A Leaky Mutation in *DWARF4* Reveals an Antagonistic Role of Brassinosteroid in the Inhibition of Root Growth by Jasmonate in Arabidopsis[1][C]

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The F-box protein CORONATINE INSENSITIVE1 (COI1) plays a central role in jasmonate (JA) signaling and is required for all JA responses in Arabidopsis (*Arabidopsis thaliana*). To dissect JA signal transduction, we isolated the *partially suppressing coi1* (*psc1*) mutant, which partially suppressed *coi1* insensitivity to JA inhibition of root growth. The *psc1* mutant partially restored JA sensitivity in *coi1-2* background and displayed JA hypersensitivity in wild-type *COI1* background. Genetic mapping, sequence analysis, and complementation tests revealed that *psc1* is a leaky mutation of *DWARF4* (*DWF4*) that encodes a key enzyme in brassinosteroid (BR) biosynthesis. Physiological analysis showed that an application of exogenous BR eliminated the partial restoration of JA sensitivity by *psc1* in *coi1-2* background and the JA hypersensitivity of *psc1* in wild-type *COI1* background. Exogenous BR also attenuated JA inhibition of root growth in the wild type. In addition, the expression of *DWF4* was inhibited by JA, and this inhibition was dependent on *COI1*. These results indicate that (1) BR is involved in JA signaling and negatively regulates JA inhibition of root growth, and (2) the *DWF4* is down-regulated by JA and is located downstream of *COI1* in the JA-signaling pathway.

The plant hormone jasmonates, which include jasmonic acid and its cyclopentanone derivatives as well as cyclopentenones, regulate a variety of plant developmental processes including root growth, pollen development, senescence, and trichome development (McConn and Browse, 1996; Li et al., 2004; Browse, 2005; Schilmiller et al., 2007; Shan et al., 2007; Wasternack, 2007; Yan et al., 2007; Balbi and Devoto, 2008). Jasmonates also mediate responses to stress, wounding, insect attack, pathogen infection, and UV damage (Reymond and Farmer, 1998; Reymond et al., 2000, 2004; Bodenhausen and Reymond, 2007; Browse and Howe, 2008; Howe and Jander, 2008; Farmer and Dubugnon, 2009).

The effects of jasmonate (JA) on Arabidopsis (*Arabidopsis thaliana*) have been defined mainly through genetic analysis of JA biosynthetic mutants such as *fad3/fad7/fad8* (McConn and Browse, 1996), *opr3/dde1* (Sanders et al., 2000; Stintzi and Browse, 2000), and *aos* (Park et al., 2002), and through genetic analysis of JA-signaling mutants including *jar1* (Staswick et al., 1992), *coronatine insensitive1-1* (*coi1-1*; Feys et al., 1994), *jin1*, and *jin4* (Berger et al., 1996). Among these mutants, *coi1-1* is completely deficient in all the JA responses (Feys et al., 1994; Xie et al., 1998; Reymond et al., 2000; Browse, 2009; Shan et al., 2009; Sun et al., 2009). The *coi1-1* mutant has defects in JA-inhibited root growth, JA-induced anthocyanin accumulation, JA-induced lateral root formation, and JA-regulated gene expression, exhibits male sterility, and is susceptible to insect attack and pathogen infection, thereby having been considered as a key regulator in the JA signal transduction pathway.

The *COI1* gene has been found to encode an F-box protein, providing the first indication that ubiquitin-mediated protein degradation is involved in JA signaling (Xie, et al., 1998). This hypothesis has been supported by the demonstration that COI1 interacts with Arabidopsis CULLIN1, RBX1, and Skp1-like proteins ASK1 or ASK2 (Liu et al., 2004) to assemble SCF^{COI1} complexes in planta (Xu et al., 2002; Wang et al., 2005), and by observations that mutations in genes required for SCF function, such as *AXR1* and *CULLIN1*, result in reduced JA responses (Tiryaki and Staswick, 2002; Ren et al., 2005). JAZ proteins were identified as the substrates of the SCF^{COI1} complex

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for degradation by the 26S proteasome in response to JA (Chini et al., 2007; Thines et al., 2007; Chico et al., 2008; Katsir et al., 2008a). The complex containing COI1 and JAZ proteins might be a reception site of the jasmonoyl Ile (Katsir et al., 2008b), which is active as a specific enantiomeric form, the (+)-7-iso-jasmonoyl-L-Ile (Fonseca et al., 2009). Recent study demonstrated that COI1 is a JA receptor (Yan et al., 2009). Together, these research findings have gradually uncovered the molecular mechanism for the role of *COI1* in JA signaling.

To understand the molecular mechanism by which *COI1* regulates JA responses, we previously conducted a genetic screen for suppressors of the *coi1* mutant and identified *cos1* as a suppressor of *coi1* (Xiao et al., 2004). To further investigate *COI1*-mediated JA responses and dissect JA-signaling pathway, we continued to carry out genetic screens for suppressors of *coi1* mutant, and isolated one mutant named *partially suppressing coi1* (*psc1*) insensitivity to JA. Genetic mapping and a complementation test revealed that *PSC1* is an allele of *DWARF4* (*DWF4*) that encodes a key enzyme in brassinosteroid (BR) biosynthesis (Choe et al., 1998). Physiological analysis showed that the *psc1* mutation partially restored JA inhibition of root growth in *coi1-2* background and displayed JA hypersensitivity in wild-type *COI1* background; the effects of *psc1* were eliminated by exogenous BR. Furthermore, we found that BR repressed JA sensitivity in wild-type seedlings and that the inhibition of *DWF4* expression by JA was dependent on *COI1*. These results indicate that BR is involved in JA signaling and negatively regulates JA inhibition of root growth, and that *DWF4* is down-regulated by JA and is located downstream of *COI1* in the JA-signaling pathway.

RESULTS

Isolation of the *coi1* Suppressors

To isolate mutants that suppress *coi1*, we screened approximately 100,000 M_2 seedlings from approximately 20,000 M_1 ethyl methanesulfonate-mutagenized seeds of *coi1-2*, a *coi1* leaky mutant resistant to JA, but partially fertile and able to produce a small quantity of seeds (Xu et al., 2002), for reduced resistance to JA. Suppressor candidates of *coi1* were selected based on seedling phenotype with shorter roots and stunted growth when grown on a medium containing 10 μM methyl JA (MeJA). One suppressor candidate exhibited partial but obvious root growth inhibition by 10 μM MeJA, which was determined to have a single recessive Mendelian locus and named *psc1*. This *psc1coi1* mutant (homozygous for both *psc1* and *coi1-2* mutations) was backcrossed to *coi1-2* four times to remove other potential mutations. Further analysis of the inhibition of root growth by JA showed that relative root elongation of *psc1coi1* was clearly less than that of *coi1-2*, though it was still higher than that of wild type (Fig. 1A). Approximately 9%, 30%, and 54% inhibition

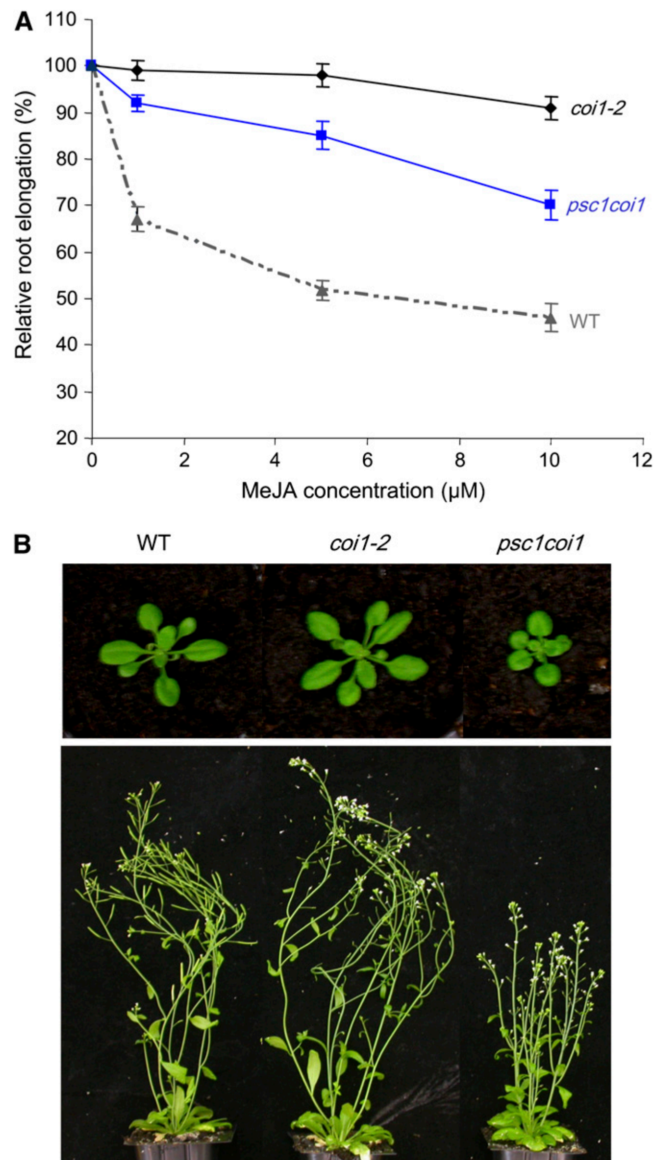

Figure 1. *psc1coi1* exhibited increased JA inhibition of root growth and morphologic alteration. A, Effect of MeJA on root growth of *psc1coi1*. Five-day-old seedlings transferred from MS to MS medium containing 0, 1, 5, and 10 μM MeJA were grown on vertically oriented plates for 3 d and increase in root length was measured. Relative root elongation is expressed as a percentage of root elongation on MS medium. Error bars represent SE ($n > 30$). B, The morphology of wild-type (WT), *coi1-2*, and *psc1coi1* seedlings at 18 d (top section) and flowering plants at 6 weeks (bottom section).

of root elongation by 10 μM MeJA was observed in *coi1-2*, *psc1coi1*, and the wild type, respectively (Fig. 1A). These results indicated that the *psc1* mutation partially restores the JA sensitivity in the *coi1* mutant background.

In addition to the partial root growth inhibition by JA, the *psc1coi1* exhibited other alterations in plant morphology. For example, the rosette leaves were smaller and round, the petioles were shorter, and the

plant height was reduced significantly compared to *coi1-2* (Fig. 1B).

Map-Based Cloning of the *PSC1* Gene

To map the *PSC1* locus, we carried out the genetic crossing between *psc1coi1*, which is of the Columbia (Col) ecotype, and *coi1-12*, a Landsberg *erecta* JA-insensitive mutant containing a single amino acid replacement (from Phe 359 to Lys) in COI1 (Xiao et al., 2004). We subsequently screened the F_2 progeny for *psc1coi1* homozygous seedlings that exhibited partial root growth inhibition by JA.

Based on the linkage analysis among molecular markers and the *psc1* phenotype of about 2,000 *psc1coi1* seedlings (about 25% of the F_2 population), we mapped the *PSC1* gene onto an approximately 45-kb interval between simple sequence length polymorphic (SSLP) marker S18800 and cleaved-amplified polymorphic sequence (CAPS) marker C18845, which were localized onto the T3A5 bacterial artificial chromosome (BAC; Fig. 2A). Sequencing verification identified a mutation within the *DWF4* gene: A single cytosine nucleotide at the 1,403rd base in the coding region of the *DWF4* gene was replaced by a thymine nucleotide, resulting in the substitution of the 468th

Figure 2. Mapping of the *PSC1* gene. A, With the SSLP markers S17854 and CIW4, CAPS marker MS-3-1, SSLP marker S18800, and CAPS marker C18845 in order, the *PSC1* locus was mapped to a approximately 45-kb interval between S18800 and C18845 on chromosome 3. The BAC T3A5 covers the *PSC1* locus region, and its two conjoint BACs, T20E23 and F18B3, are shown. The positions below the markers indicate the locations of the Arabidopsis Genome Initiative map on the chromosome. The numbers in parentheses indicate the recombinants. B, Sequence analysis of the *DWF4* gene in the *psc1coi1* mutant has a one-base change from C to T at the 1,403rd base, resulting in substitution of Val for Ala. [See online article for color version of this figure.]

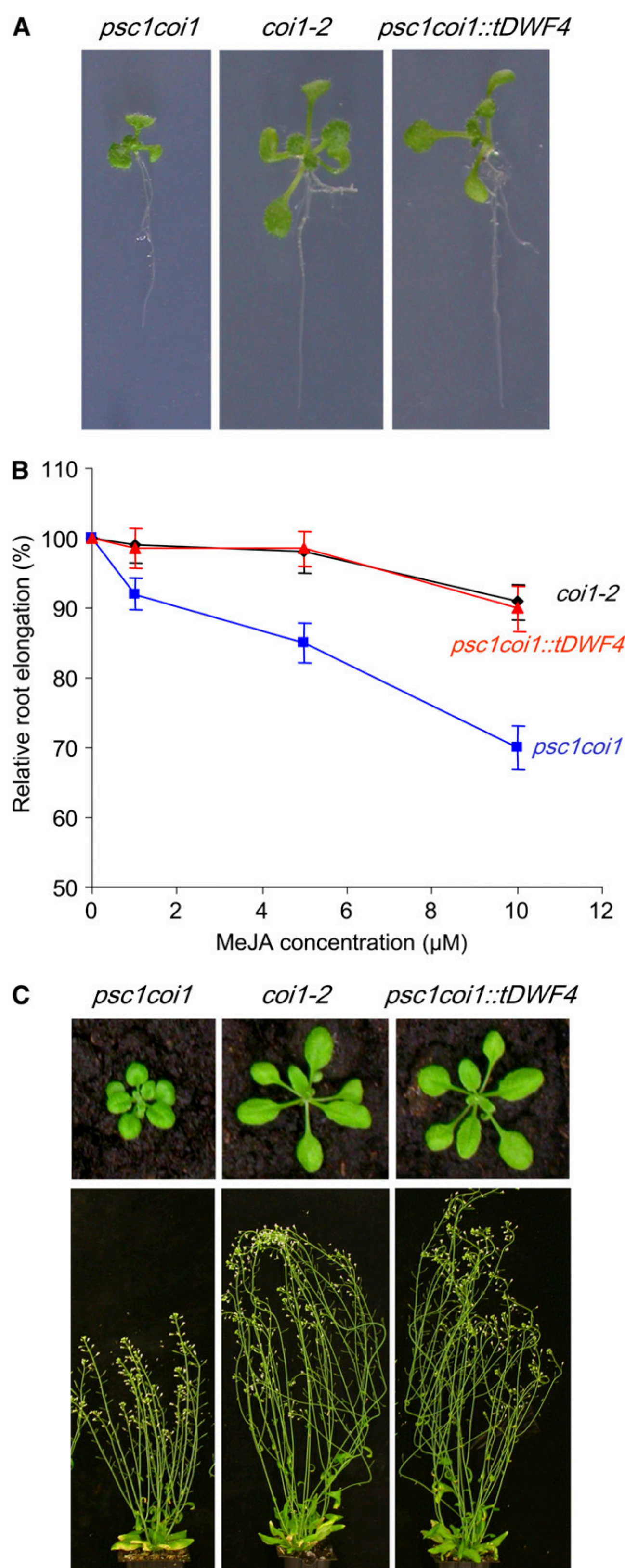

Figure 3. *DWF4* restores both JA insensitivity and morphologic phenotype in *psc1coi1*. A, The phenotype of 12-d-old seedlings grown on MS medium with 10 μM MeJA. B, Effect of MeJA on root growth of *coi1-2*, *psc1coi1*, and transgenic *psc1coi1*::*tDWF4* seedlings. Five-day-old seedlings transferred from MS to MS medium containing 0, 1, 5, and 10 μM MeJA were grown on vertically oriented plates for 3 d and increase in root length was measured. Relative root elongation is expressed as a percentage of root elongation on MS medium. Error bars represent SE ($n > 30$). C, The morphology of 18-d-old seedlings (top section) and 8-week-old flowering plants (bottom section).

amino acid in the DWF4 protein, Ala, with Val (Fig. 2B).

We transferred a genomic fragment containing wild-type *DWF4* gene with its endogenous promoter into the *psc1coi1* mutant and generated *psc1coi1*::*tDWF4* transgenic plants (see "Materials and Methods") for genetic complementation test. As shown in Figure 3A, the *psc1coi1*::*tDWF4* seedling was similar to the *coi1-2* seedling. When seedlings were grown on Murashige and Skoog (MS) supplemented with various concentrations of MeJA, relative root elongation of *psc1coi1*::*tDWF4* showed an obvious increase compared with that of *psc1coi1*, and was comparable to that of *coi1-2* (Fig. 3B). Also, other phenotypes including the size and status of leaves as well as height of plants were all restored to those of *coi1-2* (Fig. 3C). These results demonstrated that *PSC1* is an allele of *DWF4* and that the *psc1* mutation in the *psc1coi1* mutant is responsible for the partial suppression on *coil* insensitivity to JA-inhibitory root growth.

Cross Talk between JA and BR Signaling Pathways

Because the *DWF4* gene encodes a key enzyme in BR biosynthesis (Choe et al., 1998) and the mutants in BR biosynthesis are dwarf plants with short roots (Azpiroz et al., 1998; Nemhauser and Chory, 2004), we first investigated whether the short root of *psc1coi1* seedlings could be rescued by an application of exogenous BR, epibrassinolide (epi-BL; the most active BR). As expected, root elongation of *psc1coi1* was less than that of wild type or *coi1-2* when seedlings were grown on MS medium (Fig. 4A). When treated with 10 nM epi-BL, root elongation of *psc1coi1* appeared similar to that of wild type or *coi1-2* (Fig. 4A). Treatment with 10 nM epi-BL was sufficient to rescue the short root of *psc1coi1* seedlings and had no obvious effect on the root growth of the wild-type or *coi1-2* seedlings, but higher concentrations of epi-BL (100 or 1,000 nM) inhibited root growth (Fig. 4A). These results suggest that the mutation of *DWF4* in *psc1coi1* leads to a defect in BR biosynthesis.

The *psc1* mutation affected BR biosynthesis and caused JA partial sensitivity in *coi1-2* background, implying a cross talk between JA and BR. To verify the cross talk between JA and BR, we tested whether the suppression of *coi1* by *psc1* could be eliminated by 10 nM epi-BL, a concentration of epi-BL that did not affect the root growth of the wild type or *coi1-2* but rescued the root growth of *psc1coi1* to the normal wild-type level (Fig. 4A). As shown in Figure 4B, the inhibition of

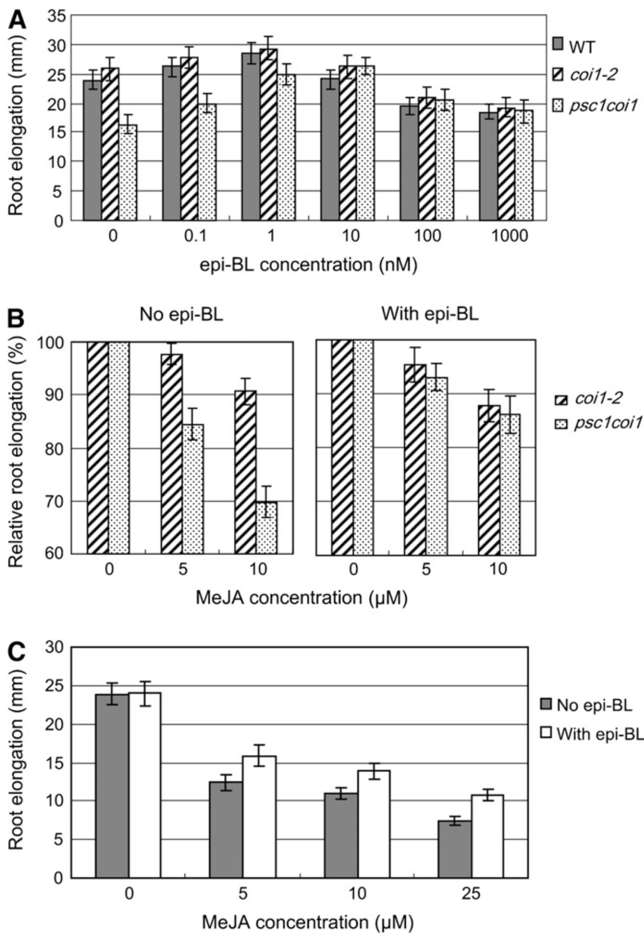

Figure 4. BR eliminates the effect of *psc1* on the sensitivity of *coi1* to JA and attenuates JA sensitivity in the wild type. A, Effect of epi-BL (the most active BR) on root growth of wild-type (WT), *coi1-2*, and *psc1coi1* seedlings. Five-day-old seedlings transferred to MS medium with 0, 0.1, 1, 10, 100, and 1,000 nM epi-BL were grown on vertically oriented plates for 3 d and increase in root length was measured. Error bars represent SE ($n > 30$). B, Effect of MeJA and epi-BL on root growth of *coi1-2* and *psc1coi1* seedlings. Five-day-old seedlings transferred to MS medium containing 0, 5, and 10 μM MeJA with or without 10 nM epi-BL were grown on vertically oriented plates for 3 d and increase in root length was measured. Relative root elongation is expressed as a percentage of root elongation on MS with (right section) or without (left section) 10 nM epi-BL. Error bars represent SE ($n > 30$). C, Effect of MeJA and epi-BL on root growth of wild-type seedlings. Five-day-old seedlings transferred to MS medium containing 0, 5, 10, and 25 μM MeJA with or without 10 nM epi-BL were grown on vertically oriented plates for 3 d and increase in root length was measured. Error bars represent SE ($n > 30$).

root growth by JA in *psc1coi1* seedlings was reduced remarkably by epi-BL and was restored to that of *coi1-2*. Therefore, BR is able to eliminate the suppression of *psc1* on *coil* insensitivity to JA-inhibitory root growth, suggesting that the BR signal might negatively regulate JA inhibition of root growth.

To further determine whether the BR signal negatively regulates JA inhibition of root growth, we investigated whether BR was able to attenuate JA inhibition of root growth in the wild type. The wild-type seedlings were grown on MS medium supplemented with 5, 10, and 25 μM MeJA with or without 10 nM epi-BL. As shown in Figure 4C, JA inhibition of root growth in the wild-type seedlings was partially attenuated by epi-BL. All seedlings treated with epi-BL exhibited less sensitivity to JA, suggesting that the BR signal partially counters JA inhibition of root growth.

The *psc1* Single Mutant Exhibits JA Hypersensitivity

We generated the *psc1* single mutant in wild-type *COI1* background (see "Materials and Methods") and found that the morphologic phenotypes of the *psc1*

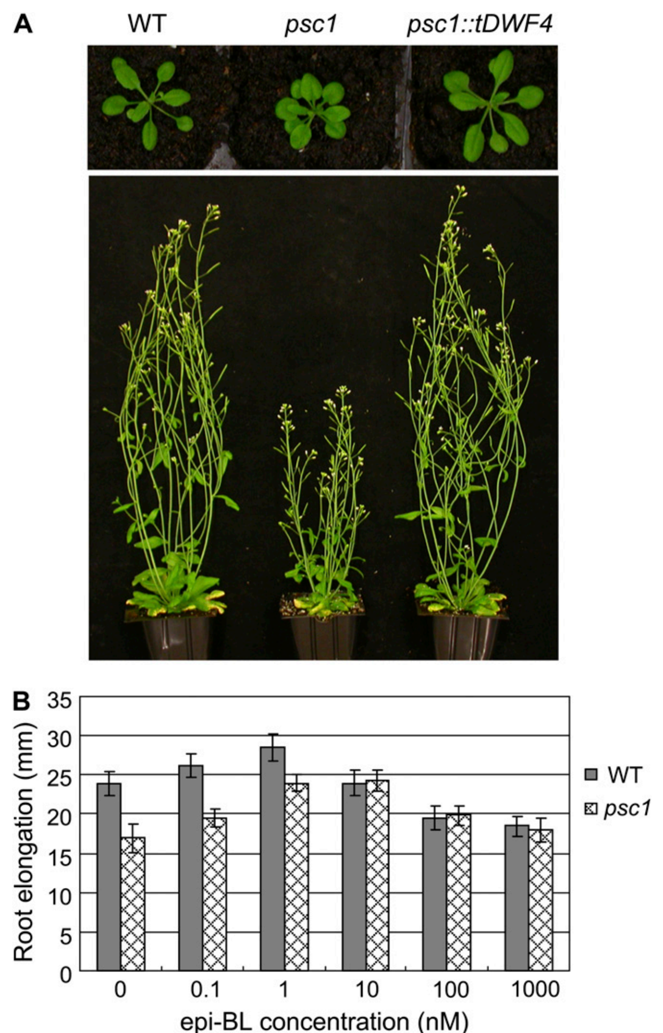

Figure 5. Phenotypes of the *psc1* single mutant. A, The morphology of wild-type (WT), *psc1*, and transgenic *psc1*::*tDWF4* seedlings at 21 d (top section) and flowering plants at 6 weeks (bottom section). B, Effect of epi-BL (the most active BR) on root growth of wild-type (WT) and *psc1* seedlings. Five-day-old seedlings transferred to MS medium with 0, 0.1, 1, 10, 100, and 1,000 nM epi-BL were grown on vertically oriented plates for 3 d and increase in root length was measured. Error bars represent SE ($n > 30$).

single mutant, such as size of seedlings, rosette leaves, and plant height, were similar to those of the *psc1coi1* double mutant except that fertility was almost normal in the *psc1* single mutant but partial in *psc1coi1* (Figs. 1B, 3C, and 5A). The transgenic plant (*psc1*::*tDWF4*) expressing wild-type *DWF4* with its endogenous promoter in *psc1* (see "Materials and Methods") displayed wild-type-like morphologic phenotypes (Fig. 5A). As shown in Figure 5B, root elongation of seedlings treated with 10 nM epi-BL was similar for *psc1* and the wild type. These results suggest that the wild-type *DWF4* gene complements the phenotypes in the *psc1* mutant and that the application of exogenous BR is able to rescue the root growth of the *psc1* mutant.

Relative root elongation was less for *psc1* than for the wild type when seedlings were grown on a medium with JA (left section of Fig. 6). Exposure to 10 μM MeJA reduced root length by 70% in the *psc1* mutant but by only 54% in the wild type, demonstrating that the *psc1* mutant deficient in BR biosynthesis was more sensitive to JA than the wild type.

To test whether the JA hypersensitivity in the *psc1* single mutant could be eliminated by exogenous BR, the seedlings were grown on MS medium supplemented with various concentrations of MeJA and without or with 10 nM epi-BL. As shown in right section of Figure 6, relative root elongation in the presence of epi-BL was similar for *psc1* and the wild type, indicating that BR can completely depress JA hypersensitivity in the *psc1* single mutant. These results further confirm that the defect of BR biosynthesis in *psc1* increases sensitivity to JA.

JA Inhibits *DWF4* Expression in *COI1*-Dependent Manner

We next investigated whether JA affects the expression of *DWF4*. Because *DWF4* transcripts were rarely detected by northern blotting (Kim et al., 2006; data not shown), reverse transcription (RT)-PCR was used to analyze the expression of the *DWF4* gene. As shown in Figure 7, the amplified transcripts of *DWF4* observably decreased upon JA treatment in wild type, suggesting that JA inhibits the expression of *DWF4*. To determine whether the inhibition on the *DWF4* expression by JA is dependent on *COI1*, we treated the null mutant *coi1-1* plants with JA for various periods. We found that level of *DWF4* expression was not significantly altered in *coi1-1* treated with or without JA (Fig. 7). These data demonstrate that JA inhibits *DWF4* expression in *COI1*-dependent manner.

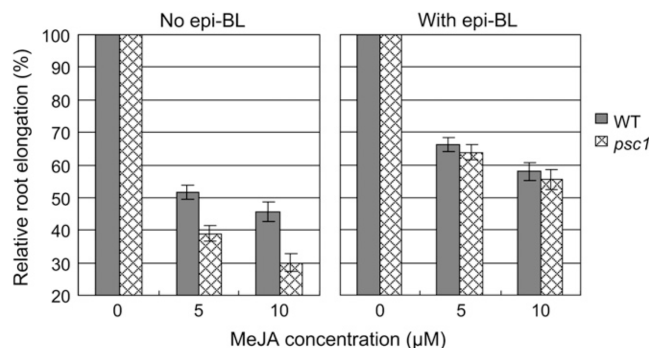

Figure 6. BR eliminates JA hypersensitivity in the *psc1* single mutant. Five-day-old seedlings transferred to MS medium containing 0, 5, and 10 μM MeJA with or without 10 nM epi-BL were grown on vertically oriented plates for 3 d and increase in root length was measured. Relative root elongation is expressed as a percentage of root elongation on MS medium with (right section) or without (left section) 10 nM epi-BL. Error bars represent SE ($n > 30$). WT, Wild type.

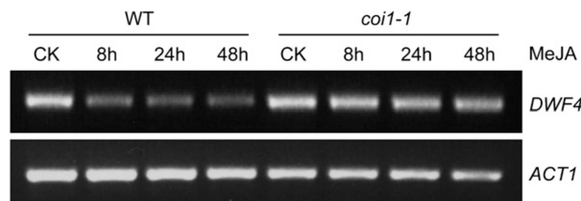

Figure 7. Analysis of *DWF4* expression by RT-PCR. The total RNA was extracted from rosette leaves of 4-week-old plants with treatment of 100 μM MeJA for 8, 24, and 48 h, or water for 8 h (CK), and then was used in RT-PCR to examine the expression of *DWF4*. The *ACT1* fragment was amplified as a control. WT, Wild type, *Col-0*.

DISCUSSION

By using genetic screens for suppressors, we isolated the *psc1* mutant that partially suppresses the JA insensitivity of *coi1* (Fig. 1A). Genetic mapping and a complementation test revealed that *PSC1* is an allele of the *DWF4* gene, in which the 468th amino acid has changed from Ala to Val (Fig. 2B). The *DWF4* gene encodes a cytochrome P450 that mediates multiple 22α-hydroxylation steps in BR biosynthesis (Choe et al., 1998).

BR is a family of polyhydroxylated steroid hormones involved in many aspects of plant growth and development (Belkhadir and Chory, 2006; Wang et al., 2006; Gendron et al., 2008; Tang et al., 2008). The growth of mutants defective in BR biosynthesis and signaling is severely retarded (Azpiroz et al., 1998; He et al., 2005; Belkhadir and Chory, 2006). Compared with *dwf4-102* (Nakamoto, et al., 2006), a null mutant of *DWF4* that is plant lethal (Nakamoto et al., 2006; data not shown), the *psc1* mutant showed a dwarf phenotype that included shorter petioles, round and smaller rosette leaves, and reduced plant height (Fig. 5A), but the fertility of the *psc1* single mutant was almost normal (Fig. 5A), indicating that *psc1* is a leaky mutation in *DWF4*.

Physiological analysis of roots revealed that *psc1* partially restored JA sensitivity in *coi1-2* background (Fig. 1A) and exhibited JA hypersensitivity in wild-type *COI1* background (Fig. 6). Both of these responses to JA were eliminated by exogenous BR (Figs. 4B and

6), whereas exogenous BR attenuated JA sensitivity in the wild type (Fig. 4C), suggesting that BR is involved in JA signaling and negatively regulates JA inhibition of root growth.

Upon BR treatment, several BR synthesis genes, including *DWF4* and *CPD* encoding C-23 hydroxylase (Szekeres et al., 1996), were down-regulated and a BR inactivation gene (*BAS1*) was up-regulated (Tanaka et al., 2005; Kim et al., 2006). However, when BR was depleted by treatment with brassinazole, a BR biosynthesis inhibitor, the expression of several BR synthesis genes, including *DWF4*, *CPD*, and *DET2*, increased (Tanaka et al., 2005). In this study, we found that JA inhibited the expression of *DWF4* (Fig. 7), which was consistent with the result generated by Genevestigator (Zimmermann et al., 2004; http://www.arabidopsis.org). Furthermore, the data from Genevestigator expression analysis (http://www.arabidopsis.org; Zimmermann et al., 2004) showed that MeJA treatment reduced the expression of *CPD* and *BAS1* but induced expression of *DET2*. In the BR-biosynthesis pathway, DWF4 locates downstream of DET2 and upstream of CPD and catalyzes the rate-limiting step (Choe et al., 1998). Therefore, we hypothesized that JA treatment might reduce endogenous BR by regulation of the expression of BR biosynthetic genes including *DWF4*.

Taken together, we proposed a model for *psc1* to exhibit a partial restoration of JA-inhibitory root growth in the *coi1-2* background and JA hypersensitivity in the *COI1* wild-type background. BR signal negatively regulates JA-inhibitory root growth. Reduction of BR synthesis would reduce the negative effect of BR signal on JA-inhibitory root growth and enhance JA sensitivity of root growth. The *psc1* mutation (a leaky mutation of *DWF4*) partially reduces normal BR synthesis, whereas partial reduction of BR synthesis in *psc1* would partially reduce the negative effect of BR signal on JA-inhibitory root growth. As a result, *psc1* shows an increased JA sensitivity of root growth in both *coi1-2* and wild-type background. The *psc1coi1* mutant exhibited partial sensitivity to JA-inhibitory root growth compared with the *coi1-2* mutant that is resistant to JA, and the *psc1* mutant is more sensitive to JA-inhibitory root growth compared with the wild type.

Generally speaking, JA inhibits plant root growth and also induces expression of many genes including *VSP1*, *LOX2*, *Thi2.1*, and *JAZs* (Xu et al., 2002; Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Chico et al., 2008; Katsir et al., 2008a), and regulates anthocyanin accumulation (Shan et al., 2009) and lateral root formation (Sun et al., 2009). However, we found that the *psc1* mutation, which partially increased sensitivity of JA-inhibitory root growth, failed to restore expression of *VSP1*, *LOX2*, *Thi2.1*, and *JAZ9* in *coi1-2* background (data not shown). In contrast, the expression of JA-induced gene *VSP1* appeared to decrease in *psc1coi1* compared with *coi1-2*, and *VSP1* expression was also reduced in the *psc1* single mutant compared with wild type (data not shown). Consistent with this observation, the data from Genevestigator expression analysis (http://www.arabidopsis.org; Zimmermann, et al., 2004) showed that BR treatment increased the expression of some JA-inducible genes including *LOX2*, *Thi2.1*, and *JAZ9*. Similarly, we also found that the *psc1* mutation failed to restore JA-induced anthocyanin accumulation or JA-induced lateral root formation in *coi1-2* background (data not shown). The cross talk between JA and BR could be very complicated, as is the case for the cross talk between JA and ethylene where these two hormones can either work cooperatively or antagonistically in the regulation of different stress responses and developmental processes (Lorenzo and Solano, 2005).

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The *coi1-2* leaky mutant was identified previously in our laboratory (Xu et al., 2002).

Seeds were surfaced sterilized, plated on plant growth medium (MS supplemented with 1% Suc; Sigma), chilled at 4°C for 3 d, and then transferred to a growth room with a 16-h-light (22°C–24°C)/8-h-dark (16°C–19°C) photoperiod.

Mutant Screening

Approximately 30,000 seeds of Arabidopsis (*Arabidopsis thaliana*) *coi1-2* were mutagenized with 0.3% ethyl methanesulfonate following routine procedures. About 70% of the mutagenized seeds (referred to as the M1 population) could grow in soil and generate M2 seeds. M2 seeds were routinely plated on MS medium containing 10 μM MeJA (Aldrich) to screen for mutants sensitive to MeJA, i.e. mutants with shorter roots and stunted growth relative to *coi1-2*.

Generation of the *psc1* Single Mutant

The *psc1coi1* mutant was crossed to the wild type, *Col-0*, and then the plants (homozygote of *psc1* and heterozygote or homozygote of *COI1*) were selected from the F_2 population based on their increased sensitivity to MeJA (relative to *psc1coi1*) and dwarf phenotype. The *psc1* single mutant was then identified by sequencing both *COI1* and *PSC1*.

Measurement of Root Elongation

Seedlings were grown on MS medium for 5 d and then transferred to MS medium supplemented with various concentrations of MeJA and/or epi-BL (Sigma). The position of the root tips was marked, and plates were placed vertically in the growth room. Three days later, increase in root length was measured for more than 30 seedlings. All experiments were repeated three to five times.

Molecular Markers

The CAPS markers MS_3_1 and C18845 show a DNA polymorphism between Col and Landsberg *erecta* when *Hin*cII (for MS_3_1) and Bsp1407I (for C18845) were used to digest the PCR fragment amplified with their corresponding primers (MS_3_1, 5′-GAGAGTAAACTTGACAATTACAAGAGA-3′ and 5′-TTCCCAATTTTTTCCAAGTTTTTAGGG-3′; C18845, 5′-ACGCATTTAGCACTCTGATG-3′ and 5′-TGTCAGCTTCTATTGGATTG-3′). The SSLP markers S17854, S18800, and CIW4 show a polymorphism of difference in size of 13, 16, and 25 bp, respectively, between Col and Landsberg *erecta* when the PCR fragment was amplified with their corresponding primers (S17854, 5′-AACATGGTAAAGCCAAAATCA-3′ and 5′-AATGCATTAGACGAATGATTCA-3′; S18800, 5′-GGAAAAGCCAGCCAATTATA-3′ and

5′-CAGTGCAATTAGTGCATATC-3′; CIW4, 5′-GTTCATTAAACTTGCG-TGTGT-3′ and 5′-TACGGTCAGATTGAGTGATTC-3′).

Complementation Test

A 5,122-bp genomic fragment (referred to as *tDWF4*) containing the *DWF4* promoter region and the coding sequence was amplified by Pfu DNA polymerase (Stratagene) from the wild type (Col-0) using forward primer P1 (5′-ACTTGAGCTCAAACATTACGGGACACTGGACTC-3′) and reverse primer P2 (5′-AAAACCCGGGCAGAATACGAGAAACCCTAATA-3′). The amplified fragment was cloned into *pFlag* (Ren et al., 2005) at the *Sac*I/*Sma*I sites.

The construct was verified by sequencing and then introduced into *psc1coi1* by the floral-dip method of in planta *Agrobacterium tumefaciens*-mediated transformation (Clough and Bent, 1998). Three independent lines were analyzed in detail and exhibited similar phenotypes to each other. Data from one of lines, *psc1coi1*::*tDWF4*, were representative and are shown in the figures. The *psc1*::*tDWF4* line was generated from the cross of *psc1coi1*::*tDWF4* with *psc1* and was screened for presence of the *tDWF4* transgene and the *psc1* mutation but absence of *coi1-2*.

RT-PCR Analysis

The rosette leaves of 4-week-old plants grown in soil were drenched in 100 μM MeJA for 8, 24, and 48 h, or in water for 8 h (control), and then harvested. RT-PCR analysis was performed following routine procedures. The *DWF4* gene was amplified with primers 5′-GGTCGATGCTTGTTCTTGTTGGT-3′ and 5′-GCTCCGTTGTTTTGCTGTTGC-3′, and the *ACT1* gene was amplified with primers 5′-TGGGTCGTCCTCGTCACA-3′ and 5′-GATACCAG-CATTCTCCATACCA-3′. The PCR program consisted of an initial denaturing at 95°C for 2 min; followed by 26 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s; and a final extension at 72°C for 10 min.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number AY090266.

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