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Spatiotemporal Phosphorus Deficiency Responses in B73 and Root Hairless *rth3* Maize Mutants

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Introduction

- The project aims to characterize and identify the molecular and physiological role of root hairs in an early developmental stage of *Zea mays.*
- Comparison of B73 maize wildtype and it's isogenic root hairless mutant *rth3*.
- Phosphorus (P) being an essential nutrient, focus will be held on P-deficiency responses.
- To investigate the function of root hairs in P-uptake and signalling, B73 root hairs will be isolated and expressed P-transporter will be analysed as expressional markers for P-deficiency.
- Root phenotyping using root system architectural (RSA) traits to reveal differences between B73 and *rth3* RSA during early growth stages after growing under P-deficiency.

Research Questions

- Is it possible to extract RNA from in soil grown root hairs and to detect P-deficiency in a molecular way by gene marker analysis?
- How do the B73 and *rht3* maize genotypes differ physiologically in a P-deficient environment?

Conclusion

- We succeeded to extract root hair RNA from B73 seedlings grown on loamy soil and established gene transcript markers reacting to P-deficiency.
- Compared to B73, *rth3* does not form a longer root system in response to P-deficiency.

How to Extract RNA from Root Hairs

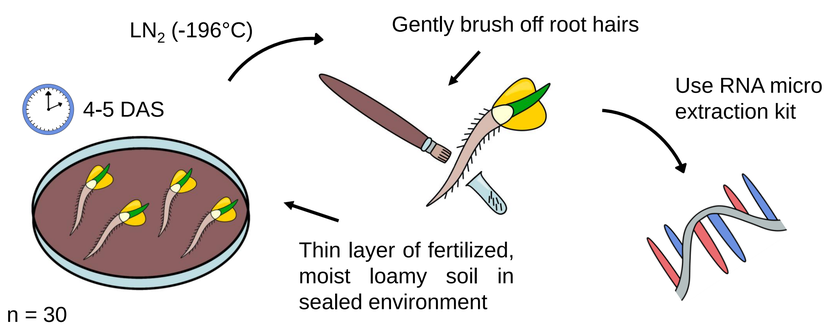

Figure 1: Collection of root hair samples for mRNA extraction. DAS: days after sowing; LN_2: liquid Nitrogen.

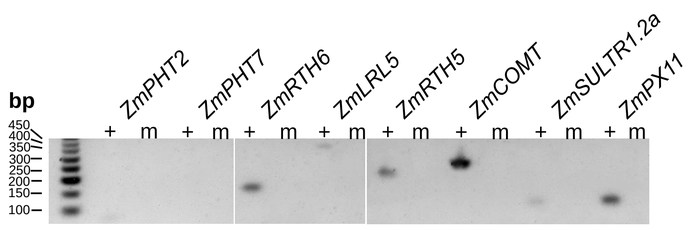

Figure 2: Gel electrophoresis of PCR results after converting B73 root hair mRNA into cDNA. P-transporter: *ZmPHT2* (82 bp), *ZmPHT7* (93 bp). Root hair marker genes: *ZmRTH6* (151 bp), *ZmLRL5* (336 bp), *ZmRTH5* (207 bp). Exodermal marker gene: *ZmCOMT* (239 bp). Epidermal marker genes: *ZmSULTR1.2a* (105 bp), *ZmPX11* (110 bp). With cDNA (+), mock (m).

- PCR shows that RNA extraction of B73 root hairs has been successful (Fig. 2).
- Exodermis, epidermis and root hair marker genes, as well as two P-transporters have been detected.
- Exclusive isolation of root hairs is not possible, as it is inevitable to remove parts of the exodermis and epidermis as well.

Expressional Markers for P Deficiency

To identify P deficiency marker genes, the P-transporter *ZmPHT2* and *ZmPHT7* were analyzed in 47 days after germination (DAG) old maize root tips using qPCR.

- **Growth conditions:** "Loamy soil" and "Fruhstorfer Nullerde" (FN), fertilized with P (plus P, 180 mg P/kg soil) and without P (minus P, 0 mg P/kg soil).
- **Results:** Expression of *ZmPHT2* and *ZmPHT7* are not significantly different for the loamy soil treatment (Fig. 3 a), b)) but are significantly higher in the FN between plus P and minus P conditions (Fig. 3, c), d)), showing that *ZmPHT2* and *ZmPHT7* react to P-deficiency by being upregulated.

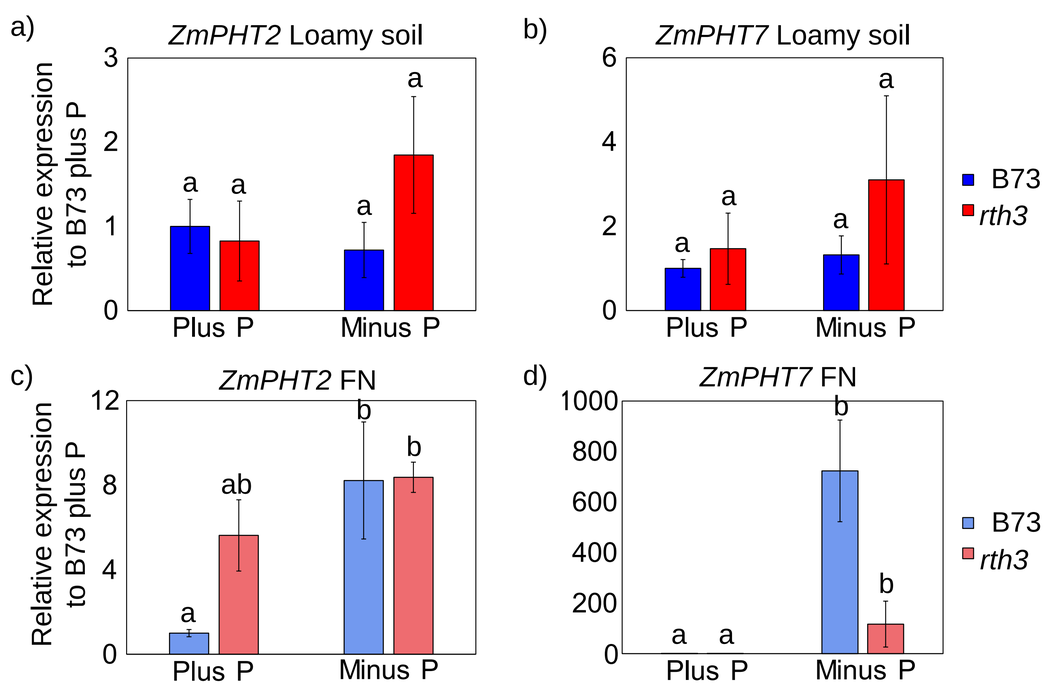

Figure 3: Relative expression of *ZmPHT2* and *ZmPHT7* in maize root tips grown in in loamy soil (a, b) or FN (c, d) measured by qPCR. Statistical analysis done with ANOVA and Tukey test. Significance means $p < 0.05$. Error bars showing SD. n = 3

B73 and *rth3* RSA Responses to P Deficiency

RSA traits were measured from 5 DAG old B73 and *rth3* seedlings growing under plus P (180mg P/kg soil) and minus P (0 mg P/kg soil) conditions.

Results: No difference in the primary root length between P treatments and neither between the genotypes in the loamy soil (Fig. 4 a)) nor in the FN (Fig. 4 c)), but a slight increased *rth3* primary root length in minus P conditions was measured. Although not significant different, this could show a reaction to minus P conditions. Seminal root length was significantly smaller in *rth3* compared to B73 (Fig. 4 b), d)). No adaptations trough an increased primary and seminal root length were observed to minus P conditions for *rth3*.

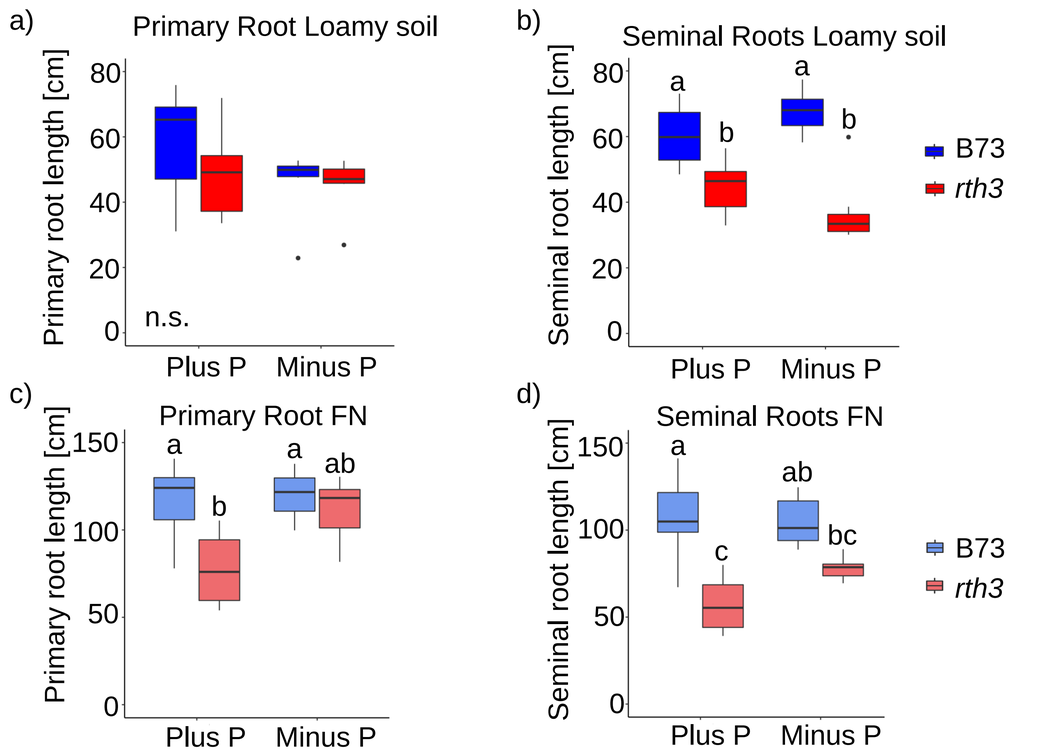

Figure 4: Root length of the primary root grown in a loamy soil (a) and FN (c) as well as the total length of seminal roots grown in loamy soil (b) and FN (d). Statistical analysis were done with two sided ANOVA and Tukey Test. Significance means $p < 0.05$. n = 6-10. Measurements were made using WinRHIZO (Regent Instruments Inc.) software.

Outlook

- RNASeq of B73 root hairs, B73 roots without root hairs and *rth3* roots grown on P-deficient, water limited and combined P-deficient and water limited conditions (loamy soil or FN) to understand transcriptome responses to adverse seedbed conditions.
- Root and shoot phenotyping of maize seedlings (3 and 5 DAG) exposed to water limitation and combined water limitation and P-deficiency (loamy soil, FN and sandy soil) to investigate physiological adaptations to adverse seedbed conditions.
- Apoplastic barriers respond to nutrient deficiency and water limitation. Therefore, apoplastic compounds of the radicle will be investigated using light microscopy.

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