Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Systems Biology

System-level understanding of plant mineral nutrition in the big data era

Hatem Rouached[1] and Seung Y. Rhee[2]

Abstract

Plants convert mineral elements from the soil into organic molecules, thereby serving as a major entry point of these elements into the food web and ultimately impacting human nutrition. Therefore, it is important to understand how plants regulate the homeostasis of these elements and how multiple mineral nutrient signals are wired to influence plant growth. The emergence of big data and methods for their analyses open new avenues for answering these questions. Here, we review the current understanding of how plants respond to single and multiple mineral nutrient limitations. We further highlight the importance of integrating omics data to gain new insights on mineral nutrition as an integrated system, which is required for devising future strategies to improve crop yield.

Addresses

[1] Biochimie et Physiologie Moléculaire des Plantes, CNRS, INRA, Montpellier SupAgro, UM, Montpellier, France

[2] Department of Plant Biology, Carnegie Institution for Science, Stanford, CA 94305, USA

Corresponding authors: Rhee, Seung Y (srhee@carnegiescience.edu); Rouached, Hatem (hatem.rouached@inra.fr)

Current Opinion in Systems Biology 2017, **4**:71–77

This review comes from a themed issue on **Big data acquisition and analysis (2017)**

Edited by **Pascal Falter-Braun and Michael A. Calderwood**

For a complete overview see the Issue and the Editorial

Available online 24 July 2017

http://dx.doi.org/10.1016/j.coisb.2017.07.008

Introduction

Mineral elements are essential nutrients for plants to complete their life cycle. Plants require many macronutrients, such as phosphorus (P) and sulfur (S), and micronutrients, such as iron (Fe) and zinc (Zn), to ensure basic cell functions [1]. Most plants acquire these mineral nutrients from the soil through their roots [2]. Plants take up P and S in their inorganic forms, phosphate (PO_4^{3-}, Pi) and sulfate (SO_4^{2-}), and convert them to essential molecules such as nucleic acids, ATP (a major energy carrier), lipids, amino acids, thiols and polysaccharides [3,4]. Fe is needed for life-sustaining processes from photosynthesis to respiration, yet it can be toxic at high levels due to its propensity to form hydroxyl radicals that can damage cellular constituents [5,6]. Plants must sense the levels of all mineral nutrients in the soil and regulate various processes, such as uptake, metabolism and sequestration, to maintain their intracellular levels within working ranges. These processes are collectively termed mineral nutrient homeostasis. Understanding how plants regulate mineral nutrient homeostasis is important for advancing our basic knowledge of plants and highly relevant for modern agriculture in order to meet the growing demand for food.

In light of emerging research findings, the canonical textbook view of mineral nutrient homeostasis regulation in which each nutrient's level is controlled by its own mechanisms and signaling pathways is too simple—there is a complex coordination between the homeostases of various mineral nutrients. For instance, Fe and S physically interact to form Fe–S clusters that are a major sink for Fe and essential for many cellular enzymatic reactions, including photosynthesis and respiration [7]. Pi is known to be an efficient chelator of Fe. Fe is mainly chelated and stored in seed vacuoles through a tight interaction with Pi ions of phytate (inositol hexakisphosphate = IP6) [8]. P and S are metabolically interdependent. For example, phospholipids are rapidly replaced by sulfolipids during P deficiency, and sulfolipids are replaced by phospholipids under S deficiency [9,10]. Consistent with their interactive roles, deficiency of one element causes changes in the accumulation of the other elements. For example, S deficiency (-S) decreases leaf Fe concentration in tomato [11]. P deficiency causes Fe and S accumulation in the leaf and root [12]. Beyond these binary interactions among P, S and Fe, there are many other interactions recognized in plants, such as Fe and zinc (Zn) [13], P and Zn [14,15], Pi and nitrate (NO_3^-) [16]•, to name a few. On a larger scale, ionome analysis showed that changing one or more nutrients from the medium affects the concentrations of many other nutrients *in planta*; some with known causes and some that are yet to be discerned. For example, theionome of *Arabidopsis thaliana* plants (Columbia-0 ecotype) grown under -P revealed significantly increased concentrations of Fe, Zn and S and decreased concentrations of P, copper and cobalt [12,17]. Ionomics thus further support the existence of relationships among mineral element homeostasis in plants. Nevertheless, the molecular basis of most of these interactions remains obscure. A major challenge is to examine mineral

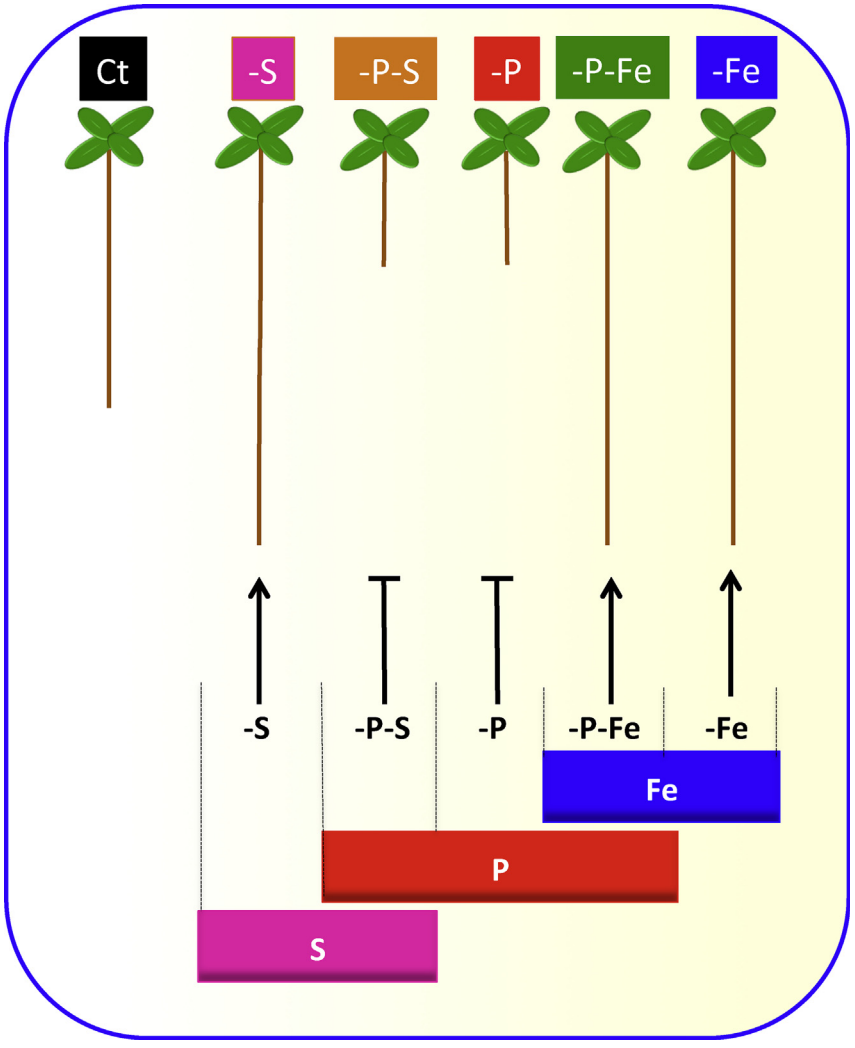
nutrition as a system and to develop tools that enable modeling and analysis of gene networks that integrate the availability of macro- and micro-elements and their interactions (bipartite, tripartite, etc.) [18].

Plant mineral nutrition has greatly benefited from the development of high-throughput measurement technologies for biomolecules such as DNA, RNA and proteins, which has generated new insights of biological systems at the molecular level. Here, we review recent evidence supporting the existence of crosstalks between P and Fe signals to adjust root growth, and provide our perspective on how best to use big data and systems biology approaches to uncover the molecular basis and gene regulatory networks that govern the coordination of mineral nutrient homeostasis. Mapping such networks is only the beginning of deciphering the molecular mechanisms that control the physiological and developmental processes to integrate mineral nutrient signals.

Mineral nutrient epistasis between phosphorus and other nutrients to regulate root growth

Plants have evolved numerous adaptive mechanisms to cope with nutrient deficiencies, including changes at morphological, physiological and molecular levels. At a morphological level, plant roots are particularly sensitive to the limiting and fluctuating availability of mineral elements in the soil. Root growth is highly plastic in response to various nutrient stresses [19], presumably to

Figure 1



Epistatic interactions between mineral nutrients to control primary root growth Phosphorus deficiency (-P) strongly inhibits primary root growth (PRG) compared to control condition (Ct) [23]. In contrast, sulfur (-S) [21] or iron (-Fe) [20] deficiency (but not removal) causes slight promotion of PRG. Simultaneous mineral nutrient deficiency stress affects PRG non-additively compared to single nutrient deficiency. Simultaneous deficiencies of P and Fe (-P-Fe) restore the PRG capacity compared to -P alone. Combined -S and -P stress (-S-P) inhibits PRG similarly to -P alone [22]••. Positive and negative effects on PRG are indicated by arrow and flat-ended lines, respectively. The effects of these deficiencies on shoot and lateral root growth are not shown in this schematic representation.

allow optimal exploration of the soil and enhance mineral nutrient uptake capacity (Figure 1). Changes in the availability of various nutrients have contrasting effects on primary root growth (PRG), likely triggered by nutrient-specific sensing and signaling pathways. In *Arabidopsis*, removing Fe (Fe starvation) reduces PRG [19]. However, reducing Fe but not completely removing it (Fe deficiency) promotes PRG [20]. This complex effect of Fe on PRG remains poorly understood. Similar to Fe deficiency, reducing S promotes PRG [21] (Figure 1). However, both P starvation and deficiency reduce PRG [20].

The effect of single element deficiency on root growth depends on the availability of other elements in the growth medium [20,22]••. In *Arabidopsis*, experiments assessing the effects of simultaneous deficiencies of two or more elements (e.g. N, P, S and potassium (K)) on root growth revealed that root system architecture was determined by the interaction of different nutrient signals [22]••. While P caused the most important single-nutrient effects, the effects of S occurred mostly through nutrient interactions in paired or multiple combinations. Simultaneous P and S deficiency (-P-S) stress inhibited PRG in a similar manner as the single P deficiency (-P) [22]•• (Figure 1). In contrast, simultaneous P and Fe deficiency (-P-Fe) modulated PRG in a similar manner as Fe deficiency (-Fe) [20] (Figure 1). It has been proposed that the increase of root Fe concentration in P-deficient plants may be the cause of PRG inhibition [20]. The hypothesis is that the accumulation of Fe is 'toxic' for root growth under -P [20]. Consistently, simultaneous P and Fe deficiencies permit the recovery of PRG (Figure 1) [20]. Taken together, these observations indicate the existence of epistatic interactions between mineral nutrients that regulate root growth. Further molecular dissection of these crosstalks will be essential to explain how plants integrate multiple nutritional stimuli into complex developmental programs.

Molecular mechanisms for P and Fe interaction in controlling root growth are emerging. Several genes that regulate PRG under P deficiency have been identified (reviewed in Ref. [23]). In *Arabidopsis*, under low P regime, mutants of these genes are characterized by their ability to maintain PRG, such as the *low phosphate root 1* (*lpr1*) mutant [24], or by a strong inhibitory effect on PRG (hypersensitivity) such as the *phosphate deficiency response 2* (*pdr2*) mutant [25]. *LPR1* encodes a cell wall targeted ferroxidase and *PDR2* encodes a P5-type ATPase (AtP5A). It is thought that PDR2 represses LPR1 [24,26]. Under low P, the PDR2−LPR1 module is proposed to mediate the accumulation of Fe, reactive oxygen species (ROS) and callose in root meristems, which is alleviated by Fe deficiency [27]••. This Fe-dependent callose deposition was proposed to be a cause for PRG inhibition through prevention of the symplastic cell-to-cell communication that causes apical root meristem differentiation [27]••. This provides circumstantial physiological and molecular evidence for a link between Fe and P homeostases to regulate PRG. Recently, a transcription factor SENSITIVE TO PROTON RHIZOTOXICITY (STOP1) and its target ALUMINUM ACTIVATED MALATE TRANSPORTER 1 (ALMT1) were identified as an important module that promotes Fe accumulation under low P in the root meristem [28]••. This Fe accumulation was dependant on malate exudation and presumably malate chelating Fe. This module was shown to inhibit cell expansion under P deficiency [29]••. These two modules, LPR1-PDR2 and STOP1-ALMT1, could serve as molecular entry points for elucidating the global gene networks that coordinate P and Fe signals to shape PRG and modulate P and Fe homeostases.

Transcriptomics data as a basis for gene regulatory networks underlying nutrient homeostasis

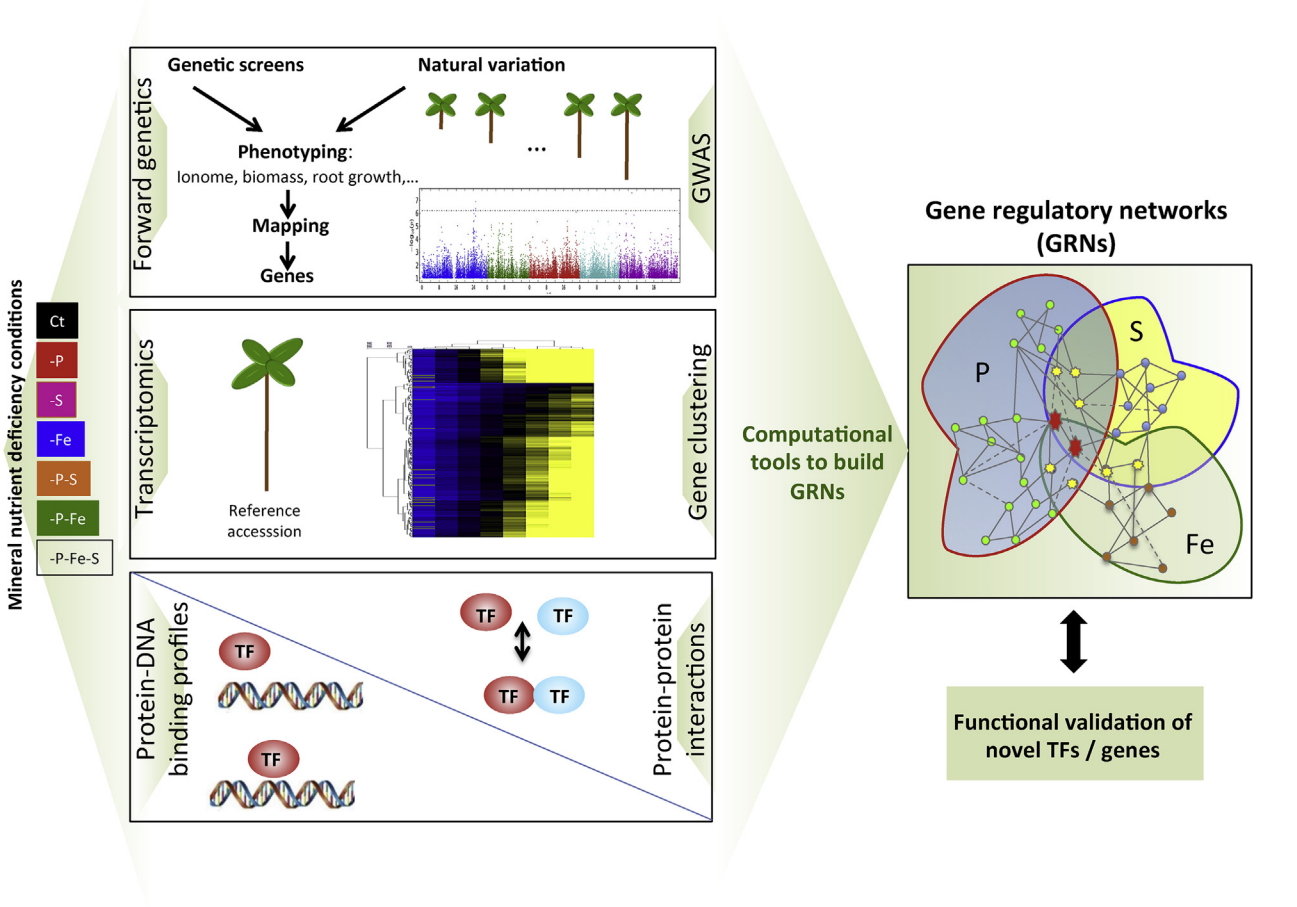
Transcriptomics experiments have identified many genes whose expression changes in response to nutrient deficiencies. But, we currently have only a limited understanding of the way these genes are organized and coordinately function to govern the plasticity of root architecture under different nutrient deficiencies. Below, we present insights gained from publically available transcriptomics data, and propose a way for how to use these data to construct gene regulatory networks (GRNs) that control individual and multiple nutrient stress responses.

Transcriptome analysis of plants subjected to various nutrient deficiencies revealed that individual nutrient deficiency imposes a large transcriptome reprogramming, which vary between leaves and roots (e.g. P [30], Fe [31] or S [32]), and vary depending on the duration of nutrient deficiency (e.g. Ref. [12]). While transcriptome analysis supports the concept of nutrient-specific deficiency responses, it also indicates the presence of interactions between nutrient homeostases. For example, transcriptome analysis of P deficient *Arabidopsis* plants revealed a down-regulation of Fe deficiency responsive genes, and reciprocally an up-regulation of excess Fe responsive genes [12,18]. The same transcriptome showed an induction of genes encoding sulfate transporters (e.g. *SULTR 1;3*, *SULTR 3;4*) that may facilitate the uptake of S, possibly to meet the metabolic demand to increase sulfolipids in replacement of phospholipids [9,12]. Transcriptome from Fe deficient *Arabidopsis* plants showed co-expression of Fe-deficiency response genes with genes involved in sulfate transport and S assimilation [33]. Beyond comparing transcriptome data obtained from plants grown under individual nutrient stresses, transcriptional responses to combined nutrient starvation

regimes have been obtained and further confirmed the presence of complex interactions between various nutrient deficiency signals [22]••. Collectively, these transcriptomics data clearly demonstrate that nutrient homeostasis interaction in plants is a general rule rather than an exception. So far, only few regulatory genes have been shown to play a role in the coordination of multiple nutrient homeostases. PHOSPHATE RESPONSE 1 (PHR1) TF has been proposed to act as an integrator of P, S and Fe signals [34,35]. Beyond its involvement in the regulation of P starvation related genes such as the high affinity Pi transporter *PHT1;1* [12], PHR1 regulates a sulfate transporter (*SULTR1;3*) [34] and induces expression of a key gene involved in Fe chelation, *Arabidopsis FERRITIN 1* (*AtFER1*) [35]. Nevertheless, PHR1 does not regulate all Pi-response genes or all genes at the interface of P-, Fe- and S-homeostasis interaction. Therefore, future research opportunities exist in identifying new TFs that integrate different nutrient deficiency signals and elaborate appropriate decision-making mechanisms to adapt growth (e.g. root growth) on the basis of the plant's nutritional status (Figure 2).

Identifying TFs involved in the regulation of nutrient deficiency stress through analysis of transcriptome data, and then establishing the nature of the interactions between these TFs and target genes is crucial for specifying gene-expression programs and for generating GRNs. There is a plethora of tools and methods to infer

Figure 2



Construction of gene regulatory networks Information collected from forward genetics, genome-wide association studies (GWAS), transcriptomics, protein–protein and promoter-binding interaction assays can be integrated to generate gene regulatory networks (GRNs). In addition to forward genetic approaches, quantitative genetic approaches that explore natural variation of plants subjected to single (e.g. -P, -Fe, -S), double (e.g. -P-Fe, -P-S, -Fe-S) and triple (e.g. -P-Fe-S) nutritional stresses can help identify new genes involved in controlling responses to these nutritional stresses. Analysis of transcriptomics data will enable the identification of exhaustive lists of genes responding to these nutrient deficiency conditions, and modules based on their expression patterns. Protein–protein interaction analysis will assess the capacity of transcription factors (TFs) to interact with each other. TF-promoter binding interaction analysis will allow the identification the genome-wide binding sites for each TF, and thus identify their potential target genes. These key genes can also be further characterized using classic genetic and biochemical approaches. Computational biology tools that build GRNs can help identify modules—sets of biologically interconnected genes underlying the same signaling pathway(s). Genes that are common between two (yellow stars) or three (red stars) stresses indicate potential key genes that integrate multi-nutrient signaling pathways.

GRNs using experimental data (reviewed in Ref. [36]•). For instance, GRN inference programs such as GENIE3 [37] or LBN [38] can be used to construct transcriptional regulatory networks as illustrated in Figure 2 for GRNs governing mineral nutrient signaling crosstalks. Generated GRNs can help identify a number of new genes and TFs involved in either individual or multiple mineral nutrient homeostases (Figure 2). The constructed GRNs can also lead to identifying transcriptionally co-regulated genes and discovering new genes that function in the same process (functional modules) in single nutrient or multi-nutrient GRNs (Figure 2). For example, the constructed GRN can generate a useful set of candidate genes that underpin the responses of primary root growth to different combinations of P, S and Fe (Figure 2). Validation of these newly identified genes requires *in planta* testing. For instance, validation of key regulatory genes such as TFs would include the analysis of expression profiles of their predicted target genes in different genetic backgrounds (e.g. wild-type plants, knock-out mutants and over-expressing lines). Methods based on protein–DNA binding profiles [39,40] and protein–protein interactions [41] enable linking these TFs to their target genes and between TFs, respectively (Figure 2). Assessing the effects of the mutations and over-expression of TFs and their target genes on PRG of plants grown under different combinations of P, S and Fe constitutes an important step to link these genes to root growth and ultimately validate the constructed GRN. Elucidation of where and when the genes and proteins in the network function at the cellular, tissue and organ system levels will be needed to enable a system-level understanding of these networks.

Combination of GWAS and omics data to identify pathways that coordinate nutrient homeostases and root growth under nutrient deficiency

Nutrient deficiency imposes changes in the transcriptome, activates molecular transport systems operating in the roots to enhance nutrient uptake capacity, and modulates root growth and development (Figure 1). For example, the root growth inhibition by -P conditions is concomitant with enhanced Pi uptake and translocation capacity, which involves two main Pi transporters PHT1;1 and PHOSPHATE1 (PHO1) respectively [3]. Nevertheless, our knowledge about how the various nutrient uptake systems are integrated to control root growth is still limited. Combination of classic genetic approaches, omics data (e.g. transcriptomics, proteomics) and genome-wide association studies (GWAS) will help identify key genes and organize them into functional modules and pathways that control nutrient homeostasis and root growth under nutrient deficiency.

The existence of genes that are involved in both nutrient uptake and root growth has been documented in *Arabidopsis*. For example, a GWAS in *Arabidopsis* showed an Fe uptake gene *FERRIC-REDUCTION OXIDASE2* (*FRO2*) to be involved in regulating PRG under -Fe condition [42]•. Furthermore, a GWAS has revealed the involvement of a Pi transporter PHO1 in regulating RSA under high nitrate conditions [43]. The availability of thousands of genotypes in a given plant species (e.g. *A. thaliana*) [44], development of methods to simultaneously evaluate thousands of roots using image analysis non-destructively [45] and methods to quantify the ionome in roots using high-throughput inductively coupled plasma mass spectrometry (ICP-MS) [46] enable obtaining multiple quantitative data sets, PRG and ionome respectively, on the same plants. Performing GWAS based on PRG and ion concentrations of plants grown on either single or multiple nutrient stress conditions will lead to the detection of candidate genes controlling these traits. The common candidate genes for both traits could be indicative of their potential roles in controlling nutrient levels and PRG, which will require testing *in planta*. It is important to go beyond detection of candidate genes by GWAS to the identification of pathways and functional modules that explain complex traits. Genome-scale gene co-function networks such as AraNet [47] can be used to predict gene modules and pathways from a list of genes such as GWAS candidates. For example, starting with a list of candidate genes detected by GWAS, AraNet2 [48] was able to effectively identify and prioritize novel candidates for complex traits (e.g. shade avoidance) in *Arabidopsis* [49]. Taken together, combining diverse big data is a promising approach to identifying pathways and functional modules that have been previously unrecognized in the regulation of nutrient homeostasis and root growth under single and combined nutrient deficiency stresses.

Conclusion

How plants integrate different stress signals to maintain mineral nutrient homeostasis and modulate their growth capacity constitutes a frontier in plant nutrition. Although plant systems biology is still in its early stages, a growing list of datasets and computational tools are becoming available. Integration of big data holds promise for constructing gene regulatory networks to help solve the mystery of mineral nutrient signaling crosstalks. Identification of these networks will represent a breakthrough in understanding the molecular mechanisms underlying nutrient signal interactions. The translation of network knowledge from model plants to crops can help develop effective and sustainable biotechnological solutions to enhance mineral nutrition in plants in natural or agricultural environments and meet the challenges facing agriculture in the 21st century.

Acknowledgements

Work in the Rhee lab is supported by grants from the National Science Foundation (IOS-1617020, IOS-1546838), National Institutes of Health (1U01GM110699-01A1), Department of Energy (grant no. DE-SC0008769) and Brigitte Berthelemot. H.R. is supported by the AgreeenSkills Plus and French National Institute for Agricultural Research.

References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. White P, Brown P: **Plant nutrition for sustainable development and global health**. *Ann Bot* 2010, **105**:1073–1080.

2. Chapin III FS: **The mineral nutrition of wild plants**. *Annu Rev Ecol Syst* 1980, **11**:233–260.

3. Heuer S, Gaxiola R, Schilling R, Herrera-Estrella L, López-Arredondo D, Wissuwa M, Delhaize E, Rouached H: **Improving phosphorus use efficiency: a complex trait with emerging opportunities**. *Plant J* 2017, **90**:868–885.

4. Kopriva S: **Regulation of sulfate assimilation in *Arabidopsis* and beyond**. *Ann Bot* 2006, **97**:479–495.

5. Curie C, Briat J-F: **Iron transport and signaling in plants**. *Annu Rev Plant Biol* 2003, **54**:183–206.

6. Briat J-F, Dubos C, Gaymard F: **Iron nutrition, biomass production, and plant product quality**. *Trends Plant Sci* 2015, **20**:33–40.

7. Couturier J, Touraine B, Briat J-F, Gaymard F, Rouhier N: **The iron-sulfur cluster assembly machineries in plants: current knowledge and open questions**. *Front Plant Sci* 2013, **4**:259.

8. Lanquar V, Lelièvre F, Bolte S, Hamès C, Alcon C, Neumann D, Vansuyt G, Curie C, Schröder A, Krämer U: **Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron**. *EMBO J* 2005, **24**:4041–4051.

9. Essigmann B, Güler S, Narang RA, Linke D, Benning C: **Phosphate availability affects the thylakoid lipid composition and the expression of *SQD1*, a gene required for sulfolipid biosynthesis in *Arabidopsis thaliana***. *Proc Natl Acad Sci U S A* 1998, **95**:1950–1955.

10. Sugimoto K, Sato N, Tsuzuki M: **Utilization of a chloroplast membrane sulfolipid as a major internal sulfur source for protein synthesis in the early phase of sulfur starvation in *Chlamydomonas reinhardtii***. *FEBS Lett* 2007, **581**:4519–4522.

11. Zuchi S, Cesco S, Varanini Z, Pinton R, Astolfi S: **Sulphur deprivation limits Fe-deficiency responses in tomato plants**. *Planta* 2009, **230**:85–94.

12. Misson J, Raghothama KG, Jain A, Jouhet J, Block MA, Bligny R, Ortet P, Creff A, Somerville S, Rolland N, *et al.*: **A genome-wide transcriptional analysis using *Arabidopsis thaliana* Affymetrix gene chips determined plant responses to phosphate deprivation**. *Proc Natl Acad Sci U S A* 2005, **102**:11934–11939.

13. Haydon MJ, Kawachi M, Wirtz M, Hillmer S, Hell R, Krämer U: **Vacuolar nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in *Arabidopsis***. *Plant Cell* 2012, **24**:724–737.

14. Khan GA, Bouraine S, Wege S, Li Y, De Carbonnel M, Berthomieu P, Poirier Y, Rouached H: **Coordination between zinc and phosphate homeostasis involves the transcription factor PHR1, the phosphate exporter PHO1, and its homologue PHO1; H3 in *Arabidopsis***. *J Exp Bot* 2014, **65**:871–884.

15. Kisko M, Bouain N, Rouached A, Choudhary SP, Rouached H: **Molecular mechanisms of phosphate and zinc signalling crosstalk in plants: phosphate and zinc loading into root xylem in *Arabidopsis***. *Environ Exp Bot* 2015, **114**:57–64.

16. • Medici A, Marshall-Colon A, Ronzier E, Szponarski W, Wang R, Gojon A, Crawford NM, Ruffel S, Coruzzi GM, Krouk G: **AtNIGT1/HRS1 integrates nitrate and phosphate signals at the *Arabidopsis* root tip**. *Nat Commun* 2015, **6**:6274.

This work proposes an interesting mechanism for how two key mineral nutrients (phosphate and nitrate) may interact to control root growth. The authors identified two early nitrate-regulated transcription factors (TFs), namely HRS1 and HHO1, which appeared to have a role in repressing primary root growth in low-phosphate conditions but only in the presence of nitrate.

17. Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot ML, Salt DE: **The leaf ionome as a multivariable system to detect a plant's physiological status**. *Proc Natl Acad Sci U S A* 2008, **105**:12081–12086.

18. Briat J-F, Rouached H, Tissot N, Gaymard F, Dubos C: **Integration of P, S, Fe, and Zn nutrition signals in *Arabidopsis thaliana*: potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1)**. *Front Plant Sci* 2015, **6**:290.

19. Gruber BD, Giehl RF, Friedel S, von Wirén N: **Plasticity of the *Arabidopsis* root system under nutrient deficiencies**. *Plant Physiol* 2013, **163**:161–179.

20. Ward JT, Lahner B, Yakubova E, Salt DE, Raghothama KG: **The effect of iron on the primary root elongation of *Arabidopsis* during phosphate deficiency**. *Plant Physiol* 2008, **147**:1181–1191.

21. Zhao Q, Wu Y, Gao L, Ma J, Li CY, Xiang CB: **Sulfur nutrient availability regulates root elongation by affecting root indole-3-acetic acid levels and the stem cell niche**. *J Integr Plant Biol* 2014, **56**:1151–1163.

22. •• Kellermeier F, Armengaud P, Seditas TJ, Danku J, Salt DE, Amtmann A: **Analysis of the root system architecture of *Arabidopsis* provides a quantitative readout of crosstalk between nutritional signals**. *Plant Cell* 2014, **26**:1480–1496.

Authors performed an extensive analysis of how different combinations of nutrient deficiencies impact root system architecture in *Arabidopsis*. They characterized 13 root architecture traits using 32 combinations of nitrate, phosphate, potassium and sulfate supply and two light regimes. Complemented with root transcriptomics and shoot ionomics, this work represents a pioneering work from which to develop a better understanding of the complex interactions between the nutritional environment of plants and their root development programs.

23. Bouain N, Doumas P, Rouached H: **Recent advances in understanding the molecular mechanisms regulating the root system response to phosphate deficiency in**. *Arab Curr Genomics* 2016, **17**:308–314.

24. Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L, Desnos T: **Root tip contact with low-phosphate media reprograms plant root architecture**. *Nat Genet* 2007, **39**:792–796.

25. Ticconi CA, Delatorre CA, Lahner B, Salt DE, Abel S: ***Arabidopsis* pdr2 reveals a phosphate-sensitive checkpoint in root development**. *Plant J* 2004, **37**:801–814.

26. Ticconi CA, Lucero RD, Sakhonwasee S, Adamson AW, Creff A, Nussaume L, Desnos T, Abel S: **ER-resident proteins PDR2 and LPR1 mediate the developmental response of root meristems to phosphate availability**. *Proc Natl Acad Sci U S A* 2009, **106**:14174–14179.

27. •• Müller J, Toev T, Heisters M, Teller J, Moore KL, Hause G, Dinesh DC, Bürstenbinder K, Abel S: **Iron-dependent callose deposition adjusts root meristem maintenance to phosphate availability**. *Dev Cell* 2015, **33**:216–230.

Based on an earlier observation describing the effect of iron on primary root elongation of *Arabidopsis* during phosphate deficiency [20], this study demonstrated that the antagonistic interactions of phosphate and iron availability control primary root growth via meristem-specific callose formation. Authors showed that phosphate deficiency in the presence of iron induces a burst of reactive oxygen species, which triggers callose deposition. Consequently, symplastic cell-to-cell communication in the stem-cell niche is inhibited, which leads to growth arrest of the primary root.

28. •• Mora-Macías J, Ojeda-Rivera JO, Gutiérrez-Alanís D, Yong-Villalobos L, Oropeza-Aburto A, Raya-González J, Jiménez-Domínguez G, Chávez-Calvillo G, Rellán-Álvarez R, Herrera-Estrella L: **Malate-dependent Fe accumulation is a critical checkpoint in the root developmental response to low phosphate**. *Proc Natl Acad Sci U S A* 2017, **114**:E3563–E3572.

The authors show that a transcription factor STOP1 and a malate transporter ALMT1 constitute a module that underlies a malate-exudation–

dependent mechanism of iron relocation in the root apical meristem to regulate primary root growth under limited phosphate conditions.

29. •• Balzergue C, Dartevelle T, Godon C, Laugier E, Meisrimler C, Teulon J-M, Creff A, Bissler M, Brouchoud C, Hagège A, *et al.*: **Low phosphate activates STOP1-ALMT1 to rapidly inhibit root cell elongation**. *Nat Commun* 2017, **8**:15300.
Authors independently show that under low phosphate conditions, STOP1 and its target ALMT1 inhibit cell expansion in the root. They hypothesize that malate-facilitated iron chelation contributes to triggering a new pathway that inhibits primary root growth in low phosphate conditions.

30. Hammond JP, Bennett MJ, Bowen HC, Broadley MR, Eastwood DC, May ST, Rahn C, Swarup R, Woolaway KE, White PJ: **Changes in gene expression in *Arabidopsis* shoots during phosphate starvation and the potential for developing smart plants**. *Plant Physiol* 2003, **132**:578–596.

31. Thimm O, Essigmann B, Kloska S, Altmann T, Buckhout TJ: **Response of *Arabidopsis* to iron deficiency stress as revealed by microarray analysis**. *Plant Physiol* 2001, **127**:1030–1043.

32. Hirai MY, Fujiwara T, Awazuhara M, Kimura T, Noji M, Saito K: **Global expression profiling of sulfur-starved *Arabidopsis* by DNA macroarray reveals the role of O-acetyl-l-serine as a general regulator of gene expression in response to sulfur nutrition**. *Plant J* 2003, **33**:651–663.

33. Schuler M, Keller A, Backes C, Philippar K, Lenhof H-P, Bauer P: **Transcriptome analysis by GeneTrail revealed regulation of functional categories in response to alterations of iron homeostasis in *Arabidopsis thaliana***. *BMC Plant Biol* 2011, **11**:87.

34. Rouached H, Secco D, Arpat B, Poirier Y: **The transcription factor PHR1 plays a key role in the regulation of sulfate shoot-to-root flux upon phosphate starvation in *Arabidopsis***. *BMC Plant Biol* 2011, **11**:19.

35. Bournier M, Tissot N, Mari S, Boucherez J, Lacombe E, Briat J-F, Gaymard F: ***Arabidopsis* ferritin 1 (*AtFer1*) gene regulation by the phosphate starvation response 1 (AtPHR1) transcription factor reveals a direct molecular link between iron and phosphate homeostasis**. *J Biol Chem* 2013, **288**:22670–22680.

36. • Banf M, Rhee SY: **Computational inference of gene regulatory networks: approaches, limitations and opportunities**. *Biochim Biophys Acta-Gene Regul Mech* 2017, **1860**:41–52.
This review comprehensively covers computational inference of gene regulatory networks (GRNs). The authors discuss current techniques, challenges and trends in GRN inference and highlight challenges and opportunities for plant science. Examples for plant-specific applications are provided, which may help researchers to select methodologies that suit their particular research questions.

37. Irrthum A, Wehenkel L, Geurts P: **Inferring regulatory networks from expression data using tree-based methods**. *PLoS One* 2010, **5**:e12776.

38. Liu F, Zhang S-W, Guo W-F, Wei Z-G, Chen L: **Inference of gene regulatory network based on local Bayesian networks**. *PLoS Comput Biol* 2016, **12**:e1005024.

39. Ouwerkerk PB, Meijer AH: **Yeast one-hybrid screening for DNA-protein interactions**. *Curr Protoc Mol Biol* 2001, **55**. 12.12.11-12.12.11.

40. Hellman LM, Fried MG: **Electrophoretic mobility shift assay (EMSA) for detecting protein–nucleic acid interactions**. *Nat Protoc* 2007, **2**:1849–1861.

41. Stephens DJ, Banting G: **The use of yeast two-hybrid screens in studies of protein: protein interactions involved in trafficking**. *Traffic* 2000, **1**:763–768.

42. • Satbhai SB, Setzer C, Freynschlag F, Slovak R, Kerdaffrec E, Busch W: **Natural allelic variation of FRO2 modulates *Arabidopsis* root growth under iron deficiency**. *Nat Commun* 2017, **8**:15603.
Using natural variation of a geographically restricted panel of *Arabidopsis thaliana* accessions, the authors showed that the *Ferric Reduction Oxidase 2* (*FRO2*) is a key gene in which natural genetic variation determines root growth in response to low iron levels. In this study, authors identified *FRO2* variants that cause higher *FRO2* expression and elevated enzymatic activities of FRO2. Furthermore, they found evidence for potential adaptive roles of *FRO2* alleles for soils with low iron content.

43. Rosas U, Cibrian-Jaramillo A, Ristova D, Banta JA, Gifford ML, Fan AH, Zhou RW, Kim GJ, Krouk G, Birnbaum KD, *et al.*: **Integration of responses within and across *Arabidopsis* natural accessions uncovers loci controlling root systems architecture**. *Proc Natl Acad Sci U S A* 2013, **110**: 15133–15138.

44. Horton MW, Hancock AM, Huang YS, Toomajian C, Atwell S, Auton A, Muliyati NW, Platt A, Sperone FG, Vilhjálmsson BJ: **Genome-wide patterns of genetic variation in worldwide *Arabidopsis thaliana* accessions from the RegMap panel**. *Nat Genet* 2012, **44**:212–216.

45. Slovak R, Göschl C, Su X, Shimotani K, Shiina T, Busch W: **A scalable open-source pipeline for large-scale root phenotyping of *Arabidopsis***. *Plant Cell* 2014, **26**:2390–2403.

46. Baxter I, Ouzzani M, Orcun S, Kennedy B, Jandhyala SS, Salt DE: **Purdue ionomics information management system. An integrated functional genomics platform**. *Plant Physiol* 2007, **143**:600–611.

47. Lee I, Ambaru B, Thakkar P, Marcotte EM, Rhee SY: **Rational association of genes with traits using a genome-scale gene network for *Arabidopsis thaliana***. *Nat Biotechnol* 2010, **28**: 149–156.

48. Lee T, Yang S, Kim E, Ko Y, Hwang S, Shin J, Shim JE, Shim H, Kim H, Kim C, *et al.*: **AraNet v2: an improved database of co-functional gene networks for the study of *Arabidopsis thaliana* and 27 other nonmodel plant species**. *Nucleic Acids Res* 2014, **43**:D996–D1002.

49. Filiault DL, Maloof JN: **A genome-wide association study identifies variants underlying the *Arabidopsis thaliana* shade avoidance response**. *PLoS Genet* 2012, **8**:e1002589.