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Original article

Effects of phosphate limitation on expression of genes involved in pyrimidine synthesis and salvaging in *Arabidopsis*

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Abstract

Arabidopsis seedlings grown for 14 d without phosphate (P) exhibited stunted growth and other visible symptoms associated with P deficiency. RNA contents in shoots decreased nearly 90%, relative to controls. In shoots, expression of *Pht1;2*, encoding an inducible high-affinity phosphate transporter, increased threefold, compared with controls, and served as a molecular marker for P limitation. Transcript levels for five enzymes (aspartate transcarbamoylase, ATCase, EC 2.1.3.2; carbamoyl phosphate synthetase, CPSase, EC 6.3.5.5); UMP synthase, EC 2.4.1.10, EC 4.1.1.23; uracil phosphoribosyltransferase, UPRTase, EC 2.4.2.9; UMP kinase, EC 2.7.1.14) increased 2–10-fold in response to P starvation in shoots. These enzymes, which utilize phosphorylated intermediates at putative regulated steps in de novo synthesis and salvaging pathways leading to UMP and pyrimidine nucleotide formation, appear to be coordinately regulated, at the level of gene expression. This response may facilitate pyrimidine nucleotide synthesis under P limitation in this plant. Expression of P-dependent and P-independent phosphoribosyl pyrophosphate (PRPP) synthases (PRS2 and PRS3, respectively) which provide PRPP, the phosphoribosyl donor in UMP synthesis via both de novo and salvaging pathways, was differentially regulated in response to P limitation. PRS2 mRNA levels increased twofold in roots and shoots of P-starved plants, while PRS3 was constitutively-expressed. PRS3 may play a novel role in providing PRPP to cellular metabolism under low P availability.
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Keywords: Arabidopsis; Gene expression; Phosphate starvation; Pyrimidine nucleotide biosynthesis

1. Introduction

Phosphate availability is one of the major factors affecting plant growth and development and plants exhibit common morphological, physiological and biochemical adaptations to P limitation [34]. Morphological changes in response to P deficiency include slowing of overall growth, increased lateral root production [25,47], an increase in the length and density of root hairs [7], and accumulation of anthocyanins [33]. P limitation also results in an increased root to shoot biomass ratio [17] and increased Pi uptake by and retention within roots [33].

A large number of biochemical changes, which serve to increase acquisition and tissue availability of P, are also seen in response to P starvation. These include the production of intracellular and extracellular RNases [5,24,29] and phosphodiesterases [1], which release P from RNA and nucleotide pools, a variety of phosphatases [17], secretion of organic acids, which acidify the rhizosphere and maximize uptake of phosphorous as $H_2PO_4^-$ [17,22], and induction of numerous high-affinity phosphate transporters [23,39].

The synthesis of nucleotides has been reported to be strongly influenced by P availability (see [45] for review). The main P flux through nucleotide pools is toward nucleic acids, representing 45% of the total P in cultured plant cells [4]. In *Catharanthus* cell cultures, NTP pools decreased three to fivefold within 24 h after inoculation into P-deficient medium [3]. Decreased nucleotide pools associated with depleted cellular P in *Datura* cell cultures results in a pro-

Abbreviations: ATCase, aspartate transcarbamoylase; CarAsp, carbamoyl aspartate; CPSase, carbamoyl phosphate synthetase; DHO, dihydroorotate; FOA, 5-fluoroorotic acid; OMP, orotate-5'-monophosphate; P, phosphate; PALA, *N*-(phosphonacetyl)-L-aspartate; Pi, orthophosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate; UMP, uridine-5'-monophosphate; UPRTase, uracil phosphoribosyltransferase..

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longed cell division phase, during which most cellular P is consumed. After addition of P to these cultures, uptake was rapid (complete within 2 d of inoculation into fresh medium) and preceded nucleotide biosynthesis [49].

We are interested in the regulation of pyrimidine biosynthesis in plants. Pyrimidines are derived from UMP, which is formed either by de novo synthesis or the "salvaging" of preformed nucleobases or nucleosides ([19,40] see Fig. 1). In the de novo pathway, ATP is the phosphoryl donor in the synthesis of carbamoyl-P, which is utilized by ATCase in the committed step in the pathway. However, ATP is later resynthesized (step 3 of de novo pathway; Fig. 1) by capturing the energy from dihydroorotate oxidation to orotate via the mitochondrial flavoenzyme dihydroorotate dehydrogenase, transferring reducing equivalents to the proximal quinone and final molecular oxygen acceptor in the respiratory chain [44]. Other ATP-utilizing steps include the salvaging of uridine to UMP, via uridine kinase, and phosphorylation of UMP to UDP by UMP kinase, which is representative of numerous other NMP/NDP kinase-mediated transformations in which P is incorporated into pyrimidine nucleotides.

The formation of phosphoribosyl pyrophosphate (PRPP), which donates the phosphoribosyl moiety in salvaging of uracil to UMP and in the synthesis of OMP by the orotate phosphoribosyltransferase (EC 2.4.2.10) activity of the bifunctional UMP synthase [35], also requires ATP. In these later PRPP-requiring steps, phosphate is directly incorporated into newly-formed nucleotides. It is known that P limitation results in markedly reduced cellular levels of ATP [45] and Ukaji and Ashihara [43] reported that Pi addition to P-starved cells resulted in a 10-fold increase in PRPP levels. Thus, it is not surprising that P limitation would negatively impact pyrimidine nucleotide synthesis by limiting the availability of the essential phosphorylated intermediates ATP and PRPP.

While metabolic studies have provided important insights into the regulation of pyrimidine synthesis under P limitation, little is known about the regulation key enzymes in these pathways, at the level of gene expression. Genes encoding ATCase (*PyrB*) and UMP synthase (*PyrE-F*), which catalyze the committed and rate-limiting steps of the de novo pathway, respectively, have been shown to be regulated in response to changes in pyrimidine availability [6,35] and we expected that they might similarly be regulated by P availability. Metabolic regulation of plant CPSase, which produces the carbamoyl-P intermediate used in both pyrimidine and arginine synthesis ([37]; see Fig. 1) has been characterized [30], but nothing is known regarding the expression of the *CarA* and *CarB* genes encoding the small (glutamine amidohydrolase [9]) and large (synthetase [46]) subunits of this enzyme. Similarly, expression of genes for UMP kinase, which catalyzes the committed step in the synthesis of pyrimidines from UMP [50], and the salvage enzyme UPRTase [39], does not appear to have been investigated. Recent studies of spinach PRPP synthases [20,21] have demonstrated that, in addition to two isoforms that display Pi dependence for activity ("class I"; PRS1, PRS2), two additional isoforms do not require Pi for activity ("class II"; PRS3, PRS4). The second class of enzymes might enable a plant experiencing limited P availability to continue synthesizing PRPP for nucleotide synthe-

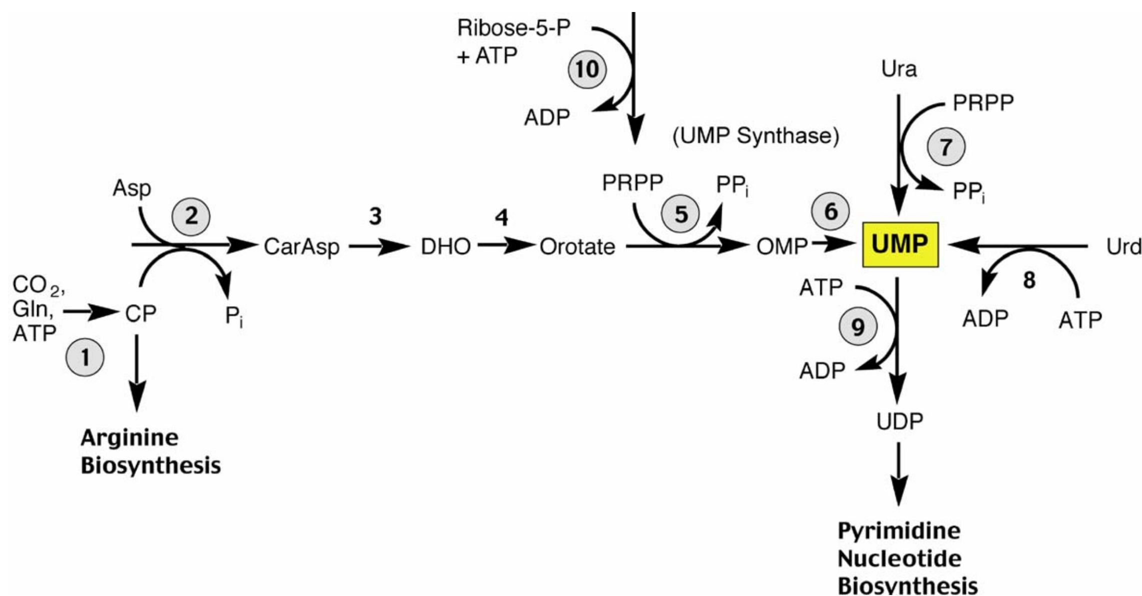

Fig. 1. Synthesis of UMP in plants via the de novo pathway (steps 1–6) or salvaging pathways (steps 7, 8). Synthesis of UDP from UMP (step 9) is the committed step leading to the synthesis of other pyrimidine nucleotides. Phosphate is required for the synthesis of PRPP (step 10), the phosphoribosyl donor in UMP synthesis, and ATP, the phosphoryl donor in reactions catalyzed by NMP and NDP kinases and carbamoyl phosphate synthetase (step 1). Carbamoyl phosphate is a common intermediate in the de novo synthesis of UMP and arginine. Numbers indicate enzymes catalyzing each step. Circled numbers represent enzymes for which gene expression was investigated in the present study. **1**, Carbamoyl phosphate synthetase (CPSase, EC 6.3.5.5); **2**, aspartate transcarbamoylase (ATCase, EC 2.1.3.2); **3**, dihydroorotase (DHOase, EC 3.5.2.3); **4**, dihydroorotate dehydrogenase (EC 1.3.3.1); **5**, **6**, orotate phosphoribosyltransferase (OPRTase, EC 2.4.2.10) and OMP decarboxylase (EC 4.1.1.23) activities residing within the bifunctional UMP synthase; **7**, uracil phosphoribosyltransferase (UPRTase, EC 2.4.2.9); **8**, uridine/cytidine kinase (EC 2.7.1.48); **9**, UMP kinase (EC 2.7.1.14); **10**, PRPP synthase (EC 2.7.6.1).

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