Root Architecture Responses: In Search of Phosphate[1]

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Soil phosphate represents the only source of phosphorus for plants and, consequently, is its entry into the trophic chain. This major component of nucleic acids, phospholipids, and energy currency of the cell (ATP) can limit plant growth because of its low mobility in soil. As a result, root responses to low phosphate favor the exploration of the shallower part of the soil, where phosphate tends to be more abundant, a strategy described as topsoil foraging. We will review the diverse developmental strategies that can be observed among plants by detailing the effect of phosphate deficiency on primary and lateral roots. We also discuss the formation of cluster roots: an advanced adaptive strategy to cope with low phosphate availability observed in a limited number of species. Finally, we will put this work into perspective for future research directions.

Plant embryogenesis generates a very primitive developmental blueprint with two apical meristems (shoot and root) that, unlike in animals, do not reflect the anatomy of the adult organism. The ability to form new organs is maintained throughout their lifecycle because of the sustained activity of these meristems as well as the presence of dedicated cells that dedifferentiate and generate new meristems. The continuous nature of plant development associated with their sessile lifestyle results in a strong dependency on their immediate environment. As a result, the study of plant development must not only focus on the fundamental molecular and cellular mechanisms but also, integrate their ability to perceive and respond to the environment. In this regard, plant root systems represent a good model, because they have a high level of developmental plasticity in response to water, nutrients, gravity, and mechanical characteristics of the soil as well as biotic interactions.

Among the essential nutrients for plant growth and development, phosphorus is a key component of nucleic acids and phospholipids and present in soil in the form of either inorganic phosphate (Pi) or organophosphates. The former strongly interacts with divalent and trivalent cations. The latter has to be hydrolyzed to release phosphate for root uptake. The high sorption capacity of phosphate to soil particles results in a very low mobility and availability for uptake by plants. Therefore, the capacity of plants to find an adequate phosphate supply is directly correlated with their ability to explore the soil. Correspondingly, phosphorus deficiency induces changes in root system architecture as a key adaptive mechanism. A general strategy has been described under the term topsoil foraging that favors a shallower root system to explore the upper part of the soil, where phosphate tends to be more available because of the presence of organic matter and animal excrements. Although this term was first introduced to describe root system adaptation in bean (*Phaseolus vulgaris*; Lynch and Brown, 2001), the set of responses behind the topsoil foraging strategy has now been described in many other species (Panigrahy et al., 2009; Péret et al., 2011; Li et al., 2012; Shi et al., 2013). We will give an up-to-date overview of recent publications on developmental adaptations to low phosphate observed in diverse monocot and dicot species by focusing on the responses of the primary root (PR) and lateral roots. Finally, we will describe the evolutionarily advanced developmental adaptation to low phosphorus that has been found in several plant families' (i.e. cluster or proteoid) root formation.

LOW PHOSPHATE AVAILABILITY INHIBITS PR GROWTH

Phosphate deficiency dramatically inhibits Arabidopsis (*Arabidopsis thaliana*) PR growth (for review, see Abel, 2011; Niu et al., 2013; Giehl et al., 2014). This growth arrest is caused by reduced cell elongation and progressive

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cessation of cell proliferation in the root meristem that ultimately exhausts the PR stem cell niche (Fig. 1). Concomitantly, cells differentiate (e.g. root hair formation in epidermal cells) toward the root apex (Sánchez-Calderón et al., 2005). By comparing the effect of different nutrient deficiencies on root system architecture, Gruber et al. (2013) and Kellermeier et al. (2014) confirmed that Pi is one of the major factors controlling the PR length. Three major hypotheses have been suggested to explain the response of the PR to low Pi. First, one hypothesis relies on a reduction in metabolic activity, resulting in such an arrest. Second, some studies have reported that low phosphate leads to a higher availability of iron that could promote toxic effects responsible for the PR response. Third, the identification of several mutants with long PRs under low phosphate supply brings evidence for a determinant genetic control.

Reduced Root Growth Caused by Reduced Phosphate Metabolism

As a means to retrieve more Pi, plants concomitantly adopt two main strategies. First, they increase Pi recovery from organic phosphate by excreting ribonucleases, phosphatases, and carboxylates. Second, they improve phosphate uptake by increasing the affinity and capacity of its transport system. This is achieved by inducing the expression of a subset of plasma membrane phosphate transporters belonging to the PHOSPHATE TRANSPORTER1 (PHT1) family in Arabidopsis (Nussaume et al., 2011). As a result, mutants affected in genes belonging to either of these two classes of adaptive responses will alter plant capacity to maintain growth in low phosphate conditions. For instance, the *no acid phosphatase activity1* (*nop1*) mutant is affected in the *PURPLE ACID PHOSPHATASE10* (*PAP10*) gene, encoding for PAP10 (Wang et al., 2011). When grown on a low Pi medium, the root development of *nop1* mutants is slightly attenuated compared with the wild type. To test the importance of *PAP10* in using an organic source of phosphorus, Wang et al. (2011) supplied the low-Pi medium with ADP. In the *nop1* mutants, the root fresh weight is improved by exogenous ADP but does not reach that of the wild type. These results show that *PAP10* participates in root growth by allowing the seedling to use exogenous organic phosphate more efficiently. *PAP12* and *PAP26* are the two closest paralogs of *PAP10* and the predominant PAPs secreted by roots of Pi-deficient Arabidopsis (Tran et al., 2010). In Pi-replete conditions, the growth of the *pap12 pap26* double mutant is similar to the wild type but reduced in low Pi (Robinson et al., 2012). When provided with organic phosphate (glycerol-3-P or DNA), the root growth of the *pap12 pap26* double mutant is slower than that of the wild type. In these conditions, the root growth of wild-type seedlings is reduced compared with that in high-Pi medium. This shows that, although organic phosphate can be metabolically used for shoot growth, the root tip still reacts to the Pi-deficient medium. The above results indicate that the reduced recovery of Pi in the external environment because of the lack of acid phosphatase activity can directly affect plant growth.

A similar reduction of the phosphorus source has been obtained in mutants of the PHT1 phosphate transporters, which results in a reduced Pi uptake capacity. The *pht1;8* and *pht1;9* mutants grown on a Pi-deficient medium display a reduced PR growth (Remy et al., 2012). Inversely, seedlings overexpressing *PHT1;8* or *PHT1;9* have a slightly better PR growth than the wild type. All of these growth differences are abolished when the seedlings are grown in high-Pi medium. This result confirms the work by Shin et al. (2004) showing that the *pht1;1 pht1;4* double mutant affected in the two bulk root uptake systems absorbs less Pi and displays an overall reduced growth, including that of roots. Therefore, reducing the ability of plants to acquire phosphate from the soil by decreasing either its recovery or its uptake capacity results in an overall reduction of plant growth that can be explained by the law of mass action.

In parallel, there is clear evidence for a role of shoot-derived carbohydrates in modulating plant root responses to low Pi availability (Hammond and White, 2011). Based on the study of the *hypersensitive to phosphate starvation1* (*hps1*) mutant, which ectopically overexpresses the Suc transporter SUC2, Lei et al. (2011a) proposed that Suc is a global regulator of phosphorus starvation. In particular, *hps1* seedlings have a reduced PR growth in low Pi. This defect is not reversed by high Pi (1.2 mM Pi; Lei et al., 2011a). By using a different strategy aimed at overexpressing SUC2,

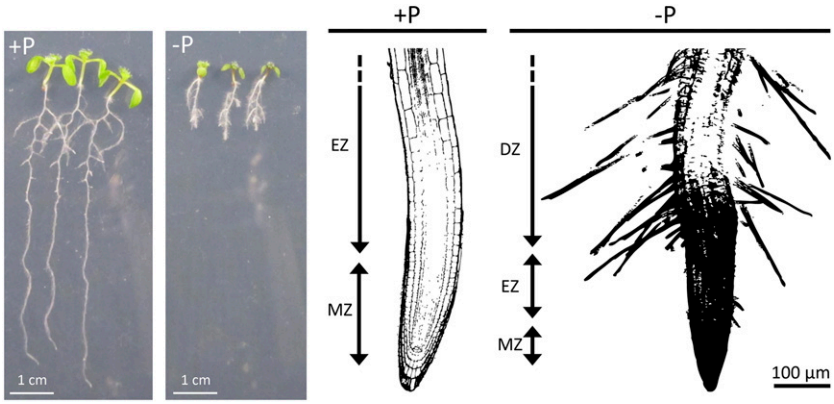

Figure 1. Arabidopsis PR response to low phosphate. The PR of the model plant Arabidopsis displays a striking phenotype in response to low phosphate supply. The lengths of the meristematic zone (MZ) and the elongation zone (EZ) are strongly reduced. The differentiation zone (DZ) is, therefore, observed much closer to the root tip. Meristem exhaustion ultimately leads to the complete arrest of PR growth. Plants were transferred to a low-phosphate medium (approximately 15 μM) for 48 h, imaged with a laser-scanning confocal microscope, and then converted to a black-and-white vector image.

Dasgupta et al. (2014) also observed a reduced PR length in a growth medium containing 0.6 mM Pi, and this altered growth was reversed at 1.2 mM Pi.

Isolated in the same genetic forward screen as the *hps1* mutant, the *hps7* mutant exhibits a hypersensitive root phenotype under Pi deficiency, but this phenotype is not reversed in high Pi (Kang et al., 2014). The *HPS7* gene corresponds to tyrosylprotein sulfotransferase, a protein required for the production of active sulfated phytosulfokine with absence that has pleiotropic consequences, including altered root meristem maintenance (Komori et al., 2009; Zhou et al., 2010) and enhanced Microbe Associated Molecular Pattern-triggered seedling growth inhibition (Igarashi et al., 2012). Surprisingly, expression of many photosynthetic genes is activated in roots of *hps7*, and their expression is further increased in low Pi; additionally, the PR tip of *hps7* accumulates chlorophyll, starch, and Suc (Kang et al., 2014). Kang et al. (2014) proposed that tyrosylprotein sulfotransferase acts as a master switch in the suppression of photosynthetic gene expression in roots. These findings extend the data for *suc2* mutants, but the molecular origin of the root growth defect of *hps7* seedlings is not yet known.

Reduced Root Growth Caused by an Indirect Low Pi-Mediated Stress Effect

An experiment aimed at uncoupling the root internal phosphorus status from the Pi content in the growth medium suggested that the local external conditions and not the phosphorus status inside the plant trigger PR growth inhibition (Thibaud et al., 2010). Indeed, foliar application of Pi could not prevent the PR growth arrest (Thibaud et al., 2010) in accordance with split root growth experiments showing that contact with a low phosphate medium is needed to trigger this response (Ticconi et al., 2004). This growth response, therefore, is likely not a consequence of reduced metabolic activity but part of a specific stress-induced morphogenic response (SIMR; Potters et al., 2007) and dependent on the iron content in the medium (Svistoonoff et al., 2007; for review, see Abel, 2011). These findings extend the data for *suc2* mutants and suggest that the root growth defect of *hps7* seedlings originates from the overaccumulation of sugar or reactive oxygen species in the root tip. It was suggested that reduction of phosphate concentration would increase the availability of iron (Ward et al., 2008), resulting in a toxic effect. However, in the absence of direct toxicity measurement, this remains speculative. SIMR is a generic term describing a set of common growth and developmental processes displayed by plants when exposed to sublethal abiotic stress conditions (Potters et al., 2007). Thus, these SIMRs are active responses that should be distinguished from toxic effects (Potters et al., 2007), which are caused by exposition to high doses of noxious compounds not tolerated by plants. Conversely, the PR response to low Pi requires a coordinated response comprising the inhibition of cell elongation, the cessation of cell division, and the stimulation of cell differentiation. The coordination of these cellular processes might involve reactive oxygen species, cell-to-cell signaling, and downstream effector targets (Potters et al., 2007) that remain to be discovered. It is possible that distinct stresses activate SIMR through specific genetic pathways, making SIMR compatible with our third hypothesis to explain the response of the PR to low Pi discussed below.

Genetic Control of the PR Response to Low Phosphate

The molecular mechanism underlying the PR growth response to low Pi is poorly understood and probably depends on many genes. However, so far, only very few candidate genes have been isolated in Arabidopsis: *LOW PHOSPHATE ROOT1* (*LPR1*), *LPR2* (encoding for multicopper oxidases; Svistoonoff et al., 2007), and *PHOSPHATE DEFICIENCY RESPONSE2* (*PDR2*; encoding a P-type 5 ATPase; Ticconi et al., 2009). Genetic and molecular analyses have shown that *LPR1* and *PDR2* are functionally related to the maintenance of the stem cell niche (for review, see Abel, 2011). Other mutants with an *lpr*- or *pdr*-like phenotype (i.e. long and short PRs, respectively) have been isolated in the past (*low phosphate-insensitive1-4*, *pdr23*, and *pdr3*), but the corresponding underlying genes have not yet been identified (for review, see Niu et al., 2013; Giehl et al., 2014).

Recently, several new mutants with an altered root growth in response to low Pi have been isolated, and the corresponding genes have been identified. The *local phosphate sensing impaired* (*lpsi*) mutant was found in an activation-tagging screen aimed at identifying seedlings with higher *PHT1;4* expression in low Pi (Karthikeyan et al., 2014). This mutant displays a long PR when grown in low Pi. Moreover, the expression of several genes involved in iron and zinc homeostasis and starch metabolism is altered in *lpsi* seedlings. In contrast to all of the other *lpr*-like mutants, the *lpsi* adult plant displays delayed growth and flowering as well as a strongly reduced fertility. In addition, *lpsi* seedlings do not overexpress the endogenous *PHT1;4* gene, suggesting that the *lpsi* phenotype has a complex genetic origin. It will be interesting to identify the molecular origin(s) of the *lpr*-like phenotype of *lpsi* and if it is functionally linked with the altered iron and zinc homeostasis.

The *ALTERED PHOSPHATE STARVATION RESPONSE1* (*APSR1*) gene is necessary for root meristem maintenance, and compared with the wild type, the *apsr1* mutants have a shorter PR under high Pi supply (González-Mendoza et al., 2013). In this condition, the root tip of the *apsr1* seedling looks much like the tip of the wild type growing under low Pi supply, with a shorter meristematic zone and differentiation of root hairs closer to the root tip. Surprisingly, this short root phenotype is not accentuated in low Pi, and the PR is similar to the wild type grown in low Pi. This conditional phenotype is correlated with a stronger root expression of *APSR1* in high- than low-Pi conditions. These results suggest that the function of APSR1 is necessary for decelerated root growth but

not under restrictive, suboptimal conditions. It would be interesting to test whether the root growth of *apsr1* is altered under other nutrient deficiencies. *APSR1* encodes a putative basic Leucine Zipper-like protein, and the APSR1-GFP fusion protein is located in the nucleus, suggesting a role in the control of transcriptional regulation.

Ethylene is a plant growth regulator modulating the amplitude and direction of root cell elongation (Nagarajan and Smith, 2012). Ethylene is also involved in controlling plant responses to biotic and abiotic stresses (Vandenbussche et al., 2012). In a forward genetic screen similar to the one used to identify *lpsi* (see above), Lei et al., (2011b) isolated the *hps2* mutant, an overexpressor of *PHT1;4*, and other Pi-related genes. In contrast to *lpsi*, *hps2* seedlings grown on low-Pi medium display a shorter root than the wild type. However, this reduced root growth is not specific to the low-Pi condition, because on high Pi, *hps2* also has a shorter root. It was reported that *hps2* is allelic to *constitutive triple response1* (*ctr1*), a key negative regulator of ethylene signaling. Corroborating this link between ethylene and Pi signaling, Wang et al. (2012) isolated two allelic mutants (*hps3-1* and *hps3-2*) with increased acid phosphatase activity in roots. Wang et al. (2012) showed that the *hps3* mutants are alleles of *ethylene overproducer1* (*eto1*), and they display altered expression of Pi-responsive genes. As seen before for *hps2*/*ctr1*, these mutants have a reduced PR length irrespective of Pi supply. In the same screen, Yu et al. (2012) isolated the *hps4* mutant, which also has increased root-associated acid phosphatase activity and a short PR irrespective of Pi supply. Cloning of HPS4 showed that *hps4* is a weak loss-of-function allele of *SABRE*, a gene necessary for cell expansion (Aeschbacher et al., 1995). The *hps4* root- and phosphate-associated phenotypes were confirmed with several other *sabre* alleles. Notably, in low Pi, the short root of *hps4* is partially reversed by Ag^+, an inhibitor of ethylene perception. Although ethylene modulates several Pi-related responses (Nagarajan and Smith, 2012), the results summarized here show that the role of ethylene in regulating PR growth is not Pi dependent. However, under phosphate starvation, ethylene biosynthesis or signaling might be increased in root tissue, which in turn, enhances auxin biosynthesis in root tips as shown by Yu et al. (2012).

PR Response in Monocot Species

Compared with Arabidopsis, in cereals, the development of the root system is more complex. For example, although in Arabidopsis, the PR is functional from germination to the senescing adult plant, the embryonic PR has significance only for seedling development in cereals (for review, see Hochholdinger and Zimmermann, 2008).

In rice (*Oryza sativa*) and barley (*Hordeum vulgare*), the effect of low Pi on PR growth is less pronounced than in Arabidopsis (Figs. 2 and 3), possibly because their seeds contain more abundant phosphorus reserves (Calderón-Vázquez et al., 2011). For example, low Pi slightly stimulates growth of the PR in maize (*Zea mays*; Li et al., 2012) and rice 'Japonica' (Zhou et al., 2008; Dai et al., 2012), although some reports are contradictory (for example, Yang et al., 2014). This may be attributed to differences in crop cultivars and experimental conditions. Both environmental adaptations and selective breeding of these crops would have contributed to these differential effects of low Pi on root growth.

Only very few genes acting on PR development of monocots in response to Pi have been identified to date. The expression of the rice *OsMYB4P* gene encoding an R2R3-type MYELOBLASTOSIS (MYB) protein is induced in the wild-type root after 7 d of Pi deprivation. Interestingly, when overexpressed, this

Figure 2. Rice developmental response to low phosphate. Rice plants from the cv Nipponbare variety were grown for 2 weeks in hydroponic conditions in one-tenth-strength Murashige and Skoog medium with high (+P; 1 mM) or low (−P; 10 μM) phosphate. Left, Entire plant. Center, Root systems. Right, Close-up view of the PR.

Figure 3. Barley developmental response to low phosphate. Barley 'OUK305' variety plants were grown for 2 weeks in hydroponic conditions in one-tenth-strength Murashige and Skoog medium with high (+P; 1 mM) or low (−P; 10 μM) phosphate.

gene increases the PR length independent of the Pi supply (Yang et al., 2014). This is reminiscent of results obtained with *OsMYB2P-1*, a closely related *MYB* transcription factor gene with expression that is also induced in roots of seedlings without phosphate (Dai et al., 2012). In the *OsMYB2P-1* overexpressor lines, the PR is longer than in the wild type, whereas in knockdown RNA interference lines, it is slightly shorter. In contrast to *OsMYB4P* overexpressors, *OsMYB2P-1*-overexpressing lines have a shorter PR than the wild type at high Pi supply, whereas knockdown lines are similar to the wild type (Dai et al., 2012).

The rice *leaf tip necrosis1* (*ltn1*) mutant was identified in a forward genetic screen, and its leaf necrosis phenotype is reminiscent of Pi toxicity (Hu et al., 2011). Similar to the mutant of its putative Arabidopsis ortholog *PHOSPHATE OVERACCUMULATOR2* (*PHO2*; Delhaize and Randall, 1995), the *ltn1* mutant exhibits increased Pi uptake and translocation from root to shoot, and it is altered in Pi signaling. In addition, the PR of *ltn1* is longer than in the wild type when grown in low-Pi medium but not when grown in high Pi (Hu et al., 2011). This growth phenotype may be caused by a stronger starvation signaling resulting from a lower phosphorus status in the *ltn1* mutant roots. Another rice gene named *NUTRITION RESPONSE AND ROOT GROWTH* (*NRR*) produces two alternatively spliced transcripts, *NRRa* and *NRRb*, coding for polypeptides of 308 and 223 amino acids, respectively. Knockdown of the expression of these genes by RNA interference resulted in enhanced rice root growth in Pi-limited conditions (Zhang et al., 2012).

The plant hormone strigolactone regulates many aspects of shoot and root development (Waldie et al., 2014). By using rice mutants altered in the biosynthesis or sensitivity to strigolactones, Sun et al. (2014) showed that strigolactones control the induction of PR growth in response to low Pi. However, this response is not specific to Pi, because similar effects were observed with nitrogen deficiency (Sun et al., 2014). Root architecture alterations resulting from Pi deficiency are also achieved by modulation of the auxin sensitivity of roots. Accordingly, some knockout lines of auxin response factor (ARF) genes impair root growth under low Pi supply. In the *osarf12* and *osarf12/25* mutants, the PR elongation was more responsive to Pi deficiency than the wild type (Wang et al., 2014b).

In conclusion, over the recent years, several new mutants with altered root growth under low Pi conditions have been isolated. However, for most of these new mutants, the root growth phenotype is not completely suppressed in Pi-replete conditions. Thus, although some of their phenotypes are caused by an alteration of the local low Pi-triggered signaling or stress response, others are probably a mere consequence of reduced metabolic activities (Péret et al., 2011).

LATERAL ROOT FORMATION IS INDUCED BY PHOSPHATE STARVATION

Concomitantly with the effect on PR growth, Pi starvation affects the formation of lateral roots. In this case, plants are faced by a dilemma: they must maximize phosphorus use efficiency while at the same time, promote exploration of the soil. As a result, the lack of Pi triggers a reduction of root growth according to the metabolic limitation, while at the same time, genetic programs will induce the development of new organs. It is, therefore, not surprising that the effect of Pi deficiency on lateral root formation is not as striking as that on the PR. Experimental setups used to reveal root responses to low Pi also may affect the phenotypic outcomes. Plants germinated on low-Pi medium may harbor a stronger metabolic limitation, whereas transferring plants from high- to low-Pi medium will reveal short-term genetically controlled responses. These changes can affect lateral root production, growth rate, and angle as well as root diameter (Bonser et al., 1996; Williamson et al., 2001; Hodge, 2004). The initial phases of lateral root development are affected by Pi starvation. A difference between plants grown in high- and low-Pi medium can be seen from 1 to 2 d after germination (Pérez-Torres et al., 2008), suggesting that both lateral root initiation driven by divisions of the pericycle cells and lateral root primordium growth and emergence through the outer tissue are affected.

Auxin Impacts Lateral Root Adaptation to Low Phosphate

The role of auxin during the formation of lateral roots has been well described (Lavenus et al., 2013),

and the involvement of auxin in the response to Pi has been shown (López-Bucio et al., 2002, 2005; Al-Ghazi et al., 2003; Nacry et al., 2005). However, most reports have relied on auxin-related mutants rather than searching for phosphate-specific lateral root mutants. For instance, the *indole acetic acid28* (*iaa28*) mutant shows resistance to the stimulatory effect of low Pi on lateral root formation (López-Bucio et al., 2002). Another example is the *aberrant lateral root formation3* (*alf3*) mutant displaying a long PR covered with many arrested lateral primordia on high phosphate (Celenza et al., 1995). However, lateral root formation of *alf3* can be rescued by either addition of exogenous auxin or transfer to a low-phosphate medium (Nacry et al., 2005). This suggested that low-phosphate conditions trigger lateral root formation by increasing the sensitivity of roots to auxin. Recently, it was shown that an increase in auxin sensitivity as a result of increased *TRANSPORT INHIBITOR RESPONSE1* (*TIR1*) expression was responsible for the increase in lateral root formation in low phosphate (Pérez-Torres et al., 2008). The mechanisms controlling the level of expression of the auxin receptor *TIR1* as a result of changes in Pi availability remain to be discovered.

Interestingly, some mutants of the Pi perception pathway are affected in their lateral root response to Pi. For instance, *pdr2* has lost the ability to produce more lateral roots on low Pi (Ticconi et al., 2004). Epistasis analysis indicates that the *LPR* and *PDR2* genes are functionally connected. Correspondingly, PDR2 colocalizes with LPR1 in the endoplasmic reticulum, which could indicate PDR2 and LPR1 functioning together in an endoplasmic reticulum-resident pathway and adjusting root meristem activity to external Pi (Ticconi et al., 2009). This would, therefore, control PR growth, whereas their combined effect on lateral root is not known.

Despite a small effect of the *pht1;8* and *pht1;9* mutants on PR growth, the *pht1;9* mutant displays an increase in lateral root number (Remy et al., 2012). The absence of this transporter results in a higher sensitivity to Pi starvation, which is further confirmed by a *PHT1;9*-overexpressing line that forms fewer lateral roots than wild-type plants, thus showing a decreased sensitivity to Pi starvation (Remy et al., 2012).

Phosphite ($H_2PO_3^-$) is a close steric but not metabolically inert analog of Pi that triggers unique physiological and developmental responses in plants and impairs Pi sensing, membrane transport, and subcellular compartmentalization. It is able to block some typical Pi starvation responses, such as an increased root-to-shoot ratio, root hair formation, anthocyanin accumulation, and phosphate starvation-related gene induction (Ticconi et al., 2001; Varadarajan et al., 2002). Surprisingly, phosphite application does not impact the induction of lateral roots by low phosphate (Berkowitz et al., 2013). This provides evidence for the existence of distinct mechanisms of phosphate perception and downstream responses to control the wide variety of physiological and developmental adaptations. All of the above findings suggest the existence of distinct pathways regulating PR and lateral responses to low phosphate.

Diverse Lateral Root Responses to Phosphate Supply in Monocot Species

Root system architectures of monocots and dicots display strong differences (Hochholdinger and Zimmermann, 2008), and their adaptation to low Pi reflects these differences. Phosphate starvation in maize inhibits lateral root formation (Li et al., 2012), whereas lateral root formation is increased in rice (Li et al., 2000). However, the selection processes used to produce these domesticated plants may have affected the way that they respond to Pi. Indeed, the common use of high levels of Pi fertilizers may have removed the selection pressure for genotypes adapted to low Pi. Increasing the efficiency of root systems to explore the soil for Pi may help reduce the amount of phosphorus needed to grow crops. Interestingly, some species have developed specific adaptations to low Pi conditions. The full genetic pathways controlling their formation are still unknown, but they may represent good strategies for future crop improvement (Veneklaas et al., 2012). A good example of such improvement is the recent cloning of a rice quantitative trait locus (QTL) conferring resistance to low phosphorus availability. This locus, called *Phosphorus uptake1* (*Pup1*), was originally identified in the traditional *aus*-type rice 'Kasalath' variety. This variety comes from a group of rice that originates from a region of India with very nutrient-poor soils and is, therefore, used as a good source of genes that are absent from other cultivated varieties. The *Pup1* QTL is the only phosphorus-related QTL available for marker-assisted breeding programs (Ramaekers et al., 2010; Calderón-Vázquez et al., 2011; Lynch, 2011; Shi et al., 2013), and it confers improved root growth under stress conditions. The molecular cloning of the *Pup1* QTL revealed that overexpression of the protein kinase PHOSPHORUS STARVATION TOLERANCE1 (PSTOL1) is responsible for the early establishment of the root system, therefore improving acquisition of phosphorus and other nutrients (Gamuyao et al., 2012). Although the molecular mechanisms of the PSTOL1 action are still unknown and seem to be nonspecific to phosphorus, this example illustrates how root developmental adaptation directly impacts the plant's capacity to acquire phosphorus and other nutrients.

CLUSTER ROOTS ARE AN EXTREME ADAPTATION TO PHOSPHORUS-LIMITED ENVIRONMENTS

Cluster roots (CRs) are specialized roots formed by densely spaced lateral rootlets that form at very low Pi supply (typically 1–5 μM Pi depending on the species) and are suppressed at higher Pi supply (Fig. 4). Their formation is an adaptive mechanism of specialist, mostly nonmycorrhizal plant species that thrive in environments with scarce nutrient availability (Shane and Lambers, 2005). Their development has, so far, largely been investigated under phosphorus-limited conditions, but it is also affected by nitrogen and iron availability (Arahou and Diem, 1997; Zaid et al., 2003; McCluskey et al., 2004; Rath et al., 2010). CR structure and physiology are geared to enlarge the surface area of the root for the

exudation of large amounts of carboxylates (exudative burst) to generate high local concentrations for the mining of insoluble forms of Pi from the soil and the efficient uptake of Pi (Neumann and Martinoia, 2002; Lambers et al., 2006).

CRs are found in a diverse range of monocot (Cyperaceae and Restionaceae) and dicot plant families and occur in two main forms: simple bottle brush like or compound mat forming (Skene, 1998; Shane and Lambers, 2005). Over the past two decades, white lupin (*Lupinus albus*; Fabaceae; Fig. 4) and harsh hakea (*Hakea prostrata*; Proteaceae; Fig. 5) have become model species for the analysis of CR development and physiology (Cheng et al., 2011; Lambers et al., 2011). CR formation is highly responsive to both abiotic and biotic factors (Lamont, 2003). Although detailed microscopic and molecular analyses of the events leading to the initiation of tens to hundreds of rootlets in close proximity to one another are scarce (Skene, 2000), evidence suggests that many of the key events leading on from the primordia foundation are very similar to the processes described for the established model plant species (Cheng et al., 2011). In white lupin, an intriguing finding is the synchronous emergence of rootlet clusters in pulses, suggesting a systemic signal linking CR formation to whole-plant phosphorus status (Watt and Evans, 1999). Correspondingly, foliar application of Pi leads to a depression of CRs, whereas sensing of Pi-rich patches induces local CR formation in white lupin (Shane et al., 2003b; Shu et al., 2007). In harsh hakea, analysis of a split root system showed that, although CR initiation occurred in regular bursts and was controlled locally, CR growth was systemically regulated (Shane et al., 2003a). Because of the Mediterranean climate in its natural habitat, phosphorus is stored in stem tissues, allowing for CR development and Pi uptake in the wetter winter months and shoot growth in summer (Shane and Lambers, 2005).

Similar to lateral root initiation in well-studied model species (Péret et al., 2009), auxin and cytokinin have been established as the key hormones regulating the spatial patterning of rootlet initiation in white lupin, whereas there is some evidence that gibberellic acid, nitrous oxide, ethylene, reactive oxygen species, and sugars also have some function in the fine tuning of CR formation (Cheng et al., 2011).

Most recently, several studies in white lupin using next generation sequencing technology have generated a de novo transcriptome assembly for white lupin. This provided the basis for global gene expression analyses of the acclimation of white lupin CRs to phosphorus deficiency and the identification of gene networks involved in CR formation at different developmental stages (O'Rourke et al., 2013; Secco et al., 2014; Wang et al., 2014a). These studies revealed known regulators of lateral root formation to also be involved in the establishment of the characteristic dense rootlet patterning. For example, genes homologous to PIN-FORMED, LIKE-AUXIN1, Aux/IAA and YUCCA are differentially expressed across mature, immature, and the PR tip of CRs likely to generate an auxin gradient. Genes coding for

Figure 4. White lupin developmental response to low phosphate. White lupin plants were grown in hydroponic conditions for 3 weeks on Hoagland medium with (+P; 100 μM) or without (−P) phosphate. Close-up image shows a CR from the low-phosphate plant.

cytokinin receptors and degrading enzymes have contrasting expression levels in different CR developmental stages, possibly controlling lateral root density (Secco et al., 2014; Wang et al., 2014a). Similarly, transcription factors involved in lateral root initiation, meristem maintenance, and cell differentiation, such as members of the ARF and PLETHORA families as well as SCARECROW and PHAVOLUTA, were more highly expressed toward the PR tip (Secco et al., 2014). By contrast, transcription factors involved in the formation of root hairs, ROOT HAIR DEFECTIVE-LIKE1 (RSL1) and RSL2, were preferentially expressed toward the mature part of the CRs, where dense root hair formation on the rootlets is taking place for efficient nutrient uptake (Watt and Evans, 1999; Secco et al., 2014).

Proteaceae show a much more complex CR morphology than white lupin (Fig. 5; Skene, 1998). Harsh hakea is endemic to the Southwest Botanical Province of western Australia that features ancient weathered soils

Figure 5. Harsh hakea developmental response to low phosphate. Four-month-old harsh hakea seedlings were transferred from soil to hydroponic solution containing 10 μM phosphate and grown for 12 weeks before being transferred to solutions with (+P; left) or without (−P; center) phosphate. Solutions were exchanged two times per week, and plants were treated for 21 d. Right, A developing 7-d-old CR (bottom) and a fully mature CR with full carboxylate exudation potential (top).

that are mostly limited by phosphorus requiring a highly specialized Pi mining strategy (Lambers et al., 2008; Hopper, 2009). This plant develops up to 1,000 rootlets per centimeter of secondary or tertiary root to a point where all pericycle cells have given rise to a rootlet and in extreme cases, two rootlets emerge from each of seven protoxylem poles (i.e. every possible rootlet initiation site is used in an all or nothing pattern along the root axis; Lamont, 1972; Skene, 2000). This massive structure poses a high carbon cost to the plant and therefore, only provides a competitive advantage in soils with very low phosphorus availability (Lambers et al., 2008). Early during harsh hakea CR development, respiration peaks before protein synthesis, which emphasizes the enormous energy cost and a need for the sequential organization of developmental processes (Shane et al., 2004a). Harsh hakea CRs are ephemeral and able to remobilize more than 95% of the phosphorus at the end of their lifecycle of about 21 d (Shane et al., 2004b). Although harsh hakea is slow growing and has a long lifespan, the first steps have been taken to develop this species into a model plant for molecular studies (Lambers et al., 2012; Shane et al., 2013; Sulpice et al., 2014). A de novo transcriptome obtained by next generation sequencing will become available in the near future to allow for the analysis of CR development on the transcriptional level (R. Jost, P.M. Finnegan, and H. Lambers, unpublished data). Harsh hakea has adapted to its phosphorus-impoverished environment in unique ways (e.g. through delayed chloroplast development in leaves and partitioning of scarce phosphorus resources between cytosolic and plastidic ribosomes; Sulpice et al., 2014). Combined with metabolome studies, the molecular characterization of CR development will elucidate the underlying regulators of CR initiation and sequential resource allocation that enable growth on extremely phosphorus-impoverished soils.

CONCLUSION

Evolution has selected several strategies to deal with the lack of readily available phosphorus sources in the soil. Most commonly represented in land plants is the establishment of mycorhizal symbioses, a subject that

has not been discussed in this Update, because it involves distinct molecular interactions and cellular differentiations, and has been extensively reviewed elsewhere (Parniske, 2008; Smith et al., 2011). However, developmental adaptations discussed here similarly represent strategies that lead to an increased capacity for soil exploration. Because of the immobile nature of phosphate, plants have to actively search for phosphate-rich soil patches, and this fact has conditioned their adaptive response to this deficiency. Additional studies in CR-forming species will increase our knowledge on how these species generate these specialized structures by using essentially very similar regulatory networks of hormones, transcription factors, and other signaling components used by plants with less complex roots. However, the unique dense formation of lateral roots is likely dependent on an added layer of regulatory and metabolic processes yet to be elucidated. Understanding of these networks might open up the possibility to engineer crops with improved root architecture able to use limited soil phosphorus more efficiently. Isolating more mutants and variants in model species, such as Arabidopsis and rice, specifically altered in the low-Pi response and signaling will be crucial for the understanding of molecular mechanisms. Screening mutants altered in root architecture is still very labor intensive, albeit plenty of imaging tools are now available (Lobet et al., 2013). QTL and Genome Wide Association analyses require less plant manipulation than mutant screenings and therefore, should help in finding new genes and their interactions more quickly. Another level of complexity will arise from studies of cross talks between nutrients to further decipher natural adaptation strategies. Among these nutrients, iron seems to play a key role in terms of both physical interactions in the soil and in planta and perception and signaling pathways. Recent studies have described that the Pi starvation-related transcription factor PHOSPHATE STARVATION RESPONSE1 (*PHR1*) can bind to the FERRITIN1 promoter. This first report on a direct molecular link between iron and phosphate homeostasis (Bournier et al., 2013) suggests the existence of a complex genetic interplay between nutrients for future research to decipher.

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