsis* COI1 F-box motif. Fragments A and B were then mixed and used as templates to amplify the chimerical gene using primers COI1-ATG and GmCOI1-END, resulting in the *CF-SL* gene.

To swap with the soybean COI1 F-box motif in the *Arabidopsis* COI1, a similar strategy was used. Primers GmCOI1-ATG and SC-R (5'-GTAGCAAAGCGCCATAGTGACGTGCTTCCGAGTGAG-3') were used to amplify fragment A from *GmCOI1*, and primers SC-F (5'-CTCACTCGGAAGCACGTC ACTATGGCGCTTTGCTAC-3') and COI1-3E (5'-TATCATAT TGGCTCCTTCAG-3') were used to amplify fragment B from COI1. Fragments A and B were mixed to serve as templates to amplify fused fragments, using primers GmCOI1-ATG and COI1-3E, resulting in the *SF-CL* gene.

The *CF-SL* and *SF-CL* genes were finally cloned in-frame to pMYC2 in sense orientation under the control of 35S promoter following routine methods (Xu et al. 2002).

All the constructs were sequence-verified. The *Arabidopsis* COI1/*coi1-1* F1 plants were used in the *Agrobacterium*-mediated floral infiltration transformation (Bechtold et al. 1993; Bent 2000).

Transient expression assay and GUS histochemical staining as well as pollen grain staining in 0.01% (wt/vol) fluorescein diacetate (FDA) were described in our previous work (Xie et al. 1998).

coi1-1-specific CAPS marker.

Primers p1 (5'-GGTTCTCTTTAGTCTTTAC-3') and p2 (5'-CAGACAACTATTTCGTTACC-3') were used to amplify the approximately 1.5-kb genomic DNA fragment containing COI1 or *coi1-1* (but not the transgene). As the *coi1-1* mutation abolishes the *XcmI* recognition site (Xie et al. 1998), the *XcmI* was unable to digest the approximately 1.5-kb PCR fragment amplified from the *coi1-1*-homozygous plants but was able to generate two bands (approximately 0.5 and 1.0 kb) for wild type (COI1/COI1) or three bands (approximately 0.5, 1.0, and 1.5 kb) for heterozygous (COI1/*coi1-1*) plants.

All the transgenic plants used in this work were confirmed to be homozygous for *coi1-1*. Three independent transgenic lines with similar expression levels of each transgene were tested and exhibited similar results. We only showed the results obtained from one transgenic line of each transgene in all the figures except for Figure 3D.

Plant growth conditions and treatment.

Seeds were surface-sterilized, were plated on plant growth (MS) medium, were chilled at 4°C for 3 days, and then, were transferred to a growth chamber under a 16-h light (21 to 23°C) and 8-h dark (16-19°C) cycle. For root-length measurement experiments, the seedlings were grown on MS medium supplemented with various concentrations of MeJA (Aldrich, St. Quentin, France) for 9 days before measurement.

Defense assay in *Arabidopsis*.

Two-week-old *Arabidopsis* seedlings were inoculated with a spore suspension from the fungus *Botrytis cinerea* (provided by Y. D. Choi) (Seo et al. 2001) at a concentration of 1×10^5 spores/ml in 1% D-Glucose (wt/vol), whereas control seedlings were sprayed with 1% D-Glucose. The plants were placed in a dark chamber for 18 h at 22°C with high relative humidity and were subsequently maintained under an 8-h light and 16-h dark cycle at 22°C and high relative humidity. The death rate of the seedlings was recorded on the 14th day after inoculation.

For inoculation of bacterium *Erwinia carotovora* subsp. *carotovora*, 1 μ l of bacterial suspension (2×10^7 CFU/ml) in LB liquid medium (pH 7.0) was added to each of two wound sites on each leaf of three-week-old seedlings, whereas control seedlings were inoculated with Luria-Bertani (LB) liquid medium. Five leaves from each seedling were inoculated. Plant growth conditions were similar to those used in the inoculation experiment with *Botrytis cinerea* described above. The death rate of the seedlings was recorded on the 14th day after inoculation.

Plant materials used in Northern blot analysis.

Two-week-old seedlings on MS medium were treated for 7 h with 100 μ M MeJA (Aldrich) or with distilled water as control, and then, were frozen in liquid nitrogen.

Two-week-old seedlings were inoculated with the fungus *Botrytis cinerea* (or with 1% D-Glucose as control). Three-week-old seedlings were inoculated with the bacterium *Er-*

winia carotovora (or with LB liquid medium as control). After inoculation (72 h), the plants were harvested and frozen in liquid nitrogen.

Total RNA was isolated from the above materials and was used in Northern hybridization with the digoxigenin-labeled probes *PDF1.2*, *Thi2.1*, *AtVSP*, and *18S* rDNA, following the method described in our previous work (Xu et al. 2001; Xu et al. 2002).

Interaction of GmCOI1 with ASK1 and ASK2 in yeast.

The *GmCOI1* was amplified with primers GmCOI1-*Bam*HI (5'-GAGGATCCTTATGACGGAGGATCGGAACGT-3') and GmCOI1-3ENcoI (5'-GACCATGGCTAGGTGTCAACGCATGTG-3') and was ligated to the vector pLexA at *Bam*HI and *Nco*I sites, generating pLexA-GmCOI1. pLexA-COI1, pB42AD-ASK1, and pB42AD-ASK2 were constructed in our previous study (Xu et al. 2002). These constructs were transferred to the yeast strain EGY48, to detect mutual interaction according to the MATCHMARKER LexA two-hybrid system (Clontech, Palo Alto, CA, U.S.A.) (Xu et al. 2002).

Coimmunoprecipitation in *Arabidopsis*.

The coimmunoprecipitation assays and antibodies were described in our previous work (Xu et al. 2002).

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