

## The role of *Arabidopsis* MYB2 in *miR399f*-mediated phosphate-starvation response

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In plants, microRNA399 (*miR399*) is a major regulator of phosphate (Pi) homeostasis by way of post-transcriptional mechanisms including transcript cleavage and transcriptional repression. Although miRNA genomic organization, biogenesis, and mode of action in plants are known, the regulatory mechanisms affecting miRNAs are poorly understood. We have shown that AtMYB2 functions as a transcriptional activator for *miR399f* expression in the context of phosphate homeostasis. AtMYB2 directly binds to a MYB-binding site in the promoter of the *miR399f* precursor and regulates *miR399f* expression. In addition, AtMYB2 transcripts are induced under Pi deficiency. The overexpression of AtMYB2 affects root system architecture (RSA), indicated by suppression of primary root growth and enhanced development of root hairs. AtMYB2 and *miR399f* are expressed and localized in the same tissues under Pi limitation. This study establishes that AtMYB2 regulates Pi-starvation responses (PSR) by activating of *miR399f* transcript, suggesting that an analysis of this miRNA promoter could reveal the existence and extent of crosstalk with other signaling mechanisms.

Phosphorus (P), a macronutrient, is an essential ingredient of plant growth and propagation, and is taken up by plant roots from the soil. A deficiency of phosphorus constitutes a severely limiting factor for crop productivity all over the world.<sup>1</sup> Plants experiencing Pi-deficient conditions employ systemic mechanisms

that absorb external Pi for maintenance of Pi homeostasis. These mechanisms include change in the root systemic architecture (RSA), enhancement of Pi uptake activities, exudation of organic acids and phosphatases in order to solubilize organically and inorganically bound Pi.<sup>2-5</sup>

Recently, identification and characterization of major systemic regulators in Pi-starvation signaling and Pi homeostasis have been accomplished. Among the regulatory mechanisms are transcript levels of several miRNAs, among them *miR156*, *miR158*, *miR163*, *miR319*, *miR399*, *miR447*, *miR778*, *miR827*, *miR866* and *miR2111* that are induced by Pi limitation.<sup>6-9</sup> However, molecular mechanisms of miRNAs are unknown with the exception of *miR399*, *miR827* and *miR2111*. *miR827* regulates anthocyanin synthesis in some cross-talk between Pi-homeostasis and homeostasis of other nutrients.<sup>7</sup> *miR2111* is involved in a regulatory role for plant survival under Pi limitation by the regulation of expression of a target, *At3g27150*, which encodes a kelch domain-containing F-box protein.<sup>8</sup> Importantly, *miR399* is a regulator of long-distance regulation of shoot-to-root communication under Pi limiting conditions. One target of *miR399* is the transcript of *PHO2/UBC24* encoding the ubiquitin-conjugating E2 enzyme in *Arabidopsis*. The *PHO2/UBC24* gene is downregulated in Pi-deficiency responses, thereby activating the expression of root Pi-uptake transporters (e.g., *PHT1;8* and *PHT1;9*).<sup>10</sup> Thus, transgenic *Arabidopsis* overexpressing *miR399* were impaired in Pi remobilization and enhanced Pi accumulation in the shoots.<sup>11</sup>

**Keywords:** *Arabidopsis* MYB2, microRNA399, phosphate, phytohormones, signaling

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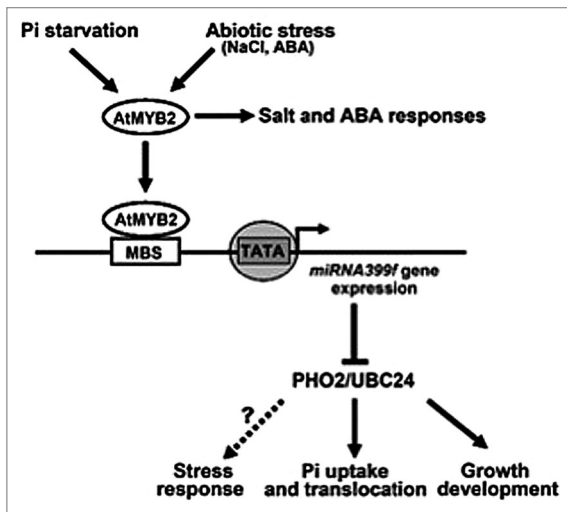
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**Figure 1.** Model proposed for AtMYB2-regulated *miR399f* expression under Pi starvation and abiotic stresses. AtMYB2 directly binds in the promoter of the *miR399f* precursor and activates *miR399f* expression. It is expected that AtMYB2 may function as a linker between the Pi homeostasis and abiotic stresses, such as NaCl and ABA or developmental regulation. MBS: MYB binding site.

The *miR399* family in *Arabidopsis* consists of six genes (*a* to *f*). All of them are strongly expressed by Pi starvation, and repress the transcript of the *UBC24* target gene. To characterize the regulatory mechanism controlling *miR399f* expression, we performed in silico analysis of the presumptive *miR399f* promoter region using the PlantCARE database. This region included various *cis*-acting regulatory elements related to biotic and abiotic-stress responses. We identified two putative MYB-binding sites (MBS, 5'-TAACTG-3') on the hypothetical *miR399f* promoter. The more distal of these MBS specifically bound to the *Arabidopsis* MYB2 transcription factor. AtMYB2 binding in the identified *miR399f* promoter led to transcriptional activation of *miR399f* expression. In addition, transcriptional expression of *AtMYB2*, like *miR399f* expression, was induced by Pi-limitation in shoot and root. Significantly, *AtMYB2* and *miR399f* were expressed in the same vascular tissues of cotyledons, leaves and roots, and their expressions were more strongly enhanced in those tissues under Pi starvation.

Several studies have provided evidence for cross-regulation between Pi-starvation responses (PSR), sugar signaling, and hormone signaling in plants.<sup>12-17</sup> Exogenous cytokinin induced Pi uptake via the regulation of Pi transporters including

*PHT1;8*, *PHT1;9*, *PHT1;4*, *PHT1;5* and *PHT3;1*. CRE1/AHK4 plays an important role in suppressing the expression of phosphate starvation induced (*PSI*) genes, suggesting cross-talk between Pi and cytokinin signaling.<sup>14</sup> Auxin and ethylene also play important roles in modulating the developmental adaptations of roots under Pi limitation.<sup>12,15</sup> Application of gibberellic acid (GA) in *Arabidopsis* showed the Pi-limited phenomena including anthocyanin accumulation, root hair growth, and root systemic architecture and regulated the expression of *PSI* genes including *AtPHT1*, *AtPHT2*, *At4* and *AtIPS1* in Pi-starvation responses (PSR).<sup>16</sup> Interestingly, ABA mediated the Pi-starvation responses (PSR) by the repression of transcriptional expression of *At4* genes encoding low-Pi-induced ribo-regulators.<sup>17</sup> These results strongly imply a connection between the Pi-starvation response and phytohormones. In addition, several transcription factors containing *PHR1*, *WRKY75*, *MYB62*, *ZAT6* and *BHLH32* have been identified as regulators in Pi-starvation responses. These transcription factors are also involved in the crosstalk between Pi-starvation signaling and signaling of phytohormones to regulate responses to Pi-limitation.<sup>13</sup>

AtMYB2 has initially been identified as a transcriptional activator of the

dehydration-responsive gene, *RD22* in ABA- and salt-stress signaling mechanisms. AtMYB2 is also involved in the cross-talk between apical dominance mechanism and auxin signaling, and it regulates cytokinin biosynthesis.<sup>18</sup> The overexpression of *AtMYB2* enhanced the expression of *miR399f*, thereby reducing the expression of *UBC24*, which serves as a target gene for *miR399f*, for Pi accumulation in the root and induced chlorosis symptoms in the leaves. In addition, *AtMYB2*-overexpressing plants showed a reduction of primary root growth under Pi-deficiency conditions and increased root hair density under Pi accumulation. Thus, these results indicate that phenotypes observed in the *AtMYB2*-overexpressing plants exhibit the same phenotypes as *miRNA399*-overexpressing plants, suggesting that *AtMYB2* is a part of a Pi signaling pathway for maintaining Pi homeostasis within plants.

Knowledge about the functions of miRNAs in plants has increased in the past few years. *miRNA399* has recently emerged as an important post-transcriptional regulator in controlling plant adaptive responses to Pi starvation. Pi starvation-induced *miR399f* promotes Pi uptake by cleavage of *UBC24* mRNA, and mediates plant growth development and stress responses (Fig. 1). However, little is known about the upstream regulatory mechanisms. The observation of *AtMYB2* to function as a transcriptional activator of *miR399f* expression is a new discovery in miRNA biogenesis for Pi homeostasis in plants. The finding suggests that *AtMYB2* may function as a linker between the Pi homeostasis and abiotic stresses, such as NaCl and ABA or developmental regulation (Fig. 1). In this context we point to a *GNATATNC* sequence that is located 84 bp upstream on the *miR399f* promoter. The *GNATATNC* element is known as a putative binding site of *PHR1*, a MYB transcription factor in Pi starvation responses (PSR).<sup>19</sup> It raises the possibility that *AtMYB2* functions as a transcriptional activator together with *PHR1* for *miR399f* expression. Also, the *miR399f* promoter region as defined by our study includes putative *cis*-acting regulatory elements related to defense-, hormone-, light- and water stress based on in silico

analysis. Therefore, further investigations into the regulation of miRNAs in plants will be necessary, especially with respect to the characterization of *cis*-regulatory elements and their transcription factors. This will provide a better understanding on the roles of miRNAs in the cross-talks between nutrient acquisition, phytohormones, and abiotic stress signaling.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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