FOCUS PAPER

The transcriptional control of plant responses to phosphate limitation

José Manuel Franco-Zorrilla, Esperanza González, Regla Bustos, Francisco Linhares, Antonio Leyva and Javier Paz-Ares*

Centro Nacional de Biotecnología-CSIC, Campus de Cantoblanco, Madrid, E-28049, Spain

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Abstract

Plants have evolved an array of responses that adapt their growth to conditions of limited phosphate (Pi) supply. These involve biochemical and developmental changes that improve Pi acquisition and recycling, and protect against the stress of Pi starvation. The induction of these responses requires a sophisticated regulatory system that integrates information on external and internal plant Pi status and the details of this regulatory system are only just beginning to be elucidated. In this review, the current knowledge of this regulatory system is summarized, the hallmark of which is the central role of transcription factor PHR1 in the co-ordinated regulation of many phosphate-starvation-responsive genes. The role of hormonal signalling is also described, including auxins, ethylene and, particularly, cytokinins in the regulation of Pi-starvation responses.

Key words: Local Pi status, long-distance systemic repression, MYB transcription factor, phosphate starvation signalling, whole-plant Pi status.

Introduction

Phosphorus is an essential macronutrient for all living organisms. This mineral is quite abundant in the lithosphere, but P nutrition is frequently a factor limiting crop productivity. It is estimated that crop yield is limited by P availability in 30–40% of arable lands (Runge-Metzger, 1995; von Uexküll and Mutert, 1995). This paradox is due to the fact that plants preferentially take up P as orthophosphate (Pi), and more than 80% of soil orthophosphate is immobile and not readily available to roots (Holford, 1997).

Plants have evolved developmental and biochemical adaptations to low and unevenly distributed phosphate supply (for reviews see Abel et al., 2002; Raghothama, 1999; Rausch and Bucher, 2002; Vance et al., 2003). Developmental responses mostly involve changes in root architecture that enhance the root surface/soil volume ratio and, consequently, the ability of the plant to access soil phosphate. These include increases in the root-to-shoot growth ratio, in the number of lateral roots and in the number and length of root hairs (Bates and Lynch, 1996; López-Bucio et al., 2002; Ma et al., 2001; Williamson et al., 2001). In addition, some plants can improve their soil scavenging capacity further by forming clusters of lateral roots (proteoid roots) or by establishing symbiotic associations with mycorrhizal fungi (for reviews see Burleigh et al., 2002; Harrison, 1999; Vance et al., 2003; Watt and Evans, 1999). Also, when the root systems establish in a soil in which Pi is distributed in patches, lateral roots proliferate in those areas that are high in Pi and are inhibited in the low Pi areas (Drew, 1975; Robinson, 1994).

Biochemical responses serve two main functions: increasing the endogenous and soil Pi availability involves increases in Pi uptake capacity through the induction of high affinity Pi transporters, and increasing Pi mobilization and recycling activity through the induction of soil-secreted and endogenous phosphatases and RNases and the increased release of organic acids and protons (Raghothama, 1999; Vance et al., 2003). The second function is metabolic adaptation to Pi stress, which involves the utilization of alternative glycolytic or respiratory pathways which circumvent metabolic steps requiring Pi or adenylate, the concentrations of which drop under prolonged Pi stress (Duff et al., 1989). Pi stress also results in an increase in anthocyanin accumulation (which may protect leaves from photoinhibition resulting from limited light) and in the expression of genes that encode enzymes involved in the synthesis of carbon skeletons for the efficient conversion of Pi to other organic phosphorus forms (Raghothama, 1999; Vance et al., 2003).

* To whom correspondence should be addressed. Fax: +34 91 5854506. E-mail: jpazares@cnb.uam.es
photochemical reactions in photosynthesis; Takahashi et al., 1991; Trull et al., 1997), in changes carbohydrate metabolism (e.g. increases in starch content), and in thylakoid lipid composition (decreased phosphatidyglycerol may be compensated by an increase in sulpholipids; Essigmann et al., 1998).

Many genes involved in Pi-starvation responses have been cloned and their Pi responsiveness has demonstrated the importance of transcriptional control in the regulation of these responses in plants, as is also the case in bacteria and yeast. A great deal of information is available on the mechanisms underlying the control of Pi-starvation responses in bacteria and yeast but, in plants, this regulatory system and its complexity is only just beginning to be elucidated. Present knowledge regarding the control of Pi-starvation responses in plants includes: (i) evidence for a complex transcriptional response to Pi starvation, as indicated from the identification of different sets of genes whose maximal activation or repression occurs at different time points after Pi-starvation stress is elicited and/or occurs in different parts of the plant; (ii) the identification of some Pi-starvation-associated transcription factors; (iii) the involvement of hormone signal transduction in the control of Pi-starvation responses, and (iv) the existence of both whole plant Pi status-dependent, long-distance, systemically controlled responses and local Pi-controlled responses.

Transcriptional changes in plants during Pi starvation

Most studies of the changes in gene expression that follow Pi starvation have involved northern analysis of genes presumed to have a role in the Pi-starvation rescue system, such as RNases, phosphatases and high affinity Pi transporters, and of genes isolated following differential screening strategies, such as genes of the Mt4/TPSI1 family, which encode RNAs with limited protein coding potential which possibly act as riboregulators (Abel et al., 2002; Raghothama et al., 1999; Table 1). These studies have shown that many genes of the Pi-starvation rescue system are controlled transcriptionally. Collectively, they display similar mRNA accumulation patterns; their transcripts are detected well before plants are Pi starved, but increase significantly with the onset of the stress. These results support the idea that, in plants, there is a Pi-starvation-inducible rescue system, the PHO regulon, under a common regulatory system (Goldstein et al., 1988). Most of these genes whose expression increases during the Pi-starvation stress, share a common motif in their promoter, the GNATATNC motif (Table 1), which is recognized by the transcription factor PHOSPHATE STARVATION RESPONSE1 (PHR1; Rubio et al., 2001).

Three recent studies, however, point to a more complex transcriptional control of the Pi-starvation response in plants. In an analysis of transcriptional changes in Arabidopsis shoots, Hammond et al. (2003) found that at least two major transcriptional programmes operate in response to Pi starvation, one transiently, during the early stages of the stress, which preferentially involves genes with the characteristics of general stress response factors. The second transcriptional programme is most highly induced later in response to Pi-starvation stress and includes all genes presumed to play specific roles in the Pi-starvation response. In line with the existence of at least two major transcriptional programmes, the promoters of early response genes are significantly enriched in two sequence motifs, the PHO-like (CDHGTGG; D: G, T or A; H: C, T or A) and the TATA box-like (TATAAATA) elements, which are different from the binding motif of PHR1 (Hammond et al., 2003). The idea that there is a less-specific, early response to Pi-starvation stress is also supported by a study of the changes in transcript levels in tomato roots following Pi, K and Fe starvation using a macroarray enriched in mineral nutrition-related genes (Wang et al., 2002). Many of the genes induced early are already expressed at all three of these types of nutrient starvation stress. All of the K-, Fe- and Pi-starvation responses also involve the early induction of genes encoding Fe, K and Pi transporters. The relevance of these findings to the potential cross-talk between different types of nutrient starvation signal transduction pathways and their roles in the maintenance of mineral homeostasis still needs to be established.

An even more complex picture of transcriptional changes in response to Pi starvation can be drawn from the study of Wu et al. (2003). These authors found that 29% of the 6172 genes analysed were altered in the root and/or in the shoot in response to Pi starvation. These genes could be subdivided in several groups (potential transcriptional programmes) according to the time point after the stress or the part of the plant in which they were maximally activated or repressed. However, the significance of all these different transcriptional programmes associated with Pi starvation in relation to the establishment of an effective Pi-starvation response also need to be evaluated.

Transcription factors in Pi-starvation responses in plants

The greatest understanding of transcriptional control of Pi-starvation responses in eukaryotes is in yeast. In this organism, it is known that transcriptional control is predominantly the function of two transcription factors Pho2 and Pho4, belonging to the homeodomain and basic-helix-loop-helix families, respectively (for review see Lenburg and O’Shea, 1996). The Pi-starvation signal transduction pathway also includes a cyclin-dependent kinase CDK (Pho85), a cyclin (Pho80) and a CDK
inhibitor (Pho81), which is possibly the Pi sensor (Lenburg and O’Shea, 1996). When Pi is high in the cytoplasm, the Pho80/Pho85 complex phosphorylates Pho4 causing its rapid export from the nucleus through a mechanism involving the cooperation of the nuclear export protein Mns5 and a small Ran-GTPase (Kaffman et al., 1998a). Phosphorylation of Pho4 also prevents the interaction of this transcription factor with Pho2. Interaction of Pho4 and Pho2 is a step necessary for transcriptional activation. As a result, transcription of Pi responsive genes is prevented when Pi is high. When Pi is low, Pho81 inhibits the Pho80/Pho85 kinase and, through an unknown phosphatase, Pho4 becomes dephosphorylated and enters the nucleus via the import protein Pse1 (Kaffman et al., 1998b) where it cooperates with Pho2 to activate transcription of Pi-starvation responsive genes.

Unfortunately, searches of the Arabidopsis genome have not revealed any closely related members of PHO regulatory genes in yeast, suggesting that plants have evolved a different regulatory system to control Pi-starvation responses and precluding extrapolation from the yeast system. In fact, although understanding of the genes regulating the pathway in plants is much more limited, molecular genetic evidence has highlighted the role of an unrelated transcription factor, PHR1, as a master transcriptional activator of the Pi-starvation response in

### Table 1. Sequences related to the PHR1-binding site found at the upstream region of many known phosphate starvation-responsive genes

The position is given with respect to the predicted ATG translation start codon.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Code</th>
<th>Sequence</th>
<th>Position</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>ACP5</td>
<td>At3g17790</td>
<td>GAATATCC</td>
<td>−290</td>
<td>del Pozo et al., 1999</td>
</tr>
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<td>−</td>
<td>−</td>
<td>Ciereszko et al., 2001</td>
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<td>AF055372</td>
<td>GTATATGC</td>
<td>−783</td>
<td>Burleigh and Harrison, 1999</td>
</tr>
<tr>
<td>DGD2</td>
<td>At4g00550</td>
<td>GAATATCC</td>
<td>−366</td>
<td>Kelly and Dormann, 2002</td>
</tr>
<tr>
<td>IPS1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Martin et al., 2000</td>
</tr>
<tr>
<td>IPS3</td>
<td>At1g23110</td>
<td>GAATATGC</td>
<td>−745</td>
<td>del Pozo et al., unpublished data</td>
</tr>
<tr>
<td>PAP1</td>
<td>U48448</td>
<td>GGTATAC</td>
<td>−149</td>
<td>Haran et al., 2000</td>
</tr>
<tr>
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<td>At2g18130</td>
<td>GGTATAC</td>
<td>−101</td>
<td>Li et al., 2002</td>
</tr>
<tr>
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<td>At2g17190</td>
<td>GGTATATCC</td>
<td>−149</td>
<td>Li et al., 2002</td>
</tr>
<tr>
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<td>GGTATATCC</td>
<td>−1301</td>
<td>Muchhal et al., 1996</td>
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<td>Ciereszko et al., 2002</td>
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<td>GGTATATCC</td>
<td>−572</td>
<td>Yu et al., 2002</td>
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<tr>
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<td>GGTATATCC</td>
<td>−1564</td>
<td>Ciereszko and Kleczkowski, 2001</td>
</tr>
<tr>
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<td>−1204</td>
<td>Burleigh and Harrison, 1998</td>
</tr>
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<td>C19881</td>
<td>GGTATATCC</td>
<td>−231</td>
<td>Wasaki et al., 2003</td>
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<td>AF356962</td>
<td>GGTATATCC</td>
<td>−343</td>
<td>Paszkowski et al., 2002</td>
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<tr>
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<td>X99214</td>
<td>GGTATATCC</td>
<td>−551</td>
<td>Liu et al., 1997</td>
</tr>
</tbody>
</table>
Arabidopsis (Rubio et al., 2001). In addition to the studies on transcriptome changes that show the induction of transcription factors from several families in response to Pi starvation and the existence of novel common sequence motifs in early-induced Pi-starvation genes (Hammond et al., 2003; Wu et al., 2003), other approaches including promoter dissection studies coupled to southwestern screening of expression cDNA libraries and mobility shift assays with nuclear extracts have suggested the involvement of homeodomain-leucine zipper (HD-ZIP) proteins in the control of some Pi-responsive genes and the existence of negative regulation of Pi-starvation responsive genes (see below).

Role of transcription factor PHR1

PHR1 was identified in a genetic screen for mutants in the Pi-starvation response in Arabidopsis, using a line harbouring the highly specific AtIPS1-GUS reporter that had been mutagenized with EMS (Rubio et al., 2001). phr1 mutants displayed reduced Pi-starvation responsiveness of five Pi-starvation responsive genes. In addition, phr1 plants showed reduced anthocyanin accumulation in response to Pi starvation and, to a lesser extent, reduced root-to-shoot growth ratio, indicating that PHR1 is a positive regulator of the Pi-starvation response. An interesting observation concerning the effect of phr1 on anthocyanin accumulation is that this effect is specific for Pi starvation, because accumulation of this pigment following other stimuli, such as nitrogen starvation, cytokinin or ABA treatment was unaffected in this mutant (Rubio et al., 2001). This indicates that responses shared between different stresses may be controlled by at least partially independent regulatory systems. It would be interesting to test whether this is the case for 'non-specific' early responses to Pi starvation.

Molecular cloning of PHR1 showed that it encodes a transcription factor of the MYB superfamily which is highly related to the Chlamydomonas reinhardtii gene, PHOSPHORUS STARVATION RESPONSE1 (PSR1; Wykoff et al., 1999). Vascular plants have, therefore, derived a system regulating Pi-starvation responses from a transcriptional regulatory system already present in their unicellular ancestors. PHR1 and PSR1 share, in addition to a MYB DNA binding domain, a second domain predicted to form a coiled coil (CC), a fold usually involved in protein–protein interactions. This class of transcription factors (MYB-CC) is specific to plants and is represented by 15 members in Arabidopsis, raising the possibility of (partial) functional redundancy. In fact, the existence of partial redundancy would explain the fact that loss of function mutations at PHR1 do not result in full impairment of any of the Pi-starvation responses.

PHR1 binds as a dimer to an imperfect palindromic DNA sequence present in the promoter of many 'late' Pi-starvation genes (Rubio et al., 2001; Table 1), and mutation of the two PHR1-binding sequences (P1BS sequences) present in the promoter of the Pi-starvation response gene, AtIPS1, impairs its Pi-starvation responsiveness, establishing the importance of PHR1 (and related transcription factors) in the control of Pi-starvation responsive genes (JM Franco-Zorrilla, E González, R Bustos, F Linhares, A Leyva, J Paz-Ares, unpublished results). PHR1 itself, is not highly Pi-responsive and is located in the nucleus independent of the Pi status of the plant, indicating that either PHR1 activity is regulated post-translationally or that a second Pi-starvation regulatory protein is also needed to mount a proper Pi-starvation response.

Possible role of HD-ZIP proteins

The possible involvement of HD-ZIP proteins in the transcription control of some Pi-starvation responsive genes has been suggested from promoter dissection experiments on the vegetative storage protein B gene from soybean (Tang et al., 2001). A 50 bp fragment of VspB promoter can confer Pi-starvation responsiveness to a minimal CaMV 35S promoter. This fragment lacks a PHR1 binding site and contains two motifs recognized by proteins present in soybean and pea nuclear extracts. One of these motifs is recognized by two soybean HD-ZIP proteins in southwestern experiments, raising the possibility that this transcription factor type plays a role in Pi-starvation responses. However, experiments to evaluate the effect of mutating the HD-ZIP binding motif of VspB on its responsiveness to Pi starvation are needed to substantiate this possibility.

Possible role of transcriptional repressors in the control of Pi-starvation responsive genes

The possibility that transcriptional repressors are involved in the control of Pi-starvation responsive genes has been raised by the fact that mobility shift assays, with promoter fragments from two Pi-starvation responsive genes, detected DNA binding proteins in nuclear extracts from plants grown on Pi-rich medium, which were lacking in Pi-starved plants (Mukatira et al., 2001). The finding of high Pi-status specific DNA binding proteins, that specifically interact with promoter sequences of Pi-starvation responsive genes suggests the involvement of transcriptional repressors in the regulation of Pi-starvation responses, but again additional work, such as mutagenesis and analysis of Pi responsiveness of the mutant promoter, is required to substantiate this possibility.

Hormonal signalling and Pi-starvation responses

Hormones play an important role in plant development and in acclimating responses to stress and several studies have addressed the involvement of the hormones ABA,
ethylen, auxin and cytokinin in the control of Pi-starvation responses. These studies have shown that ABA signalling does not play a major role in Pi-starvation responses; abi1 and abi2-1 mutants do not significantly modify morphological responses to Pi starvation and Pi-starvation-induced phosphatase activity is unaffected in these mutants (Trull et al., 1997). However, some degree of cross talk between ABA and Pi-starvation signalling is suggested by the fact that abi2-1 mutants accumulate less anthocyanin in response to Pi starvation. Additionally, it was recently reported that the expression of rab18 under Pi-starvation conditions is partially reduced in the abal mutant (Ciereszko and Kleczkowski, 2002).

The involvement of ethylene and auxin signalling has been studied in the context of Pi-starvation changes in root morphology. The most remarkable observation is that Pi starvation results in an alteration in ethylene and auxin responsiveness in the root (Borch et al., 1999; López-Bucio et al., 2002; Ma et al., 2003). The effects of ethylene on the root are opposite for Pi-rich and Pi-starved plants (Borch et al., 1999; Ma et al., 2003). Thus, ethylene decreases root elongation in Pi-rich plants and has an opposite effect on Pi-starved plants. Reciprocally, inhibitors of ethylene synthesis or action promote increased root elongation in Pi-rich plants and decreased elongation in Pi-starved plants. Likewise, inhibition of ethylene synthesis or activity results in root hair initiation closer to the root tip in Pi-starved plants and farther from the root tip in Pi-rich plants (Ma et al., 2003). It is also noteworthy that the ethylene signalling that influences root hair formation in Pi-rich roots is not involved in the increase in root hair density and size that occurs in Pi-starved plants, probably because it involves a different cellular mechanism (Ma et al., 2001; Schmidt and Schikora, 2001). In case of auxin, Pi starvation increases sensitivity to this hormone in Arabidopsis, a change which could explain the increased number of lateral roots observed in response to Pi starvation (López-Bucio et al., 2002).

A more general role in the control of Pi-starvation responses has been proposed for cytokinins (Martín et al., 2000). Cytokinin concentration is reduced in Pi-starved plants, a situation which could also contribute to the increased root-to-shoot growth ratio and in lateral root proliferation in these plants (Horgan and Wareing, 1980; Salama and Wareing, 1979; Wagner and Beck, 1993). It was observed here that the exogenous addition of cytokinins repressed the expression of several Pi-starvation responsive genes, such as those encoding the ACPS phosphatase and the AtPT1 Pi transporter in the roots (Martín et al., 2000). This effect of cytokinin on the expression of Pi-starvation response genes is impaired in the mutants of the cytokinin receptor gene, CRE1, indicating that the repressing effect of this hormone on Pi-starvation responses shares at least the first signalling step with other types of cytokinin response (Franco-Zorrilla et al., 2002). These effects of cytokinin on Pi-starvation responses make the signalling pathway of this hormone a possible component of the long-distance systemic signalling pathway of Pi-starvation responses, described below.

**Phosphate sensing**

Little is known about the molecular mechanisms underlying Pi sensing in plants. However, some information on the physiological aspects of Pi sensing is currently available and this indicates that the mechanisms of sensing Pi starvation in plants are much more complex than in micro-organisms. This situation is probably due to the fact that plants are multicellular organisms and, in addition to requiring Pi-sensing mechanisms at the cellular level, need to communicate information about Pi levels between different organs and to co-ordinate an integrated organismal response to Pi status.

The nature of the Pi-sensing system at the cellular level has been analysed by Köck et al. (1998) using tomato cell cultures to study the expression of genes encoding RNases which are typical genes of the Pi-starvation rescue system. Tomato cells grown in a Pi-rich medium, were incubated with compounds that sequester intracellular Pi (by being phosphorylated after uptake). Under these conditions, the Pi-starvation responsive RNase genes were rapidly induced. These results suggest that Pi sensing itself is intracellular (Köck et al., 1998).

At the whole plant level, two types of response appear to operate, one dependent on whole plant Pi status involving long-distance signalling and the second dependent on external Pi concentration involving local signalling.

**Long-distance signalling**

The existence of long-distance signals that regulate Pi-starvation responses has been inferred from split root experiments, in which the roots of Pi-starved plants were divided and one part was exposed to a high Pi medium, while the other was left in a low Pi medium. Pi-starvation responses are systemically repressed in the parts of the roots exposed to low Pi medium (Burleigh and Harrison, 1998; Liu et al., 1997; Baldwin et al., 2001). This has led to the proposal that it is the shoot Pi status that controls Pi-starvation responses in the root (Burleigh and Harrison, 1998; Liu et al., 1997; Baldwin et al., 2001). Additional responses controlled by this system are the lateral root elongation that occurs in response to Pi starvation (Linkohr et al., 2002).

The nature of the long-distance systemic signal does not appear to be Pi itself, because repression precedes the increase in Pi in the Pi-starved root (Burleigh and Harrison, 1998). However, it seems that the signal has a repressor function and originates from the Pi-rich parts of the plant, rather than an activation function originating from Pi-
starved shoots. This interpretation is based on the fact that in the pho1 mutant (which is impaired in xylem loading and, therefore, has a low overall Pi content; Poirier et al., 1991), non-starved cells in the root (such those of the epidermis and cortex) do not express Pi-starvation genes (Martín et al., 2000). A candidate component of this systemic repression signalling system is the cytokinin signalling pathway because cytokinins are able to repress long-distance-controlled Pi responses, but not external Pi-controlled responses (Martín et al., 2000). However, external addition of cytokinin to one part of the root cannot repress Pi-starvation responsive genes systemically in the non-cytokinin-treated roots (JM Franco-Zorrilla, E González, R Bustos, F Linhares, A Leyva, J Paz-Ares, unpublished results). Therefore, further work is necessary to evaluate the importance of cytokinin signalling in long-distance systemic repression of Pi-starvation responses.

The above-mentioned comments about long-distance systemic repression signalling refer to the 'late' Pi-starvation-induced genes which are controlled by PHR1. Whether such systemic signalling acts through PHR1 or through other, as yet to be identified, transcription factors requires further investigation. However, Pi starvation elicits changes in gene expression in shoots as early as 4 h after starvation, an insufficient interval to allow significant changes in the shoot Pi pool (Hammond et al., 2003; Wu et al., 2003). Therefore, the possibility that there are, in addition to systemic signalling systems of a repressive nature, long-distance activation signals in Pi-starved roots, that induce gene expression in the shoot, should be kept in mind.

**Local Pi-controlled responses**

Despite the fact that most of the well established Pi-starvation responses and Pi-starvation-responsive genes are controlled by whole plant Pi status, there are some responses which depend on local Pi availability. For instance, changes in root hair number and length have been shown to occur in the Pi-starved roots of a plant independent of whether part of the root system of the same plant is in a Pi-rich environment (Bates and Lynch, 1996).

It is also possible that other responses, represented by genes whose expression in the root is elicited shortly after the onset of Pi starvation, are controlled by external Pi concentration (Wang et al., 2002; Wu et al., 2003). Again, it seems unlikely that, only 1 h after Pi starvation, shoot Pi-status could be decreased sufficiently to prevent long-distance repression.

Finally, an intermediate situation appears to occur in root tips, which are controlled both by external and whole plant Pi status. Growth through a high-Pi patch will reduce primary root growth after the root tip has left the patch, indicating that the root tip is responsive to local Pi status (Linkohr et al., 2002). However, the pho2 mutant, which hyperaccumulates Pi in the shoot while having a similar Pi content to wild type in its roots (Delhaize and Randall, 1995), displays a higher primary root growth than the wild type indicating the influence of shoot Pi status on root growth (Williamson et al., 2001).

**Outlook**

Significant progress has been made over the last few years in the understanding of Pi-starvation responses in plants and their regulation. The transcriptional changes that occur in response to Pi starvation are beginning to be revealed, although much is left to understand about their significance. A major regulatory gene of the Pi-starvation response has also been characterized molecularly. Current knowledge has already been used to design better strategies of management of Pi fertilization, as elegantly illustrated by Hammond et al. (2003) and their concept of the smart plant. The concept of smart plants is built on the finding that many of the Pi-starvation-responsive genes are induced well before plant growth is limited by Pi starvation. Therefore, plants harbouring reporter genes responsive to Pi starvation (smart plants) can be used to monitor plant Pi status and allow precision management of Pi fertilization, reducing application requirements without compromising crop yield.

Some success has been obtained in engineering plants that acquire Pi more efficiently, and thus require less fertilizer input, through ectopic expression of citrate synthases or secreted phytases (Koyama et al., 2000; López-Bucio et al., 2000; Richardson et al., 2001). However, given the complexity of the Pi-starvation response, the exploitation of the full potential of this response will depend on the manipulation of its regulatory system. Current knowledge of the control of Pi-starvation responses is, however, insufficient to make this possibility feasible. For instance, it is known that ectopic expression of PHR1 does not result in transgenic plants that perform better when grown under low Pi regimens (JM Franco-Zorrilla, E González, R Bustos, F Linhares, A Leyva, J Paz-Ares, unpublished results). Therefore, further work to identify additional regulatory components of the Pi-starvation response is needed before rational manipulation of the control system of Pi-starvation responses can contribute to sustainable agriculture. The molecular characterization of some Pi-starvation response mutants that have been identified, such as pho2, pho3, and the psr1-psr22 mutants (Chen et al., 2000; Dong et al., 1998; Zakhleniuk et al., 2001) and the further exploitation of the genetic screen based on transgenic lines harbouring different Pi-responsive reporters, such as that carried out with AtIPS1::GUS by Rubio et al. (2001) should prove valuable in this respect. The better understanding of the regulatory system of the Pi-starvation response will also shed light on the significance of the different transcrip-
tional programmes associated to Pi starvation in relation to the Pi-starvation response per se, and on the relevance of potential interrelations between the sensing, assimilation and metabolism of Pi, and that of carbon, nitrogen and other types of nutrient.

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