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Publication date:
2009

Document version
Publisher's PDF, also known as Version of record

Plant Biology 2009 Final Program

Phycological
Society of America

Final program and abstracts of symposia, plenaries, minisymposia, talks, and poster presentations at Plant Biology 2009

**Joint Annual Meetings of the
American Society of Plant Biologists
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Hawaii Convention Center, Honolulu, Hawaii

Saturday July 18 thru Wednesday July 22, 2009

Transcriptional regulation of the phosphate starvation response via members of the transcription factor family GARP-CC

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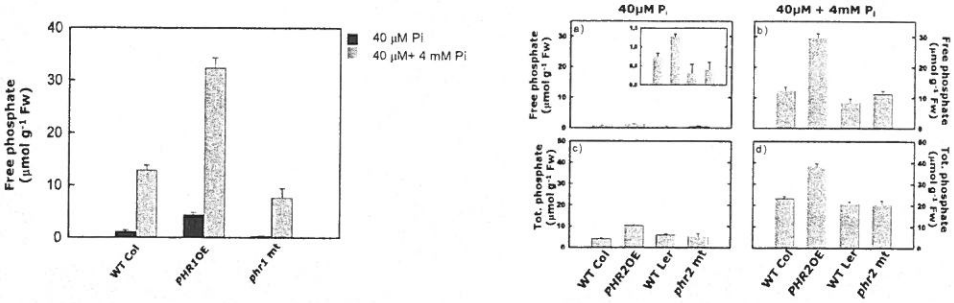
Introduction

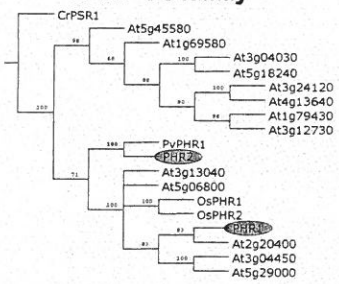
Phosphorous acquisition and homeostasis is fundamental for growth and performance in plants. Since the bioavailability of inorganic phosphate (Pi) is limited several adaptations have evolved aimed at increasing the scavenging and the use efficiency. Recently the existence of signaling networks has been demonstrated. Much of this research has been focused on the transcription factor PHR1 and its signaling cascade, including microRNA399, IPS/At4 and PHO2. In *Arabidopsis* PHR1 (At4g28610) belongs to a MYB-transcription factor gene family with 15 members. The function of the other members is largely unknown. Here we show new results concerning one of the members, PHR2 (At2g01060). We also present microRNA microarray data showing that, in addition to miR399d, other miRNAs are involved in regulation of the phosphate starvation response.

The expression level of both *PHR1* and *PHR2* affects the phosphate status of the plant



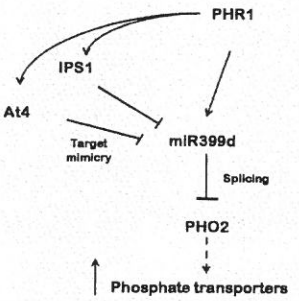
A clear correlation between expression of both transcription factors and P-uptake was observed, such that increased expression resulted in increased amount of phosphate in shoots.

Phylogenetic relationship of the GARP-CC family



Strict consensus of the 2 most parsimonious trees with bootstrap support plotted at respective internodes (1000 replications)

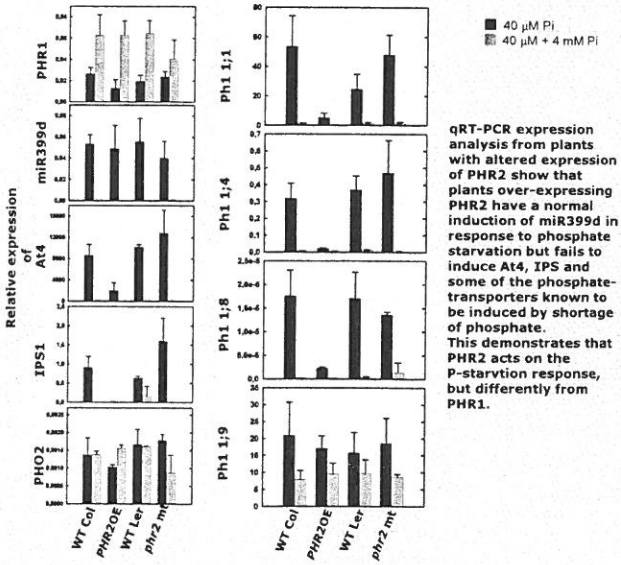
The PHR 1 signaling pathway



The transcription factor PHR1 has been shown to enhance the expression of miR339d in response to shortage of Pi. The miR posttranscitionally cleaves the mRNA of PHO2 and thus relase the negative regulation of downstream genes (1).
The pathway is also subjected to target mimicry, where the noncoding RNA of IPS1 and At4 binds and sequesters miR399d (2).

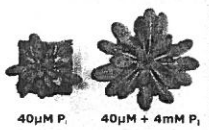
(1) Bari *et al. (2006) Plant Physiol* 141: 988-999
(2) Franco-Zorrilla *et al.* (2007) *Nature Genet* 39: 1033-1037.

Over expression of PHR2 affects the expression of the genes involved in the PHR1 signaling pathway

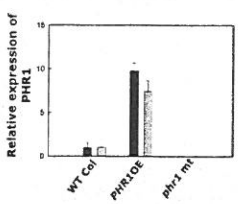


qRT-PCR expression analysis from plants with altered expression of PHR2 show that plants over-expressing PHR2 have a normal induction of miR399d in response to phosphate starvation but fails to induce At4, IPS and some of the phosphate-transporters known to be induced by shortage of phosphate.
This demonstrates that PHR2 acts on the P-starvtion response, but differently from PHR1.

Plant material

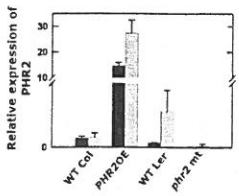


All genotypes were grown on Rockwool and supplied with a limited amount of Pi (40µM) for 5 weeks after which half of the plants were resupplied with Pi (4mM) for 1 week. Except for experiments involving roots in which case the plants were grown hydroponically with either 1 µM or 40µM Pi.



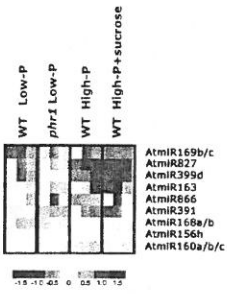
Transgenic plants with altered expression of PHR1 and PHR2 was used.

The alterations in expression was consistent regardless of P status of the plants.



The PHR1 overexpressor (*PHR1*OE), *phr1* mutant and the *PHR2* overexpressor (*PHR2*OE) was made in Columbia background while the tDNA *phr2 mutant* is in a Landsberg background.

MicroRNA microarray-there is more to it than miR399d



By employing a novel Arabidopsis microarray platform from Exiqon*, with a unique sensitivity and specific detection of mature miRNA the induction of miRNA 399d in response to limited phosphate supply could be corroborated. The expression of miRNA 399d was strongly induced in WT plants starved for phosphate while the induction was attenuated in the phr1 mutant, demonstrating the necessity of the transcription factor PHR1 for the complete induction. Furthermore we were able to identify novel phosphate responsive miRNAs demonstrating that the miRNA-based regulation of the phosphate starvation response goes beyond the PHR1-miRNA399 loop.

* Exiqon A/S, www.exiqon.com

The MYB-related transcription factor PHR1 is known to be involved in the regulation P-dependent gene expression, and overexpression of PHR1 in Arabidopsis leads to accumulation of phosphate in shoots. The regulatory mechanism involves miR399

Conclusions:

- An expression study using novel microarrays demonstrate that several other miRNAs can be involved in the P-starvation response.
- A homolog to PHR1, here termed PHR2, is also shown to be involved in P-dependent gene regulation.
- Overexpression of PHR2 result in accumulation of more phosphate in shoots.
- Overexpression of PHR2 leads to dramatic changes in expression for the regulatory genes At4 and IPS1 and for several genes encoding P-transporters.