

Plant age and soil texture rather than the presence of root hairs cause differences in maize resource allocation and root gene expression in the field

Plants

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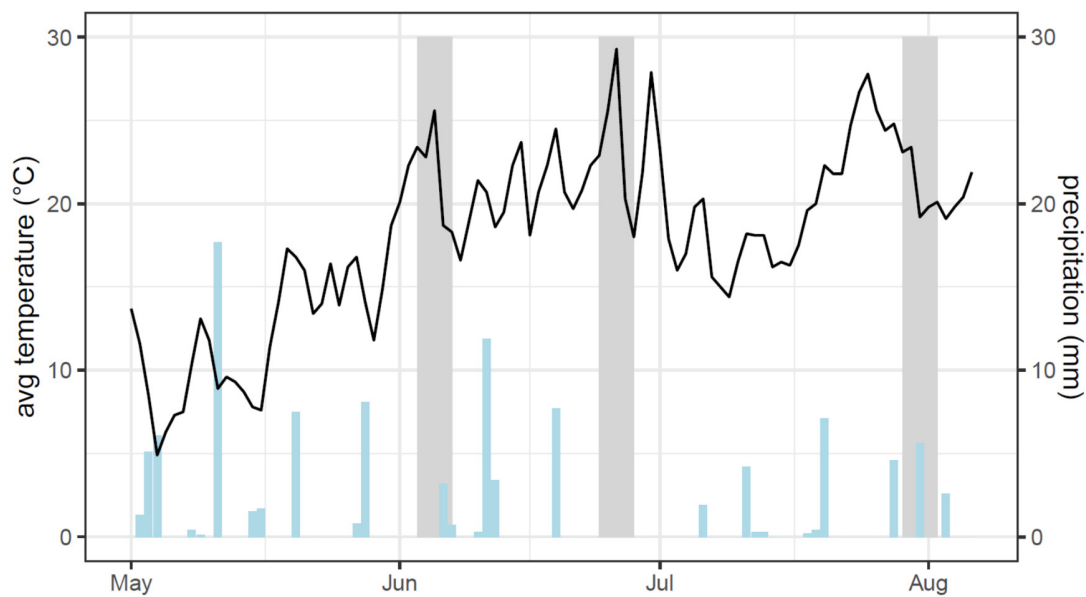


Figure S1. Above-ground daily average temperature and precipitation data for the months May to August at the maize sampling site in Bad Lauchstädt, Germany. Sampling time points (BBCH14: first week of June, BBCH19: last week of June, BBCH59: last week of July) are highlighted with grey bars. Weather data were collected from the DWD Open Data archive (opendata.dwd.de/climate_environment/CDC/observations_germany/climate/ - Location ID 02878)

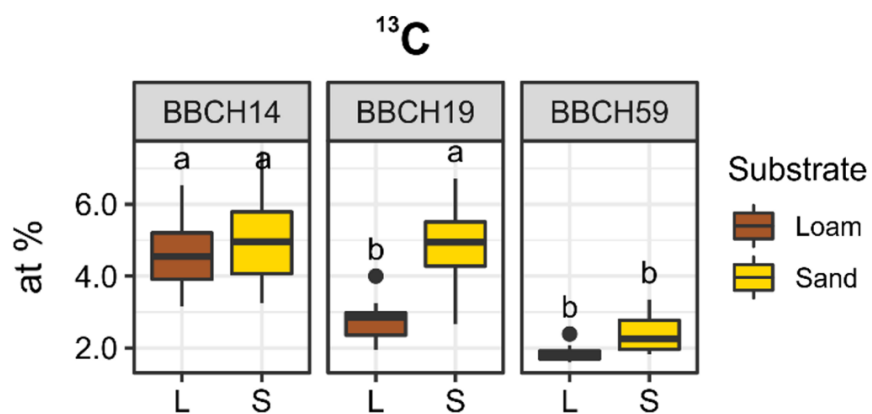


Figure S2. Recently incorporated carbon from air (¹³C atomic %) in the shoot remainder. Differences between treatments (P<0.05) across all stages according to ANOVA and Tukey's HSD test are indicated by different letters. L: loam, S: sand

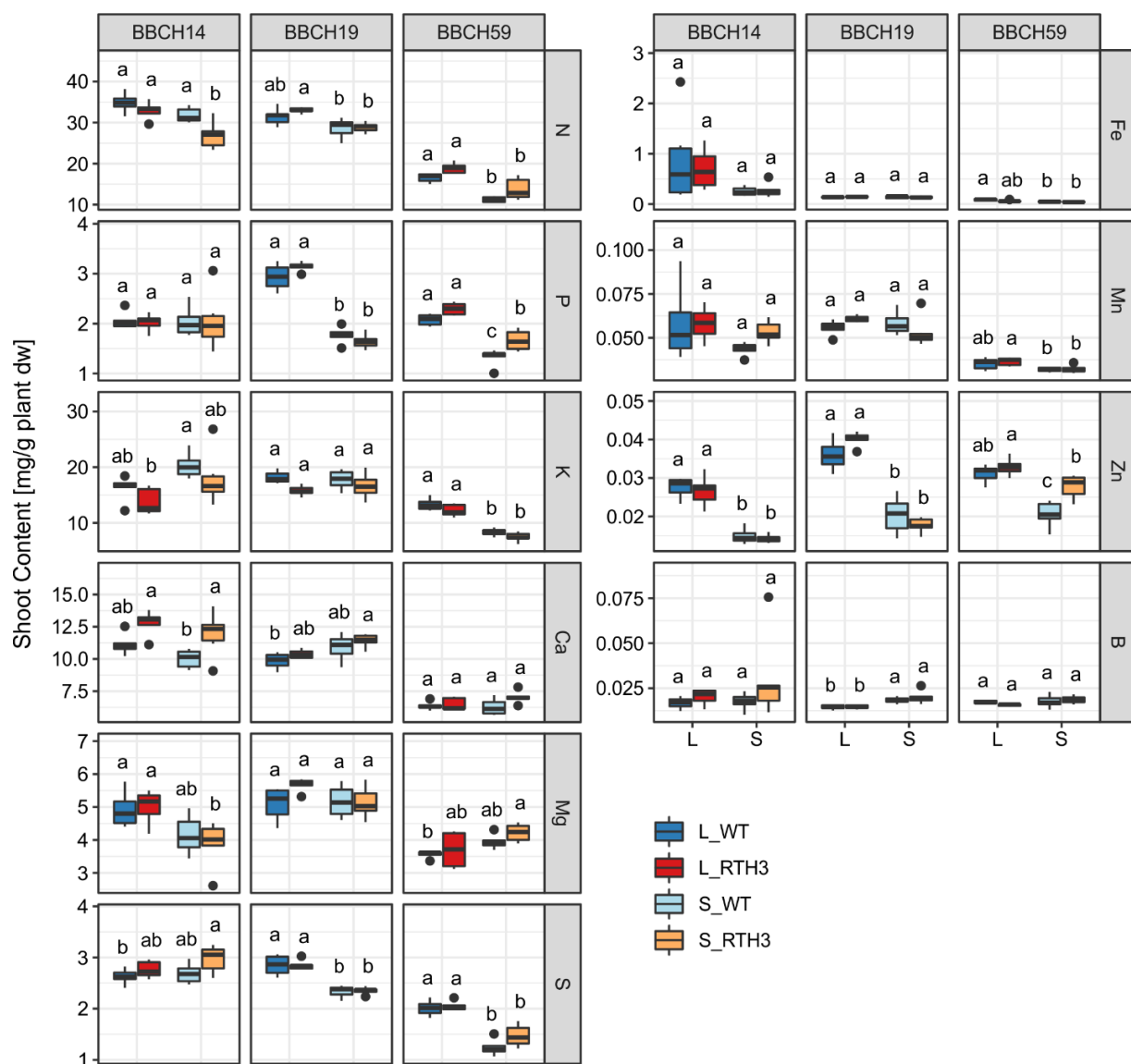


Figure S3. Mineral element concentration (mg/g plant dry weight) in the remaining shoot (RS). The amounts of the mineral nutrients are given by mg per g plant dry weight (dw). Note the different scales for the different mineral nutrients. Differences between treatments ($P < 0.05$) within one stage according to ANOVA and Tukey's HSD test are indicated by different letters. L, loam; S, sand; WT, wild type; RTH3, root hair mutant

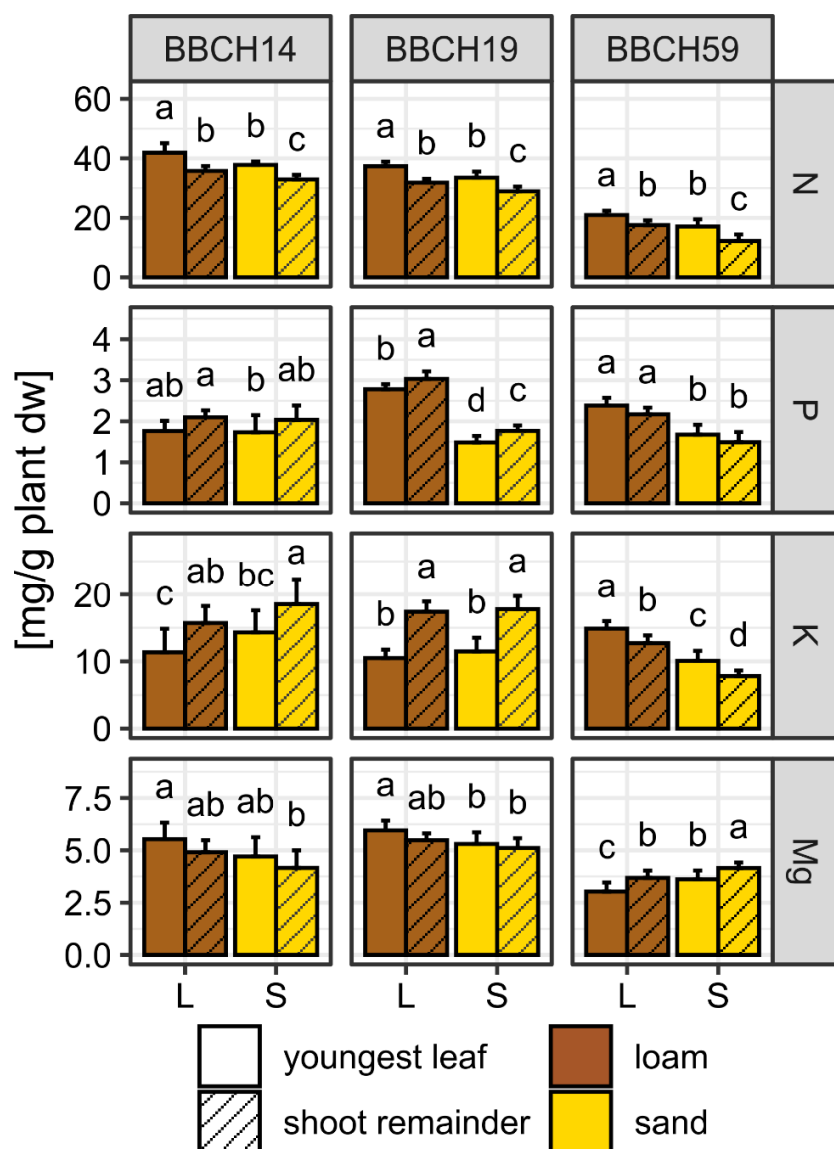


Figure S4. Comparison of mobile element concentration in the youngest leaf (YL) and the remaining shoot (RS) for mobile elements N, P, K, and Mg. The amounts of the mineral nutrients are given in mg/g plant dry weight (dw). Note the different scales for the different mineral nutrients. Differences between treatments ($P < 0.05$) within one stage according to ANOVA and Tukey's HSD test are indicated by different letters. L, loam; S, sand

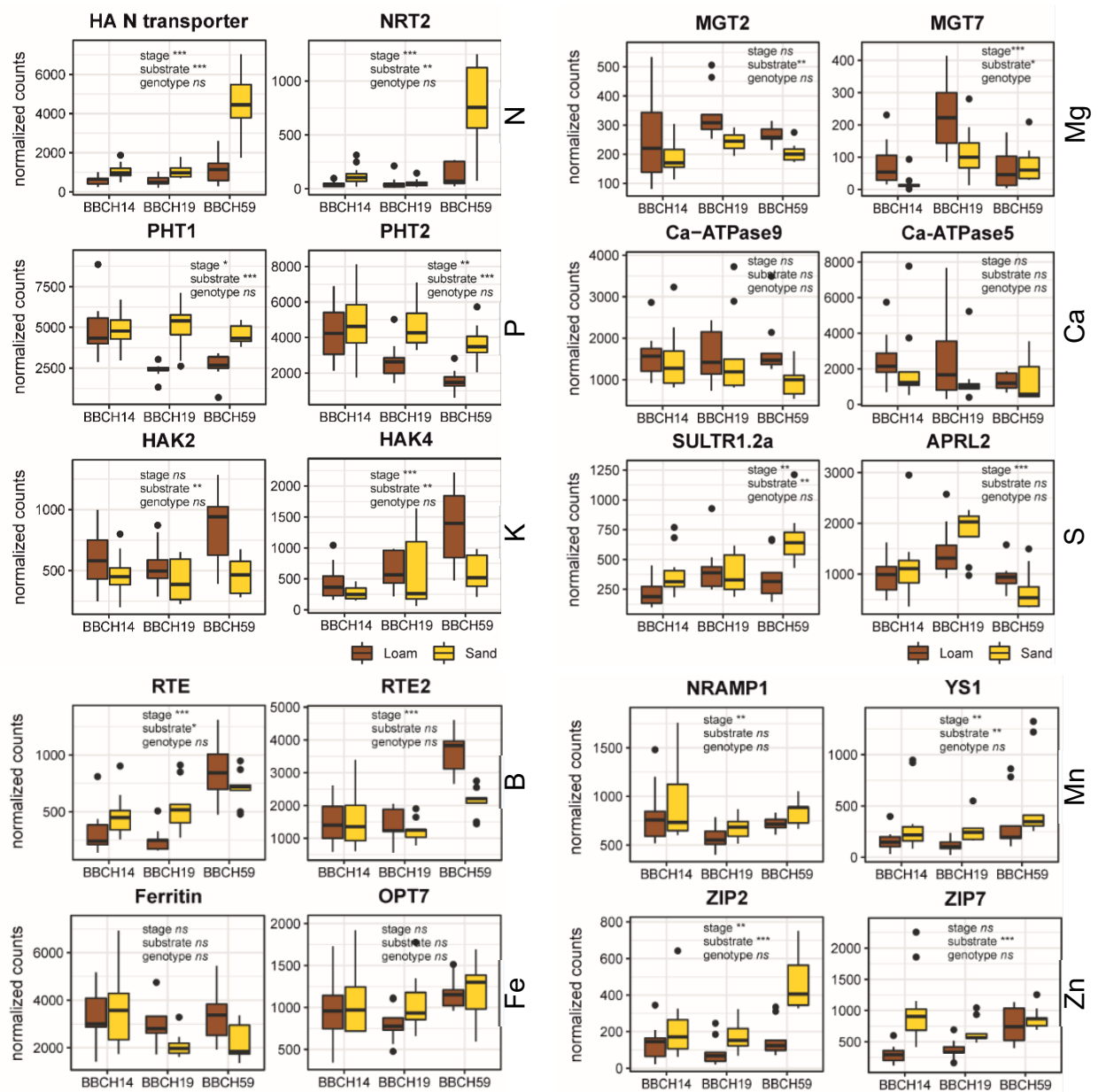


Figure S5. Mineral nutrient transporter expression in maize roots over time and substrate. Treatments with different letters are significantly different (ANOVA, Tukey's HSD test, $P < 0.05$). Asterisks indicate significant impact of grouping factor (Likelihood ratio test, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns not significant). L, loam; S, sand. The presence of root hairs did not affect the transporter gene expression levels.

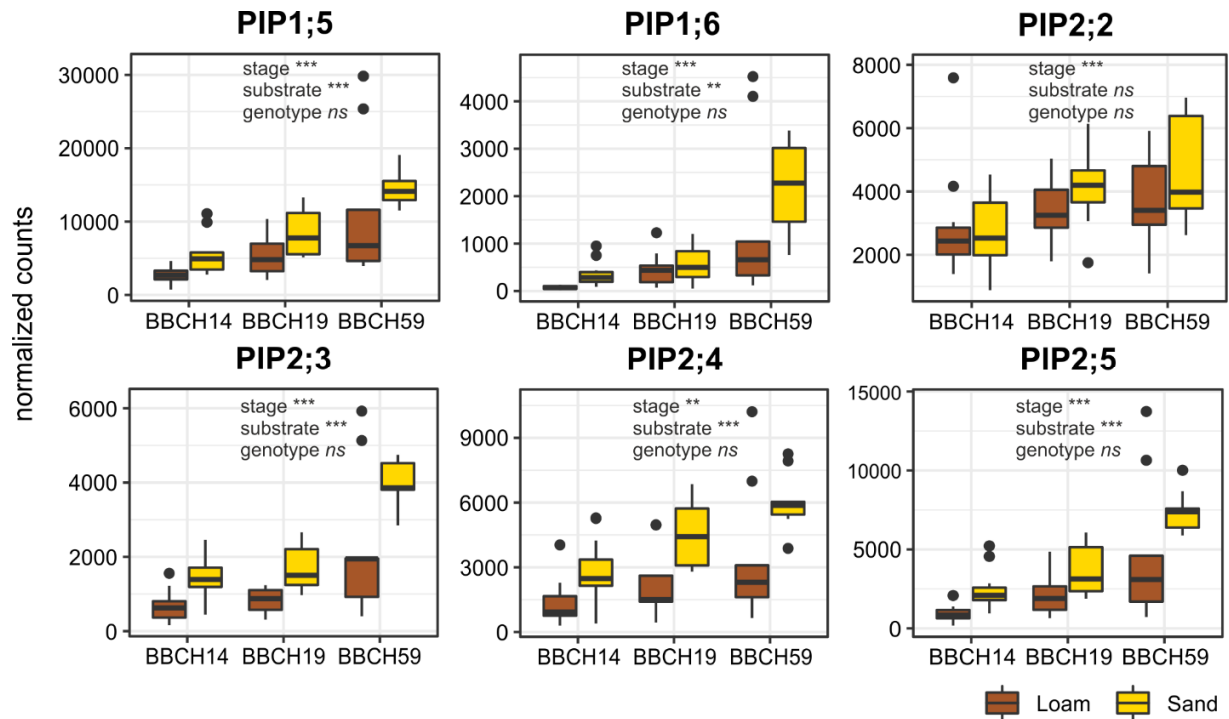


Figure S6. Root plasma membrane aquaporin expression in relation to plant age and substrate. Root gene expression levels (normalized counts) for selected related aquaporin genes. RNA sequencing analysis of mRNA in roots of maize. Data are represented as DESeq2 normalized counts. Due to low variation between the *rth3* and wild type maize the data from the two genotypes were combined for this analysis. Asterisks indicate significance of the respective experimental factor (Likelihood ratio test, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns not significant)

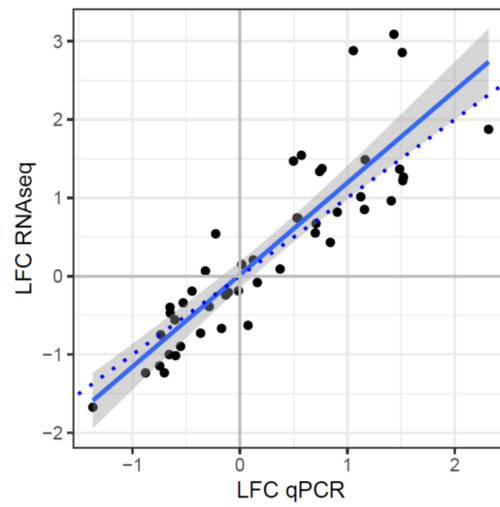


Figure S7. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) confirmation of differential gene expression. Comparison of log₂ fold changes (LFC) for RNAseq and qRT-PCR data. The plotted data points were calculated from the sand vs loam contrast for 41 surveyed qPCR array genes. The blue solid line represents a linear regression model, whereas the dotted line represents the ideal outcome with equal values for RNAseq and qPCR data. LFC values from RNAseq data were calculated with the DESeq package (Wald test), and LFC values from qPCR data were derived by using the $\Delta\Delta\text{CT}$ method. GAPDH, Actin1 and EF-1a served as reference genes of qPCR analysis

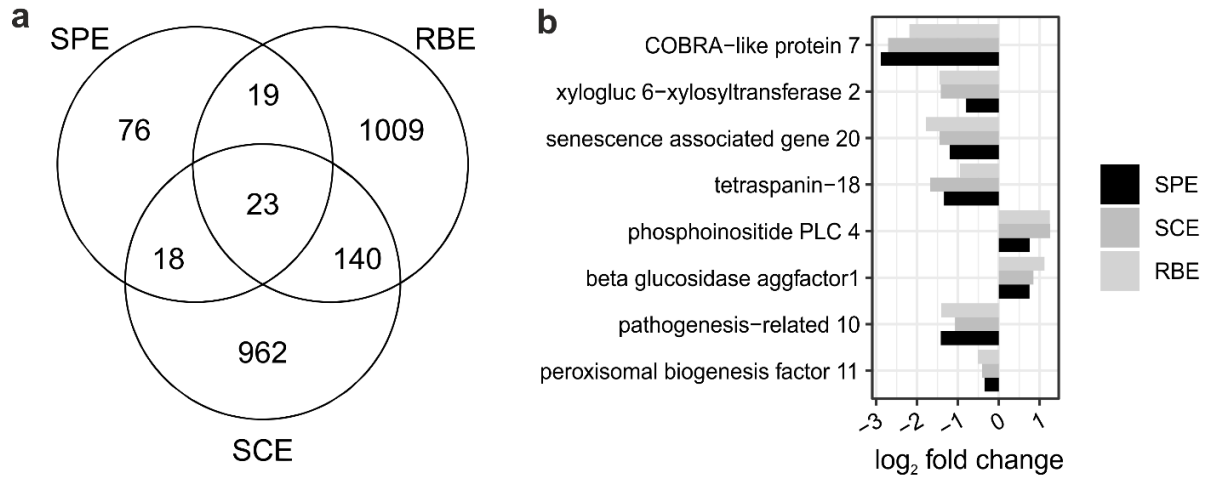


Figure S8. Differential gene expression between root hairless *rth3* and wild type maize in different experiments. Differential expression (RNAseq data) between *rth3* and wild type was assessed for three different experiments. SPE: field-scale, developmental stage: BBCH14-BBCH59, mix of total roots from 0-20 cm topsoil. SCE: lab-scale, soil column, developmental stage: BBCH14, total roots from 1.4 cm soil slices along the soil column. RBE: lab-scale, rhizobox, developmental stage: 10 days after germination, primary roots. (a) Venn diagram showing the overlap of differentially expressed genes by root hair formation in the three experiments. (b) Comparison of expression level changes (\log_2 fold changes) of eight genes between the three experiments (taken from the overlap in a)). The displayed genes related to processes in cell wall organization, signaling and stress response

Table S1. Effect of substrate and maize genotype on the concentrations of macro- and micronutrients in maize shoot. Significance of the experimental factor was determined by ANOVA and Tukey's HSD test. Level of significance: *** P<0.001, ** P<0.01, * P<0.05, *ns* not significant

| | | Effect significance during growth stages | | |
|----------------|--------------------|--|--------|--------|
| Element | Factor | BBCH14 | BBCH19 | BBCH59 |
| Macronutrients | | | | |
| Ca | Substrate | ns | ** | ns |
| Ca | Genotype | ** | ns | * |
| Ca | Substrate:Genotype | ns | ns | ns |
| K | Substrate | ** | ns | *** |
| K | Genotype | ns | * | * |
| K | Substrate:Genotype | ns | ns | ns |
| Mg | Substrate | ** | ns | ** |
| Mg | Genotype | ns | ns | ns |
| Mg | Substrate:Genotype | ns | ns | ns |
| N | Substrate | *** | *** | *** |
| N | Genotype | ** | ns | ** |
| N | Substrate:Genotype | ns | ns | ns |
| P | Substrate | ns | *** | *** |
| P | Genotype | ns | ns | *** |
| P | Substrate:Genotype | ns | * | ns |
| S | Substrate | ns | *** | *** |
| S | Genotype | * | ns | * |
| S | Substrate:Genotype | ns | ns | ns |
| Micronutrients | | | | |
| B | Substrate | ns | *** | ns |
| B | Genotype | ns | ns | ns |
| B | Substrate:Genotype | ns | ns | ns |
| Fe | Substrate | * | ns | *** |
| Fe | Genotype | ns | ns | * |
| Fe | Substrate:Genotype | ns | ns | ns |
| Mn | Substrate | ns | ns | ** |
| Mn | Genotype | ns | ns | ns |
| Mn | Substrate:Genotype | ns | * | ns |
| Zn | Substrate | *** | *** | *** |
| Zn | Genotype | ns | ns | *** |
| Zn | Substrate:Genotype | ns | * | * |

Table S2. Effect of substrate and maize genotype on the total amounts of macro- and micronutrients in maize shoot. Significance of the experimental factor was determined by ANOVA and Tukey's HSD test. Level of significance: *** P<0.001, ** P<0.01, * P<0.05, *ns* not significant

| | | Effect significance during growth stages | | |
|----------------|--------------------|--|--------|--------|
| Element | Factor | BBCH14 | BBCH19 | BBCH59 |
| Macronutrients | | | | |
| Ca | substrate | ns | *** | ** |
| Ca | genotype | ** | *** | *** |
| Ca | substrate:genotype | ns | ns | ns |
| K | substrate | ns | *** | *** |
| K | genotype | *** | *** | *** |
| K | substrate:genotype | ns | ns | * |
| Mg | substrate | * | *** | *** |
| Mg | genotype | *** | *** | *** |
| Mg | substrate:genotype | ns | ns | ns |
| N | substrate | ns | *** | *** |
| N | genotype | *** | *** | ** |
| N | substrate:genotype | ns | * | ns |
| P | substrate | ns | *** | *** |
| P | genotype | ** | *** | ** |
| P | substrate:genotype | ns | * | * |
| S | substrate | ns | *** | *** |
| S | genotype | ** | *** | ** |
| S | substrate:genotype | ns | * | * |
| Micronutrients | | | | |
| B | substrate | ns | *** | ** |
| B | genotype | ns | *** | *** |
| B | substrate:genotype | ns | ns | ns |
| Fe | substrate | * | *** | *** |
| Fe | genotype | ns | *** | *** |
| Fe | substrate:genotype | ns | ns | * |
| Mn | substrate | * | *** | *** |
| Mn | genotype | ** | *** | *** |
| Mn | substrate:genotype | ns | ns | ns |
| Zn | substrate | *** | *** | *** |
| Zn | genotype | *** | *** | ** |
| Zn | substrate:genotype | * | * | * |

Table S3. Expression of mineral element transporters, acid phosphatases and plasma membrane aquaporins by stage, substrate, and genotype (file Table S3.xlsx)

Table S4. Numbers of differentially expressed genes between the treatments. Differential gene expression was identified by Wald tests in DESeq2. Multiple testing correction was performed using Benjamini-Hochberg adjustment of p-values. Genes expression differences with $P < 0.05$ and a two-fold difference in gene expression were considered as significant. Treatments refer to a contrast of condition1 vs condition 2 (baseline). LFC, log₂ fold change

| treatment | up-regulated | down-regulated |
|----------------------|---------------------|-----------------------|
| BBCH19 vs BBCH14 | 789 | 752 |
| BBCH59 vs BBCH19 | 743 | 586 |
| BBCH59 vs BBCH14 | 1187 | 1350 |
| sand vs loam | 780 | 568 |
| rth3 vs WT | 76 | 21 |
| BBCH14: sand vs loam | 283 | 390 |
| BBCH19: sand vs loam | 800 | 444 |
| BBCH59: sand vs loam | 2218 | 997 |

Table S5. Differentially expressed genes by stage, substrate, and genotype (file Table S5.xlsx)

Table S6. Enriched GO terms for differentially expressed genes by stage and substrate (file Table S6.xlsx)

Table S7. qPCR gene expression in relation to stage, substrate and depth (file Table S7.xlsx)

Table S8. Differentially expressed genes (DEG) by root hair genotype (*rth3* vs WT) in three independent experiments (file Table S8.xlsx)

Table S9. Primers for RT-qPCR analysis. Primers were constructed for maize genes associated with mineral nutrient or water acquisition, root exudation, stress and immunity. Selection was based on their expression level in the field and laboratory experiments and differential gene expression between the substrates loam and sand, determined by RNA sequencing

| gene_id | primer sequence (5' - 3') | direction | description |
|----------------|---------------------------|-----------|--|
| Zm00001d033862 | GTCAAGGGCGTGCTCATC | forward | ACC synthase6 |
| | AAGTCCACCAGCATCTCCAG | reverse | |
| Zm00001d003712 | ACCACCAACGACGACGAATA | forward | ABA stress ripening3 |
| | CGGGAGGAGCCGTACTCT | reverse | |
| Zm00001d019406 | TGAAAGGGTACAGCAACTCCG | forward | Acidic endochitinase |
| | ACCGACCCAGCTATCAGGAA | reverse | |
| Zm00001d026102 | CAGCAGCTCACCAAACCACT | forward | Al-activated malate transporter 10 |
| | ACGAGAAGAGACACACCACC | reverse | |
| Zm00001d003749 | AATCTGTCGGTACCCTTGCG | forward | Al-activated malate transporter 8 |
| | AGCGTTGTTCTCATGGTCGT | reverse | |
| Zm00001d048709 | CTGAGGAGGCTGGAGGAGTAT | forward | benzoxazinless1 |
| | TTTATTGGTCGGTCGTCTTCTC | reverse | |
| Zm00001d043988 | CAGAGCAGCGACATCCAGAC | forward | Chitinase chem5 |
| | GTTGTCCACAGGTAGTCGG | reverse | |
| Zm00001d017292 | ATCCCCCTTTCTGCTCCTGT | forward | Defensin-like protein 6 |
| | CTCTGTACTCACTGGCTCGC | reverse | |
| Zm00001d035129 | GATGCACAGGGAGGGATCT | forward | Disease resistance RPP13-like 4 |
| | TGCCTCGTCTTATTGGTCTCAG | reverse | |
| Zm00001d025050 | AGAAACCGGTCACATTAGCC | forward | Ethylene receptor homolog2 |
| | TGAGAACCGTCAGGCTCTTT | reverse | |
| Zm00001d002762 | GACGACATGGTGGTGTTCGG | forward | Ethylene-responsive TF2 |
| | GGAGTAGTAGGAGTCGGGGG | reverse | |
| Zm00001d042611 | GCGGCCTGGTGTTCTTTTC | forward | Gibberellin 20-oxidase3 |
| | TGAACTATCCATCGTCCATAGG | reverse | |
| Zm00001d041656 | CACCACCAACGCCAGTAGTA | forward | Jasmonate-induced protein |
| | TTGACAGCATCGACCGACAT | reverse | |
| Zm00001d033623 | GCACGAGACGGACCATGTAT | forward | Lipoxygenase |
| | GGAGCACTGCGAAACGACTA | reverse | |
| Zm00001d035115 | TCAATGGTCCTTGTGCCGT | forward | MATE1 |
| | GCCGTCCCTATCCTCCAAAA | reverse | |
| Zm00001d028815 | CAGCTGGACTGTTGAGATCG | forward | Pathogenesis-related protein 10 |
| | GTGTGCCAGTCCATCACG | reverse | |
| Zm00001d048947 | ACCTTCACITTTCTCCTGCT | forward | Pathogenesis-related protein3 |
| | ACACCGTCTCGTAGTCCAGG | reverse | |
| Zm00001d011080 | TGATCCTCAAGGACAAGCCG | forward | Protease inhibitor; Proteinase inhibitor |
| | AAGATGCGGACACGGTTAGG | reverse | |
| Zm00001d012660 | CCGAAGTGCTCTATGCGAGT | forward | NPR1 |
| | TGCAACCGAAGGTCTCAGTG | reverse | |
| Zm00001d027854 | TGTGGCTATGCGGTCTATTG | forward | sucrose transporter1 |
| | CAGTGCAGGAGGTGGAAGGA | reverse | |
| Zm00001d041067 | TTGAGACAGCAGAGGACACT | forward | SWEET13c |
| | CGAATGCCAACAGTTGCAGA | reverse | |
| Zm00001d015905 | GCGCGACTTGTTATTGGTGT | forward | SWEET4a |
| | GAGAGACCGTGTGATGCGAT | reverse | |
| Zm00001d041111 | AGGAGTTCTTCTTCGTCGCA | forward | transporter of mugineic acid1 |

| | | | |
|----------------|---------------------------|---------|--------------------------|
| Zm00001d051872 | GCACCAAGAAAACCAGCGTA | reverse | |
| | CACGTGGTCATCATCAGG | forward | PIP1;5 |
| | CGTATGCTGCATGGTTGCT | reverse | |
| Zm00001d051174 | AGTACGTGCTGAGAGCCAGC | forward | PIP2;3 |
| | CGTACGTATCTACACTTGGATCGAT | reverse | |
| Zm00001d019565 | TTTAAGGTGAACGGAGAAGGAGA | forward | PIP2;6 |
| | GAAAGCTACTGCTGCTGTGGAT | reverse | |
| Zm00001d032850 | CATTGTCACGCTCGTCATCT | forward | PHT2 |
| | GGTGGAGTTGAAGTGGTCGT | reverse | |
| Zm00001d031875 | AGTCCAAGGGCAAATCCCTG | forward | PHT7 |
| | GCACTGTGCGGTTGTTGTAG | reverse | |
| Zm00001d004308 | TACTTCGGCACCAACGAAGC | forward | PHT12 |
| | CTCTTGTTGGCGTACTCGGA | reverse | |
| Zm00001d038252 | CGACGGCTGAATTCTGAACC | forward | KCH2 |
| | GGCGATCAGGTAGTACAGGC | reverse | |
| Zm00001d037289 | ATGTTAAGTCGCCCCAGGTC | forward | ZORK |
| | GCGAACATCCCACTGGCTA | reverse | |
| Zm00001d003859 | AGGTGCTCAAAGTGGGGATG | forward | HAK20 |
| | CTGGCTGCCAAGCAAGTACA | reverse | |
| Zm00001d017249 | TCTTCCTCTTCCAGTGGGCT | forward | AMT2 |
| | GCGGAGTAGATGAGGTACGC | reverse | |
| Zm00001d012261 | CATACCGGACTGGCTGAACA | forward | AMT7 |
| | GGCCCCACTTCTTTCACGA | reverse | |
| Zm00001d054057 | CCTATGGGCGGCATCATGT | forward | NRT2 |
| | CCAGATGTTCCAGAGGCGAG | reverse | |
| Zm00001d014976 | CGCATGGTAGCTCGCTGGA | forward | NRT3 |
| | GGATGGTCTTGGCCTTGTGC | reverse | |
| Zm00001d011679 | GCATCGTCCCGTTCGTCTC | forward | NRT5 |
| | CCGTCTCCGTCTTGTACTTGG | reverse | |
| Zm00001d026590 | TGACGTTTCAGGTGTTCTCTG | forward | Auxin response factor 8 |
| | GCAACTGTATTTTCGTGGTC | reverse | |
| Zm00001d032883 | ACATCACGTACACCAGATTGC | forward | Expansin-A11 |
| | GTCTGCACATTGTTGGTAGA | reverse | |
| Zm00001d003368 | GGCTCCCATGAAAACGCAA | forward | Kinesin KP1 |
| | CCACTGGAACGAGATGCTGC | reverse | |
| Zm00001d014507 | CGACGAGCCTTTCAGTGAGT | forward | Auxin response factor 16 |
| | AGACTGGACTCTACCTCGCT | reverse | |
| Zm00001d010159 | GCCCTGCTGTATGAAATGGA | forward | Actin1 |
| | AAAGGAACCAGCTAAAAGCAAAC | reverse | |
| Zm00001d049641 | TTGTTTCCCTTCCTGCTACC | forward | GAPDH |
| | AAACTGCAACCTCACCACAAG | reverse | |
| Zm00001d046449 | TGGGCCTACTGGTCTTACTACTGA | forward | EF1-alpha |
| | ACATACCCACGCTTCAGATCCT | reverse | |
