The GCR1 and GPA1 Participate in Promotion of *Arabidopsis* Primary Root Elongation Induced by *N*-Acyl-Homoserine Lactones, the Bacterial Quorum-Sensing Signals

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Many gram-negative bacteria use *N*-acyl-homoserine lactones (AHL) as quorum-sensing signals to coordinate their collective behaviors. Accumulating evidence indicates that plants can respond to AHL. However, little is known about the molecular mechanism of plants reacting to these bacterial signals. In this study, we show that the treatment of *Arabidopsis* roots with *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL) and *N*-3-oxo-octanoyl-homoserine lactone (3OC8-HSL) resulted in significant root elongation. The genetic analysis revealed that the T-DNA insertional mutants of *gcr1*, encoding a G-protein-coupled receptor GCR1, were insensitive to 3OC6-HSL or 3OC8-HSL in assays of root growth. The loss-of-function mutants of the sole canonical G α subunit GPA1 showed no response to AHL promotion of root elongation whereas G α gain-of-function plants overexpressing either the wild type or a constitutively active version of *Arabidopsis* G α exhibited the exaggerated effect on root elongation caused by AHL. Furthermore, the expression of *GCR1* and *GPA1* were significantly upregulated after plants were contacted with both AHL. Taken together, our results suggest that GCR1 and GPA1 are involved in AHL-mediated elongation of *Arabidopsis* roots. This provides insight into the mechanism of plant responses to bacterial quorum-sensing signals.

Quorum sensing (QS) is a cell density-dependent signaling system by which bacteria can coordinate gene expression. The most common QS molecules found in gram-negative bacteria are *N*-acyl-homoserine lactones (AHL). These compounds all contain a conserved homoserine lactone ring and an amide (*N*)-linked acyl side chain. The acyl groups of naturally occurring AHL range from 4 to 18 carbons in length; they can be saturated or unsaturated and with or without a C-3 substitution (Raffa et al. 2004; Vannini et al. 2002). Excretion (release or secretion) of these chemical signals occurs in a cell-density- and growth-phase-dependent manner and facilitates their adaptation to changing environmental conditions. Bacterial QS coordinates gene expression in many physiological processes, such as symbiosis, virulence, exopolysaccharide production, resistance to oxidative stress, antibiotic production, motility, and

biofilm formation (Parsek et al. 1999; Pearson et al. 1994). In recent years, accumulating evidence indicates that AHL molecules are also perceived by eukaryotes which, in turn, specially respond to these bacterial QS signals. *N*-3-oxo-dodecanoyl homoserine lactone (3OC12-HSL), a QS signal produced by *Pseudomonas aeruginosa* (an opportunistic pathogen in cystic fibrosis patients), can stimulate various host-signaling pathways and activate cell immune responses (Kravchenko et al. 2006). *Medicago truncatula* roots respond to AHL with significant changes in accumulation of over 150 proteins, which were found to be involved in host defense, stress responses, energetic and metabolic activities, transcriptional regulation, protein processing, cytoskeletal activities, and plant hormone responses (Mathesius et al. 2003). *N*-hexanoyl-DL-homoserine lactone (C6-HSL) can induce transcriptional changes in *Arabidopsis* roots and shoots and induce root elongation (Von Rad et al. 2008). Microarray analysis gave evidence that C6-HSL induces systemic accumulation of salicylic acid and ethylene-dependent defense gene expression (Schuegger et al. 2006). Furthermore, Ortiz-Castro and associates (2008) found that *N*-decanoyl-DL-homoserine lactone (C10-HSL) effectively altered root development in *Arabidopsis*. In a previous study, we found that *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL) and *N*-3-oxo-octanoyl-homoserine lactone (3OC8-HSL) promoted primary root growth in *Arabidopsis* (unpublished data). All of these results suggested that plants can sense the bacterial QS signals and respond to them. However, little is known about how plants perceive bacterial AHL and subsequently transduce these signals into the cells.

Heterotrimeric G-protein-mediated cell signaling is one of the most highly conserved signaling mechanisms in eukaryotes (Hamm et al. 1998). In plants, heterotrimeric G-protein signaling has been implicated in several processes in plant growth and development which transduce extracellular environmental signals into cells. Pharmacological and genetic studies have identified a role of heterotrimeric G-protein in signaling pathways induced by a pathogen (Trusov et al. 2006), ozone (Joo et al. 2005), touch (Weerasinghe et al. 2009), and light (Okamoto et al. 2001; Warpeha et al. 2007). Analysis of G-protein mutants have confirmed a role of G proteins in plant responses to phytohormones such as gibberellin (GA) (Ullah et al. 2002), abscisic acid (ABA) (Pandey et al. 2006; Ullah et al. 2002; Wang et al. 2001), auxin (Ullah et al. 2002, 2003), brassinolides (Chen et al. 2004), and jasmonic acid (Trusov et al. 2006).

The *Arabidopsis* genome contains genes encoding a canonical G-protein-coupled receptor (GPCR), GCR1, and a simple

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heterotrimeric G-protein system comprising a single canonical $G\alpha$ gene, *AtGPA1*; a $G\beta$ gene, *AtAGB1*; and two $G\gamma$ genes, *AtAGG1* and *AtAGG2* (Gudermann et al. 1997; Josefsson and Rask 1997; Ma et al. 1990; Mason and Botella 2000).

Mutations in *GCR1* or *GPA1* exhibit increased sensitivity to ABA (Perfus-Barbeoch et al. 2004) and, in some experiments, mutants have shown ABA sensitivity in specific cells and tissues, or even insensitivity (Mishra et al. 2006; Wang et al. 2001). Pandey and Assmann (2004) demonstrated a specific physical interaction between GPA1 and GCR1 by in vitro pull-down assays, yeast split-ubiquitin assays, and co-immuno-precipitation in *Arabidopsis*. Furthermore, Pandey and associates (2006) found that GCR1, GPA1, and AGB1 are each components of ABA signaling and affect germination and early development, particularly in roots. Warpeha and associates (2006) reported that GCR1-GPA1 signaling may participate in blue-light-induced production of phenylalanine in etiolated *Arabidopsis*.

Based on the previous finding that G-protein signaling is involved in the signal transduction in response to a variety of phytohormones in plants, we hypothesized that GPA1 and GCR1 might participate in the regulation of root growth that is mediated by bacterial QS signals in *Arabidopsis*. To test this hypothesis, we analyzed the $G\alpha$ -deficient mutants *gpa1-1* and *gpa1-2* and the GCR1-deficient mutant *gcr1-1*. To our knowledge, a relationship between AHL and the G-protein signaling pathway has not been shown before. The present results provide genetic evidence that GCR1 and GPA1 play an important role in AHL-mediated root elongation in *Arabidopsis*.

RESULTS

AHL regulate *Arabidopsis* primary root growth.

In literature, it has been reported that *Arabidopsis* root system architecture is regulated by several AHL in a dose- and structure-dependent manner (Ortiz-Castro et al. 2008; Von Rad et al. 2008). To further confirm the effects of AHL on *Arabi-*

Fig. 1. Effect of *N*-acyl-homoserine lactones (AHL) on *Arabidopsis* primary root length. *Arabidopsis* seedlings were germinated on Murashige and Skoog (MS)-agar medium and grown on $1/2$ MS agar medium containing 10 μ M different types of AHL for 7 days. Then, the primary root lengths of seedlings were measured. Values shown in the figure represent the mean $\pm$ standard error ($n = 30$). Asterisks indicate statistically significant differences compared with the control at $P < 0.05$. Two asterisks indicate statistically significant differences compared with the control at $P < 0.01$. The experiment was replicated three times with similar results.

dopsis root growth, we used eight AHL with different chain lengths and modifications at the C3 position to treat *Arabidopsis*. The results revealed AHL structure-dependent effects on root growth. *N*-butyryl-homoserine lactone (C4-HSL), C6-HSL, 3OC6-HSL, and 3OC8-HSL significantly promoted root growth up to over 1.2-fold compared with the control plants (Fig. 1), whereas C10-HSL did not. Contact with *N*-dodecanoyl-DL-homoserine lactone (C12-HSL) and *N*-DL-tetradecanoyl-homoserine lactone (C14-HSL) strongly inhibited root growth. A 60% reduction in root length was observed when *Arabidopsis* was treated with 10 μ M C12-HSL (Fig. 1).

3OC6-HSL and 3OC8-HSL regulate *Arabidopsis* primary root growth in a concentration-dependent manner.

To further investigate the concentration effects of 3OC6-HSL and 3OC8-HSL on root growth, we applied these two signal molecules at a variety of concentrations to two ecotypes of *Arabidopsis*, Col-0 and Wassilewskija (Ws). Root lengths increased as the concentration of 3OC6-HSL increased from 100 nM up to 1 μ M (Fig. 2A and C). However, an inhibitory effect on root growth was observed when the higher concentration of 3OC6-HSL (e.g., 100 μ M) was used. The concentration-dependent regulation of root growth by 3OC6-HSL was identical in the two ecotypes of *Arabidopsis* Col-0 and Ws (Fig. 2A and C). The promoting effect of 3OC8-HSL on root growth depended on the concentration applied while the maximum promoting effect was observed with the concentration of 10 μ M. Exposure of the roots to 100 μ M 3OC8-HSL resulted in a reduction in root length. We obtained similar results in Ws and Col-0 for the treatments with 3OC8-HSL (Fig. 2B and C).

GCR1 is involved in plant root response to 3OC6-HSL and 3OC8-HSL.

To investigate the involvement of G-protein signaling in root growth response to bacterial AHL, we examined the responses of the *gcr1-1* mutant to 3OC6-HSL and 3OC8-HSL. The growth of wild-type and *gcr1-1* plants was identical under normal growth conditions. However, in the presence of 3OC6-HSL or 3OC8-HSL, root growth of wild-type seedlings was significantly promoted, whereas the promoting effect on root growth by 3OC6-HSL or 3OC8-HSL was completely impaired in the *gcr1-1* seedlings (Fig. 3A and B).

We also monitored the expression profiles of *GCR1* in the absence or presence of 3OC6-HSL or 3OC8-HSL in wild-type *Arabidopsis* by real-time quantitative polymerase chain reaction (qPCR). Results obtained are plotted as ratios of fold-change in expression level of *GCR1* with or without induction by 1 μ M 3OC6-HSL or 10 μ M 3OC8-HSL. 3OC6-HSL induced an increase of 2.8-fold in *GCR1* gene expression at 6 h after exposure in ecotype Col-0 *Arabidopsis* while 3OC6-HSL increased *GCR1* expression twofold from 12 to 24 h after addition of 3OC6-HSL in ecotype Ws *Arabidopsis* (Fig. 4A). A concentration of 10 μ M 3OC8-HSL was similar to 1 μ M 3OC6-HSL in its effect on *GCR1* gene expression (Fig. 4B).

GPA1 participates in the *Arabidopsis* root response to 3OC6-HSL and 3OC8-HSL.

In order to illuminate the role of $G\alpha$ in AHL signaling in *Arabidopsis*, AHL-dependent root growth promotion was measured in the *gpa1* mutant, and the expression profile of *GPA1* induced by 3OC6-HSL or 3OC8-HSL in wild-type *Arabidopsis* was analyzed by real-time qPCR. Whereas 3OC6-HSL-treated wild-type plants had a 24% increase in root length, the root elongation responses to 3OC6-HSL in *gpa1-1* and *gpa1-2* mutants were completely inhibited (Fig. 5A and C). The induction of root growth by 3OC8-HSL was fully blocked in *gpa1-1* and *gpa1-2* plants whereas 3OC8-HSL sig-

nificantly promoted the root growth in wild-type *Arabidopsis* (Fig. 5B and C). These results indicated that the *gpa1* mutant was insensitive to 3OC6-HSL and 3OC8-HSL.

Real-time qPCR analysis revealed that *GPA1* was induced by 3OC6-HSL with the expression peak at 6 h after 3OC6-HSL application in *Arabidopsis* ecotype Col-0 (Fig. 4A). Express-

sion level of *GPA1* in wild-type plants was increased 3.5-fold over the untreated control by 3OC6-HSL induction (Fig. 4A). However, the maximal expression level of *GPA1* induced by 3OC6-HSL occurred 24 h after addition of 3OC6-HSL in *Arabidopsis* ecotype Ws (Fig. 4A). The *GPA1* expression was induced after 12 h of exposure (Fig. 4B) and this induction lasted until

Fig. 2. Effect of *N*-acyl-homoserine lactone (AHL) concentration on primary root length in two ecotypes of *Arabidopsis*. *Arabidopsis* seedlings were grown on $1/2$ Murashige and Skoog medium supplemented with the indicated concentration of AHL for 7 days. **A**, Effects of *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL) with changing concentrations on primary root length. **B**, Effects of *N*-3-oxo-octanoyl-homoserine lactone (3OC8-HSL) with changing concentrations on primary root length. Root lengths were measured 7 days after treatment, and the relative root lengths were calculated by comparison with control plants treated with only solvent. Values shown represent the mean $\pm$ standard error ($n = 30$). Asterisks indicate statistically significant differences compared with the control at $P < 0.05$. **C**, Representative photographs were taken of plants grown under the indicated conditions. The experiment was replicated three times with similar results.

24 h after treatment with 10 μ M 3OC8-HSL in Col-0 *Arabidopsis*. However, when Ws *Arabidopsis* was treated with 10 μ M 3OC8-HSL, the *GPA1* expression was obviously induced to approximately 5.5 fold at 6 h compared with that of the untreated control (Fig. 4B). The level of *GPA1* expression remained 2.5-fold higher than the untreated control at 24 h after treatment (Fig. 4B).

In contrast to the $G\alpha$ mutants, two transgenic lines (wG α and cG α) were used to further evaluate the function of $G\alpha$ in the response of roots to AHL in *Arabidopsis*. wG α and cG α plants refer to transgenic *Arabidopsis* harboring the wild-type full-length gene *Atgpa1* and a constitutively active form of AtGPA1, respectively. Root length of wild-type *Arabidopsis* increased to 120 to 135% of the control when treated with 3OC6-HSL or 3OC8-HSL. However, the promoting effects of AHL on root elongation were further increased in $G\alpha$ -overexpressing lines. The root lengths of wG α and cG α plants reached 150 to 180% of the control (Fig. 5A–C), indicating that $G\alpha$ overexpression resulted in increased sensitivity to bacterial signals.

DISCUSSION

Here, we demonstrate that the short-acyl-chain AHL promote *Arabidopsis* primary root growth and present genetic evidence that supports a role for the *Arabidopsis* GCR1 and the hetero-

trimeric $G\alpha$ subunit GPA1 in modulating AHL-mediated root elongation during seedling development. To the best of our knowledge, this is the first report of the involvement of G-protein signaling in the response of plants to bacterial QS signals. This provides insight into the molecular mechanism of plant sensing of bacterial AHL.

Eight different types of AHL were tested for their abilities to influence *Arabidopsis* root growth in this study. We found that AHL with acyl chain lengths up to eight carbon atoms, particularly 3OC6-HSL and 3OC8-HSL at an appropriate concentration, had promoted plant root growth whereas the longer-chain AHL C12-HSL and C14-HSL inhibited root growth (Fig. 1). Treatment with C10-HSL resulted in a root length comparable with that of untreated control plants. Von Rad and associates (2008) evaluated the effects of six AHL without C3-position modification and pointed out that the short-chain AHL C4-HSL and C6-HSL significantly enhanced root length in *Arabidopsis* while the long-chain AHL C10-HSL, C12-HSL, and C14-HSL did not. In contrast, inoculation with C10-HSL even led to a significant reduction in root length (Von Rad et al. 2008). Ortíz-Castro and associates (2008) reported that C4-HSL and C6-HSL did not significantly affect primary root growth among seven different types of AHL. In contrast, C10-HSL, C12-HSL, and C14-HSL at concentrations above 12 μ M

Fig. 3. Effects of *GCR1* knockout on *N*-acyl-homoserine lactone promotion of root elongation of *Arabidopsis*. **A**, Relative root elongation of seedlings treated with *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL) (1 μ M) and *N*-3-oxo-octanoyl-homoserine lactone (3OC8-HSL) (10 μ M). Values shown represent the mean $\pm$ standard error ($n = 30$). Asterisks indicate statistically significant differences compared with the control at $P < 0.05$. **B**, Representative photographs of primary root length of 7-day-old *Arabidopsis* seedlings. The experiment was replicated three times with similar results.

Fig. 4. Expression of genes in response to *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL) (1 μ M) and *N*-3-oxo-octanoyl-homoserine lactone (3OC8-HSL) (10 μ M) treatment. Wild-type *Arabidopsis* (Col-0 and Wasilewskija [Ws]) were water cultivated until they were 3 weeks old. Then, the expression of *GPA1* and *GCR1* were detected by real-time polymerase chain reaction. There were three biological repeats with similar results in these experiments. **A**, Expression of genes *GPA1* and *GCR1* in response to 3OC6-HSL. **B**, Expression of genes *GPA1* and *GCR1* in response to 3OC8-HSL. Values shown represent the mean $\pm$ standard deviation ($n = 3$).

remarkably inhibited *Arabidopsis* root growth. The discrepancy in the effects of different AHL on *Arabidopsis* root growth from these reports may be due to differences in the method of AHL application, AHL concentration, incubation time after AHL treatment, and experimental conditions. Nevertheless, the short-chain AHL seem to enhance root elongation while the long-chain AHL seem to inhibit the primary root growth to some degree but alter the root architecture. We observed that C12-HSL and C14-HSL inhibited root elongation while they promoted lateral root formation (data not shown). These results suggest that short- and long-chain AHL act on plants through distinct signaling pathways. Here, we focused on the mechanism underlying the plant's response to short-chain AHL.

It is now well established that G-protein signaling is involved in the responses of plants to several phytohormones (Chen et al. 2004; Pandey et al. 2006; Trusov et al. 2006; Ullah et al. 2002, 2003) as well as to biotic and abiotic environmental signals such as pathogens (Trusov et al. 2006), ozone (Joo et al. 2005), touch (Weerasinghe et al. 2009), and light (Okamoto et al. 2001; Warpeha et al. 2007). The fact that heterotrimeric G-proteins transduce a variety of extracellular environmental signals into cells suggests that early signaling mechanisms for different stimuli can have shared signaling components. This prompted us to speculate that G proteins may mediate AHL signaling in plant cells. We first took a pharmacological ap-

proach. We found that the presence of pertussis toxin, a specific inhibitor of heterotrimeric G protein, blocked the promotion effects of 3OC6-HSL and 3OC8-HSL on primary root length (data not shown). This suggested the involvement of a heterotrimeric G protein in the plant's response to AHL. Here, we used a genetic approach to investigate the participation of G-proteins in AHL signaling in plants. Our results clearly showed that the loss of *GCR1* function in a knockout mutant altered sensitivity to short-chain AHL such as 3OC6-HSL and 3OC8-HSL for root elongation when compared with wild-type plants (Fig. 3). *Gα* loss- and gain-of-function plants showed an opposite and complementary response to exogenous application of AHL for root elongation (Fig. 5). Null mutants of the sole canonical *Gα* subunit *GPA1* no longer exhibited the AHL promotion of root length, whereas both *wGα*- and *cGα*-overexpressing plants showed increased sensitivity to both 3OC6-HSL and 3OC8-HSL for induction of root growth (Fig. 5A to C). Moreover, the expression levels of *GCR1* and *GPA1* were significantly upregulated by 3OC6-HSL and 3OC8-HSL (Fig. 4A and B). Collectively, our data demonstrate that *GCR1* and *GPA1* might be involved in transducing AHL signals in plant cells.

GCR1 belongs to the secretin (so-called 7-TM2) class of G-protein-coupled receptors. The secretin class tends to bind ligands on the extracellular surface in part through an N-terminal extracellular extension. The interaction between *GCR1* and

Fig. 5. Effects of *Gα* knockout and overexpression on *N*-acyl-homoserine lactone promotion of root elongation of *Arabidopsis*. **A**, Relative root elongation of *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL)-treated (1 μM) seedlings and **B**, relative root elongation of *N*-3-oxo-octanoyl-homoserine lactone (3OC8-HSL)-treated (10 μM) seedling. Values shown represent the mean ± standard error ($n = 30$). Asterisks indicate statistically significant differences compared with the control at $P < 0.05$. **C**, Representative photographs of primary root length of 7-day-old *Arabidopsis* seedlings. The experiment was repeated three times with similar results.

GPA1 has been demonstrated physically and genetically (Pandey and Assmann 2004; Pandey et al. 2006; Warpeha et al. 2006). Warpeha and associates (2007) reported that GCR1 and GPA1 act in concert for both blue light and ABA responses in *Arabidopsis*, and GCR1 could be a common receptor for blue light and ABA. GCR1 also functions in brassinosteroids and GA signaling for regulation of seed germination in *Arabidopsis* (Chen et al. 2004). Liu and associates (2007) demonstrated that the only other reported GPCR in *Arabidopsis*, GCR2, encodes a seven-transmembrane receptor for ABA. Our results revealed that *GCR1* knockout mutants and *GPA1* knockout mutants exhibited similar root growth phenotypes, suggesting that GCR1 and GPA1 may act in concert to mediate AHL signaling in *Arabidopsis* root cells.

Recently, we found that the short-chain AHL C4-HSL is able to induce a transient elevation in the cytosolic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{cyt}}$) in plants, and the $[\text{Ca}^{2+}]_{\text{cyt}}$ increase induced by C4-HSL was mainly due to a Ca^{2+} influx from the extracellular space into the cells. This suggested that Ca^{2+} signaling is involved in the response of plants to bacterial QS signals (Song et al. 2011). The link between G-protein and Ca^{2+} signaling has been well established. Thus, our present data lead to a hypothesis that AHL might first interact directly or indirectly with GCR1 and then activate $\text{G}\alpha$. Subsequently, the activated $\text{G}\alpha$ activates the Ca^{2+} channels in plasma membranes, resulting in an extracellular Ca^{2+} influx. The elevation of $[\text{Ca}^{2+}]_{\text{cyt}}$ triggers the downstream cascades that ultimately lead to the specific cellular reactions. However, because AHL can enter and are functional within eukaryotic cells (Gotz et al. 2007; Williams et al. 2004), they can also directly interact with intracellular signaling intermediates without the involvement of transmembrane proteins. Therefore, identification of the putative AHL receptors and other components in their signaling pathways will be of great importance for understanding AHL signal transduction pathways in plants.

MATERIALS AND METHODS

Plant growth conditions and treatments.

Arabidopsis wild-type plants (Col-0 or Ws ecotype), transgenic line wG α (overexpressed GPA1 protein induced by dexamethasone), cG α (constitutive overexpressed GPA1 protein) (Okamoto et al. 2001), and the T-DNA insertion null mutants *gpa1-1*, *gpa1-2*, and *gcr1-1* were used for all experiments. Seed were surface sterilized with 75% (vol/vol) ethanol for 1 min and 30% (vol/vol) NaClO for 5 min. After washing with sterile distilled water five times, seed were germinated on agar plates containing Murashige and Skoog (MS) medium (Murashige and Skoog 1962) or $1/2$ MS medium. The pH of MS medium was kept at 5.8. Plants were placed in a plant growth chamber with a photoperiod of 16 h of light and 8 h of darkness, light intensity of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, and temperature of 22°C . For real-time qPCR experiment, a hydroponic system was employed to cultivate *Arabidopsis*. Seedlings were grown until two leaves and roots reached 2 cm in length, after which they were transplanted in a sterile plastic basin (18 by 11 cm)

containing 500 ml of $1/2$ MS medium and the indicated concentration of AHL. Plants grown under these conditions were more vigorous than those grown in potting media, uniformly absorbing solution with AHL. To ensure that the hydroponic system is free of bacterial contamination, the medium and AHL solution were sterilized by passing them through a $0.22\text{-}\mu\text{m}$ filter just prior to use. The untreated plants were taken as the control. After harvest, an aliquot of the growth medium was plated on bacterial growth medium and incubated overnight to check for contaminations that occurred during the experiment. Any contaminated samples were discarded.

The eight AHL detected in this study included five shorter acyl chain AHL (C4-HSL, C6-HSL, 3OC6-HSL, C8-HSL, and 3OC8-HSL), and three longer acyl chain AHL (C10-HSL, C12-HSL, and C14-HSL). They were all purchased from Sigma-Aldrich (Taufkirchen, Germany), stored dry, and diluted as 10 mM stock solution in sterile distilled water or ethanol adjusted to pH 5.0 just before use. For all experiments, $1/2$ MS nutrient medium was supplemented with AHL (concentration ranging from 10 nM to 100 μM). Dissolved compounds were added to cooled (50°C) molten medium and poured onto plates. Control plates were supplied with the distilled water or ethanol only.

Analysis of root growth and statistical analysis.

Growth of primary roots was recorded using a ruler. After germination, seedlings with similar length were transferred to $1/2$ MS medium and grown for 7 days with the same growth condition as described above. For all experiments, the overall data were statistically analyzed in the DPS 3.0 program. Univariate and multivariate analyses with a Tukey's post hoc test were used for testing different root growth responses of different ecotype, wild-type, and mutant lines to AHL.

RNA extraction.

The plant tissues for real-time PCR were collected from hydroponics. A 12-day-old seedling was transferred to the Hoagland solution and grown further for 10 days. Total RNA of homogenized plant tissues was extracted using RNA plus reagent purchased from TaKaRa (Dalian, China). Tissue material (100 mg) was mixed with 1 ml of RNA plus reagent, incubated for 5 min on a shaker (900 rpm), and supplemented with 200 μl of chloroform. Samples were carefully mixed, incubated for 2 to 3 min at room temperature, and centrifuged for 15 min at 4°C ($10,000 \times g$). A colorless upper aqueous phase was transferred to a fresh tube. RNA was precipitated by mixing with isopropanol and incubating for 45 min at 4°C . Samples were centrifuged (10 min, 4°C , $10,000 \times g$) and washed with 70% ethanol twice. The air-dried RNA pellet was dissolved in sterile water.

Real-time qPCR of *GPA1* and *GCR1*.

Plants were harvested at 0, 6, 12, 24, and 48 h after 3OC6-HSL, 3OC8-HSL treatment or from the untreated plants. Target RNA from 3OC6-HSL, 3OC8-HSL-treated plants or the control were extracted as described above. For cDNA synthesis, 0.5 μg of RNA was mixed with 1 μl of oligo dT primer (50 μM), 1 μl of random 6 primer (100 μM), 4 μl of 5 $\times$ PrimeScript Buffer, and 1 μl of PrimeScript RT Enzyme Mix I; adjusted to a volume of 10 μl ; and incubated for 15 min at 37°C and 5 s at 85°C . For quantitative reverse-transcription (qRT)-PCR, received cDNA was diluted 15 times in diethylpyrocabonate-treated water. PCR amplification was done in a total volume of 20 μl containing 5 μl of diluted cDNA, 0.4 μl of each primer (10 μM), and 10 μl of SYBR Premix Ex Taq (TaKaRa, Shiga, Japan). For relative quantification of gene expression the comparative cycle threshold method (Livak and

Table 1. Primer information of genes investigated in quantitative reverse-transcription polymerase chain reaction

Primer	Sense	Antisense
ACTIN2/8	5' GGTAACATTGTGCT CAGTGGTGG 3'	5' AACGACCTTAATCT TCATGCTGC 3'
GPA1	5' GCACAACCTGGTGTC GTGGAA 3'	5' GCGTTTGGTCTGACT CGCTGAT 3'
GCR1	5' ATGGGCGATTCGGCA TTATTA 3'	5' TGGAAGCCATCGAT ATAGCC 3'

Schmittgen 2001) with the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, U.S.A.) was used. The following qPCR thermal cycling program was employed: 10 s at 95°C and 40 cycles for 5 s at 95°C and 31 s at 60°C. The amount of target was normalized to the endogenous reference gene β -actin. Each data point represents the average of three independent experiments. For technical control, each qRT-PCR experiment was repeated four times on the same 96-well plate.

For qRT-PCR, we used specific primers shown in Table 1.

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