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REVIEW

Transcriptional Regulatory Components Responding to Macronutrient Limitation

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Abstract The mechanisms of how plants sense nutrient availability in soils and then send the signals through downstream signal cascades are essential processes for plant survival. Although understanding the regulatory mechanisms of macronutrient sensing and signaling are very important, many components still remain unknown. This review describes the recent progress in identifying the transcriptional regulatory components that respond to phosphorus, nitrogen, potassium, and sulfur limitation. Under phosphorus limited conditions, a MYB transcription factor, *PHR1,* functions as a key transcriptional regulator of the phosphorus deficient responsive genes via binding to the P1BS *cis* element. Similarly, *SLIM1* plays crucial roles in the expression of the sulfur starved responsive genes through binding to the sulfur responsive *cis* element. The nitrogen deficient responsive MYB transcription factors control nitrogen metabolism. Cytokinins and microRNAs (miRNAs) also mediate macronutrient metabolic processes. Much less is known about the potassium deficient signaling components, as compared to the other nutrient signaling pathways. Multiple recent studies have suggested that phytohormones, such as ethylene, auxin, jasmonic acid, and abscisic acid (ABA), and reactive oxygen species (ROS) play roles as transcriptional regulatory components in potassium deficient signaling.

Keywords Macronutrient · Nitrogen · Phosphorus · Potassium · Sulfur · Transcription factors

Abbreviations

bHLH	helix–loop–helix
miRNA	microRNA
ROS	reactive oxygen species

Introduction

Management of macronutrients is crucial in agricultural systems due to the depletion of soil nutrients by mono-crop cultures, reduced water availability, and other factors. When plants are starved for nutrients, plant growth is repressed and their morphologies become modified. To increase crop yields, an optimal nutrient supply is essential. Therefore, we have relied on fertilizers to provide nutrients to plants to ensure that they obtain sufficient nutrients year after year (Amtmann et al. 2006; Armengaud et al. 2009). Nitrogen, phosphorus, potassium, and sulfur are the core components of fertilizers, but an understanding of the regulatory mechanisms that govern the sensing and signaling in response to the availability of these nutrients has been relatively limited (Schachtman and Shin 2007).

Our former understanding of nutrient sensing and signaling was mainly based on physiological and biochemical studies. The phenotypic alterations in response to nutrient limitations were analyzed and nutrient transporters and metabolic enzymes were isolated and characterized. However, the detailed regulatory mechanisms have remained largely unknown for many years. Much of the recent progress in the study of each nutrient deficient signaling component has been accomplished through transcriptome analyses (Hammond et al. 2003, 2004; Armengaud et al. 2004; Hilson et al. 2004; Tohge et al. 2005; Yi et al. 2005; Bi et al. 2007; Peng et al. 2007a; Calderon-Vazquez et al. 2008; Saito et al. 2008).

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These studies have shown that each nutrient deficient signaling pathway has a unique mechanism, but also many shared regulatory components.

This review mainly focuses on the transcriptional regulatory components of nutrient deficient signaling. Several types of transcription factors were identified as transcriptional regulators of nutrient deficient signaling. Phosphorus and sulfur deficient responsive *cis* elements on the promoter regions of responsive genes and microRNAs (miRNAs) involved in macronutrient signaling (excluding potassium) were also identified and characterized. Finally, phytohormones were identified as important regulators of transcriptional regulation in nutrient deficient signaling.

Phosphorus

Phosphorus is one of the most essential nutrients necessary for plant growth and development. Plant phosphorus deprivation mostly results from a lack of bioavailability rather than a deficiency of phosphorus contents in soil (Schachtman et al. 1998). Phosphorus-deficient signaling is one of the most studied areas of nutrient utilization among the macronutrient deficient signaling pathways.

Several studies have described the transcription factors which alter their expression in response to phosphorus limitation. These transcription factors belong to various transcription factor families, such as zinc finger, WRKY, MYB, and bHLH (basic helix–loop–helix) (Burleigh and Harrison 1997; Rubio et al. 2001; Wang et al. 2002; Todd et al. 2004; Yi et al. 2005; Devaiah et al. 2007a, b, 2009; Devaiah and Raghothama 2007). An *Arabidopsis* cysteine-2/histidine-2 zinc finger type transcription factor, *ZAT6,* was isolated as a phosphate deficiency inducible transcription factor. The overexpression of *ZAT6* resulted in altered root architecture and higher phosphate uptake and accumulation. From these studies, it was suggested that ZAT6 negatively controls primary root growth and is involved in phosphate homeostasis (Devaiah et al. 2007b). Another *Arabidopsis* transcription factor, *WRKY75,* which was upregulated by phosphate starvation, also regulated phosphorus starvation responses. When *WRKY75* expression was suppressed, the expression of multiple phosphorus deficient responsive genes, such as *PS2-1*, *AT4*, *IPS1,* and the phosphate transporters *Pht1,1* and *Pht1,4*, were attenuated (Devaiah et al. 2007a). Additionally, an in silico analysis showed that there are multiple WRKY-binding W boxes (TTGACC/T) in the promoter regions of these genes (Devaiah et al. 2007a).

Functional roles for several MYB type transcription factors have been linked to phosphorus starvation responses. Phosphorus deficient conditions induced two R2R3 MYB transcription factors, *MYB75* (*PAP1*) and *MYB90* (*PAP2*), which are known to be involved in the control of flavonoid biosynthesis (Baudry et al. 2004). Another *Arabidopsis* R2R3 type MYB transcription factor, *MYB62,* regulates phosphorus starvation responses through an alteration of gibberellic acid (GA) metabolism and signaling (Devaiah et al. 2009). Also, multiple MYB-CC (coiled coil) type transcription factors, including *PHR1*, *PHR2,* and *NSR1,* were identified as essential transcription factors in the phosphorus deficient signaling cascade (Rubio et al. 2001; Todd et al. 2004). The expression levels of MYB-CC transcription factors were upregulated by phosphorus starvation and act as positive regulators for phosphorus signaling (Rubio et al. 2001; Todd et al. 2004). In addition, *NSR1* has also been shown to provide a regulatory role for nitrogen and phosphorus assimilatory genes (Todd et al. 2004). Among these MYB-CC transcription factors, *PHR1* is one of the major transcription factors controlling the various phosphorus deficient responses, including the regulation of target gene expression (Rubio et al. 2001; Todd et al. 2004). The *PHR1*-recognized *cis* element (GNATATNC; P1BS element) is present in the promoter regions of many phosphorus starvation responsive genes, including phosphate transporters, protein kinases, RNases, and enzymes involved in lipid metabolism (Rubio et al. 2001; Hammond et al. 2003, 2005; Todd et al. 2004; Hou et al. 2005).

A rice bHLH domain containing transcription factor, *OsPTF1*, which was induced under phosphorus-deficient conditions, appears to be involved in the tolerance to phosphate starvation (Yi et al. 2005). An *Arabidopsis* bHLH domain containing transcription factor, *PHO4*, was also linked to phosphorus deficient signaling via an interaction with *PHO2* (O’Neill et al. 1996). *Arabidopsis* bZIP transcription factors, including *bZIP1*, *bZIP2*, *bZIP11*, *bZIP44*, and *bZIP53,* were associated with phosphorus starvation through experiments that controlled carbohydrate metabolism (Lloyd and Zakhleniuk 2004; Amtmann et al. 2006).

MiRNAs are another regulatory component of phosphorus starved responsive genes. The levels of miR778, miR827, miR2111, and miR399 were induced and the level of miR398 was repressed when plants sensed a phosphorus limitation (Pant et al. 2009). These miRNAs controlled multiple phosphorus deficient responses where miR399 inhibited *PHO2,* which is crucial for phosphorus homeostasis (Bari et al. 2006) and miR2111 targeted *NAC24* (NAC domain transcription factor 24) (Pant et al. 2009) (Fig. 1). Phosphorus deficient conditions induced miR827 targeting of the E3 ligase, *NLA* (At1g02860), which is important for anthocyanin synthesis. Nitrogen-limited anthocyanin accumulation was also controlled by miR827 (Peng et al. 2007a, b, 2008; Kant et al. 2011).

Phytohormones are important regulatory components of phosphorus starvation responses. Specifically, cytokinins

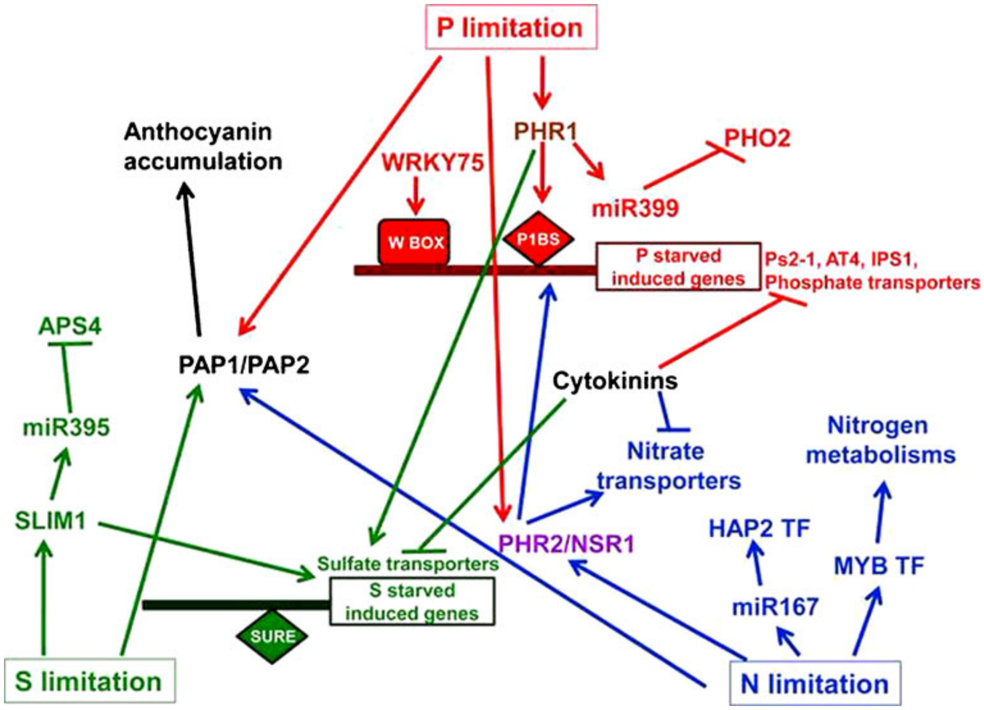

Fig. 1 Transcriptional regulatory components involved in plant responses and signaling under phosphate, nitrogen and sulfur limitation as described in the text. *Red* components linked to phosphorus limitation; *blue* components of nitrogen limitation; *green* components of sulfur limitation. *Purple* (*PHR2/NSR1*) component linked to nitrogen and phosphorus limitation; *brown* (*PHR1*) components linked to phosphorus and sulfur limitation; *black* components involved in all three nutrient limitations. The *arrow line* indicates the positive regulation and the *closed bar line* indicates the negative regulation

have been shown to control systemic and local responses to phosphorus limitation (Franco-Zorrilla et al. 2004, 2005). Cytokinins repressed the expression of phosphate transporters, which were induced by phosphorus starvation through CRE1/AHK4 receptors, and inhibited low phosphorus-induced anthocyanin accumulation (Franco-Zorrilla et al. 2002). Auxin is also involved in lateral root development which was promoted by low phosphorus status (Lopez-Bucio et al. 2002, 2005; Perez-Torres et al. 2008, 2009; Rubio et al. 2009); however, detailed mechanisms of this response are still unknown.

Nitrogen

Various forms of nitrogen, nitrate (NO_3^-), ammonium (NH_4^+), peptides, amino acids, urea, etc., are essential macronutrients that plants need to acquire from the soil to ensure their growth. Among them, nitrate is a key molecule for nitrogen signaling that influences plant metabolic processes and development (Krouk et al. 2010a, b). Nitrogen signaling has mostly been studied by testing the influence of exogenously applied nitrogen, rather than looking at the effects of nitrogen limitation.

Several transcription factors related to nitrogen signaling have been isolated and functionally characterized. The expression of *PAP1*, *PAP2*, *WRKY54*, *WRKY43*, *WRKY70,* and *WRKY38* were clearly upregulated by nitrogen starvation (Scheible et al. 2004). An *Arabidopsis* bZIP domain containing transcription factor, NLP7 (Nodule inception-like protein 7), was shown to play roles in the regulation of nitrate metabolism and alterations of root architecture (Castaings et al. 2009). Another *Arabidopsis* bZIP transcription factor, bZIP1, functions in the regulation of the response to nitrogen levels. The expression of *bZIP1* was upregulated by nitrogen treatment and controlled several nitrogen responsive genes (Gutierrez et al. 2008; Obertello et al. 2010). The MADS-box containing transcription factors were also isolated as possible signaling components for nitrogen deficient signaling and ANR1 was shown to play a role in regulating nitrogen metabolism (Gan et al. 2005). The expression of phosphorus deficiency induced *PHR2* and *NSR1* MYB transcription factors was also induced by nitrogen deprivation and these genes were also shown to control the expression of nitrate transporters as well (Todd et al. 2004) (Fig. 2). During nitrogen deficiency, ABI4 (abscisic acid insensitive4), an AP2 (activator protein-2) type transcription factor, activated the expression of the *DGAT1* (acyl-coenzyme A:diacyglycerol acytransferase 1) gene, which is the rate-limiting enzyme in triacylglycerol biosynthesis. ABI4 binds the CE1-like *cis* element (CACCG) on the *DGAT1* promoter that leads to an acceleration of triacylglycerol accumulation in nitrogen limited plants (Yang et al. 2011).

Several MYB transcription factors were also identified as regulatory components of nitrogen signaling from other plant species. Three lotus R2R3-MYB transcription factors (*LjMYB101*, *LjNYB102* and *LjMYB103*) and a soybean R2R3-MYB transcription factor (*GmMYB101*) were induced by low nitrogen conditions. It was also shown that lotus chalcone synthase and lotus glutamine synthetase were coincidently activated along with *LjMYB101* under

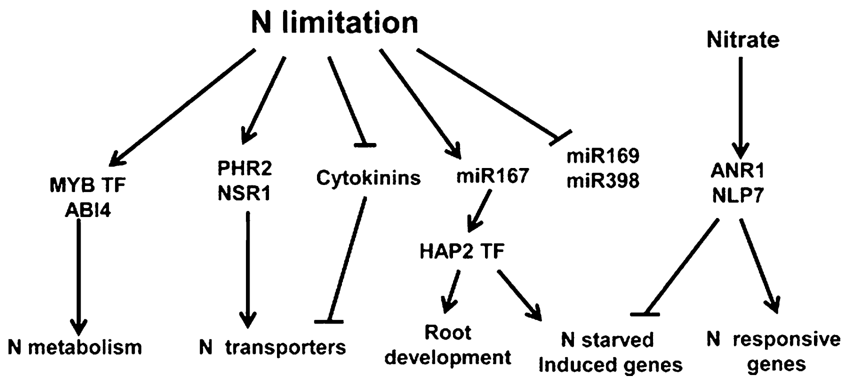

Fig. 2 Overview of nitrogen availability regulated transcriptional regulatory components described in the text. The *arrow line* indicates the positive regulation and the *closed bar line* indicates the negative regulation

nitrogen limited conditions and that they shared several *cis* elements in their promoters. These results suggested that these MYB transcription factors are linked to nitrogen metabolism (Miyake et al. 2003).

In addition to transcription factors, other regulatory mechanisms of nitrogen signaling have been studied. Specifically, the control of nitrogen responsive genes by miRNAs is an important regulatory mechanism. MiR169 and miR398 were repressed during nitrogen limitation (Pant et al. 2009), while MiR167 has been linked to lateral root development in response to nitrogen starvation (Gifford et al. 2008). MiR167 also targets several *HAP2* type transcription factors (Gifford et al. 2008) (Fig. 2). An SBP-box containing transcription factor, *SPL9*, was identified as an important component for controlling nitrogen assimilation genes, including nitrate reductase. MiR156 targeted the *SPL9* transcription factor that leads to an alteration in the nitrate response (Krouk et al. 2010b).

Reactive oxygen species (ROS) may represent another group of signals which control nitrogen sensing and signaling. Microarray data suggested that the addition of nitrate altered a large set of genes involved in redox (reduction–oxidation) status (Scheible et al. 2004). In roots, ROS levels were increased when plants were starved for nitrogen (Shin et al. 2005). However, the detailed regulatory mechanisms of ROS-nitrogen signaling are still unknown. Phytohormones have also been shown to function in nitrogen signaling. Cytokinins, specifically, have been shown to regulate nitrogen-mediated root-to-shoot allocation (Takei et al. 2002) and to negatively regulate the expression of nitrate transporters.

Sulfur

Sulfur, which is one of the major macronutrients, is generally taken up by roots via sulfate transporters (Takahashi et al. 1996, 1997; Leustek and Saito 1999; Saito 2000). When exposed to conditions limited sulfur, plants accumulate anthocyanin, alter their morphology, and exhibit earlier flowering (Nikiforova et al. 2004). Sulfur metabolism, especially the assimilation of sulfur, is tightly linked to sulfur availability. A key transcription factor, *SLIM1* (Sulfur Limitation 1), which controls the sulfate transporter1;2 (SULTR1;2), has been identified using ethyl methanesulfonate-mutagenesis of SULTR1;2 fused GFP expressing plants (Maruyama-Nakashita et al. 2006). SLIM1 is an EIL3 (ethyelene-insensitive3 (EIN3)-like3) transcription factor and controls several sulfur responsive genes, such as multiple sulfate transporters, a cysteine synthesis related serine acetyltransferase, a methyl(thio) alkymalate synthase, an ATP sulfurylase, and a key enzyme of sulfur assimilation, 5′-adenylylsulfate reductase. Furthermore, two rice *SLIM1* homologues were able to complement the *Arabidopsis slim1* mutant, suggesting a conserved function between plant species. Another regulatory component of *SLIM1* is the sulfate deficient induced miR395 (Maruyama-Nakashita et al. 2006; Kawashima et al. 2011) which targeted several sulfate metabolic process related genes, including sulfate transporters and APS4 (ATP sulphyrulases4) (Kawashima et al. 2009) (Fig. 1).

The *Arabidopsis* MYB transcription factors, MYB28 and MYB29, are involved in the regulation of aliphatic glucosinolate biosynthesis, a branch of sulfate metabolism (Hirai et al. 2007). Several other *Arabidopsis* R2R3 MYB transcription factors, such as MYB16, MYB56, MYB69, MYB75, MYB90, MYB93, and MYB94, were linked to sulfur starvation (Nikiforova et al. 2004, 2005). Since sulfate starvation also leads to an accumulation of anthocyanin, *PAP1* and *PAP2* MYB transcription factors were regulated by sulfur availability as well (Hirai et al. 2004). In addition, phosphorus deficiency induced *PHR1* also regulated the expression of sulfate transporter genes (Rouached et al. 2011).

The auxin-responsive transcription factors, *IAA28*, *IAA13*, and *ARF2*, were induced in response to sulfur starvation, but seem to be indirectly involved in sulfur metabolic processes. This may be mediated through SAM (*S*-adenosyl-methionine) degradation during sulfur starvation via ethylene signaling (Falkenberg et al. 2008). Similar to phosphorus deficiency responses, cytokinins function negatively in sulfur uptake by repressing the expression of sulfate transporters (Maruyama-Nakashita et al. 2004a, b).

The sulfur responsive *cis* element (SURE) was isolated from a deletion analysis of the *Arabidopsis* sulfate transporter1;1 (*SULTR1;1*) promoter (Maruyama-Nakashita et al. 2005). SURE elements have been identified in the promoters of many sulfur starved responsive genes and are essential for their induction (Maruyama-Nakashita et al. 2005, 2006), however, the transcription factors that bind to these elements have not yet been identified.

Potassium

Potassium is one of most abundant inorganic cations in plants and is a crucial macronutrient for plant growth and metabolism (Leigh and Jones 1984). Even though many types of macronutrients signaling and regulations are similar, potassium is not metabolized or incorporated differently from nitrogen, phosphorus and sulfur (Schachtman and Shin 2007). This difference may be one of the main reasons why less is known about the signaling components of potassium than other macronutrients.

Some of the bHLH, WRKY, NAM, AP2, and E2F type transcription factors, which were differentially regulated by external potassium resupply, were isolated from an *Arabidopsis* microarray analysis (Armengaud et al. 2004). An *Arabidopsis* R2R3-MYB transcription factor, *MYB77*, was isolated based on its down regulation by potassium starvation. MYB77 interacted with the auxin transcriptional activator, ARF7, and controlled the low potassium dependent lateral root development through auxin signaling (Shin et al. 2007). Abiotic stress, including the potassium deficient induced nuclear protein, NPX1, which interacted with the *Arabidopsis* NAC transcription factors, has also been linked with enhancing ABA biosynthesis under potassium deficient conditions (Kim et al. 2009).

The calcium binding proteins, CBL1 and CBL9, and their target kinase, CIPK23, were found to have crucial roles in low potassium responses. Together, they activated an inward potassium channel, AKT1, suggesting that the calcium signal induced potassium uptake via AKT1 (Li et al. 2006).

ROS is a well-known crucial signal for low potassium signaling, uptake, gene expression, and root growth (Shin and Schachtman 2004; Shin et al. 2005; Jung et al. 2009). In potassium deficient signaling, ethylene plays a positive role and works upstream of ROS signaling (Jung et al. 2009). In addition, jasmonic acid is involved in potassium signaling and nutrient storage (Armengaud et al. 2004) (Fig. 3). However, no miRNA, plant potassium receptor/sensor, or potassium responsive *cis* element has been identified to date.

Concluding Remarks

Over the years, a number of studies have helped our understanding of the transcriptional regulation of macronutrient deficient signaling. When plants sense phosphorus limitation, several transcription factors, including PHR1, NSR1, and WRKY75, activate phosphorus starvation induced genes via binding to P1BS and/or W box *cis*-promoter elements. In the case of sulfur limited conditions, SLIM1 was identified as an essential transcription factor which controls sulfur responsive genes, including several sulfate transporters and metabolic enzymes. A MADS-box containing transcription factor, ANR1, and multiple MYB transcription factors were shown to play roles in nitrogen sensing and signaling. Cytokinins and miRNAs were also identified as important regulators of nitrogen, sulfur and phosphorus signaling. During potassium deficiency, phytohormones, such as ethylene, jasmonic acid, ABA, and auxin, were linked to modulations in the expression of downstream transcription factors. Finally, ROS were recog-

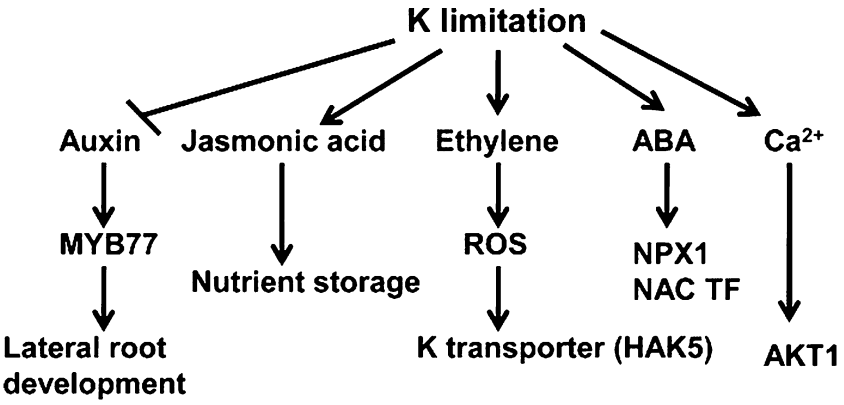

Fig. 3 Overview of the potassium-deficient dependent transcriptional regulatory components described in the text. The *arrow line* indicates the positive regulation and the *closed bar line* indicates the negative regulation

nized as key signaling molecules for potassium deficient signaling and other nutrient deficient signaling pathways.

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