Auxin Overproduction in Shoots Cannot Rescue Auxin Deficiencies in Arabidopsis Roots

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Auxin plays an essential role in root development. It has been a long-held dogma that auxin required for root development is mainly transported from shoots into roots by polarly localized auxin transporters. However, it is known that auxin is also synthesized in roots. Here we demonstrate that a group of *YUCCA* (*YUC*) genes, which encode the rate-limiting enzymes for auxin biosynthesis, plays an essential role in Arabidopsis root development. Five *YUC* genes (*YUC3*, *YUC5*, *YUC7*, *YUC8* and *YUC9*) display distinct expression patterns during root development. Simultaneous inactivation of the five *YUC* genes (*yucQ* mutants) leads to the development of very short and agravitropic primary roots. The *yucQ* phenotypes are rescued by either adding 5 nM of the natural auxin, IAA, in the growth media or by expressing a *YUC* gene in the roots of *yucQ*. Interestingly, overexpression of a *YUC* gene in shoots in *yucQ* causes the characteristic auxin overproduction phenotypes in shoots; however, the root defects of *yucQ* are not rescued. Our data demonstrate that localized auxin biosynthesis in roots is required for normal root development and that auxin transported from shoots is not sufficient for supporting root elongation and root gravitropic responses.

Keywords: Auxin • Biosynthesis • Hormone • Root development • Transport.

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; GFP, green fluorescent protein; GUS, β-glucuronidase; IPA, indole-3-pyruvate; NPA, 1-*N*-naphthylphthalamic acid; TAA, TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS; TAR, TRYPTOPHAN AMINOTRANSFERASE RELATED; YUC, YUCCA flavin monooxygenase.

Introduction

Auxin is known to regulate almost every aspect of root development. In Arabidopsis, auxin has been shown to play a key role in primary root elongation, lateral root initiation, root hair development and root gravitropism (Benjamins and Scheres 2008, Petricka et al. 2012). Arabidopsis seedlings grown on auxin-containing media develop shorter primary roots, more lateral roots and more and longer root hairs than plants grown on media without exogenous auxin (Hobbie and Estelle 1994).

The leading hypothesis regarding how auxin regulates root development is that the formation of an auxin gradient is a key step for several processes of root development (Petricka et al. 2012). An auxin gradient is often visualized using the auxin reporters DR5–green fluorescent protein (GFP)/β-glucosidase (GUS) or DII-VENUS (Sabatini et al. 1999, Brunoud et al. 2012). During embryogenesis, a maximum of *DR5-GFP* activity is observed in the uppermost suspensor cells (Friml et al. 2003). The auxin maximum as shown by DR5–GFP is proposed to play an essential role for the specification of the hypophysis, which is the precursor for the root meristem. During seedling stages, DR5–GFP is highly expressed in the quiescent center, the adjacent columella cells and root cap. It is suggested that the observed auxin gradient instructs the patterning of the distal part of the roots (Sabatini et al. 1999). Lateral root initiation is also known to depend on the formation of an auxin maximum centered at the founder cells (Benkova et al. 2003). It has long been recognized that polar auxin transport, particularly the auxin transport mediated by PIN (PINFORMED) proteins, plays an essential role in creating and maintaining an auxin gradient (Wisniewska et al. 2006). Mathematic modeling based on the polarity of PIN proteins suggests that polar auxin transport is sufficient for generating an auxin maximum/gradient that guides Arabidopsis root growth (Grieneisen et al. 2007). It is generally believed that auxin required for root development is first synthesized in shoots and then transported into roots, mainly through the action of directional transporters such as the PINs (Petricka et al. 2012). However, recent studies have clearly demonstrated that auxin is also synthesized locally in roots (Ljung et al. 2005, Stepanova et al. 2008). It is important to determine whether root-produced auxin actually plays any physiological roles. Interestingly, localized auxin biosynthesis has been recognized to play essential roles in other developmental processes

including embryogenesis, seedling growth, vascular patterning, phyllotaxis and flower development (Cheng et al. 2006, Cheng et al. 2007, Pinon et al. 2013).

In Arabidopsis, auxin is mainly synthesized from the TAA (TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS)/ YUCCA (YUC) pathway, in which tryptophan is first converted to indole-3-pyruvate (IPA) by the TAA family of aminotransferases (Zhao 2012). Subsequently, IAA is synthesized from IPA by the YUC family of flavin-containing monooxygenases (Zhao 2012). The founding member of the TAA family of aminotransferases was identified from three different genetic screens for mutants with: (i) altered shade avoidance responses; (ii) insensitivity to the ethylene biosynthetic precursor 1-aminocyclopropane-1-carboxylic acid (ACC); and (iii) resistance to the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) (Stepanova et al. 2008, Tao et al. 2008, Yamada et al. 2009). The latter two screens were based on root phenotypes. The taa1 mutants have longer primary roots than wild-type plants when grown on media containing ACC or NPA. Inactivation of TAA1 and its close homolog TRYPTOPHAN AMINOTRANSFERASE RELATED2 (TAR2) leads to short and agravitropic roots. Both TAA1 and TAR2 are expressed in roots and shoots, and the taa1 tar2 double mutants display pleiotropic phenotypes in both roots and shoots (Stepanova et al. 2008, Tao et al. 2008, Yamada et al. 2009). Therefore, it is difficult to determine whether the root defects in taa1 tar2 are caused by deficiency of local auxin biosynthesis in roots or decreased auxin transport from the shoots to roots, or both. The YUC genes were identified in an activation-tagging screen for mutants with long hypocotyls, and the YUCs have been suggested as a rate-limiting enzyme for auxin biosynthesis (Zhao et al. 2001). There are 11 YUC genes in Arabidopsis, and the expression of each YUC gene is temporally and spatially regulated (Cheng et al. 2006, Cheng et al. 2007). So far, YUC genes have been shown to be essential for embryogenesis, seedling growth, vascular pattern formation and flower development. The physiological roles of YUC genes correlate well with their expression patterns. For example, YUC1 and YUC4 have overlapping expression patterns during flower development. Consequently, the yuc1 yuc4 double mutants have dramatic defects in flower development, whereas the single mutants do not have obvious defects (Cheng et al. 2006, Cheng et al. 2007).

Herein, we identified five YUC genes (YUC3, YUC5, YUC7, YUC8 and YUC9) that had detectable expression in Arabidopsis roots at seedling stages. Simultaneous inactivation of the five YUC genes (yucQ mutants) caused auxin deficiency in roots and led to the development of short primary roots and abnormal gravitropic responses. The root defects of yucQ were rescued either by adding auxin to growth media or by expressing a YUC gene in roots. However, overproduction of auxin in shoots did not rescue yucQ. We conclude that localized auxin biosynthesis in roots plays an essential role in root development and that the shoot-produced auxin is not sufficient to support normal root development.

Results

YUC genes are expressed in root cells

We analyzed the microarray data (Birnbaum et al. 2003, Toufighi et al. 2005) that are available to the general public to determine whether YUC genes are expressed in Arabidopsis roots. As shown in **Fig. 1A**, four YUC genes (YUC3, YUC5, YUC8 and YUC9) are highly expressed in root cells. YUC7 is also expressed in roots, but the expression levels are much lower compared with that of the above four YUC genes. The microarray data also revealed that the other six YUC genes had very low or no expression in the roots (**Fig. 1A**). Interestingly, the five YUC genes that are expressed in roots cluster together in a phylogenetic tree (**Fig. 1B**) (Cheng et al. 2006).

We further tested the expression patterns of the five YUC genes in roots by generating promoter::GUS reporter lines. Consistent with the microarray data analysis (**Fig. 1A**), four of the five YUC::GUS constructs showed expression in roots (**Fig. 1C**). We did not detect the expression of our YUC7::GUS construct. It is likely that the YUC7 promoter we used did not contain all of the necessary elements because other groups have shown that YUC7::GUS displayed expression in roots (Lee et al. 2011).

Overexpression of the root YUC genes leads to auxin overproduction phenotypes

We overexpressed the five YUC genes that are expressed in roots, using the Cauliflower mosaic virus (CaMV) 35S promoter to determine whether the YUC genes have the capacity to mediate auxin biosynthesis. We have previously shown that overexpression of YUC1, YUC2, YUC4 or YUC6 leads to the characteristic auxin overproduction phenotypes (Cheng et al. 2006, Cheng et al. 2007). The YUC1 overexpression lines have long hypocotyls and epinastic cotyledons when grown in light (Zhao et al. 2001). It was reported that overexpression of YUC5, YUC7, YUC8 and YUC9 using the CaMV 35S promoter also leads to auxin overproduction phenotypes similar to those observed in YUC1 overexpression lines (Woodward et al. 2005, Lee et al. 2011, Hentrich et al. 2013). We confirmed that overexpression of YUC5, YUC7, YUC8 and YUC9 caused auxin overproduction phenotypes (**Fig. 2A**). Moreover, we also observed the auxin overproduction phenotypes in the YUC3 overexpression lines (**Fig. 2A**). Our analyses of the YUC overexpression lines indicated that the five YUC genes in this study participate in auxin biosynthesis.

Inactivation of the YUC genes causes dramatic defects in root development

We obtained mutants for the YUC genes from publicly available sources (see the Materials and Methods). Single mutants of the YUC genes did not display obvious developmental defects. However, simultaneous inactivation of the five YUC genes (YUC3, YUC5, YUC7, YUC8 and YUC9) (yucQ mutants) led to
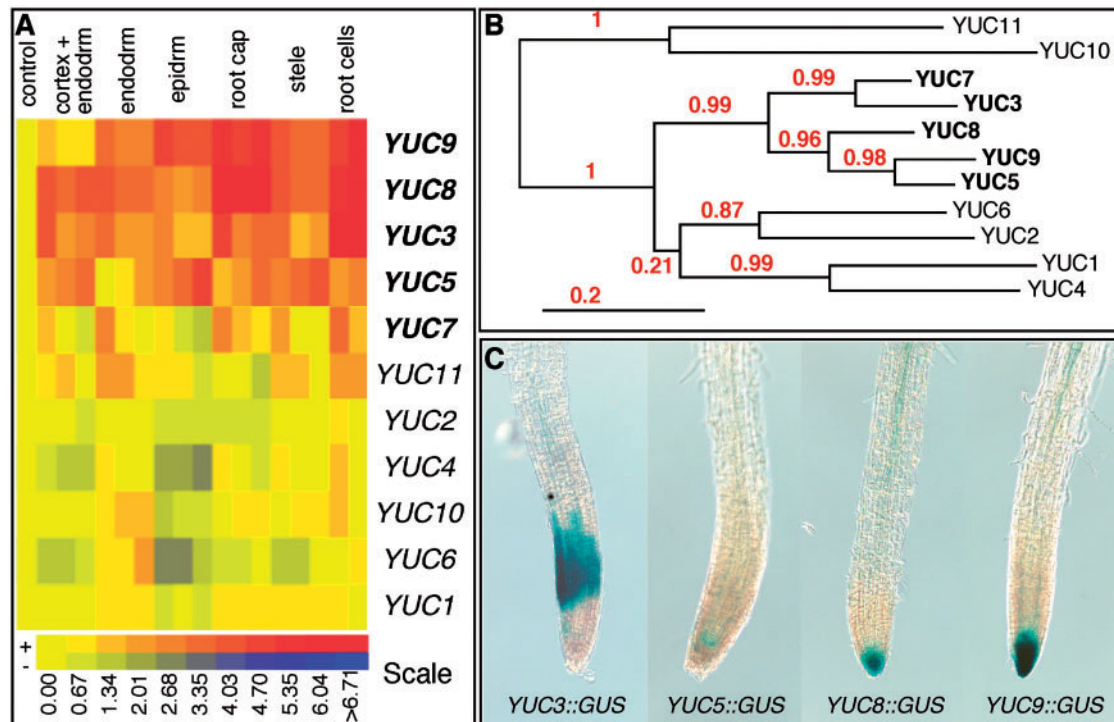
Fig. 1 Identification of auxin biosynthesis genes in Arabidopsis roots. (A) Cluster analysis of the expression patterns of *YUC* genes in root cells. Microarray data from the public domain were clustered using methods previously described in (Birnbaum et al. (2003) and Toufighi et al. (2005). Note that *YUC3*, *YUC5*, *YUC7*, *YUC8* and *YUC9* are expressed in root cells whereas other *YUC* genes have low or no expression in roots. (B) Arabidopsis has 11 YUC flavin monooxygenases that can be divided into five clades (Cheng et al. 2006). The *YUC* genes with expression in roots form two close clades in the phylogenetic tree. (C) The patterns of GUS expression in roots of Arabidopsis transgenic plants that harbor *YUC promoter::GUS* constructs.

severe developmental defects in roots (**Fig. 2B**). The *yucQ* mutants developed very short primary roots and had agravitropic root growth (**Fig. 2B**; **Supplementary Fig. S1**). Compared with wild-type plants, the primary roots of *yucQ* had much smaller meristems (**Fig. 2C**), and the cells in the meristem region in *yucQ* were enlarged and appeared to be partially differentiated (**Fig. 2D**), supporting the conclusion that auxin plays a key role in root meristem maintenance. Overall, the root cap of *yucQ* was small and less organized (**Fig. 2D**). We conclude that these *YUC* genes play critical roles in root development, including root meristem maintenance, and cell division/differentiation in the root cap.

The root defects are caused by partial auxin deficiency in roots

Because overexpression of each *YUC* gene led to auxin overproduction (**Fig. 2A**), we hypothesized that these *YUC* genes participate in auxin biosynthesis in roots and that the phenotypes of *yucQ* mutants are caused by auxin deficiency in roots. We first measured auxin levels in the *yucQ* roots and discovered that *yucQ* had about 55% less free IAA than wild-type roots (**Fig. 3A**). The observed low auxin levels were also consistent with the findings that the expression levels of the auxin reporter DR5–GFP were decreased in *yucQ* roots (**Fig. 3B**). More

importantly, adding 5 nM free IAA into the growth media rescued the root growth phenotypes of *yucQ* (**Fig. 3C**), demonstrating that the root defects of *yucQ* were caused by partial auxin deficiency in roots. We noticed that the IAA-treated *yucQ* were still slightly agravitropic compared with wild-type plants (**Supplementary Fig. S1**). This could be due to the fact that the rescue conditions such as IAA concentrations were not optimum.

Shoot-produced auxin is not sufficient for root development

Early experiments showed that exogenously applied auxin is transported from shoots into roots (Eliasson 1972). However, the physiological functions of such a long-distance transport have not been thoroughly assessed. The *yucQ* mutants, which display defects in root development caused by partial auxin deficiency in roots, provide a system to test the role of shoot-produced auxin in root development. We used a shoot-specific promoter to express *YUC3* in *yucQ* to determine whether shoot-produced auxin can rescue the root defects observed in *yucQ*. Because overexpression of *YUC* genes in shoots leads to dramatic auxin overproduction phenotypes and the resulting low seed set, we used an inducible system (Zuo et al. 2000) to overexpress *YUC3* in shoots. *YUC3* was under the control of a
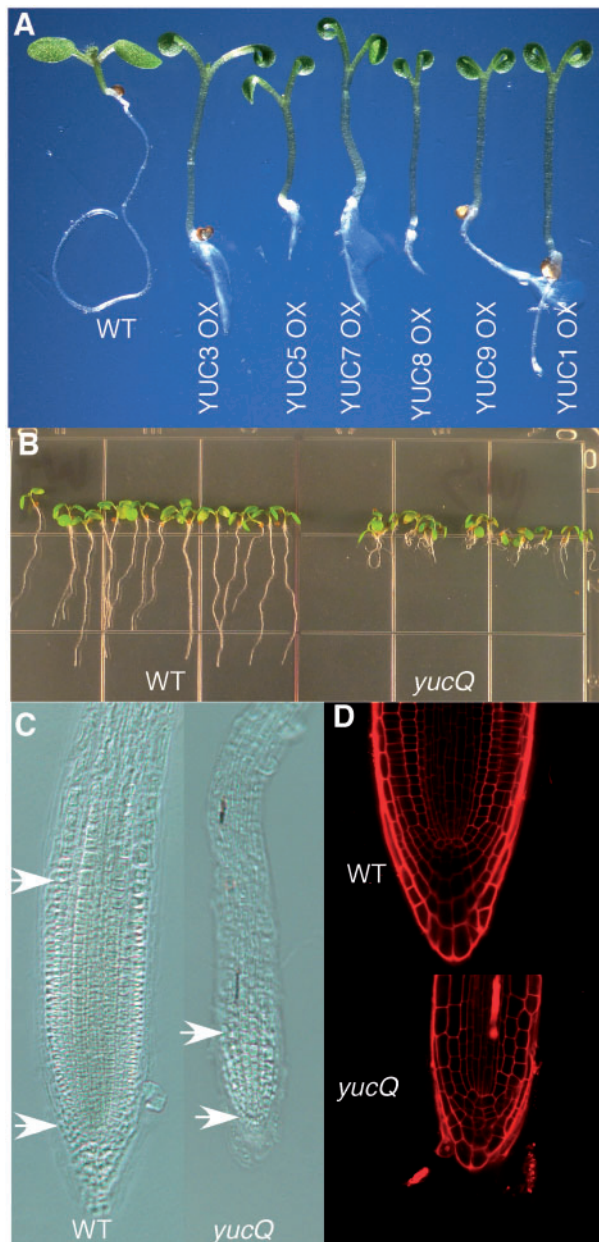
Fig. 2 A group of *YUC* genes are important for auxin biosynthesis and root development. (A) Overexpression of the *YUC* genes causes auxin overproduction phenotypes. (B) Simultaneous inactivation of *YUC3*, *YUC5*, *YUC7*, *YUC8* and *YUC9* leads to dramatic defects in Arabidopsis root development. The *yucQ* mutants develop short and agravitropic primary roots. (C). Roots of *yucQ* are smaller in diameter and have a smaller meristem. (D) The *yucQ* roots have a smaller root cap and an abnormal meristem.
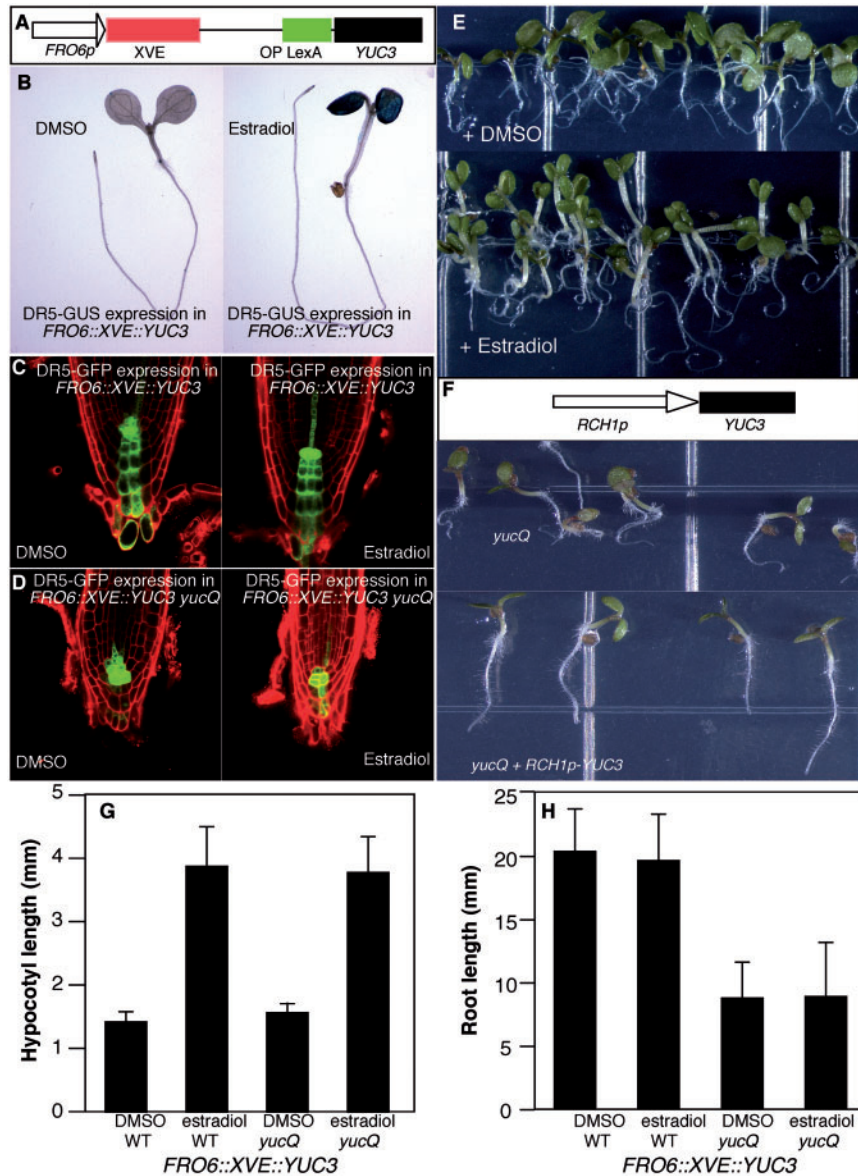
synthetic promoter and the synthetic transcription factor XVE (Zuo et al. 2000) (**Fig. 4A**). The shoot-specific expression was achieved by using the promoter FRO6 (Feng et al. 2006) to drive the expression of XVE. In the presence of estradiol, XVE moves into the nucleus and specifically activates the expression of *YUC3* in shoots.
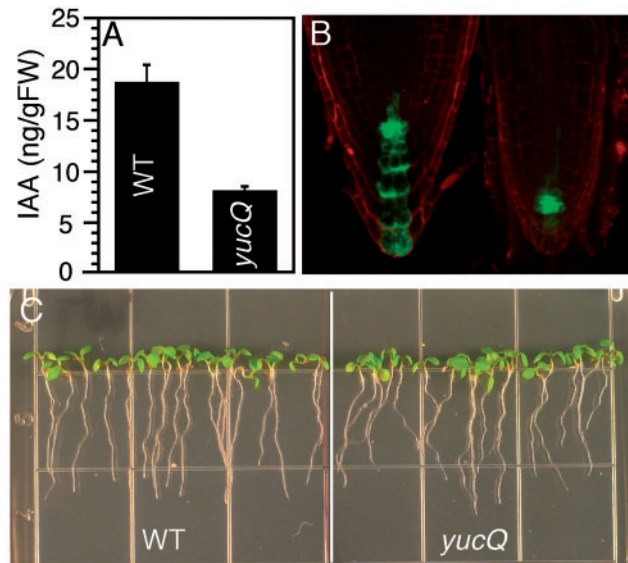
Fig. 3 The root defects in *yucQ* are caused by partial auxin deficiency in roots. (A) Lower auxin levels are detected in *yucQ* roots than in wild-type roots. (B) The expression level of the auxin reporter DR5–GFP is dramatically decreased in *yucQ*. (C) The root defects of *yucQ* are partially rescued by 5 nM IAA.

We also analyzed the effects of overexpressing *YUC3* in shoots in wild-type plants. Induction of *YUC3* expression in shoots resulted in the development of long hypocotyls and epinastic cotyledons, which are the characteristic auxin over-production phenotypes (**Fig. 4B**). Consistent with auxin over-production in the estradiol-treated plants, we observed elevated and ectopic expression of the auxin reporter DR5–GUS in the shoots (**Fig. 4B**). However, the DR5–GUS expression levels in root tips in estradiol-treated and mock-treated plants were very similar (**Fig. 4B**). We further obtained high-resolution images of DR5–GFP in both estradiol-treated and mock-treated plants (**Fig. 4C**). Consistent with our DR5–GUS data, induction of *YUC3* expression in shoots in wild-type plants did not dramatically alter the expression of DR5–GFP expression in the root tip (**Fig. 4C**).

We then introduced the *FRO6::XVE::YUC3* construct into *yucQ* to investigate whether shoot-produced auxin can rescue *yucQ* (**Fig. 4E**). As expected, estradiol treatments induced *YUC3* expression and led to the development of long hypocotyls and epinastic cotyledons in *yucQ* (**Fig. 4E**). However, the roots of estradiol-treated plants still displayed the same agravitropic growth as *yucQ* plants (**Fig. 4E**). Furthermore, the expression levels of DR5–GFP in the estradiol-treated plants were not restored to the wild-type level (**Fig. 4D**). The root morphology of *yucQ* was not rescued either (**Fig. 4D**). We further measured the hypocotyl and the root length of wild-type/ *FRO6::XVE::YUC3* and *yucQ/FRO6::XVE::YUC3* in the presence and absence of estradiol treatment (**Fig. 4G, H**). Induction of *YUC3* expression in shoots by estradiol led to long hypocotyls in both wild-type and *yucQ* plants (**Fig. 4G**). Overexpression of *YUC3* in shoots did not affect root length in either wild-type or

Fig. 4 Shoot-produced auxin is not sufficient for rescuing the root defects of *yucQ*. (A) A construct for inducible expression of *YUC3* in Arabidopsis shoots. The synthetic transcription factor XVE is under the control of a shoot-specific promoter *FRO6p*. Upon estradiol treatment, *YUC3* is specifically expressed in the shoots. (B) Overexpression of *YUC3* in wild-type shoots causes auxin overproduction in shoots. Note that the estradiol-treated seedling has long hypocotyls, epinastic cotyledons and elevated expression of the auxin reporter DR5–GUS in cotyledons. (C) DR5–GFP expression in roots in the mock-treated plants in the wild-type background and in the estradiol-treated plants. Although induction of *YUC3* expression in shoots caused auxin overproduction in shoots, the expression of DR5–GFP was similar to that of the mock-treated plants. (D) DR5–GFP levels were reduced in *yucQ*, and overexpression of *YUC3* in shoots did not lead to an increase in expression of DR5–GFP in roots. (E) Overproduction of auxin in shoots in *yucQ* did not rescue the root defects of *yucQ*. (F) The root phenotypes of *yucQ* (3 days old) were reversed when *YUC3* was expressed using a root-specific promoter *RCH1p*. (G) Hypocotyl length of wild-type/*FRO6::XVE::YUC3* and *yucQ*/*FRO6::XVE::YUC3* plants treated with DMSO (dimethylsulfoxide) or estradiol. (H) The effects of induction of *YUC3* expression in wild-type/*FRO6::XVE::YUC3* and *yucQ*/*FRO6::XVE::YUC3* plants on root length.

yucQ plants (**Fig. 4H**). Moreover, auxin measurements in shoot and root tissues further demonstrated that overexpression of *YUC3* in shoots led to auxin overproduction in shoots, but did not change the IAA concentrations in roots (**Supplementary Fig. S2**). Our results demonstrated that shoot-produced auxin is not sufficient to support normal root development.

We expressed *YUC3* under the root-specific promoter *RCH1p* (Casamitjana-Martinez et al. 2003) in the *yucQ* background (**Fig. 4F**). As shown in **Fig. 4F**, the root defects of *yucQ* were rescued when *YUC3* was expressed in roots. The root length of the rescued *yucQ* was very similar to that of the wild type.

Discussion

Spatial and temporal regulation of auxin levels is believed to be essential for normal root development. The predominant dogma regarding auxin in root development is that polar auxin transport, particularly auxin transport mediated by the PIN auxin efflux carriers, is necessary and sufficient for controlling auxin dynamics during root development (Petricka et al. 2012). The main auxin source for root development has been hypothesized to come from shoots. In this study, we clearly demonstrate that a group of *YUC* genes, which encode the rate-limiting enzymes for tryptophan-dependent auxin biosynthesis, were expressed in Arabidopsis roots. We show that auxin synthesized in roots by the YUC flavin-containing monooxygenases is required for normal root development and root gravitropic responses. Furthermore, we provide evidence that the shoot-produced auxin is not sufficient to support the development of roots.

The Arabidopsis genome contains 11 *YUC* genes, which have distinct and overlapping expression patterns (**Fig. 1**). Interestingly, the physiological functions of the *YUC* genes appear to correlate well with their expression patterns. We have previously shown that *YUC1*, *YUC2*, *YUC4* and *YUC6*, which form two close subclades in a phylogenetic tree (**Fig. 1B**), are expressed in discrete groups of cells in seedlings, leaves and flowers (Cheng et al. 2006, Cheng et al. 2007). Consequently, *YUC1*, *YUC2*, *YUC4* and *YUC6* had overlapping functions in vegetative growth, the formation of veins and flower development (Cheng et al. 2006, Cheng et al. 2007). During flower development, the *YUC2* and *YUC6* subclade is involved in stamen development, whereas *YUC1* and *YUC4* play a more dominant role in the development of the carpel and other floral organs (Cheng et al. 2006, Cheng et al. 2007). We also showed previously that *YUC1*, *YUC4*, *YUC10* and *YUC11* are essential for embryogenesis (Cheng et al. 2006, Cheng et al. 2007). We have shown that *YUC1*, *YUC2*, *YUC4*, *YUC6*, *YUC10* and *YUC11* are expressed during embryogenesis and in shoots, but have very low or no expression in roots (**Fig. 1A**). The *yuc1 yuc2 yuc4 yuc6* quadruple mutants have very severe defects in vascular patterning and flower development, but the quadruple mutants did not have obvious root defects, which is consistent with the expression patterns of the *YUC* genes (**Fig. 1A**). The five *YUC* genes that are expressed in roots are clustered together in a phylogenetic tree (**Fig. 1B**), and are essential for root development. The existence of multiple *YUC* genes in the Arabidopsis genome provides an effective way to control auxin levels temporally and spatially during development.

It is clear that Arabidopsis uses different sets of *YUC* genes to synthesize auxin for root and shoot development. Inactivation of the root *YUC* genes caused dramatic defects in root development (**Fig. 2B**), but did not cause obvious defects in shoots, indicating that local auxin production by the YUC proteins is required for normal root development. On the other hand, mutations in the shoot *YUC* genes led to defects in shoot development without severely affecting root development,

suggesting that shoot-produced auxin may not be necessary for normal root development. Our studies on various *yuc* combinations suggest that auxin transport between shoots and roots cannot compensate local auxin deficiency in shoots or in roots. We further showed clearly that overproduction of auxin in shoots is not sufficient to rescue the root defects observed in *yucQ* mutants (**Fig. 4**), which are defective in auxin biosynthesis in roots.

YUC genes have been shown to function non-cell autonomously, suggesting that auxin is transported from the source cells to target cells (Tobena-Santamaria et al. 2002). The five *YUC* genes studied in this work were expressed in roots, but their expression patterns are distinct and overlapping (**Fig. 1**). The fact that single *yuc* mutants did not display obvious root phenotypes indicates that defects in auxin biosynthesis in some cells can be compensated by auxin synthesized in other cells. However, it is not clear how far auxin can travel to compensate auxin deficiency in cells. We have previously shown that auxin from both localized auxin biosynthesis and polar auxin transport contributes to the initiation of true leaves (Cheng et al. 2007). Development of Arabidopsis roots has been studied extensively, and many tools for root research are available. Further studies using cell type-specific promoters to drive auxin biosynthesis in roots of *yucQ* and in auxin transport mutants such as *aux1* and *pin2* will provide insights into the distance and directions of auxin transport required for root development.

Materials and Methods

The *yuc* mutants

The *yuc* mutants were genotyped using the following genotyping primer sets. Primers for *yuc3* (GABI_376G12) are: yuc3LP, TTATAGCCCCTAAAACAGAGCATC; yuc3RP, GAAA TGTGAAATACAACGACG; and the T-DNA primer CCCATTT GGACGTGAATGTAGACAC. The *yuc5* (CSHL_GT6160) mutant was genotyped with primers: yuc5LP, CGGACTCTA ATCAAAGTCCC; yuc5RP, GGAGATTTCAAAACTAGATTTG; and the T-DNA primer Ds3_1, ACCCGACCGGATCGTATC GGT. Primers for the *yuc7* (SALK_059832) mutant are: yuc7LP, CATGGAGTGGGCTTATCTCTTTG; yuc7RP, ACGAA AAACAGAGCACCCTG; and the T-DNA primer JM-LB1, GGC AATCAGCTGTTGCCCGTCTCACTGGTG. Primers for *yuc8* (SM_3.23299) are: the T-DNA primer spm32, TACGAATAA GAGCGTCCATTTTAGAGTGA; Yuc8LP, CATCCTCTCCACGT GGCTTCC; and yuc8RP, GAACTGACGCTTCGTCGGGTAC. Genotyping primers for *yuc9* (SAIL_762_D07) are: yuc9-LP, GCTCGGTAAGCAAAACAAAACTG; yuc9RP, GAAGGAAAT GCCCAATGAGAC; and the T-DNA primer SAIL-LB1, GCCT TTTCAGAAATGGATAAATAGCCTTGCTTCC. The genotyping results are shown in **Supplementary Fig. S3**.

The *YUC::GUS* reporter lines

The lines were constructed by amplifying the *YUC* promoters using the primers specified below. The *YUC* promoters then

were cloned into the pBI101.3 vector with *Sal*I at the 5′ end and *Sma*I at the 3′ end to drive the expression of the *GUS* reporter. Primers for *YUC3* promoters are: Y3pro5p, TCAATCAACGAGAA TGGAACACGCC; and Y3pro3p, CTTCCACAGAGTTTAGAGTTT GC. The *YUC5* promoter was amplified using Y5pro5p, CCAAACT CAAGTGCCTATGG; and Y5pro3p, CTTTAGGGGTGAGTTTGA TCG. Y7pro5p, GCCCGATTCAACTCCACTACG; and Y7pro3p, CTTATTGGGAGCTTTTTAGTG were used to amplify the *YUC7* promoter. Primers for the *YUC9* promoter are: Y9pro3p, TTTCTT GAGTGAGTTTTTGAATG; and Y9pro5p, CACAAAGATCAATAC AAAAGCC. We cloned the *YUC8* promoter using primers: Y8pro5p, TCTTTTTTTATAAGTTTCTTTAATAAG; and Y8pro3p, CGCATGACTCAGTTGTTGCGATC.

Plant growth conditions

Seeds were surface-sterilized and sown on vertical square plates with half-strength Murashige and Skoog (MS) medium (1/2 MS salts, 1.5% sucrose, 0.65% agar), stratified in the dark at 4°C for 2 d, germinated, and grown at 22°C under a 16 h light/8 h dark cycle for 5 d or for times specifically mentioned.

Microscopic analysis

Propidium iodide staining was performed as previously described (Helariutta et al. 2000). Fluorescence was visualized under a Zeiss LSM 710 confocal microscope. DIC (differential interference contrast) pictures were taken with a Leica DM5000 microscope. GUS staining and measurements of the gravitropic response were performed as previously described (Li et al. 2010).

Auxin measurements

Endogenous levels of IAA in Arabidopsis were analyzed by a previously reported method (Okumura et al. 2013).

Tissue-specific auxin production

Root-specific auxin production was achieved by expressing *YUC3* under the control of the *RCH1* promoter (Casamitjana-Martinez et al. 2003). The *RCH1* (*At5g48940*) promoter and *YUC3* genomic DNA were fused together and cloned into the *Xho*I and *Spe*I sites of the pER8 plasmid (Zuo et al. 2000). The *RCH1* promoter was amplified using the primers: RCH1pF, AGC TAGTCGACTCTAGCCTGTGTGAGAAGCGAGGAGGGGT; and RCH1pR, TGATAGACTTCTTGTTATTATTTCCATACATA AGAGTTTTTTTCTTTGCATTTGGCTCT. The primers for amplifying *YUC3* were: YUC3F1, AGAGCCAAATGCAAAGAAAAAA ACTCTTATGTATGGAAATAATAACAAGAAGTCTATCA; and YUC3R1, CTGGGAGGCCTGGATCGATTAGAAATGTGAAAT ACAACGACGATGA.

To express *YUC3* in Arabidopsis shoots, the *FRO6* promoter was used. The *FRO6* (*At5g49730*) promoter (Feng et al. 2006) was amplified using the following two primers: FRO6pF, 5′-CA ATATATCCTGTCAAACCGATGCTCTCAAGGCCAAAGAAC A-3′; and FRO6pR, 5′-GCCCGTTAACGCTTTCATCTTTATTTGA ATTTCCACTTCTCAGTG-3′. The G10-90 promoter in pER8

was removed and an *Mfe*I site was introduced before XVE. The *FRO6* promoter was inserted at the *Mfe*I site. *YUC3* genomic DNA was cloned into *Xho*I and *Spe*I sites using the primers YUC3F2, AGCTAGTCGACTCTAGCCATGTATGGAAATAATA ACAAGAAGTCTATCA, and YUC3R1.

Disclosures

The authors have no conflicts of interest to declare.

References

Benjamins, R. and Scheres, B. (2008) Auxin: the looping star in plant development. *Annu. Rev. Plant Biol.* 59: 443–465.

Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G. et al. (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115: 591–602.

Birnbaum, K., Shasha, D.E., Wang, J.Y., Jung, J.W., Lambert, G.M., Galbraith, D.W. et al. (2003) A gene expression map of the Arabidopsis root. *Science* 302: 1956–1960.

Brunoud, G., Wells, D.M., Oliva, M., Larrieu, A., Mirabet, V., Burrow, A.H. et al. (2012) A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature* 482: 103–106.

Casamitjana-Martinez, E., Hofhuis, H.F., Xu, J., Liu, C.M., Heidstra, R. and Scheres, B. (2003) Root-specific CLE19 overexpression and the sol1/2 suppressors implicate a CLV-like pathway in the control of Arabidopsis root meristem maintenance. *Curr. Biol.* 13: 1435–1441.

Cheng, Y., Dai, X. and Zhao, Y. (2006) Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. *Genes Dev.* 20: 1790–1799.

Cheng, Y., Dai, X. and Zhao, Y. (2007) Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in Arabidopsis. *Plant Cell* 19: 2430–2439.

Eliasson, L. (1972) Translocation of shoot-applied indolylacetic acid into the roots of Populus tremula. *Physiol. Plant.* 27: 412–416.

Feng, H., An, F., Zhang, S., Ji, Z., Ling, H.Q. and Zuo, J. (2006) Light-regulated, tissue-specific, and cell differentiation-specific expression of the Arabidopsis Fe(III)-chelate reductase gene AtFRO6. *Plant Physiol.* 140: 1345–1354.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T. et al. (2003) Efflux-dependent auxin gradients establish the apical–basal axis of Arabidopsis. *Nature* 426: 147–153.

Grieneisen, V.A., Xu, J., Maree, A.F., Hogeweg, P. and Scheres, B. (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* 449: 1008–1013.

Helariutta, Y., Fukaki, H., Wysocka-Diller, J., Nakajima, K., Jung, J., Sena, G. et al. (2000) The SHORT-ROOT gene controls radial patterning of the Arabidopsis root through radial signaling. *Cell* 101: 555–567.

Hentrich, M., Bottcher, C., Duchting, P., Cheng, Y., Zhao, Y., Berkowitz, O. et al. (2013) The jasmonic acid signaling pathway is linked to auxin homeostasis through the modulation of YUCCA8 and YUCCA9 gene expression. *Plant J.* 74: 626–637.

Hobbie, L. and Estelle, M. (1994) Genetic approaches to auxin action. *Plant Cell Environ.* 17: 525–540.

Lee, M., Jung, J.H., Han, D.Y., Seo, P.J., Park, W.J. and Park, C.M. (2011) Activation of a flavin monooxygenase gene YUCCA7 enhances drought resistance in Arabidopsis. *Planta* 235: 923–938.

Li, Y., Dai, X., Cheng, Y. and Zhao, Y. (2010) NPY genes play an essential role in root gravitropic responses in Arabidopsis. *Mol. Plant* 4: 171–179.

Ljung, K., Hull, A.K., Celenza, J., Yamada, M., Estelle, M., Normanly, J. et al. (2005) Sites and regulation of auxin biosynthesis in Arabidopsis roots. *Plant Cell* 17: 1090–1104.

Okumura, K., Goh, T., Toyokura, K., Kasahara, H., Takebayashi, Y., Mimura, T. et al. (2013) GNOM/FEWER ROOTS is required for the establishment of an auxin response maximum for Arabidopsis lateral root initiation. *Plant Cell Physiol.* 54: 406–417.

Petricka, J.J., Winter, C.M. and Benfey, P.N. (2012) Control of Arabidopsis root development. *Annu. Rev. Plant Biol.* 63: 563–590.

Pinon, V., Prasad, K., Grigg, S.P., Sanchez-Perez, G.F. and Scheres, B. (2013) Local auxin biosynthesis regulation by PLETHORA transcription factors controls phyllotaxis in Arabidopsis. *Proc. Natl Acad. Sci. USA* 110: 1107–1112.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J. et al. (1999) An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. *Cell* 99: 463–472.

Stepanova, A.N., Robertson-Hoyt, J., Yun, J., Benavente, L.M., Xie, D.Y., Dolezal, K. et al. (2008) TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* 133: 177–191.

Tao, Y., Ferrer, J.L., Ljung, K., Pojer, F., Hong, F., Long, J.A. et al. (2008) Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* 133: 164–176.

Tobena-Santamaria, R., Bliek, M., Ljung, K., Sandberg, G., Mol, J.N., Souer, E. et al. (2002) FLOOZY of petunia is a flavin mono-oxygenase-like protein required for the specification of leaf and flower architecture. *Genes Dev.* 16: 753–763.

Toufighi, K., Brady, S.M., Austin, R., Ly, E. and Provart, N.J. (2005) The Botany Array Resource: e-Northerns, Expression Angling, and promoter analyses. *Plant J.* 43: 153–163.

Wisniewska, J., Xu, J., Seifertova, D., Brewer, P.B., Ruzicka, K., Blilou, I. et al. (2006) Polar PIN localization directs auxin flow in plants. *Science* 312: 883.

Woodward, C., Bemis, S.M., Hill, E.J., Sawa, S., Koshiba, T. and Torii, K.U. (2005) Interaction of auxin and ERECTA in elaborating Arabidopsis inflorescence architecture revealed by the activation tagging of a new member of the YUCCA family putative flavin monooxygenases. *Plant Physiol.* 139: 192–203.

Yamada, M., Greenham, K., Prigge, M.J., Jensen, P.J. and Estelle, M. (2009) The TRANSPORT INHIBITOR RESPONSE2 gene is required for auxin synthesis and diverse aspects of plant development. *Plant Physiol.* 151: 168–179.

Zhao, Y. (2012) Auxin biosynthesis: a simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. *Mol. Plant* 5: 334–338.

Zhao, Y., Christensen, S.K., Fankhauser, C., Cashman, J.R., Cohen, J.D., Weigel, D. et al. (2001) A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* 291: 306–309.

Zuo, J., Niu, Q.W. and Chua, N.H. (2000) Technical advance: an estrogen receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants. *Plant J.* 24: 265–273.