

*Supplementary Material*

**Superior root hair formation confers root efficiency in some,  
but not all, rice genotypes upon P deficiency**

**Josefine Nestler\*, Matthias Wissuwa\***

\* Correspondence:

Matthias Wissuwa [wissuwa@affrc.go.jp](mailto:wissuwa@affrc.go.jp)

Josefine Nestler [j.nestler@fz-juelich.de](mailto:j.nestler@fz-juelich.de)

Included are:

Supplementary tables: 3

Supplementary figures: 8

**Figure S1. Root hair properties on lateral roots of Nutrient solution-grown plants.**

Root hair length (left) and density (middle) means ( $n=5$ ) +/- SE. The derived root hair factor (RHF, right) is shown as mean ( $n=5$ ) +/- maximal/minimal replicate value as diamond. Low P (light bars) and high P (dark bars). [%] values represent the increase in low P compared to high P. Statistical differences are shown per genotype with  $P$ -value \* $\leq 0.05$ , \*\* $\leq 0.01$ , \*\*\* $\leq 0.001$  for P level differences; and LSD bars representing the least significant difference among genotypes within each P level. Second order LRs are not depicted as no root hairs were found. n.d. = not determined.

**Figure S2. Rooting depth in the upland field at 50 DAS.****Figure S3. Root hair properties on lateral roots of Rhizobox-grown plants.**

Root hair length (left) and density (middle) means ( $n=5$ ) +/- SE. The derived root hair factor (RHF, right) is shown as mean ( $n=5$ ) +/- highest / lowest replicate (diamond). Low P (light bars) and high P (dark bars). [%] values represent the increase in low P compared to high P. Statistical differences are shown per genotype with  $P$ -value \* $\leq 0.05$ , \*\* $\leq 0.01$ , \*\*\* $\leq 0.001$  for P level differences; and LSD bars representing the least significant difference among genotypes within each P level.

**Figure S4. Root hair properties on lateral roots of upland field-grown plants.**

Root hair length (left) and density (middle) means ( $n=5$ ) +/- SE. The derived root hair factor (RHF, right) is shown as mean ( $n=5$ ) +/- maximal / minimal replicate value (diamond). Low P (light bars) and high P (dark bars). [%] values represent the increase in low P compared to high P. Statistical differences are shown per genotype with  $P$ -value \* $\leq 0.05$ , \*\* $\leq 0.01$ , \*\*\* $\leq 0.001$  for P level differences; and LSD bars representing the least significant difference among genotypes within each P level.

**Figure S5. Root hair properties on all measured root types of lowland-grown plants.**

Root hair length (left) and density (middle) means ( $n=5$ ) +/- SE. The derived root hair factor (RHF, right) is shown as mean ( $n=5$ ) +/- highest / lowest replicate (diamond). Low P (light bars) and high P (dark bars). [%] values represent the increase in low P compared to high P. Statistical differences are shown as least significant difference (LSD) bars among genotypes within high P. n.d. = not determined.

**Figure S6. Root hair liveliness on lateral roots in the upland field.**

Depicted are means ( $n=3$ ) of the relative amount of living root hairs on lateral roots in increasing field depth.

**Figure S7. Expression of selected genes in the Rhizobox experiment.**

The relative expression [D c(t)] of selected P deficiency- (*OsPT1*, *OsPT6*, *OsPHO2.1*, *OsPHR1*, *OsPHR2*, *OsPHR3*) or root hair- (*OsAux1*, *OsARF1*, *OsCSLD1*, *OsFH1*, *OsSND1*) related genes is depicted relative to the expression of the reference genes *OsEIF1a* and *OsACT7*. Shown are mean values ( $n=3$ ) +/- standard error. The student's T-test was employed to calculate statistical differences between high and low P condition per genotype and gene ( $P$ -value: \*  $\leq 0.05$ , \*\*  $\leq 0.01$ ).

**Figure S8. Total root hair surface area in upland soil under low P conditions.**

The mean root hair surface area / cm root length was calculated by determining the surface area of a single hair per genotype and root type followed by multiplication of the specific root hair density. By integration of the average root type length composition in the upland field low P conditions (Nestler *et al.*, 2016) the total root hair surface area was calculated for Nerica4 and DJ123.

**Table S1.** Oligonucleotide primer sequences.

Gene name	Accession number	Forward primer sequence	Reverse primer sequence	Product size [bp]
<i>OsACT7</i>	Os05g0438800	TCCAGCCATCTCTTGGTGGT	TCTTCTGGTGGTCAATCACT	221
<i>OsARF16</i>	Os06g09660	TAGACGAGAGGCCACGAGAA	TCGACATCCCTGTGATCCCT	199
<i>OsAUX1</i>	Os01g63770	ATCATGCACGCGATGTGGAA	CTGAGGCAGATGCTTTGGT	293
<i>OsCSLD1</i>	Os10g0578200	GACGTCCCTCATGATACCGC	CAGACGTAGACGATGGTGGG	213
<i>OsEIF1a</i>	Os02g0300700	CAAGTACATGAACGACGAGGC	CCATTGGACTGATGAAGCAGC	193
<i>OsEXPA8</i>	Os01g0248900.1	CCTCCAAACTACGCCCTGT	GTCCCGGACCCCTTGATC	198
<i>OsEXPA17</i>	Os06g0108600	CACTACTGGTTGCTGCTCTAC	GTGCTGTAGGTGGTGAGCTTG	188
<i>OsFH1</i>	Os01g67240	AGATCACTGAGGTGTTGCGG	CGCAGAGTCACCATGGAAGT	185
<i>OsPHO2.1</i>	Os05g48390	AACTGCTGCTGCCCTCAA	TCTCGAAATGCTTGGTGGC	186
<i>OsPHO2.2</i>	Os05g48390	CGGGTACATGGTTGTGTGGA	CCCTCGCAATCCGAAAGAGT	232
<i>OsPHR1</i>	Os03g21240	ACAGCAACTCCATCTGAGCC	TGCACTCTACGACGCTTCTC	203
<i>OsPHR2</i>	Os07g25710	TTGAGGAGCAAGGGAAGTGC	TGGATTCTCATGCCTGTTGT	193
<i>OsPHR3</i>	Os02g04640	TGCCTCCATGAGTTAGTGCAG	TGTACTGGCAGAGATAGCACA	229
<i>OsPT1</i>	Os03g0150600.02	TCGTCATGTACGGATTCAACC	ATCGTCCCAGGAAGTTGTG	160
<i>OsPT2</i>	Os03g0150800	TCGGTATGCTCATGACGCTG	ACCCCCAAATCCACAACGTGA	208
<i>OsPT6</i>	Os08g0564000	CAGGTGGTGGAGATGCGTAAT	CTGGAACAGGTTCTGGCTGT	162
<i>OsRHL1</i>	Os06g0184000	AGCGGAGCGGATGAAATCG	GAGAACCTTGACTGGAGCTG	121
<i>OsSNDP1</i>	Os10g03400	GTTAGCCGAAACATGCCAAA	CCTCGATATCAGATAGGCGCT	154
<i>OsSQD2</i>	Os01g0142300	GCGATCTGGTACTGGAGGAA	TCTGTTGACCTCAGGTGCTG	93

Table S1

**Tables S2. Correlation analysis of Rhizobox-grown root hair properties.**

Depicted are correlation coefficients of root hair length, density, root diameter, and root hair longevity proportions grown in Rhizoboxes. Low P (white) and High P (grey).

Correlation (R) between Root hair length on different root types

	MR	L-type LR	S-type LR	2 <sup>nd</sup> Ord. LR
MR		0.2	0.04	0.15
L-type LR	0.88		0.73	0.57
S-type LR	0.65	0.8		0.65
2 <sup>nd</sup> Ord. LR	0.62	0.64	0.68	

For n (Low P: 24, High P: 23) the *P*-value of correlation is  $\leq 0.05$  at  $R > 0.41$ .

Correlation (R) of root hair length with Rh number & root diameter on different root types

	MR	L-type LR	S-type LR	2 <sup>nd</sup> Ord. LR
Rh number	0.83	0.62	0.39	0.28
Rh number	0.8	0.58	0.52	0.35
Rt diameter	0.82	0.69	0.66	0.46
Rt diameter	0.58	0.27	0.41	0.36

For n (Low P: 24, High P: 23) the *P*-value of correlation is  $\leq 0.05$  at  $R > 0.41$ .

Correlation (R) of root hair length with Rh number & root diameter per genotype

	DJ123	STM	Nerica4	Taichung	Santhi
Rh number	0.83	0.25	0.76	0.51	0.51
Rh number	0.41	-0.18	0.26	0.25	0.06
Rt diameter	0.73	0.88	0.47	0.82	0.54
Rt diameter	0.88	0.86	0.08	0.75	0.86

For n (Low P: 19, High P: 18) the *P*-value of correlation is  $\leq 0.05$  at  $R > 0.47$ .

Table S2

**Tables S3. Correlation analysis of upland field-grown root hair properties.**

Depicted are correlation coefficients of root hair length, density, root diameter, and root hair longevity proportions grown in the upland field for 100 DAS. Low P (white) and High P (grey).

Correlation (R) between Root hair length on different root types

	MR	L-type LR	S-type LR	2 <sup>nd</sup> Ord. LR
MR		0.26	0.58	0.26
L-type LR	0.59		0.33	0.39
S-type LR	0.63	0.59		0.36
2 <sup>nd</sup> Ord. LR	-0.16	0.30	0.33	

For n (Low P: 15, High P: 19) the *P*-value of correlation is  $\leq 0.05$  at  $R > 0.5$ .

Correlation (R) between Root hair liveliness on different root types

	MR	L-type LR	S-type LR	2 <sup>nd</sup> Ord. LR
MR		0.76	0.74	n.d.
L-type LR	0.87		0.69	n.d.
S-type LR	0.93	0.90		n.d.
2 <sup>nd</sup> Ord. LR	n.d.	n.d.	n.d.	

For n (Low P: 29, High P: 24) the *P*-value of correlation is  $\leq 0.05$  at  $R > 0.38$ .

Correlation (R) of root hair length with Rh number & liveliness on different root types

	MR	L-type LR	S-type LR	2 <sup>nd</sup> Ord. LR
Rh number	0.74	0.83	0.82	0.84
Rh number	-0.8	0.40	0.27	0.67
Rh liveliness	-0.55	-0.24	-0.43	n.d.
Rh liveliness	-0.54	-0.72	-0.49	n.d.

For n (Low P: 29, High P: 24) the *P*-value of correlation is  $\leq 0.05$  at  $R > 0.42$ .

Correlation (R) of root hair length with Rh number & liveliness per genotype

	DJ123	Mudgo	Nerica4	Taichung	Santhi
Rh number	0.68	0.89	0.81	0.66	0.73
Rh number	0.81	0.73	0.47	0.84	0.83
Rh liveliness	0.29	-0.26	-0.08	-0.26	-0.06
Rh liveliness	-0.54	-0.45	-0.42	-0.05	0.60

For n (Low P: 18, High P: 21) the *P*-value of correlation is  $\leq 0.05$  at  $R > 0.46$ .

Table S3

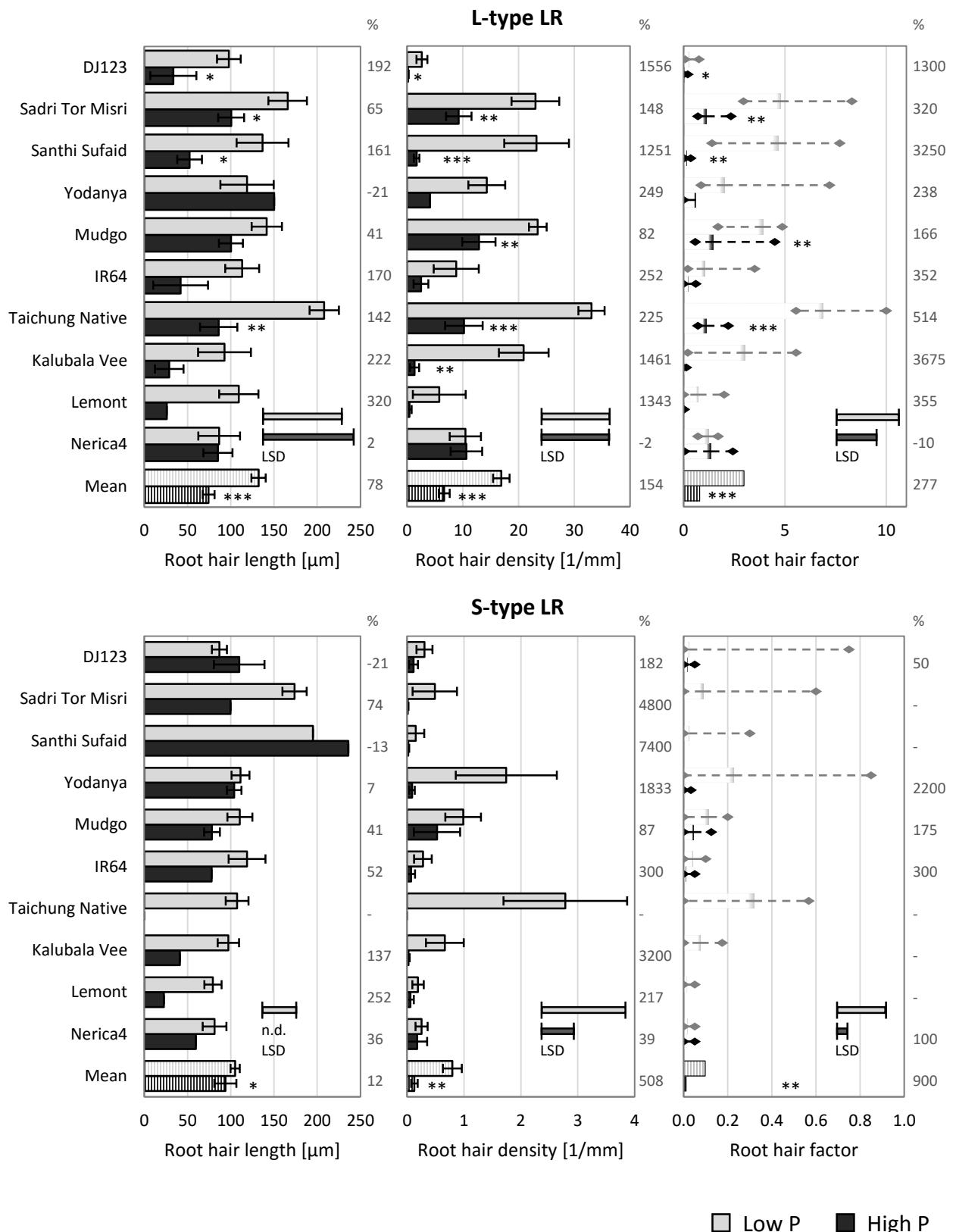


Figure S1

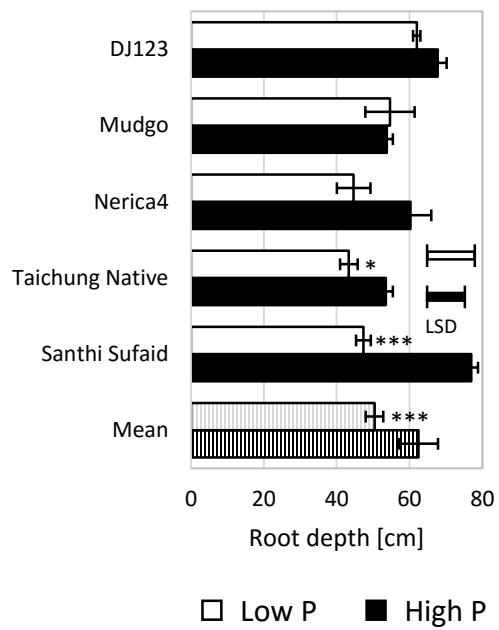


Figure S2

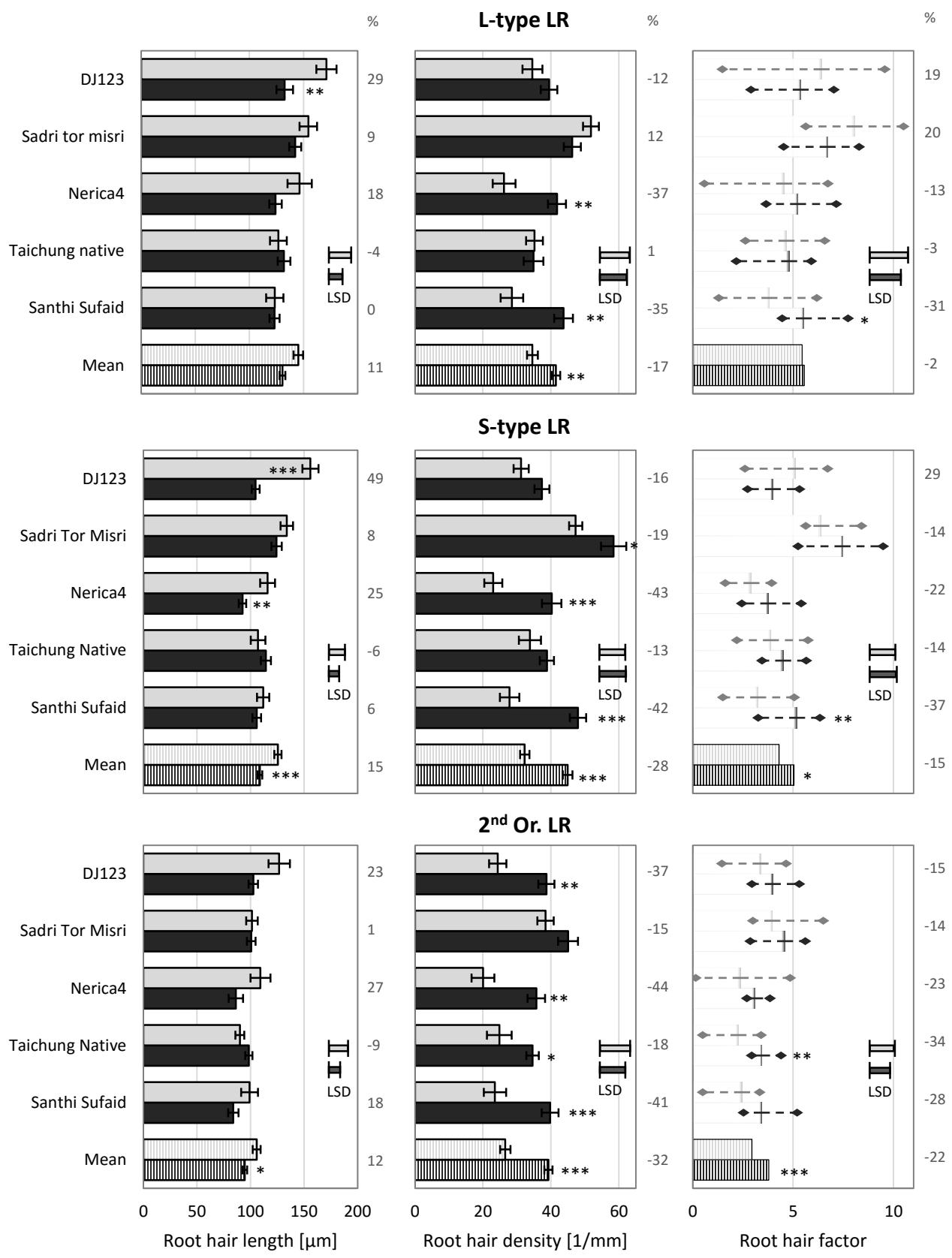


Figure S3

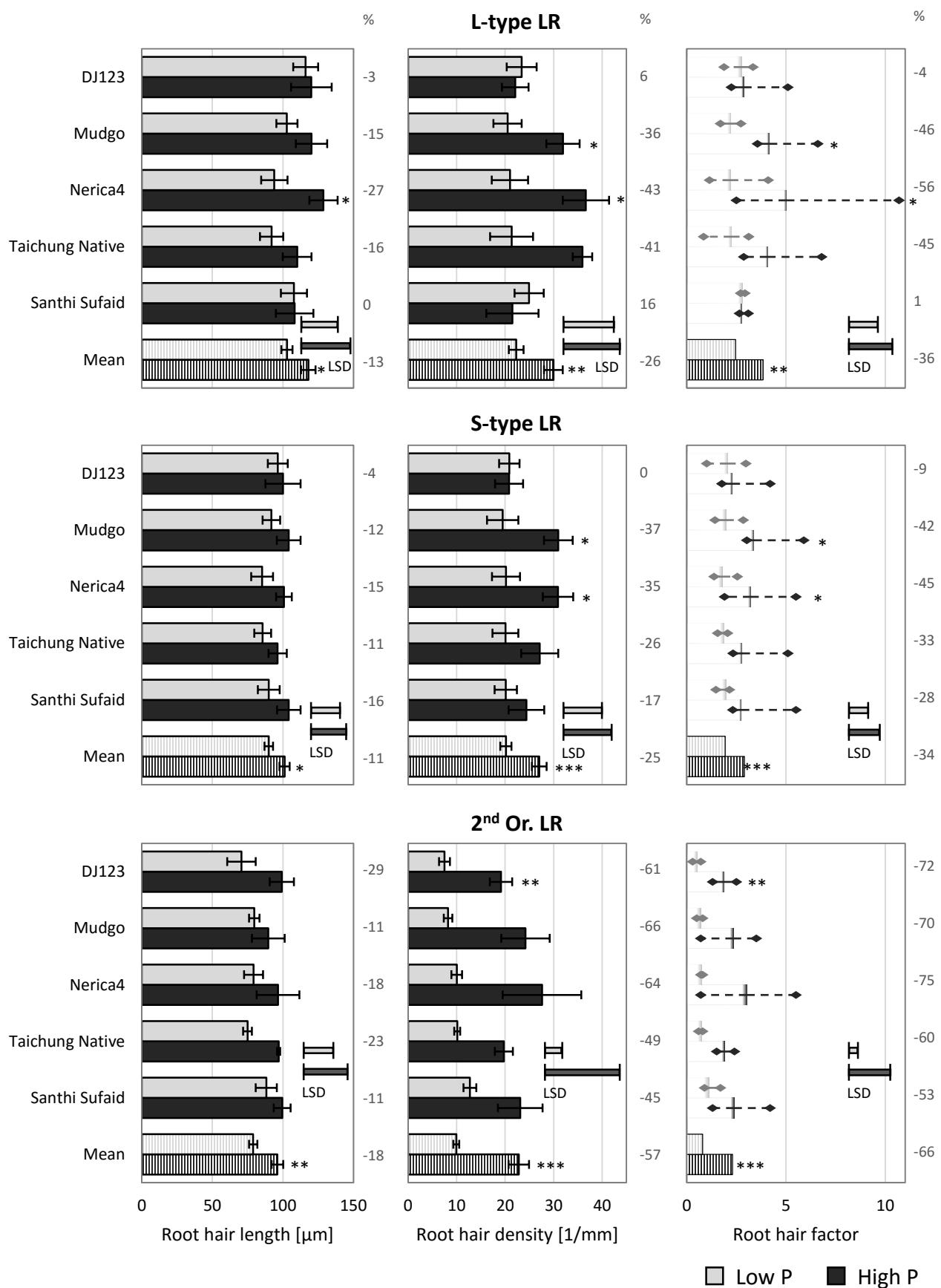


Figure S4

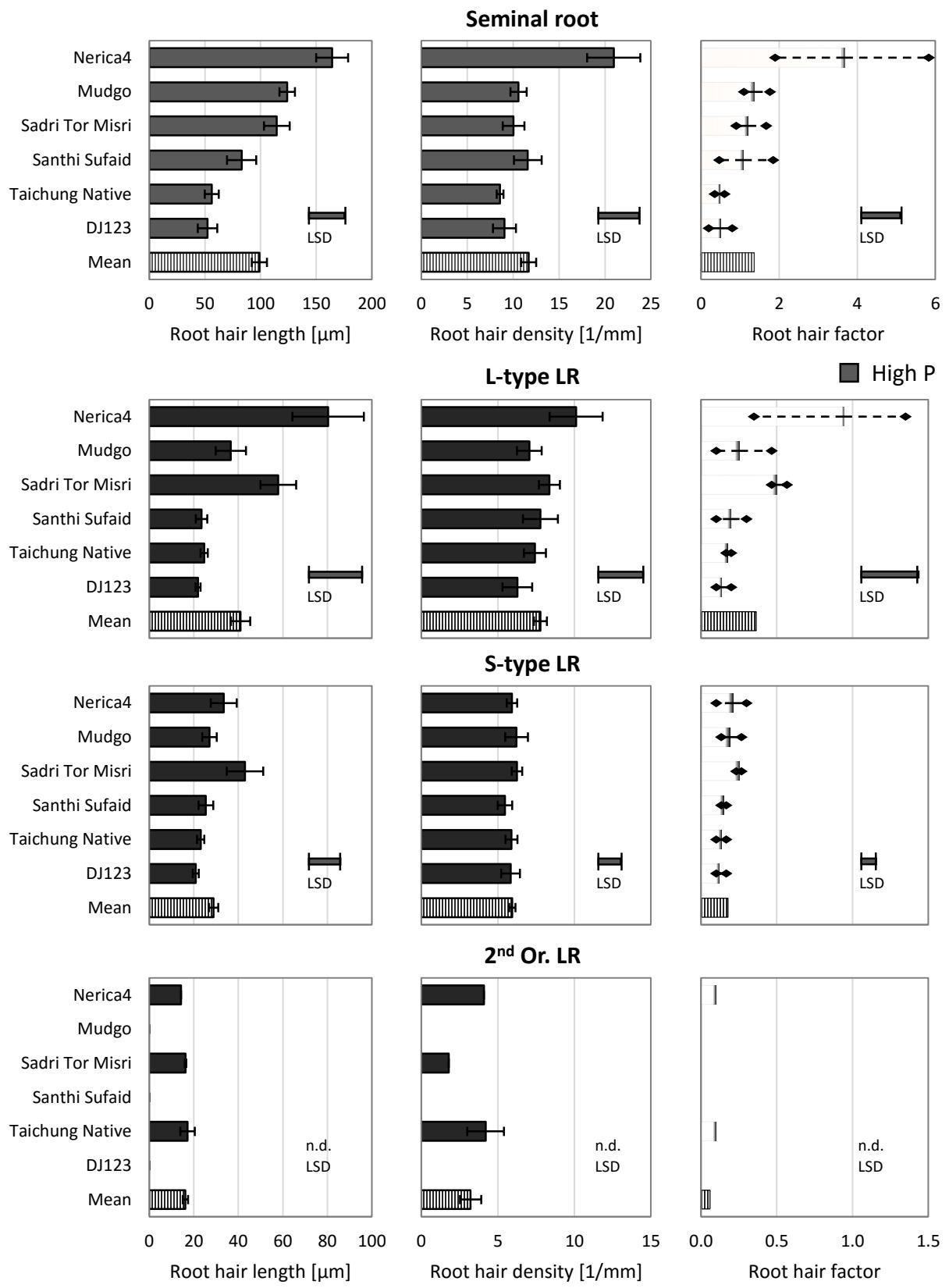
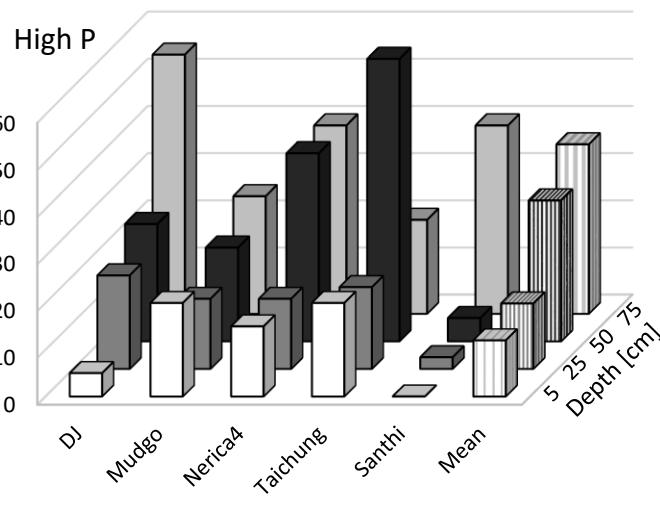
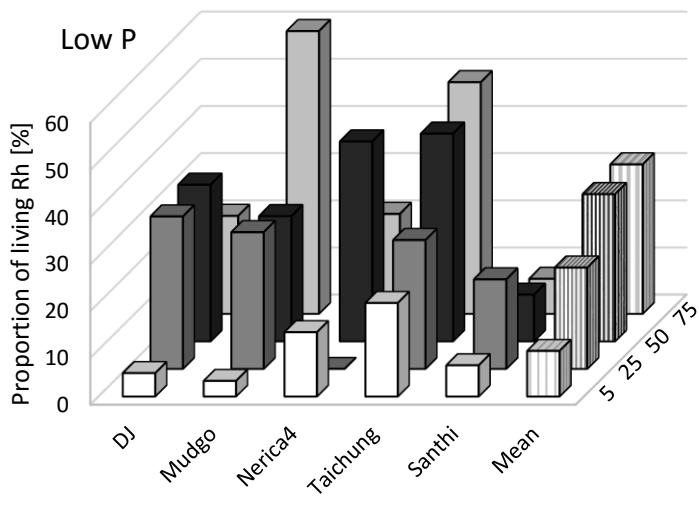


Figure S5

### L-type LR



### S-type LR

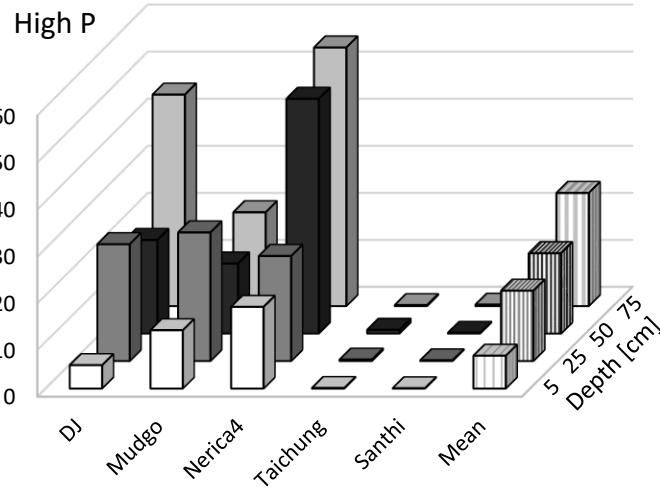
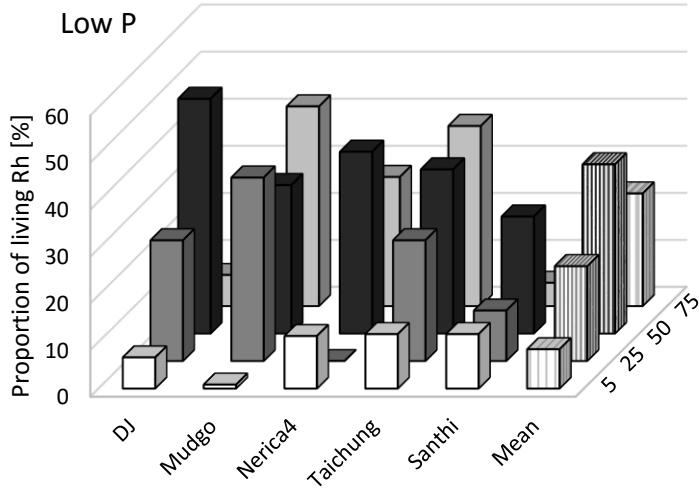


Figure S6

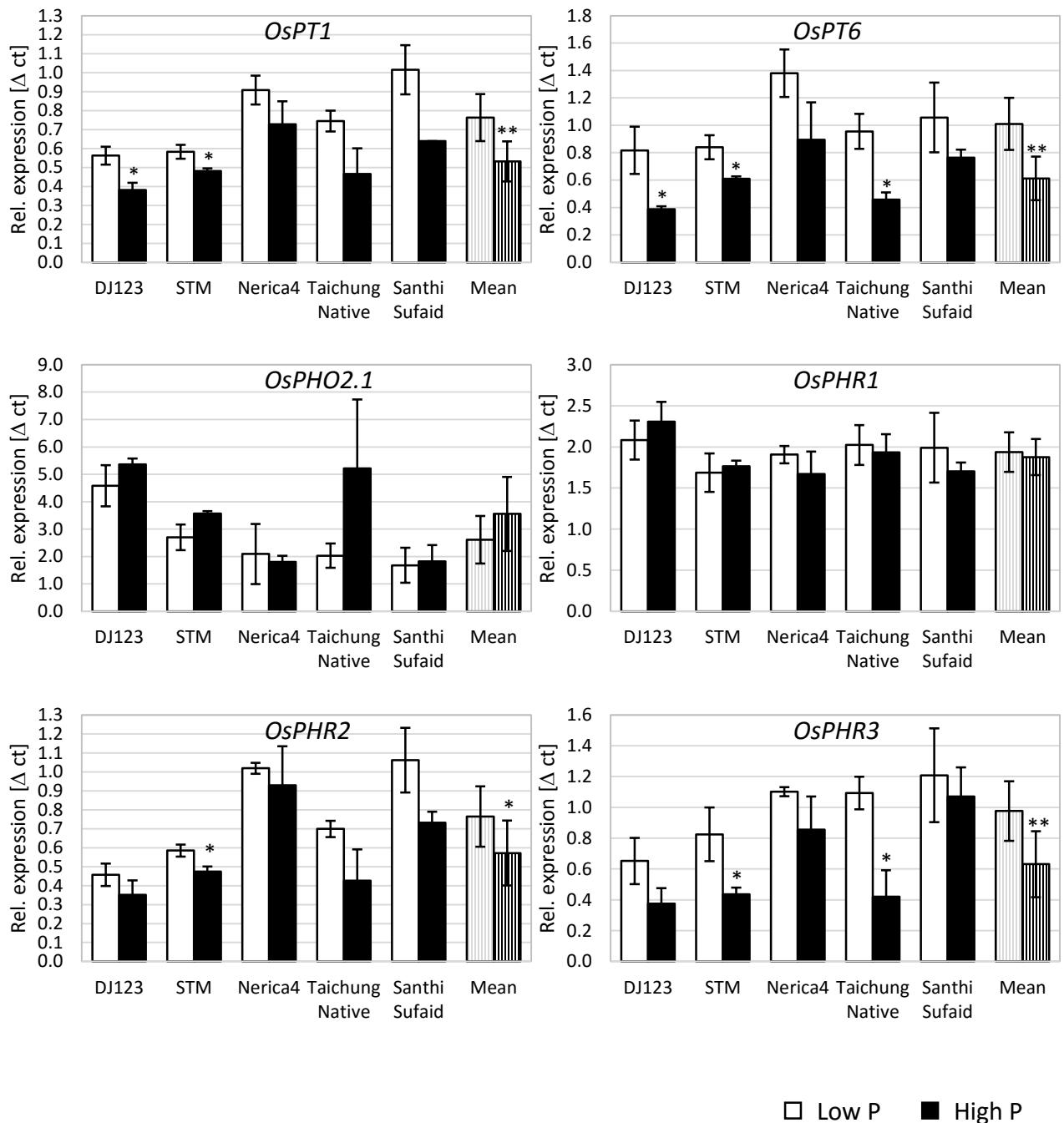


Figure S7

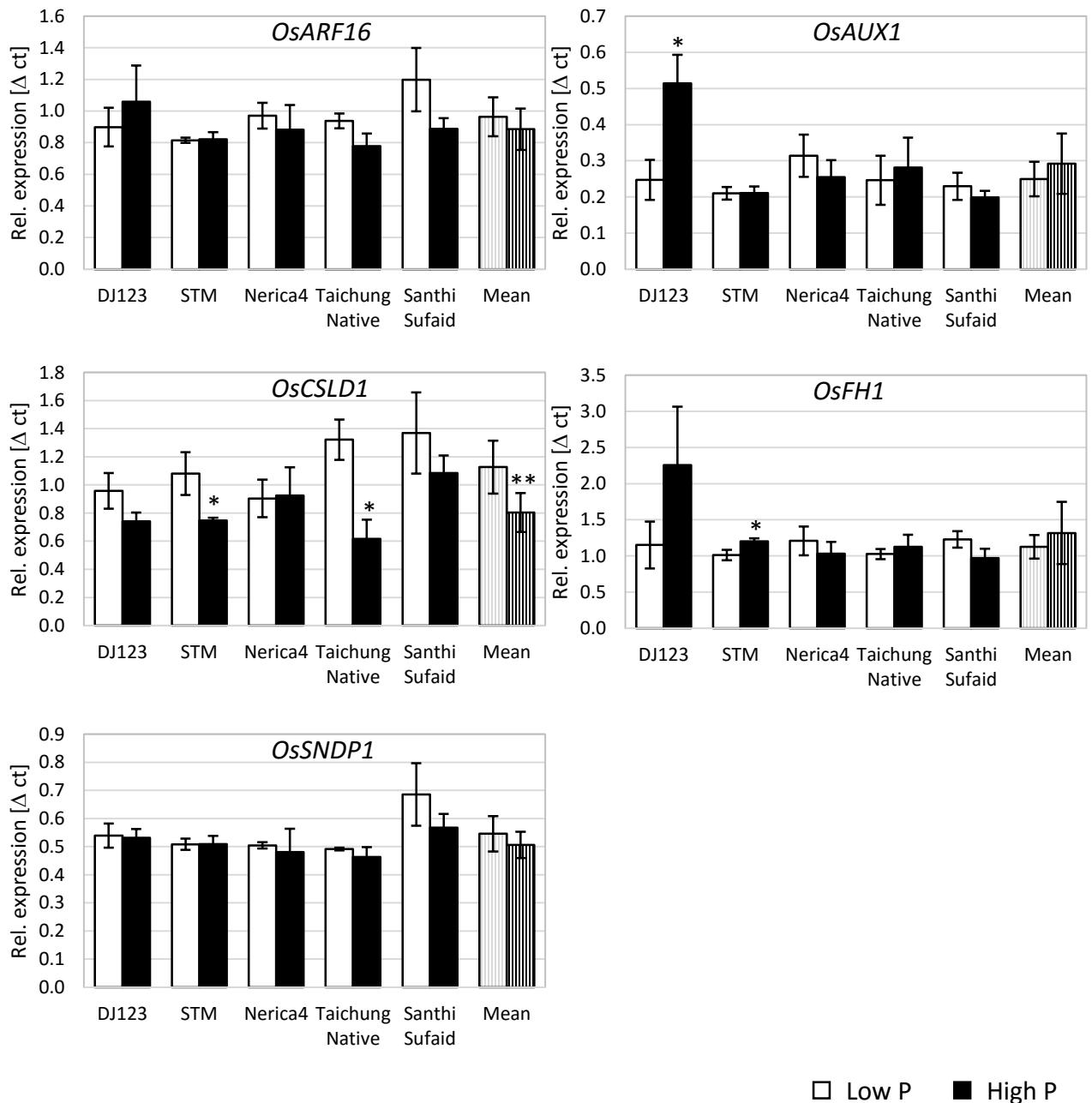


Figure S7 cont.

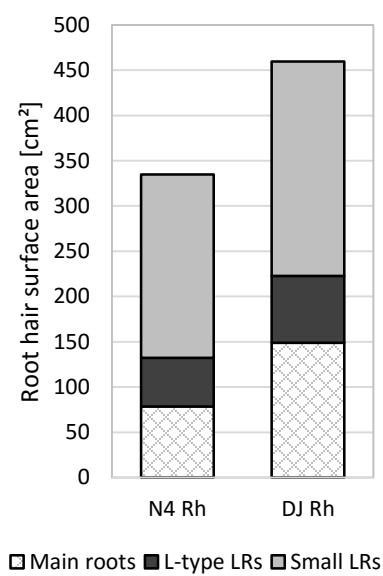


Figure S8