

## Supplementary Material

### **Overexpression of a putative nitrate transporter (*StNPF1.11*) increases plant height, leaf chlorophyll content and tuber protein content of young potato plants**

*Michiel T. Klaassen<sup>A,B</sup>, Dianka C. T. Dees<sup>A</sup>, Rommel M. Garrido<sup>A</sup>, Jorge Alemán Báez<sup>A</sup>, Michiel Schrijen<sup>A</sup>, Pablo G. Baldeón Mendoza<sup>A</sup> and Luisa M. Trindade<sup>A,C</sup>*

<sup>A</sup>Wageningen University and Research, Plant Breeding, PO Box 386, 6700 AJ Wageningen, The Netherlands.

<sup>B</sup>Aeres University of Applied Sciences, Department of Applied Research, PO Box 374, 8250 AJ Dronten, The Netherlands.

<sup>C</sup>Corresponding author. Email: luisa.trindade@wur.nl

**Table S1** Forward (F) and reverse (R) primer sequences for PCR amplification of the putative potato nitrate transporter (*StNPF1.11*) encoding fragment.

Gene	Gene ID	Primers sequence 5'-3'
<i>StNPF1.11</i>	XM_006355891	F: caccATGAACTGAAAATGGGCACAGAACAC R: TCAAGATTGATGTAAGAGTCTATAATCACATTCTTC

**Table S2** Forward (F) and reverse (R) primer sequences for qRT-PCR of the overexpressed putative potato nitrate transporter (*StNPF1.11*) and potato elongation factor (*EF1α*).

Gene	Gene ID	Primers sequence 5'-3'
<i>StNPF1.11</i>	XM_006355891	F: TCCTATGTGGTCTGCTGGTT R: AGGGACAAAAACACGGTCGTA
Elongation factor ( <i>EF1α</i> )*	AB061263	F: ATTGGAAACGGATATGCTCCA R: TCCTTACCTGAACGCCGTCA

\*Sequences of primers for potato elongation factor (*EF1α*) from (Nicot et al. 2005).



**Fig. S1** Schematic diagram of the pK7WG2-*StNPF1.11* construct used for transformation (not to scale). The potato nitrate transporter (*StNPF1.11*) gene was cloned in the pK7WG2 destination vector (Karimi et al. 2002) and under control of the CaMV 35S promotor for constitutive expression in all plant tissues.

RB: right border; 35S-P: CaMV35S promotor; AttR1: recombination site 1;

*StNPF1.11*: potato nitrate transporter gene (ID: XM\_006355891;

PGSC0003DMG400015591); AttR2: recombination site 2;

35S-T: CaMV35S terminator; KAN: kanamycin resistance

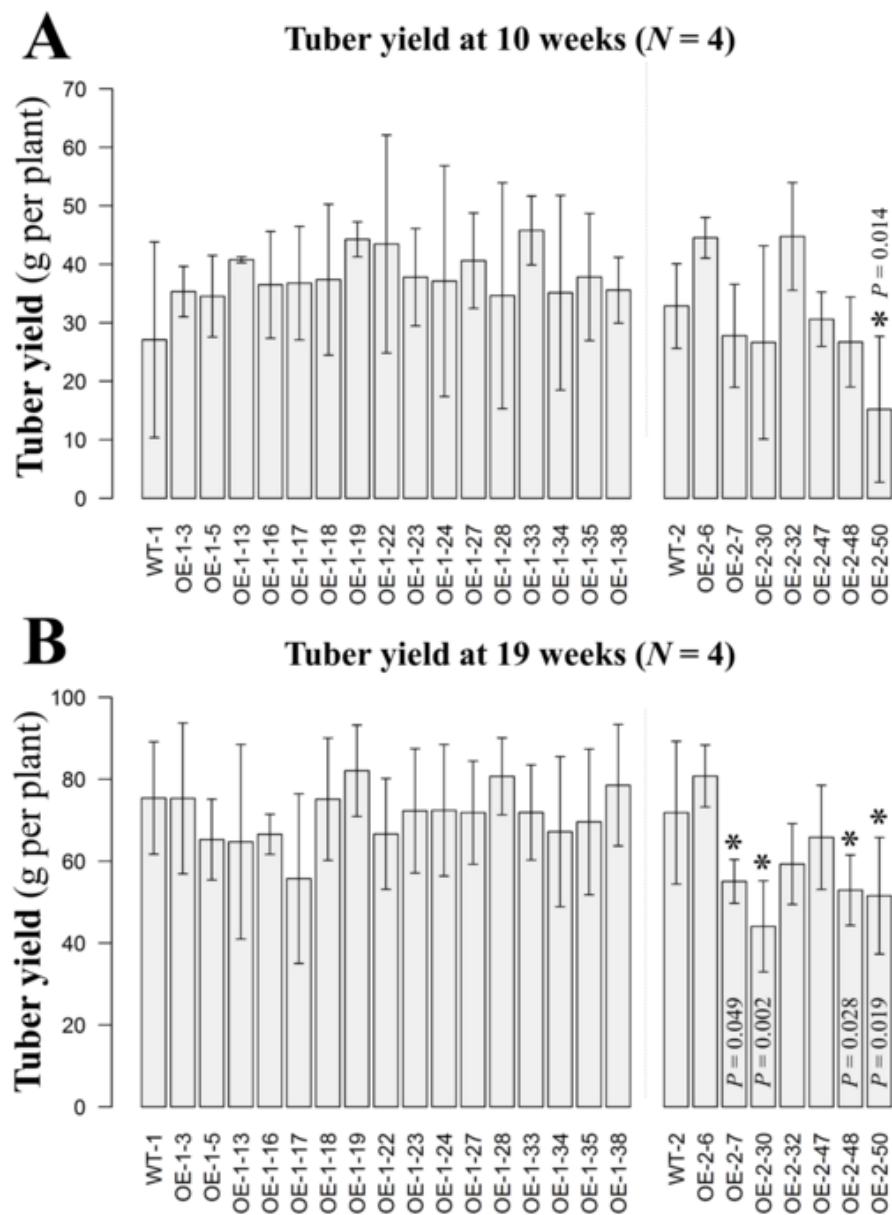
gene; LB: left border.



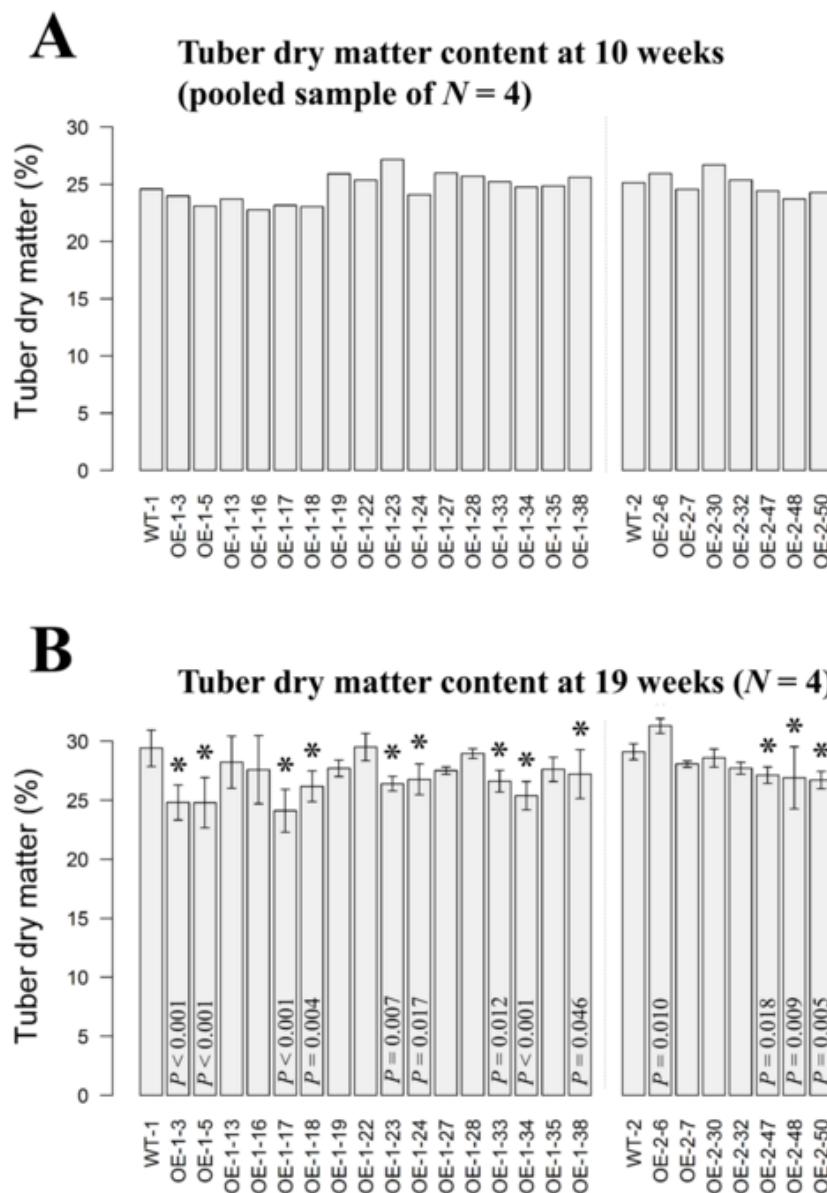
**Fig. S2** Plant height of selected transformants at 10 weeks. Several transformants were taller than the wild type control. OE = overexpression line. WT = wild type. 1 = Series 1. 2 = Series 2.



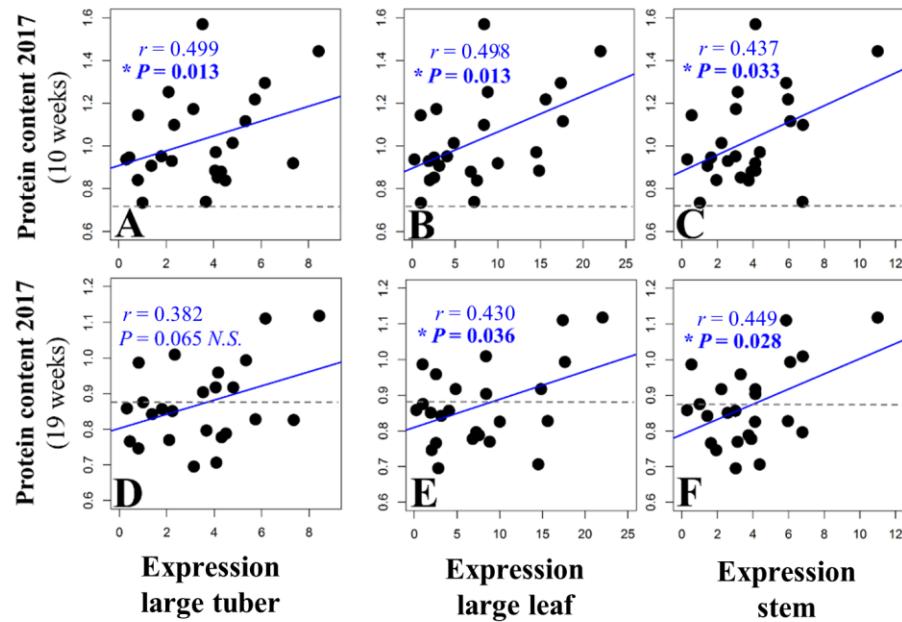
**Fig. S3** Darker pigmented (brown) stems observed in selected transformants, whereas normal (light green) coloured stems were observed for the wild type (WT) controls. OE = overexpression line. 1 = Series 1. 2 = Series 2.



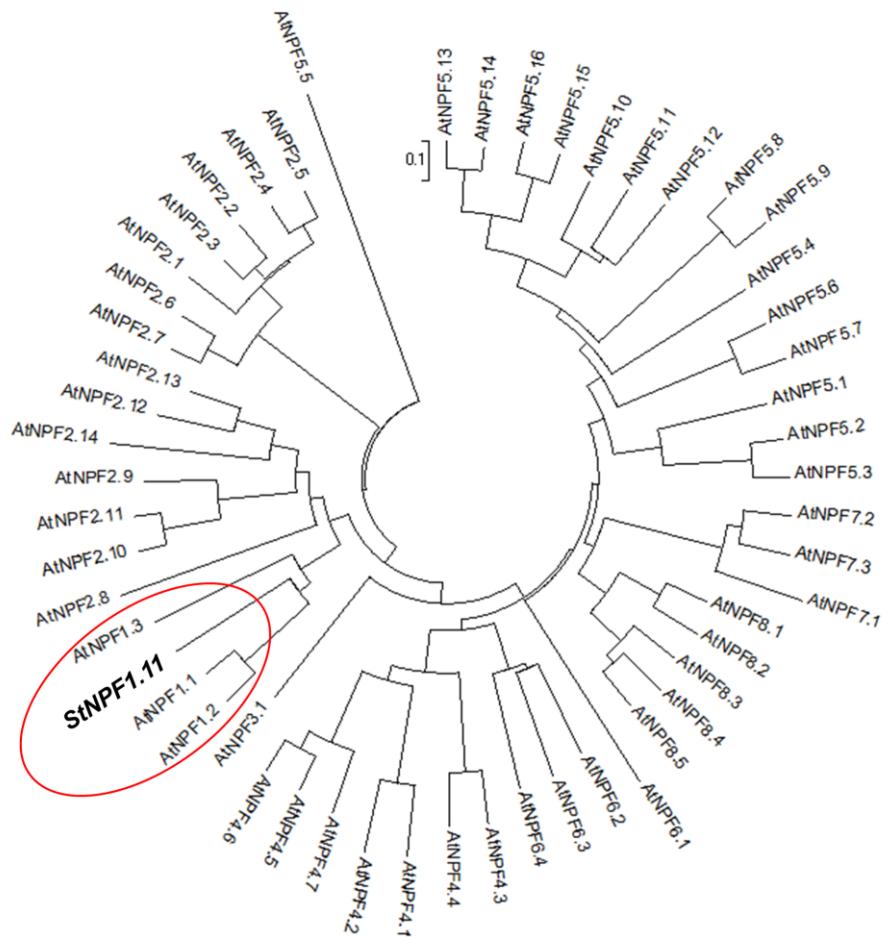
**Fig. S4** Tuber yield (fresh weight) of the transformants at (A) 10 weeks and (B) 19 weeks. Data (mean  $\pm$  SD) represent samples collected from four biological replicates ( $N = 4$ ).  $P$  denotes Fisher's least significant difference (LSD) probability values from one-way ANOVA. Asterisks denote significant differences at  $\alpha = 0.05$ .



**Fig. S5** Tuber dry matter content (mean  $\pm$  SD) at (A) 10 weeks and (B) 19 weeks. Data represent (A) pooled samples from four biological replicates ( $N = 4$ ).  $P$  represent Fisher's least significant difference (LSD) probability values from one-way ANOVA. Asterisks denote significant differences at  $\alpha = 0.05$ .



**Fig. S6** Bi-variate scatter plots for *StNPF1.11* expression in large leaves (10 weeks), stems (10 weeks) and large tubers (19 weeks) of the transformants versus tuber protein content at 10 and 19 weeks. The grey dashed lines represent the values of protein content for the wild type (WT) control. Simple linear regression lines are shown in blue.  $P$  represents the probability values. Asterisks denote significant relationships at  $\alpha = 0.05$ . *N.S.* represent non-significant relationships



**Fig. S7** Phylogenetic ties between potato *StNPF1.11* and *Arabidopsis* NPF orthologs. Protein sequences were collected from NCBI Genbank. Analyses and alignments were performed using MEGA version 7.0 (Kumar, Stecher and Tamura, 2016). The *StNPF1.11* sequence is orthologous to *AtNPF1.1*, *AtNPF1.2* and *AtNPF1.3* (shown in the red oval sphere). At = *Arabidopsis thaliana*.