AtMYB44 Positively Regulates the Enhanced Elongation of Primary Roots Induced by *N*-3-Oxo-Hexanoyl-Homoserine Lactone in *Arabidopsis thaliana*

Qian Zhao,^{1,2} Man Li,¹ Zhenhua Jia,^{1,2} Fang Liu,^{1,2} Hong Ma,^{1,2} Yali Huang,^{1,2} and Shuishan Song^{1,2}

¹Biology Institute, Hebei Academy of Sciences; and ²Hebei Engineering and Technology Center of Microbiological Control on Main Crop Disease, 46th South Street of Friendship, Shijiazhuang, 050051, China

Accepted 6 September 2016.

N-acyl-homoserine lactones (AHL) are the quorum-sensing (QS) signal molecules used by many gram-negative bacteria to coordinate their collective behavior in a population. Recent evidence demonstrates their roles in plant root growth and defense responses. AtMYB44 is a multifaceted transcriptional factor that functions in many physiological processes in plants but whether AtMYB44 modulates the plant response to AHL with aspects of primary root elongation remains unknown. Here, we show that the expression of *AtMYB44* was upregulated upon treatment with *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL). The stimulatory effect of 3OC6-HSL on primary root elongation was abolished in the *AtMYB44* functional-deficiency mutant *atmyb44*. In contrast, an enhanced promoting-impact of 3OC6-HSL on primary root growth was observed in *AtMYB44*-overexpressing plant *MYB44OTA*. Cellular analysis indicated that the prolonged primary root elicited by 3OC6-HSL is the consequence of increased cell division in the meristem zone and enhanced cell elongation in the elongation zone, and AtMYB44 may act as a positive regulator in this process. Furthermore, we demonstrated that AtMYB44 might participate in the 3OC6-HSL-mediated primary root growth via regulating the expression of cytokinin- and auxin-related genes. The data establish a genetic connection between the regulatory role of AtMYB44 in phytohormones-related gene expression and plant response to the bacterial QS signal.

Many gram-negative bacteria use *N*-acyl-homoserine lactones (AHL) to communicate with each other and to synchronize their social behavior—a process commonly called quorum sensing (QS). Bacterial QS plays roles in many bacterial physiological processes, including symbiosis, virulence, bioluminescence, sporulation, biofilm formation, antibiotic production, swarming, and plasmid conjugal transfer (Bassler 1999; Fuqua et al. 2001; Pearson et al. 1994; Quiñones et al. 2005). Recent evidence has accumulated that AHL also can influence plant physiological responses. Mathesius and coworkers (2003) demonstrated that treatment of *Medicago truncatula* roots with two types of AHL cause significant changes in the accumulation of 150 proteins. Our proteomic analysis showed that treatment of *Arabidopsis*

seedlings with *N*-3-oxo-octanoyl-homoserine lactone (3OC8-HSL) alters the accumulation level of 53 proteins. These proteins are implicated in a variety of physiological process, including metabolism of carbohydrates and energy, protein biosynthesis and quality control systems, defense response, signal transduction, and cytoskeleton remodeling (Miao et al. 2012). Increasing evidence indicated that bacterial AHL can regulate plant defense response. Inoculation of the roots with *Serratia liquefaciens* MG1 protected tomato from the infection of leaf-pathogenic fungus *Alternaria alternata* while the AHL-negative *S. liquefaciens* mutant MG44 did not (Schuhegger et al. 2006). It is likely that colonization with *S. plymuthica* HRO-C48 increased the resistance of cucumber and tomato against pathogens *Pythium aphanidermatum* and *Botrytis cinerea*, respectively (Pang et al. 2009). However, the negative *spII* mutant of *S. plymuthica* which cannot synthesize AHL could not confer the resistance against both pathogens. Zarkani and colleagues (2013) reported that the induction of resistance in *Arabidopsis thaliana* against pathogen *Pseudomonas syringae* by inoculation of *Ensifer meliloti* was depended on AHL accumulation of *E. meliloti*. Furthermore, the resistance-inducing effects of *E. meliloti* were demonstrated in crop plants, including barley, wheat, and tomato, and these effects were attributed to the presence of *N*-3-oxo-tetradecanoyl-homoserine lactone (3OC14-HSL) produced by *E. meliloti* (Hernández-Reyes et al. 2014). Similarly, application of commercial 3OC14-HSL increased plant resistance to bacterial infection by accumulation of phenolic compounds, lignin and callose deposition in plant cell walls, and promotion of stomatal closure (Schenk et al. 2014). In addition, transgenic plants expressing bacterial AHL-synthesis-encoding gene *expI* exhibited enhanced resistance to the infection of bacterial pathogens (Lee et al. 2008; Mäe et al. 2001). The opposite result was reported for potato, where transgenic potato producing *N*-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL) and *N*-hexanoyl-homoserine lactone (C6-HSL) showed increased susceptibility to soft rot *Erwinia* spp. (Toth et al. 2004). Nevertheless, these data suggest that bacterial AHL play a role in plant immune responses. On the other hand, bacterial AHL are proved to regulate plant growth. von Rad and colleagues (2008) pointed out that *N*-butyryl-homoserine lactone (C4-HSL) and C6-HSL can promote primary root elongation of *A. thaliana*. Previously, we found that exposure of *Arabidopsis* roots to 3OC6-HSL and 3OC8-HSL enhanced primary root growth, and that G-protein and Ca²⁺/CaM signaling were involved in the regulation of primary root growth by bacterial AHL (Jin et al. 2012; Liu et al. 2012; Song et al. 2011; Zhao et al. 2015). In contrast, long-chained AHL *N*-decanoyl-homoserine lactone (C10-HSL) and *N*-3-oxo-dodecanoyl-homoserine lactone (3OC12-HSL) strongly inhibited primary root growth

Q. Zhao and M. Li contributed equally to this work.

Corresponding author: S. Song; E-mail: shuishans620@163.com

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table and three supplementary figures are published online.

instead of stimulating lateral root and root hair development when high concentrations were applied (Ortíz-Castro et al. 2008; Ortiz-Castro et al. 2011). Bai and coworkers (2012) found that *N*-3-oxo-decanoyl-homoserine lactone (3OC10-HSL) promoted the formation of adventitious roots in mung bean. More recently, Palmer and colleagues (2014) demonstrated that it is L-homoserine, yielded through amidolysis by a plant-derived fatty acid amide hydrolase, which regulated the root growth. All these data indicate that bacterial AHL are important mediators for the interaction between plant and bacteria (Hartmann et al. 2014). However, little is known about the molecular mechanism by which bacterial AHL regulate the physiological responses in plant cells.

Transcription factors are critical regulators for gene expression that drive growth, developmental processes, and environmental stimuli responses. Over 1,600 transcription factors, representing approximately 6% of the total number of genes, have been identified in the *Arabidopsis* genome (Gong et al. 2004; Riechmann et al. 2000). The MYB family, which comprises 163 genes, is one of the largest transcription factor families in *Arabidopsis* (Chen et al. 2006). The *Arabidopsis* R2R3-type MYB transcription factors have been categorized into subgroups on the basis of conserved amino acid sequence motifs present on the carboxy-terminal side of the MYB domain. Subgroup 22 includes AtMYB44, AtMYB70, AtMYB73, and AtMYB77, and shares two conserved motifs: TGLYMSPxSP and GxFMxVVQEMLxxEVRSYM (Kranz et al. 1998; Romero et al. 1998; Stracke et al. 2001). The genes encoding subgroup 22 proteins have similar expression patterns and are associated with stress responses. AtMYB44, one member of subgroup 22 of the AtMYB family, is found to be involved in embryogenesis (Kirik et al. 1998), seed maturation (Kirik et al. 1998), and response to pathogens (Kranz et al. 1998; Liu et al. 2010; Pitzschke et al. 2009), insects (Liu et al. 2010), and environmental stresses (Jung et al. 2007, 2008, 2010) in *Arabidopsis*. Several studies indicated that AtMYB44 is induced by a number of phytohormones, including abscisic acid (ABA), gibberellin acid (GA), jasmonic acid (JA), ethylene, and auxin (Jung et al. 2008, 2010; Zou et al. 2013). Recently, Nguyen and coworkers (2012) found that AtMYB44 is the target protein for mitogen-activated protein kinase (MPK) 3 and 6, and the phosphorylation of AtMYB44 by AtMPK3 and AtMPK6 is required for inhibition of seed germination in response to ABA-reduced GA biosynthesis. On the other hand, Schikora and colleagues (2011) showed that AtMPK6 is required for AHL-induced resistance to the obligate biotrophic fungi *Golovinomyces orontii* in *Arabidopsis*. These data give a clue that AtMYB44 might play a role in AHL signaling in plant cells. However, the direct evidence for the participation of AtMYB44 in AHL-mediated root elongation is still missing.

In the present study, we found that the expression of *AtMYB44* is induced by treatment with 3OC6-HSL. The promoting effects of 3OC6-HSL on primary root elongation were abolished and exaggerated in the *AtMYB44* functional-deficiency mutant *atmyb44* and *AtMYB44*-overexpressing transgenic line *MYB44OTA*, respectively. The increased cell division in the meristem zone and enhanced cell elongation in the elongation zone caused by 3OC6-HSL resulted in a prolonged primary root and AtMYB44 might positively regulate this process. The transcriptions of several genes involved in auxin and cytokinin signaling pathways were regulated by AtMYB44 upon AHL treatment. Our results suggested that AtMYB44 may play a role in 3OC6-HSL-mediated primary root elongation via regulating the expression of auxin- and cytokinin-related genes.

RESULTS

Transcriptomic profile in *Arabidopsis* roots and induction of *AtMYB44* after treatment with 3OC6-HSL.

Previously, von Rad and colleagues (2008) reported that the contact of *Arabidopsis* roots with C6-HSL resulted in

distinct gene transcriptional changes in roots and leaves. To further determine the contribution of bacterial QS signals to the regulation of plant growth and development, we used a 29k *Arabidopsis* Genome Array to examine the effects of 3OC6-HSL, a derivative of C6-HSL in which the C3 position is substituted with a ketone group, on expressing profiles in *Arabidopsis* roots. Transcriptomic analysis revealed that 2,873 genes, in total, were differentially expressed in *Arabidopsis* roots in response to 3OC6-HSL for 24 h, of which 1,271 were upregulated and 1,602 were downregulated (National Center for Biotechnology Information [NCBI] Gene Expression Omnibus [GEO] accession number GSE78079). The data indicated that approximately 10% of the known genomic genes in *Arabidopsis* are 3OC6-HSL responsive.

The differentially expressed genes (DEG) were grouped into 15 functional categories based on the Gene Ontology tool at The *Arabidopsis* Information Resource (TAIR). Except for unclassified or unknown genes, most genes could be assigned to biotic or abiotic stress adaptation (16.7%), DNA or RNA processing (13.2%), metabolism (7.9%), protein processing (7.9%), transcription/translation (6.7%), signal transduction (4.6%), and hormone response (3.1%) (Supplementary Fig. S1). It was noteworthy that approximately 7% of DEG could contain transcriptional factor activity. These differentially expressed transcription factors include MYB family, zinc finger family, zinc ion-binding protein, AP2 domain-containing protein, NAC domain-containing protein, TCP family, COL family, IAA family, CDF family, WRKY family, CRF family, and so on (Table 1). Among these transcription factors, we are interested in the MYB family because the proteins of this family function in the control of plant growth and development, hormone response, abiotic stress response, and gene expression (Kranz et al. 1998). The microarray data revealed that the expression of six MYB genes, including MYB44, MYB70, MYB77, At5g17300, At1g19510, and At3g13040, was increased at least twofold by treatment with 3OC6-HSL, while three genes (MYB2, MYB29, and MYB60) were downregulated after a treatment of 3OC6-HSL (Table 1). AtMYB44, together with AtMYB70 and AtMYB77, belong to R2R3 MYB subgroup 22 and share two conserved motifs (Kranz et al. 1998; Romero et al. 1998; Stracke et al. 2001) and have similar expression patterns. AtMYB44 is reported to be phosphorylated by AtMPK3 and AtMPK6 (Nguyen et al. 2012), while AtMPK6 is required for AHL-mediated resistance to bacterial pathogens in *Arabidopsis* (Schikora et al. 2011), suggesting that AtMYB44 might play roles in AHL-mediated root elongation in *Arabidopsis*. Hereinafter, we focused on AtMYB44 and its role in primary root growth regulated by bacterial AHL.

To validate the microarray analysis and further investigate the induction of AtMYB44 by 3OC6-HSL, we used a real-time quantitative polymerase chain reaction (qRT-PCR) approach to check the expression similarity between microarray and qRT-PCR for *AtMYB44* and several other selected genes. We found that microarray and qRT-PCR data showed a consistent trend, and the upregulated expression of genes identified by microarray that were also detected by qRT-PCR (Table 2), confirming the authenticity of transcriptomic changes caused by 3OC6-HSL treatment. Furthermore, the time course of *AtMYB44* expression was monitored by qRT-PCR after contact of *Arabidopsis* roots with 3OC6-HSL. The results indicated that the transcript accumulation of *AtMYB44* increased as the treatment time was prolonged and the level of *AtMYB44* transcript reached the maximum of 3.5-fold over that of the untreated control 12 h after treatment (Fig. 1). Then, the expression of AtMYB44 declined. This result demonstrated that *AtMYB44* was induced in the response of *Arabidopsis* roots to 3OC6-HSL.

Table 1. Differentially expressed genes related to transcription factors (TF)

TF family, <i>Arabidopsis</i> locus	3OC6-HSL (ratio)	Description ^a
MYB		
At5g17300	57.60	Regulation of transcription
At1g19510	4.51	Regulation of transcription
At3g13040	2.45	Regulation of transcription
At3g50060	2.44	MYB77, response to ABA, JA, GA, SA, salt stress
At5g67300	2.18	MYB44, response to ABA, JA, GA, SA, salt stress
At2g23290	2.16	MYB70, response to salt stress, cadmium ion
At4g00540	0.48	PC-MYB2, regulation of transcription
At1g08810	0.45	MYB60, response to ABA, JA, GA, SA, salt stress
At5g07690	0.10	MYB29, response to JA, GA, SA
Zinc finger		
At1g26790	65.94	Dof-type, regulation of transcription
At3g21150	26.28	B-box type, response to chitin
At1g73870	24.82	B-box type, regulation of transcription
At3g21890	21.16	B-box type, response to UV-B
At4g27310	11.24	B-box type, regulation of transcription
At4g26150	10.84	GATA type, response to cytokinin stimulus
At2g21320	9.28	B-box type, regulation of transcription
At1g69570	4.74	Dof-type, regulation of transcription
At3g55980	4.25	CCCH-type, response to chitin
At1g27730	3.99	STZ, salt tolerance zinc finger
At1g51700	2.91	Dof-type, response to chitin
At1g67030	2.86	ZFP6, regulation of transcription
At2g41940	2.25	ZFP8, regulation of transcription
At1g25440	0.49	B-box type, regulation of transcription
At1g32360	0.49	CCCH-type, regulation of transcription
At1g24625	0.39	ZFP7, regulation of transcription
At1g78600	0.37	B-box type, anthocyanin biosynthesis
At2g47890	0.35	B-box type, regulation of transcription
At1g68520	0.32	B-box type, regulation of transcription
At3g29035	0.26	B-box type, development
At1g68190	0.17	B-box type, regulation of transcription
At1g28050	0.12	B-box type, regulation of transcription
Zinc ion binding		
At2g31380	23.55	STH, salt tolerance homolog
At4g38960	17.95	Regulation of transcription
At2g28200	6.63	Regulation of transcription
At1g06040	3.19	STO, salt tolerance
At2g02070	0.45	IDD5, indeterminate (ID)-domain 5
At3g50700	0.30	IDD2, indeterminate (ID)-domain 2
At1g49130	0.02	Regulation of transcription
AP2 domain containing		
At5g51190	4.21	Response to chitin, ethylene-mediated signaling pathway
At1g21910	3.18	Ethylene-mediated signaling pathway
At2g44940	2.83	Ethylene-mediated signaling pathway
At1g22190	2.40	Ethylene-mediated signaling pathway
At4g28140	0.18	Response to chitin, ethylene-mediated signaling pathway
At5g61590	0.15	Response to water deprivation, ethylene-mediated signaling pathway
NAC domain containing		
At5g13180	3.04	ANAC083, development
At1g77450	2.88	ANAC032, development
At1g01720	2.73	ATAF1, ABA-mediated signaling, development
At3g15510	2.64	ATNAC2, development
At2g02450	0.41	ANAC034/ANAC035, development
At3g04070	0.34	ANAC047, development
TCP		
At2g45680	4.40	Regulation of transcription
At5g23280	2.39	Regulation of transcription
At4g18390	0.44	Development
At1g53230	0.40	TCP3, positive regulation of development
At1g30210	0.35	TCP24, positive regulation of development
COL		
At3g02380	57.53	COL2, regulation of flower development
At5g15850	32.02	COL1, regulation of flower development
At3g07650	0.12	COL9, negative regulation of long-day photoperiodism
IAA		
At3g23050	2.59	IAA7, auxin-mediated signaling pathway
At4g14550	2.42	IAA14, auxin-mediated signaling pathway
At4g32280	0.18	IAA29, auxin-mediated signaling pathway
CRF		
At2g46310	4.24	CRF5, cytokinin-mediated signaling
At4g23750	2.03	CRF2, cytokinin-mediated signaling
CDF		
At3g47500	5.17	CDF3, regulation of timing of transition from vegetative to reproductive phase
At5g39660	4.94	CDF2, regulation of timing of transition from vegetative to reproductive phase
WRKY		
At1g68150	0.42	WRKY9, regulation of transcription
At4g23550	0.37	WRKY29, defense response, response to chitin
At2g03340	0.23	WRKY3, regulation of transcription

^a ABA = abscisic acid, JA = jasmonic acid, GA = gibberellin acid, and SA = salicylic acid.

Role of AtMYB44 in the 3OC6-HSL-mediated growth of primary roots in *Arabidopsis*.

Recently, we reported that an appropriate concentration of 3OC6-HSL can promote *Arabidopsis* primary root elongation (Jin et al. 2012; Liu et al. 2012); also, others indicated the impact of C6-HSL on the plant's growth (von Rad et al. 2008). To investigate the physiological function of AtMYB44 in AHL promotion of primary root growth, we compared the primary root length in AtMYB44 functional-deficiency mutant *atmyb44*, AtMYB44-overexpressing transgenic line *MYB44OTA*, and wild-type plants after 3OC6-HSL treatment. As previously reported, inoculation with 1 μ M 3OC6-HSL led to a 13% increase in primary root length over the untreated control in wild-type plants (Fig. 2A and B). However, the stimulating-effect of 3OC6-HSL on primary root elongation was abolished in *atmyb44* plants (Fig. 2A and B). In contrast, primary roots of *AtMYB44OTA* were hypersensitive to 3OC6-HSL treatment. Application of 3OC6-HSL caused a 31% increase in primary root length over that of the untreated plants in *AtMYB44OTA*, exhibiting a stronger promoting effect of 3OC6-HSL on primary root growth in the *AtMYB44*-overexpressing line than the wild-type plants (Fig. 2A and B). RT-PCR analysis confirmed that no transcript of *AtMYB44* was detected in *atmyb44* plants and the augmented expression of *AtMYB44* was observed in *AtMYB44OTA* plants (Fig. 2C). These data indicated that AtMYB44 plays a positive role in 3OC6-HSL-mediated primary root elongation in *Arabidopsis*.

Participation of AtMYB44 in the 3OC6-HSL-mediated primary root growth via changes in cell division and elongation.

Cell division in the meristem zone and the cell expansion in the elongation zone decide the root length. In a previous study, we demonstrated that promotion of primary root growth by 3OC6-HSL was due to the increased number of cortical cells in the meristem and enlarged cells in the elongation zone of roots, suggesting that 3OC6-HSL enhanced cell division in the meristem zone and cell expansion in the elongation zone (Zhao et al. 2015). To determine whether AtMYB44 played roles in the regulation of cell division and cell elongation in the root meristem and elongation zones by 3OC6-HSL, we also monitored the length of the root meristem zone and elongation zone and the number of the cortical cells in the respective zones in 7-day-old seedlings of wild-type plants, mutant *atmyb44*, and AtMYB44-overexpressing line *MYB44OTA* after exposure to 3OC6-HSL. As previously found (Zhao et al. 2015), 3OC6-HSL caused an accelerated cell division and cell expansion in the meristem and elongation zones, respectively, and resulted in a prolonged primary root in wild-type plants (Fig. 3). However, no difference was observed in the length of and cell number in root meristem and elongation zones between 3OC6-HSL-treated and untreated *atmyb44* plants (Fig. 3), indicating that mutation of the *AtMYB44* gene diminished the effects of 3OC6-HSL on root

meristem and elongation zones. On the other hand, the stimulating effect of 3OC6-HSL on the length and cell number of the root meristem zone was pronounced in *AtMYB44OTA* if this effect was compared with that in wild-type plants (Fig. 3B and C), whereas no difference in cortical cell length of the root meristem zone between treatment and untreated in *AtMYB44OTA* was observed, and the size of cells in the meristem was comparable between *AtMYB44OTA* and wild-type plants (Fig. 3D). By comparing the length of the root elongation zone between the 3OC6-HSL treatment and the untreated control in wild-type and *AtMYB44*-overexpressing plants, we found that the promoting effect of 3OC6-HSL on the size of the elongation zone in *AtMYB44OTA* was stronger than that in wild-type plants (Fig. 3E). Furthermore, this enlarged impact of 3OC6-HSL on elongation zone length in *AtMYB44OTA* resulted from the expanded cell in the root elongation zone (Fig. 3G), because 3OC6-HSL caused no difference in cell number of the elongation zone in both *AtMYB44OTA* and wild-type plants (Fig. 3F). These results indicated that overexpression of *AtMYB44* could upregulate the promoting effect of 3OC6-HSL on cell number in the meristem and cell volume in the elongation zone of plant roots. Taken together, we can conclude that AtMYB44 participates in 3OC6-HSL promotion of primary root growth via increasing cell division in the meristem and cell expansion in the elongation zone.

Fig. 1. Relative expression of *AtMYB44* after treatment with 3OC6-HSL in *Arabidopsis* roots. Total mRNA was extracted from plants grown in hydroponic system with or without 1 μ M 3OC6-HSL at the indicated time interval. Quantitative real-time polymerase chain reaction was conducted with specific primers for gene *MYB44* using the expression of *ACTIN2* for normalization. Experiments were performed in triplicate and data are expressed as the mean $\pm$ standard deviation. Asterisks indicate statistically significant differences compared with the control at $P < 0.05$ in Student's *t* test.

Table 2. Differentially expressed genes induced by 3OC6-HSL in microarray and quantitative real-time polymerase chain reaction (qRT-PCR) assay

Gene	<i>Arabidopsis</i> locus	Description ^a	Microarray (ratio)	qRT-PCR (ratio)
MKK4	At1g51660	MAPKKK cascade	3.62	2.23
CPK32	At3g57530	ABA-mediated signaling	2.06	2.40
ARR15	At1g74890	Cytokinin-mediated signaling	9.04	2.02
MYB44	At5g67300	Oxidation reduction	2.18	2.27
MAPKKK14	At2g30040	MAPKKK cascade	4.01	3.13
PIN3	At1g70940	Auxin-mediated signaling pathway	3.31	1.77
PAL2	At3g53260	Response to oxidative stress	6.44	2.04
CAMP25	At2g41010	Regulation of SA metabolic process	6.76	6.25
LCR78/PDF1.4	At1g19610	Defense response	2.37	2.04
CAM9	At3g51920	Calcium-mediated signaling	2.49	2.03

^a MAPKKK = mitogen-activated protein kinase kinase kinase, ABA = abscisic acid, and SA = salicylic acid.

Modulation of 3OC6-HSL-promoted primary root elongation by AtMYB44 via regulating cytokinin- and auxin-related gene expression.

Phytohormones such as auxin, cytokinin, and strigolactone play an essential role in regulating plant root and shoot growth. To further elucidate the way by which AtMYB44 regulates the 3OC6-HSL-mediated primary root growth in *Arabidopsis*, we analyzed the expression pattern of several genes involved in auxin, cytokinin, and strigolactone signaling under different genetic backgrounds of AtMYB44 after exposure to AHL using a qRT-PCR approach. CyCB2, a cyclin-dependent protein kinase regulator, is implicated in regulation of cell cycles and positively regulates cytokinin signaling (Lee et al. 2003; Renaudin et al.

1996). We found that the expression of *CyCB2* was significantly downregulated by treatment with 3OC6-HSL in wild-type *Arabidopsis* (Fig. 4A). In contrast to wild-type Col-0, no significant repression of transcript accumulation of *CyCB2* by 3OC6-HSL was detected in mutant *atmyb44* plants during the treating period. On the other hand, the transcription of *CyCB2* was found to be more repressed in *MYB44OTA* plants than in wild-type plants in response to 3OC6-HSL (Fig. 4A).

ARR4 and *ARR15*, two two-component response regulator (ARR) genes, function as the negative mediator in cytokinin signaling. After treatment with 3OC6-HSL for 24 h, the expression of *ARR4* significantly increased in wild-type and *AtMYB44*-overexpressing plants (Fig. 4B). However, no obvious induction in expression of *ARR4* by 3OC6-HSL was detected in mutant *atmyb44* plants even up to 48 h of exposure to 3OC6-HSL. Notably, much more induction in transcript accumulation of *ARR4* was detected in *MYB44OTA* plants compared with the wild-type plants after 48 h of treatment with 3OC6-HSL (Fig. 4B). Upon treatment with 3OC6-HSL, the level of *ARR15* transcript increased in wild-type plants while no marked induction of *ARR15* expression was observed in mutant *atmyb44* plants (Fig. 4C). In contrast, much higher accumulation of *ARR15* transcript was monitored in *MYB44OTA* plants than in wild-type plants 12 h after treatment with 3OC6-HSL (Fig. 4C). By 24 and 48 h post 3OC6-HSL treatment, the transcript level of *ARR15* increased up to 9- and 49-fold over that at the 0 time point (Fig. 4C). When the plants were grown on medium without addition of 3OC6-HSL, the expression of the respective genes in *atmyb44* mutants or *AtMYB44OTA* exhibited a comparable or slightly altered level compared with those of the wild-type, except *ARR15* (Fig. 4D, E and F), indicating that no impact was exerted on the expression of these genes by knockout or overexpression of *AtMYB44*, except *ARR15*. Overexpression of *AtMYB44* exerted impact on *ARR15* expression to some extent (Fig. 4F). However, this promoting effect on *ARR15* transcription was negligible if compared with the induction of *ARR15* expression by 3OC6-HSL treatment under the background of *AtMYB44* overexpression (Fig. 4C). These results indicated that AtMYB44 is involved in the regulation of cytokinin-related gene expression by 3OC6-HSL in planta.

MAX2, an F-box protein, is a component of strigolactone signaling which functions in root growth and development via cross-talk with auxin (Koren et al. 2013; Nelson et al. 2011). The results showed that the induction of *MAX2* by 3OC6-HSL was repressed in *AtMYB44* mutant *atmyb44* whereas 3OC6-HSL promoted the transcript accumulation of *MAX2* as the treating time increased to 12 h in wild-type and *AtMYB44OTA* plants. After 12 h of treatment with 3OC6-HSL, the level of *MAX2* transcript continued to increase up to 11-fold over that at the 0 time point in *AtMYB44OTA*, while the expression of *MAX2* kept constant in wild-type plants (Fig. 5A). Although mutation or overexpression of *AtMYB44* exerted an impact on expression of *MAX2* to some extent (Fig. 5D), these effects could be ignored when compared with that in 3OC6-HSL-induced *MAX2* expression in *AtMYB44OTA* plants. These data suggested a role of AtMYB44 in AHL-mediated expression of *MAX2*.

IAA7 and IAA14, two auxin-responsive proteins, play a central role in auxin-regulated transcriptional activation and repression (Fukaki et al. 2002; Nagpal et al. 2000; Timpote et al. 1994). In a qRT-PCR experiment, we found that treatment with 3OC6-HSL induced *IAA14* and *IAA7* transcription in wild-type *Arabidopsis* while the induction of *IAA14* and *IAA7* by 3OC6-HSL was repressed and exaggerated significantly in *atmyb44* mutants and *AtMYB44OTA* plants, respectively (Fig. 5B and C). Mutation of *AtMYB44* did not affect the transcription of *IAA7* and *IAA14*, whereas overexpression of *AtMYB44* upregulated the expression of *IAA7* but had no impact on *IAA14* transcription

Fig. 2. Primary root growth assay for *Arabidopsis* wild-type plant (Col-0), *MYB44* functional-deficiency mutant (*atmyb44*), and *MYB44*-overexpressing transgenic line *MYB44OTA* after treatment with 3OC6-HSL. **A**, Representative images of *Arabidopsis* seedlings grown on vertically oriented plates with or without 1 μ M 3OC6-HSL after 7 days of cultivation. Scale bars = 1 cm. **B**, Impacts of 3OC6-HSL on primary root length in *Arabidopsis* seedlings. The experiment was replicated three times with similar results. Data shown represent the mean $\pm$ standard error ($n = 30$). Asterisks indicate statistically significant differences compared with the control (Student's t test, * and ** indicate $P < 0.05$ and 0.01 , respectively). **C**, Identification of *MYB44* mutant and *MYB44*-overexpressing transgenic line by reverse transcription-polymerase chain reaction (RT-PCR). Total mRNA were extracted from 1-month-old seedlings of wild-type Col-0, *atmyb44*, and *MYB44OTA*, and RT-PCR was done to amplify the *AtMYB44* gene. *ACTIN* was used as the endogenous reference gene.

Fig. 3. Analysis of root meristem zone and root elongation zone in *Arabidopsis* wild-type plant (Col-0), *AtMYB44* functional-deficiency mutant (*atmyb44*), and *AtMYB44*-overexpressing transgenic line *MYB44OTA* after treatment with 3OC6-HSL. **A**, Representative images of *Arabidopsis* root meristem zone and elongation zone grown on vertically oriented plates with or without 1 μM 3OC6-HSL after 7 days of cultivation. Scale bars = 50 μm . **B**, Length of the root meristem zone; **C**, number of cortical cells in the meristem zone; **E**, length of the root elongation zone; and **F**, number of the cortical cells in the elongation zone were detected in *Arabidopsis* plants grown on vertically oriented plates with or without 1 μM 3OC6-HSL for 7 days. Lengths of the cortical cell in **D**, the root meristem zone and **G**, the root elongation zone were calculated according to Zhao and colleagues (2015). Values shown are the average of three independent biological replications. Each experiment included at least 30 seedlings. Asterisks indicate statistically significant differences relative to the control (Student's *t* test, * and ** indicate $P < 0.05$ and 0.01 , respectively).

(Fig. 5E and F). In the case of *IAA7*, the level of *IAA7* transcript caused by 48 h of 3OC6-HSL induction in *AtMYB44OTA* plants was much higher than that caused by overexpression of *AtMYB44* in wild-type plants (Fig. 5B and E). These data implied that *AtMYB44* participates in the modulation of auxin-related gene expression regulated by bacterial AHL.

DISCUSSION

The *Arabidopsis AtMYB44* gene is a nuclear-localized transcriptional factor that regulates many aspects of development (Kirik et al. 1998) and also regulates defense responses to pathogens (Kranz et al. 1998; Liu et al. 2010; Pitzschke et al. 2009), herbivorous insects (Liu et al. 2010), and environmental stresses (Jung et al. 2007, 2008, 2010) and the plant responses to several phytohormones (Chen et al. 2006; Jung et al. 2008,

2010; Kranz et al. 1998). Whether *AtMYB44* functions in the responses of plants to bacterial QS signals is unknown thus far. The present study provides the first molecular and genetic evidence for the role of *AtMYB44* in primary root elongation modulated by bacterial QS signal 3OC6-HSL.

In the literature, several reports suggested that distinct transcriptional changes occurred in plants after exposure to bacterial QS signal molecules. In *Arabidopsis* treated with 10 μ M C6-HSL for 4 h, 1 day, and 4 days, 721 genes were differentially expressed in leaves and 1,095 in root tissues (von Rad et al. 2008). Recently, Schenk and colleagues (2014) reported that 305 genes were differentially expressed in *Arabidopsis* seedlings after application of 6 μ M C6-HSL to roots for 3 days. The difference in total quantity of differentially expressed genes between the above two data sets may be due to the different concentration of C6-HSL used, treatment duration, sampling tissues,

Fig. 4. Effects of 3OC6-HSL on the expression of cytokinin-mediated signaling genes in *Arabidopsis* roots. Total mRNA was extracted from the plants grown in a sterile hydroponic system with or without 1 μ M 3OC6-HSL. Relative expressions of **A**, *CYCB2*; **B**, *ARR4*; and **C**, *ARR15* genes at indicated time points and the initial expression level of **D**, *CYCB2*; **E**, *ARR4*; and **F**, *ARR15* genes in indicated genotype plants without 3OC6-HSL treatment were detected by quantitative real-time polymerase chain reaction with specific gene primers. Values were normalized to the 0-h time point (A, B, and C) and the wild type (D, E, and F) as control. Data shown represent the mean $\pm$ standard deviation from three independent biological replications. Asterisks indicate statistically significant differences compared with the control at $P < 0.05$ in Student's *t* test.

and the set threshold for data analysis. In our study, we used the derivative of C6-HSL in which the C3 position was substituted with a ketone group, 3OC6-HSL, to treat *Arabidopsis* roots for 24 h and monitored the alteration in the transcriptional profile in root tissues. We found that 2,873 differentially regulated genes (NCBI GEO accession number GSE 78079). By comparing the root transcriptome between von Rad's study and ours, only 151 genes were found to overlap each other while the majority of the differentially expressed genes responded only to C6-HSL or 3OC6-HSL, respectively, indicating that both AHL regulate a specific set of host genes even though they are structurally similar (Supplementary Fig. 2A). Notably, of the 151 genes that are responsive to both AHL, 19.2% (29 genes) were related to the functional category "biotic or abiotic stress" (Supplementary Fig. 2B). A similar finding was reported by the Schikora group when they directly compared the effects of three

types of AHL on transcriptional reprogramming in *Arabidopsis* (Schenk et al. 2014). All these data suggested that plants respond to distinct AHL in a specific manner and biotic or abiotic stress remains the major functional category.

The involvement of AtMYB44 in AHL-mediated primary root elongation has been elucidated by several lines of evidence. First, the expression of AtMYB44 in *Arabidopsis* is induced by 3OC6-HSL. In a microarray-based screening experiment, we found that AtMYB44, together with AtMYB70 and AtMYB77, which are assigned to subgroup 22 of the *Arabidopsis* R2R3-MYB family, are among the 3OC6-HSL-responsive genes (Table 1). The time profile of AtMYB44 expression confirmed the induction of transcript accumulation after exposure to 3OC6-HSL using a qRT-PCR approach (Fig. 1). Second, mutation of AtMYB44 blocks the promoting effect of 3OC6-HSL on primary root elongation. *Arabidopsis* mutant *atmyb44*, with a

Fig. 5. Effects of 3OC6-HSL on the expression of auxin-mediated signaling genes in *Arabidopsis* roots. Total mRNA was extracted from the plants grown in sterile hydroponic system with or without 1 μ M 3OC6-HSL. Relative expressions of **A**, MAX2; **B**, IAA7; and **C**, IAA14 genes at indicated time points and the initial expression level of **D**, MAX2; **E**, IAA7; and **F**, IAA14 genes in indicated genotype plants without 3OC6-HSL treatment were measured by quantitative real-time polymerase chain reaction with specific gene primers. Values were normalized to the 0-h time point (A, B, and C) and the wild type (D, E, and F) as control. Data shown represent the mean $\pm$ standard deviation from three independent biological replications. Asterisks indicate statistically significant differences compared with the control at $P < 0.05$ in Student's t test.

T-DNA insertion that interrupts the promoter region of *AtMYB44* (Liu et al. 2011), was insensitive to 3OC6-HSL treatment with regard to primary root elongation (Fig. 2). Third, overexpression of *AtMYB44* confers 3OC6-HSL hypersensitivity in promotion of primary root elongation. We found that application of 3OC6-HSL caused an enhanced promotion of primary root elongation in *AtMYB44*-overexpressing *MYB44OTA* plants when compared with that in wild-type plants (Fig. 2). Taken together, these results demonstrated the requirement of *AtMYB44* for the stimulatory effect of 3OC6-HSL on *Arabidopsis* primary root growth.

Based on the accumulating evidence, Hartmann and colleagues (2014) recently pointed out that bacterial QS signals are important modulators of plant–microbe interactions. On the other hand, *AtMYB44* is found to function as a key regulator in the interaction between plant and microbe. Infection of bacterial pathogen and treatment with bacterial elicitors induced the expression of *AtMYB44* in *Arabidopsis* (Liu et al. 2011; Lü et al. 2013; Zou et al. 2012). *AtMYB44* is required for the induction of resistance to *P. syringae* pv. *tomato* DC3000 in *Arabidopsis* treated with HrpN_{Ea}, a harpin protein from a bacterial plant pathogen, *Erwinia amylovora* (Liu et al. 2011; Wei et al. 1992). Subsequent studies have revealed that *AtMYB44* is regulated by an MPK cascade. Following stress treatment, *AtMPK3* is activated and phosphorylates *VIP1*, which then rapidly activates the expression of *AtMYB44* (Pitzschke et al. 2009). Further results have shown that *AtMYB44* interacts directly with and is phosphorylated by *AtMPK3*, and the phosphorylation of *AtMYB44* is required for its function in plant response to ABA treatment with regard to seed germination and survival under salt stress (Jaradat et al. 2013; Nguyen et al. 2012; Persak and Pitzschke 2013). It is noteworthy that *AtMPK3* and *AtMPK6* are strongly activated by the model elicitor flg22 after pretreatment with 3OC12-HSL or *N*-3-oxo-tetradecanoyl-homoserine lactone (3OC14-HSL), and *AtMPK6* is required for AHL-induced resistance (Schikora et al. 2011). Furthermore, 3OC14-HSL can act as a priming agent to induce plant resistance, possibly through the salicylic acid (SA) signaling pathway (Schenk et al. 2014). Zou and coworkers (2013) demonstrated that *AtMYB44* positively modulates disease resistance through the SA signaling pathway in *Arabidopsis*. Taking all of these lines of evidence into account, we speculate that the connection between AHL and *AtMYB44* might exist in regulating the elongation of plant primary roots even though *AtMYB44* or *AtMPK3/6* are known to mainly play roles in modulation of plant resistance and stress responses. The present results provide a strong support for our hypothesis.

It has been known that short-chain AHL, including C4-HSL, C6-HSL, 3OC6-HSL, and 3OC8-HSL, can promote plant root growth (Liu et al. 2012; von Rad et al. 2008). Recently, we found that the 3OC6-HSL-elicited primary root elongation appears to be the consequence of increased cell division activity in the meristem zone and promoted cell elongation in the elongation zone (Zhao et al. 2015). The effects of AHL on cell division in *Arabidopsis* roots were also reported by Ortíz-Castro and colleagues (2008). They monitored the expression of pPRZ1: *udiA*, which marks only active meristem (Sieberer et al. 2003) and *CycB1: udiA*, which is expressed only in cells in the G2/M phase of the cell cycle and is a marker of mitotic activity (Colón-Carmona et al. 1999) after application of C10-HSL at a concentration of 48 μ M. They found that the strong inhibition of C10-HSL on primary root growth correlated to the loss of the expression of PRZ1 and *CycB1* and the decreased size of PRZ1 expression. C10-HSL with the tested concentration significantly affected primary root growth while 3OC6-HSL at a concentration of 1 μ M stimulated remarkably the primary root elongation (Liu et al. 2012; Ortíz-Castro et al. 2008; Zhao et al. 2015).

Nevertheless, it is likely that different types and different concentrations of AHL alter primary root growth through regulating cell division and cell elongation in roots. In this study, we compared the cell number and cell length in the root meristem zone and root elongation zone between different genotypes regarding *AtMYB44* and the wild-type plants grown in the presence of 3OC6-HSL. We observed increased effects of 3OC6-HSL on cell division in the meristem zone and cell elongation in the elongation zone in *AtMYB44*-overexpressing transgenic lines compared with wild plants. In contrast, mutation of *AtMYB44* resulted in resistance to 3OC6-HSL treatment in respect to cell division in the meristem zone and cell elongation in the elongation zone. Therefore, these observation implied that *AtMYB44* participates in the pathways of 3OC6-HSL in regulating cell division and cell elongation in the meristem zone and elongation zone, respectively.

Plant hormones (especially auxins and cytokinins) play critical roles in plant growth and development, including root growth. Previous studies revealed that accumulations of auxin-responsive proteins and the transcriptional level of auxin- or cytokinin-related genes were altered after AHL treatments (Mathesius et al. 2003; von-Rad et al. 2008). Exposure of *Arabidopsis* roots to C6-HSL could even change the ratio of auxin to cytokinin in roots (von-Rad et al. 2008). In microarray analysis, we also found some phytohormone-responsive genes, including *ARR15*, *ARR4*, *IAA7*, *IAA14*, and *MAX2*, whose transcript levels were changed by treatment with 3OC6-HSL. In contrast, Ortíz-Castro and colleagues (2008) stated that auxin may not be involved in *Arabidopsis* responses to C10-HSL because C10-HSL failed to induce auxin-regulated gene expression, and several auxin-related mutants showed a similar reaction to C10-HSL in root development as the wild type. To explore the mechanism by which *AtMYB44* regulates 3OC6-HSL-induced primary root elongation, we determined the effects of *AtMYB44* on induction of auxin- and cytokinin-related gene expression by 3OC6-HSL using qRT-PCR approaches. Our results showed that the transcript inductions of *ARR4* and *ARR15*, two negative regulators of cytokinin signaling, by treatment of 3OC6-HSL were blocked and strengthened in loss- and gain-of-function mutation, respectively, in *AtMYB44* (Fig. 4B and C). A contrary result was obtained, where the mutation of *AtMYB44* conferred plant resistance to 3OC6-HSL-induced repression of transcript accumulation of *CyCB2*, which encodes a positive regulator of cytokinin signaling, while overexpression of *AtMYB44* enhanced the inhibition of 3OC6-HSL on *CyCB2* expression (Fig. 4A). On the other hand, we found that the upregulation of *IAA7* and *IAA14* transcription, two auxin-inducible genes, caused by 3OC6-HSL treatment is diminished and augmented in the *AtMYB44* knock-out mutant and overexpressing transgenic plants, respectively (Fig. 5B and C). In the present study, we did not monitor a change in concentration of cytokinin and auxin in plant roots after application of 3OC6-HSL. However, von Rad and colleagues (2008) observed that auxin concentrations are higher in C6-HSL-treated leaf and root tissues compared with untreated control plants. Notably interesting was that C6-HSL treatment resulted in an obvious shift of auxin/cytokinin ratio in both leaf and root tissues toward higher auxin (von Rad et al. 2008). Hence, although we could not conclude that the stimulatory effect of 3OC6-HSL on primary root growth is the direct function of auxin and cytokinin, our results and those of others at least demonstrated two important points. One is that 3OC6-HSL might promote primary root elongation via auxin and cytokinin signaling. Another is that *AtMYB44* participates in 3OC6-HSL-mediated primary root growth via regulating the auxin- and cytokinin-related gene expression. It has been known that MYB77 can recognize *cis*-elements MBSI (CNGTTR) and MBSII (GTTAGTTA) and preferentially bind to the MBSI motif while MYB44 has a clear

preference for MBSII but still binds to the MBSI motif (Persak and Pitzschke 2013; Zhao et al. 2014). After checking some DNA fragments for MBSI contents Xing and colleagues (2016), found that several auxin-inducible genes, including *IAA1*, *IAA7*, *IAA17*, and *IAA19*, harbored this *cis*-element. Furthermore, they demonstrated that the ABA receptor PYL9, a close relative of PYL8, could form a protein complex with MYB77 and then bind to the MBSI motif in the promoter region of *IAA7* (Xing et al. 2016). Accordingly, they speculated that ABA might function in plant root growth via MYB transcription factors (Xing et al. 2016). In the present study, we observed that the transcriptions of *IAA7* and *IAA14* were regulated by 3OC6-HSL through AtMYB44. AtMYB44 was also reported to directly interact with PYL8 and PYL9 (Li et al. 2014; Xing et al. 2016). Taking these clues into account, we put forward a possibility that the unidentified intracellular receptor perceives the presence of 3OC6-HSL and forms a protein complex with AtMYB44, subsequently binding the *cis*-element MBSI in the promoter of *IAA*, regulating their transcription, and mediating the primary root growth. Additionally, AtMYB44 also likely plays a role in 3OC6-HSL promoting an effect on primary root growth via strigolactone signaling because the induction of *MAX2* by 3OC6-HSL was repressed and enhanced in *AtMYB44* knockout lines *atmyb44* and *MYB44OTA*, respectively, compared with the wild-type plants. Based on these data, we speculate that 3OC6-HSL may activate transcriptional factor AtMYB44 through an unidentified pathway, and then activated AtMYB44 regulates phytohormones, including auxin, cytokinin, and even the strigolactone signal transduction pathway, to modulate the elongation of primary roots.

Overall, we have demonstrated a role for AtMYB44 in 3OC6-HSL-mediated primary root elongation in *Arabidopsis*. Further study should be conducted to obtain more physiological and genetic evidence.

MATERIALS AND METHODS

Plant growth conditions and treatments.

Genotypes of the *Arabidopsis* ecotype Col-0 used in this study were the wild-type plant, the T-DNA insertion null mutant *atmyb44* (SALK_008606), and *AtMYB44*-overexpressing transgenic *Arabidopsis* MYB44OTA. Seed of the Col-0 wild type were obtained from the TAIR stock center (www.arabidopsis.org) while seed of *atmyb44* and *AtMYB44OTA* were provided by H. Dong in Nanjing Agriculture University, China (Liu et al. 2011; Zou et al. 2012). Seed were surface sterilized with 75% (vol/vol) ethanol for 1 min and 25% (vol/vol) bleach for 5 min. After five washes with sterile distilled water, seed were germinated on agar plates containing Murashige and Skoog (MS) medium (Murashige and Skoog 1962) adjusted to pH 5.8. The plates were stratified at 4°C for 2 days and then placed in a plant growth chamber with a photoperiod of 12 h of light, 12 h of darkness, light intensity of 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and temperature of $22 \pm 2^\circ\text{C}$.

3OC6-HSL was purchased from Sigma-Aldrich, stored dry, and diluted as 10 mM stock solutions in distilled H₂O just prior to use. For primary root growth assay, the seed were grown vertically for 3 days on MS agar plates, and the seedlings with similar root length were transferred to ½ MS agar plates containing 1 μM 3OC6-HSL (Supplementary Fig. S3). For microarray and qRT-PCR assay, a hydroponic system was employed to cultivate *Arabidopsis*. Seedlings germinated on MS agar plates for 10 days were transplanted into a sterile plastic basin (18 by 11 cm) containing 500 ml of sterile Hoagland medium. After 7 days, the seedlings were used for 1 μM 3OC6-HSL treatments. 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 was free of

bacterial contamination, the medium and 3OC6-HSL solution were sterilized by passing them through a 0.22- μm 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.

Microarray analysis.

Seventeen-day-old seedlings were cultured in Hoagland medium with or without 1 μM 3OC6-HSL. Roots of plants were harvested at 24 h after 3OC6-HSL treatment. Total RNA was extracted from treated and control plants using the RNAiso Plus reagent (TaKaRa) and purified using the NucleoSpin RNA clean-up kit (Macherey-Nagel), according to the manufacturers' instructions. The probes were prepared using the CapitalBio cRNA amplified labeling kit (CapitalBio Corp.) and fluorescently labeled with Cy5-dCTP and Cy3-dCTP (GE Healthcare). The 29k *Arabidopsis* Genome Arrays (CapitalBio Corp.) were prepared according to the *A. thaliana* Genome Oligo Set (version 3.0; Operon). After hybridization, the arrays were scanned with the LuxScan 10KA two-channel laser scanner (CapitalBio Corp.) and analyzed using the LuxScan 3.0 software (CapitalBio Corp.). For extraction of the data from the individual channels, faint spots with intensity <400 units after subtraction of the background were removed. A space- and intensity-dependent normalization method based on a LOWESS program (Yang et al. 2002) was used. Each data point represents the average of three independent experiments. A twofold increase (ratio > 2.0) or a twofold decrease (ratio < 0.5) in expression in treated plants compared with untreated plants was considered as differential expression corresponding to upregulation or downregulation, respectively, in response to the AHL. The gene annotation and functional classification were performed using the Molecule Annotation System v. 3.0 and based on the Gene Ontology tool at TAIR. The data discussed in the present study have been deposited in NCBI GEO and are accessible through GEO Series accession number GSE78079.

qRT-PCR.

Seventeen-day-old seedlings were cultured in Hoagland medium with or without 1 μM 3OC6-HSL. Roots of the treated plants were harvested at 0, 3, 6, 12, 24, and 48 h after 3OC6-HSL treatment and total RNA was extracted from the treated and untreated roots using the RNAiso Plus reagent (TaKaRa) according to the manufacturer's instructions. The cDNA was synthesized using the PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa) according to the manufacturer's instructions. For the relative quantification of gene expression, the comparative cycle threshold method (Livak and Schmittgen 2001) was used with the 7500 Real Time PCR System (Applied Biosystems). For qRT-PCR, received cDNA was diluted 15 times in sterile double-distilled H₂O. 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). The following qRT-PCR 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 *ACTIN2* (AT3G18780). Values were normalized to the 0-h time point. For technical control, each qRT-PCR experiment was repeated four times on the same 96-well plate. Each data point represents the average of three independent experiments. A 1.5-fold increase (ratio > 1.5) or 1.5-fold decrease (ratio < 0.8) in expression in the treated plants compared with the untreated plants was considered as upregulation or downregulation, respectively, related to 3OC6-HSL response. The specific primers for qRT-PCR are shown in Supplementary Table S1.

Identification of *MYB44* mutant and *MYB44*-overexpressing transgenic lines.

The leaves of a 1-month-old *Arabidopsis* wild-type plant (Col-0), *MYB44* functional-deficiency mutant (*atmyb44*), and *MYB44*-overexpressing transgenic line *MYB44OTA* cultured in vermiculite were harvested and total RNA was extracted using the RNAiso Plus reagent (TaKaRa) according to the manufacturer's instructions. The cDNA was synthesized using the PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa) according to the manufacturer's instructions. The full-length sequence of *AtMYB44* were amplified by RT-PCR. For RT-PCR, amplification was done in a total volume of 20 µl containing 1 µl of received cDNA, 1 µl of each primer (10 µM), 2 µl of 10× PCR buffer, 2 µl of dNTP (2.5 mM each), 0.2 µl of rTaq DNA Polymerase (5 units/µl) (TaKaRa), and 12.8 µl of sterilized distilled water. The following RT-PCR thermal cycling program was employed: initial denaturation for 5 min at 94°C; 30 cycles for 40 s at 94°C, 50 s at 56°C, and 1 min at 72°C; then extension for 10 min at 72°C. *ACTIN* was used as the endogenous reference gene. The specific primers for RT-PCR are shown in Supplementary Table S1.

Analysis of primary root growth.

The seed of wild-type Col-0, mutant *atmyb44*, and overexpressing transgenic line *MYB44OTA* germinated on MS agar plates vertically. After 3 days, seedlings with similar root length were transferred to ½ MS agar plates containing 1 µM 3OC6-HSL and cultured vertically for another 7 days in the same growth condition as described above. The untreated plants were taken as the control. Primary root length was assessed using Image J software. The experiment was replicated three times and each biological replication included at least 30 seedlings for each genotype and treatment.

For dissecting the root elongation, the length of root meristem zone and root elongation zone were measured on the 7-day-old seedlings fixed in ethanol/acetic acid (3:1) and mounted in choral hydrate. Microscopy was performed on a Leica DM4000 B (CMS GmbH) equipped with a Leica DFC 420C camera. Root meristem zone length was assessed as the distance between the quiescent center and the first elongating cell with a vacuole. The length of elongation zone was measured as the distance from the first elongating cell to the first cell with a root hair. The number of the cortical cells in the root meristem and elongation zones was obtained by counting the cortical cells under the high-resolution images of the root tips using Image J software. The length of cortical cells in the root meristem or elongation zone was calculated by dividing the root meristem or elongation zone length by the number of the cortical cells in root meristem or elongation zone. At least 30 seedling were processed, and at least three independent experiments were conducted, giving similar statistically significant results.

Statistical analysis.

For all experiments, the overall data were statistically analyzed in the DPS v7.05 program. Univariate and multivariate analyses with a Duncan's new multiple-range method were used for testing different root growth responses of different ecotype, wild-type Col-0, *atmyb44*, and *MYB44OTA* lines to 3OC6-HSL.

ACKNOWLEDGMENTS

We thank H. Dong from Nanjing Agriculture University, China for providing the seed of *AtMYB44* mutant line *atmyb44* and *AtMYB44*-overexpressing transgenic line *MYB44OTA*. This work was financially supported by a project from the National Basic Research Program of China (number 2015CB150600), the National Natural Science Foundation of China (number 31270880), and the Natural Science Foundation of Hebei Province (number C2015302020).

LITERATURE CITED

- Bai, X., Todd, C. D., Desikan, R., Yang, Y., and Hu, X. 2012. *N*-3-oxo-decanoyl-L-homoserine-lactone activates auxin-induced adventitious root formation via hydrogen peroxide- and nitric oxide-dependent cyclic GMP signaling in mung bean. *Plant Physiol.* 158:725-736.
- Bassler, B. L. 1999. How bacteria talk to each other: Regulation of gene expression by quorum sensing. *Curr. Opin. Microbiol.* 2:582-587.
- Chen, Y., Yang, X., He, K., Liu, M., Li, J., Gao, Z., Lin, Z., Zhang, Y., Wang, X., Qiu, X., Shen, Y., Zhang, L., Deng, X., Luo, J., Deng, X.-W., Chen, Z., Gu, H., and Qu, L.-J. 2006. The MYB transcription factor superfamily of *Arabidopsis*: Expression analysis and phylogenetic comparison with the rice MYB family. *Plant Mol. Biol.* 60:107-124.
- Colón-Carmona, A., You, R., Haimovitch-Gal, T., and Doerner, P. 1999. Technical advance: Spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. *Plant J.* 20:503-508.
- Fukaki, H., Tameda, S., Masuda, H., and Tasaka, M. 2002. Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of *Arabidopsis*. *Plant J.* 29:153-168.
- Fuqua, C., Parsek, M. R., and Greenberg, E. P. 2001. Regulation of gene expression by cell-to-cell communication: Acyl-homoserine lactone quorum sensing. *Annu. Rev. Genet.* 35:439-468.
- Gong, W., Shen, Y. P., Ma, L. G., Pan, Y., Du, Y. L., Wang, D. H., Yang, J. Y., Hu, L. D., Liu, X. F., Dong, C. X., Ma, L., Chen, Y. H., Yang, X. Y., Gao, Y., Zhu, D., Tan, X., Mu, J. Y., Zhang, D. B., Liu, Y. L., Dinesh-Kumar, S. P., Li, Y., Wang, X. P., Gu, H. Y., Qu, L. J., Bai, S. N., Lu, Y. T., Li, J. Y., Zhao, J. D., Zuo, J., Huang, H., Deng, X. W., and Zhu, Y. X. 2004. Genome-wide ORFeome cloning and analysis of *Arabidopsis* transcription factor genes. *Plant Physiol.* 135:773-782.
- Hartmann, A., Rothballer, M., Hense, B. A., and Schröder, P. 2014. Bacterial quorum sensing compounds are important modulators of microbe-plant interactions. *Front. Plant Sci.* 5:131.
- Hernández-Reyes, C., Schenk, S. T., Neumann, C., Kogel, K. H., and Schikora, A. 2014. *N*-acyl-homoserine lactones-producing bacteria protect plants against plant and human pathogens. *Microb. Biotechnol.* 7:580-588.
- Jaradat, M. R., Feurtado, J. A., Huang, D., Lu, Y., and Cutler, A. J. 2013. Multiple roles of the transcription factor AtMYB1/AtMYB44 in ABA signaling, stress responses, and leaf senescence. *BMC Plant Biol.* 13:192.
- Jin, G., Liu, F., Jia, Z., Bian, Z., and Song, S. 2012. Two G-protein-coupled-receptor candidates, Cand2 and Cand7, are involved in *Arabidopsis* root growth mediated by the bacterial quorum-sensing signals *N*-acyl-homoserine lactones. *Biochem. Biophys. Res. Commun.* 417:991-995.
- Jung, C., Lyou, S. H., Yeu, S., Kim, M. A., Rhee, S., Kim, M., Lee, J. S., Choi, Y. D., and Cheong, J. J. 2007. Microarray-based screening of jasmonate-responsive genes in *Arabidopsis thaliana*. *Plant Cell Rep.* 26:1053-1063.
- Jung, C., Seo, J. S., Han, S. W., Koo, Y. J., Kim, C. H., Song, S. I., Nahm, B. H., Choi, Y. D., and Cheong, J. J. 2008. Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic *Arabidopsis*. *Plant Physiol.* 146:623-635.
- Jung, C., Shim, J. S., Seo, J. S., Lee, H. Y., Kim, C. H., Choi, Y. D., and Cheong, J. J. 2010. Non-specific phytohormonal induction of AtMYB44 and suppression of jasmonate-responsive gene activation in *Arabidopsis thaliana*. *Mol. Cells* 29:71-76.
- Kirik, V., Kölle, K., Miséra, S., and Bäumllein, H. 1998. Two novel MYB homologues with changed expression in late embryogenesis-defective *Arabidopsis* mutants. *Plant Mol. Biol.* 37:819-827.
- Koren, D., Resnick, N., Mayzlish Gati, E., Belausov, E., Weininger, S., Kapulnik, Y., and Koltai, H. 2013. Strigolactone signaling in the endodermis is sufficient to restore root responses and involves SHORT HYPOCOTYL 2 (SHY2) activity. *New Phytol.* 198:866-874.
- Kranz, H. D., Denekamp, M., Greco, R., Jin, H., Leyva, A., Meissner, R. C., Petroni, K., Urzainqui, A., Bevan, M., Martin, C., Smeeckens, S., Tonelli, C., Paz-Ares, J., and Weisshaar, B. 1998. Towards functional characterisation of the members of the R2R3-MYB gene family from *Arabidopsis thaliana*. *Plant J.* 16:263-276.
- Lee, J., Das, A., Yamaguchi, M., Hashimoto, J., Tsutsumi, N., Uchimiya, H., and Umeda, M. 2003. Cell cycle function of a rice B2-type cyclin interacting with a B-type cyclin-dependent kinase. *Plant J.* 34:417-425.
- Lee, J. H., Hong, J. B., Hong, S. B., Choi, M. S., Jeong, K. Y., Park, H. J., Hwang, D. J., Lee, S., Ra, D., and Heu, S. 2008. Disease-resistant transgenic *Arabidopsis* carrying the *exp1* gene from *Pectobacterium carotovorum* subsp. *carotovorum* SL940. *Plant Pathol. J.* 24:183-190.
- Li, D., Li, Y., Zhang, L., Wang, X., Zhao, Z., Tao, Z., Wang, J., Wang, J., Lin, M., Li, X., and Yang, Y. 2014. *Arabidopsis* ABA receptor RCAR1/PYL9 interacts with an R2R3-type MYB transcription factor, AtMYB44. *Int. J. Mol. Sci.* 15:8473-8490.
- Liu, F., Bian, Z., Jia, Z., Zhao, Q., and Song, S. 2012. The GCR1 and GPA1 participate in promotion of *Arabidopsis* primary root elongation induced

- by *N*-acyl-homoserine lactones, the bacterial quorum-sensing signals. *Mol. Plant-Microbe Interact.* 25:677-683.
- Liu, R., Chen, L., Jia, Z., Lü, B., Shi, H., Shao, W., and Dong, H. 2011. Transcription factor AtMYB44 regulates induced expression of the ETHYLENE INSENSITIVE2 gene in *Arabidopsis* responding to a harpin protein. *Mol. Plant-Microbe Interact.* 24:377-389.
- Liu, R., Lü, B., Wang, X., Zhang, C., Zhang, S., Qian, J., Chen, L., Shi, H., and Dong, H. 2010. Thirty-seven transcription factor genes differentially respond to a harpin protein and affect resistance to the green peach aphid in *Arabidopsis*. *J. Biosci.* 35:435-450.
- Livak, K. J., and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* 25:402-408.
- Lü, B.-B., Li, X.-J., Sun, W.-W., Li, L., Gao, R., Zhu, Q., Tian, S.-M., Fu, M.-Q., Yu, H.-L., Tang, X.-M., Zhang, C.-L., and Dong, H.-S. 2013. AtMYB44 regulates resistance to the green peach aphid and diamondback moth by activating EIN2-affected defences in *Arabidopsis*. *Plant Biol.* 15:841-850.
- Mäe, A., Montesano, M., Koiv, V., and Palva, E. T. 2001. Transgenic plants producing the bacterial pheromone *N*-acyl-homoserine lactone exhibit enhanced resistance to the bacterial phytopathogen *Erwinia carotovora*. *Mol. Plant-Microbe Interact.* 14:1035-1042.
- Mathesius, U., Mulders, S., Gao, M., Teplitski, M., Caetano-Anolles, G., Rolfe, B. G., and Bauer, W. D. 2003. Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proc. Natl. Acad. Sci. U.S.A.* 100:1444-1449.
- Miao, C., Liu, F., Zhao, Q., Jia, Z., and Song, S. 2012. A proteomic analysis of *Arabidopsis thaliana* seedling responses to 3-oxo-octanoyl-homoserine lactone, a bacterial quorum-sensing signal. *Biochem. Biophys. Res. Commun.* 427:293-298.
- Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
- Nagpal, P., Walker, L. M., Young, J. C., Sonawala, A., Timpte, C., Estelle, M., and Reed, J. W. 2000. AXR2 encodes a member of the Aux/IAA protein family. *Plant Physiol.* 123:563-574.
- Nelson, D. C., Scaffidi, A., Dun, E. A., Waters, M. T., Flematti, G. R., Dixon, K. W., Beveridge, C. A., Ghisalberti, E. L., and Smith, S. M. 2011. F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U.S.A.* 108:8897-8902.
- Nguyen, X. C., Hoang, M. H. T., Kim, H. S., Lee, K., Liu, X. M., Kim, S. H., Bahk, S., Park, H. C., and Chung, W. S. 2012. Phosphorylation of the transcriptional regulator MYB44 by mitogen activated protein kinase regulates *Arabidopsis* seed germination. *Biochem. Biophys. Res. Commun.* 423:703-708.
- Ortiz-Castro, R., Díaz-Pérez, C., Martínez-Trujillo, M., del Río, R. E., Campos-García, J., and López-Bucio, J. 2011. Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. *Proc. Natl. Acad. Sci. U.S.A.* 108:7253-7258.
- Ortiz-Castro, R., Martínez-Trujillo, M., and López-Bucio, J. 2008. *N*-acyl-L-homoserine lactones: A class of bacterial quorum-sensing signals alter post-embryonic root development in *Arabidopsis thaliana*. *Plant Cell Environ.* 31:1497-1509.
- Palmer, A. G., Senechal, A. C., Mukherjee, A., Ané, J. M., and Blackwell, H. E. 2014. Plant responses to bacterial *N*-acyl L-homoserine lactones are dependent on enzymatic degradation to L-homoserine. *ACS Chem. Biol.* 9:1834-1845.
- Pang, Y., Liu, X., Ma, Y., Chernin, L., Berg, G., and Gao, K. 2009. Induction of systemic resistance, root colonisation and biocontrol activities of the rhizospheric strain of *Serratia plymuthica* are dependent on *N*-acyl homoserine lactones. *Eur. J. Plant Pathol.* 124:261-268.
- Pearson, J. P., Gray, K. M., Passador, L., Tucker, K. D., Eberhard, A., Iglewski, B. H., and Greenberg, E. P. 1994. Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proc. Natl. Acad. Sci. U.S.A.* 91:197-201.
- Persak, H., and Pitzschke, A. 2013. Tight interconnection and multi-level control of *Arabidopsis* MYB44 in MAPK cascade signalling. *PLoS One* 8:e57547.
- Pitzschke, A., Djamei, A., Teige, M., and Hirt, H. 2009. VIP1 response elements mediate mitogen-activated protein kinase 3-induced stress gene expression. *Proc. Natl. Acad. Sci. U.S.A.* 106:18414-18419.
- Quiñones, B., Dulla, G., and Lindow, S. E. 2005. Quorum sensing regulates exopolysaccharide production, motility, and virulence in *Pseudomonas syringae*. *Mol. Plant-Microbe Interact.* 18:682-693.
- Renaudin, J. P., Doonan, J. H., Freeman, D., Hashimoto, J., Hirt, H., Inzé, D., Jacobs, T., Kouchi, H., Rouzé, P., Sauter, M., Savouré, A., Sorrell, D. A., Sundaresan, V., and Murray, J. A. 1996. Plant cyclins: A unified nomenclature for plant A-, B- and D-type cyclins based on sequence organization. *Plant Mol. Biol.* 32:1003-1018.
- Riechmann, J. L., Heard, J., Martin, G., Reuber, L., Jiang, C., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O. J., Samaha, R. R., Creelman, R., Pilgrim, M., Broun, P., Zhang, J. Z., Ghandehari, D., Sherman, B. K., and Yu, G. 2000. *Arabidopsis* transcription factors: Genome-wide comparative analysis among eukaryotes. *Science* 290:2105-2110.
- Romero, I., Fuertes, A., Benito, M. J., Malpica, J. M., Leyva, A., and Paz-Ares, J. 1998. More than 80R2R3-MYB regulatory genes in the genome of *Arabidopsis thaliana*. *Plant J.* 14:273-284.
- Schenk, S. T., Hernández-Reyes, C., Samans, B., Stein, E., Neumann, C., Schikora, M., Reichelt, M., Mithöfer, A., Becker, A., Kogel, K. H., and Schikora, A. 2014. *N*-acyl-homoserine lactone primes plants for cell wall reinforcement and induces resistance to bacterial pathogens via the salicylic acid/oxylin pathway. *Plant Cell* 26:2708-2723.
- Schikora, A., Schenk, S. T., Stein, E., Molitor, A., Zuccaro, A., and Kogel, K. H. 2011. *N*-acyl-homoserine lactone confers resistance toward biotrophic and hemibiotrophic pathogens via altered activation of AtMPK6. *Plant Physiol.* 157:1407-1418.
- Schuhegger, R., Ihring, A., Gantner, S., Bahnweg, G., Knappe, C., Vogt, G., Hutzler, P., Schmid, M., Van Breusegem, F., Eberl, L., Hartmann, A., and Langebartels, C. 2006. Induction of systemic resistance in tomato by *N*-acyl-L-homoserine lactone-producing rhizosphere bacteria. *Plant Cell Environ.* 29:909-918.
- Sieberer, T., Hauser, M. T., Seifert, G. J., and Luschig, C. 2003. PROPORZ1, a putative *Arabidopsis* transcriptional adaptor protein, mediates auxin and cytokinin signals in the control of cell proliferation. *Curr. Biol.* 13:837-842.
- Song, S., Jia, Z., Xu, J., Zhang, Z., and Bian, Z. 2011. *N*-butyryl-homoserine lactone, a bacterial quorum-sensing signaling molecule, induces intracellular calcium elevation in *Arabidopsis* root cells. *Biochem. Biophys. Res. Commun.* 414:355-360.
- Stracke, R., Werber, M., and Weisshaar, B. 2001. The R2R3-MYB gene family in *Arabidopsis thaliana*. *Curr. Opin. Plant Biol.* 4:447-456.
- Timpte, C., Wilson, A. K., and Estelle, M. 1994. The axr2-1 mutation of *Arabidopsis thaliana* is a gain-of-function mutation that disrupts an early step in auxin response. *Genetics* 138:1239-1249.
- Toth, I. K., Newton, J. A., Hyman, L. J., Lees, A. K., Daykin, M., Ortori, C., Williams, P., and Fray, R. G. 2004. Potato plants genetically modified to produce *N*-acylhomoserine lactones increase susceptibility to soft rot *Erwinia*. *Mol. Plant-Microbe Interact.* 17:880-887.
- von Rad, U., Klein, I., Dobrev, P. I., Kottova, J., Zazimalova, E., Fekete, A., Hartmann, A., Schmitt-Kopplin, P., and Durner, J. 2008. Response of *Arabidopsis thaliana* to *N*-hexanoyl-DL-homoserine-lactone, a bacterial quorum sensing molecule produced in the rhizosphere. *Planta* 229:73-85.
- Wei, Z. M., Laby, R. J., Zumoff, C. H., Bauer, D. W., He, S. Y., Collmer, A., and Beer, S. V. 1992. Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. *Science* 257:85-88.
- Xing, L., Zhao, Y., Gao, J., Xiang, C., and Zhu, J. K. 2016. The ABA receptor PYL9 together with PYL8 plays an important role in regulating lateral root growth. *Sci. Rep.* 6:Article 27177.
- Yang, Y. H., Dudoit, S., Luu, P., Lin, D. M., Peng, V., Ngai, J., and Speed, T. P. 2002. Normalization for cDNA microarray data: A robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res.* 30:e15.
- Zarkani, A. A., Stein, E., Röhrich, C. R., Schikora, M., Evguenieva-Hackenberg, E., Degenkolb, T., Vilcinskas, A., Klug, G., Kogel, K. H., and Schikora, A. 2013. Homoserine lactones influence the reaction of plants to rhizobia. *Int. J. Mol. Sci.* 14:17122-17146.
- Zhao, Q., Zhang, C., Jia, Z., Huang, Y., Li, H., and Song, S. 2015. Involvement of calmodulin in regulation of primary root elongation by *N*-3-oxo-hexanoyl homoserine lactone in *Arabidopsis thaliana*. *Front. Plant Sci.* 5:807.
- Zhao, Y., Xing, L., Wang, X., Hou, Y. J., Gao, J., Wang, P., Duan, C. G., Zhu, X., and Zhu, J. K. 2014. The ABA receptor PYL8 promotes lateral root growth by enhancing MYB77-dependent transcription of auxin-responsive genes. *Sci. Signal.* 7:ra53.
- Zou, B., Jia, Z., Tian, S., Wang, X., Gou, Z., Lü, B., and Dong, H. 2013. AtMYB44 positively modulates disease resistance to *Pseudomonas syringae* through the salicylic acid signaling pathway in *Arabidopsis*. *Funct. Plant Biol.* 40:304-313.

AUTHOR-RECOMMENDED INTERNET RESOURCES

Gene Ontology tool at TAIR: <http://www.arabidopsis.org>

Image J software: <https://imagej.nih.gov/ij>

Molecule Annotation System v. 3.0: <http://bioinfo.capitalbio.com/mas3>

NCBI GEO database, accession number GSE78079:

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE78079>