INTRODUCTION

Phosphate (Pi) is an essential macronutrient that plays a central role in virtually all metabolic processes in plants, including photosynthesis and respiration. Despite its importance, Pi is one of the least available nutrients in many ecosystems, and is a frequent limiting factor for plant productivity. Although plentiful in the earth’s crust, soil Pi often exists in insoluble mineral forms that render it unavailable to plants. Agricultural Pi deficiency is alleviated by the massive application of Pi fertilizers, currently estimated to be about 40 million metric tons per year worldwide. However, the assimilation of Pi fertilizers by crops is quite inefficient, as a large proportion of applied Pi becomes immobile, or may runoff into and thereby pollute nearby surface waters. Moreover, the world’s reserves of rock Pi (mined for production of Pi fertilizers) are expected to be depleted within the next century. Thus, studies of the complex mechanisms whereby plants acclimate to nutritional Pi deficiency are of great importance. This could lead to the development of rational strategies for engineering Pi-efficient transgenic crops that would reduce or eliminate our current overreliance on expensive, polluting, and nonrenewable Pi fertilizers. The aim of this article is to provide a brief overview of the fascinating biochemical adaptations of Pi-starved (-Pi) plants.

THE PLANT PHOSPHATE-STARVATION RESPONSE

Plants have evolved the ability to acclimate, within species-dependent limits, to extended periods of Pi deficiency. Pi deprivation elicits a complex array of morphological, physiological, and biochemical adaptations, collectively known as the Pi-starvation response. Plant morphological/physiological adaptations that enhance the acquisition of limiting Pi from the soil include increased root growth relative to shoot growth, as well as root coloniziation by mycorrhizal fungi.[1,2]

Mycotrophic Versus Nonmycotrophic Plants

Symbiotic mycorrhizal fungi help mycotrophic plants increase Pi-uptake from Pi deficient soils. In return, the host plant supplies sucrose to fuel the energy demands of the mycorrhizal symbiont. Mycorrhizae colonize the roots of most plants, except for the nonmycotrophic minority that include members of the Cruciferae, Chenopodiaceae, and Proteaceae families. It is notable that many nonmycotrophs, such as buckwheat and white lupin, are notorious for their ability to thrive on infertile soils. This reflects the view that relative to mycotrophic plants, the nonmycotrophs appear to have evolved to allow more efficient acclimation to low Pi conditions.[2]

BIOCHEMICAL ADAPTATIONS OF PHOSPHATE-STARVED PLANTS

Adaptation #1: Increased Efficiency of Cellular Phosphate Uptake

Multiple plasmalemma Pi transporters are differentially expressed under varying Pi nutritional regimes.[1] The widely accepted dual Pi uptake model is characterized by constitutive low-affinity and Pi-starvation inducible (PSI) high-affinity Pi transporters that respectively function at high (mM) and low (µM) concentrations of external Pi. High-affinity Pi transporters likely play a crucial role in the acquisition of limiting external Pi by -Pi plants.[1,2] Genome sequencing has indicated that Arabidopsis contains nine members of the high-affinity Pi transporter gene family, but only a single low-affinity Pi transporter gene.

Adaptation #2: Induction of Phosphate Scavenging and Recycling Enzymes

2A: Acid phosphatase

Acid phosphatase (APase) induction is a universal symptom of plant Pi stress.[2,3] APases function as intracellular
(vacuolar) or extracellular (secreted) Pi salvage systems that catalyze the hydrolysis of Pi from phosphate-monoesters (Fig. 1(a)). The existence of 22 Arabidopsis genes that putatively encode different APases indicates that plant APase biochemistry is relatively complex.

2B: Secreted nuclease and phosphodiesterase

Nucleic acids present in decaying organic matter represent an important source of extracellular Pi that may be exploited by −Pi plants. Degradation of extracellular DNA and RNA by PSI-secreted nucleases, phosphodiesterase, and APase liberates Pi from nucleic acids for its subsequent uptake by PSI high-affinity Pi transporters of −Pi plant roots (Fig. 1(b)).[2,4]

2C: Replacement of membrane phospholipids with nonphosphorus galacto- and sulfonyl-lipids

−Pi plants can scavenge and conserve Pi by replacing their membrane phospholipids with amphipathic galacto- and sulfonyl lipids. Arabidopsis mutants defective in sulfolipid synthase (the terminal enzyme of sulfonyl lipid synthesis) were recently reported to show impaired growth during Pi deprivation[5]

2D: Induction of metabolic phosphate recycling enzymes

Several PSI glycolytic bypass enzymes such as PPI-dependent phosphofructokinase (PPI-PFK), phosphoenol-

pyruvate (PEP) phosphatase, and PEP carboxylase (PEPCase) may facilitate intracellular Pi recycling, because Pi is a by-product of the reactions catalyzed by each of these enzymes (Fig. 2). Their reactions may also facilitate respiration and/or organic acid excretion, while generating free Pi for its reassimilation into the metabolism of the −Pi cells.[2]

2E: Organic acid excretion

Scavenging of Pi from extracellular sources may be aided by the enhanced excretion of organic acids due to PEPCase induction. Roots and suspension cell cultures of −Pi plants have been demonstrated to markedly up-regulate PEPCase.[1,2,6–8] PEPCase induction during Pi stress has been correlated with the excretion of significant levels of organic acids such as malate and citrate. This leads to acidification of the rhizosphere, which thereby contributes to the solubilization and assimilation of mineral Pi from the environment.[1,2,7]

**Adaptation #3: Induction of Alternative Pathways of Cytosolic Glycolysis**

As a consequence of the large decline (up to 50-fold) in cytoplasmic Pi levels that follows severe Pi stress, large reductions in intracellular levels of ATP and related nucleoside phosphates can also occur (Table 1).[2,7,8] This may hinder carbon flux through the enzymes of classical glycolysis that are dependent upon adenylates or Pi as cosubstrates (Fig. 2). Despite depleted intracellular Pi
Fig. 2 A model suggesting various adaptive metabolic processes (indicated by bold arrows) that may promote the survival of \(-\text{Pi}\) plants. Alternative pathways of cytosolic glycolysis and mitochondrial electron transport, and tonoplast H\(^+\)-pumping facilitate respiration and vacuolar pH maintenance by \(-\text{Pi}\) plant cells because they negate the dependence on adenylates and Pi, the levels of which become markedly depressed during severe Pi starvation. Organic acids produced by PEPCase may also be excreted by roots to increase the availability of mineral-bound Pi (by solubilizing Ca-, Fe- and Al-phosphates). A key component of this model is the critical secondary role played by metabolic Pi recycling systems during Pi deprivation. Enzymes that catalyze the numbered reactions are as follows: 1) hexokinase; 2) fructokinase; 3) nucleoside diphosphate kinase; 4) UDP-glucose pyrophosphorylase; 5) phosphoglucone isomerase; 6) phosphoglucone mutase; 7) NAD-dependent G3P dehydrogenase (phosphorylating); and 8) 3-phosphoglycerate kinase. Abbreviations are as in the text and as follows: DHAP, dihydroxyacetone-phosphate; Fru, fructose; Glu, glucose; MDH, malate dehydrogenase; OAA, oxaloacetate; 3-PGA, 3-P-glycerate; PK, pyruvate kinase; UQ, ubiquinone.
and adenylate pools, –Pi plants must continue to generate energy and carbon skeletons for key metabolic processes. As indicated in Fig. 2, at least six Pi- and adenylate-independent glycolytic bypass enzymes (sucrose synthase (SuSy), UDP-glucose pyrophosphorylase, PPI-PFK, nonphosphorylating NADP-glyceraldehyde-3-phosphate (G3P) dehydrogenase, PEPCase, and PEP phosphatase) have been reported to be plant PSI enzymes.[2,6–8] These enzymes are hypothesized to represent PSI by-passes to the adenylate or Pi-dependent glycolytic enzymes (i.e., invertase/hexokinase, ATP-PFK, phosphorylating NAD-G3P dehydrogenase, and pyruvate kinase), thereby facilitating glycolysis during severe Pi stress. Furthermore, as Pi exerts reciprocal allosteric effects on the activity of ATP-PFK (potent activator) and PPI-PFK (potent inhibitor) (Fig. 2),[7] the large reduction in cytoplasmic Pi in –Pi plants should promote the in vivo activity of PPI-PFK while curtailing that of ATP-PFK.[2,8]

Pyrophosphate helps Pi-starved plants to conserve ATP

PPI is a byproduct of a host of anabolic reactions, including the terminal steps of macromolecule synthesis. In animals, the high energy phosphoanhydride bond of PPI is never utilized because PPI is always hydrolyzed by abundant inorganic pyrophosphatase (PPIase), making macromolecule synthesis thermodynamically favorable.[7] However, the large amounts of PPI produced during biosynthesis may be employed by plants to enhance the energetic efficiency of several cytosolic processes. In contrast to animals, the plant cytosol lacks soluble PPIase and thus contains PPI concentrations of up to about 0.5 mM.[2,7–9] Furthermore, plant cytosolic PPI levels are remarkably insensitive to abiotic stresses such as anoxia or Pi starvation, which elicit significant reductions in cellular ATP pools (Table 1).[5,7–9]

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<tr>
<th>Metabolite</th>
<th>+ Pi cells</th>
<th>– Pi cells</th>
<th>Change due to Pi-deprivation</th>
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<tbody>
<tr>
<td>Pi</td>
<td>17,400 ± 1,200</td>
<td>400 ± 60</td>
<td>44-fold decrease</td>
</tr>
<tr>
<td>ATP</td>
<td>138 ± 12</td>
<td>36 ± 5</td>
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<td>ADP</td>
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<td>PPI</td>
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<td>Not significant</td>
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*Metabolite levels in Brassica nigra (black mustard) suspension cells cultured for 7 days in the presence and absence of 10 mM Pi (+Pi and –Pi, respectively). All values represent means ± S.E.M. of duplicate determinations performed on three separate cell cultures.
(Data from Ref. 8. Reproduced with Permission of The American Society of Plant Biologists.)

Adaptation #4: Induction of Tonoplast H+-Pumping Pyrophosphatase

In addition to the SuSy pathway of sucrose conversion to hexose-monophosphates, and PPI-PFK, PPI could be employed as an alternative energy donor for the active transport of protons from the cytosol into the vacuole (Fig. 2). That PPI-powered processes may be a crucial facet of the metabolic adaptations of plants to environmental extremes causing depressed ATP (but not PPI) pools is further indicated by the significant induction of the tonoplast H+-PPIase by anoxia, or by severe Pi starvation.[7,9] As indicated in Fig. 2, the induction of PPI-dependent cytosolic bypasses (i.e., tonoplast H+-PPIase, PPI-PFK, and SuSy) may serve –Pi plants by: 1) circumventing ATP-limited reactions; 2) conserving limited cellular pools of ATP; while 3) recycling valuable Pi from PPI.

Adaptation #5: Induction of Alternative Pathways of Respiratory Electron Transport

Respiratory O2 consumption by plant mitochondria can be mediated by the phosphorylating cytochrome pathway or by nonphosphorylating alternative pathways (Fig. 2). The significant reductions in cellular Pi and ADP pools that follow extended Pi deprivation will impede respiratory electron flow through the cytochrome pathway at the sites of coupled ATP synthesis. However, the presence of nonphosphorylating pathways of electron transport provides a mechanism whereby respiratory flux can be maintained under conditions when the availability of ADP and/or Pi are restrictive. Plants acclimate to Pi stress by increased engagement of the nonphosphorylating (i.e., rotenone- and cyanide-insensitive) alternative pathways of respiratory electron transport.[2,10,11] (Fig. 2). Moreover, increased levels of alternative oxidase protein may occur in –Pi plants.[10,11] This allows continued functioning of

Table 1 Metabolite levels in black mustard suspension cells

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the citric acid cycle and respiratory electron transport chain with limited ATP production. By preventing severe respiratory restriction, the alternative oxidase has been hypothesized to prevent undesirable redirections in carbon metabolism as well as the excessive generation of harmful reactive O₂ species in the mitochondrion of −Pi plants.¹⁰,¹¹ This has been corroborated by the impaired growth and metabolism of −Pi transgenic tobacco plants that are unable to synthesize a functional alternative oxidase protein.¹⁰

CONCLUSION

Studies of plant responses to nutritional Pi deprivation have revealed some remarkably adaptive mechanisms that contribute to the survival of −Pi plants. Although these adaptations are not identical in all plants, certain aspects are conserved in a wide variety of plants from very different environments. The biochemical adaptations of −Pi plants provides an excellent example of how the unique flexibility of plant metabolism and energy transduction helps them cope in a typically stressful environment. These adaptations also provide a useful system for studies of plant signal transduction and gene expression. Future investigations of these pathways should provide further links between the biochemical and molecular control of plant metabolism. A better understanding of the extent to which changes in flux through alternative enzymes and pathways influences plant stress tolerance is of significant practical interest. This knowledge is relevant to the ongoing efforts of agricultural biotechnologists to engineer transgenic crops that have improved resistance to environmental extremes, including Pi starvation.

ACKNOWLEDGMENTS

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ARTICLES OF FURTHER INTEREST

Glycolysis, p. 547
Mitochondrial Respiration, p. 729

REFERENCES