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Sucrose transport in the phloem: integrating root responses to phosphorus starvation

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Abstract

Sugars in plants, derived from photosynthesis, act as substrates for energy metabolism and the biosynthesis of complex carbohydrates, providing sink tissues with the necessary resources to grow and to develop. In addition, sugars can act as secondary messengers, with the ability to regulate plant growth and development in response to biotic and abiotic stresses. Sugar-signalling networks have the ability to regulate directly the expression of genes and to interact with other signalling pathways. Photosynthate is primarily transported to sink tissues as sucrose via the phloem. Under phosphorus (P) starvation, plants accumulate sugars and starch in their leaves. Increased loading of sucrose to the phloem under P starvation not only functions to relocate carbon resources to the roots, which increases their size relative to the shoot, but also has the potential to initiate sugar-signalling cascades that alter the expression of genes involved in optimizing root biochemistry to acquire soil phosphorus through increased expression and activity of inorganic phosphate transporters, the secretion of acid phosphatases and organic acids to release P from the soil, and the optimization of internal P use. This review looks at the evidence for the involvement of phloem sucrose in co-ordinating plant responses to P starvation at both the transcriptional and physiological levels.

Key words: Deficiency, mineral, nutrient, phloem, phosphate, phosphorus, signal, starvation, sucrose.

Defining sucrose as a signal

The purpose of this review is to explore the potential for shoot-derived carbohydrate signals to initiate acclimatory responses in roots to phosphorus (P) starvation. In this context, these carbohydrates act as systemic plant growth regulators. For shoot-derived carbohydrates to act as causal intermediary signals in co-ordinating root responses to P starvation they must meet the following criteria (*sensu* White, 2000): (i) root physiological and biochemical responses must be preceded by an increase in the biosynthesis of shoot carbohydrates and their translocation via the phloem to the root, (ii) blocking the biosynthesis or translocation of shoot carbohydrates must eliminate, or attenuate, the root physiological and biochemical responses to P starvation, and (iii) artificial changes in carbohydrate concentrations in the root, similar to those experienced in P-starved plants, must initiate similar responses to those induced by P starvation. This review will test each criterion and establish the potential for shoot-derived carbohydrates to act as systemic signals co-ordinating root responses to P starvation. Whilst exploring these criteria the mechanism by which sucrose might act as a signal will also be considered. For example, is there a change in phloem sucrose concentration or is there increased phloem flux to the roots? Is any change in phloem sucrose transient or sustained throughout P starvation? Is any change reversed on re-supply of P or is a further signal required? Although it may not be possible to answer all these questions, they will serve as a focus for future research into phloem sucrose and its ability to co-ordinate root responses to P starvation.

Sensing and signalling P availability

Phosphorus is the second most limiting mineral nutrient in crop production after nitrogen (Vance *et al.*, 2003). It is thought that a mechanistic understanding of how plants sense and respond to P starvation might facilitate selection, breeding, and GM approaches to improve crop production,

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and reduce our reliance on non-renewable inorganic P fertilizers (Vance *et al*., 2003; Hammond *et al*., 2004; Jain *et al*., 2007*b*). This may ultimately lower production costs, our reliance on mineral fertilizers, and P pollution to surface and groundwaters (Hammond *et al*., 2004).

Phosphorus starvation in plants initiates a myriad of transcriptional, biochemical, and physiological responses that serve either to enhance the plant's ability to acquire P from the soil or improve the efficiency with which plants utilize P internally (Fig. 1; Vance *et al*., 2003; Franco-Zorrilla *et al*., 2004; Hammond *et al*., 2004; Jain *et al*., 2007*b*). Our knowledge of how plants sense their P status and initiate responses to P starvation is increasing rapidly, although much still remains to be discovered. It is probable that plants can detect both whole plant P status, enabling efficient use of P internally, and local variations in P availability, enabling the proliferation of roots in P-rich patches (Fig. 1; Forde and Lorenzo, 2001; Williamson *et al*., 2001; Amtmann *et al*., 2006).

A complex series of signalling cascades is emerging that control plant responses to P starvation. These include many transcription factors. The first transcription factor implicated in regulating plant P starvation responses was PHR1, a myb transcription factor (Rubio *et al*., 2001). The PHR1 protein was shown to bind to an imperfect-palindromic sequence (P1BS; GNATATNC), which is present in the promoter regions of many P starvation-induced genes (Rubio *et al*., 2001; Hammond *et al*., 2004). The expression of *PHR1* appears to be constitutive, irrespective of plant P status, however, recent evidence suggests that the PHR1 protein may be targeted by a small ubiquitin-like modifier (SUMO) E3 ligase (SIZ1), whose expression is increased by P starvation (Miura *et al*., 2005). Sumoylation appears to modify the function of proteins in distinct ways; by altering their cellular location, activity, stability, or susceptibility to degradation by ubiquitination (Müller *et al*., 2001; Johnson, 2004; Kerscher *et al*., 2006). Interestingly, the *Arabidopsis siz1* knockout mutant is hypersensitive to P starvation, suggesting SIZ1 acts as a repressor of plant responses to P starvation. *Arabidopsis siz1* maintains many characteristic phenotypic responses to P starvation, including reduced primary root growth and increased lateral root and root hair number and length, increased root:shoot ratio, and increased anthocyanin accumulation (Miura *et al*., 2005). Interestingly, the expression of three P starvation-responsive genes, *AtPT2*, *AtPS2*, and *AtPS3* are greater in *siz1* compared with wild-type plants under P-replete conditions, and still show increases in expression during P starvation. However, the transcript accumulation of two other P-responsive genes, *AtIPS1* and *AtRNS1*, occurs at a reduced rate in *siz1* seedlings compared with wild-type seedlings, despite the presence of the P1BS sequence in their promoter regions (Miura *et al*., 2005).

As well as direct effects of PHR1 on the transcriptional regulation of P starvation-induced genes, a second

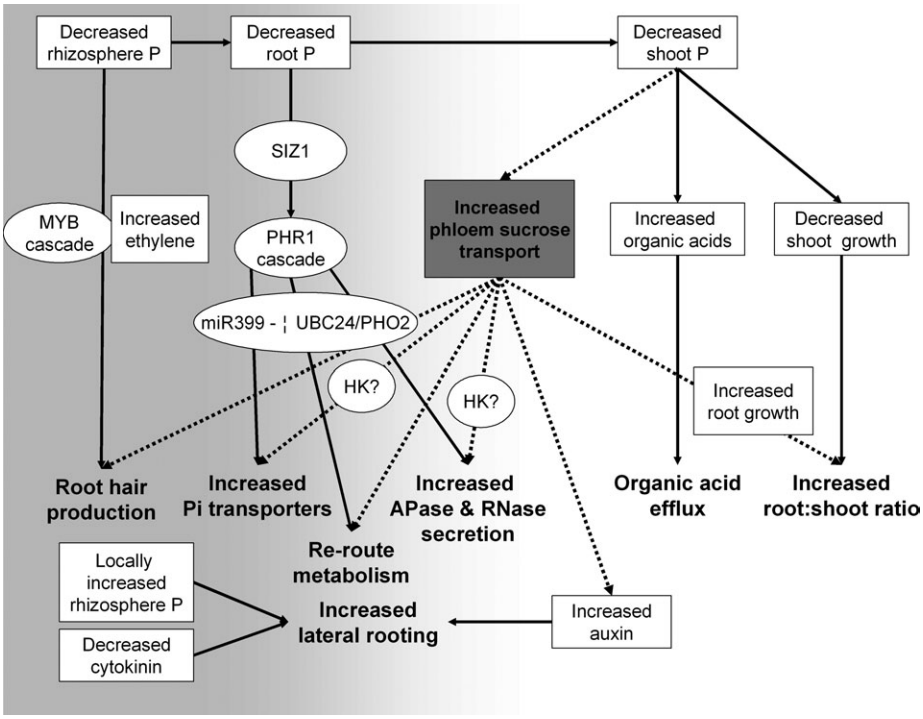

Fig. 1. Signalling cascades impacting on root system morphology, root Pi transport capacity, and the exudation of organic acids and enzymes into the rhizosphere. Bold arrows represent known steps in signalling cascades; Dotted arrows represent putative signalling cascades discussed in the text.

intermediate component of this signalling cascade has recently been identified. The microRNA family, miR399, contains six members in *Arabidopsis*, with orthologues in rice, and contain P1BS elements in their upstream regions (Sunkar and Zhu, 2004; Bari *et al*., 2006). Members of this family are specifically and rapidly up-regulated by P starvation, but are not detectable under P-replete conditions (Fujii *et al*., 2005; Bari *et al*., 2006; Chiou *et al*., 2006). The target gene for miR399 is a ubiquitin E2 conjugating enzyme, also identified as the gene responsible for the *pho2* mutant phenotype (*AtUBC24*; At2g33770; Aung *et al*., 2006; Bari *et al*., 2006). The *AtUBC24* gene contains five miR399 target sites in the 5′ UTR. The expression of *AtUBC24* has been shown to be reciprocal to that of miR399 during P starvation (Fujii *et al*., 2005; Bari *et al*., 2006; Chiou *et al*., 2006), although Bari *et al*. (2006) suggest some *AtUBC24* transcripts persist during P starvation and that a second, miR399-independent, post-transcriptional control mechanism acts on *AtUBC24*. It is thought that *AtUBC24* has a negative role in the control of a subset of P starvation-responsive genes, possibly through an intermediary transcription factor (Chiou, 2007). Interestingly, there is some sequence similarity between miR399 and the TPSI1/Mt4/At4 family of non-coding transcripts. This family of transcripts also responds rapidly and specifically to P starvation (Burleigh and Harrison, 1997, 1998, 1999; Liu *et al*., 1997; Martin *et al*., 2000; Shin *et al*., 2006). The recent characterization of the *At4* T-DNA knockout mutant suggests that it functions in the internal redistribution of P from the shoots to the roots (Shin *et al*., 2006). This family of non-coding transcripts contains a conserved 24 nucleotide sequence, which does not result in a consensus amino acid sequence, but does have partial complementarity to miR399 (Shin *et al*., 2006; Chiou, 2007). The functional significance of this has recently been resolved by Franco-Zorrilla *et al*. (2007), who show that these non-coding transcripts bind imperfectly to miR399, which prevents their degradation, sequestering miR399, and acting to attenuate the miR399-mediated P starvation response.

Global transcriptional profiling of plant responses to P starvation have also implicated many other transcription factors in plant responses to P starvation (Hammond *et al*., 2003; Wu *et al*., 2003; Misson *et al*., 2005; Morcuende *et al*., 2007; Müller *et al*., 2007). However, few have been characterized in detail and placed in specific signalling cascades. OsPTF1, a bHLH transcription factor from rice, with similarity to the *PHO4* gene in yeast, has been shown to enhance tolerance to P starvation. The expression of *OsPTF1* increases in the roots of P-starved rice plants, but is constitutively expressed in the shoots (Yi *et al*., 2005). However, transcriptional comparisons between wild type and *35S::OsPTF1*-over-expressing mutant lines revealed no direct regulation of Pht1 transporters or acid phosphatases (Yi *et al*., 2005). More recently, a WRKY transcription factor from *Arabidopsis*, AtWRKY75, has been implicated in regulating some P starvation responses in plants (Devaiah *et al*., 2007). RNAi suppression of this nuclear localized transcription factor resulted in impaired plant responses to P starvation and early accumulation of anthocyanin. The authors propose that AtWRKY75 acts as a positive regulator of plant P starvation responses, and also functions in the regulation of root architecture, independent of plant P status (Devaiah *et al*., 2007).

The signals that regulate, or are regulated by, these transcriptional cascades and initiate plant P starvation responses are a topic of current debate. Since it is likely that plants can detect both tissue P status and local variations in soil Pi availability, signalling molecule(s) that initiate plant P starvation responses may be different for local and systemic responses, with the potential for cross-talk between local and systemic signals at the point of action. López-Bucio *et al*. (2003) suggested that Pi is sensed locally and acts as a signal to control root hair and lateral root formation in response to plant P starvation. In addition, studies utilizing phosphite, a non-metabolized form of P, suggest that P may act directly as a signal and regulate some aspects of plant P starvation responses, including root meristem activity, anthocyanin accumulation, and root hair development, although many of these are local to the site of perception (McDonald *et al*., 2001; Ticconi *et al*., 2001, 2004; Varadarajan *et al*., 2002; Bozzo *et al*., 2004). Also some root responses to P starvation, such as root hair development, are regulated independently of shoot-derived signals (Bates and Lynch, 1996; Ma *et al*., 2001). However, studies in which the root system is split between compartments with contrasting Pi availabilities, suggest that Pi does not act as a systemic signal to control root system responses to P starvation (Burleigh and Harrison, 1999). The fine balance between auxin, ethylene, and local cytokinin concentrations, their transport from the shoot to the root or changes in the sensitivity of target tissues to these PGRs may be involved in the control of systemic responses to P starvation (Table 1; Forde and Lorenzo, 2001; Abel *et al*., 2002; López-Bucio *et al*., 2002; Vance *et al*., 2003; Hammond *et al*., 2004; Franco-Zorrilla *et al*., 2005). In addition, many recent publications have implicated the involvement of shoot-derived carbohydrate signals, and in particular sucrose, in the systemic control of plant P starvation responses (White *et al*., 2005; Amtmann *et al*., 2006; Hermans *et al*., 2006; Karthikeyan *et al*., 2007; Müller *et al*., 2007). It is speculated that sucrose not only functions as the primary compound for delivery of C to sink tissues, but also as a signal that alters gene expression (Chiou and Bush, 1998; Smeekens, 2000; Rolland *et al*., 2002). Unfortunately, the study of phloem-sucrose signalling is problematic given the central role of

Table 1. *The effects of plant growth regulators on roots during P starvation*

Plant growth regulator	Change in concentration, sensitivity or transport during P starvation	Effect on root system during P starvation	References
Auxin	Increased sensitivity	Increased lateral root formation and reduced primary root growth	López-Bucio *et al*., 2002
	Increased transport	Increased initiation of lateral root primodia and elongation	López-Bucio *et al*., 2005; Nacry *et al*., 2005
	Increased concentration	Increased formation of proteoid roots	Gilbert *et al*., 2000
	Increased concentration	Increased root hair density	Ma *et al*., 2001
	Increased concentration	Increased lateral root formation and elongation	Al-Ghazi *et al*., 2003
Cytokinin	Decrease in root concentrations	Reduced inhibition of lateral root initiation	Martín *et al*., 2000; Franco-Zorrilla *et al*., 2002; López-Bucio *et al*., 2002
Ethylene	Increased root concentrations	Increased root hair initiation: although not in all studies	Gilbert *et al*., 2000; Ma *et al*., 2001, 2003; Michael, 2001; Schmidt and Schikora, 2001
ABA	Increased biosynthesis	None: mainly exported via the xylem to the shoot	Jeschke *et al*., 1997

sucrose in driving phloem transport, delivering C skeletons to sink tissues and impacting tissue osmotic status, the rapid breakdown of sucrose into glucose and fructose, and potential cross-talk with other signalling pathways (Léon and Sheen, 2003; Franco-Zorrilla *et al*., 2005; Gibson, 2005).

These signals ultimately initiate and control plant responses to P starvation, including morphological, biochemical and physiological adaptation in the roots.

Root responses to P starvation

Plants acquire P from the soil solution via their roots, in the form of phosphate (Pi; Ullrich-Eberius *et al*., 1981). However, the complex chemistry of P in the soil often results in poor availability and supply of Pi to the root surface. To increase the availability and supply of Pi under P starvation, plants must adapt the biochemistry, physiology, and morphology of their root systems to maximize the acquisition of Pi (Fig. 1; Vance *et al*., 2003; Hammond *et al*., 2004; Raghothama and Karthikeyan, 2005; Jain *et al*., 2007*b*). Direct sensing of low rhizosphere P and increased ethylene production are thought to induce the initiation and elongation of root hairs (Föhse and Jungk, 1983; Bates and Lynch, 1996, 2001; Zhang *et al*., 2003). The increased proliferation of fine root hairs increases the root surface area and enables a greater volume of soil to be exploited (Bates and Lynch, 1996, 2001; Jungk, 2001; Zhang *et al*., 2003). Root hairs can contribute over 70% of a plant's root surface area, and maximize the soil volume a root can exploit, for a minimal input of biomass (Bates and Lynch, 1996; Jungk, 2001). Mutant lines exhibiting defects in root hair formation are less able to acquire available P and grow more slowly than wild-type plants (Bates and Lynch, 2000, 2001; Gahoonia *et al*., 2001). Recent evidence also suggests a strong influence of the availability of P and sucrose on the development of root hairs, with P-starved roots supplied with sucrose having three times more root hairs (of substantially longer length) than P-starved roots that were not supplied with sucrose (Jain *et al*., 2007*a*).

To complement the increase in root hair production initiated by low rhizosphere P, plants also alter the morphology of their root system in response to plant P status, and allocate more resources to their roots, increasing their root:shoot ratio (Fig. 1; Lynch, 1995; de Groot *et al*., 2001; Williamson *et al*., 2001; Wissuwa *et al*., 2005). However, these changes are not universal and vary between plant species. In *Arabidopsis*, P starvation results in the cessation of primary root growth, through a decrease in meristematic activity (Ticconi *et al*., 2004; Sánchez-Calderón *et al*., 2005). Contact of the root cap with P-deficient media is thought to be necessary and sufficient to initiate this response and multicopper oxidases have been implicated in effecting the response (Svistoonoff *et al*., 2007). This is not thought to be dependent on sucrose availability or auxin (Jain *et al*., 2007*a*). An increase in the initiation and elongation of lateral roots is also observed, although this is very variable (Williamson *et al*., 2001; Linkohr *et al*., 2002; López-Bucio *et al*., 2002, 2003, 2005; Al-Ghazi *et al*., 2003; Nacry *et al*., 2005; Jain *et al*., 2007*a*). Recent evidence suggests that a switch from an indeterminate root development programme to a determinate root development programme is responsible for this highly branched root morphology in *Arabidopsis* roots (Sánchez-Calderón *et al*., 2005, 2006). In *Phaseolus vulgaris*, P starvation results in a change in the growth angle of basal roots. The resulting root system has a shallower phenotype, allowing it to forage for available Pi in the top soil (Bonser *et al*., 1996; Lynch and Brown, 2001; Rubio *et al*., 2003). It is thought that the growth angle of basal roots in these plants

is modulated by the sensitivity of the basal roots to ethylene, which increases with P starvation (Basu *et al*., 2007). Many species also form specialized root clusters in response to P starvation (Lambers *et al*., 2006). These root structures are compact clusters of short branched roots or root hairs, which function to increase the surface area of the root within a small volume of soil, although their morphology and anatomy vary between species (Lambers *et al*., 2006). Root clusters also allow root exudates, including carboxylic acids and enzymes with the ability to release P from organic sources, to be concentrated to exploit patches of insoluble organic and inorganic sources of P (Gerke *et al*., 1994; Keerthisinghe *et al*., 1998; Gilbert *et al*., 1999; Neumann *et al*., 2000; Miller *et al*., 2001; Kihara *et al*., 2003; Shen *et al*., 2005). Split-root studies using white lupin suggest that root cluster formation and release of exudates are regulated by shoot P status (Shen *et al*., 2005). These alterations in root growth and morphology, and the increased production of organic acids are likely to be dependent on an increased allocation of resources from the shoot, including sucrose.

The proliferation of lateral roots in P-starved plants in response to localized Pi availability is also well documented (Fig. 1; Drew, 1975; Robinson, 1994; Forde and Lorenzo, 2001; López-Bucio *et al*., 2003). This phenomenon is probably a result of local nutrient sensing by the root tip growing through the Pi rich patch, initiating increased lateral root formation in the Pi rich patch.

Changes in either the local concentration, transport or sensitivity of auxin, ethylene, and cytokinin have been implicated in effecting root responses to low rhizosphere or plant P status, including the development of root hairs, lateral roots, and root clusters, although some studies provide conflicting results (Table 1; Martín *et al*., 2000; Williamson *et al*., 2001; Linkohr *et al*., 2002; López-Bucio *et al*., 2002, 2003, 2005; Al-Ghazi *et al*., 2003; Casimiro *et al*., 2003; Casson and Lindsey, 2003; Malamy, 2005; Nacry *et al*., 2005; Karthikeyan *et al*., 2007). Most recently, detailed analyses of root system architecture in P-starved *Arabidopsis*, have suggested that a change in the transport of auxins has an important role in initiating lateral root primodia (López-Bucio *et al*., 2005; Nacry *et al*., 2005), and a role of sucrose in making the root system more responsive to auxin during P starvation has been proposed (Jain *et al*., 2007*a*). Nevertheless, evidence for the involvement of auxin in reducing primary root growth and in stimulating root hair growth is still equivocal (López-Bucio *et al*., 2005; Nacry *et al*., 2005).

Ethylene has also been implicated in the stimulation of root elongation and root hair growth under P starvation (Gilbert *et al*., 2000; Michael, 2001; Ma *et al*., 2003). Experiments treating *Arabidopsis* plants with ethylene inhibitors or ethylene precursors suggests ethylene is important for stimulating lateral root elongation and reducing primary root elongation, but is not required for lateral root initiation during P starvation (López-Bucio *et al*., 2002; Ma *et al*., 2003). However, the analysis of root development in ethylene-insensitive or ethylene-resistant mutants has revealed responses to P starvation similar to wild-type plants (López-Bucio *et al*., 2002). The treatment of roots with ethylene synthesis inhibitors results in roots devoid of root hairs, whilst treatment of roots with the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), promotes the formation of root hairs (Masucci and Schieflbein, 1994; Tanimoto *et al*., 1995; Zhang *et al*., 2003). However, the treatment of P-starved plant roots with an ethylene biosynthesis inhibitor had no effect on root hair density, suggesting that during P starvation, other signal transduction pathways may be responsible for altering root morphology (Ma *et al*., 2001). This is supported by the analysis of ethylene-insensitive or -resistant mutants, which were shown to develop normal root hairs in response to P starvation (Schmidt and Schikora, 2001).

Finally, cytokinin concentrations have been observed to decrease in the roots of P-starved plants (Salama and Wareing, 1979; Horgan and Wareing, 1980; Kuiper *et al*., 1988). Cytokinins are traditionally associated with stimulating shoot growth and inhibiting root growth (Skoog and Miller, 1957; Aloni *et al*., 2006). Cytokinins have been shown to suppress lateral root initiation in *Arabidopsis* plants during P starvation (López-Bucio *et al*., 2002). Therefore, a decrease in root cytokinin concentration during P starvation may serve to release the inhibition of root growth and act as a negative control mechanism for increased root growth and other P starvation responses (Martín *et al*., 2000; Franco-Zorrilla *et al*., 2002). However, changes in cytokinin signalling during P starvation appear to be a secondary response, as a consequence of cross-talk between sugar and P signalling cascades (Franco-Zorrilla *et al*., 2005).

To extend their exploration of the soil further, most plant species also form symbiotic relationships with mycorrhizal fungi. The symbiosis between host and fungus is generally mutual, with the fungus receiving C from its host and the host receiving P and other soil nutrients from the fungi (Harrison, 1999; Smith *et al*., 2003; Morgan *et al*., 2005; Bucher, 2007). The smaller diameter of the fungal hyphae compared to roots (two to five times smaller) gives them a greater surface area: volume ratio, making them more efficient at acquiring P and enabling them to explore soil pores too small for roots to enter (Bolan, 1991). Mycorrhizal fungi may also have the ability to increase the availability of Pi, by acidifying the soil or secreting acid phosphatases or inducing P starvation responses in the roots, causing the roots to modify the rhizosphere (Koide and Kabir, 2000; Miyasaka and Habte, 2001; Ezawa *et al*., 2005). Consequently, as a result of mycorrhizal symbiosis, plants can often acquire

between three to five times more Pi than non-mycorrhizal plants, when grown in low P soils (Smith and Read, 1997; Smith *et al*., 2003). An increased allocation of C resources to the root under P starvation could promote and support these beneficial relationships.

Plant roots also have the ability to modify their rhizosphere during P starvation, to release Pi from immobilized organic and inorganic forms. The release of organic acids (e.g. citrate, malate, or oxalate) into the rhizosphere serves to release Pi bound to clay particles by ligand exchange (Lipton *et al*., 1987; Holford, 1997; Raghothama, 1999; López-Bucio *et al*., 2000*a*, *b*; Shane and Lambers, 2005). Since, for many soils, a significant portion (20–80%) of soil P is in organic forms, plants have evolved the ability to secrete enzymes capable of releasing Pi from organic P, making the P available for uptake (Fig. 1; Goldstein, 1992; Bariola *et al*., 1994; Duff *et al*., 1994; del Pozo *et al*., 1999; Haran *et al*., 2000; Baldwin *et al*., 2001; Miller *et al*., 2001; Li *et al*., 2002).

To maximize the effect of the adaptations to P starvation described above, plants also increase production and availability of Pi transporters to optimize the uptake and remobilization of tissue Pi (Muchhal *et al*., 1996; Daram *et al*., 1999; Muchhal and Raghothama, 1999; Kai *et al*., 2002; Karthikeyan *et al*., 2002; Mudge *et al*., 2002; Versaw and Harrison, 2002; Misson *et al*., 2004; Schünmann *et al*., 2004; Shin *et al*., 2004). Three subfamilies of Pi transporters have been identified (Pht1, Pht2, and Pht3; Daram *et al*., 1999; Takabatake *et al*., 1999; Mudge *et al*., 2002; Rausch and Bucher, 2002) in addition to the plastid Pi translocator family (Flügge *et al*., 2003). The Pht1 subfamily of Pi transporters is principally responsible for the uptake of Pi from the soil solution. Several members of the Pht1 subfamily have been identified within individual plant species (Karandashov and Bucher, 2005; Bucher, 2007), showing a range of expression profiles both spatially and in response to P starvation, with many having high expression in the roots and specifically in the tips of root hair cells (Karthikeyan *et al*., 2002; Mudge *et al*., 2002; Gordon-Weeks *et al*., 2003; Misson *et al*., 2004; Schünmann *et al*., 2004; Shin *et al*., 2004). There is also evidence for some redundancy within this subfamily (Shin *et al*., 2004). Recently, the presence of a specific secretory pathway from the ER to the plasma membrane for proteins of the Pht1 subfamily, involving an accessory protein, PHosphate transporter traffic Facilitator 1 (PHF1), has been identified. This suggests that a novel mechanism for localizing proteins of the Pht1 subfamily to the plasma membrane might exist, and provides an additional control point in controlling plant P starvation responses (González *et al*., 2005).

Plants also improve their ability to utilize their tissue P more efficiently when the availability of P is low. This involves increasing the activity of enzymes that replace P in metabolites (Fig. 2; Malboobi and Lefebvre, 1997; Plaxton and Carswell, 1999; Ciereszko *et al*., 2001) and structural compounds, for example, the proteins involved in the replacement of phospholipids with sulpho- and galacto-lipids (Essigmann *et al*., 1998; Härtel *et al*., 2000; Kelly and Dörmann, 2002; Yu *et al*., 2002; Hammond *et al*., 2003; Andersson *et al*., 2005; Cruz-Ramírez *et al*., 2006; Li *et al*., 2006).

The following sections of this review will attempt to synthesize the evidence for the involvement of sucrose in signalling plant P starvation responses in the roots using the criteria set out previously.

Plant responses to low P availability are preceded by increased carbohydrate concentrations in the shoot and phloem

Phosphorus starvation has immediate and direct consequences for photosynthesis, glycolysis, and respiration (Fig. 2; Plaxton and Carswell, 1999). Intracellular Pi is selectively distributed between the cytoplasm and the vacuole, with excess Pi being stored in the vacuole and used to maintain the Pi homeostasis in the cytoplasm (Foyer and Spencer, 1986; Lee *et al*., 1990; Mimura *et al*., 1990). During P starvation, when the available vacuole Pi reserves are depleted, the lack of cytoplasmic Pi often inhibits photosynthesis, although some research suggests that photosynthesis can be sustained under P deficiency for some time (Wissuwa *et al*., 2005). This has direct effects on the energy transducing systems in the thylakoids, causing inhibition of Calvin cycle enzymes and feedback inhibition through changes in the pH gradient across the thylakoid membrane or by the redox state of electron carriers (Preiss, 1984; Foyer and Spencer, 1986; Fredeen *et al*., 1990; Jacob and Lawlor, 1992; Natr, 1992; Plaxton and Carswell, 1999). The reduction in cellular Pi concentration reduces the activity of ATP synthases in the thylakoid membrane and the activity of ribulose-1,5-*bis*phosphate carboxylase (RuBisCo), consequently reducing carbon assimilation, but not terminating it (Fredeen *et al*., 1990; Usuda and Shimogawara, 1991; Natr, 1992; Rao *et al*., 1993; Cakmak, 1994; Cakmak *et al*., 1994*a*; Barret and Gifford, 1995). As a consequence, chlorosis is not observed during P starvation, in contrast to K and Mg starvation (Cakmak *et al*., 1994*a*). Although carbon assimilation is restricted during P starvation and the export of triose-Pi from the chloroplast is reduced, resulting in its conversion to starch, a sustained, and in some species an increased translocation of mobile carbohydrates via the phloem to the roots is observed, primarily in the form of sucrose (Fig. 2; Cakmak *et al*., 1994*b*; Hermans *et al*., 2006). The increased translocation of sucrose to the root may be driven by a reduced shoot demand, or an increased root demand, for sucrose. Sucrose may be utilized immediately

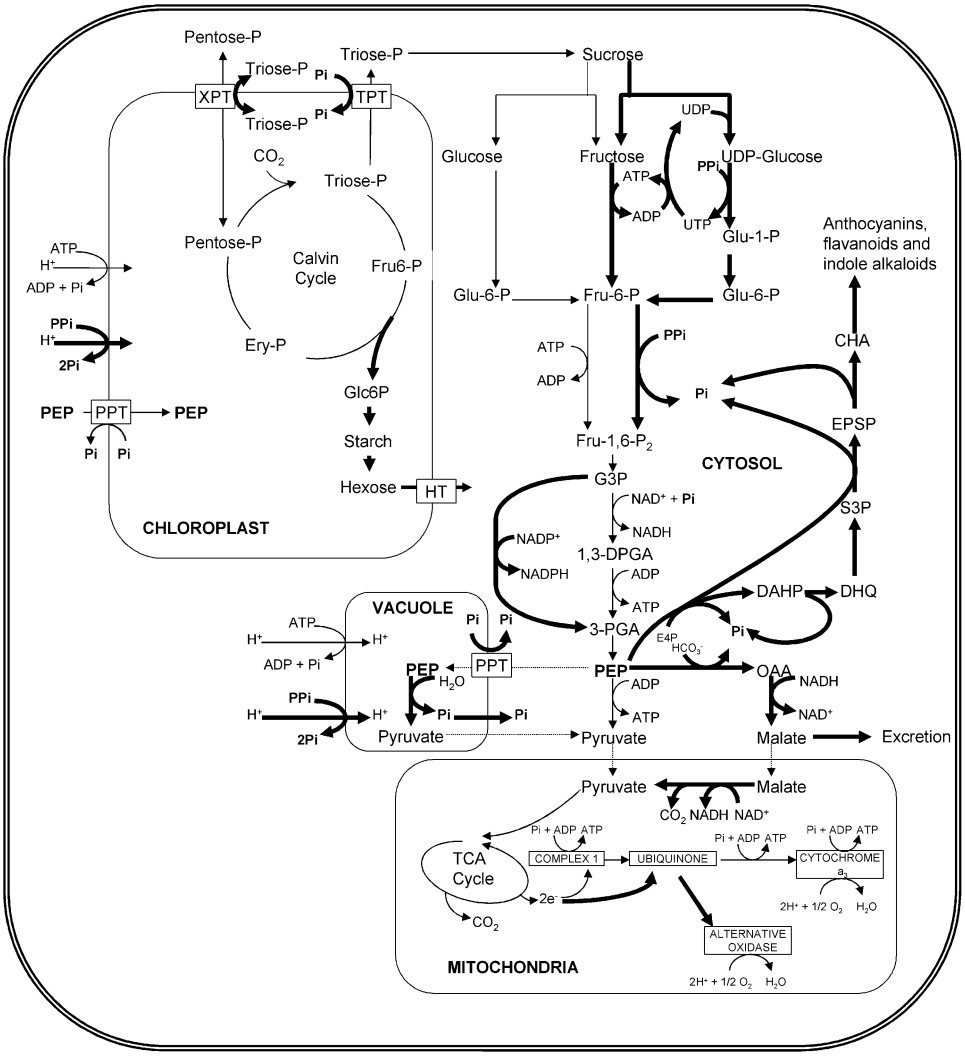

Fig. 2. Alternative metabolic processes for cytosolic glycolysis, mitochondrial electron transport, chloroplast processes, and tonoplast H^+ pumping (bold arrows) that may enable plants to survive under P-limiting conditions. Abbreviations for compounds are as follows; Glu-1-P, glucose 1-phosphate; Glu-6-P, glucose 6-phosphate; Fru-6-P, fructose 6-phosphate; Fru-1,6-P_2, fructose 1,6-*bis*phosphate; G3P, glyceraldehyde-3-phosphate; 1,3-DPGA, 1,3-dephosphoglycerate; OAA, oxaloacetate; E4P, erythrose 4-phosphate; S3P, shikimate-3-phosphate; HT, hexose translocator; PEP, phosphoenolpyruvate; PPT, phosphoenolpyruvate/phosphate translocator; Pi, inorganic phosphate; TPT, triose phosphate/phosphate translocator; TrioseP, triose phosphates; XPT, xylulose 5-phosphate/phosphate translocator. Figure redrawn from Plaxton and Carswell (1999) and Flügge *et al.* (2003) and reproduced with permission.

by roots or converted to starch (Wissuwa *et al.*, 2005). An increase in sucrose biosynthesis in the leaves of P-starved *Arabidopsis*, bean, barley, spinach, and soya plants has been observed (Foyer and Spencer, 1986; Cakmak *et al.*, 1994*b*; Ciereszko *et al.*, 1996; Ciereszko and Barbachowska, 2000; Morcuende *et al.*, 2007; Müller *et al.*, 2007). However, there appears to be considerable variation in the responses of shoot carbohydrate metabolism during P starvation (Foyer and Spencer, 1986; Kondracka and Rychter, 1997; Fujita *et al.*, 2004). Some of this variation may result from the different light intensities used in different studies, impacting on photosynthetic capacity. In addition, no common tissue P status has been defined for studies of plant responses to P starvation. This makes true comparisons between studies difficult, especially considering the potential cross-talk with pathways that respond to other environmental cues. Differences between species in their intrinsic C metabolism or photosynthetic responses to light intensity could also result in contrasting shoot carbohydrate metabolism during P starvation.

It is noteworthy that the increased export of sucrose via the phloem may not be sustained, since the export of sucrose from P-starved bean leaves returned to basal levels within 9 d (Cakmak *et al.*, 1994*b*). Nevertheless, for changes in sucrose biosynthesis and export to affect plant P starvation responses, and implicate sucrose in signalling low shoot P status, these changes must be an early response. The earliest temporal measurement of changes

in phloem sucrose in response to low P availability show an increase after 6 d and it has been suggested therefore, that the accumulation of sugars is an early response to P starvation (Ciereszko *et al*., 2005). However, some plant responses to P starvation will be initiated prior to this timepoint. Consequently, further research is required to monitor early changes in sucrose transport to the root following transfer to low P conditions to elucidate its potential role in signalling plant P starvation further. Nevertheless, changes in root morphology and architecture appear to occur in *Arabidopsis* seedlings after 6–9 d of P starvation (Al-Ghazi *et al*., 2003; Nacry *et al*., 2005). These include a decrease in primary root growth, and increases in lateral root length and elongation rate. This is consistent with the increased flux of phloem sucrose from the shoots to the roots observed in this time frame. In addition, the root sucrose concentration of P-starved bean and soya plants is higher than the root sucrose concentration of P-replete plants (Fig. 3; Fredeen *et al*., 1989; Ciereszko *et al*., 1996; Ciereszko and Barbachowska, 2000), although this was not the case in *Arabidopsis* roots (Ciereszko *et al*., 2001).

Although physiological measurements of phloem sucrose flux are lacking during early P starvation, transcriptional studies of plant P starvation responses indicate a rapid change in shoot carbohydrate metabolism (Hammond *et al*., 2003, 2005; Uhde-Stone *et al*., 2003; Wasaki *et al*., 2003; Wu *et al*., 2003; Misson *et al*., 2005; Morcuende *et al*., 2007; Müller *et al*., 2007). The reduction in photosynthesis is evident in the down-regulation of many genes encoding proteins involved in photosynthesis, including photosystem subunits, small subunits of RuBisCo, and chlorophyll synthesis (Wu *et al*., 2003; Morcuende *et al*., 2007). Phosphorus starvation also impacts on the export of triose-Pi from the chloroplast, which is subsequently converted to starch. Several genes encoding enzymes involved in starch synthesis and degradation are known to have altered expression during P starvation including *AGPase*, β-*AMY*, *BAM5*, and *GWD3* (Nielsen *et al*., 1998; Wu *et al*., 2003; Müller *et al*., 2005, 2007; Morcuende *et al*., 2007). Changes in the synthesis, translocation, and degradation of sucrose are also apparent from these transcriptional studies. Transcripts from genes encoding invertases, sucrose synthases, sucrose-phosphate synthases, and sucrose-phosphate phosphatase have been shown to be differentially expressed in transcriptional studies of plant P starvation responses (Hammond *et al*., 2003, 2005; Uhde-Stone *et al*., 2003; Wu *et al*., 2003; Misson *et al*., 2005; Müller *et al*., 2007). In addition, the expression patterns of several carbohydrate transporters are modified in the shoots and roots of plants responding to changes in P availability (Hammond *et al*., 2003; Wu *et al*., 2003, Misson *et al*., 2005; Müller *et al*., 2007). However, it is noteworthy that whilst some transcriptional profiling studies monitor gene expression in response to P starvation, others are monitoring gene expression in P-starved plants following the re-supply of P. Because removing P from P-replete plants and supplying P to P-starved plants are likely to induce different transcriptional, biochemical, and morphological changes, events that occur when P is supplied to P-starved plants do not necessarily represent plant responses to P starvation.

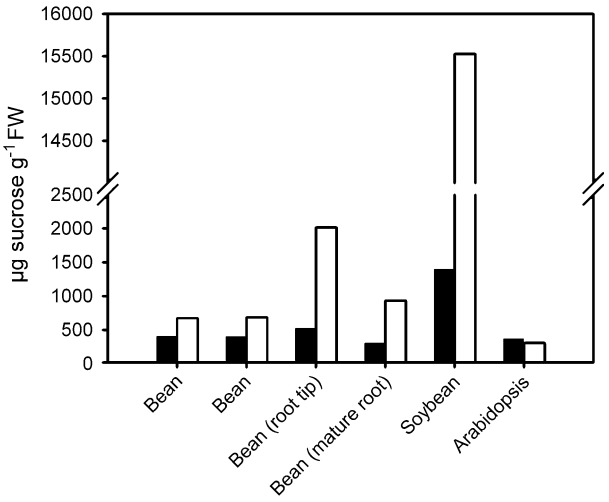

Fig. 3. Sucrose concentrations in the roots of P-starved (open bars) and P-replete (filled bars) bean (Ciereszko *et al*., 1996; Ciereszko and Barbbachowska 2000), soybean (Fredeen *et al*., 1989), and *Arabidopsis* (Ciereszko *et al*., 2001) plants.

Inhibition of sucrose biosynthesis and/or translocation attenuates plant responses to P starvation

An increase in extracellular phosphatase activity is a characteristic response of plants to P starvation, and functions to release Pi from exogenous organic compounds (Duff *et al*., 1994; del Pozo *et al*., 1999; Li *et al*., 2002). The *pho3* mutant was isolated by screening for root acid phosphatase activity, and was identified as having 30% less acid phosphatase activity than wild-type plants and showing attenuated responses to P starvation (Zakhleniuk *et al*., 2001). The inability of *pho3* to respond to low internal P concentrations led to the suggestion that it might encode a Pi transporter or a signalling component involved in the regulation of P nutrition (Zakhleniuk *et al*., 2001). However, when the *PHO3* mutation was cloned it was identified as *SUC2*, a sucrose transporter involved in phloem loading (Lloyd and Zakhleniuk, 2004), although the SUC2 transporter is also capable of transporting other carbohydrate substrates (Chandran *et al*., 2003). Consequently, the *pho3* mutant has constitutively high shoot carbohydrate concentrations compared with wild-type plants due to its limited ability

to translocate sucrose to the roots (Lloyd and Zakhleniuk, 2004). In addition, the application of ^{14}C-labelled sucrose to the leaves of *suc2* plants resulted in very little translocation of the labelled sucrose to the roots compared with wild-type plants (Gottwald *et al*., 2000). Since the mutant was originally identified in a screen for mutants with impaired root phosphatase activity, this suggests that a restriction in phloem sucrose loading interferes with at least some aspects of plant P starvation responses. Two contrasting studies have compared the gene expression profiles of this mutant with the gene expression profiles of the *pho1* mutant (Usadel *et al*., 2005) and with a combined list of genes differentially regulated during P starvation, including genes from the *pho1* mutant (Hermans *et al*., 2006). Whilst Usadel *et al*. (2005) concluded there was little overlap between the gene expression profiles of the *pho3* and *pho1* mutants, Hermans *et al*. (2006) observed that 22% of genes responding to P starvation were also differentially expressed in the shoots of the *pho3* mutant. This overlap in differentially expressed genes includes many genes involved in carbohydrate metabolism, suggesting a common component in signalling P starvation.

Reducing photosynthetic capacity by growing plants under low light or in the dark can also reduce shoot sucrose concentrations and potentially impact on the ability of sucrose to signal shoot P status. Liu *et al*. (2005) hypothesized that photosynthates may play a role in regulating plant responses to low P availability. To test this, white lupin plants grown under low Pi availability in a 16/8 h photoperiod were transferred to continuous darkness for 24 h. The level of expression of three P responsive genes, *LaPT1*, *LaSAP1*, and *LaMATE*, in the cluster roots of white lupin plants was then monitored. These genes were expressed highly in the cluster roots of plants grown with low P availability under a normal photoperiod. Transferring plants to darkness for 24 h resulted in a dramatic reduction in the expression of all three transcripts. The dramatic reduction in the expression of these transcripts was also shown to be reversible. The levels of transcript observed for all three genes returned to levels observed in plants grown under a normal photoperiod following 48 h of continuous light (Liu *et al*., 2005). Thus, it was demonstrated that the expression of these genes in the cluster roots of white lupin were not only regulated by low P availability, but also by light and potentially photosynthates. In *Arabidopsis*, the expression of the P starvation responsive Pi transporter, Pht1;4, has been assessed using a transgenic plants transformed with a *Pht1;4* promoter–luciferase construct. Phosphorus-starved transgenic plants were grown under light and dark conditions, with and without exogenous sucrose (Karthikeyan *et al*., 2007). A significant decrease in luciferase activity was observed when plants were transferred to dark conditions, but the presence of exogenous sucrose in the growth medium sustained some luciferase activity. This suggests that active photosynthesis or exogenous sucrose is important for the expression of this gene during P starvation (Karthikeyan *et al*., 2007). It is noteworthy, that the expression of the P starvation responsive Pi transporter, *Pht1;4*, is also regulated diurnally, suggesting a role for photosynthesis and its products in regulating its expression (Lejay *et al*., 2003).

To test the involvement of phloem sucrose in signalling low shoot P status, Liu *et al*. (2005) restricted the phloem flux by girdling the stems of P-starved white lupin plants and monitored the expression of three P starvation responsive genes, *LaPT1*, *LaSAP1*, and *LaMATE*, in the cluster roots. Stem girdling resulted in a 95% reduction in the translocation of sucrose from the shoot to the root. Transcript accumulation for all three genes was severely reduced in girdled compared to non-girdled P-starved plants, indicating a requirement for shoot-derived carbohydrates in activating these genes in response to low P availability (Liu *et al*., 2005). However, restricting phloem flux to the root could potential block other phloem mobile signals reaching the root that signal shoot P status.

Interestingly, phloem sucrose loading has also been shown to be dependent on phloem K channels, with the phloem sap from the loss-of-function mutant, *akt2/3*, containing half the amount of sucrose of wild-type plants (Deeken *et al*., 2002). It is suggested that these K channels stabilize the phloem membrane potential, preventing sucrose-induced depolarization caused by the sucrose/proton symporter SUC2 (Deeken *et al*., 2002). Hence, in the absence of K, or an active K channel in the phloem, sucrose loading to the phloem is restricted. It is thought that this will reduce the delivery of sucrose to the roots and prevent sucrose-dependent changes in root morphology and root/shoot biomass ratio. Consistent with this hypothesis, in comparison to P-starved plants, in which root biomass and root/shoot biomass ratio increases, K-starved plants rarely alter their biomass partitioning (Cakmak *et al*., 1994*a*, *b*; White, 1997; Hermans *et al*., 2006).

Consequently, reducing phloem sucrose transport from the shoot to the root impacts on a plant's ability to respond appropriately to P starvation. As seen in the *pho3/suc2* mutant, acid phosphatase activity and root biomass allocation are reduced. Similarly, growth of P-starved plants under dark conditions attenuates the expression of genes induced during P starvation, suggesting a need for active photosynthesis and transport of photosynthates to the root to facilitate plant responses to P starvation. Nevertheless, the reduction in sucrose loading of the phloem might also reduce the transport of other phloem-mobile signalling molecules capable of initiating root responses to P starvation. In this context, the loading of sucrose into the phloem in the shoot would act as a facilitator rather than a primary signal. The exogenous application of carbohydrates to the roots of P-starved

plants, which should not increase the movement of sucrose in the phloem, provides a test of whether sucrose is acting merely as a facilitator.

Increasing root carbohydrate concentrations enhances its P starvation responses

Manipulating plant sucrose concentrations *in vitro* can be achieved by controlling photosynthesis or by application of exogenous carbohydrates. Increasing photosynthetic activity by growing plants under high light intensities has been shown to increase phloem translocation of sucrose to the roots and increased root growth and root:shoot ratio under P replete conditions (Nagel *et al*., 2006). However, direct comparisons between P-starved plants under high and low light conditions have not been made, although contrasting photosynthetic activity of dark- and light-grown plants as discussed above, demonstrates a need for active photosynthesis to enable a plant to respond to P starvation.

Addition of carbohydrates to the growth medium to simulate increased sucrose supply to the root during P starvation has a marked effect on its P starvation responses. *Arabidopsis* root growth and development was influenced by the exogenous supply of Pi and sucrose (Nacry *et al*., 2005; Karthikeyan *et al*., 2007). The density of lateral roots in *Arabidopsis* was greater when plants were grown in media supplemented with sucrose, irrespective of plant P status, although lateral root density was greater in P-starved plants (Karthikeyan *et al*., 2007). Interestingly, this effect was further enhanced by the addition of IAA to the growth media. However, the presence of sucrose in the growth media appears to have no effect on controlling primary root length, suggesting this P starvation response is regulated by local P availability or another signalling molecule. Karthikeyan *et al*. (2007) further investigated the transcript abundance of five P starvation responsive genes, *Pht1;1*, *Pht1;4*, *PAP*, *RNase2*, and *At4,* in response to exogenous sucrose concentration at high and low external P concentrations. The transcript abundance of all five genes increased with increasing sucrose concentration under P starvation, but were not detected under P replete conditions (Karthikeyan *et al*., 2007). The expression of *Pht1;4* was also monitored in more detail, using a transgenic line containing a *Pht1;4* promoter–luciferase construct, in response to various sugars. Exogenous sucrose increased luciferase activity by more than 250-fold, with glucose and fructose also increasing luciferase activity slightly. However, there was no increase in activity when trehalose, palatinose, turanose, lactose, sorbitol, 3-*O*-methylglucose (3OMG) or osmotic controls were applied (Karthikeyan *et al*., 2007). The expression of two genes induced by P starvation in lupin, *LaPT1* and *LaSAP1*, both showed enhanced expression in seedlings when exogenous sugars were supplied. The expression of *LaPT1* increased the most when glucose or fructose was supplied, but the expression of *LaSAP1* increased the most when sucrose was supplied (Liu *et al*., 2005). The expression of several P starvation induced genes, *IPS1*, *Pht1;1*, *ACP5*, *PHF*, and *SPX*, also increased in the roots of P-starved *Arabidopsis* plants when supplied with 3% sucrose, compared with control plants supplied with 0.2% sucrose (Franco-Zorrilla *et al*., 2005). This suggests that increases in the abundance of these transcripts during P starvation are influenced by sugar availability, particularly sucrose.

Recently, microarrays have been employed to investigate the interaction between P availability and exogenous sucrose at the whole genome level in the leaves of *Arabidopsis* (Müller *et al*., 2007). Leaves from P-replete and P-starved *Arabidopsis* plants were incubated with or without sucrose, and the abundance of 21 500 transcripts was measured. The abundance of 187 transcripts changed more than 2-fold in response to P starvation, with 171 increasing and 16 decreasing. The abundance of 644 transcripts changed more than 2-fold in response to sucrose supply, with 337 increasing and 307 decreasing (Müller *et al*., 2007). The interaction between the two experimental factors, in which genes will respond to either factor alone, but the response is dependent on the other factor identified 149 transcripts whose abundance was influenced by the interaction, with the abundance of 37 transcripts varying by more than 2-fold. Analysis of the 149 genes identified a specific cluster of genes whose transcripts responded primarily to P starvation but whose abundance was accentuated by sucrose. This cluster included purple acid phosphatases, phosphoesterases, a sucrose phosphate kinase, pyruvate kinase, a sucrose transporter, and several genes regulated by auxin, ethylene, and GA (Müller *et al*., 2007). Interestingly, over 42% of the genes in this cluster contained the PHR1 binding site (Rubio *et al*., 2001) within 1000 bp upstream of the gene, compared with only 18% of the genes in the whole genome (Müller *et al*., 2007), suggesting that many of the genes within this cluster are regulated by PHR1.

Potential sensing mechanisms for shoot-derived carbohydrate signals

Since sucrose can be rapidly hydrolysed, by either sucrose synthase or invertase, to its component sugars, glucose and fructose, it can be difficult to determine if sucrose or glucose (or fructose) is acting as a potential signalling molecule when sucrose is supplied to responsive tissues (Table 2; Smeekens, 2000). To elucidate the signalling pathway influencing the expression of P starvation responsive genes, P-starved *Arabidopsis* seedlings have been supplied with the glucose analogue, 3OMG, which can alter the expression of glucose-regulated genes (Smeekens, 2000). This compound failed to induce the

Table 2. *Influence of shoot-derived carbohydrate signals on root responses to P starvation*

Carbohydrate signal	Root response during P starvation	References
Sucrose	Biomass partitioning: increased root:shoot ratio	Cakmak *et al*., 1994*b*; Hermans *et al*., 2006
	Increased expression of Pht1 phosphate transporters	Lejay *et al*., 2003; Franco-Zorrilla *et al*., 2005; Karthikeyan *et al*., 2007
	Increased expression of ribonucleases and phosphatases	Franco-Zorrilla *et al*., 2005; Liu *et al*., 2005; Müller *et al*., 2005; Karthikeyan *et al*., 2007
Glucose	Increased expression of Pht1 phosphate transporters	Liu *et al*., 2005
	Increased lateral root density	Karthikeyan *et al*., 2007
	Increased expression of ribonucleases	Müller *et al*., 2005

expression of the Pi transporter, *Pht1;4* (Karthikeyan *et al*., 2007). Similarly, the addition of another glucose analogue, 2-deoxyglucose, to the leaves of P-starved *Arabidopsis* plants failed to alter the expression of *ACP5*, *IPS1*, and *RNS1* genes induced by P starvation (Müller *et al*., 2005). This indicates that sucrose itself, rather than glucose, is acting as the causal intermediary signal.

Following the breakdown of sucrose into glucose and fructose, it is thought that hexokinase (HK) acts as a sensor for glucose in subsequent glucose signalling pathways (Moore *et al*., 2003). It is noteworthy that during P starvation, HK activity decreases (Rychter and Randall, 1994). Müller *et al*. (2005) used the *gin2* mutant of *Arabidopsis*, which contains a mutation in the *HK* gene, to test the involvement of HK-dependent signalling during P starvation. The expression of the P starvation inducible genes, *RNS1* and *IPS1*, showed higher expression in the leaves of P-starved *gin2* mutants compared to wild-type plants (Müller *et al*., 2005), suggesting that HK-dependent signalling was not regulating the plant-responses to P starvation. However, this does not preclude the involvement of HK-dependent signalling affecting the expression of other P starvation inducible genes, or the expression of these P starvation inducible genes in other tissues. To investigate this further, Karthikeyan *et al*. (2007), studied the root system of the *gin2* mutant and the expression of the P starvation inducible genes, *Pht1;1*, *Pht1;4*, *At4*, *AtIPS1*, and *RNase2*, in whole seedlings. They observed a decrease in the number of lateral roots produced in the *gin2* mutant, compared with wild-type plants, but no difference in primary root length. Interestingly, the expression of the genes induced by P starvation showed a decrease in expression in P-starved *gin2* mutant seedlings compared to P-starved wild-type seedlings, including *IPS1* (Karthikeyan *et al*., 2007). Furthermore, under P-replete conditions, root elongation and branching has been correlated with local hexose concentration (Freixes *et al*., 2002).

The failure of glucose and glucose analogues to alter the expression of genes induced by P starvation, but the reduction in the expression of some of these genes in the roots of the *gin2* mutant, suggests both signalling pathways might be operating in P-starved plants. Further work to tease apart any spatial or temporal differences between these pathways in signalling P starvation is therefore required.

Conclusions

Plants acclimate to P starvation through many diverse transcriptional, biochemical, and physiological changes. The changes observed in roots of P-starved plants are controlled by a variety of local and systemic signals. In this paper, we have provided an overview of the hormonal signals implicated in the acclimatory responses of plant roots to P starvation, and also reviewed the literature to determine whether sucrose translocation in the phloem meets the criteria set out in the Introduction for a causal intermediary signal linking P starvation to acclimatory responses in the root. Changes in shoot carbohydrate metabolism occur rapidly in response to P starvation, although it is unclear how quickly these changes are signalled to the root, and whether changes in sucrose supply to the root precede transcriptional and morphological responses to P starvation. Further research is required to determine the temporal and spatial changes in sucrose delivery to the root in response to P starvation and their impact on biomass partitioning, gene expression, and root morphology. Nevertheless, reducing the availability of sucrose to the roots, either in the *pho3* mutant or by growing plants in the dark, has a dramatic effect on the expression of genes induced by P starvation, suggesting that sucrose could influence root gene-expression patterns during P starvation. Finally, in many cases, supplying sucrose to plants increases the expression of genes induced by P starvation. Taken together there is strong evidence for sucrose transport in the phloem playing a role in the systemic regulation of responses to P starvation in plant roots.

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