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Getting to the root of plant mineral nutrition: combinatorial nutrient stresses reveal emergent properties

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In nature, plants have to handle daily fluctuations in light and temperature. In addition, plants face biotic and abiotic stresses that often come in various combinations. For instance, the availability of various nutrients in soil is heterogeneous, resulting in combined nutrient stress. Recent studies reveal that plant responses to multiple nutrient stresses are not the summation of the plant responses to each individual stress. Here, we present and discuss the interactions between phosphate, nitrogen and zinc to illustrate the effect of macro- and micronutrient interactions on plant growth and ion homeostasis. Solving the mystery of these interactions will pave the way to develop strategies to improve crop productivity.

Mineral nutrient interactions

As sessile organisms, plants must cope with constantly changing environments that are often stressful or unfavorable for growth and development, such as fluctuations in the bioavailability of essential mineral nutrients. The nutrient bioavailability can be strongly influenced by physico-chemical properties of the surrounding soil (*e.g.* pH [1]) and by the presence of microorganisms [2]. Plants require 16 essential nutrients to complete their life cycle and achieve yield including macronutrients, *e.g.* phosphorus (P) and nitrogen (N), as well as micronutrients, *e.g.* zinc (Zn^{2+} hereafter Zn) [3]. Plant root systems take up P and N mainly in their inorganic forms, *i.e.* phosphate (HPO_4^- , Pi) and nitrate (NO_3^-) or ammonium (NH_4^+), which are then converted to essential molecules such as amino acids and nucleic acids [4, 5]. Zn is also acquired by plant roots in the form of Zn^{2+} , although it is not metabolized [6]. Instead, Zn is required for the proper functioning of numerous metalloenzymes involved in different processes such as energy metabolism, protein synthesis and catabolism [6]. Therefore, it is not surprising to see in agriculture that Pi, N or Zn deficiencies severely affect crop growth and productivity, which can threaten food safety. Thus, plants must sense the nutrient levels in the surrounding soil and inside their cells and consequently adjust various transport steps that lead to nutrient accumulation within cells. Advancing our basic knowledge of how plants regulate and coordinate these processes is therefore critical for designing strategies to improve crop growth, development and productivity.

Early agronomical/physiological studies reported the existence of strong relationships between different nutrients (*e.g.* two-by-two; ref [7]). This is exemplified by the link of two elements, where a deficiency in one element causes changes in the

accumulation of the second element [8, 9]. On a larger scale, ionomic studies further support the existence of relationships between mineral element homeostases in plants [10], and genetic, physiological and chemical interactions between elements have been reported [11, 12]. Ionome analysis has shown that changing one or more nutrients in the medium affects the concentrations of many other nutrients *in planta* [10]. For example, Zn, iron (Fe) and sulfur concentrations increase in *Arabidopsis thaliana* plants (Columbia-0 ecotype) grown under -Pi conditions, whereas the concentrations of P, copper and cobalt decrease [10]. Furthermore, interactions between mineral nutrients induce developmental changes such as adjustments in root system architecture (RSA) [9, 13]. Studying the effects of combined nutrient deficiencies on RSA as compared to single nutrient deficiencies revealed various types of antagonistic, synergistic, or null effects [9]. Taken together, it is clear that these nutrient homeostasis interactions are a general rule rather than the exception. Whether this phenomenon is adaptive (advantageous for plant performance) or is simply a defect (disadvantageous for plant performance) remains to be proven. The manner in which plants detect, make sense of and adapt to various nutrient signals are fundamental biological questions that deserve to be addressed in order to better understand the regulation of plant nutrition. In this context, the study of plant nutrient interactions provides a powerful tool to conduct molecular studies on nutrient signaling crosstalk [9]. Progress is currently underway in deciphering the molecular mechanisms that control the physiological and developmental processes that integrate mineral nutrient signals, thanks to the development of high-throughput omics technologies, and systems biology approaches including genome-wide association studies (GWAS) and network biology driven search [14]. Here, we review recent progress in our understanding of the crosstalk between mineral nutrient signals in plants. Here we focus on the homeostases interactions between Pi, N, Zn and Fe to exemplify how such interactions influence the RSA, particularly primary root [2] growth (PRG), and the accumulation of these minerals in plants.

Phosphate and metal homeostases crosstalk

Phosphorus (P) in the form of Pi can influence the bioavailability and mobility of metals (e.g. Fe and Zn) in soils. This interaction also occurs *in planta*, and manifests itself at physiological and molecular levels. For example, Pi and Zn homeostasis interact to control their respective contents [15, 16], and Pi and Fe homeostasis interact to regulate root growth [13]. While root morphological changes that occur under nutrient deficiency are believed to be an adaptive strategy to explore greater soil volume and improve the acquisition of the deficient element [17, 18], the physiological and biological relevance of ionomic changes in absence of one or more elements for plants remains poorly understood.

Plant root responses to -Pi or -Zn have been studied in many species, particularly in the model plant *Arabidopsis thaliana* [19, 20]. Pi and Zn starvations have opposite effects on root architecture in the Col-0 ecotype: -Pi causes a reduction in primary root growth (PRG; for review, [21, 22]), whereas -Zn slightly promotes primary root growth [23, 24](Figure 1). Why plants evolved different developmental strategies in response to different mineral nutrient deficiency remains to be elucidated.

At the molecular level, a handful of genes involved in regulating PRG under -Pi have been identified including *LOW PHOSPHATE ROOT 1* (*LPR1* encodes a cell wall targeted ferroxidase [25]), *PHOSPHATE DEFICIENCY RESPONSE 2* (*PDR2* encodes a P5-type ATPase (AtP5A) [26]),

and the transcription factor SENSITIVE TO PROTON RHIZOTOXICITY (STOP1) and its target *ALUMINUM ACTIVATED MALATE TRANSPORTER 1 (ALMT1)* [27, 28]. These genes appear to form two functional modules that both operate under -Pi: STOP1-ALMT1 and PDR2-LPR1 [27, 28]. Each module promotes Fe accumulation in the root meristem, which in turn presumably leads to the inhibition of primary root elongation [13]; this primary root elongation is consistently restored in -Pi/-Fe medium [13] (Figure 1). This observation is surprising in light of the fact that -Fe causes a reduction of the primary root growth [29]. Studying the effect of combined stress (*i.e.* -Pi/-Fe) on plant growth has thus revealed a so-called “emergent property” that would have gone undetected by studying the effect of each element in isolation (*i.e.* -Pi or -Fe) (Figure 1). Furthermore, this combined nutrient stress is a new stress for the plant, and more than the sum of the two stresses. This is supported by GWAS analyses that have revealed that there are distinct genetic architectures that control the response to single (-Pi or -Fe) and combined nutrient (-Pi/-Fe) stresses in plants [24]. Several reviews have addressed the detailed mechanism of the Pi and Fe interaction in controlling primary root elongation [21, 22], and are recommended for further reading on this topic. Nevertheless, it should be noted that a recent study has provided new data that mitigate this statement and show that the degree of PRG inhibition induced by Pi deficiency in *Arabidopsis* Col-0 is not linked to the level of Fe accumulated in the root apical meristem or the elongation zone [30]. Future work should aim to determine the precise role of the Fe signal in regulating root growth in -Pi conditions. Another layer of complexity regarding ionic changes that take place in roots to respond to -Pi stress was recently reported in *Arabidopsis*. In -Pi conditions the cytosolic calcium (Ca^{2+}) concentration significantly changes the root [31]. The presence of Ca^{2+} was shown essential for the activity of malate channel (ALMT12) activity in *Brachypodium* [32]. In conclusion, although recent research uncovered the presence of interaction between phosphate and metal homeostases to regulate root growth under -Pi conditions, how other key players such as hormones or light signaling pathways are integrated in this process is not sorted out yet.

Contrary to the -Pi response, the molecular bases of Zn-dependent root growth changes are limited. Recent GWAS analyses have provided novel insight into the molecular mechanisms used by plants to control PRG under -Zn conditions (from increase in some genotypes to decrease root growth for most of them), resulting in the identification of a causal gene *AZELAIC ACID INDUCED (AZI1)* [23]. *AZI1* belongs to the lipid transfer protein family [33], and is an important player of the innate immune signaling system [34]. In -Zn conditions, *azi1* KO mutants display shorter roots as compared to wild-type plants (Col-0) [23]. Remarkably, the combination of -Pi and -Zn alleviates the effect of -Pi on root growth rate in most *Arabidopsis* accessions [24]. This trait appeared to be genetically controlled and is governed by genetic architectures distinct from those that control the response to -Pi, -Zn single stress [24]. Finally, numerous candidate genes associated with root growth rate under -P (e.g. *RECEPTOR LIKE PROTEIN 10, CLV2*), -Zn (e.g. *BRASSINAZOLE-RESISTANT 1, BZR1*) or -Pi/-Zn (e.g. *MYB88*) conditions have been identified using GWAS approach [24]. Consequently, these genes can now be used to identify the underlying molecular components of these interactions through mutant studies.

The underlying mechanisms of the effect of Pi and Zn interaction on Pi homeostasis, which takes place in roots and shoots, are now being revealed [15, 16]. In roots, Pi uptake is achieved through a suite of high-affinity transporters of the PHT1 family [35] that are

regulated via a signaling pathway that comprises the transcription factor (TF) PHOSPHATE RESPONSE 1 (PHR1) (a master regulator of Pi starvation responses), the micro-RNA miR399, and the ubiquitin-conjugating E2 enzyme (PHO2) (a key player in the regulation of Pi uptake and translocation transporter proteins abundance) [36-38]. Remarkably, -Zn can induce the expression of *PHT1* transporters in a PHR1-miR399-PHO2-independent manner, despite the presence of a sufficient Pi concentration in the medium [15]. Importantly, this is helping to pave the way for the discovery of new pathways that regulate Pi acquisition and accumulation in plants. The authors in [15] used a candidate gene approach in arabidopsis to identify the Pi exporter *PHO1;H3* as a key player in Pi xylem loading, specifically under -Zn conditions. The *PHO1;H3* mutation consistently leads to further accumulation of Pi in leaves as compared to wild-type plants grown in -Zn [15]. The induction of *PHO1;H3* in -Zn conditions is controlled by several TFs: MYB15, MYB84, bHLH35 and ICE1 [39], which together form a functional module. The involvement of these TFs in regulating Pi accumulation in a Zn-dependent manner was confirmed using functional genomic approaches [39]. Although the precise biological role of *PHO1;H3* in controlling Pi loading into xylem in plants awaits examination, one recent study showed that *PHO1;H3* expression increases with the expansion of the passage cells [40]. As these passage cells are believed to enable uptake across an otherwise impermeable endodermal barrier, it is tempting to propose that the xylem pole-associated endodermal cells can dynamically adapt passage cell numbers in response to -Zn conditions, and that the PHO1;H3 in these cells can fine-tune the activity of the expressed Pi transporters. Whether this hypothesis for PHO1;H3 function holds true also following hormones application (e.g cytokinin) that are known to control Casparian strip formation as well as passage cell density [40] remains to be tested.

After it is loaded into the root xylem, Pi is translocated and accumulated in the shoots. Pi accumulation increases under -Zn conditions [16]. Recently, a GWAS approach allowed the identification of the *Lyso-PhosphatidylCholineAcylTransferase 1 (LPCAT1)* as a causal gene involved in the regulation of Pi accumulation in shoots in Zn-dependent manner [16]. Mutation of *LPCAT1* gene in a Col-0 background promotes Pi accumulation under -Zn [16]. Transcriptionally, *LPCAT1* is under the control of the TF bZIP23 [16], which is a master regulator of the -Zn response in plants [41], thus providing a plausible mechanistic link between the -Zn signal and the regulation of *LPCAT1* expression. Furthermore, the mutation of *LPCAT1* changes the LPC/PC ratio to ultimately induce *PHT1;1* expression, which in turn leads to Pi overaccumulation in the shoots [16] [42]. Taken together, these recent results indicate that under -Zn conditions, phospholipids likely regulate the Pi content by controlling *PHT1;1* expression, but the precise mechanism remains to be elucidated. These new data thus provide a foundation for exploring the role of phospholipid signals in ion homeostasis interactions and the response to environmental changes in plant cells.

The identification of key molecular players acting at the interface of the P and Fe homeostasis in one hand (e.g. LPR1, PDR2, STOP1, ALMT1), and Pi and Zn (*LPCAT1*, PHR1, PHO1;H3) in other hand indicates that manipulating Fe or Zn may present a novel approach for improving the P nutrition of crops (and *vice versa*).

Phosphate and nitrogen signaling crosstalks

Plants require large amount of N and Pi to grow, and deficiency in one of these two nutrients severely limits crop yield. Studying the effect of single (-N or -Pi) and combined (-N and -Pi) deficiency stress on plant growth revealed that they have developed highly sophisticated mechanisms to co-regulate N and P homeostasis [43].

Under natural conditions, N and Pi are spatiotemporally heterogeneous. It has been well documented that plants use different strategies to regulate their root growth in -N as compared to -Pi conditions [18, 44]. Nevertheless, determining how the interaction between P and N signals controls root growth is only in its earliest stages. For example, it has been shown that the arabidopsis NO_3^- transporter NPF7.3/NRT1.5 modulates the root architecture in response to -Pi conditions [45]. The *npf7.3/atnrt1.5* KO mutants displayed conspicuously longer primary roots along with a significantly reduced lateral root density under Pi-deficient conditions as compared to wild-type plants, which is consistent with its high expression in the roots of Pi-deficient plants [45]. Additional molecular players that connect N and Pi signaling to the control of the primary root response have been identified, namely the TFs HRS1 and its homologs HHOs [46]. Mutation of HRS1 and/or HHO1 affects the expression of genes coding for proteins involved in Pi nutrition such as the phosphate transporters *PHT3;4* and *PHT1;6* [46]. The HRS1/NIGT subfamily of GARP TFs [47] is well known for being highly transcriptionally up-regulated by NO_3^- [48-50]. This has led to the demonstration that HRS1 and its homolog HHO1 are responsible for primary root repression on Pi-depleted media, but only in the presence of NO_3^- [46]. This combinatorial phenotype is thought to be maintained by the double control of the HSR1 protein that is both transcriptionally induced by NO_3^- and post-translationally repressed by the Pi starvation signal [46]. Finally, the effect of the combined N and Pi deficiency (-N/-Pi) on the root system architecture has been assessed in Arabidopsis, where it appears to have a potentiating effect on apical length inhibition (defined as main root path length from last lateral root to main root tip [9]), as compared to either single -N or -Pi stress [9].

The manner in which root architecture changes affect the nutrient status of aboveground tissues and vice versa is another important question, which cannot be fully understood if the nutrients are investigated individually [9]. The aboveground physiological adaptive responses to -N conditions (*e.g.* senescence) have been studied in many plant species, although the molecular bases remain poorly understood [51]. A genetic screen in arabidopsis resulted in the identification of *NITROGEN LIMITATION ADAPTATION (NLA)* gene as an essential component in a ubiquitination-dependent pathway that controls plant adaptability to -N conditions [52]. *nla* mutants display maintained growth in -N conditions. The *NLA* gene encodes an E3 ubiquitin ligase that works with PHO2 to control the trafficking of Pi transporters and consequently the Pi transport and accumulation. Indeed, the Pi accumulation in shoots appeared to be N-dependent, which is detrimental in N-depleted conditions, thereby explaining the *nla* mutant phenotype [52-54]. In addition, a reciprocal control of NO_3^- transporters by the P-signaling pathway in plants has been reported, in which AtNIGT1/HHOs were found to be central regulators of *NRT2.1* [55, 56]. These NIGT/HHO TFs seem to function as the molecular hub by which Pi signaling controls NO_3^- transport. The central Pi starvation response regulator PHR1 directly regulates NIGT1/HHOs, which confers P regulation to *NRT2.1*, leading to a P-dependent control of the NO_3^- high affinity transport system [57]. Very recently, work by [43] showed that N/Pi cross-talk is dependent on several key molecular actors belonging at the same time to i) the phosphate starvation responses

(PSR) itself including PHR1 and PHO2, and ii) the NO_3^- sensing pathway via NPF6.3/NRT1.1, which is an established NO_3^- nitrate transceptor [58, 59]. Interestingly, N signal controls the abundance and the stability of PHR1 protein [43]. N tightly controls PHO2 as well. The *pho2* mutants are affected in the N control of PSR. The transcriptional N regulation of PHO2 is dependent on NRT1.1, and in turn PHO2 controls NRT1.1 protein accumulation [43]. The fact that PSR genes get activated only when N and not Pi signals are combined it is tempting to propose a resemblance between N/Pi signals crosstalk and an “electronic logic gate”. A better understanding of how plant integrate of N and Pi signals will help gaining insight on the possibly evolved logic gate in plant cells.

Finally, it is worth to mention that ionome analysis revealed that -N/-Pi combined deficiency alters the accumulation of other essential nutrients. For instance, -N/-Pi causes a remarkable increase of Fe accumulation (10-20 fold) as compared to the small (if any) increase observed under -N or -Pi single stress [9]. This result affirms that future studies should: (i) include Fe in the combinatorial analysis of P and N deficiency effects and (ii) aim to identify the novel mechanisms that influence Fe accumulation in plants through the unexpected crosstalk of N and Pi signals.

Altogether, these studies outline the existence of signaling pathways involved in the combinatorial response to different levels of N and Pi in plants. This knowledge will have important consequences in agricultural practices, or biotechnology, to adapt genotypes to particular agricultural conditions, and to decrease our dependency to N and P fertilizers.

Nitrogen and zinc homeostasis interplay

After N, Zn is the second most yield-limiting nutrient for some plant species, such as rice (*Oryza sativa*) [60]. To cope with low Zn levels in the soil, current practices rely on excessive Zn application [61]. Although this method is considered effective, it has been shown that N is a critical player in the uptake and accumulation of Zn in plants [62]. Interestingly, combined N and Zn application is proven to be even more efficient than Zn application alone [63-65]. Simultaneous application of N and Zn improves root development and Zn uptake capacity, especially in -Zn conditions. However, few studies have investigated how the combined application of N and Zn affects the Zn fractions in the soil in order to increase its absorption through the plant root systems. The current proposal is that this combined application likely improves the bioavailability of Zn in the soil by changing the soil pH [63]. This recent Zn and N interaction study echoes an earlier report showing that -Zn conditions cause a drastic decrease in NO_3^- uptake in plants (*e.g.* cotton, sunflower and buckwheat), in which the decreased NO_3^- uptake capacity in -Zn is associated with an increase in the cation-anion uptake ratio and a corresponding decrease in external pH [66]. It is clear that despite its primary importance, the interaction between Zn and N has retained less interest at the molecular level, and discovery of mechanisms that coordinate Zn and N homeostasis in plants will help improving Zn nutrition in plants.

Transcriptome analyses can provide useful data that might help to both understand Zn and N homeostasis interactions at the molecular level. In the context of this review, we considered transcriptomic data published for NO_3^- [67] and for Zn deficiency [68] in *A. thaliana*, and the expression levels of NO_3^- and Zn-related genes were compared. Interestingly, the analysis of these transcriptomes has provided additional information on

the N and Zn interaction in plants. For example, a large number of genes involved in N homeostasis was found to be up-regulated in shoots of Zn-starved arabidopsis plants. For instance, *NITRATE TRANSPORTER 2.4* (*NRT2.4*, AT5G60770) [69], a marker of N-deficiency, was strongly induced by -Zn conditions, and its induction is dependent on two key TFs, namely bZIP19 and bZIP23 without which plants are unable to respond to Zn starvation [70]. Indeed *NRT2.4* was not induced in the *bzip19bzip23* double mutant background [41]. It is worth to mention that bZIP19 and bZIP23 play also a negative regulatory role on the expression of genes coding for ammonium (NH₄⁺) transporters under -Zn conditions. For instance, the expression of the *AMMONIUM TRANSPORT 1.1* (*AMT1.1*, AT4G13510) and *AMMONIUM TRANSPORTER 2.1* (*AMT2.1*, AT2G38290) [69] genes were strongly induced in *bzip19bzip23* double mutant compared to *Arabidopsis* wild type plants in -Zn conditions [68]. Taken together, the induction of these N-related genes may reflect that the molecular response to N deficiency in plants induced by Zn deficiency. In another hand, the gene expression analysis revealed that the expression of important genes involved in the regulation of Zn homeostasis is repressed by N deficiency, such as the key gene *FERRIC REDUCTASE DEFECTIVE 3* (*FRD3*, AT3G08040) that is involved in loading Zn into xylem [71]. Aforementioned genes reported can be used as molecular entry points to investigate different branches of a complex signaling crosstalk between N and Zn. Future research will benefit from obtaining transcriptomic data from plants exposed to combined N and Zn deficiency, which is necessary to investigate the functional categories of genes that are specifically involved in Zn and N signaling crosstalk. Finally, how the N and Zn signals are relayed to the transcription machinery deserves further investigations.

Taken together, the physiological and molecular data reviewed here support the presence of interplay between Zn and N interplay in plants, which deserves special attention in improving plant growth and also in biofortification of food crops with Zn.

Concluding remarks and future perspectives

How plants adapt to a changing environment is an important question in biology. Combined nutrient stress is one of the environmental challenges that plants are facing in nature. Typically, ion homeostasis interactions in plants are manifested through changes in their growth capacity. Few examples exist in literature on changes in the RSA of plants subjected to combinatorial nutrient limitations [9, 13, 24], and perhaps can be best illustrated in this review by the interaction between Pi and Fe [13]. As mentioned earlier, -Pi/-Fe restores the plant's capacity for primary root elongation, in comparison to the negative effect of -Pi or -Fe alone on the roots [13] (Figure 1). The examples (Pi/Fe or Pi/Zn) illustrate how a deficiency in two elements can overcome the growth defect caused by a single nutrient deficiency. This also demonstrates how important it is to study nutrients as combinatorial signals that are perceived by the plant as interconnected signals requiring tightly controlled mechanisms, yet to be discovered. This is especially the case when we know that there is a distinct genetic architecture in response to a combined stress, with limited (if any) overlap between the genetic architectures controlling each individual stress response [24]. Therefore, future research aiming to answer the question about how plant nutrient homeostasis is wired to control plant root growth and how it evolves, should take into account the co-occurrence of different nutrient stress. Approaches should make the best use of the modern RSA phenotyping facilities in combination with the most advanced molecular and genetics tools. This field of research will likely also benefit from applying "big data" to

investigations of the molecular mechanisms that control the developmental processes used by plants to integrate mineral nutrient signals.

Nutrient-nutrient interaction and its influence on ion accumulation are widely recognized. Whether these responses have an underlying signaling or nutrition basis is a topic of debate. The interaction between Pi and Zn shows nicely how a deficiency in one element (herein Zn) can cause the over-accumulation of the other ion (herein Pi). This observation provides a great opportunity to go beyond focusing solely on the use of a Pi starvation related genes to improve the Pi response in plants. As such, this approach could be used to identify a new route for Pi transport and accumulation in plants in Zn-dependent manner. This strategy holds true for many identified ion bipartite interactions (Pi/N, N/Zn, etc) and can be extended for the growing list of ion tripartite interactions (Pi/N/Zn, Pi/N/Fe). For instance, through studying P-N interaction raise an opportunity to discover the molecular mechanism underlying an emergent property (*i.e.* the increase in Fe accumulation under -Pi/-N) and use it in an essential metal biofortification strategy. Together, the examples used in this review indicate that future research will need to consider mineral nutrition as a complex three-dimensional network, that will require the development of modelling tools to predict the ionome for any genotype for any given combination of nutrients, at a given time and space. The use of big data and systems biology approaches to its full potential will help to construct gene regulatory networks that (i) control individual and multiple nutrient stress responses, and (ii) govern the coordination of mineral nutrient homeostasis and root growth. Such an integrative gene network will take into account the availability of a maximum number of minerals and their combinations. In this context, a dialog between farmers, breeders, and molecular biologists is needed to prioritize which particular nutritional stress combinations should be examined first, and which are applicable to the crops of interest in specific regions. Understanding how plants integrate different nutrient signals to modulate their ionome and growth capacity, and pathways that co-regulates ions homeostasis and roots growth is in its early stage (Figure 3). These nutrient signals are likely to be wired with the immune signaling pathways triggered by the wide range of microbes that cohabitate soil with plants and compete for capturing the limited nutrients in soil. This interplay between nutrient signal and immunity interactions is an emerging research topic, as it is crucial for plants to find a correct balance between growth and defense. Taken Therefore, developing system level understanding of plant mineral nutrition is needed (Figure 3) to help unravel the underlying molecular mechanisms to design crop ideotype depending on environmental scenarios in the precision farming era (see also outstanding questions).

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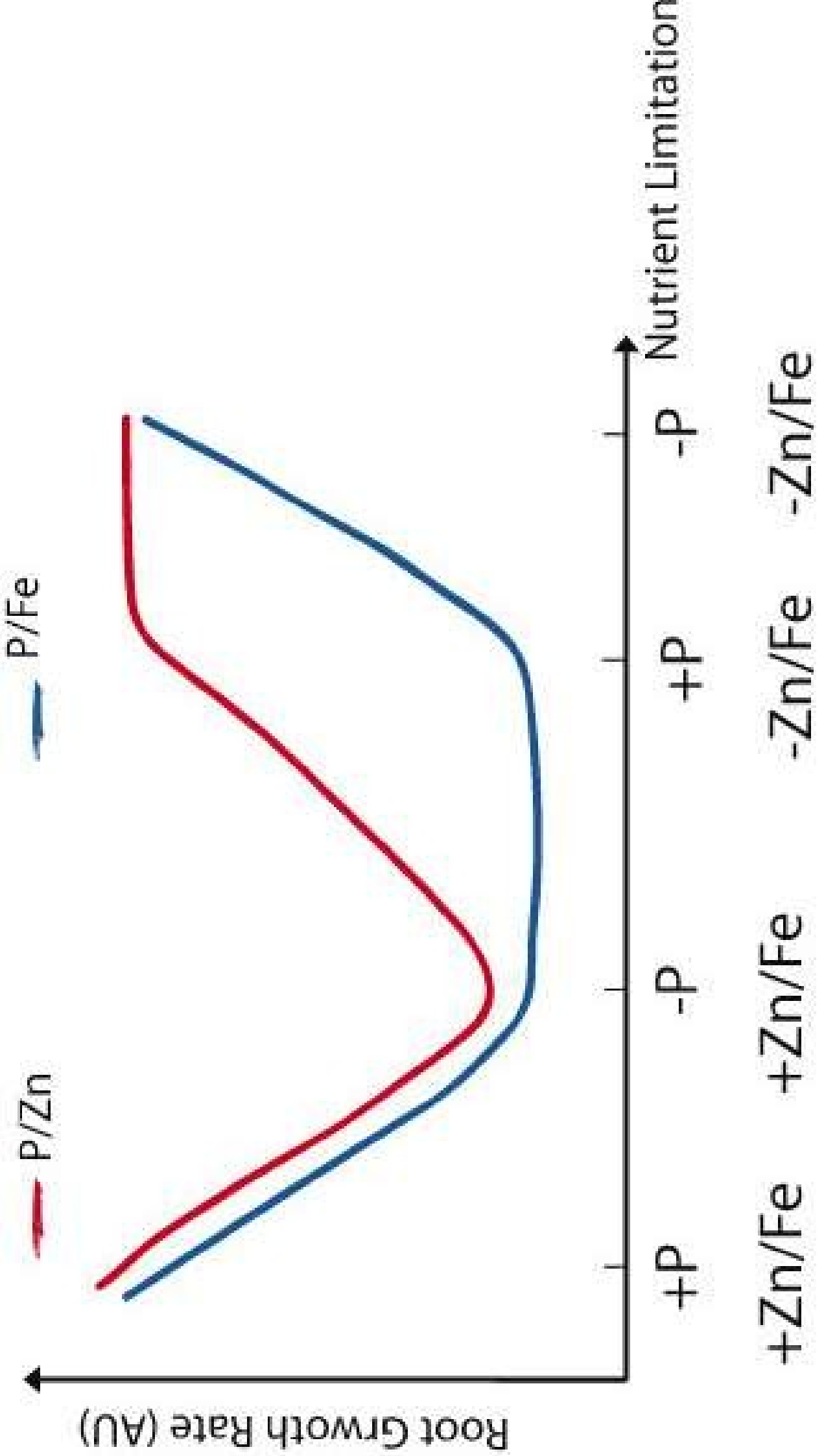
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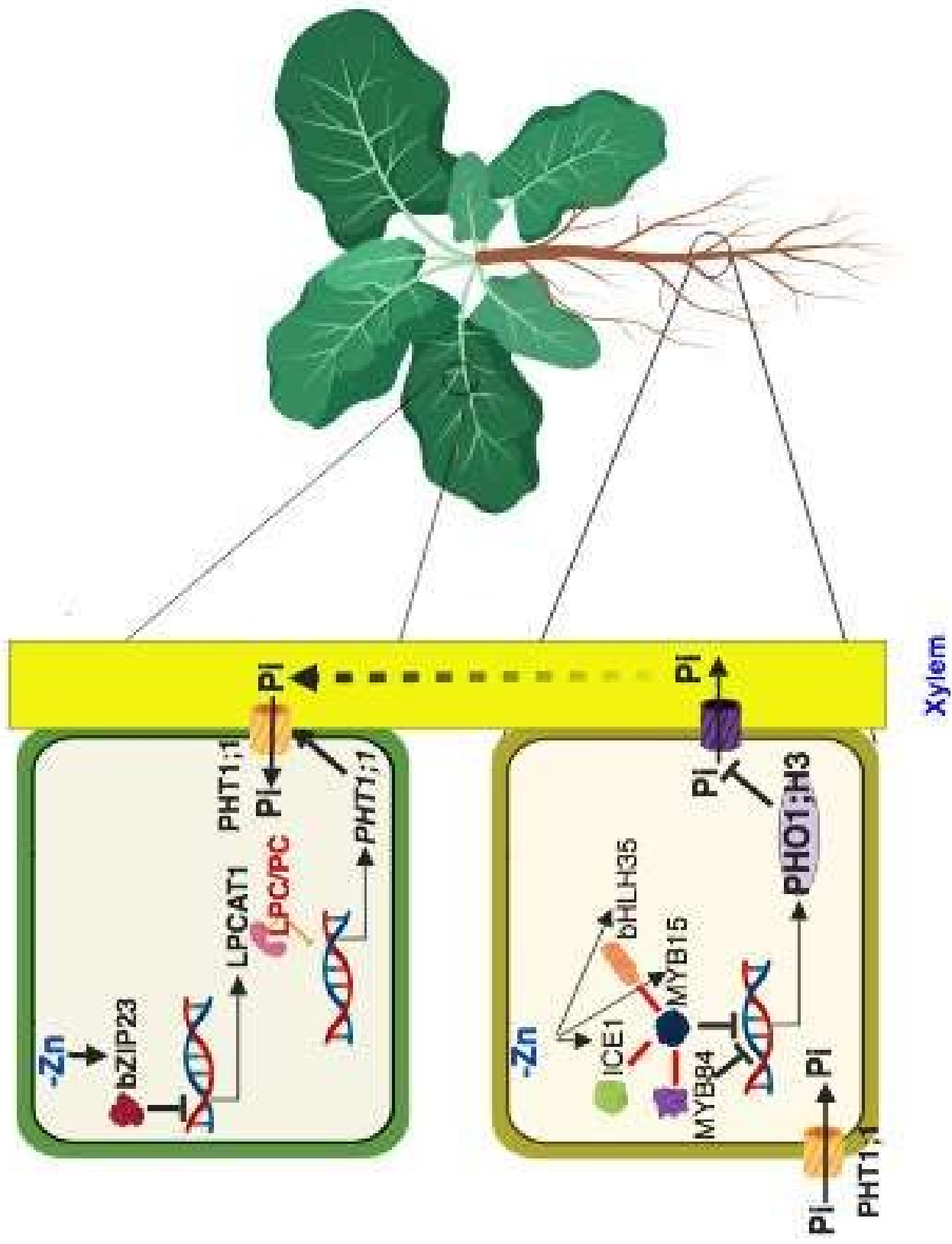
Figure 1. Schematic representation of combinatorial nutrient limitation effects on early root growth rate (RGR). The limitation of phosphorus (P) or iron (Fe) inhibits RGR and the deficiency of both abolishes this inhibition. Whereas, zinc (Zn) limitation alone slightly promotes RGR and alleviates the negative effect of P limitation.

Figure 2

Figure 2. Schematic representation of Signaling pathways controlling phosphate accumulation under zinc deficiency. In roots, phosphate (Pi) is acquired by the high affinity Pi transporters such as PHT1;1. After it is radial transport, Pi is loaded into the xylem. Under -Zn conditions, the Pi loading into xylem is negatively regulated by PHO1;H3 protein. *PHO1;H3* expression is induced under -Zn conditions [15]. Under this condition (-Zn), the expression of *PHO1;H3* is controlled by four transcription factors, namely bHLH35, ICE1, MYB15, and MYB84. Three of them (bHLH35, ICE1, MYB15) are induced by -Zn signal [39]. MYB15 interacts physically (red line) with ICE1 et MYB4. MYB15 and MYB84 repress the expression of *PHO1;H3* via a direct binding to the *PHO1;H3* promoter represented as headed black line. After being loaded into the xylem, Pi is transferred to shoots represented by dashed arrow. In shoots, the regulation of Pi accumulation involves the PhosphatidylCholine (PC) AcylTransferase 1 (LPCAT1), the transcription factor bZIP23 and PHT1;1. Under -Zn conditions, the expression of LPCAT1 is mediated by bZIP23. LPCAT1 regulates the PhosphatidylCholine/PhosphatidylCholine (LPC/PC) ratio, which in turn increases the expression of Pi transporter PHT1;1 leading thus to the accumulation of Pi in shoots [16].

Figure 3. Schematic representation of the effect of mineral nutrient availability on ions homeostasis, gene regulatory network and plant growth and development. This presentation has been arranged arbitrarily and does not reflect any sequential order. Black arrows indicate the effects of nutrient availability on ions homeostasis, gene regulatory network and plant growth and development. Blue double head arrows indicate the related effect of ions homeostasis, gene regulatory network and plant growth and development on each other.





Xylem

Zinc Deficiency

Mineral nutrient availability

