Arbuscular Mycorrhizal Symbiosis and Phosphate Homeostasis: Systemic Induction of miRNA399 in Mycorrhizal *Medicago truncatula* plants

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Abstract

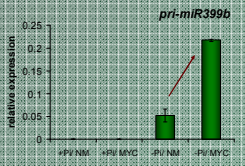
The formation of the symbiotic association between plants and arbuscular mycorrhizal (AM) fungi increases the phosphate (Pi) availability and is probably regulated by key components of the plant Pi homeostasis pathway.

MicroRNAs (miRs) of the 399-family are systemic Pi-starvation signals important for maintenance of Pi-homeostasis in *Arabidopsis thaliana*, and might also qualify as signals regulating AM development in response to Pi availability. MiR399s could either represent the systemic low Pi-signal promoting or required for AM formation, or they could act as counter players of systemic Pi-availability signals that suppress AM symbiosis.

To test either of these assumptions, we analyzed the miR399-family in the AM-capable model plant *Medicago truncatula*.

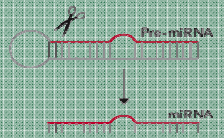
shoot

Expression analysis of miR399 primary transcripts



Despite of increasing levels of Pi in the roots, 7 members of the 399-gene family are stronger induced in leaves of mycorrhizal plants (e.g. pri-miR399b)[1]

3



miRNA precursor

miR399 transcription → processing and maturation of miR399 → **mature miR399**

xylem

putative systemic myc signal

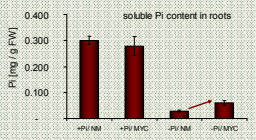
phloem

translocation of mature miR399 to the roots[2]

root

1

Pi↑ ← **AM symbiosis**



Pi availability is locally improved in mycorrhizal roots

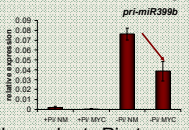
NM: non mycorrhizal roots

MYC: *G. intraradices* inoculated roots, 5 wpi

2

miR399 transcription

Increasing Pi-level lead to decreasing expression levels of pri-miR399...

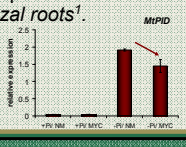


relative expression to the reference gene *MtEf1-α*

NM: non inoculated

MYC: inoculated with Glomus intraradices, 5 wpi

...and PHO2 independent Pi-stress marker genes such as *MtPID in mycorrhizal roots*[1].

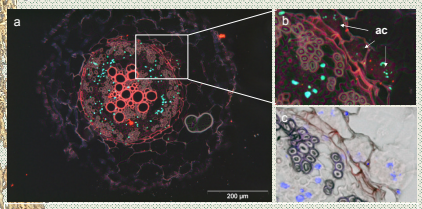


relative expression to the reference gene *MtEf1-α*

NM: non inoculated

MYC: inoculated with Glomus intraradices, 5 wpi

Higher abundance of mature miR399 signals in arbuscule containing cells of mycorrhizal roots



In situ hybridization with miR399b LNA detection probes. *Medicago truncatula* root cross section 10 µm. Visualization of probe signals using Fast Red staining, nuclei were stained with DAPI blue. a) and b) root cross section treated with miR399b LNA detection probe, c) root cross section treated with LNA scramble probe as negative control.(ac) arbuscule containing cell

On single cell level:
***MtPho2* transcript is significantly down-regulated in arbuscule containing cells**

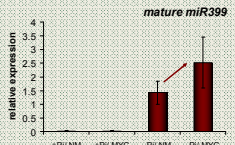
Sampling of arbuscule/adjacent single cells (LMD) → Affymetrix GeneChip® *Medicago* genome array

log_2-fold change (arb/adj) **- 3,8834**

mature miR399

4

Accumulate to higher levels in mycorrhizal roots [1]



relative expression to the reference gene *MtEf1-α*
Amplification of mature miR399 using stemloop-qRT-PCR

NM: non inoculated

MYC: inoculated with Glomus intraradices, 5 wpi

MtPho2 ⇢ **Target proteins**

***MtPho2* transcript cleavage leads to the activation of Pi-starvation response**

- **Support of AM mediated Pi uptake and translocation**
- **Sustain AM symbiosis despite of increasing Pi availability**

Summary

Pi-depleted plants showed increased expression of mature miR399 and pri-miR399s, and unexpectedly, seven of the fifteen pri-miR399 species were higher in leaves of mycorrhizal plants than in leaves of non-mycorrhizal plants. Expression levels of *MtPho2* remained low whereas PHO2-independent Pi-stress marker transcript levels indicate the increasing Pi status in mycorrhizal roots. Hence, an AM symbiosis-related signal appears to increase miR399 expression and decrease PHO2 activity. On single cell level, we could detect miR399 signals concentrating in arbuscule containing cells which is further supplemented by a significant decrease of *MtPho2* transcripts in those cells.

These results indicate that the miR399 might act as systemic signal keeping Pi starvation responses on a high level to sustain AM symbiosis despite of locally improved Pi availability in mycorrhizal roots.

Ref.erences. [1] Branscheid *et al.*, Expression pattern suggests a role of miR399 in the regulation of the cellular response to local Pi increase during arbuscular mycorrhizal symbiosis, MPMI Vol. 53, pp. 915-926, 2010

[2] Pant *et al.*, MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis, Plant Journal Vol. 53, pp. 731-738, 2008