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The involvement of auxin in root architecture plasticity in *Arabidopsis* induced by heterogeneous phosphorus availability

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Abstract

Homogeneous low phosphorus availability was reported to regulate root architecture in *Arabidopsis via* auxin, but the roles of auxin in root architecture plasticity to heterogeneous P availability remain unclear. In this study, we employed auxin biosynthesis-, transport- and signalling-related mutants. Firstly, we found that in contrast to low P (LP) content in the whole medium, primary root (PR) growth of *Arabidopsis* was partially rescued in the medium divided into two parts: upper with LP and lower with high P (HP) content or in the reverse arrangement. The down part LP was more effective to arrest PR growth as well as to decrease density of lateral roots (DLR) than the upper LP, and effects were dependent on polar auxin transport. Secondly, we verified that auxin receptor TIR1 was involved in the responses of PR growth and lateral root (LR) development to P supply and loss of function of *TIR1* inhibited LR development. Thirdly, effects of heterogeneous P on LRD in the upper part of PR was dependent on PIN2 and PIN4, and in the down part on PIN3 and PIN4, whereas density of total LRs was dependent on auxin transporters PIN2 and PIN7. Finally, heterogeneous P availability altered the accumulation of auxin in PR tip and the expression of auxin biosynthesis-related genes *TAA1*, *YUC1*, *YUC2*, and *YUC4*. Taken together, we provided evidences for the involvement of auxin in root architecture plasticity in response to heterogeneous phosphorus availability in *Arabidopsis*.

Additional key words: auxin biosynthesis-related genes, lateral roots, PIN, primary root, TIR.

Introduction

Phosphorus is a macronutrient essential for root growth and development. The P uptake is closely associated with root morphology and, on the other hand, the P content and its heterogeneous distribution in soil dramatically affects root growth and root system architecture (Pothuluri *et al*. 1986, Lynch 1995, Williamson *et al*. 2001, López-Bucio *et al*. 2002, 2003). Auxin affects development and growth of both primary roots (PR) and lateral roots (LR) and root hair proliferation (Casimiro *et al*. 2001, Mendes *et al*. 2011, Park *et al.* 2011). As documented in literature, the polar auxin transport (PAT) forms gradient of auxin concentration that controls LR development (Casimiro *et al*. 2001). When N-1-naphthyl-phthalamic acid (NPA), an auxin efflux transport inhibitor, was applied to *Arabidopsis* plants, content of auxin in roots was reduced and LR development was inhibited (López-Bucio *et al*. 2002). The transports of auxin in plants are basipetal and acropetal. Previous

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Abbreviations: DLR - density of lateral roots; DLRP - density of lateral root primordia; DMSO - dimethyl sulfoxide; DTLR - density of total lateral roots; HP - high P content; IAA - indole acetic acid; IPA - indole-3-pyruvic acid; LP - low P content; LRP - lateral root primordium; NLR - number of lateral roots; NPA - N-1-naphthylphthalamic acid; PIN - auxin transporter; PR - primary root; PRL - primary root length; TAA1 - tryptophan aminotransferase of *Arabidopsis* 1; TIBA - 2,3,5-triiodobenzoic acid; TNLR - total number of lateral roots; TIR - auxin receptor.

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studies have demonstrated that the basipetal auxin transport promotes LR initiation and acropetal transport appears to regulate the emergence of LR (Casimiro *et al*. 2001). LR development is also regulated by environmental conditions, such as irradiance, water, and nutrition. Auxin signalling-related *Arabidopsis* mutants *axr1-3*, *axr2-1*, and *axr4-1* form more root hairs and longer LRs, the phenotype typical for P stress in wild type (López-Bucio *et al*. 2002, Nacry *et al*. 2005). These observations suggest some of P starvation responses independent of AXR.

Although more studies have concern about the roles of auxin in root architecture remodeling in response to homogeneous P availability in past decades, the details about the involvement of auxin in root architecture plasticity under heterogeneous P conditions remains unclear. In this study, using *CYCB1;1::GUS* and different mutants such as auxin biosynthesis-, auxin signalling-, and auxin transport-related mutants, we tried to clarify the involvement of auxin in root architecture plasticity in response to heterogeneous P availability in *Arabidopsis*.

Materials and methods

Plants and growth conditions: Seeds of *Arabidopsis thaliana* (L.) Heynh. wild type (Col-0) and *CycB1;1::GUS*, *DR5::GUS*, *tir1*, *YUC1-OX*, *yuc1 yuc6*, *aux1-7*, *pin1-1*, *pin2*, *pin3-1*, *pin4-1*, and *pin7-3* tranformants/mutants were stratified at 4 °C for 48 h in darkness to promote germination. Cold-treated seeds were then surface-sterilized in 70 % (v/v) ethanol for 1 min followed by 5 times washing with autoclaved deionized water. Further, these seeds were treated with 5 % (m/v) NaClO for 5 min, followed by 5 times washing in autoclaved water. Sterilized seeds were sown on 50 cm^3 of agar-solidified medium in Petri dishes. The medium containing a high concentration of P, called HP medium, was composed of 10 mM NH_4NO_3, 9.5 mM KNO_3, 1.5 mM $CaCl_2 . 2\,H_2O$, 0.75 mM $MgSO_4 . 7\,H_2O$, 25 µM KI, 125 µM H_3BO_3, 0.5 mM $MnSO_4 . 1\,H_2O$, 1.5 mM $ZnSO_4 . 7\,H_2O$, 5 µM $Na_2Mo0_4 . 2\,H_2O$, 0.5 µM $CuSO_4 . 5\,H_2O$, 0.5 µM $CoCl_2 . 6\,H_2O$, 0.5 mM $FeSO_4 . 7\,H_2O$, 0.5 mM NaFe-EDTA . $2\,H_2O$, 1 mM KH_2PO_4, and 5 g dm^{-3} sugar. The pH of medium was adjusted to 5.8 with 1 M KOH and then *Phytagel* (*Sigma*, St. Louis, USA; 3 g dm^{-3}) was added. In medium with low P concentration, LP medium, the concentration of KH_2PO_4 was only 10 µM, other nutrients were the same as in the HP medium. Sterilized seeds were germinated and grown for 7 d in HP or LP media under a 16-h photoperiod, irradiance of 100 µmol m^{-2} s^{-1}, day/night temperature of 22/20 °C, and air humidity of 75 %. Then seedlings were moved to homogeneous or heterogeneous P media for further treatments as described below.

Heterogeneous P treatments: The LP and HP media was initially made as above mentioned, then a 2 - 3 mm gap was cut by a sterilized knife in 1/4 of the Petri dish to divide the growth medium into two parts. As each Petri dish contained 50 cm^3 of growth medium, the upper part should be 12.5 cm^3 and the down part should be 37.5 cm^3. The KH_2PO_4 stock was dropped into the stratified medium according to the treatments and spread evenly with glass rod. Accordingly, four stratified P treatments were made: LP/LP, HP/LP, LP/HP, and HP/HP.

The 7-d-old seedlings with approximately a 1.5 - 2 cm long primary root (PR) grown in homogeneous HP or LP medium were transplanted in HP/HP and HP/LP or LP/LP and LP/HP media, respectively, to keep PR tip contacted with the down part of the medium. Each Petri dish contained 2 plants. Then these seedlings were allowed to grow 14 d in media containing 5 µM 2,3,5-tri-iodobenzoic acid (TIBA, *Sigma*) or 0 µM TIBA (control) under conditions described above. TIBA was dissolved in dimethyl sulfoxide (DMSO) to make stock solution.

Histochemical analysis: For histochemical analysis of GUS activity, *Arabidopsis* seedlings were fixed in 90 % (v/v) acetone under -20 °C for 2 h, washed two times in sodium phosphate buffer (pH 7.5) on the ice, and subsequently incubated in a GUS staining buffer (0.5 mM $K_3Fe(CN)_6$, 0.5 mM $K_4Fe(CN)_6$, 10 mM EDTA, 1 % (v/v) *Triton X-100*, and 0.5 g dm^{-3} X-Glu in 100 mM sodium phosphate (pH 7.5) at 37 °C for 12 h. The stained seedlings were observed and photographed using the fluorescent microscope (*DM5000B*, Leica, Somme, Germany).

Root trait analysis: Roots of plants grown in the Petri dishes were scanned (*Epson 1460XL*, Nagano, Japan) with a resolution of 400 dpi. The length of PR was measured with the *ImageJ 1.32* program (National Institutes of Health, Bethesda**,** USA). The length of total lateral (LR) length was measured with the *WinRHIZO* software (*Regent Instrument*, Quebec**,** Canada). The stained PR tips and LR primordia (LRP) were photographed under a microscope (*Leica DM5000B*) connected to a video camera (*Leica DFC480*).

RNA extraction and quantitative real-time PCR: Total RNA was extracted from the whole *Arabidopsis* seedlings with a standard method. RNA samples were treated with RNase-free DNase I (*Invitrogen*, Carlsbad**,** USA). The first cDNA strand was synthesized from 1 µg total RNA with reverse transcriptase (*Promega*, Madison**,** USA) according to the manufacture's protocol. Quantitative real-time PCR (qRT-PCR) was performed

using *SYBR® Premix EX Taq™* (*TaKaRa*, Shiga**,** Japan). All the reactions were completed in a *Rotor-Gene 3000* (*Corbett Research*, Sydney, Australia). Specific PCR primer pairs were designed to guarantee the specific amplification for each tested gene. The qRT-PCR thermal cycle used was 40 cycles consisting of 95 °C for 15 s, 56 - 60 °C for 15 s, and 72 °C for 30 s. Fluorescence data were analyzed with *Rotor-Gene* software. *AtEF1a* was used as a reference gene to normalize samples, the forward primer and reverse primer for *AtEF1a* were GTCGATTCTGGAAAGTCGACC and AATGTCAAT GGTGATACCACGC, respectively. The primer pairs used for amplification of cDNA of *TAA1*, *YUC1*, *YUC2*, *YUC4*, *YUC5*, and *YUC6* were the same as reported previously (Tao *et al*. 2008, Eklund *et al*. 2010).

Statistical analysis: Data were statistically analyzed through *SAS 8.01* program (*SAS Institute Inc.*, Chicago, USA). A one-way *ANOVA* analysis was carried out to analyze PR length (PRL), lateral root primordium (LRP) number in two layers, density of LRP (DLRP), and total lateral root length.

Results

CycB1 encodes a protein controlling the transition from G2 to M phase in a cell cycle. One *CycB1;1::GUS Arabidopsis* line was generated to study the development of LRP (Ferreira *et al*. 1994, Malamy and Benfey 1997). We found that *CycB1;1::GUS* line displayed similar responses to P availability as Col-0 (data not shown). This indicates that *CycB1;1::GUS* line we can employ to study effects of heterogeneous P availability on PR growth and LR development (Fig. 1). In HP/HP, the PR was significantly longer than in other treatments, followed by LP/HP and then HP/LP. By contrast, the entire root system in LP/LP medium was weak, the length of PR was 49.6 % of that in HP/HP medium (Table 1). Interestingly, the upper layer P availability had a little role in the upper part PR growth and conversely, the down layer P availability significantly affected the down part PR growth and down part LP significantly inhibited PR growth (Table 1). The length of PR in LP/HP was 11.7 ± 0.27 cm but that in HP/LP was 7.6 ± 0.35 cm. These results indicated that local root tip P signal but not the systematic P signal determined the PR growth.

Seedlings exhibited different LR patterns in four P treatments (Fig. 1). We divided the whole developmental stage of LRP into three stages, namely stage I (initiation stage, the early stage of primordium development), stage II (establishment stage, the primordium was developed but not penetrated epidermis), and stage III (emergence stage, LR penetrated from epidermis). In the upper part of PR, nearly 100 % of LRs were in stage III. Relative to HP/HP, HP/LP treatment promoted emergence of LRs (11.8 to 9.5), and in contrast to LP/HP, LP/LP stimulated LR emergence (11.7 to 8.0). LP in the down part decreased the number of LRP in stage I, II, and III. For instance, the emerged LRP in HP/HP was 53.6 but that in HP/LP was 28.6, and for LP/HP and LP/LP, the emerged LRP was 35.7 and 16.9, respectively. But when we compared the percentage of total LRP in stage III from the four P treatments, we found that the down part LP treatment increased the percentage of emerged LRP in whole PR. The percentage of total LRP in

Table 1. Effects of heterogeneous phosphorus availability (see Materials and methods for a detail) on primary root growth, lateral root development, and growth in *CycB1;1::GUS* line. Means ± SE from four independent experiments, for each at least 12 samples. Different letters associated with the same parameter indicate the significant difference between P treatments ($P < 0.05$).

Parameters	HP/HP	HP/LP	LP/HP	LP/LP
LPR in upper part [cm]	1.7 ± 0.10a	1.9 ± 0.09a	1.7 ± 0.05a	1.8 ± 0.06a
LPR in lower part [cm]	12.2 ± 0.40a	5.7 ± 0.43c	9.4 ± 0.24b	5.1 ± 0.27d
Total length of PR [cm]	13.9 ± 0.30a	7.6 ± 0.35c	11.7 ± 0.26b	6.9 ± 0.28d
Number of stage I LRP in upper part	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a	0.0 ± 0.00a
Number of stage II LRP in upper part	0.0 ± 0.00a	0.0 ± 0.00a	0.2 ± 0.02a	0.0 ± 0.00a
Number of stage III LRP in upper part	9.5 ± 0.27b	11.8 ± 0.75a	8.0 ± 0.68c	11.7 ± 0.75a
Number of stage I LRP in down part	10.2 ± 1.50a	6.5 ± 0.93b	7.7 ± 1.00b	1.6 ± 0.62c
Number of stage II LRP in down part	12.6 ± 2.11a	7.0 ± 0.76b	7.9 ± 1.10ab	4.0 ± 1.99c
Number of stage III LRP in down part	53.6 ± 6.30a	28.6 ± 5.06b	35.7 ± 3.12ab	16.9 ± 1.74c
DLRP in upper part [cm^{-1}]	5.5 ± 0.28ab	6.3 ± 0.27a	4.8 ± 0.38b	6.4 ± 0.44a
DLRP in down part [cm^{-1}]	6.2 ± 0.52a	7.3 ± 0.48a	6.8 ± 0.56a	6.7 ± 0.48a
Total length of LRs in upper part [cm]	18.9 ± 1.27a	18.4 ± 2.30a	6.2 ± 1.28b	9.5 ± 1.62b
Total length of LR in down part [cm]	72.7 ± 10.29a	33.7 ± 1.45b	29.4 ± 6.33c	11.9 ± 3.09d

Table 2. Effects of heterogeneous phosphorus availability on primary root length, DLR, and DTLR in wild type Col-0, *YUC1-OX, yuc1 yuc6,* and *tir1* mutants. Col-0 plants were either treated or not treated with 5 μM TIBA. Means ± SE, $n \geq 8$, different letters associated with the same parameter in each genotype group indicate the significant difference between P treatments ($P < 0.05$).

Genotype	Treatment	Length of PR in the upper part [cm]	Length of PR in the lower part [cm]	Total length of PR [cm]	DLR in upper part [cm^{-1}]	DLR in lower part [cm^{-1}]	DTLR [cm^{-1}]
Col-0	HP/HP	1.0 ± 0.07a	12.2 ± 0.79a	13.2 ± 0.83a	6.0 ± 0.35a	3.6 ± 0.38a	3.8 ± 0.35a
	HP/HP +TIBA	1.4 ± 0.14b	2.6 ± 0.42d	3.9 ± 0.43cd	2.7 ± 0.38a	0.8 ± 0.25c	1.5 ± 0.17c
	HP/LP	1.3 ± 0.07b	8.6 ± 0.37b	9.8 ± 0.35b	5.9 ± 0.32ab	4.2 ± 0.39ab	4.4 ± 0.36ab
	HP/LP+TIBA	1.2 ± 0.12ab	2.6 ± 0.16d	3.7 ± 0.20cd	2.1 ± 0.25a	0.6 ± 0.28c	1.1 ± 0.17c
	LP/HP	0.9 ± 0.27a	9.9 ± 0.45b	10.8 ± 0.46b	7.9 ± 0.21a	2.6 ± 0.23ac	3.1 ± 0.22ac
	LP/HP+TIBA	1.0 ± 0.07a	2.5 ± 0.27d	3.5 ± 0.25d	3.8 ± 0.55ab	0.5 ± 0.17c	1.4 ± 0.28c
	LP/LP	0.7 ± 0.03a	5.2 ± 0.40c	5.9 ± 0.39c	8.5 ± 0.81b	5.4 ± 0.91b	5.7 ± 0.81b
	LP/LP+TIBA	0.7 ± 0.02a	1.9 ± 0.13d	2.6 ± 0.11d	4.7 ± 0.44ab	1.0 ± 0.34c	2.0 ± 0.32ac
YUC1-OX	HP/HP	1.0 ± 0.12a	11.8 ± 1.25a	12.8 ± 1.33a	7.4 ± 0.81a	4.2 ± 0.68a	4.5 ± 0.60a
	HP/LP	1.3 ± 0.12a	6.1 ± 0.45b	6.1 ± 0.52b	6.2 ± 0.56a	5.1 ± 0.60a	6.4 ± 0.55a
	LP/HP	0.8 ± 0.07a	8.7 ± 0.88b	9.5 ± 0.91b	6.2 ± 1.46a	2.7 ± 0.69a	3.0 ± 0.74a
	LP/LP	0.7 ± 0.05a	3.8 ± 0.36b	4.5 ± 0.37b	9.1 ± 0.86a	6.4 ± 0.68a	6.8 ± 0.55a
yuc1 yuc6	HP/HP	1.1 ± 0.09a	12.1 ± 0.43a	13.3 ± 0.07a	3.5 ± 0.52a	3.7 ± 0.57a	3.7 ± 0.49a
	HP/LP	1.3 ± 0.05a	9.3 ± 0.40b	10.6 ± 0.38b	1.9 ± 0.42a	3.9 ± 0.15a	3.7 ± 0.14a
	LP/HP	0.8 ± 0.15b	9.7 ± 0.78b	10.5 ± 0.71b	2.8 ± 1.11a	1.2 ± 0.52b	1.3 ± 0.52b
	LP/LP	0.7 ± 0.07b	5.5 ± 0.35c	6.2 ± 0.37c	5.1 ± 1.01a	4.5 ± 0.48a	4.6 ± 0.46a
tir1	HP/HP	0.7 ± 0.03a	5.7 ± 1.35a	6.5 ± 1.37a	4.1 ± 0.88a	2.3 ± 0.54a	2.5 ± 0.40a
	HP/LP	1.1 ± 0.08b	8.5 ± 0.23b	9.6 ± 0.28b	3.4 ± 0.59a	3.2 ± 0.21a	3.2 ± 0.13a
	LP/HP	0.7 ± 0.06a	4.4 ± 0.63a	5.2 ± 0.92a	0.0 ± 0.00b	1.8 ± 0.23a	1.6 ± 0.15a
	LP/LP	0.8 ± 0.06a	6.0 ± 0.34a	6.8 ± 0.36a	2.3 ± 0.54ab	3.5 ± 0.29a	3.3 ± 0.28a

Fig. 1. Effects of heterogeneous phosphorus availability on root architecture in *Arabidopsis* transgenic *CycB1;1::GUS* seedlings. The representatives of at least 12 plants for each treatment.

stage III for HP/HP, HP/LP, LP/HP, and LP/LP were 73.5, 75.0, 73.4, and 83.6, respectively, indicating that low P promoted LR development.

Compared with HP/HP treatment, the DLRP in the upper part of PR in HP/LP media was slightly but not significantly higher, whereas the DLRP in the upper part of PR in LP/LP medium was significantly higher than in LP/HP medium (Table 1).

The length of LRs in the down part of PR in HP/HP media was the highest (72.7 cm), followed by HP/LP (33.7 cm), LP/HP (29.4 cm), and LP/LP (11.9 cm) (Table 1). When compared the length of LRs in the upper part of media, no difference can be found between HP/HP and HP/LP, or LP/HP and LP/LP. In addition, the percentage of the total LR length in the upper part of PR to the whole PR in HP/HP, HP/LP, LP/HP, and LP/LP were 20.6, 35.3, 17.4, and 44.4, respectively.

To link heterogeneous P uptake with auxin content, we explored the auxin distribution in PR of *DR5::GUS* transgenic line, a classic marker line for auxin content (López-Bucio *et al*. 2002). Immediately after transfer (0 h), the GUS signalling in PR tips in HP/HP (Fig. 2*A*) was the highest followed by HP/LP (Fig. 2*D*), LP/HP (Fig. 2*G*), and LP/LP (Fig. 2*J*). Further, the GUS staining in PR slightly decreased under all the four P treatments, but the signalling in HP/HP (Fig. 2*B*) and HP/LP (Fig. 2*E*) was always higher than that in LP/HP (Fig. 2*H*) and LP/LP (Fig. 2*K*). Moreover, 10 d after transfer, we found very strong signals in the PR grown in HP/HP (Fig. 2*C*) and HP/LP (Fig. 2*F*), and mild staining in LP/HP (Fig. 2*I*), but almost no staining in PR grown in LP/LP (Fig. 2*L*).

To link polar auxin transport (PAT) with PR growth under different P treatments, wild type seedlings (Col-0) were treated with TIBA. Results showed that TIBA significantly inhibited PR growth under all P treatments but more in HP (Table 2). The inhibition of PR length in LP/LP was 56 %, but that in HP/HP was as high as 70.5 % indicating that P availability altered the sensitivity of PR to exogenous TIBA.

As we verified that the PAT was involved in PR growth, we further tested the role of auxin biosynthesis. *YUCCAs*, encoding the flavin monooxygenases, play important roles in auxin biosynthesis, and the over-expression of *YUCCA1* (*YUC1*) or *YUCCA6* (*YUC6*) results in the over-accumulation of auxin in *Arabidopsis* (Cheng *et al*. 2006). Interestingly, compared with Col-0, over-expression of *YUC1* resulted in shorter PR under all P treatments (Table 2). The *yuc1 yuc6* is T-DNA insertion knock-out double mutant (Cheng *et al*. 2006). Relative to Col-0, the responses of PR growth in *yuc1 yuc6* to heterogeneous P was similar (Table 2), but the PRs in *yuc1 yuc6* were longer than those in Col-0 under the same P conditions implying that YUC1 and/or YUC6 participated in PR growth.

TIR1 is the most important auxin receptor gene. Accordingly, TIR1 plays crucial roles in auxin signalling (Dharmasiri *et al.* 2005a,b). To test the role of auxin signalling in PR growth under heterogeneous P treatment, we employed the *tir1* loss of function mutant. Compared with Col-0, mutation of *TIR1* significantly decreased PR growth excluding the LP/LP treatment (Table 2). The length of whole PR in *tir1* was 49.2, 97.9, 48.1, and 115 % of that in Col-0 at HP/HP, HP/LP, LP/HP, and LP/LP treatments, respectively (Table 2). Moreover, in contrast to Col-0, loss-of-function in *TIR1* results in the no difference of PR length between LP/HP and LP/LP.

PAT is dependent on auxin influx and efflux carriers. AUX1 is an important auxin influx carrier (Marchant *et al.* 2002) and PINs are auxin efflux carriers (Paponov *et al*. 2005). Results in Table 3 indicate that under the same upper P conditions (HP or LP), the down part LP decreased PR growth in wild type, *aux1-7*, *pin2*, *pin3-1*, and *pin7-3*, but not in *pin4-3* ($P < 0.05$). In addition, mutation of *AUX1* resulted in slightly longer PR in contrast to Col-0 (Table 3). These results verified the involvement of auxin influx system in the regulation of PR growth.

Application of TIBA decreased DLR in the upper part of PR, lower part of PR, and the whole PR in Col-0 under the four P treatments (Table 2). And the DLR in the upper part of PR was higher than that in the lower part of PR under the same P supply (Table 2). For instance, TIBA reduced the density of total lateral roots (DTLR)

Table 3. Effects of heterogeneous phosphorus availability on primary root length, DLR, and DTLR in auxin transport-related mutant. Means ± SE from four independent experiments, for each at least 12 samples. Different letters associated with the same parameter in each genotype group indicate the significant difference among P treatments ($P < 0.05$)

Genotype	Treatment	Length of PR [cm]	DLR in upper part [cm^{-1}]	DLR in lower part [cm^{-1}]	DTLR [cm^{-1}]
Col-0	HP/HP	8.7 ± 0.17a	4.9 ± 0.72 ab	2.1 ± 0.09a	2.6 ± 0.05b
	HP/LP	5.2 ± 0.61b	4.3 ± 0.32b	1.8 ± 0.32a	2.6 ± 0.28b
	LP/HP	7.8 ± 0.48a	6.1 ± 0.38a	1.3 ± 0.33a	2.3 ± 0.16b
	LP/LP	4.2 ± 0.34b	5.9 ± 0.48a	2.3 ± 0.50a	3.7 ± 0.50a
aux1-7	HP/HP	10.4 ± 0.49a	5.0 ± 0.26a	1.2 ± 0.17a	1.9 ± 0.15b
	HP/LP	8.9 ± 0.58b	4.0 ± 0.28a	1.4 ± 0.06a	2.0 ± 0.07ab
	LP/HP	7.9 ± 0.44b	5.3 ± 1.08a	1.7 ± 0.28a	2.4 ± 0.36b
	LP/LP	4.7 ± 0.29c	5.1 ± 0.49a	1.9 ± 0.28a	3.1 ± 0.1a
pin2	HP/HP	9.6 ± 0.83a	5.4 ± 0.53c	1.8 ± 0.18a	2.4 ± 0.04b
	HP/LP	7.3 ± 0.86b	7.0 ± 0.73ab	1.7 ± 0.14a	2.5 ± 0.13b
	LP/HP	5.2 ± 0.20c	8.1 ± 0.52a	2.8 ± 0.30a	4.4 ± 0.25a
	LP/LP	3.2 ± 0.13d	7.7 ± 0.75a	2.5 ± 0.56a	4.2 ± 0.52a
pin3-1	HP/HP	10.7 ± 0.06a	6.1 ± 0.57ab	2.0 ± 0.24b	2.6 ± 0.15bc
	HP/LP	5.9 ± 0.61b	5.8 ± 0.82b	1.0 ± 0.20b	1.9 ± 0.23c
	LP/HP	5.5 ± 0.53b	7.7 ± 0.25a	3.2 ± 0.54a	4.5 ± 0.50a
	LP/LP	3.8 ± 0.22c	7.8 ± 0.48a	1.1 ± 0.35b	3.1 ± 0.28b
pin4-1	HP/HP	10.0 ± 0.80a	6.1 ± 0.54ab	2.0 ± 0.12ab	2.6 ± 0.14b
	HP/LP	8.6 ± 0.38a	5.6 ± 0.43ab	1.5 ± 0.15bc	2.1 ± 0.15b
	LP/HP	5.1 ± 0.22b	7.3 ± 0.89a	2.4 ± 0.20a	4.0 ± 0.32a
	LP/LP	4.5 ± 0.20b	4.7 ± 0.64b	1.4 ± 0.18c	2.4 ± 0.19b
pin7-3	HP/HP	11.1 ± 0.12a	5.5 ± 0.13b	2.1 ± 0.04a	2.7 ± 0.05b
	HP/LP	8.2 ± 0.70b	5.2 ± 0.46b	1.8 ± 0.08a	2.3 ± 0.07b
	LP/HP	5.7 ± 0.23c	6.8 ± 0.41ab	2.6 ± 0.15a	3.7 ± 0.10a
	LP/LP	3.7 ± 0.26d	7.3 ± 0.89a	2.5 ± 0.56a	3.9 ± 0.24a

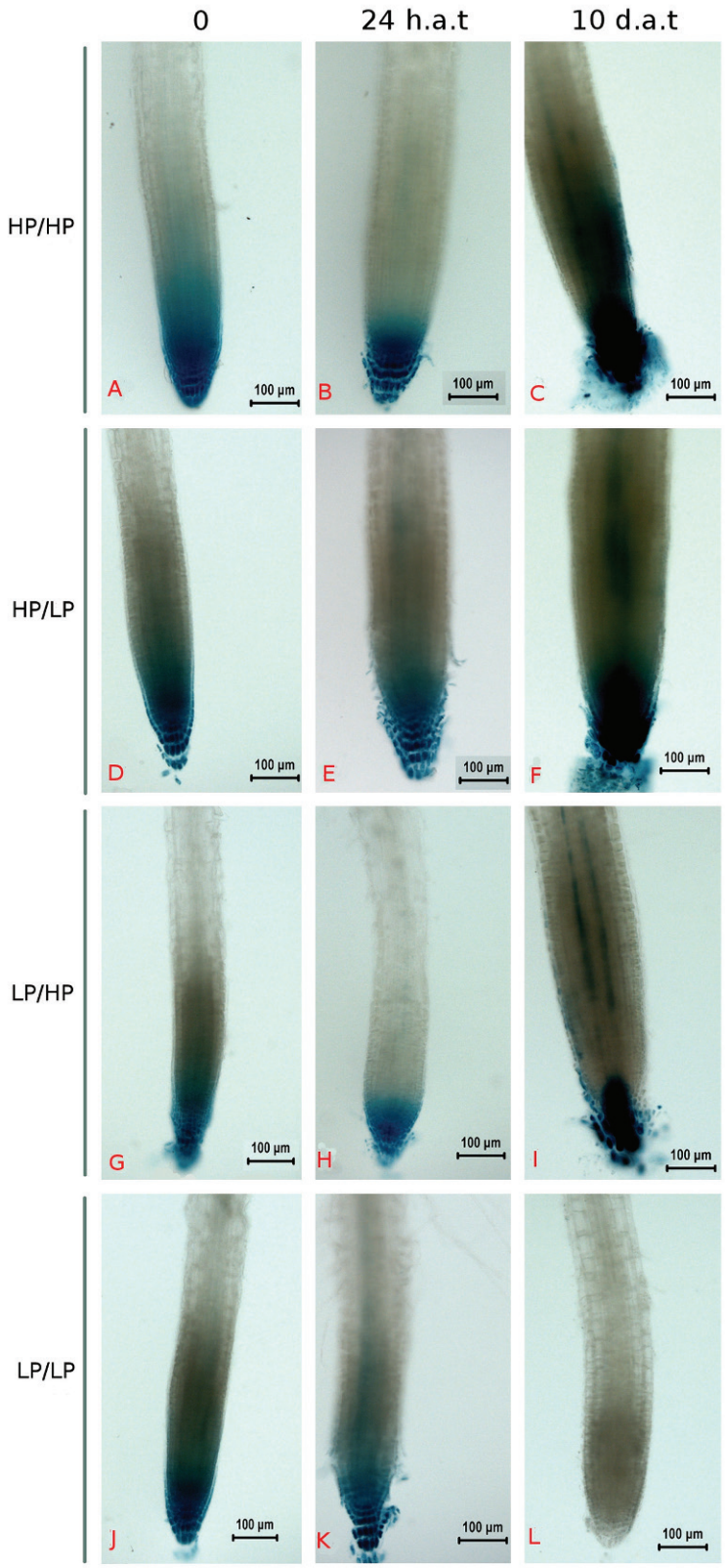

Fig. 2. Effects of heterogeneous phosphorus availability on auxin distribution in the primary root of transgenic *DR5::GUS Arabidopsis* seedlings harvested at 0 h (*A*, *D*, *G*, and *J*), 24 h (*B*, *E*, *H*, and *K*), and 10 d (*C*, *F*, *I*, and *L*) after transplantation (24 h.a.t and 10 d.a.t, respectively). Histochemical GUS staining was carried out as described in Materials and methods. Representative seedlings of at least 20 plants were selected to take a photograph.

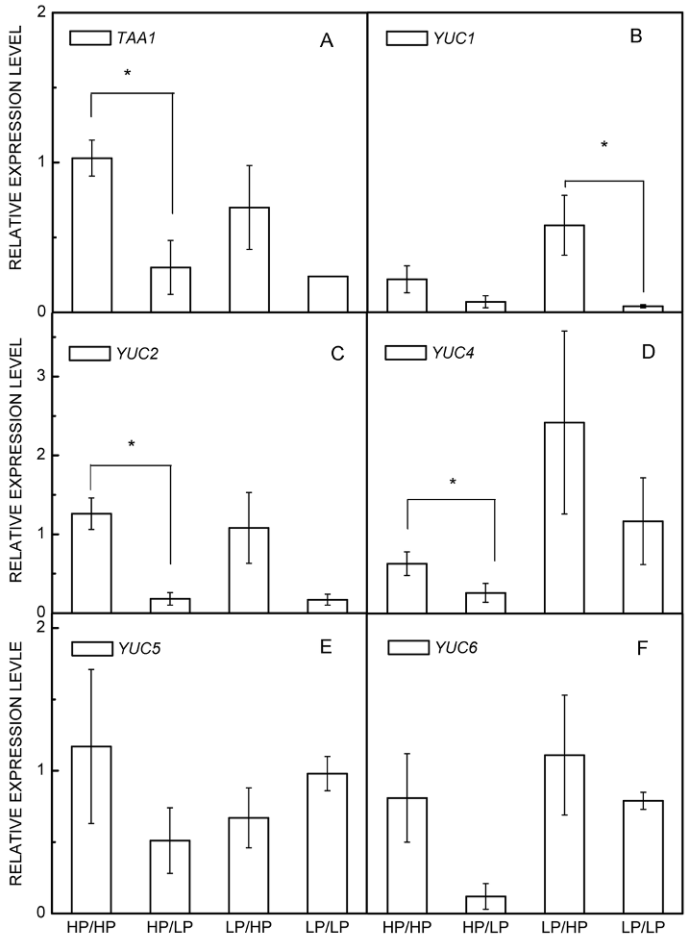

Fig. 3. Effects of heterogeneous phosphorus availability on the expression of auxin biosynthesis-related genes in Col-0 seedlings. cDNA was extracted from the whole plants. The relative expressions of *TAA1* (*A*), *YUC1* (*B*), *YUC2* (*C*), *YUC4* (*D*), *YUC5* (*E*), and *YUC6* (*F*) were determined by qRT-PCR. Data are means ± SE from three independent experiments and *asterisks* indicate the significant differences between HP/HP and HP/LP, or LP/HP and LP/LP treatments ($P < 0.05$).

from 3.8 ± 0.35 to 1.5 ± 0.17 LR per cm PR in HP/HP and from 5.7 ± 0.81 to 2.0 ± 0.32 LR per cm PR in LP/LP at day 14 after treatment. In addition, TIBA significantly down-regulated the DLTR in HP/LP and slightly decreased DLTR in LP/HP. When considering the inhibitory effects of TIBA on DLR in the upper part and lower part of PR, it was obvious that application of TIBA in the lower part was more effective than in the upper part (Table 2). In contrast to the control (no TIBA application), the DLR of Col-0 was decreased to 18.5 % in LP/LP but to 22.2 % in HP/HP. In controls, the differences of DLR in the upper part, lower part, and the whole PR between HP/HP and LP/LP treatments were significant, however, the application of TIBA masked the difference of DLR between HP/HP, HP/LP, LP/HP, and LP/LP treatments (Table 2).

In contrast to Col-0, *YUC1-OX* showed denser LR in the upper part, lower part, and the whole PR under HP/HP, HP/LP, and LP/LP conditions, respectively (Table 4). Consistently, loss-of-function of *YUC1* and *YUC6* decreased the DLR in the upper part, lower part, and the whole PR under all P treatments. In comparison with Col-0, number of LR in *yuc1 yuc6* were fewer. Unlike in Col-0, LP/LP treatment did not increase DTLR in *yuc1 yuc6* (Table 2).

Compared to Col-0, loss-of-function of *TIR1* significantly inhibits LR formation in all tested seedlings. Moreover, unlike Col-0, DLR in the lower part and the whole PR of *tir1* mutant showed no difference among the four P treatments (Table 2). Relative to Col-0, mutations of *AUX1* and *PIN3* did not alter the response pattern of LR development to heterogeneous P availability based on DLR in different parts of PR (Table 3). We also noticed that relative to Col-0 and other mutants, HP/LP treatment increased the DLR in the upper part of PR in the *pin2* mutant. In contrast to LP/HP, LP/LP increased DLR of the down part of PR in Col-0, *aux1-7*, *pin2*, and *pin7-3* but not in *pin3-1* and *pin4-3* (Table 3). When considering the difference of DLR in whole PR, unlike Col-0, *aux1-7*, *pin3-1*, and *pin4-3*, the difference between LP/HP and LP/LP in *pin2* and *pin7-3* was insignificant (Table 3).

We verified that heterogeneous P availability affected

auxin accumulation in PR (Fig. 2). To reveal the underlying molecular mechanisms, qRT-PCR was employed to understand expression pattern of genes involved in auxin biosynthesis. Six candidate genes analyzed included *TAA1*, *YUC1*, *2*, *4*, *5*, and *6*. TAA1 catalyzes the conversion of tryptophan to indole-3-pyruvic acid (Stepanova *et al*. 2008; Tao *et al*. 2008). Data in Fig. 3 indicate that in contrast to HP/HP, HP/LP significantly repressed the expression of *TAA1*, *YUC2*, and *YUC4* (Fig. 3). Compared to LP/HP treatment, LP/LP decreased the transcripts of *YUC1*. In addition, compared to HP/HP, HP/LP slightly but not significantly inhibited the expressions of *YUC1*, *YUC5*, and *YUC6* . Relative to LP/HP, LP/LP slightly decreased the abundances of *TAA1*, *YUC2*, *YUC4*, and *YUC6*. These results suggest that spatial P availability altered the expression of auxin biosynthesis-related genes.

Discussion

In this study, we found that low P availability significantly inhibited PR growth (Table 1), which is consistent with previous studies (Williamson *et al.* 2001, López-Bucio *et al.* 2002, Jain *et al.* 2007). More interestingly, PRs in LP/HP media were longer than those in HP/LP media (Fig. 1, Tables 1 and 2) similarly as reported previously by Svistoonoff *et al.* (2007). Hence, we concluded that down part low P more effectively arrested PR growth than upper part low P. The possible reason is that the meristem and elongation zone of PR is crucial for PR growth and the important signalling site for P availability is localized in a root tip as suggested previously (Svistoonoff *et al.* 2007, Arnaud *et al*. 2010).

Application of TIBA suppressed the growth of PR under all P treatments but increased the ratio of PRL in the upper and lower parts (data not shown). This strongly implies that response of PR growth to heterogeneous P availability is dependent on PAT. On the other hand, over-expression of *YUC1*, the key enzyme for auxin biosynthesis, suppressed PR growth under all P treatments (Table 2). These results indicated that auxin homeostasis regulates PR growth. Moreover, the PRs of *tir1* under HP/HP and LP/HP conditions were shorter than PRs of Col-0 (Table 2). This demonstrated that locally high P concentration might promote PR growth *via* TIR1.

Single *pin* mutant shows slight difference in PR growth (Blilou *et al*. 2005). This is in accordance with our data (Table 3). Auxin content is increased in *pin4* root meristem especially in the root tips (Friml *et al*. 2002). It seems that the mutation in *PIN4* might alter the content of auxin in *Arabidopsis* roots under the four P treatments.

Application of TIBA inhibited LR formation under all P treatments (Table 2). This is consistent with the study of López-Buci *et al.* (2002). More interestingly, application of TIBA increased the ratio of DLR of the upper part to DLR of the down part, and masked the stimulatory effects LP/LP on DLTR in contrast to other three P treatments (Table 2). Hence, we concluded that PAT was involved in the regulation of LR development in response to heterogeneous P availability.

Over-expression of *YUC1* stimulated DTLP in PR and loss-of-function of *YUC1* and *YUC6* reduced DTLP in PR under the four P treatments (Table 2) confirming that auxin biosynthesis machinery modulates LR development. Relative to Col-0, loss-of-function of *TIR1* attenuated the stimulatory effects of LP/LP on DTLR in contrast to LP/HP and decreased the DLR under HP/HP (Table 2). This verified that auxin signalling was also involved in LR development in response to both homogeneous and heterogeneous P availability. It has been established that LP stimulates the expression of *TIR1* at transcription level in PR, and in turn, increases the degradation of IAA17, a negative regulator of LR formation, leading to more LRs under low P (Pérez-Torres *et al*. 2008).

AUX1 is one of auxin transporters (Teale *et al.* 2006). Compared with Col-0, *aux1-7* mutant show similar responses to P availability based on LR development and DLRP (Table 3). Hence, we concluded that effects of spatial P availability on LR development is independent of AUX1. This is in agreement with the previous studies (Williamson *et al.* 2001, López-Bucio *et al.* 2002). In *Arabidopsis* root, PIN2 is localized apically in epidermal and lateral root cap cells and predominantly basally in cortical cells. PIN7 is expressed at lateral and basal membranes of provascular cells in the meristem and elongation zone (Paponov *et al*. 2005). In this study, loss-of-function of *PIN2* and *PIN7* masked the stimulatory effects of LP/LP on DTLR in contrast to LP/HP (Table 3) indicating the involvement of them in LR development under homogeneous P conditions. Moreover, PIN3 and PIN4 were involved in LR development in response to spatial P availability in terms of DLR in the lower part of PR under LP/HP and LP/LP (Table 3). *PIN3* is largely located in columella and stele of the elongation zone and *PIN4* is expressed in the central root meristem (Vieten *et al*. 2005). Hence, the involvement of PIN4 and PIN3 in response of root architecture to heterogeneous P availability might be ascribed to their activity in meristem and lateral transport of auxin, respectively. Consistently, the mutation of *PGP19* results in altered P deficiency responses in LR development (Jain *et al*. 2007), further supporting the notion that the involvement of auxin transport in the adaptations to heterogeneous P availability. It is important to explore the localization domains of auxin efflux genes under heterogeneous P treatment in contrast

to homogeneous P treatment in the near future.

Here, we found that LP increased the number of stage III lateral root primordium (LRP) in the upper part of PR, but decreased that in the down part of PR (Table 1). Moreover, the total LR number in LP/LP was less than that in HP/HP (Table 1). However, previous studies pointed out that homogeneous LP increases LR number and DLR in contrast to homogeneous HP (Ticconi *et al.* 2004, Jain *et al.* 2007, Pérez-Torres *et al*. 2008). The reasons may be that Ticconi *et al* (2004) firstly cultured *Arabidopsis* in high P media for 5 d and then treated with +P/-P, or –P/+P, respectively, for 6 d; Jain *et al*. (2007) cultured *Arabidopsis* under very low irradiance (25 to 30 μmol m^{-2} s^{-1}) in +P (1.25 mM) or –P (20 μM) media; and Pérez-Torres *et al.* (2008) planted *Arabidopsis* in high P and low P up to 8 d; however, we grew *Arabidopsis* for 21 d. As was reported, the long-term low P stress resulted in the decreased growth of PR due to the complete loss of meristem activity in the root tip (Sánchez-Calderón *et al.* 2005), so LR formation was drastically inhibited during the late phase of low P treatment, but LRs in HP/HP media developed continually leading to the higher LR number in HP/HP media than in LP/LP media (Table 1). More interestingly, we found that DTLR in LP/LP and HP/LP were higher than in LP/HP and HP/HP (Table 1), implying that locally low P signal is enough to boost density of LR on the whole PR.

Consistent with the *DR5::GUS* staining (Fig. 2), the down part LP, in contrast to down part HP, reduced the expressions of *TAA1*, *YUC1*, *YUC2*, and *YUC4* (Fig. 3). This indicated that heterogeneous P availability altered auxin accumulation *via* controlling auxin biosynthesis-related gene transcription. However, we extracted total RNA from the whole *Arabidopsis* seedlings and treated *Arabidopsis* with spatial P for 14 d; it is necessary to extensively explore the expression patterns of *TAA1* and *YUCs* in roots and shoots under short-term and long-term P treatments. Based on our results, we suppose that heterogeneous P availability possibly alters auxin content or distribution *via* transcriptional approach. Accordingly, identification of transcription factors that bind the *cis*-elements in the promoter region of those genes will facilitate the understanding of crosstalk of auxin and P signalling. Extensive studies on the activity of *TAA1* and *YUCs* at tissue or even cellular levels will deepen our understanding on the roles of auxin biosynthesis machinery in root architecture remodelling.

Recently, it was reported that P starvation inhibited LR formation and promotes PR growth in maize (Li *et al*. 2012). P starvation down-regulated *AUX1* and up-regulated tryptophan synthase α-chain 1.94-fold and the anthranilate synthase α2 subunit 1.64-fold indicating that LP affects auxin biosynthesis *via* Trp-dependent pathway (Li *et al*. 2012). In addition, P deprivation induced the expression of *ARF19* in *Arabidopsis* root (Woo *et al*. 2012). ARF19 promotes LR formation and is induced by high auxin content (Okushima *et al*. 2007). It appears that regulation of the auxin biosynthesis-related gene expression by low P availability is universal across plants. Taken together, we verified the involvement of auxin in the root plasticity to P availability: *1*) the effects of P availability on PR growth and DLR was dependent on PAT (Tables 2 and 4); *2*) TIR1 was involved in the responses of PR growth and LR development and density to P availability (Table 2); *3*) the effects of P on LR density was dependent on *YUC1*, *PIN2*, *PIN3*, *PIN4*, and *PIN7* (Tables 2 and 3); *4*) P availability altered the accumulation of auxin in PR (Fig. 2) and the expressions of *TAA1*, *YUC1*, *YUC2*, and *YUC4* (Fig. 3). Auxin is not only the integrator of root architecture plasticity response to homogeneous P availa-bility but also the modulator of root architecture remodeling in response to heterogeneous P availability.

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