

Identification of QTL controlling root growth response to phosphate starvation in *Arabidopsis thaliana*

MATTHIEU REYMOND^{1*}, SERGIO SVISTOONOFF¹, OLIVIER LOUDET², LAURENT NUSSAUME¹ & THIERRY DESNOS¹

¹CEA Cadarache, DSV DEVM Laboratoire de Biologie du Développement des Plantes, UMR 6191 CNRS-CEA, Aix-Marseille II, F-13108, France and ²Station de Génétique et d'Amélioration des Plantes, INRA Versailles, route de St Cyr, 78026 Versailles, France

ABSTRACT

One of the responses of plants to low sources of external phosphorus (P) is to modify root architecture. In *Arabidopsis thaliana* plantlets grown on low P, the primary root length (PRL) is reduced whereas lateral root growth is promoted. By using the Bay-0 × Shahdara recombinant inbred line (RIL) population, we have mapped three quantitative trait loci (QTL) involved in the root growth response to low P. The Shahdara alleles at these three QTL promote the response of the primary root to low P (i.e. root length reduction). One of these QTL, *LPRI*, located in a 2.8 Mb region at the top of chromosome 1, explains 52% of the variance of the PRL. We also detected a single QTL associated with primary root cell elongation in response to low P which colocalizes with *LPRI*. *LPRI* does not seem to be involved in other typical P-starvation responses such as growth and density of root hairs, excretion of acid phosphatases, anthocyanin accumulation or the transcriptional induction of the P transporter *Pht1;4*. *LPRI* might highlight new aspects of root growth that are revealed specifically under low P conditions.

Key-words: accessions; natural variation; root architecture.

INTRODUCTION

Plant growth habit and physiology are profoundly altered by low availability of environmental phosphorus (P). The main features of P starvation syndrome include an increase in the root/shoot ratio; a modification of the root architecture to explore P-richer soil horizons; an increase in root hair length and density; the induction of P transporters; a higher level of root secretion of organic acids, phosphatases and nucleases and the accumulation of anthocyanins etc. (Raghothama 1999). Some of these responses are part of an adaptive strategy of plants for better acquisition of the different forms of P in the rhizosphere and an efficient utilization of the P absorbed.

Correspondence: Thierry Desnos. Fax: +33 4 42 25 45 97; e-mail: desnos@dsvsud.cea.fr

*Present address: Max Planck institut for plant breeding research, Carl-von-Linné Weg 10, 50829 Köln, Germany.

The alteration of root system architecture in response to P starvation is a long-known phenomenon. In some species such as *Lupinus albus* and other Proteacea, rather specialized roots are generated. They emerge along the main roots in bottlebrush-like clusters of lateral rootlets, called 'proteoid' roots, and exude organic acids that serve in the mobilization of the insoluble P chelated with divalent or trivalent ions (Johnson, Vance & Allan 1996). This example represents an extreme specific response to very poor soils, and the general trend is a growth stimulation of the whole root system to the detriment of the shoot. The elongation of roots reaching P-rich localized volumes of soil is stimulated (Drew 1975).

P-starved *Arabidopsis* exhibit a short primary root growth concomitantly associated with a stimulation of lateral root initiation and growth (Williamson *et al.* 2001; Linkohr *et al.* 2002). One can question the role of growth hormones in this root architecture response. It seems, however, that it is largely independent of auxin, ethylene (Poirier & Bucher 2002; Ma *et al.* 2003) and abscisic acid (ABA) (Trull *et al.* 1997).

The genetics of the low P-induced architectural modification are not well developed, and until now no mutant specifically altered in this response has been isolated. As for the many traits that have now been analysed in *Arabidopsis*, the observation of accessions of worldwide origin has revealed the natural variation of responses to P starvation. With the purpose of analysing the P acquisition efficiency of 36 accessions, Narang, Bruene & Altmann (2000) selected five accessions with contrasting responses. They noted that these lines also displayed different shoot/root mass ratios, root hair lengths and densities depending on the nature (solubility) of the P in the nutrient solution. In a work more focused on the root architecture related to P nutrition, Chevalier *et al.* (2003) reported the diversity of *Arabidopsis* accessions. With two growth traits – primary root length (PRL) and number of lateral roots – they categorized 73 accessions. Compared with a P-rich medium, the P-poor medium reduced both the PRL and the number of lateral roots for 50% of the accessions. On the other hand, 25% were not affected while the remaining lines were affected in only one trait. These data may reflect different physiological strategies that plants exhibit under P starvation. These works also show that accessions are a rich

source of genetic diversity to analyse P-starvation responses. Overall, these observations suggest that a deeper understanding of the root architecture of *Arabidopsis* in response to low exogenous P should result from the power of quantitative trait loci (QTL) analysis.

MATERIALS AND METHODS

Plant material

Accessions of *A. thaliana* used were: Bay-0 (Bayreuth, N57923), Columbia (Col-0 CS60000), Cape Verde Islands (Cvi, N902), Shahdara (N57924) and Wassilevskaja (WS, N5390). Progenies of seeds were provided by the NASC (<http://nasc.nott.ac.uk>). Landsberg *erecta* was from Lehle (Round Rock, TX, USA).

For QTL analysis, we used the Core-Pop set of 165 RILs (N57921) selected from the original 411 RIL population as an optimal set for QTL mapping experiments (Loudet *et al.* 2002; see <http://www.inra.fr/qtlat/for> details).

For the confirmation and fine analysis of *LPR1*, we generated near-isogenic lines (NILs) by segregating F6 RIL (194^B and 194^S). These are called heterogeneous inbred family (HIF) lines (Tuinstra, Ejeta & Goldsbrough 1997). *LPR1* maps between molecular markers MSAT1.10 and NGA248 at the top of chromosome 1. We examined the whole collection of 411 Bay-0 × Shahdara RILs and we found that the F6 generation of RIL194 contains a residual heterozygosity at markers MSAT1.10 and NGA248 only (Loudet *et al.* 2002). From the progeny of this RIL, we selected the HIF couple (fixed for the Bay-0 194^B allele or the Shahdara 194^S allele) by genotyping individuals with markers MSAT1.10 and NGA248.

For the dissection of epistasis between *LPR1*, *LPR2* and *LPR3*, RILs that carry a recombinant event between the markers flanking one of these QTL were withdrawn because the exact QTL genotype could not be asserted.

Plant growth and measurements

The *in vitro* nutrient solution (MS) was prepared based on the Murashige & Skoog (1962) protocol; it was diluted 10 times with 8 g L⁻¹ agar (Sigma, catalogue number A-7002; Saint Louis, MO, USA) and 1% sucrose. For the high P condition, 500 μM NaH₂PO₄ was added to the medium, while for the low P condition, 5 μM NaH₂PO₄ and 495 μM NaCl were added to the medium. The pH was buffered at 5.6–5.8 with 3.5 mM of MES. Forty millilitres of the autoclaved medium were poured into 12 × 12 cm Petri dishes. For the iron (Fe) starvation experiments, no FeSO₄ was added to the media while 300 μM FerroZine [3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine sulphonate; Sigma] was added to chelate any Fe as previously described (Yi & Guerinot 1996). For sulphur (S) starvation, MgSO₄ was replaced by MgCl₂. Media deprived of nitrogen (N) contained 2.07 mM NaCl and 1.88 mM KCl instead of 2.07 mM NH₄NO₃ and 1.88 mM KNO₃.

The seeds were surface sterilized for 5 min in a solution of ethanol (90%)-Bayrochlore™ (Bayrol Mundolsheim, France) (10%), and was rinsed twice with ethanol (100%). Five seeds of each line were placed on each plate. The plates were stored at 4 °C overnight and then vertically disposed in a growing chamber for 10 d (16 h photoperiod; 24.5/21 °C day/night, respectively). For QTL mapping, the plates (two per line) were randomly moved every 2 d within the same shelf of the growth chamber.

Ten-day-old plantlets were scanned (Agfa Snap Scan 1236s, Ridgefield Park, NJ, USA) and the roots were measured with Bioscan Optimas V6.10 image analyser (Edmonds, WA, USA). This software allowed us to measure the PRL (in 5 or 500 μM P) and the total length of the root system (in 500 μM only) for each plant. We were also able to count the number of visible (emerged) lateral roots for each plant (in 500 μM only).

The length of the primary root epidermis cells was measured only in experiment 1 (Exp1). A 1–2 cm piece of primary root from a 10-day-old plantlet was mounted between a lame and lamella and sealed with nail varnish (Gemey, Paris, France). Cell length was measured under the microscope (Leica-Leitz DM RXA; Wetzlar, Germany) by using a micrometer. Ten cells, located above the elongation zone, were measured per root for each RIL. Statistical analysis was then performed by using SPSS (SPSS 11.0.1), general linear model, univariate. The response of PRL and primary root cell length to P starvation was estimated with the following formula:

$$\text{Response} = (\text{trait in high P} - \text{trait in low P}) / \text{trait in high P}$$

QTL mapping and analysis

For the parental lines and the 165 RILs, the root traits of five individual plantlets were measured. The growth traits measured were PRL and the number of lateral roots for both the low and the high P plantlets, and the total length of lateral roots (ΣLRI) for the high P plantlets only. We repeated the QTL experiment in the same growth chamber on two different dates.

Broad sense heritability (h^2_b) was estimated for each trait by using the following equation:

$$h^2_b = VG / (VG + VE)$$

where VG is the variance between RILs and VE is the variance within RIL.

QTL detections were performed by using QTL Cartographer (Basten, Weir & Zeng 1995). Briefly, QTL were detected successively by interval mapping (IM) and composite interval mapping (CIM). IM (Lander & Botstein 1989) was used first to determine the putative QTL involved in the variation of the considered trait. CIM model 6 of QTL Cartographer was then performed. The closest marker to each local logarithm-of-odds (LOD) score peak was considered as a cofactor while testing the presence of a novel QTL elsewhere in the genome. The number of cofactors in our models varied from three to six, depending

on the trait. The same cofactors were used for identical traits measured in the two QTL experiments. When a cofactor was also a flanking marker of the tested region, the cofactor was excluded from the model. The walking speed was 0.5 cm. As suggested by Churchill & Doerge (1994), the LOD significant threshold was estimated from several test permutation analyses. One thousand permutations of phenotypic data were analysed by using the CIM model for each trait as described earlier. On average, the experiment-wise threshold obtained ($\alpha = 5\%$) for all the traits that were studied corresponds to a 2.5 LOD.

The additive effects of the detected QTL were estimated from CIM results. Additive effect represents the mean effect of the replacement of the Shahdara allele by the Bay-0 allele at a particular locus. The contribution of each detected QTL to the total variance (R^2) was estimated by variance component analyses. Significant QTL–QTL interactions (epistatic effect) were also added to the genetic model via the corresponding marker interactions.

Analysis of low P response

In order to quantify the anthocyanin content, the plants were grown for 20 d on MS media with 5 or 500 μM P. Anthocyanins were extracted from 20 mg of pooled rosette leaves of either HIF 194B or 194S, and five independent extractions were performed for each growth condition. Anthocyanins were extracted and quantified as described by Ticconi, Delatorre & Abel (2001).

For *in vivo* visualization of acidic phosphatase activity, 10-day-old plants grown on MS media with 5 or 500 μM P were transferred to MS media containing 0.008% BCIP (Trull & Deikman 1998) and 5 or 500 μM P, respectively, and were replaced in normal growth conditions for 48 h. The plants were then observed and photographed with a stereomicroscope MZ16A (Leica).

For reverse transcription-polymerase chain reaction (RT-PCR) analysis of gene expression, RNA was extracted from roots of 10-day-old plants grown on an MS medium with either 5 or 500 μM P. RNA-Easy plant extraction kit (Qiagen, Valencia, CA, USA) was used according to the manufacturer's instructions. Six micrograms of total RNA were treated with DNaseI (Roche Diagnostics, Indianapolis, IN, USA) for 15 min at 37 °C and were used for the retro-transcription reaction using the AMV reverse transcriptase (Roche Diagnostics) according to the manufacturer's instructions. Aliquots of each preparation were used as templates after dilution for the PCR. The first strand cDNA amount was adjusted in order to have semi-quantitative responses. PCR was performed with specific oligonucleotides for *Phl1*; 4 (F: CGATCATTCCACTTCCTTCTTCTT, R: CCACGTGGTAATAAATACGACCG). Thermal cycling consisted of an initial denaturation at 94 °C for 5 min, followed by 25 cycles of denaturation step at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 1 min. A final extension at 72 °C was performed for 7 min. Normalization of samples was performed by using the actin gene *At3g18780* with primers ACT2F:

CTTCCCTCAGCACATTCCAG and ACT2R: AACAT TGCAAAGAGTTTCAAGGT. For actin, the PCR consisted of 25 thermal cycles as described above.

RESULTS

Natural variation in response to low P

To gain further insight into the natural variety of root system response to P starvation in *Arabidopsis*, we compared six commonly used accessions grown under low P and under high P (5 and 500 μM P, respectively – see Materials and methods). Typical 10-day-old seedlings are shown in Fig. 1. A rapid examination of these seedlings shows that accessions have distinct root architecture. These accessions are also contrasted for their lateral roots. The ΣLRI for Shahdara, on average, was 14 cm whereas that of Bay-0 was 11 cm. In our growth conditions, Shahdara always exhibited the most developed lateral roots and Bay-0 was one of the accessions showing the least developed lateral roots (Fig. 1a).

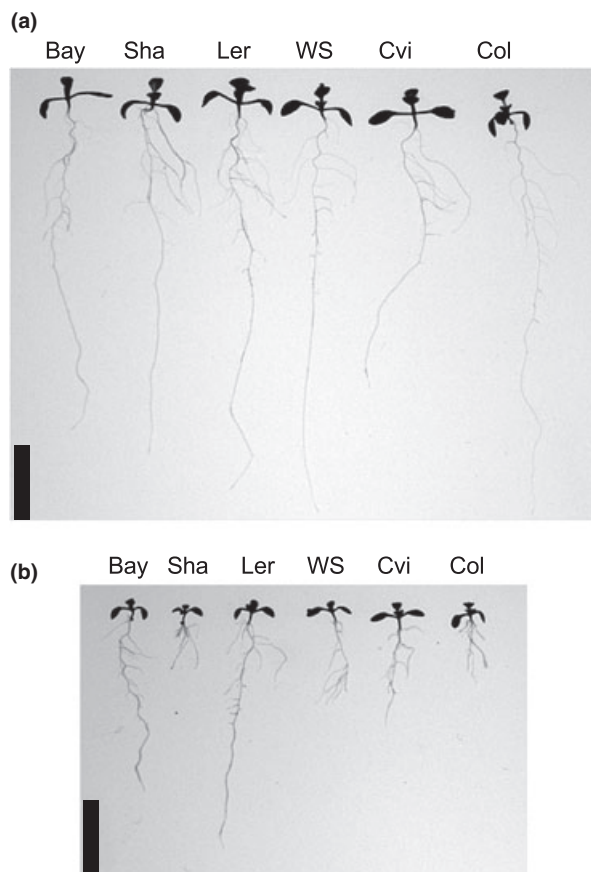


Figure 1. Natural variation in root architecture response to phosphate (P) starvation. Pictures of different *Arabidopsis* accessions grown under (a) high P conditions or +P and (b) low P conditions or -P (scale bars = 1 cm). Bay, Bay-0; Sha, Shahdara; Col, Columbia; Ler, Landsberg *erecta*; WS, Wassilevskaja; Cvi, Cape Verde Islands.

By comparison with the high P medium, the general trend on low P medium shows a reduction of root length (Fig. 1b). The range of response is wide and PRL is dramatically reduced in Shahdara (75% reduction) while PRL is less affected in Bay-0 and *L.er* (20 and 19% reduction, respectively). Low P medium also restrained lateral root growth in each of these accessions (Fig. 1b).

In order to better characterize the Shahdara and Bay-0 root architectures, we plotted for each seedling the Σ LRL as a function of PRL (Fig. 2). This representation allows us to visualize the kinetics of the settling up of root architecture of 1–15-day-old seedlings. On a high P medium, the length of lateral roots of Shahdara was always higher than that of Bay-0. For instance, when the primary root reached 8 cm, the length of lateral roots was more than 20 cm for Shahdara and only 15 cm for Bay-0. On the low P medium, the root architecture of Bay-0 and Shahdara were remarkably different (Fig. 2). The PRL of Shahdara did not exceed 2 cm whereas that of Bay-0 went beyond 8 cm. A polynomial of degree 2 was adjusted on the Σ LRL plotted against PRL for each accession, with a high correlation (R^2 between 0.65 and 0.94 depending on line and P supply; see Fig. 2). These contrasted growth habits of Bay-0 and Shahdara accessions, especially under low P conditions, led us to characterize the genetics of these growth responses.

Primary and lateral root length in the Bay-0 \times Shahdara RIL population

A series of 165 Bay-0 \times Shahdara RILs (Loudet *et al.* 2002; see also Materials and methods) has been grown twice [Exp1 and experiment 2 (Exp2)] in a climatic chamber. For each experiment, the environmental conditions in the climatic chamber were set up in the same way and the RILs were grown on a low P and high P medium.

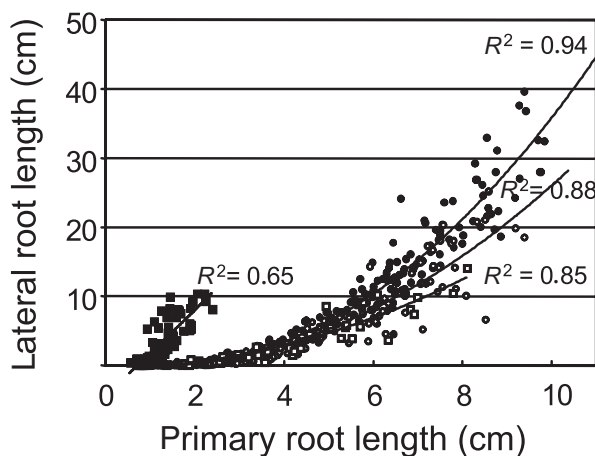


Figure 2. Graphical representation of root architecture of the Bay-0 and Shahdara accessions grown under two phosphate (P) conditions. The total length of lateral roots is plotted against the primary root length (PRL). Full symbols: Shahdara accession; open symbols: Bay-0 accession; squares: plantlets grown under P starvation ($5 \mu\text{M P}$); circles: plantlets grown in high P medium ($500 \mu\text{M P}$). Each dot represents a plant between 1 and 10 d old.

Under high P conditions, the parental lines showed a well-developed primary root and did not greatly differ from each other. Bay-0 had a PRL ranging from 6.9 to 5.2 cm and Shahdara from 7.71 to 5.36 cm in Exp1 and Exp2, respectively (data not shown).

For the RILs grown under high P, the distribution of the PRL is wide and there is a transgressive variation towards shorter as well as longer roots. In Exp1, a twofold amplitude of variation of PRL (from 5.6 to 10.9 cm) was observed among RILs (data not shown). In Exp2, on the average, the PRLs were smaller than in Exp1. This might be due to a slightly lower temperature of the growth chamber when this experiment was running. Nevertheless, during Exp2, we observed the same amplitude of lengths as in the Exp1 within the population of RILs with a twofold variation (from 4.2 to 9.2 cm) of PRL. In accordance with the results presented in Fig. 2, the PRL of the parental accessions differed considerably under low P conditions. For Shahdara, it did not reach 1 cm in both experiments, whereas for Bay-0 it was 2.3 and 3.3 cm in Exp1 and Exp2, respectively. For all the RILs, PRL was shorter when grown on low P medium than on high P medium, and it ranged from 0.2 to 4.8 cm depending on the RIL and the experiment (data not shown). The number of transgressive RILs, as well as the extent of their transgression beyond the Shahdara value, was small (data not shown). This indicates that the Shahdara parental accession already carries almost the best (higher) combination of alleles that increase this response.

Root growth depends of the number of cells produced in the root meristem and the extent of their elongation. To determine whether smaller PRL under low P conditions results from a reduction of cell extension, we measured the length of epidermis cells (Materials and methods). These measurements were performed for the two P conditions only in Exp1. In a high P environment, Shahdara and Bay-0 cell lengths were close (364 and $317 \mu\text{m}$, respectively) and for RIL they ranged from 167 to $456 \mu\text{m}$. Therefore, this population exhibits a large transgression in both directions for this trait. Surprisingly, in this P condition, there is a weak correlation ($r = 0.21$) between cell length and PRL (data not shown).

The primary root cells were significantly shorter under low P conditions than under high P conditions and ranged from 22 to $189 \mu\text{m}$. The length of primary root cells of parental lines differed considerably (48.8 and $200 \mu\text{m}$ for Shahdara and Bay-0, respectively). In addition, either no transgression or a very small transgression was observed; Bay-0 had the longest cells and Shahdara had one of the shortest cells. Unlike what we observed under high P conditions, there was a higher correlation between cell length and PRL at low P conditions ($r^2 = 0.58$, data not shown).

The Σ LRL was measured for all the 165 RILs grown in high P conditions for 10 d (data not shown). This variable ranged from 0.4 to 31.8 cm in Exp1 and from 0.6 to 20.9 cm in Exp2. As for PRL, the Σ LRL of almost all the RILs in Exp2 were smaller than those measured in Exp1, and a correlation is observed for this trait between the two exper-

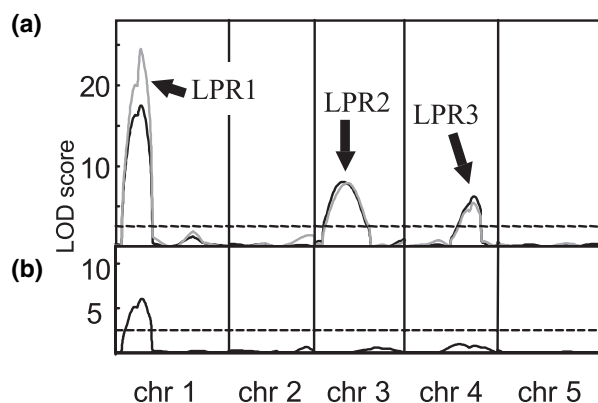


Figure 3. LOD score values from composite interval mapping (CIM) analyses of traits plotted against a linear representation of the *Arabidopsis* genome. Black lines represent LOD score values obtained in Exp1 and grey lines represent LOD score values obtained in Exp2. (a) Primary root growth response to phosphate (P) starvation. (b) Primary root cell length response to P starvation.

iments ($r = 0.52$). In the two experiments, the parental lines differed considerably from each other and Bay-0 always had a smaller Σ LR1 than Shahdara. The transgression of Σ LR1 is essentially beyond Shahdara, suggesting that Bay-0 carries one of the least favourable allelic combinations for this trait.

Mapping QTL for response to P starvation

In order to understand the root growth response to P environment, we have mapped QTL for PRL response.

In both experiments, the genetic model is composed of three QTL (*LPR1*, *LPR2* and *LPR3* on chromosomes 1, 3 and 4, respectively (Fig. 3a). *LPR1* has a major effect because it accounts for more than 52% of the phenotypic variation of PRL under low P conditions with an LOD value between 17.4 and 24.7 (Table 1). *LPR1* and *LPR2* did not colocalize with QTL of PRL in the high P environment (data not shown), underlining that these QTL are specific to PRL under P starvation. On the other hand, *LPR3* colocalizes with a QTL, affecting PRL in the high P environment (data not shown), suggesting that this QTL is involved in the PRL regardless of the P environment. Significant epistatic interactions were found between *LPR1* and *LPR2* and between *LPR1* and *LPR3* (Table 1).

The heritability of the PRL response to P environment was high (0.91, Table 1). The same QTL and epistatic interactions for PRL in the low P environment were found for the PRL response (data not shown and Table 1).

In order to better understand the epistatic interactions between QTL affecting the response to P, we grouped the RIL according to their genotype (Materials and methods). According to its QTL alleles, each of the 165 RIL has been categorized into one of eight genotypic classes. Figure 4 shows that the highest response to P occurs when the allele at *LPR1* is from Shahdara (columns 1–4), regardless of the genotype at the *LPR2* or *LPR3*. Moreover, the effects of *LPR2* and *LPR3* could be unravelled only when the allele at *LPR1* is from Bay-0 (columns 5–8). In addition, the effect of *LPR2* and *LPR3* appears to be additive (compare columns 5, 6 and 7 with 8). On the other hand, one could consider that the effect of *LPR1* is expressed only when Bay-0 allele is present at *LPR2* and/or *LPR3*.

Table 1. Quantitative trait loci (QTL) analysis results

| | QTL ^a | Chromosome | Marker ^b | Position (cM) ^c | 2a ^d | LOD score | r ^{2e} | r ² Model ^f | h ^{2g} | r ^h |
|--|------------------|------------|---------------------|----------------------------|-----------------|-----------|-----------------|-----------------------------------|-----------------|----------------|
| Response of PRL to P starvation | | | | | | | | | | |
| Exp1 | LPR1 | 1 | MSAT1.10 | 18.7 | -0.20 | 17.4 | 52 | 89 | 0.91 | 0.76 |
| | LPR2 | 3 | ATHCHIB2 | 20.8 | -0.10 | 7.9 | 13 | | | |
| | LPR3 | 4 | MSAT4.18 | 50.8 | -0.08 | 6.6 | 8 | | | |
| | LPR1*LPR2 | | | | | | 14 | | | |
| | LPR1*LPR3 | | | | | | 2 | | | |
| Exp2 | LPR1 | 1 | MSAT1.10 | 17.7 | -0.26 | 24.7 | 52 | 83 | 0.91 | |
| | LPR2 | 3 | ATHCHIB2 | 26.0 | -0.14 | 8.7 | 12 | | | |
| | LPR3 | 4 | MSAT4.18 | 49.8 | -0.10 | 6.3 | 7 | | | |
| | LPR1*LPR2 | | | | | | 10 | | | |
| | LPR1*LPR3 | | | | | | 0.5 | | | |
| Response of primary root cell length to P starvation | | | | | | | | | | |
| Exp1 | LCP1 | 1 | MSAT1.10 | 19.2 | -0.14 | 6.0 | 29 | 28 | - | - |

^aThe name given to a local LOD score peak contains the trait name suffixed with an order number.

^bThe closest left marker flanking the LOD score peak.

^cThe position of the QTL is expressed in cM from the first marker of the chromosome.

^dThe mean effect (in trait unit) of the replacement of both Shahdara alleles by Bay-0 alleles at the QTL.

^ePercentage of variance explained by the QTL or by QTL-QTL interaction, when significant.

^fPercentage of variance explained by the sum of the QTL or by QTL-QTL interaction detected.

^gHeritability of the trait (see Materials and methods).

^hCoefficient of correlation of the trait measured between the two experiments carried out.

LOD, logarithm-of-odds; PRL, primary root length; P, phosphate.

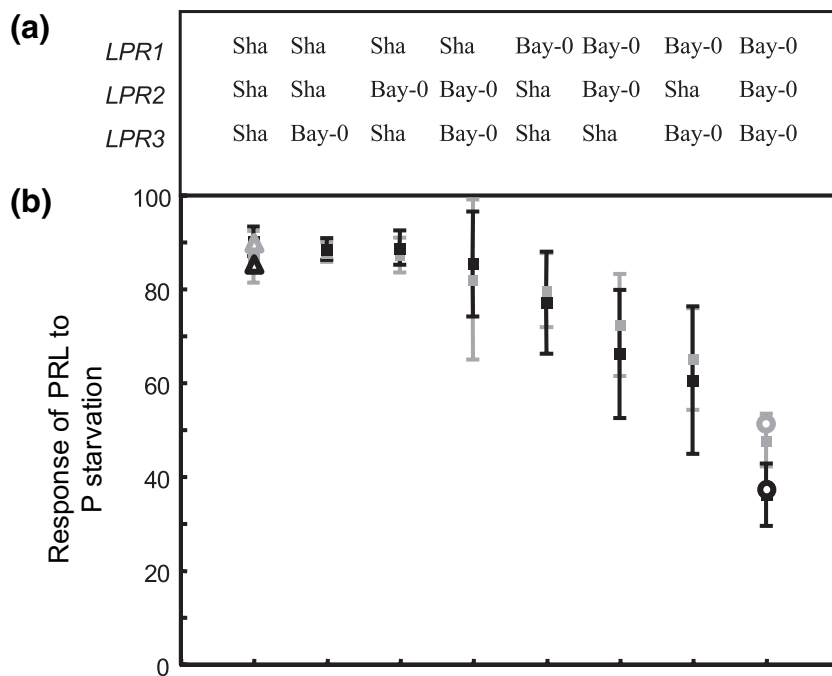


Figure 4. Relationship between the genotype of recombinant inbred line (RIL) at the different quantitative trait loci (QTL) and the response of primary root to phosphate (P) starvation. (a) RILs were classified according to their genotype at the three QTL for response of primary root length (PRL) to P starvation. (b) Phenotype of each class of RIL from Exp1 (black symbols) and from Exp2 (grey symbols). Shahdara (triangles) and Bay-0 (circles) are plotted in their respective genotypic class. Five to 22 RILs were measured per genotypic class. Error bars are standard deviations.

The heritability of cell length under low P conditions was high (0.76). Only one QTL of root cell length in response to low P was detected, at the top of chromosome 1, which explains 29% of the phenotypic variation for this trait (*LPC* in Table 1). Interestingly, this QTL colocalizes with the major QTL for the PRL response to phosphate environment (*LPR1*, Fig. 3b). These two QTL are most probably the same QTL because the data were not independent. Therefore, the small primary root under low P results, at least partially, from the effect of *LPR1* on cell elongation.

The crucial effect of *LPR1* on the primary root growth response to low P

To confirm and better understand the role of *LPR1* in the root response to low P, we used NIL in the form of a HIF that differ only for their allele at the *LPR1* region (Materials and methods): either Bay-0 (194^B) or Shahdara (194^S). These two lines were grown in low P and in high P environments and the PRL of 10-day-old plantlets was measured (Fig. 5).

In low P, 194^B looks like the parental Bay-0 accession and 194^S looks like the Shahdara accession (Fig. 5a). Figure 5c shows that the PRL of 194^S was less than 1 cm whereas in 194^B it reached 3 cm. This result further confirms the major role of *LPR1* in the variation of this trait. We tested whether *LPR1* also affects Σ LR in response to low P. When grown under low P environment, Σ LR ranged from 0.6 to 7.9 cm for 194^S and from 0.5 to 12.4 cm for 194^B (Fig. 5d). In contrast to PRL, the distribution of Σ LR of the HIFs is largely overlapping. Therefore, this supports the view that in a low P environment, *LPR1* is involved to a much greater extent in the growth response of primary root than lateral roots.

The cell length of primary and lateral roots was measured for the two NILs and the parental accessions (Fig. 5e).

Under low P conditions, the primary root cells were much shorter in Shahdara and 194^S than in Bay-0 and 194^B. This is in good accordance with results of root length presented in Fig. 5c. On the other hand, the cell length of lateral roots was not dependent on the *LPR1* allele. Once again, this corroborates data in Fig. 5d. Altogether, the NIL analysis confirmed that *LPR1* is a major QTL of primary root cell length in response to the low P environment.

The Shahdara dominant allele of *LPR1* is responsible for the low P response

The two NILs 194^B and 194^S were studied together with the original heterozygous (194^{B/S}) plants. Interestingly, in the low P environment, the primary root of 194^{B/S} is slightly longer than that of 194^S (Fig. 5f). In other words, the heterozygote strongly responded to P starvation. The degree of dominance (d/a , with d as the dominance deviation and a as the additivity; Tanksley 1993) of the Shahdara allele on this trait is 0.6. These data convincingly show that the Shahdara allele of *LPR1* is largely dominant over the Bay-0 allele. This suggests that Bay-0 carries a non-functional or a hypomorphic allele at *LPR1* that is not or only poorly able to trigger the response to low P. Thus, the short primary root in low P is most probably due to a functional allele of *LPR1*. Alternatively, the Bay-0 allele is inappropriately expressed.

Classical traits used to characterize the P-starvation response are not under the control of *LPR1*

We have shown above that the primary root growth response to P starvation is strongly compromised in plants homozygous for the Bay-0 allele at *LPR1*. In order to know

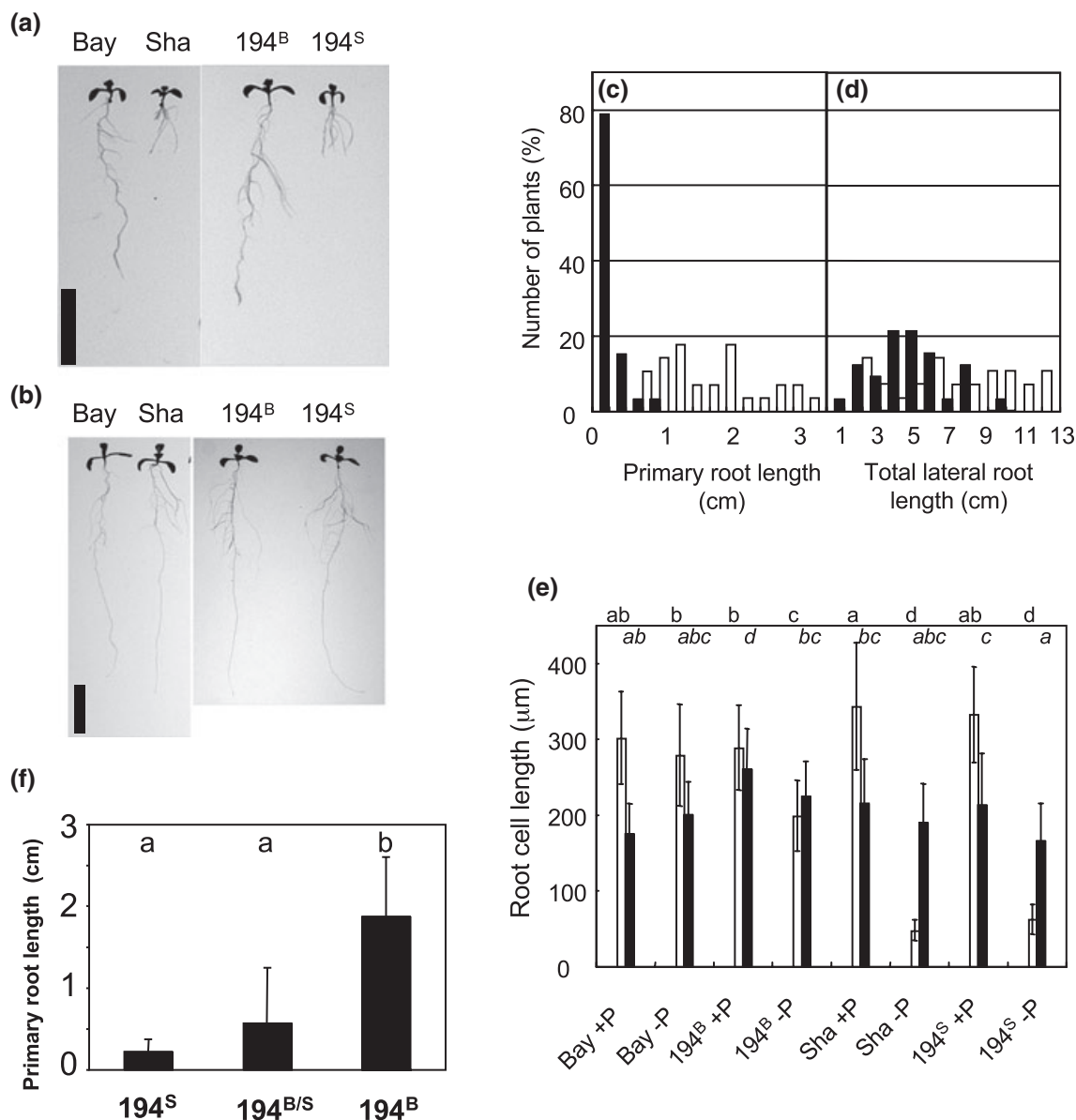


Figure 5. Effect of *LPR1* on primary and lateral root lengths. Pictures of the Bay-0 (Bay), Shahdara (Sha) and the NIL 194^B and 194^S grown for 10 d on (a) low P medium and (b) high P medium (scale bars = 1 cm). Frequency distributions (%) of the (c) primary root length (PRL) and (d) total lateral root length of the NIL 194^B and 194^S grown in low P for 10 d. Values from NIL 194^S and 194^B are represented by black and white bars, respectively. (e) Root cell length of the parental accessions (Bay-0 and Shahdara) and the NIL 194^B and 194^S grown for 10 d on a high P (+P) or low P (-P) medium. Bars represent the average length (and its standard error) of primary root cells (white) and lateral root cells (black). Bars labelled with different letters are significantly different at $P = 0.005$. (f) PRL of the 194^{B/S} grown on a low P medium. Progeny of a 194^{B/S} heterozygote has been grown on low P medium. For each seedling, the primary root length has been measured and the genotype at *LPR1* determined with the MSAT1.10 and NGA248 markers. Bars labelled with different letters are significantly different at $P = 0.01$. Error bar represents the standard error.

whether this allele reduces the whole P signalling pathway, we performed a comparative analysis of 194^B and 194^S lines for several typical low P responses.

Among the morphological responses to P starvation is the increase in the density and length of root hair (Zhang, Lynch & Brown 2003). The 194^B and 194^S plantlets increased their root hair length by 33 and 54%, respectively, in low compared to high P conditions (Fig. 6a). The root

hair density (defined as the number of observable root hairs per mm of root) was increased by 97% for 194^S grown in low P compared to 194^S grown in high P. For 194^B, the density was only slightly increased (20%) (Fig. 6b). Nevertheless, the Bay-0 allele at *LPR1* does not interfere with root hair growth stimulation by low P.

P starvation also enhances the anthocyanin content in shoot (Ticconi *et al.* 2001). Anthocyanins were extracted

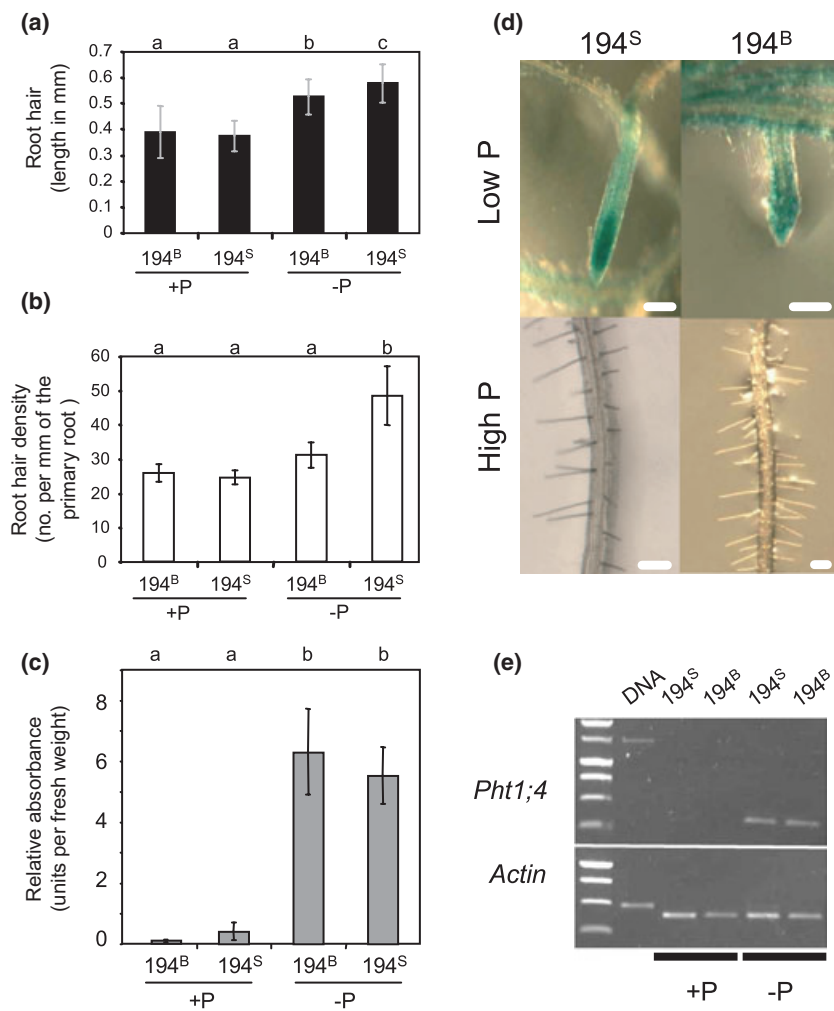


Figure 6. *LPR1* does not affect other typical low-phosphate (P) responses. Root hair length (a) and density (b) of the primary root from NIL grown on high P (+P) or low P (-P) medium. Bar labelled with different letters are significantly different at $P = 0.01$. Error bar represents the standard error. (c) Anthocyanins of 10-day-old seedlings grown on high P (+P) or low P (-P) medium were extracted and absorbance reported to the fresh weight. Bar labelled with different letters are significantly different at $P = 0.01$. Error bar represents the standard error. (d) *In vivo* BCIP revelation of acidic phosphatases. Ten-day-old 194^B and 194^S seedlings grown on low or high P medium were stained with BCIP before picture (scale bars = 150 μm). (e) Expression of the P transporter *Pht1;4* in NIL 194^B and 194^S RT-PCR have been performed on RNA extracted from 10-d-old seedlings grown on high P (+P) or low P (-P) medium. PCR on genomic DNA is a control of RT-PCR specificity. The expression of actin served to control the amount of template used for PCR (Misson *et al.* 2004).

from 194^B and 194^S plantlets grown either on low or high P. When grown on similar P conditions, both NILs accumulated similar anthocyanin content (Fig. 6c). In particular, under P starvation, the anthocyanin content of both lines was 10–60 times higher compared to high P, thus demonstrating that *LPR1* does not play a major role in this response.

Excretion of acidic phosphatases (APs) is also a landmark of P starvation (Trull & Deikman 1998). We used BCIP staining (Trull & Deikman 1998) to monitor *in vivo* the AP activity in plants cultivated either on low or high P. We observed an equivalent strong blue staining on the roots of P-starved 194^B and 194^S seedlings (Fig. 6d) whereas no staining could be detected with the high P seedlings. This strongly suggests that *LPR1* is not crucial for the excretion of APs in response to P starvation.

Finally, we monitored the expression of *Pht1;4*, a gene coding for a high-affinity phosphate transporter whose transcript level is strongly increased under P-deficient conditions (Muchhal, Pardo & Raghothama 1996; Misson *et al.* 2004). The same expression levels were observed for 194^B

and 194^S; in both lines, the RT-PCR product of *Pht1;4* was detectable only in low P plants (Fig. 6e).

***LPR1* does not affect root growth in response to N, Fe or S deficiencies**

Root architecture is also modulated by the availability of other mineral nutrients including N, Fe and S (Forde & Lorenzo 2001; Lopez-Bucio, Cruz-Ramirez & Herrera-Estrella 2003). In order to investigate whether *LPR1* is also involved in the control of architectural responses triggered by other nutrient deficiencies, NILs 194^B and 194^S were grown on separate MS media lacking N, lacking Fe and lacking S. N-starved plants began to show chlorosis symptoms 4 d after germination and developed a longer primary root compared to non-starved plants. Fe starvation resulted in a severe chlorosis and poor growth of both the shoot and roots. Finally, S deficiency also reduced growth and induced a strong development of root hairs (data not shown). For all of these growth conditions, we were unable to detect any difference between 194^B and 194^S. This sug-

gests that *LPR1* is not involved in a general nutrient-deficiency response (data not shown).

DISCUSSION

QTL for root growth response to low P

In the field, plants are exposed to spatially and time-heterogeneous environments. These conditions hinder the precise QTL detection of any growth trait. It is not difficult to imagine that mapping QTL for root growth in soil is an even more challenging task. In a pioneer study, Reiter *et al.* (1991) reported that P starvation reduces root weight of maize grown in the field. They have also mapped several QTL, one of which specifically reduces root dry weight (i.e. not shoot dry weight). As far as we know, no other QTL for root growth related to P starvation have been described in maize or any other species.

With the adoption of *Arabidopsis* growing *in vitro*, root development can be standardized. This opens the way to fine mapping and analysis of QTL controlling root growth. Examination of the Bay-0 and Shahdara accessions showed their particularly contrasted phenotype in low P.

We have carried out two independent QTL experiments, and three QTL involved in the PRL response to P starvation have been detected in each experiment. These are the first described QTL of the growth response to P starvation in *Arabidopsis*. We have also mapped the QTL of root growth in low P as well as in high P (M. Reymond, S. Svistoonoff, L. Nussaume & T. Desnos, unpublished results). One of the QTL of root growth in a high P medium is located on chromosome 4 and colocalizes with *LPR3*. It might correspond to PRL3, a QTL mapped using the same set of Bay-0 × Shahdara RILs and grown in N-enriched medium (Loudet *et al.* 2005). *LPR3* displays the same allelic effect as PRL3; thus, it is possible that they correspond to the same locus. Alternatively, these two QTL are distinct but are located in the same chromosome region. No other QTL of PRL are colocalized near *LPR1* or *LPR2* (Loudet *et al.* 2005 and this study). Therefore, we think these two QTL are specific in their response to the P environment. In addition, when the NILs 194^B and 194^S were grown on a medium depleted in N, Fe or S, they behaved similarly, suggesting that the response controlled by *LPR1* is not triggered by other nutrient deficiencies. This further supports the view that *LPR1* is a specific QTL of the response to low P. Although *LPR1* is involved in two architectural responses to low P – PRL and length of primary root cells – it does not affect other responses to P starvation. Our results with the HIF, which differ only in the region of *LPR1*, demonstrate that the other typical P-starvation responses like the increase in root hair length, the accumulation of anthocyanins, the excretion of acidic phosphatases or the expression of the P-transporter gene *Phl1;4* are not modulated by *LPR1*. In particular, plants homozygous for the Bay-0 allele at the QTL, the root growth of which is almost insensitive to low P, still activate these other low P-induced responses. Therefore, *LPR1* seems to be

involved in a specific low P response, resulting in a reduced primary root growth rather than a general pathway of low P sensing.

P starvation reduces cell elongation

Under P starvation, by comparison to high P conditions, the Bay-0 and Shahdara accessions behaved quite differently. Bay-0 cell length was reduced by 52% whereas that of Shahdara was reduced by 90%. A similar range of variation was observed in the RIL population for which low P reduces primary root and cell lengths. By contrast to high P conditions, there is a significant correlation ($R^2 = 0.33$) between the primary root and the cell lengths (data not shown). This indicates that, in this RIL population, cell elongation is one of the main targets of P starvation in reducing the PRL. This result corroborates with the data presented by Sánchez-Calderón *et al.* (2005) and shows that, in the Columbia genetics background, P starvation inhibits root cell elongation and meristem activity.

We have detected three QTL for the primary root response to low P. One of these, *LPR1*, also controls the primary root cell length. This QTL explains more than 52% of the variation of PRL and around 30% of the variation of primary root cell length. Therefore, *LPR1* is a major QTL controlling cell elongation of the primary root growing under P starvation. *LPR1* might represent one of the loci that mediate the low P induced short root phenotype described by Sánchez-Calderón *et al.* (2005). For the three QTL, the Shahdara allele enhances the response to low P (reduction of root length). Because we did not find QTL for cell length colocalizing with *LPR2* and *LPR3*, these last two QTL must affect growth in another way; they may control the rate of cell production in the meristem. Altogether, these data show that P starvation has several targets when reducing root elongation. These targets are cell elongation, as revealed by *LPR1*, and perhaps the activity of the root meristem.

Only a very few genes/QTL that control specifically the elongation of root cells have been described. Recently, Mouchel, Briggs & Hardtke (2004) mapped a major effect QTL that controls the primary root growth through cell proliferation and elongation. They have isolated the corresponding gene (*BREVIX*) and T-DNA mutants. These mutants do not display abnormal growth response to low P (Mouchel *et al.* 2004). *BREVIX* maps at the top of chromosome 1 but not within the MSAT1.10/NGA248 interval where *LPR1* is located.

The Shahdara dominant allele of *LPR1* is responsible for the low P response

The fact that Bay-0 is still able to trigger several other typical low P responses demonstrates that it is not affected in a general low P-sensing mechanism. Instead, it may be altered in a root-specific growth signal/process. The recessiveness of the Bay-0 allele of *LPR1*, compared to the Shahdara allele, supports the idea that Bay-0 has lost a

function. Taken together, these data support the idea that *LPRI* is a positive factor that contributes in inhibiting or reducing root growth in low P.

We showed that the Shahdara allele of *LPRI* is largely dominant over the Bay-0 allele and is responsible for the response of the primary root to low P. This situation is in contrast with that of *PDR2* for which the recessive mutant allele (*pd2*) hypersensitizes root growth response to low P (Ticconi *et al.* 2004). Both *LPRI* and *PDR2* specifically affect the root. This supports the speculative view according to which these two functions operate in a common molecular process, but in opposite direction. *PDR2* maps on chromosome five, thus it is not an allele of *LPRI*. *PDR2* is supposed to function at a low P-sensitive checkpoint control during root development (Ticconi *et al.* 2004). In terms of molecular mechanisms, *LPRI* would be a positive sensor/transducer and *PDR2* a negative one. It would be interesting to test whether the function of *LPRI* needs that of *PDR2* in mediating the response to low P.

Natural diversity in the P-starvation response

In this work, we investigated the natural variation in root length and growth of roots in response to low P. In a small panel of accessions, we observed contrasting root growth responses. A previous study has already unravelled the natural variation in this response (Chevalier *et al.* 2003). However, our growth conditions gave marked differences between accessions compared to those of Chevalier *et al.* (2003). For example, Col exhibits a strong response to our low P conditions (5 μM P), whereas in Chevalier *et al.* (2003) (1 μM P) the primary root is only slightly affected. In addition, comparing Col, *L.er* and WS shows that WS exhibits the strongest response in Chevalier *et al.* (2003), whereas in our conditions this is exhibited in Col. These discrepancies may be caused by the different ways the plantlets were grown. Firstly, our growth medium contained 1% sucrose and the mineral solution was more diluted than the one used in Chevalier *et al.* (2003). Secondly, Chevalier *et al.* (2003). their lines were germinated on a 1 mM P medium for 6 d before transferring the plantlets either to a 1 mM P or a 1 μM P medium. The root lengths were then measured 2 weeks after the transfer. In our protocol, the lines were continuously grown on a low (5 μM) and a high P (500 μM) medium, and the lengths measured at day 10. Finally, the light sources were also different. It is therefore possible that some accessions are more sensitive than the others to some aspects of these growth conditions.

The results that we present here confirm the wide range of response to low P in natural accessions of *A. thaliana*. Unfortunately, there are no data available on soil composition, particularly in P availability, from which these accessions originated. Therefore, it remains to be clarified whether these growth responses to low P have ecological significance. In particular, whether the poor response of accessions like Bay-0 and *L.er* result from their tolerance or insensitivity to low P is yet to be determined.

CONCLUSION

We have mapped three QTL controlling the primary root response to low P. *LPRI*, which maps at the top of chromosome 1, is a major QTL of primary root cell elongation in response to low P, and the Shahdara allele is dominant over the Bay-0 allele. *LPRI* is not involved in other classical P-starvation responses, and neither is it involved in the growth response to other mineral (N, S and Fe) deficiencies.

ACKNOWLEDGMENTS

We thank Audrey Creff, Serge Chiarenza and Renaud Blervaque for their help in the microscope slide mounting used for the cell length measurements, Séverine Boiry and the team of the GRAP for taking care of our plants, Xenie Johnson for reading the manuscript and the NASC for providing the accession seeds.

REFERENCES

- Basten C.J., Weir B.S. & Zeng Z.B. (1995) *QTL Cartographer: A Reference Manual and Tutorial for QTL Mapping*. Center for Quantitative Genetics, North Carolina State University, NC, USA.
- Chevalier F., Pata M., Nacry P., Doumas P. & Rossignol M. (2003) Effects of phosphate availability on the root system architecture: large-scale analysis of the natural variation between Arabidopsis accessions. *Plant, Cell and Environment* **26**, 1839–1850.
- Churchill G.A. & Doerge R.W. (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963–971.
- Drew M.C. (1975) Comparison of the effects of a localised supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytology* **75**, 479–490.
- Forde B. & Lorenzo H. (2001) The nutritional control of root development. *Plant Soil* **232**, 51–68.
- Johnson J.F., Vance C.P. & Allan D.L. (1996) Phosphorus deficiency in *Lupinus albus*. Altered lateral root development and enhanced expression of phosphoenolpyruvate carboxylase. *Plant Physiology* **112**, 31–41.
- Lander E.S. & Botstein D. (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–199.
- Linkohr B.I., Williamson L.C., Fitter A.H. & Leyser H.M. (2002) Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. *Plant Journal* **29**, 751–760.
- Lopez-Bucio J., Cruz-Ramirez A. & Herrera-Estrella L. (2003) The role of nutrient availability in regulating root architecture. *Current Opinion in Plant Biology* **6**, 280–287.
- Loudet O., Chaillou S., Camilleri C., Bouchez D. & Daniel-Vedele F. (2002) Bay-0 \times Shahdara recombinant inbred line population: a powerful tool for the genetic dissection of complex traits in Arabidopsis. *Theoretical and Applied Genetics* **104**, 1173–1184.
- Loudet O., Gaudon V., Trubuil A. & Daniel-Vedele F. (2005) Quantitative trait loci controlling root growth and architecture in Arabidopsis thaliana confirmed by heterogeneous inbred family. *Theoretical and Applied Genetics* **110**, 742–753.
- Ma Z., Baskin T.I., Brown K.M. & Lynch J.P. (2003) Regulation of root elongation under phosphorus stress involves changes in ethylene responsiveness. *Plant Physiology* **131**, 1381–1390.
- Misson J., Thibaud M.-C., Bechtold N., Raghothama K.G. & Nussaume L. (2004) Transcriptional regulation and functional prop-

- erties of Arabidopsis Pht1;4, a high affinity transporter contributing greatly to phosphate uptake in phosphate deprived plants. *Plant Molecular Biology* **55**, 727–741.
- Mouchel C.F., Briggs G.C. & Hardtke C.S. (2004) Natural genetic variation in Arabidopsis identifies BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root. *Genes and Development* **18**, 700–714.
- Muchhal U.S., Pardo J.M. & Raghothama K.G. (1996) Phosphate transporters from the higher plant *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the USA* **93**, 10519–10523.
- Murashige T. & Skoog F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473–497.
- Narang R.A., Bruene A. & Altmann T. (2000) Analysis of phosphate acquisition efficiency in different Arabidopsis accessions. *Plant Physiology* **124**, 1786–1799.
- Poirier Y. & Bucher M. (2002) Phosphate transport and homeostasis in Arabidopsis. In *The Arabidopsis Book* (eds C.R. Somerville & E.M. Meyercowitz). American Society of Plant Biologists, Rockville, MD, doi/10.1199/tab.0024.
- Raghothama K.G. (1999) Phosphate acquisition. *Annual Review of Plant Physiological Plant Molecular Biology* **50**, 665–693.
- Reiter R.S., Coors J.G., Sussman M.R. & Gabelman W.H. (1991) Genetics analysis of tolerance to low-phosphorus stress in maize using restriction fragment length polymorphisms. *Theoretical and Applied Genetics* **82**, 561–568.
- Sánchez-Calderón L., López-Bucio J., Chacón-López A., Cruz-Ramírez A., Nieto-Jacobo F., Dubrovsky J.G. & Herrera-Estrella L. (2005) Phosphate starvation induces a determinate developmental program in the roots of *Arabidopsis thaliana*. *Plant and Cell Physiology* **46**, 174–184.
- Tanksley S.D. (1993) Mapping polygenes. *Annual Review of Genetics* **27**, 205–233.
- Ticconi C.A., Delatorre C.A. & Abel S. (2001) Attenuation of phosphate starvation responses by phosphite in Arabidopsis. *Plant Physiology* **127**, 963–972.
- Ticconi C.A., Delatorre C.A., Lahner B., Salt D.E. & Abel S. (2004) Arabidopsis pdr2 reveals a phosphate-sensitive checkpoint in root development. *Plant Journal* **37**, 801–814.
- Trull M.C. & Deikman J. (1998) An Arabidopsis mutant missing one acid phosphatase isoform. *Planta* **206**, 544–550.
- Trull M.C., Guiltinan M.J., Lynch J.P. & Deikman J. (1997) The response of wild-type and ABA mutant *Arabidopsis thaliana* plants to phosphorus starvation. *Plant, Cell and Environment* **20**, 85–92.
- Tuinstra M.R., Ejeta G. & Goldsbrough P.B. (1997) Heterogeneous inbred family (HIF) analysis: a method for developing near-isogenic lines that differ at quantitative trait loci. *Theoretical and Applied Genetics* **95**, 1005–1011.
- Williamson L.C., Ribrioux S.P., Fitter A.H. & Leyser H.M. (2001) Phosphate availability regulates root system architecture in Arabidopsis. *Plant Physiology* **126**, 875–882.
- Yi Y. & Guerinot M.L. (1996) Genetic evidence that induction of root Fe(III) chelate reductase activity is necessary for iron uptake under iron deficiency. *Plant Journal* **10**, 835–844.
- Zhang Y.J., Lynch J.P. & Brown K.M. (2003) Ethylene and phosphorus availability have interacting yet distinct effects on root hair development. *Journal of Experimental Botany* **54**, 2351–2361.

Received 25 May 2005; received in revised form 21 June 2005; accepted for publication 26 June 2005