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Shoot phytochrome B modulates reactive oxygen species homeostasis in roots via abscisic acid signaling in *Arabidopsis*

Jun-Ho Ha[1], Ju-Heon Kim[1], Sang-Gyu Kim[2], Hee-Jung Sim[3], Gisuk Lee[4], Rayko Halitschke[4], Ian T. Baldwin[4], Jeong-Il Kim[5] and Chung-Mo Park[1,6,*]

[1]*Department of Chemistry, Seoul National University, Seoul 08826, Korea,*
[2]*Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 34141, Korea,*
[3]*Gyeongnam Department of Environmental Toxicology and Chemistry, Korea Institute of Toxicology, Gyeongnam 52834, Korea,*
[4]*Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Jena 07745, Germany,*
[5]*Department of Biotechnology and Kumho Life Science Laboratory, Chonnam National University, Gwangju 61186, Korea, and*
[6]*Plant Genomics and Breeding Institute, Seoul National University, Seoul 08826, Korea*

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*For correspondence (e-mail cmpark@snu.ac.kr).

SUMMARY

Underground roots normally reside in darkness. However, they are often exposed to ambient light that penetrates through cracks in the soil layers which can occur due to wind, heavy rain or temperature extremes. In response to light exposure, roots produce reactive oxygen species (ROS) which promote root growth. It is known that ROS-induced growth promotion facilitates rapid escape of the roots from non-natural light. Meanwhile, long-term exposure of the roots to light elicits a ROS burst, which causes oxidative damage to cellular components, necessitating that cellular levels of ROS should be tightly regulated in the roots. Here we demonstrate that the red/far-red light photoreceptor phytochrome B (phyB) stimulates the biosynthesis of abscisic acid (ABA) in the shoots, and notably the shoot-derived ABA signals induce a peroxidase-mediated ROS detoxification reaction in the roots. Accordingly, while ROS accumulate in the roots of the *phyb* mutant that exhibits reduced primary root growth in the light, such an accumulation of ROS did not occur in the dark-grown *phyb* roots that exhibited normal growth. These observations indicate that mobile shoot-to-root ABA signaling links shoot phyB-mediated light perception with root ROS homeostasis to help roots adapt to unfavorable light exposure. We propose that ABA-mediated shoot-to-root phyB signaling contributes to the synchronization of shoot and root growth for optimal propagation and performance in plants.

Keywords: phytochrome, ABA signal, shoot-to-root signaling, light signaling, ROS homeostasis, *Arabidopsis*.

INTRODUCTION

The root system is responsible for anchoring the plant body to the soil, mechanical support, absorption of water and nutrients, and storage of metabolites (Smith and De Smet, 2012). The roots also monitor changes in the surrounding environment, including water level, salinity and light (Sun *et al.*, 2008; Mo *et al.*, 2015). Light influences virtually all aspects of root growth and the developmental process. It has been reported that all photoreceptors are expressed in the roots and their specific function has been identified. For example, red/far-red photoreceptor phytochromes (phys) mediate primary root elongation, gravitropism and hormone responses. In particular, phyB senses stem-piped light to modulate root growth and tropic responses (Lee *et al.*, 2016a,b). In addition, UV-B light also triggers root photomorphogenic development (Mo *et al.*, 2015).

Moreover, shoot-to-root light signals via hormones also influence root growth and developmental processes, and phyA and phyB regulate lateral root production by mediating shoot-to-root auxin transport (Salisbury *et al.*, 2007). In *Lotus japonicus*, phyB regulates jasmonic acid (JA) signaling, which controls root nodulation (Suzuki *et al.*, 2011). In

addition, it has been shown that shoot-derived abscisic acid (ABA) promotes primary root growth in tomato and pea (McAdam *et al.*, 2016).

On the other hand, in most cases direct light illumination is unfavorable to roots. In nature, roots normally reside the dark; however, they are often exposed to ambient light transmitted through small cracks in the soil layers which occur due to drought, heavy rain, wind or high temperatures (Yokawa *et al.*, 2014, 2016). Light-exposed roots produce reactive oxygen species (ROS) to promote root growth, allowing rapid escape of the roots from non-natural light illumination (Yokawa *et al.*, 2014). Since prolonged exposure to light provokes an ROS burst that cause photooxidative damage to cellular components in the roots, the levels of ROS should be tightly controlled. However, it is largely unknown how plant roots control the levels of ROS under direct exposure to light.

Here, we demonstrate that phyB facilitates induction of ABA biosynthesis genes in the shoot, and notably that the shoot-derived ABA signals modulate ROS homeostasis through a peroxidase-mediated ROS detoxification reaction in the roots. In *phyb* mutants, the amount of ABA hormone was reduced and ROS accumulated to a high degree, resulting in reduction of primary root growth. Our findings indicate that ABA-mediated phyB signaling prevents the over-accumulation of ROS in the roots under unfavorable light conditions, contributing to the synchronization of shoot and root growth for optimal propagation and performance in plants.

RESULTS

The primary root growth of *phyb* seedlings is sensitive to light

The red and far-red light-sensing phytochrome photoreceptors are known to modulate production of ROS (Wei *et al.*, 2008; Chai *et al.*, 2015). They play a role in root development (Salisbury *et al.*, 2007; Costigan *et al.*, 2011). To investigate whether phy photoreceptors are associated with the ROS-mediated promotion of root growth, we examined the root growth phenotypes of *Arabidopsis* mutants that are defective in either phyA or phyB. While the primary root growth of *phya* mutant seedlings was similar to that of wild-type Col-0 seedlings it was significantly reduced in *phyb* mutant seedlings (Figure 1a). Anatomical analysis of the roots revealed that the reduced primary root growth of *phyb* seedlings is caused by a reduction in cell number in the meristematic zones (Figure 1b). A similar reduction of primary root growth was also observed in Landsberg *erecta* (L*er*) plants lacking phyB (Figure S1 in the online Supporting Information), supporting the functional linkage between phyB and primary root growth. To investigate the effects of direct light illumination on root growth, the root parts of the seedlings grown on vertical half-strength Murashige and Skoog agar (MS-agar) plates were sealed with aluminum foil before exposure to light. Notably, the reduction of primary root growth did not occur when the roots were kept in the dark (Figure 1c). On the other hand, lateral root formation was suppressed in *phyb* seedlings both in the light and the dark, indicating that the phyB-mediated light response is associated specifically with primary root growth.

The phyB photoreceptors exist in both shoots and roots, where they play distinct roles (Lee *et al.*, 2016b). To identify which phyB regulates primary root growth in the light, we conducted grafting experiments using Col-0 and *phyb* seedlings. It was found that the reduction of primary root growth was observed in chimeric seedlings having the *phyb* scion but not in those having the Col-0 scion (Figure 1d). These observations indicate that shoot-localized phyB is important for the light-mediated regulation of primary root growth.

Shoot phyB suppresses the accumulation of ROS in light-exposed roots

It is known that light-exposed roots produce low concentrations of ROS to promote primary root growth, which would help the roots rapidly escape from unfavorable light exposure (Yokawa *et al.*, 2014, 2016). Meanwhile, high concentrations of ROS impose photo-oxidative stress on cellular components, causing physiological impairments and growth arrest (Tsukagoshi, 2016). We therefore anticipated that the growth reduction of the *phyb* primary roots under light conditions would be caused by the accumulation of high levels of ROS.

To confirm that ROS accumulation provokes the reduction of primary root growth in *phyb* mutants, we employed a chromogenic dye, 3,3′-diaminobenzidine (DAB), for hydrogen peroxide and a fluorogenic reagent, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA), for the detection of ROS intermediates in the primary roots (Mubarakshina *et al.*, 2010; Tsukagoshi *et al.*, 2010). The staining assays revealed that ROS levels were similar in Col-0 and *phyb* roots when the roots were kept in the dark (Figure 2a,b). In contrast, when the roots were exposed to light, ROS levels were discernibly higher in the *phyb* roots than in Col-0 roots. Meanwhile, nitro blue tetrazolium (NBT) staining revealed that the levels of superoxide were similar in Col-0 and *phyb* roots regardless of light illumination (Figure S2). Together with the reduced primary root growth in *phyb* seedlings (Figure 1c), these observations support that shoot phyB-mediated light signals possibly suppress the accumulation of ROS in the roots under prolonged exposure to light.

The phyB-mediated induction of ABA biosynthesis in the shoots is associated with root growth

A critical question is how primary root growth is modulated by phyB signals from shoots. It is known that phyB

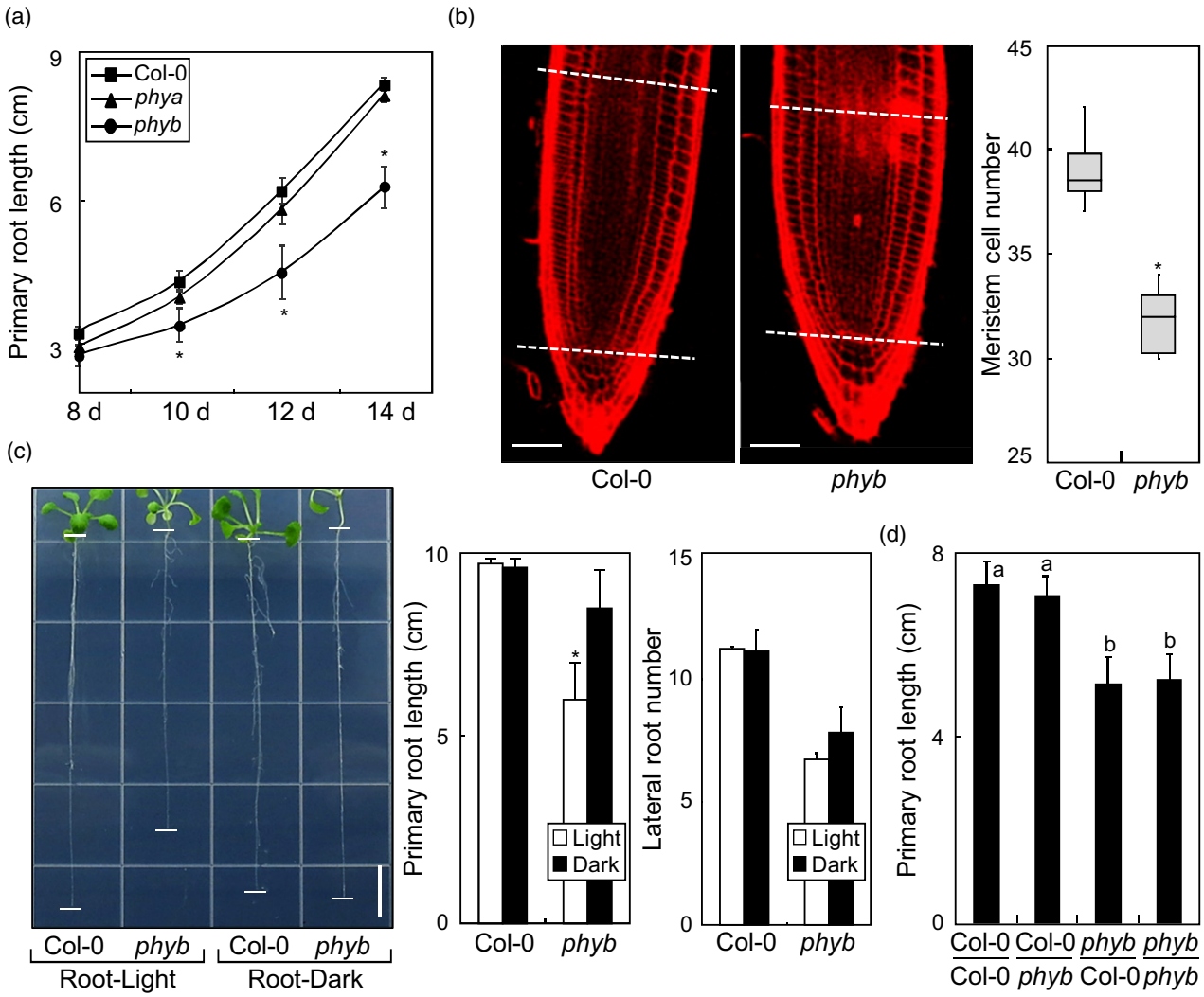

Figure 1. Primary root growth of *phyb* seedlings is sensitive to light.
Seedlings were grown on vertical MS-agar plates with the roots either exposed to light or kept in the dark. In (a), (c) and (d), 15 measurements were statistically analyzed using Student's *t*-test (*$P < 0.01$). Bars indicate standard error of the mean (SEM).
(a) Growth kinetics of *phya* and *phyb* primary roots (d, days after germination).
(b) Meristem cell numbers in *phyb* primary roots. The roots of 2-week-old seedlings were stained with propidium iodide. Dotted lines mark the boundaries of elongation zones. Scale bars = 50 μm. Seven counts were statistically analyzed (*t*-test, *$P < 0.01$). Bars indicate SEM.
(c) Light sensitivity of *phyb* roots. Primary root lengths and lateral root numbers of 2-week-old seedlings were measured. Scale bar = 1 cm.
(d) Primary root growth of grafted seedlings between Col-0 plants and the *phyb* mutant. The grafted seedlings were grown for 2 weeks in the light before measurements. Different letters represent a significant difference ($P < 0.01$) determined by one-way analysis of variance (ANOVA) with the post hoc Tukey test. Bars indicate standard deviation of the mean. [Colour figure can be viewed at wileyonlinelibrary.com]

plays a role in shoot-to-root transmission of diverse hormonal signals (Salisbury *et al.*, 2007; Suzuki *et al.*, 2011). Direct measurements of phytohormone content showed that the levels of ABA in the *phyb* shoots were more than 50% lower than those in Col-0 shoots (Figure 3a). In contrast, ABA levels were similar in the roots of *phyb* and Col-0 seedlings. In addition, feeding with ABA in a physiological concentration range (0.1–1 μM) efficiently restored the reduced primary root growth (Figures 3b and S3). These observations indicate that a shoot-derived ABA signaling mediator rather than ABA itself modulates the control of primary root growth by shoot phyB. Notably, high concentrations of ABA (>10 μM) inhibited primary root growth (Ghassemian *et al.*, 2000) (Figure S3), showing that ABA plays dose-dependent, contrasting roles in regulating primary root growth.

The levels of salicylic acid (SA) were also about 50% lower in the *phyb* shoots (Figure S4). However, SA feeding did not recover the reduced primary root growth of *phyb* seedlings (Figure S5), indicating that SA is not related to shoot phyB function in modulating primary root growth.

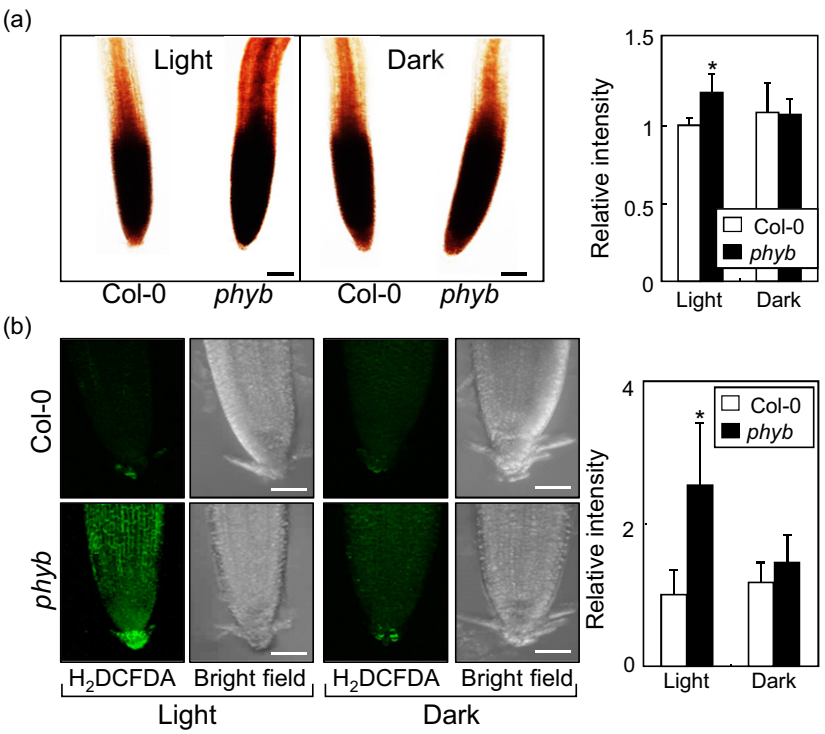

Figure 2. Levels of reactive oxygen species (ROS) are elevated in the light-exposed primary roots of *phyb* seedlings.
(a) The roots of 2-week-old seedlings grown on vertical MS-agar plates with the roots either exposed to light or kept in the dark were subjected to staining with 3,3′-diaminobenzidine for H_2O_2. Scale bars = 100 μm. Relative intensities were quantified using ImageJ software. Seven quantifications were statistically analyzed using Student's *t*-test (*$P < 0.01$). Bars indicate SEM.
(b) Root samples were subjected to confocal 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) staining for ROS. Seven quantifications were statistically analyzed as described above. Scale bars = 50 μm. [Colour figure can be viewed at wileyonlinelibrary.com]

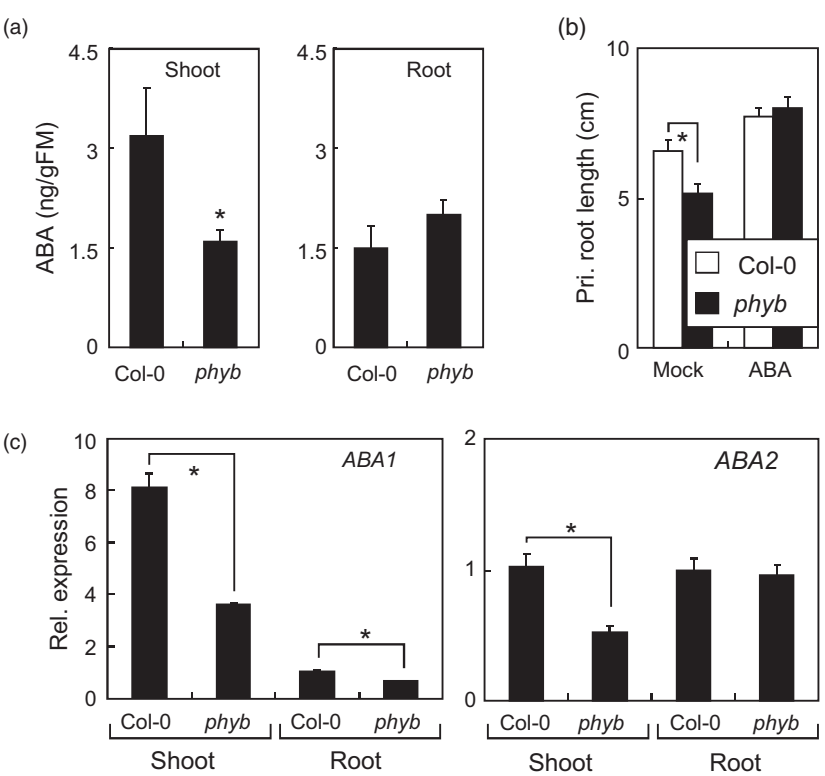

Figure 3. The phyB-mediated induction of ABA biosynthesis in shoots is associated with root growth.
Seedlings were grown on vertical MS-agar plates for 2 weeks in the light. Measurements were statistically analyzed using Student's *t*-test (*$P < 0.01$). Bars indicate SEM.
(a) Endogenous ABA contents. Shoot and root samples were used for the measurements ($n = 5$).
(b) Effects of ABA feeding on primary root growth. Seedlings were grown in the presence of 1 μM ABA. Fifteen measurements were statistically analyzed.
(c) Relative levels of *ABA1* and *ABA2* transcripts. Total RNA was prepared from shoot and root samples. Transcript levels were analyzed by RT-qPCR. Biological triplicates were statistically analyzed.

Consistent with the ABA feeding data, reverse transcription-mediated real-time PCR (RT-qPCR) assays revealed that the transcription of ABA biosynthetic genes, such as ABA DEFICIENT 1 (*ABA1*), *ABA2* and *ABA3*, was reduced by about 60% in *phyb* shoots (Figures 3c and S6). In contrast, the transcript levels were not discernibly different in

the roots, further supporting the notion that phyB signals promote ABA biosynthesis in the shoots. In conjunction with our data on ABA measurements and feeding assays, these observations strongly support that shoot phyB signals induce ABA biosynthesis and perhaps its signaling mediator in the shoots, promoting primary root growth when the roots are illuminated.

Shoot phyB signals mediate root ROS homeostasis via ABA signaling

We next asked whether shoot ABA signals are associated with the accumulation of ROS in the roots. Histochemical staining assays with DAB and H_2DCFDA revealed that treatment of *phyb* roots with 1 μM ABA attenuates the elevated content of hydrogen peroxide to a level comparable to that in Col-0 roots (Figure 4a,b), showing that low concentrations of ABA suppress the accumulation of ROS in the roots. This is in contrast to the stimulation of ROS accumulation by high concentrations of ABA (Kwak *et al.*, 2003; Böhmer and Schroeder, 2011).

Notably, a reduction of primary root growth was also observed in the ABA-defective mutant *aba1*-6 in the light (Barrero *et al.*, 2005) and the root phenotype was efficiently rescued by exogenous application of 1 μM ABA (Figure 5a), similar to what was observed with the *phyb* mutant. Likewise, ROS levels were higher in the *aba1-6* roots than in Col-0 roots, but their levels were decreased to a level comparable to that in Col-0 roots in the presence of 1 μM ABA (Figure 5b,c). These observations further support the functional linkage between shoot phyB-mediated ABA accumulation and ROS metabolism in modulating primary root growth.

To investigate how shoot ABA signals detoxify ROS accumulation in the roots, we examined the potential roles of ABA signaling mediators that modulate ROS detoxification in this signaling pathway. It is known that ABA promotes the expression of the ABA INSENSITIVE 5 (*ABI5*) gene encoding a basic leucine zipper transcription factor that directly activates PEROXIDASE1 (*PER1*) (Lee *et al.*, 2015; Zhou *et al.*, 2015), which encodes a hydrogen peroxidase that detoxifies ROS (Lee *et al.*, 2015). The RT-qPCR assays showed that the transcript levels of *ABI5* and *PER1* were lower in the *phyb* roots with a more prominent reduction of *PER1* transcripts compared with those in Col-0 roots (Figure 6a). However, the transcript levels of *ABI5* and *PER1* were similar in Col-0 and *phyb* roots when grown in the presence of 1 μM ABA. It is known that ABA signals stabilize ABI5 protein (Lopez-Molina *et al.*, 2001; Seo *et al.*, 2014). Immunoblot assays revealed that the levels of ABI5 protein are significantly lower in *phyb* roots than in Col-0 roots but in the presence of 1 μM ABA they are greatly elevated to a level comparable to those in Col-0 roots (Figures 6b and S7). These observations indicate that ABA signals activate ABI5 at both the transcriptional and protein levels in the roots. Furthermore, the reduction of *ABI5* and *PER1* transcription and ABI5 abundance is consistent with the

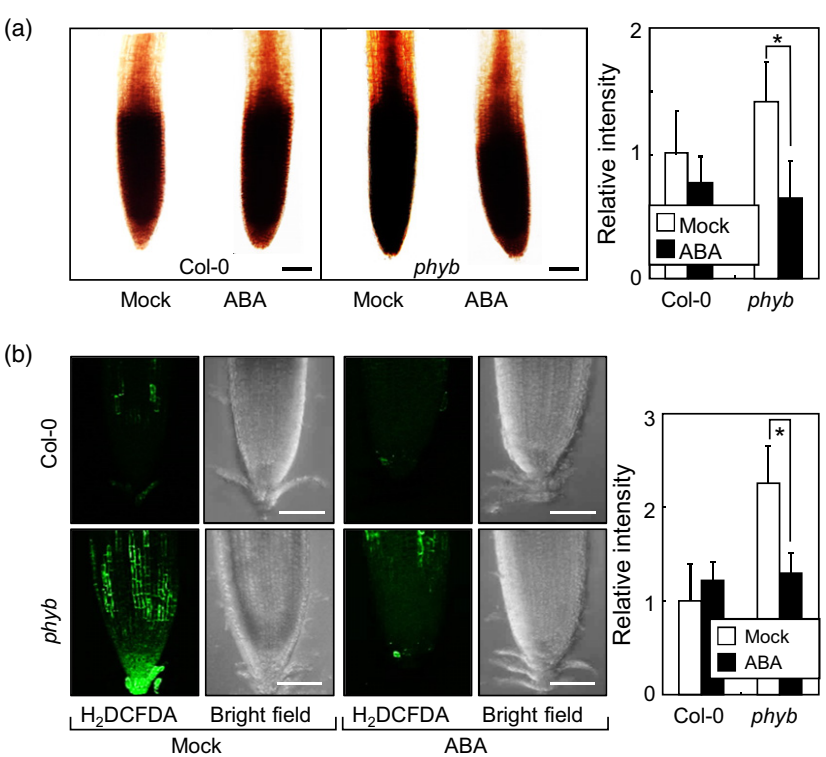

Figure 4. Abscisic acid (ABA) is associated with over-accumulation of root reactive oxygen species (ROS) in *phyb* seedlings.
Seedlings were grown on vertical MS-agar plates for 2 weeks in the light. Measurements were statistically analyzed using Student's *t*-test (*$P < 0.01$). Bars indicate SEM.
(a), (b) Detection of ROS by 3,3′-diaminobenzidine staining (a) and 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) staining (b) in *phyb* primary roots. Seedlings were fed with 1 μM ABA. Seven quantifications were statistically analyzed. Scale bars = 100 μm (a) and 50 μm (b). [Colour figure can be viewed at wileyonlinelibrary.com]

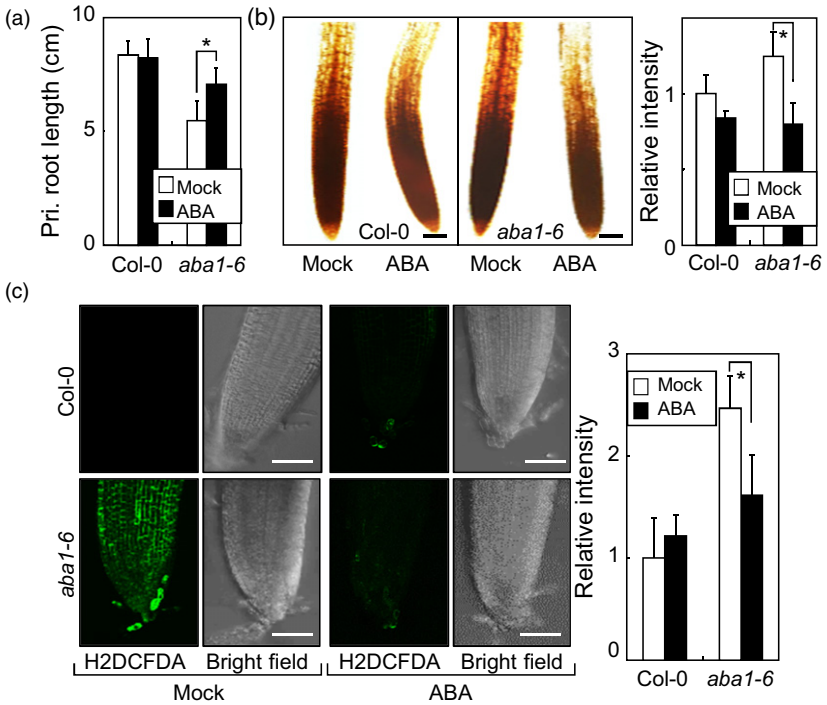

Figure 5. The primary root phenotype of *aba1-6* seedlings is sensitive to ABA.
Seedlings were grown on vertical MS-agar plates for 2 weeks in the light. Measurements were statistically analyzed using Student's *t*-test (**P* < 0.01). Bars indicate SEM.
(a) Primary root length of *aba1-6* mutant. Primary root growth of *aba1-6* seedlings. Seedlings were grown in the presence of 1 μM ABA. Fifteen measurements were statistically analyzed.
(b), (c) Detection of reactive oxygen species by 3,3′-diaminobenzidine staining (b) and 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) staining (c) in *aba1-6* primary roots. Chemical staining and quantification were performed as described above. Scale bars = 100 μm (b) and 50 μm (c). [Colour figure can be viewed at wileyonlinelibrary.com]

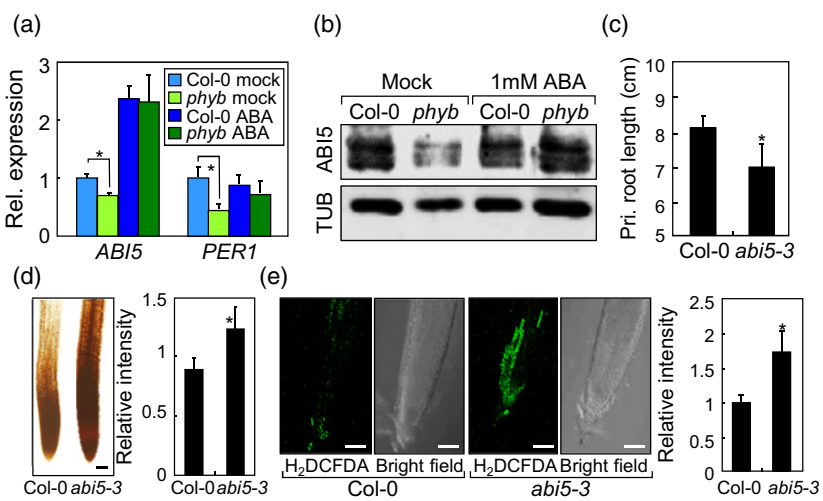

Figure 6. Shoot phyB signals mediate homeostasis of reactive oxygen species in roots via ABA signaling.
(a) Relative levels of *ABI5* and *PER1* transcripts in *phyb* primary roots. Seedlings were grown in the light for 2 weeks in the presence of 1 μM ABA. Total RNA was prepared from root samples, and biological triplicates of RT-qPCR runs were statistically analyzed (*t*-test, **P* < 0.01). Bars indicate SEM.
(b) Accumulation of ABI5 protein in *phyb* roots. Protein extracts were prepared from the roots of 2-week-old seedlings. ABI5 protein was immunologically detected using an anti-ABI5 antibody. Tubulin (TUB) protein was also immunodetected for loading control.
(c) Primary root growth of *abi5-3* seedlings. The primary roots of 2-week-old seedlings were measured and statistically analyzed (*n* = 15, *t*-test, **P* < 0.01).
(d) 3,3′-Diaminobenzidine staining and (e) 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) staining of light-grown primary roots. Scale bars = 100 μm (d) and 50 μm (e). Seven quantifications were statistically analyzed (*t*-test, **P* < 0.01). Bars indicate SEM. [Colour figure can be viewed at wileyonlinelibrary.com]

elevation of ROS in the *phyb* roots. Accordingly, the ABI5-deficient *abi5-3* mutant and PER1-deficient *per1-2* mutant exhibited reduced primary root growth in the light (Figures 6c and S8). In addition, DAB staining and H_2DCFDA fluorescence imaging revealed that ROS levels are higher in the mutant roots than in Col-0 roots (Figure 6d,e), further supporting the notion that ABA signals reduce the accumulation of ROS in the light-exposed roots.

Altogether, our findings describe a phyB-mediated light signal transduction pathway that involves shoot-to-root ABA signaling. The shoot-derived ABA signals transcriptionally and post-transcriptionally elevate the abundance

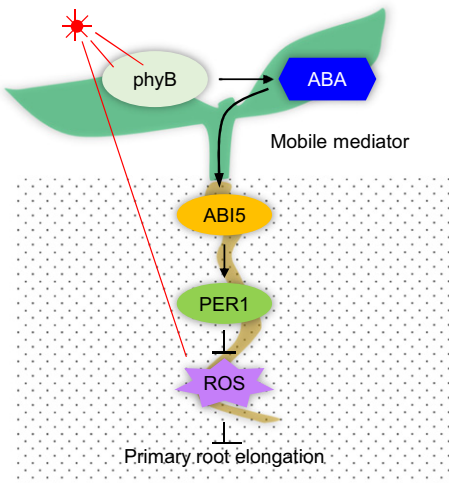

Figure 7. Schematic model for shoot phyB function in modulating homeostasis of reactive oxygen species (ROS) in roots.
Shoot phyB signals induce the biosynthesis of ABA, which triggers downstream signaling to the roots. The shoot-derived ABA signals activate ABI5, leading to the induction of the *PER1* gene for ROS detoxification. [Colour figure can be viewed at wileyonlinelibrary.com]

of ABI5 to activate the *PER1* gene, leading to ROS detoxification in the roots (Figure 7).

DISCUSSION

It has been reported that phyB-mediated light signals inhibit ABA biosynthesis (Kim *et al.*, 2008). The phyB-mediated inhibition of ABA biosynthesis is modulated by SOMNUS, a CCCH-type zinc finger protein that is expressed specifically in seeds (Kim *et al.*, 2008). Our data demonstrated that phyB from shoots induces the expression of ABA biosynthetic genes in the shoots of growing seedlings. Previous data and our own data support the occurrence of phyB-mediated regulation of ABA biosynthesis through multiple signaling pathways, depending on growth and developmental stages and varying environmental conditions.

Recently, it has been suggested that low concentrations of ABA stimulate primary root growth via interactions with auxin signaling (Li *et al.*, 2017). It has been observed that auxin transport inhibitors block the effects of ABA on primary root growth and auxin transport mutants, which exhibit reduced primary root growth, are insensitive to ABA (Li *et al.*, 2017). It has also shown that phyB-mediated light signals affect auxin transport (Salisbury *et al.*, 2007). However, we found that ABA feeding restored the reduced primary root growth phenotype of the *phyb* mutant, indicating that ABA also affects primary root growth through an auxin-independent process. It is likely that ABA modulates primary root growth through at least two distinct routes: one through signaling interactions with auxin and the other through a phyB-mediated ROS detoxification process.

We found that ABA synthesized in the shoots is not directly transported to the roots to trigger ROS detoxification. Instead, an as-yet unidentified ABA signaling mediator produced in the shoots is transported to the roots. Meanwhile, it is known that ABA is transported from the shoots to the roots under stress conditions (Ikegami *et al.*, 2009). It is notable that low concentrations of ABA (<1 μM) are required for the activation of the ABI5–PER1 pathway for the induction ROS detoxification in the roots. Therefore it is anticipated that under conditions when a low concentration of ABA is effective, ABA would be synthesized in those plant tissues that respond to environmental stimuli. However, when a large amount of ABA is required for its responses, such as under drought stress conditions, the plants tissues that respond to environmental stimuli would need more ABA transported from other tissues.

While our data strongly support that an ABA signaling mediator transmits light signals to the roots, it is still possible that ABA itself acts, at least in part, as the shoot-to-root mobile signal. We propose that phyB-mediated light signaling modulates root ROS homeostasis to sustain root growth, thus providing an adaptive strategy that coordinates the growth of shoots and roots to achieve optimal growth and performance in nature.

EXPERIMENTAL PROCEDURES

Plant materials and growth conditions

All *Arabidopsis thaliana* lines used in this work were in the Columbia (Col-0) background except for the L*er phyb-5* mutant. The *phyb-9*, *phyb-5*, *aba1-6*, *per1-2* and *abi5-3* mutants have been described previously (Barrero *et al.*, 2005; Lee *et al.*, 2015, 2016b; Jeong *et al.*, 2016).

Sterilized *Arabidopsis* seeds were cold-imbibed at 4°C for 3 days and germinated in a controlled growth chamber at 22°C with relative humidity of 60%. Plants were grown on MS-agar) plates under long-day conditions (LDs; 16-h light/8-h dark) with white-light illumination (120 μmol m^{-2} sec^{-1}) provided by FLR40D/A fluorescent tubes (Osram, https://www.osram.com/). Seedlings grown for 3 days on horizontal MS-agar plates were transferred to vertical MS-agar (0.75%) plates and grown for an additional 10 days.

Analysis of gene transcript levels

Gene transcript levels were analyzed by RT-qPCR. The RT-qPCR reactions were performed according to the experimental guidelines that have been previously published (Udvardi *et al.*, 2008). Total RNA samples were extracted from appropriate plant materials homogenized in liquid nitrogen with the TRIzol reagent (Invitrogen, http://www.invitrogen.com/). The suspension was centrifuged at 15 000 ***g*** for 8 min at 4°C. The supernatant was mixed with 200 μl of chloroform and the mixture was centrifuged under the same conditions. The aqueous part of the mixture was transferred to a fresh microcentrifuge tube and mixed with 200 μl of isopropanol and 200 μl of high-salt solution containing 0.8 M trisodium citrate and 1.2 M sodium chloride. The RNA pellet was collected by centrifugation at 15 000 ***g*** for 8 min at 4°C following incubation for 10 min at 25°C. The RNA pellet was rinsed with

80% ethanol and dissolved in triple-distilled water. The RNA sample was pre-treated with RNase-free DNase to eliminate contaminating genomic DNA.

The RT-qPCR reactions were conducted in 384-well plates with the Applied Biosystems QuantStudio 6 Flex Real-Time PCR System (ThermoFisher Scientific, https://www.thermofisher.com) using 10 μl of SYBR Green I Master Mix. The entire PCR process has been described previously (Lee *et al.*, 2016b). Primers used are listed in Table S1. An *eIF4A* gene (*At3 g13920*) was included as an internal control in the PCR reactions.

The PCR reactions were conducted in biological triplicates using total RNA samples prepared from three independent plant materials grown under the same conditions. The comparative $\Delta\Delta C_T$ method was employed to measure the levels of gene transcripts. The threshold cycle (C_T) value was calculated for each reaction by a process using default parameters.

Propidium iodide staining

Arabidopsis roots grown for 2 weeks were used for measurements. Roots were incubated in 10 mg ml^{-1} of propidium iodide solution (Sigma-Aldrich, http://www.sigmaaldrich.com/) for 5 min, and washed with water. Washed roots were mounted under a coverslip and detected with a confocal microscope (LSM710, Carl Zeiss, https://www.zeiss.com/).

Detection of ROS

DAB staining solution (1 mg ml^{-1}) and H_2DCFDA fluorescent staining solution (10 μM) were used for analyzing hydrogen peroxide and NBT staining solution (1 tablet per 50 ml) was used for superoxide detection. For DAB and NBT staining, roots grown for 2 weeks on MS-agar plates in the presence or absence of direct root illumination were incubated in each staining solution for 2 h at room temperature in complete darkness. The plant samples were destained by water and visualized with an optical microscope (Olympus, https://www.olympus-lifescience.com/). The ImageJ system (http://rsb.info.nih.gov/ij/) was used for intensity quantification.

For H_2DCFDA staining, roots grown for 2 weeks on MS-agar plates under LDs at 22°C in the presence or absence of direct root illumination were incubated in 10 μM of H_2DCFDA staining solution for 20 min at room temperature. The roots were washed with water and used for fluorescent imaging measurements by confocal microscopy. The fluorescence intensity was quantified using ImageJ software.

Micrografting

Seedlings were grown vertically on MS-agar plates for 4 days under short days (8-h light/16-h dark) at 22°C prior to grafting experiments. Grafting was conducted as described previously (Marsch-Martínez *et al.*, 2013; Lee *et al.*, 2016b). Grafted plants were grown vertically on MS-agar plates with 0.5% sucrose for an additional 2 weeks at 22°C under LDs.

Immunoblot assay

The roots of 2-week-old plants were harvested for the assays. Plant materials homogenized in liquid nitrogen were mixed with protein extraction buffer. The mixtures were boiled for 10 min before centrifugation at 15 000 ***g*** for 15 min. The supernatants were loaded onto 10% SDS-PAGE gel before transfer to a polyvinylidene difluoride membrane. An anti-ABI5 antibody (ab98831; Abcam, http://www.abcam.com/) was used for the immunological detection of ABI5 protein.

Hormone quantification

Seedlings were grown vertically on MS-agar plates for 10 days under LDs in the presence of root-light. At least 50 mg of shoot and root samples were harvested for the extraction of hormones. Hormone quantification was performed as reported previously (Schäfer *et al.*, 2016). Briefly, 50 mg of plant materials homogenized in liquid nitrogen was aliquoted into 96-well biotubes (Arctic White LLC, http://arcticwhiteusa.com/) and closed with strips of eight-plug caps (Arctic White LLC). For extraction, 800 μl of pre-cooled (−20°C) acidified methoanol [MeOH:H_2O:HCOOH 15:4:1 (v:v:v); MeOH = methanol] was added to each sample containing the internal standards (for each sample 10 ng D4-ABA, 10 ng D6-JA, 10 ng D6-JAIle, 10 ng D6-SA and 1.5 ng D5-IAA). The tubes were sealed with a Seal Mate and incubated overnight at −20°C. After incubation the samples were homogenized, centrifuged and 600 ml of the supernatant was transferred to a new vial. Analysis was performed on a Bruker Elite EvoQ Triple Quad-MS (https://www.bruker.com/) equipped with a heated electrospray ionization ion source. Samples were analyzed in multi-reaction-monitoring mode. Post-run analysis was done with the 'MS data Review' software of the 'Bruker MS Workstation' (version: 8.1.2).

Growth hormone treatment

Seeds were germinated and grown on horizontal MS-agar plates for 3 days under LDs to synchronize germination. Three-day-old seedlings were transferred to vertical MS-agar plates containing various concentrations of ABA and SA and further grown for 10 more days before measurement of primary root lengths.

ACCESSION NUMBERS

The accession numbers of genes involved in this study are ABA1 (AT5G67030), ABA2 (AT1G52340), ABA3 (AT1G16540), ABI5 (AT2G36270), NCED9 (AT1G78390), PER1 (AT1G05240), PHYB (AT2G18790).

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AUTHOR CONTRIBUTIONS

C-MP conceived and designed the experiments. C-MP prepared the manuscript with the contribution of J-HH. J-HH, J-HK and H-JL performed molecular and biochemical assays. S-GK and H-JS provided scientific discussion on light responses of the roots. GL, RH and ITB measured hormone contents and associated phenotypic assays. J-IK provided *phyb-5* plants and scientific discussion on biochemical activity of phyB.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Primary root growth of *phyb*-5 seedlings in the light.

Figure S2. Nitro blue tetrazolium staining of *phyb* primary roots.

Figure S3. Effects of different concentrations of ABA on primary root growth.

Figure S4. Quantification of endogenous growth hormones in *phyb* seedlings.

Figure S5. Effects of salicylic acid on primary root growth.

Figure S6. Transcript levels of ABA biosynthesis genes in *phyb* seedlings.

Figure S7. Evaluation of the anti-ABI5 antibody used.

Figure S8. Primary root length of *per1-2* mutant.

Table S1. Primers used in this work.

REFERENCES

Albertos, P., Romero-Puertas, M.C., Tatematsu, K., Mateos, I., Sánchez-Vicente, I., Nambara, E. and Lorenzo, O. (2015) S-nitrosylation triggers ABI5 degradation to promote seed germination and seedling growth. *Nat. Commun.* **6**, 8669.

Barrero, J.M., Piqueras, P., González-Guzmán, M., Serrano, R., Rodríguez, P.L., Ponce, M.R. and Micol, J.L. (2005) A mutational analysis of the *ABA1* gene of *Arabidopsis thaliana* highlights the involvement of ABA in vegetative development. *J. Exp. Bot.* **56**, 2071–2083.

Böhmer, M. and Schroeder, J. (2011) Quantitative transcriptomic analysis of abscisic acid-induced and reactive oxygen species-dependent expression changes and proteomic profiling in *Arabidopsis* suspension cells. *Plant J.* **67**, 105–118.

Chai, T., Zhou, J., Liu, J. and Xing, D. (2015) LSD1 and HY5 antagonistically regulate red light induced-programmed cell death in *Arabidopsis*. *Front. Plant Sci.* **6**, 292.

Costigan, S.E., Warnasooriya, S.N., Humphries, B.A. and Montgomery, B.L. (2011) Root-localized phytochrome chromophore synthesis is required for photoregulation of root elongation and impacts root sensitivity to jasmonic acid in *Arabidopsis*. *Plant Physiol.* **157**, 1138–1150.

Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y. and McCourt, P. (2000) Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis*. *Plant Cell*, **12**, 1117–1126.

Ikegami, K., Okamoto, M., Seo, M. and Koshiba, T. (2009) Activation of abscisic acid biosynthesis in the leaves of *Arabidopsis thaliana* in response to water deficit. *J. Plant. Res.* **122**, 235–243.

Jeong, A.R., Lee, S.S., Han, Y.J. *et al.* (2016) New constitutively active phytochromes exhibit light-independent signaling activity. *Plant Physiol.* **171**, 2826–2840.

Kim, D.H., Yamaguchi, S., Lim, S., Oh, E., Park, J., Hanada, A., Kamiya, Y. and Choi, G. (2008) SOMNUS, a CCCH-Type zinc finger protein in *Arabidopsis*, negatively regulates light-dependent seed germination downstream of PIL5. *Plant Cell*, **20**, 1260–1277.

Kwak, J.M., Mori, I.C., Pei, Z.M., Leonhardt, N., Torres, M.A., Dangl, J.L., Bloom, R.E., Bodde, S., Jones, J.D. and Schroeder, J.I. (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. *EMBO J.* **22**, 2623–2633.

Lee, S., Lee, H.J., Jung, J.H. and Park, C.M. (2015) The *Arabidopsis thaliana* RNA-binding protein FCA regulates thermotolerance by modulating the detoxification of reactive oxygen species. *New Phytol.* **205**, 555–569.

Lee, H.J., Ha, J.H. and Park, C.M. (2016a) Underground roots monitor aboveground environment by sensing stem-piped light. *Commun. Integr. Bio.* **9**, e1261769.

Lee, H.J., Ha, J.H., Kim, S.G. *et al.* (2016b) Stem-piped light activates phytochrome B to trigger light responses in *Arabidopsis thaliana* roots. *Sci. Signal.* **9**, ra106.

Li, X., Chen, L., Forde, B.G. and Davies, W.J. (2017) The biphasic root growth response to abscisic acid in *Arabidopsis* involves interaction with ethylene and auxin signalling pathways. *Front. Plant Sci.* **8**, 1493.

Lopez-Molina, L., Mongrand, S. and Chua, N.H. (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proc. Natl Acad. Sci. USA*, **98**, 4782–4787.

Marsch-Martínez, N., Franken, J., Gonzalez-Aguilera, K.L., de Folter, S., Angenent, G. and Alvarez-Buylla, E.R. (2013) An efficient flat-surface collar-free grafting method for *Arabidopsis thaliana* seedlings. *Plant Methods*, **9**, 14.

McAdam, S.A., Brodribb, T.J. and Ross, J.J. (2016) Shoot-derived abscisic acid promotes root growth. *Plant Cell Environ.* **39**, 652–659.

Mo, M., Yokawa, K., Wan, Y. and Baluška, F. (2015) How and why do root apices sense light under the soil surface? *Front. Plant. Sci.* **6**, 775.

Mubarakshina, M.M., Ivanov, B.N., Naydov, I.A., Hillier, W., Badger, M.R. and Krieger-Liszkay, A. (2010) Production and diffusion of chloroplastic H_2O_2 and its implication to signalling. *J. Exp. Bot.* **61**, 3577–3587.

Salisbury, F.J., Hall, A., Grierson, C.S. and Halliday, K.J. (2007) Phytochrome coordinates *Arabidopsis* shoot and root development. *Plant J.* **50**, 429–438.

Schäfer, M., Brütting, C., Baldwin, I.T. and Kallenbach, M. (2016) High-throughput quantification of more than 100 primary- and secondary-metabolites, and phytohormones by a single solid-phase extraction based sample preparation with analysis by UHPLC-HESI-MS/MS. *Plant Methods*, **12**, 30.

Seo, K.I., Lee, J.H., Nezames, C.D., Zhong, S., Song, E., Byun, M.O. and Deng, X.W. (2014) ABD1 is an *Arabidopsis* DCAF substrate receptor for CUL4-DDB1-based E3 ligases that acts as a negative regulator of abscisic acid signaling. *Plant Cell*, **26**, 695–711.

Smith, S. and De Smet, I. (2012) Root system architecture: insights from *Arabidopsis* and cereal crops. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **367**, 1441–1452.

Sun, F., Zhang, W., Hu, H., Li, B., Wang, Y., Zhao, Y., Li, K., Liu, M. and Li, X. (2008) Salt modulates gravity signaling pathway to regulate growth direction of primary roots in *Arabidopsis thaliana*. *Plant Physiol.* **146**, 178–188.

Suzuki, A., Suriyagoda, L., Shigeyama, T. *et al.* (2011) *Lotus japonicus* nodulation is photomorphogenetically controlled by sensing the red/far red (R/FR) ratio through jasmonic acid (JA) signaling. *Proc. Natl Acad. Sci. USA*, **108**, 16837–16842.

Tsukagoshi, H. (2016) Control of root growth and development by reactive oxygen species. *Curr. Opin. Plant Biol.* **29**, 57–63.

Tsukagoshi, H., Busch, W. and Benfey, P.N. (2010) Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell*, **143**, 606–616.

Udvardi, M.K., Czechowski, T. and Scheible, W.R. (2008) Eleven golden rules of quantitative RT-PCR. *Plant Cell*, **20**, 1736–1737.

Wei, Q., Zhou, W., Hu, G., Wei, J., Yang, H. and Huang, J.Y. (2008) Heterotrimeric G-protein is involved in phytochrome A-mediated cell death of *Arabidopsis* hypocotyls. *Cell Res.* **18**, 949–960.

Yokawa, K., Fasano, R., Kagenishi, T. and Baluška, F. (2014) Light as stress factor to plant roots - case of root halotropism. *Front. Plant Sci.* **5**, 718.

Yokawa, K., Kagenishi, T. and Baluška, F. (2016) UV-B induced generation of reactive oxygen species promotes formation of BFA-induced compartments in cells of *Arabidopsis* root apices. *Front. Plant Sci.* **13**, 1162.

Zhou, X., Hao, H., Zhang, Y. *et al.* (2015) SOS2-LIKE PROTEIN KINASE5, an SNF1-RELATED PROTEIN KINASE3-type protein kinase, is important for abscisic acid responses in *Arabidopsis* through phosphorylation of ABSCISIC ACID-INSENSITIVE5. *Plant Physiol.* **168**, 659–676.