

Multilevel coordination of phosphate and sulfate homeostasis in plants

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Phosphate and sulfate are two macro-elements essential for plant growth and development. Both elements play a central role in numerous aspects of plant metabolism and their deficiencies have profound effects on the transcriptome as well as on numerous metabolic pathways. The research emphasis so far has been on elucidating the molecular physiology of these individual nutritive elements. Recent data proved the existence of complex connections between the various regulatory layers of the homeostasis of these elements, but the molecular bases and biological significance of such interconnections remains poorly understood. This review provides an update on recent advances to identify the components involved in phosphate and sulfate homeostasis crosstalk. In light of this case study, developing a comprehensive understanding of the coordination of the ion homeostasis and identifying genes which can be used as good molecular markers for monitoring the "integrative ionic status" of plants is not only of great scientific interest, but also crucial for biotechnological and agronomic applications.

Phosphate (Pi) and sulfate (SO_4^{2-}) are involved in numerous aspect of plant metabolism and their deficiencies have profound effects on numerous metabolic pathways resulting in limitations in plant growth, development and productivity. Shoot growth is effected by both phosphorus (P) or sulfur (S) deficiencies, which manifests itself as a reduction in the total biomass and accumulation of anthocyanins. Root development is also influenced by P or S availability, where deficiencies in either macro-nutrient cause striking changes in the root architecture.¹ When challenged with a SO_4^{2-} deficiency, the primary root continues to grow, but the lateral roots form closer to the root tip with an increase in density.¹ In contrast, when plants are grown in very low Pi concentrations, the primary roots stop growing and lateral root density increases.¹ Besides the aforementioned phenotypic differences of Pi- or SO_4^{2-} -deficient plants, an interconnection of responses to the internal concentration of these two elements has been reported at the metabolic level. One such response is the rapid replacement of sulfolipids by phospholipids under S

deficiency and a replacement of phospholipids by sulfolipids during P deficiency.^{2,3} Transcriptome analysis of plants grown with different Pi or SO_4^{2-} concentrations reveals extensive reprogramming of the gene expression in both the shoots and the roots.^{4,5} Interestingly, a deficiency or surplus of only one of the elements often involves modifications in the expression of genes encoding for proteins specific to the homeostasis of others elements, including proteins that control the flux of these elements and their assimilation or regulatory molecules acting in the signaling pathways. These observations strongly indicate the existence of multi-level coordination in the regulation of homeostasis of the two ions in which currently unidentified key elements are actively cross talking between these signaling pathways. Such connections are likely to provide flexibility and plasticity for the adaptation to ever-fluctuating environmental conditions, which is vital for sessile organisms, such as plants, to complete their life cycle. How these connections work at molecular level is the challenge for future research.

In the last decade, research efforts have provided us with a comprehensive view on the adaptive strategies employed by plants to cope with deficiencies of these elements. In Arabidopsis and other plant species, many key genes/proteins involved in the Pi or SO_4^{2-} uptake and inter-organ transport have been cloned, and functionally characterized by the complementation of mutants or by expression in heterologous systems, including *Saccharomyces* and/or *Xenopus Oocyte*.⁶⁻⁹ The number of signaling molecules and cis- and trans-regulatory elements that play an important role in the regulation of either Pi or SO_4^{2-} homeostases have been also identified and extensively described in references 10 and 11.

It is interesting to note that in addition to the similarities between the topology of the SO_4^{2-} and the Pi transporters¹² there are comparable molecular mechanisms that exist which regulate the expression these transporters, including metabolites such as carbohydrate, phytohormones and regulatory miRNAs. It is likely that molecular mechanisms involved in photosynthetic activity and carbon status also influence the regulation of Pi and SO_4^{2-} homeostasis in order to support the metabolic demands of the plant.^{13,14} Remarkably, the expression profiles of the two major up-take transporters (*PHT2* for Pi and *SULTRI;2* for SO_4^{2-}) appeared to follow a similar diurnal rhythm^{13,14} and are sensitive to the carbon status, either upstream or downstream, of the hexokinase (HXK) activity in glycolysis.¹⁵

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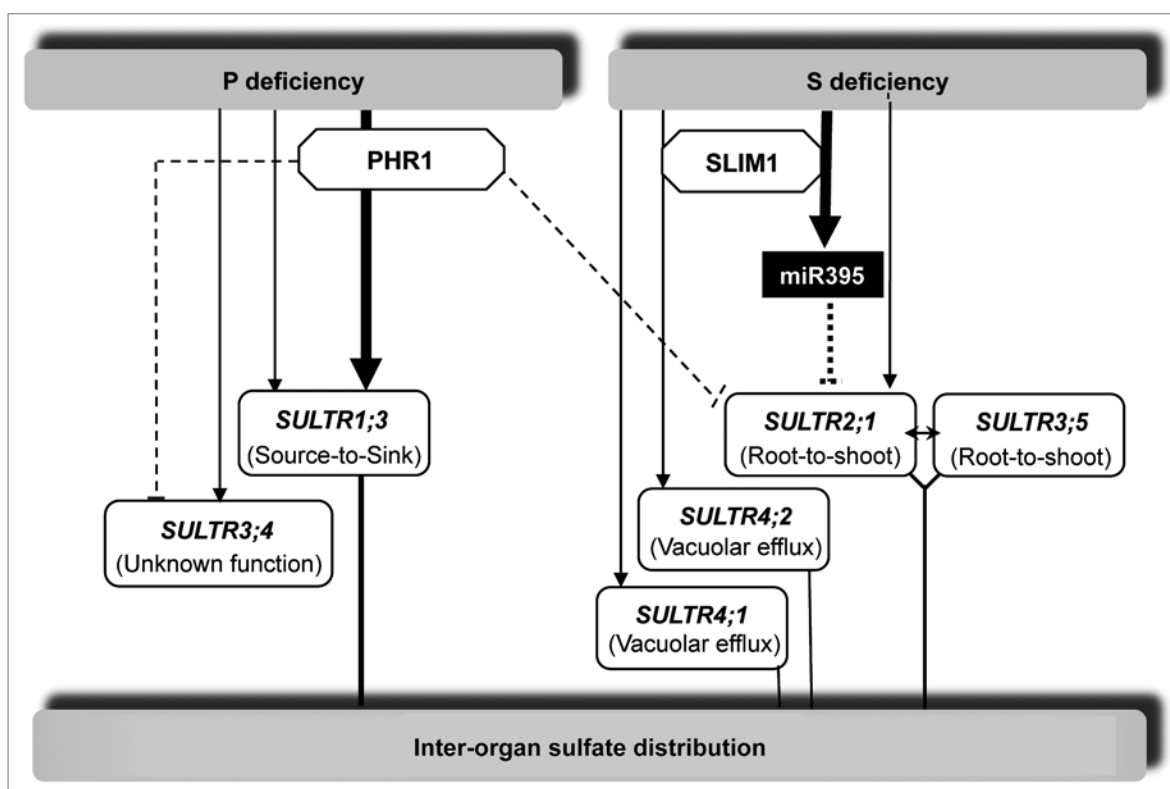


Figure 1. Schematic representation for the regulation of the expression of the *SULTR* genes involved in the internal sulfate distribution in response to sulfate and Pi nutritional status. In this model, the expression of *SULTR1;3* and *SULTR3;4* are controlled by Pi starvation.²⁶ The upregulation of *SULTR1;3* expression upon Pi limitation is partially controlled by the transcription factor PHR1, while PHR1 plays a repressive role on *SULTR3;4* overexpression under Pi-deficiency in shoots.²⁶ Limitation in sulfate external availability mainly results in the activation of *SULTR2;1* (in roots), *SULTR4;1* and *SULTR4;2* expression. The repression of *SULTR2;1* in shoots is downstream of the trans-acting factor SLIM1 and targeted by miR395.²¹ SLIM1 also controls the expression of *SULTR4;2* in response to S deficiency.²³ Double black arrow between *SULTR3;5* and *SULTR2;1* indicates the synergy between these proteins in mediating sulfate uptake.³² Solid lines with arrows and dotted lines symbolize positive and negative effects, respectively. In this scheme, the thick and thin arrows indicate major and minor signaling networks, respectively.

We have learned that the Pi and the SO_4^{2-} homeostasis are also coordinated by the status of diverse phytohormones.^{16,17} The similarity in homeostatic regulation of these two elements by phytohormones is best illustrated by viewing their relationships to the plant's response to cytokinins.^{16,17} The frequency of cross-talk between either Pi or SO_4^{2-} , and the cytokinin signal transduction pathways is extensive beginning with the uptake of these elements from the media into the plants. Indeed, both uptake systems for Pi and the SO_4^{2-} acquisition are similarly regulated by the plant hormone cytokinin. Exogenous application of cytokinin was shown to repress the expression of transporters involved in Pi and SO_4^{2-} uptake.^{17,18} We now know that cytokinins and the cytokinin receptor CYTOKININ RESPONSE 1/WOODEN LEG/ARABIDOPSIS HISTIDINE KINASE 4 (CRE1/WOL/AHK4) pathways not only play an important role in suppressing the upregulation of several genes following Pi deficiency,¹⁶ but also the downregulation of the two higher affinity SO_4^{2-} transporters in Arabidopsis.¹⁷

As mentioned above, an additional important similarity is revealed by the discovery of the molecular mechanism employing regulatory microRNAs (miRNAs) for both Pi and SO_4^{2-} homeostasis.^{19,21} In Arabidopsis, our knowledge on the role of two

miRNA molecules is particularly advanced on the miRNA395 and the miRNA399, which are characterized for their involvement in SO_4^{2-} and Pi starvation signaling pathways, respectively.^{19,21} The expression of miRNA399 is controlled by the MYB transcription factor, PHR1.²² The mature miRNA399 plays a key role in the shoot-to-root Pi-deficiency signaling pathway by targeting the transcript of an E2-conjugase (PHO2), which results in Pi transport in throughout plant starting with Pi-uptake in the roots to its translocation into the shoots.^{19,20} Likewise, regulation of the SO_4^{2-} homeostasis involves miR395,²² which acts downstream of the transcription factor SLIM1,²³ and contributes to the regulation of *SULTR2;1* transcript,²⁴ which is involved in the SO_4^{2-} root-to-shoot transfer.

In addition to the aforementioned mechanistic similarities in the regulation of the Pi and SO_4^{2-} transport systems, the presence of cross talk between the signaling pathways of these two elements are starting to surface in the face of new data. Recently, it has been shown that miRNAs and the transcription factor PHR1 (Phosphate Response 1) play an important role in the cross talk between the SO_4^{2-} and Pi signaling pathways. Also, Hsieh et al. (2009),²⁵ showed that the miRNA (miRNA395), which is known to be upregulated by SO_4^{2-} starvation and to

trigger *SULTR2;1*, is suppressed in Pi-deficient plants. An attractive explanation for this phenomenon could be that this acts as a means to increase the expression of *SULTR2;1*, which would serve to increase SO_4^{2-} translocation and increase sulfolipid biosynthesis to replace phospholipids under Pi-deficient conditions. Although the physiological impact of the regulation of miR395 on sulfate metabolism in Pi-deficient plants is currently unknown, these observations strengthen the hypothesis that there is a genetic program allowing the plant to respond to the starvation of one of the elements, while enhancing the availability and utilization of both by orchestrating the compensation of one for the other.

As already mentioned, the PHR1 (phosphate starvation response 1) transcription factor plays a role in the regulation of phosphate homeostasis in plants. The universal PHR1-binding sequence (PIBS) has been defined as an 8 bp imperfect palindrome in the promoter of many genes.²² In the context of cross talk between the SO_4^{2-} and Pi response pathways, the PHR1 appear to play an important role. For example, two genes involved in the replacement of phospholipids by sulfolipids in Pi-deficient plants, *SQD1* and *SQD2*, contain a PHR1 binding site in their promoter and are upregulated by Pi deficiency in a PHR1-dependant manner.^{22,26-28} The earlier observation, in which the MYB transcription factor PHR1 is an active molecular component linking the sulfate homeostasis and the phosphate signaling, has recently been strengthened by Rouached et al. (2011).²⁶ The authors showed that upon Pi deficiency, the expression of *SULTR1.3*, which encodes a transporter involved in shoot-to-root SO_4^{2-} distribution,²⁹ appeared to be dependent on PHR1. In agreement with this finding, structural analysis revealed that

SULTR1.3 possesses in its promoter a PHR1-binding sequence.²⁶ In line with this observation, the flux of sulfate from shoots to roots is compromised in the Pi-deficient *phr1* mutant.²⁶ It is also important to note that in addition to its positive regulatory role on *SULTR1.3* expression, PHR1 inhibits the expression of a distinct group of *SULTR* genes known to be upregulated by sulfate starvation (e.g., *SULTR2;1*).²⁶ Interestingly, a similar repressive effect of the *Chlamydomonas reinhardtii* PHR1 orthologue PSR1 on the expression of genes involved in SO_4^{2-} scavenging and acquisition has recently been reported in reference 30. These results on PSR1 in *C. reinhardtii* and PHR1 in *Arabidopsis* reveal an unexpected level of complexity and interconnection in the regulation of sulfate and Pi homeostasis and highlight the conservation of the importance of the PSR1/PHR1 transcription factor in these processes through evolution.

Taken together, we have reviewed the evidence for the genetic basis of the multilevel regulation that mediates the adaptation of the plant to the available phosphate and sulfate. Based on this evidence, obtained mainly from studies in *Arabidopsis thaliana*, it is becoming possible to propose an integrative model for the regulation of genes involved in intracellular and inter-organ sulfate homeostasis upon Pi deficiency. It therefore can be proposed that SLIM1 and miR395 are major regulators of gene expression upon sulfate deficiency, while PHR1 acts both positively to regulate *SULTR1.3* and shoot-to-root sulfate transfer, and negatively to suppress the induction of other sulfate transporters upon Pi deficiency (Fig. 1). The future challenge will be to develop a comprehensive understanding of the coordination of the homeostasis of these two elements by discovering new signaling and regulatory networks.³¹

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