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Phosphate Starvation Induces a Determinate Developmental Program in the Roots of *Arabidopsis thaliana*

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When growing under limiting phosphate (P) conditions, *Arabidopsis thaliana* plants show dramatic changes in root architecture, including a reduction in primary root length, increased formation of lateral roots and greater formation of root hairs. Here we report that primary root growth inhibition by low P is caused by a shift from an indeterminate to a determinate developmental program. In the primary root, the low P-induced determinate growth program initiates with a reduction of cell elongation followed by the progressive loss of meristematic cells. At later stages, cell proliferation ceases and cell differentiation takes place at the former cell elongation and meristematic regions of the primary root. In low P, not only the primary but also almost all mature lateral roots enter the determinate developmental program. Kinetic studies of expression of the cell cycle marker *CycB1;1:uidA* and the quiescent center (QC) identity marker *QC46:GUS* showed that in low P conditions, reduction in proliferation in the primary root was preceded by alterations in the QC. These results suggest that in *Arabidopsis*, P limitation can induce a determinate root developmental program that plays an important role in altering root system architecture and that the QC could act as a sensor of environmental signals.

Keywords: *Arabidopsis* — Cell division — Determinate growth — Phosphate availability — Root meristem.

Abbreviations: APase, acid phosphatase; GUS, β-glucuronidase; HPM, high P medium; LPM, low P medium; QC, quiescent center

Introduction

Plant root systems perform many essential adaptive functions including water and nutrient uptake, anchorage to the soil and the establishment of biotic interactions in the rhizosphere (Malamy and Benfey 1997a). Changes in the architecture of the root system, therefore, can profoundly affect the nutrient and water uptake capacity of plants. There are various processes that can affect the overall root system architecture: (i) cell division and elongation rates at the primary root tip, which determine the extent of primary root growth; (ii) lateral root formation, which increases the exploratory capacity of the root system; and (iii) root hair formation, which increases the total root surface of primary and lateral roots (Barlow 1976, Dolan et al. 1994, Celenza et al. 1995). Alterations in any of these processes can have profound effects on the architecture of the root system, thus altering the capacity of plants to grow in soils with limiting nutrient resources (Bates and Lynch 2000). Although root development is of key agronomic importance, little is understood about the processes and signals that regulate the architecture of the root system.

Root development is highly sensitive to environmental cues. Among these, temporal and spatial variations in the supply of soil nutrients such as nitrogen (N), phosphorus (P) and iron (Fe) have a major influence on root growth and architecture. These nutrients alter root patterning through particular signal transduction pathways (Zhang and Forde 1998, Zhang et al. 1999, López-Bucio et al. 2003).

Because of its low mobility in the soil solution, P is one of the most limiting nutrients for plant growth and crop productivity. Low P availability is a major constraint for plant productivity in alkaline and acid soils which make up >70% of the total arable land in the world (Schachtman et al. 1998). As an adaptive response to low P availability, many wild and cultivated plant species including tomato (*Lycopersicum esculentum* L.), bean (*Phaseolus vulgaris* L.) and white lupin (*Lupinus albus* L.) alter their root developmental programs towards the formation of branched root systems (Lorenzo and Forde 2001). In white lupin, low P conditions stimulate the formation of cluster roots, also termed proteoid roots, which have determinate growth and are densely covered by root hairs (Johnson et al. 1996). Determinate growth is a particular developmental process in which meristematic cells divide only for a limited period of time and then differentiate (Barlow 1976). This pattern of root development has also been reported in lateral roots of maize (Varney and McCully 1991), in proteoid roots of *Grevil-*

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lea robusta (Skene et al. 1996), in *Allium porrum* roots colonized by mycorrhizal fungi (Berta et al. 1990) and in adventitious roots of some Pteridophytes (Gunning et al. 1978). Determinate growth of the primary root as a stable developmental program has been reported only for certain desert Cactaceae (Dubrovsky 1997, Rodríguez-Rodríguez et al. 2003). Little is known of the signals that cause determinate growth in plants and the biological significance of this process.

Alterations in root system architecture in response to P deficiency have been reported recently for *Arabidopsis*, opening up the possibility of using this model plant to investigate the molecular mechanisms controlling nutrient perception and their developmental consequences. In *Arabidopsis*, P deficiency stimulates lateral root formation and root hair elongation but inhibits primary root growth, suggesting that P availability acts by controlling the activity of the primary root meristem by an as yet unknown mechanism (Williamson et al. 2001, López-Bucio et al. 2002). In the present work, we studied the cellular processes responsible for the inhibition of root growth induced by P deprivation. Our results show that P deprivation alters cell division and elongation and induces an irreversible shift from an indeterminate to a determinate root growth program in which the quiescent center (QC) seems to play a central role.

Results

Low P availability alters root system architecture and the structure of the root meristem

Arabidopsis seeds (ecotype Col-0) were germinated on vertically oriented agar plates containing 0.1× Murashige and Skoog (MS) medium supplied with contrasting P concentrations. Plants growing in 1 mM P (high P medium, HPM) developed long primary roots with secondary roots forming close to the root–shoot junction and short root hairs. In contrast, plants grown in 1 μM P (low P medium, LPM) showed a decreased primary root length, a highly branched root system including secondary, tertiary and quaternary lateral roots and an abundance of long root hairs (Fig. 1A). After 16 days of growth, starch grains were present in columella cells of plants grown in HPM, suggesting that columella initials were active and continuously give rise to columella cells, which progress in their differentiation (Fig. 1B). In contrast, in 16-day-old plants grown in LPM, no starch grains were observed in the root cap, suggesting that the development of columella cells is arrested (Fig. 1C). Moreover, the primary root tips of these plants were 40–60% thicker than those of high P-grown plants and root hair cell differentiation progressed towards the root tip until it reached the meristem region (Fig. 1B, C, and data not shown).

To determine more carefully the cellular changes caused by P limitation, we carried out histological analyses of 12-day-old primary root tips of plants grown in HPM and LPM. In HPM, the structure of the root apical meristem was normal, having a well-defined quiescent centre, initial cells and starch grains clearly visible in the differentiated columella cells (Fig. 1D, F). In contrast, after 12 days of growth in LPM, dramatic changes were observed within the root apical meristem. Cells in the former meristematic area were weakly basophilic and highly vacuolated, no clearly defined QC and initial cells could

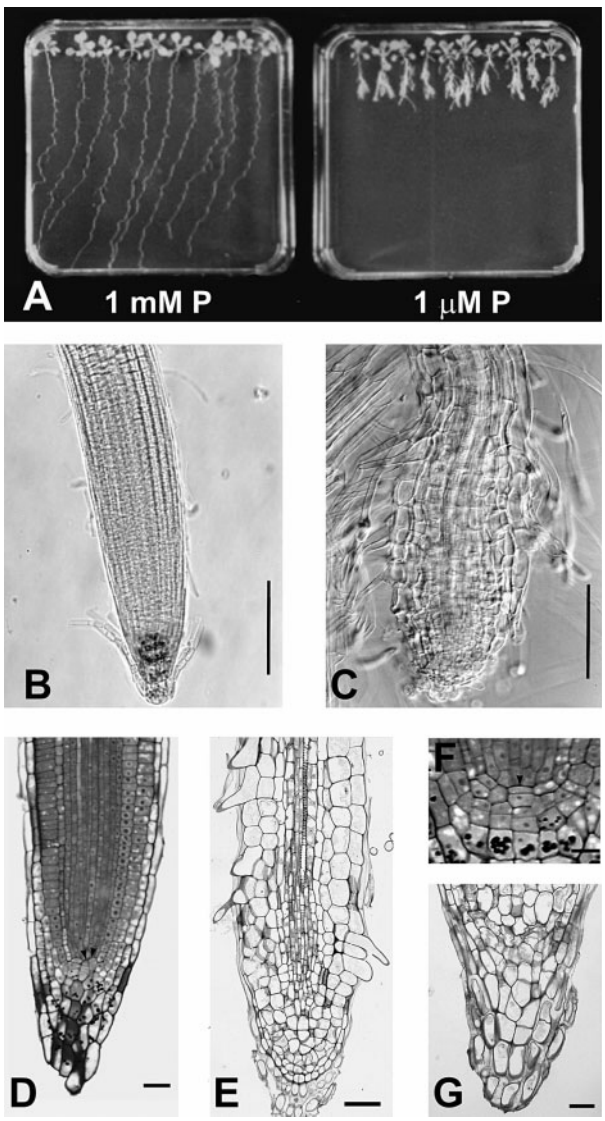


Fig. 1 Effects of P availability on *Arabidopsis* root architecture. (A) Photograph of *Arabidopsis* plants growing in low (right) and high (left) P media. Starch grains and root hairs observed in whole mounts of *Arabidopsis* primary roots grown in high (B) and low (C) P medium. Longitudinal sections of *Arabidopsis* (Col-0) root tips grown in media with high (D) and low phosphate (E). Photograph showing the QC region and columella cells of a high P plant (F). Note that no starch grains are present in the root cap in low P (G). Arrowheads indicate QC cells that were subjected to a periclinal division. (A–C) are from 16-day-old and (D–G) are from 12-day-old *Arabidopsis* seedlings. Scale bars in (B) and (C) = 100 μm. Scale bars in (D and E), (F) and (G) are equal to 20, 40 and 10 μm, respectively.

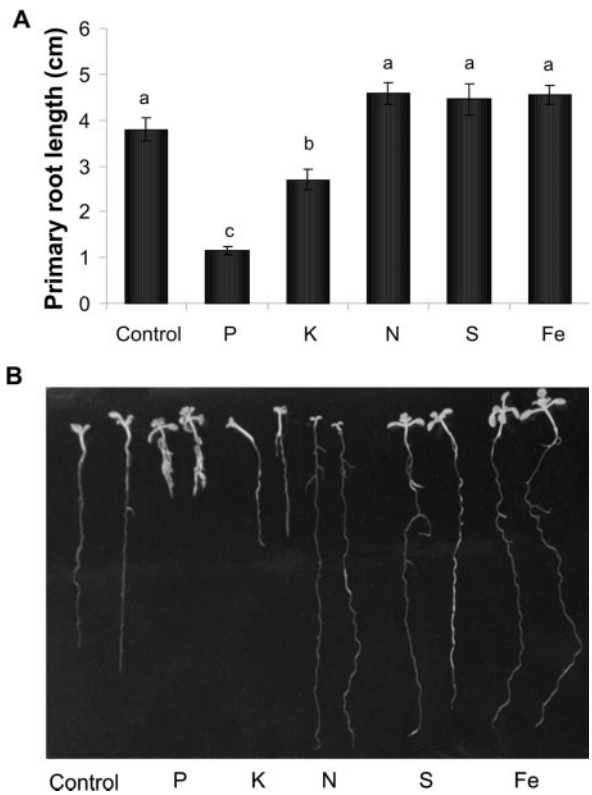


Fig. 2 Changes in *Arabidopsis* root architecture in response to various nutrient deficiencies. WT (Col-0) seedlings were grown for 14 d on media deprived of the indicated nutrients. (A) Effect of low nutrient availability on *Arabidopsis* primary root growth. (B) Photograph of plants showing the distinctive developmental responses to several nutrient deficiencies. Values shown in (A) represent the mean of 30 seedlings ± SE. Different letters indicate statical differences at $P < 0.05$ (Student's *t*-test).

be identified and no mitosis was observed (Fig. 1E, G). It was also noticed that differentiation occurred in the zone where previously meristematic cells were located (Fig. 1E). For instance, differentiated protoxylem was present within the first 140 μm from the root body–root cap junction (the region previously occupied by meristematic cells), and trichoblast differentiation to form root hairs occurred already within the first 10 epidermal cells from the initial cell and no starch grains were present in the root cap (Fig. 1E). Cell viability assays, using propidium iodide and Evans blue staining, showed that cells present in the root tip of P-deprived plants were alive at least 15 d after germination (data not shown).

We next examined the nutrient specificity of the primary root growth inhibition. Removal of N, Fe or S from the media did not inhibit primary root growth (Fig. 2A, B). Media lacking K decreased root growth rate, but did not lead to a complete arrest of primary root elongation or the formation of root hairs close to the root meristem (Fig. 2 and data not shown). This suggests that primary root growth inhibition and premature cell differentiation is specific for P deprivation.

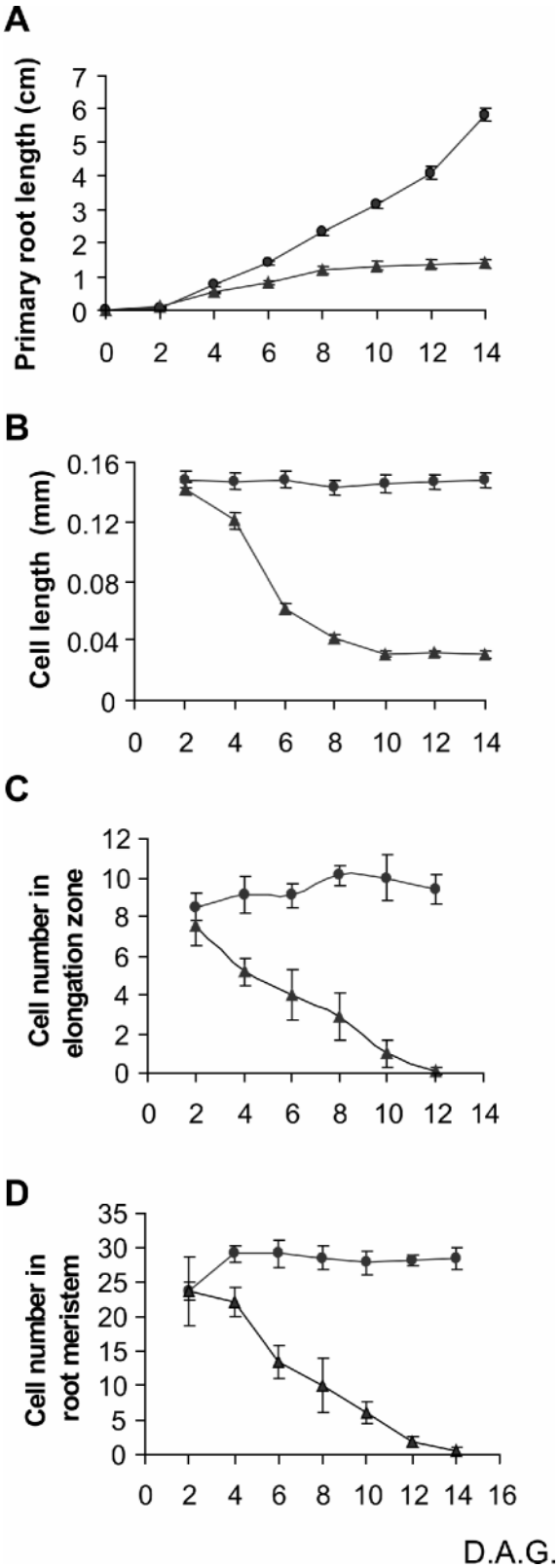
P deficiency alters cell elongation and cell division

To understand further the effect of low P conditions on primary root elongation, we carried out kinetic assays to compare the early temporal effects of P availability on primary root growth. During the first 4 d after germination, the primary roots of high and low P-grown *Arabidopsis* plants had similar root growth rates. However, 4 d after germination, a reduction in primary root elongation in low P-grown plants was observed when compared with high P-grown plants, which sustained a relatively constant growth rate (Fig. 3A). No further primary root growth was observed in 10-day-old, low P-grown plants, attaining lengths of only 1.3–1.5 cm by day 14 (Fig. 3A).

Root elongation is mediated by two components, the production of new cells by the root apical meristem and a rapid cell expansion in the elongation zone. Therefore, it was important to determine whether the short root phenotype observed in plants subjected to low P treatment was due to a reduction in cell size, reduced cell division or a combination of both. We first measured the cell length of recently differentiated epidermal cells in the differentiation zone of plants grown in high and low P treatments. In HPM, the length of differentiated epidermal cells remained constant over a 14 d period (Fig. 3B). In contrast, as early as 4 d after germination, an increasing reduction in the cell size of epidermal cells was observed in low P-grown plants. By day 10, the length of differentiated epidermal cells of LPM plants was 70% shorter than that of HPM plants (Fig. 3B).

Since epidermal cell differentiation occurred closer to the root meristem in plants grown in LPM, it was important to determine whether this was due exclusively to a reduction in the elongation zone as a consequence of a reduced cell length or to a premature differentiation of cells leaving the meristematic zone. Therefore, we counted the epidermal cells present in the elongation zone. We established the limits of the elongation zone along the root from the first cell that had twice the length of meristematic cells, to the first cell in which root hair initiation was clearly observed. In HPM, from day 2 onwards, the root elongation zone comprised 8–10 cells. In contrast, in LPM, not only was the cell length attained during the cell elongation process affected but also a dramatic reduction in the number of cells comprising the root elongation zone was observed. By day 12, the root elongation region was completely absent (Fig. 3C).

To study whether low P conditions could also affect cell division, we counted the cells present in the root meristematic zone and analyzed the temporal expression pattern of the cell cycle marker *CycB1;1:uidA* during a 14 d time course in transgenic plants growing in HPM and LPM. It was observed that the number of cells in the meristematic zone of plants growing in HPM was maintained relatively constant over time, whereas that of the plants grown in LPM started to decrease as early as 4 d after germination and was reduced further with time until no meristematic cells were identified (Fig. 3D).



To investigate the pattern of mitotic activity under low P conditions, we analyzed the activity of *CycB1;1:uidA* which, owing to a mitotic degradation signal, marks only actively dividing cells (Colón-Carmona et al. 1999). In seedlings grown in HPM, the root meristematic region showed a typical, patchy *CycB1;1:uidA* expression pattern in dividing cells covering an approximately 200 μm region (Fig. 4A–E). In LPM, *CycB1;1:uidA* expression was similar to that of HPM seedlings until day 4 (Fig. 4F,G). In contrast, at later times, the length of the cell division zone was gradually reduced until no mitotic activity was detected by day 12 (Fig. 4H–J).

P deficiency alters QC maintenance

A failure in root meristem maintenance can be caused by the loss of stem cell division potential, a more rapid differentiation of stem cell daughters or the lack of QC activity. To assess whether low P alters QC activity, we analyzed the expression of a QC identity marker (*QC46:GUS*) in high and low P conditions. In plants grown in HPM, typical QC expression of the *QC46:GUS* marker comprising 2–3 cells was observed over a 12 d period (Fig. 5A–F, Table 1). In contrast, in plants grown in LPM, as early as 2 d after germination, the QC46-expressing cells underwent a high frequency of periclinal or sometimes oblique cell divisions that were not observed in HPM plants. This was reflected in a 2-fold increase in the number of cells expressing *QC46:GUS* at 4 d after germination (Fig. 5G, Table 1). Division of QC cells continued up to day 6, and by day 8 a reduced number of small cells expressing the *QC46:GUS* marker was found (Fig. 5J, Table 1). By day 10, no expression of the QC marker was detected (Fig. 5K, L). A detailed microscopic analysis showed that in LPM, periclinal divisions occurred in an increasing number of plants, ranging from 24% at day 2 to 100% at day 4 (Table 1). Later in development in LPM, the percentage of plants expressing *QC46:GUS* gradually decreased with time from day 6, until no expression was detected by day 11 (Table 1).

It has been shown that an auxin maximum in the distal root tip is required for the correct specification of distal pattern elements and cell division programs. Alteration of this auxin maximum in auxin response mutants or by altering auxin transport produces defects in the cell fate and formation pattern (Sabatini et al. 1999). To determine whether alterations in QC cell specification correlated with changes in the auxin maximum, we examined the expression of the *DR5:GUS* marker in plants subjected to low or high P conditions (Ulmasov et al. 1997). The *DR5:GUS* auxin response reporter allows the cor-

Fig. 3 Root growth and cellular parameters in high and low P-grown *Arabidopsis* plants. Seedlings were grown on the surface of agar plates containing 0.1× MS medium with high and low P for the indicated number of days. (A) Kinetic assay of primary root growth. Mean cell length ± SE (B), epidermal cell number in the root elongation region (C) and epidermal cell number in the root meristem (D). Mean values were plotted at the indicated days after seed germination in the kinetic experimental ($n = 30$).

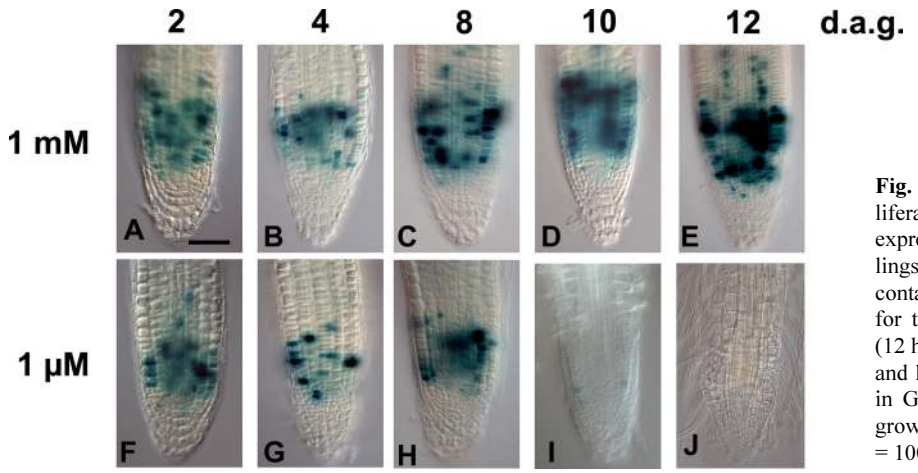


Fig. 4 (A–J) Effect of P availability on cell proliferation activity. Kinetics of *CycB1;1:uidA* expression in *Arabidopsis* primary root tips. Seedlings were grown on the surface of agar plates containing 0.1× MS medium with high and low P for the indicated number of days. GUS staining (12 h) of *CycB;1:uidA* roots grown in high (A–E) and low (F–J) P. Note the most dramatic decrease in GUS expression in root meristems of low P-grown plants at 12 d after germination. Scale bar = 100 µm.

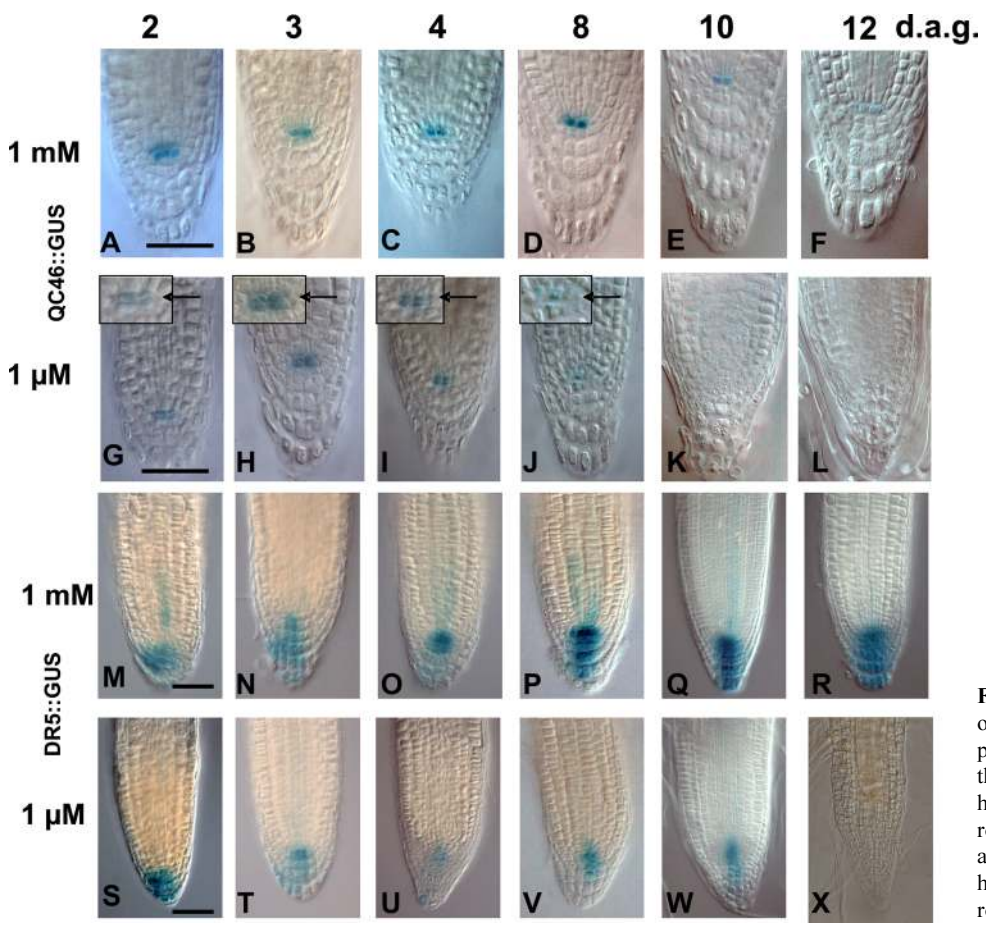


Fig. 5 (A–X) Effect of P availability on QC identity and auxin response in primary roots. Temporal expression of the QC-specific marker *QC46:GUS* in high (A–F) and low (G–I) P-grown roots. Kinetics of expression of the auxin response marker *DR5:GUS* in high (M–R) and low (S–X) P-grown roots. Scale bars = 100 µm.

rect visualization of the distal auxin maximum (Sabatini et al. 1999). In HPM, the expression of *DR5:GUS* displayed β-glucuronidase (GUS) activity in the columella initials, the QC and the mature columella root cap (Fig. 5M–R), similar to that previously reported (Sabatini et al. 1999). In seedlings grown in LPM, *DR5:GUS* expression was very similar to the HPM plants until day 3 after germination (Fig. 5S, T). However, at later stages of growth, a decrease in intensity and in the number of cells expressing GUS was detected and by day 12, *DR5:GUS* expression was totally absent (Fig. 5U–X).

Phosphate deficiency induces a determinate growth program in lateral roots

To investigate whether determinate growth also occurs in lateral roots of plants grown in LPM, we conducted analyses of the morphology and expression of the *CycB1;1:uidA* marker in

Table 1 Effect of P availability on cell division frequency of quiescent centre cells

DAG	No. of cells expressing QC46 marker		Plants presenting QC cell division (%)		Plants expressing QC46 marker (%)	
	High	Low	High	Low	High	Low
2	2.38 ± 0.10	2.46 ± 0.17	0	23.7	100	100.0
3	2.23 ± 0.09	3.61 ± 0.19	0	76.0	100	100.0
4	2.23 ± 0.09	4.77 ± 0.20	0	100.0	100	100.0
5	2.23 ± 0.09	4.46 ± 0.17	0	100.0	100	100.0
6	2.15 ± 0.37	4.66 ± 0.20	0	100.0	100	100.0
7	2.15 ± 0.37	4.45 ± 0.16	0	100.0	100	74.6
8	2.23 ± 0.05	4.57 ± 0.30	0	100.0	100	53.9
9	2.38 ± 0.10	4	0	100.0	100	15.4
10	2.30 ± 0.12	4	0	100.0	100	7.7
11	2.15 ± 0.37	0	0	100.0	100	0.0
12	2.07± 0.05	0	0	100.0	100	0.0

Transgenic seedlings expressing the *QC46:GUS* marker were grown on nutrient media with contrasting P concentrations on vertically oriented agar plates. At several days after germination (DAG), the seedlings were stained for *GUS* activity. Cells expressing *GUS* were counted at the indicated days ($n = 13 \pm$ SE).

lateral roots at different developmental stages in 12- to 14-day-old plants. A 4 d visual survey for lateral root growth in high P root systems starting at day 12 after germination failed to identify any primary or lateral root showing root hairs close to the root tip (data not shown). Expression of *CycB1;1:uidA* was normal in all lateral roots of plants grown at high P up to day 14, showing a patchy pattern of expression in the meristem (Fig. 6A). Similarly to HPM, in LPM root systems we observed elongating lateral roots at different developmental stages ranging from recently emerged (0–5 mm; Fig. 6B) to mature (5–10 mm; Fig. 6C) roots that showed a strong mitotic activity in the meristematic region. However, in older lateral roots ranging from 15 to 20 mm in length, little or no *CycB1;1:uidA* activity was detected (Fig. 6D and E). Lateral roots lacking *CycB1;1:uidA* expression had fully exhausted meristems (Fig. 6E). Analysis of 14-day-old root systems in LPM revealed that 52% of the lateral roots had arrested growth, produced root hairs at the tip and showed absence of *CycB1;1:uidA* expression (data not shown).

Effect of low P on phosphatase activity and on the expression directed by the promoters of high-affinity P transporter genes

It has been shown that limiting P conditions trigger a low P rescue system that includes enhanced excretion of acid phosphatase (APase) and the transcriptional activation of P transport genes (Raghothama 1999). To investigate the relationship between the determinate root developmental program triggered by limiting P conditions and this low P rescue system, we monitored the induction of two phosphate-regulated processes, namely the excretion of APase and the expression directed by the promoters of the *AtPT2* and *AtPT1* P transporter genes.

To analyze whether determinate growth correlated with APase production in the root system of *Arabidopsis*, a kinetic analysis of phosphatase excretion was performed in plants grown in HPM and LPM. In order to visualize APase activity without disturbing the entire root system, the colorless substrate 5-bromo-4-chloro-3-indolylphoshate (BCIP). was applied in an agarose overlay to the roots of plants growing over the agar surface (Zakhleniuk et al. 2001). Under our experimental conditions, no APase activity was observed in *Arabidopsis* plants grown in HPM up to day 16 after germination (Fig. 7A) and in LPM-grown plants up to day 8 (Fig. 7B). In contrast, 10 d after germination, a clear blue staining pattern indicative of induced APase activity was detected in the root tip of plants grown in LPM (Fig. 7C, D). The greatest increase in APase activity was observed in the root tip and root hairs of primary roots with exhausted meristems (Fig. 7E).

pAtPT2:uidA plants supplied with 1 mM P showed no detectable GUS activity in the root system, including the root meristem during the course of the experiment (16 d) (Fig. 7F) and in LPM-grown plants up to day 8 (Fig. 7G). However, starting from 10 d of growth in LPM, the plants showed high

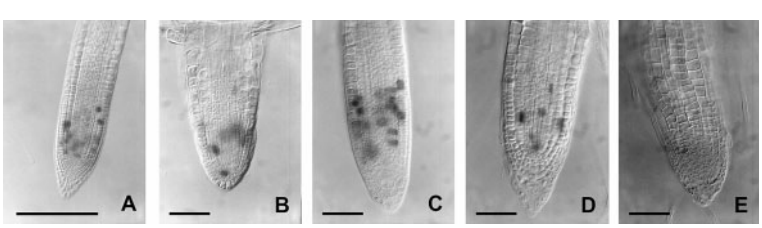


Fig. 6 (A–E) Effect of P availability on *CycB1;1:uidA* expression in lateral roots. GUS staining (12 h) of *CycB1:uidA* on a lateral root at high P (A) and in lateral roots at different stages of development in 14-day-old *Arabidopsis* plants grown under low P (B–E). Scale bars = 100 μm.

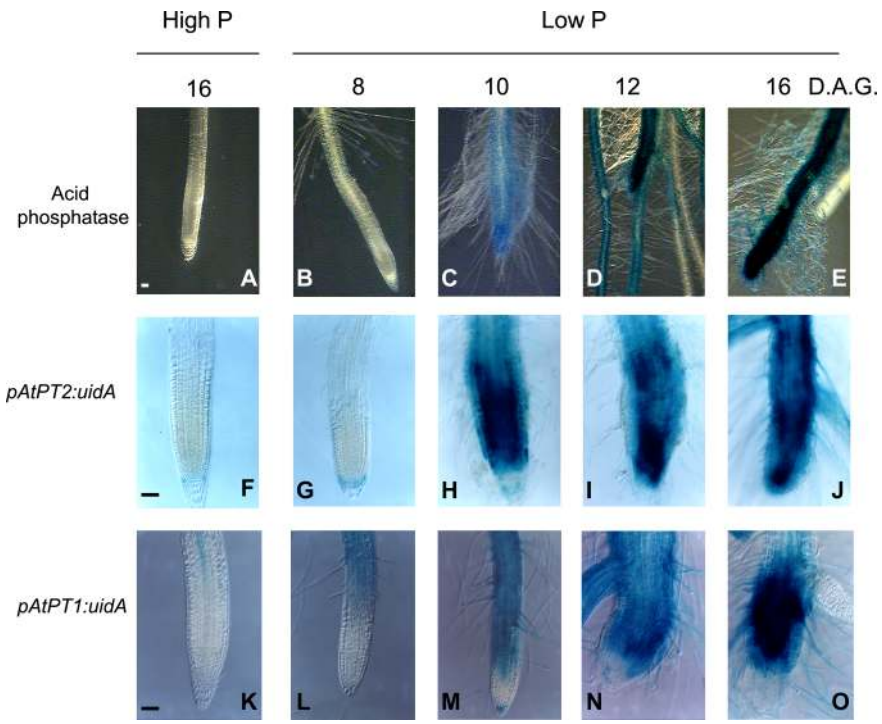


Fig. 7 (A–J) Effects of P availability on phosphatase activity and P transporter expression in primary roots of *Arabidopsis*. Staining for acid phosphatase activity in *Arabidopsis* plants grown in 1 mM (A) and 1 μM (B–E) P. GUS staining for *pAtPT2:uidA* expression in *Arabidopsis* roots grown in 1 mM (F) and 1 μM (G–J) P. GUS staining for *pAtPT1:uidA* expression in *Arabidopsis* roots grown in 1 mM (K) and 1 μM (L–O) P. Note the dramatic increase in phosphatase activity and P transporter expression in root meristems of low (1 μM) P-grown plants between 10 and 16 d after germination Scale bar = 100 μm.

pAtPT2:uidA expression in the entire root system except the most distal part of the root tip (Fig. 7H, I), and by day 16 all the root system including the entire root tip and root hairs presented strong GUS activity (Fig. 7J).

As previously reported (Karthikeyan et al. 2002), *pAtPT1:uidA* plants grown in HPM showed GUS expression in the entire root system, except in the root tips (Fig. 7K). In plants grown in LPM, *pAtPT1:uidA* activity was also detected in the root system except the root tip until day 10. The intensity of GUS staining became stronger after 8 d of growth in LPM, at day 10 GUS activity was detected closer to the columella and after day 12 GUS activity was detected in the entire root tip including the root hairs formed in the former meristematic zone (Fig. 7M–O). These results suggest that low phosphate availability induces a determinate primary root growth program that correlates with increased phosphatase activity and enhanced expression of P transporters.

Low P-induced root meristem exhaustion is not reversed by a high P treatment

To investigate whether the determinate root growth induced by low P is an irreversible process, *Arabidopsis* seedlings were germinated in LPM and, after 6, 7 and 8 d of growth, transferred to HPM. Seven days after the transfer, primary root growth was measured. It was observed that when 6- and 7-day-old seedlings grown in LPM were transferred to HPM, the elongation of their primary root was reactivated (Fig. 8A, B). In contrast, the length of the primary root of seedlings transferred to HPM after 8 d of growth in LPM remained the same; no further growth was recorded and no cell proliferation activity was observed in the primary root meristem (Fig. 8C and data not shown). Interestingly, a comparison of the growth of distal (closest to the root apex) and proximal (closest to the root base) lateral roots in plants transferred from LPM to HPM revealed that at day 8, primary root meristem exhaustion was accompanied by activation of the growth of the distal lateral root growth contrary to the proximal one (Fig. 8A–C).

Discussion

Plant meristems control the development of plant organs through balanced cell proliferation and differentiation. Root meristems contain a distinctive region of mitotically relativey inactive cells, the QC, surrounded by initial cells that perform stem cell divisions to generate a new initial cell and a daughter cell. The daughters that are no longer in contact with the QC differentiate according to positional cues, carry out a small number of cell divisions producing new cells that undergo a rapid axial elongation and then differentiate to produce all the cell types present in a mature root (Nakajima and Benfey 2002). Although the whole plant and the structure of the meristem are pre-established during embryo development, meristematic activity can be significantly altered by environmental cues during post-embryonic development. Among the nutrients that alter post-embryonic root development, low P availability has been shown to have an important effect on root architecture in many plant species (Neumann and Martinoia 2002, López-Bucio et al. 2003).

One of the most conspicuous effects of low P availability on root development is the inhibition of primary root growth

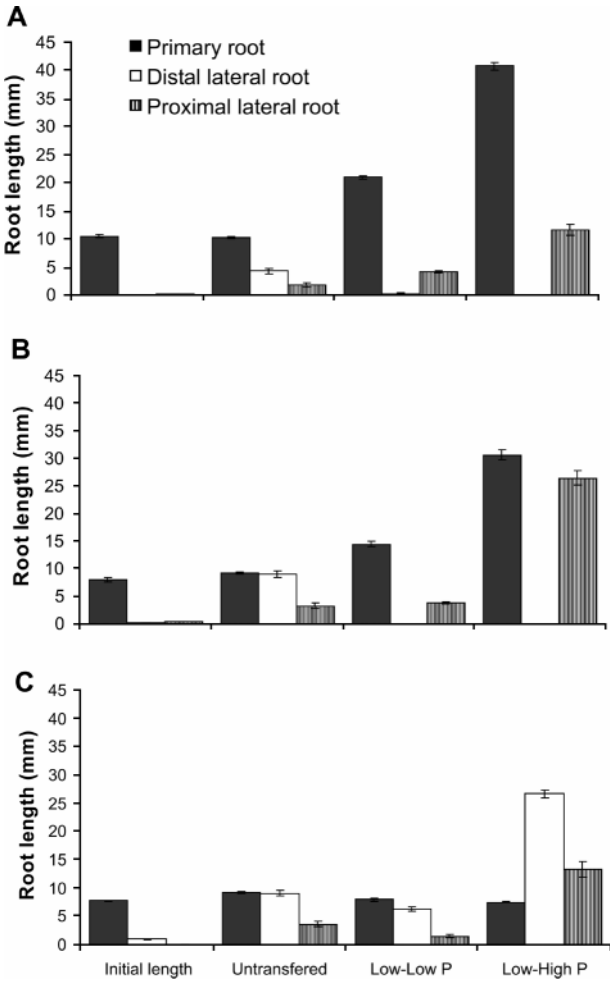


Fig. 8 Local response of primary and lateral root growth to P availability. At days 6 (A), 7 (B) and 8 (C), plants were transferred from LPM to either LPH or HPM or not transferred. Root length was measured 7 d after the transfer. Each bar represents the mean plus the SD of 10 plants. The experiment was repeated three times with similar results.

and the increased formation of lateral roots. Our results show that the reduction of primary root elongation observed in P-deprived seedlings is a complex process that involves a reduction in cell elongation and a progressive reduction in the length of the meristem. A reduction in mature epidermal cell length was observed initially 4 d after germination and further decreased, until at day 10 epidermal cell length was only 20% of that in plants grown in HPM. The roots of low P-stressed plants progressively lose cells in the meristem as a consequence of decreased cell division events and premature cell differentiation. The reduction of cell proliferation was caused by a reduction in the number of meristematic cells and a decrease in mitotic activity as observed using the *CycB1,1::uidA* reporter gene. A reduction in cell length in the root tip of plants grown in LPM has been reported previously (Williamson et al. 2001). We also observed that the number of cells in the root elongation zone decreased in low P conditions and that this reduction correlated with a premature differentiation of relatively short cells that exit the meristem. For example, trichoblasts of low P-grown plants formed root hairs much closer to the root tip than in high P-grown plants (Fig. 1B, C), and protoxylem differentiation was observed to occur very close to the meristematic zone (Fig. 1E). Taken together, these results show that P deprivation induced *Arabidopsis* roots to enter a determinate developmental program in which cell division is arrested and cell differentiation is promoted. Low P-induced determinate root growth as an adaptive response to low P availability has been reported previously in the proteoid roots of white lupin and *G. robusta* (Johnson et al. 1996, Skene et al. 1996).

The earliest alteration observed in P-deprived seedlings was the periclinal cell divisions of QC cells, which occurred at day 2 of growth and preceded changes in cell elongation and meristem size reduction. QC cells later became smaller; no QC activity was detected as indicated by the expression of the QC identity marker *QC46:GUS* and anatomical analysis. These observations suggest that the effect of low P availability on cell proliferation and accelerated cell differentiation in the meristematic zone could be due to a direct or indirect effect of P deprivation on the activity and/or maintenance of the QC. In the *Arabidopsis* root meristem, positional signals for proper differentiation appear to derive from more mature cells to guide the cell fate of the same cell type, whereas the QC primarily arrests cell differentiation to allow cell division of stem cells (van den Berg et al. 1995, van den Berg et al. 1997). Since the earliest detected event of low P availability is on changes in identity of the QC followed by a progressive decrease in cell number within the meristem and cell differentiation closer to the root tip, we propose that the QC acts as a sensor of environmental signals that affect meristem maintenance. The role of the QC as a sensor of environmental signals could involve regulation of proliferative activity and the timing of the differentiation of cells that exit the meristem. However, it is also possible that P availability could regulate other signaling pathways involved in the regulation of root meristem maintenance, such as those involving CLAVATA 19 (CLE19) or the cyclin-dependent kinase-activating kinase *Cak1At* (Umeda et al. 2000, Casamitjana-Martínez et al. 2003). For instance, it has been reported that overexpression of CLE19 specifically in the root apical meristem reduces root meristem length and induces premature cell differentiation without affecting QC specification or stem cell status (Casamitjana-Martínez et al. 2003). CLE19 is a member of the CLAVATA family of secreted proteins, of which CLE3 has been shown to be involved in the activation of the CLV1–CLV2 receptor complex that negatively regulates the size of the organizing center of the shoot meristem. Therefore, it is possible that the reduction of root meristem size and premature cell differentiation in P-deprived seedlings could also involve the direct up-regulation of CLE19 by P availability.

Because the overexpression of CLE19 does not affect QC specification or the differentiation of columella cells and the first effect of P deprivation that we observed is the periclinal cell division of the QC, it is possible that the effect of low P availability on the loss of meristem activity and premature cell differentiation involves two independent mechanisms or that the QC, acting as a sensor of environmental signals, regulates the expression of genes involved in meristem maintenance. Nevertheless, these results indicate that meristem exhaustion induced by low P availability is probably the result of a complex signaling mechanism that independently affects QC specification and meristem maintenance.

It has been shown that in parallel, or as a consequence of primary root growth inhibition, many lateral roots emerge in P-deprived plants. Analysis of the root systems of 16-day-old *Arabidopsis* plants grown at low P revealed that 52% of the lateral roots had arrested growth, produced root hairs at the tip and showed absence of *CycB1;1:uidA* and *DR5:uidA* expression (data not shown). It is important to note that as observed for primary roots, when first-order lateral roots entered the determinate growth program, second-order lateral roots emerged, resulting in the production of a more branched root system. The finding that low P induces a determinate growth program not only in primary but also in lateral roots has a major implication for the establishment of root architecture that increases the exploratory capacity of the root system, particularly within the upper layers of the soil.

Dynamic analyses showed that P deficit first induced a decrease in root elongation prior to day 8, which was later followed by morphogenetic changes within the root apical meristem and the induction of the expression directed by the promoters of high affinity P transporters and the detection of APase by day 10, and a complete meristem exhaustion and cell proliferation arrest by day 14. These results suggest that one of the earliest effects of P deficiency is the decrease in primary root growth. Whether this root growth decrease is the result or a cause of a slight decrease in meristematic activity and whether induced determinate root growth triggers the expression of genes involved in increased P uptake remains to be determined.

Although our system to assess the low P response differs from that reported by Karthikeyan et al. (2002), i.e. solid versus liquid medium and different times of P starvation, the results obtained on the expression of *pAtPT2:uidA* are similar, showing a high level of induction by low P in the entire root system including the root tips. However, in contrast to the data reported by these authors who found that in their conditions *pAtPT1:uidA* was only expressed in differentiated cells of the root but not in the root tip, we found a high level of expression of this marker gene in the exhausted meristems of low P-grown plants. This difference can be explained by the observed differentiation of meristematic cells as a consequence of the establishment of a determinate root growth developmental program under low P conditions.

To date, low P-induced root growth has only been described for a limited number of plant species in a narrow range of plant families. This report extends our knowledge of the cellular and physiological mechanisms involved in this particular root developmental pathway in *Arabidopsis*. The use of *Arabidopsis* as a model system will facilitate the identification of the genes responsible for regulating the establishment of root determinate growth programs and is expected to increase our understanding of the precise role of this root architectural trait as a part of the low P response rescue system in plants.

Materials and Methods

Plant material and growth conditions

Arabidopsis ecotype Col-0, the *Arabidopsis* transgenic lines *CycB1;1:uidA* (Colón-Carmona et al. 1999), *DR5:uidA* (Ulmasov et al. 1997), *QC46:uidA* (Sabatini et al. 1999), and *AtPT1:uidA* and *AtPT2:uidA* (Karthikeyan et al. 2002) were used for all experiments. Seeds were surface sterilized with 95% (v/v) ethanol for 5 min and 20% (v/v) bleach for 7 min. After five washes in distilled water, seeds were germinated and grown on agar plates containing low (1 μM) or high (1 mM) NaH_2PO_4 in a 0.1× MS medium, as described by López-Bucio et al. (2002). For Fe-free medium, $FeSO_4$ and Na-EDTA were replaced by Na_2SO_4 in the nutrient solution. The N-free medium was prepared by omitting NH_4NO_3 and KNO_3 from the nutrient solution, and supplying the K source with $KHCO_3$. For S-free medium, $MgSO_4$, $MnSO_4$, $ZnSO_4$ and $CuSO_4$ were substituted by their respective chloride salts. The K-free medium was made by substituting KI with NaI and omitting KNO_3. Phytagar (commercial grade) was purchased from Gibco-BRL (Gaithersburg, MD, U.S.A.). Plates were placed vertically at an angle of 65° to allow root growth along the agar surface and to allow unimpeded aerial growth of the hypocotyl. Plants were placed in a plant growth chamber (Percival Scientific, Perry, IA, U.S.A.), with a photoperiod of 16 h of light, 8 h of darkness, light intensity of 300 μmol m^{-2} s^{-1} and temperature of 22–24°C.

Transfer experiments

To determine whether determinate primary root growth induced by low P conditions was reversed by a high P treatment, transfer experiments were carried out. Wild-type (Col-0) *Arabidopsis* plants were grown in low (1 μM) P medium for several days. This provided sufficient time for low P-grown plants to enter the determinate growth pathway and terminate their growth. In these studies, plants were carefully removed from low P medium and transferred to plates with either identical or contrasting P concentrations. At day 7 after the transfer, the lengths of primary and lateral roots were measured. The lengths of the lateral roots closest to the base of the hypocotyls (proximal lateral root) and to the root tip (distal lateral root) were measured.

Histochemical analysis

For histochemical analysis of GUS activity, *Arabidopsis* seedlings were incubated overnight at 37°C in a GUS reaction buffer (0.5 mg ml^{-1} of 5-bromo-4-chloro-3-indolyl-β-D-glucuronide in 100 mM sodium phosphate, pH 7). The stained seedlings were cleared by using the method of Malamy and Benfey (1997b). For each marker line, and for each time point of assay, at least 10 transgenic plants were analyzed. For visualization of starch granules, roots were incubated for 3 min in Lugol solution (Sigma), washed three times with deionized water and mounted in chloral hydrate solution [74% (w/w) chloral hydrate, 7.4% (w/w) glycerol]. A representative plant was chosen for

each P treatment and photographed using Nomarski optics on a Leica DMR microscope.

Tissue sectioning and whole mounts

Roots were fixed overnight in 1.5% (v/v) glutaraldehyde and 0.3% paraformaldehyde (v/v) in 25 mM PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]) buffer pH 7.2. The fixed material was gradually (10% increase per step, 15 min per step) dehydrated [starting with 10% (v/v) ethanol] and then embedded in historesin (Leica Instruments GmbH, Heidelberg) by incubation in an ethanol–historesin mixture in proportions 3 : 1, 1 : 1, 1 : 3 (2 h in each), and then in pure historesin overnight. Plastic blocks were sectioned on a Leica RM 2155 microtome (Leica Microsystems Nussloch GmbH). Sections of 3 μm were mounted on gelatin-coated slides (Baum and Rost 1996) and stained by the PAS reaction (periodic acid, 40°C, 20 min; Schiff reagent at room temperature for 60 min, modified from O'Brien and McCully 1981) and counterstained with 0.05% toluidine blue at room temperature for 30 s. Root sections were analyzed under an Olympus BX-51 microscope (Olympus America Inc., NY, U.S.A.) and photographed with a Nikon Coolpix 5000 digital camera (Nikon Corporation, Tokyo, Japan) attached to the microscope; image size was 2,560×1,704 pixels.

Data analysis

Arabidopsis root systems were viewed with an AFX-II-A stereomicroscope (Nikon, Tokyo). All lateral roots emerging from the primary root and observed under the 3× objective were taken into account for lateral root number data. Primary root length was determined for each root using a ruler. The lengths of 20 epidermal cells (only those cells that give rise to root hairs) were measured on images of cleared roots taken with a digital camera connected to a microscope using the Scion Image Free software (Scion Corporation, U.S.A.; www.scioncorp.com).

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