

SUPPLEMENTARY INFORMATION

Functional characterization of the PHT1 family transporters of foxtail millet with development of a novel *Agrobacterium*-mediated transformation procedure

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Supplementary Table S1: Details of cloning primers used to clone SiPHT1 transporters into *pDDGFP-2* plasmid for yeast complementation assay experiments

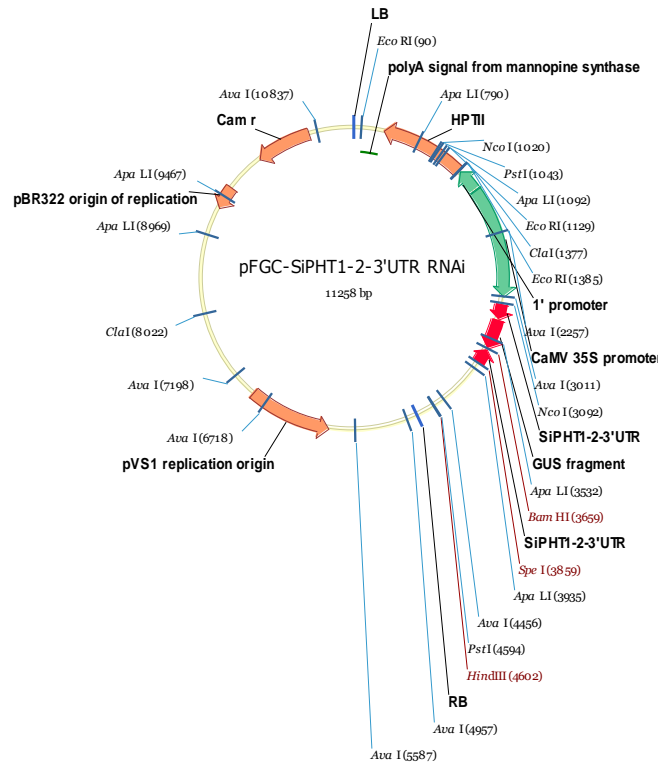
Name of the gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	T _m	Product length
SiPHT1;1	GATCACTAGTATGGCGAGGCAGGAGCGG <i>SpeI</i>	GGTTACCGGTCACCATTTCAAGTCCGGAAGGC <i>AgeI</i>	63.46	1629
SiPHT1;2	GATCACTAGTATGGCGCGTGGGGGCGAC <i>SpeI</i>	GGTTACCGGTCACCATCTGGGTCTGGGACGG <i>AgeI</i>	66.46	1623
SiPHT1;3	GATCACTAGTATGGCCCACGATCACAAGG <i>SpeI</i>	GGTTACCGGTTTCGCTCAAATTGGTCGGAAC <i>AgeI</i>	59.77	1608
SiPHT1;4	GATCACTAGTATGGCGGGAGCTCAGCTC <i>SpeI</i>	GGTTACCGGTGACTCTGGCCGGAGCATC <i>AgeI</i>	60.87	1581
SiPHT1;7	GATCACTAGTATGGCTGGCGGCGACCTG <i>SpeI</i>	GGTTACCGGTCACGGGCACGGTGCGGTTG <i>AgeI</i>	66.32	1617
SiPHT1;8	GATCACTAGTATGGCGCGGGAGAAGCTGC <i>SpeI</i>	GGTTACCGGTCAGCGGTAGAATCTGGGAGTCG <i>AgeI</i>	64.17	1623
PHO84	GATCACTAGTATGAGTTCCGTCAATAAAGATACT <i>SpeI</i>	GGTTCCCCGGGTGCTTCATGTTGAAGTTGAGATG <i>XmaI</i>	56.66	1761

Supplementary Table S2: Details of cloning primers used to clone SiPHT1 transporter fragments into *pFGC1008* plasmid for down regulation of these genes

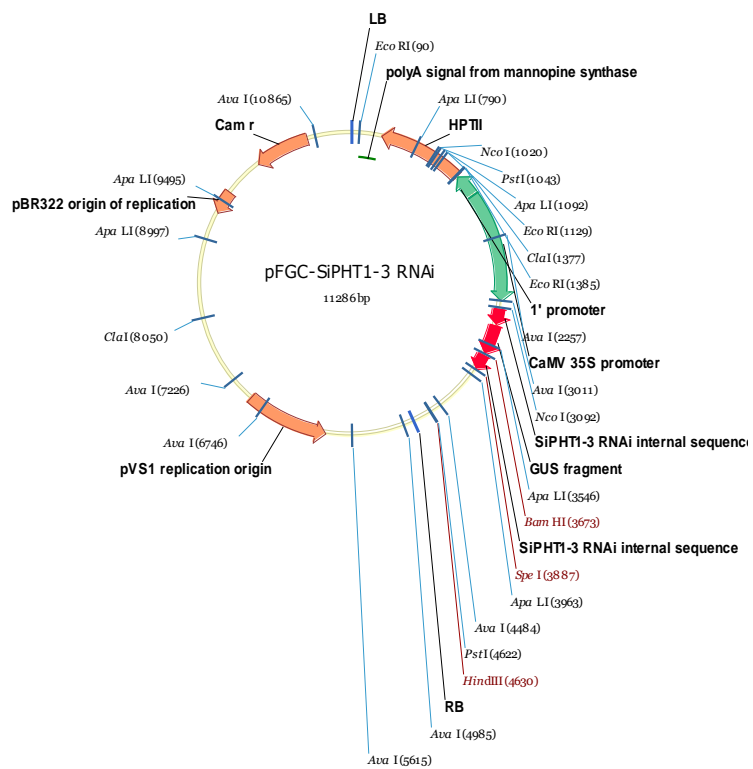
Name of the gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
SiPHT1;2	GC <u>ACTAGT</u> <u>GGCGCGCC</u> GGTGTAGTATGACCGTCCGTG <i>SpeI</i> <i>AscI</i>	GA <u>GGATCC</u> <u>ATTTAAAT</u> GCGTGGTATGTTACCAATG <i>BamHI</i> <i>SwaI</i>
SiPHT1;3	GC <u>ACTAGT</u> <u>GGCGCGCC</u> CAAGACGGATGCCGGTTAC <i>SpeI</i> <i>AscI</i>	GA <u>GGATCC</u> <u>ATTTAAAT</u> GTCGGAACAGTCTGCTGGATG <i>BamHI</i> <i>SwaI</i>
SiPHT1;4	GC <u>ACTAGT</u> <u>GGCGCGCC</u> CAGAGTCTAGGGGCCTGCAG <i>SpeI</i> <i>AscI</i>	GA <u>GGATCC</u> <u>ATTTAAAT</u> GGAATGCTGAAGTACAACAGC <i>BamHI</i> <i>SwaI</i>

Supplementary Table S3. Details of primers used for RT-PCR analysis of T1 progenies RNAi transgenic lines

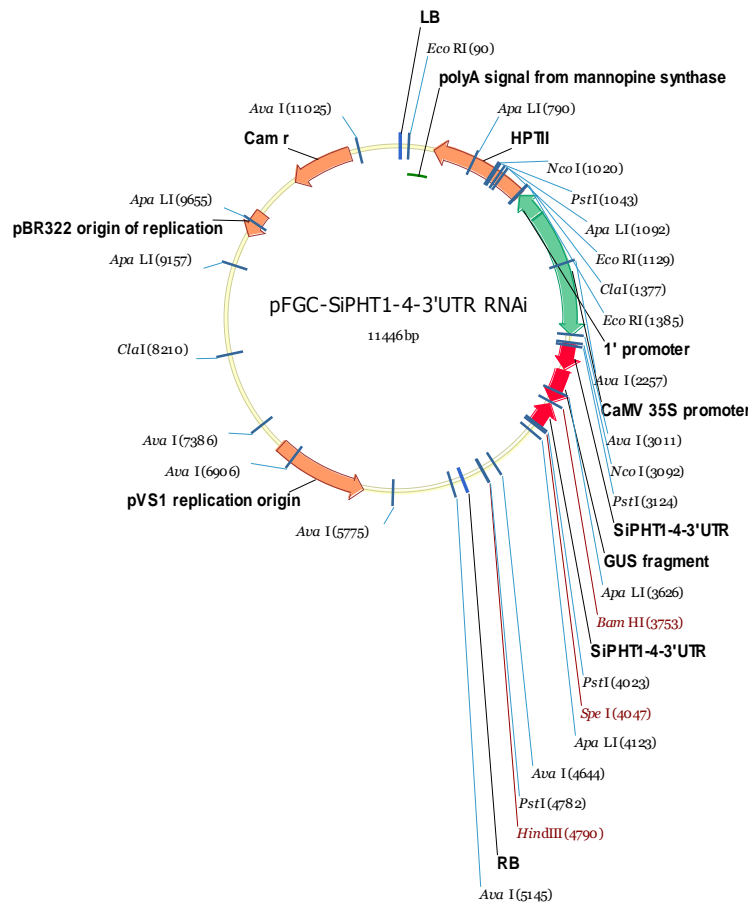
Name of the gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Product length (bp)	Ann. Temp
SiPHT1;2	ACCAGGACAAGAGCAAGGTG	GGCACGAGGAACGTGAGTAT	115	60.22
SiPHT1;3	TGTCATCGGGTTCTTGTTCA	AATTGGTCGGAACAGTCTGC	128	60.10
SiPHT1;4	CAGAAGGAGATCCAGGACGA	CGATATCGAGCAGGAACCAC	145	60.48
Si-actin-2	ACGACCATGTTCCCTGGTATT	ATCGTACTCCGCCTTTGAGAT	183	59.0



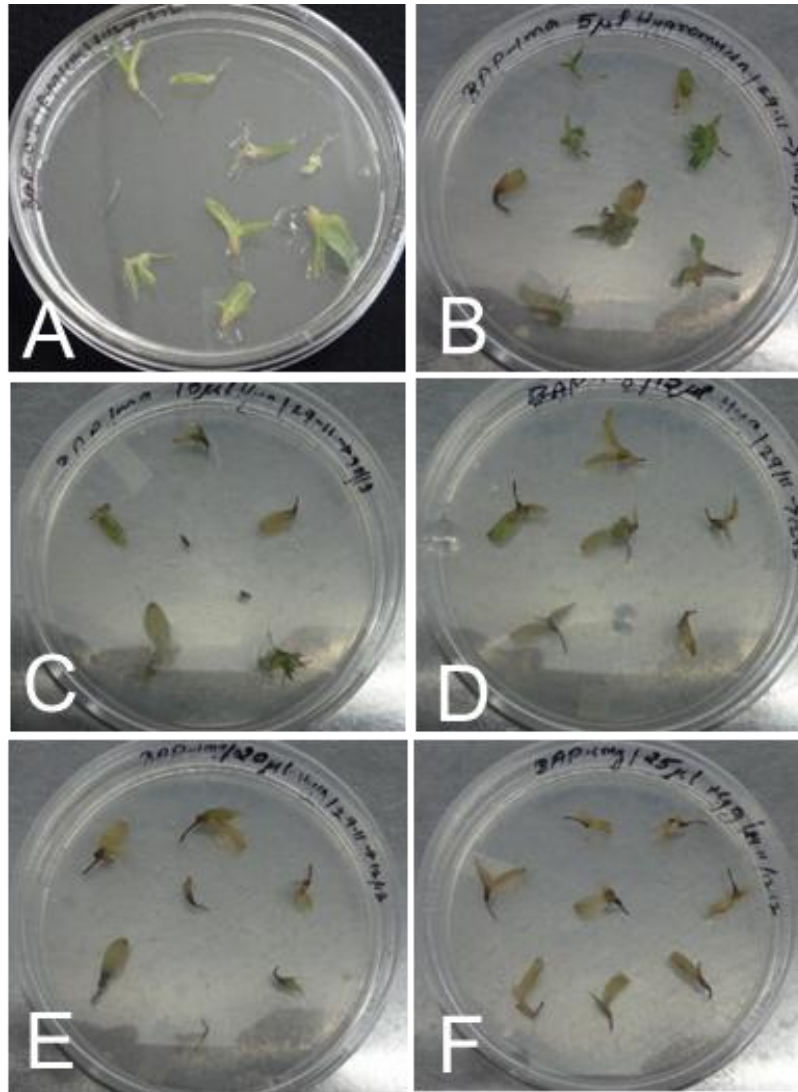
Supplementary Figure S1. Map of the plasmid *pFGC-SiPHT1;2-3'UTR-RNAi*.



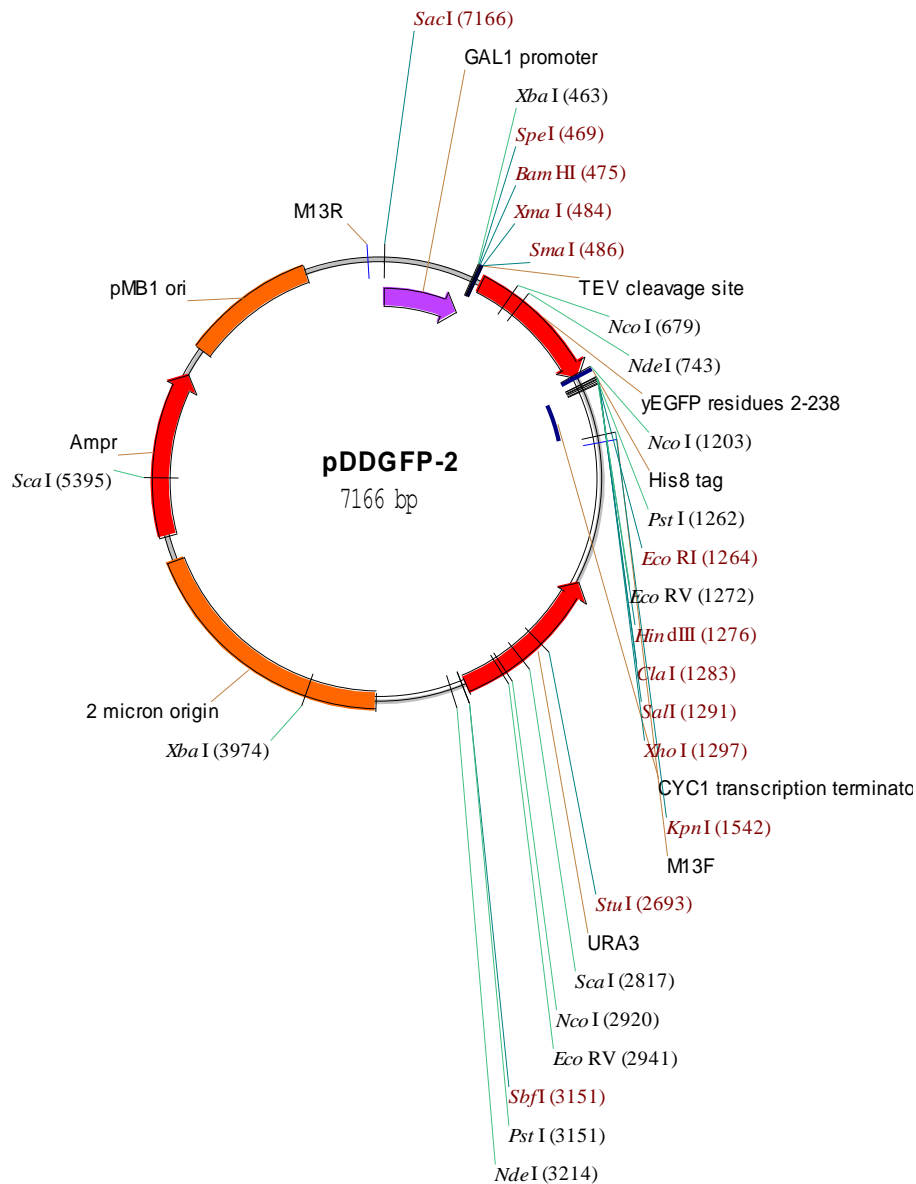
Supplementary Figure S2. Map of the plasmid *pFGC-SiPHT1;3-RNAi*.



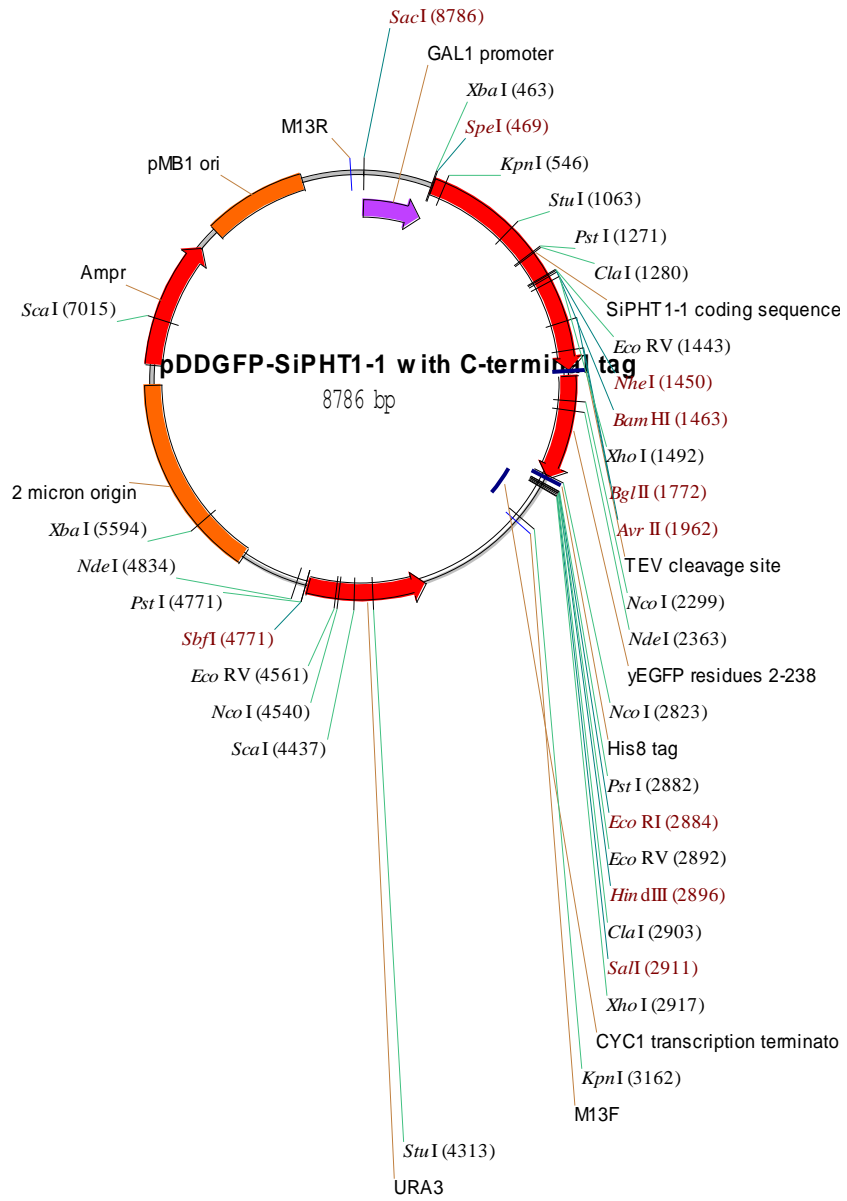
Supplementary Figure S3. Map of the plasmid *pFGC-SiPHT1;4-3'UTR-RNAi*.



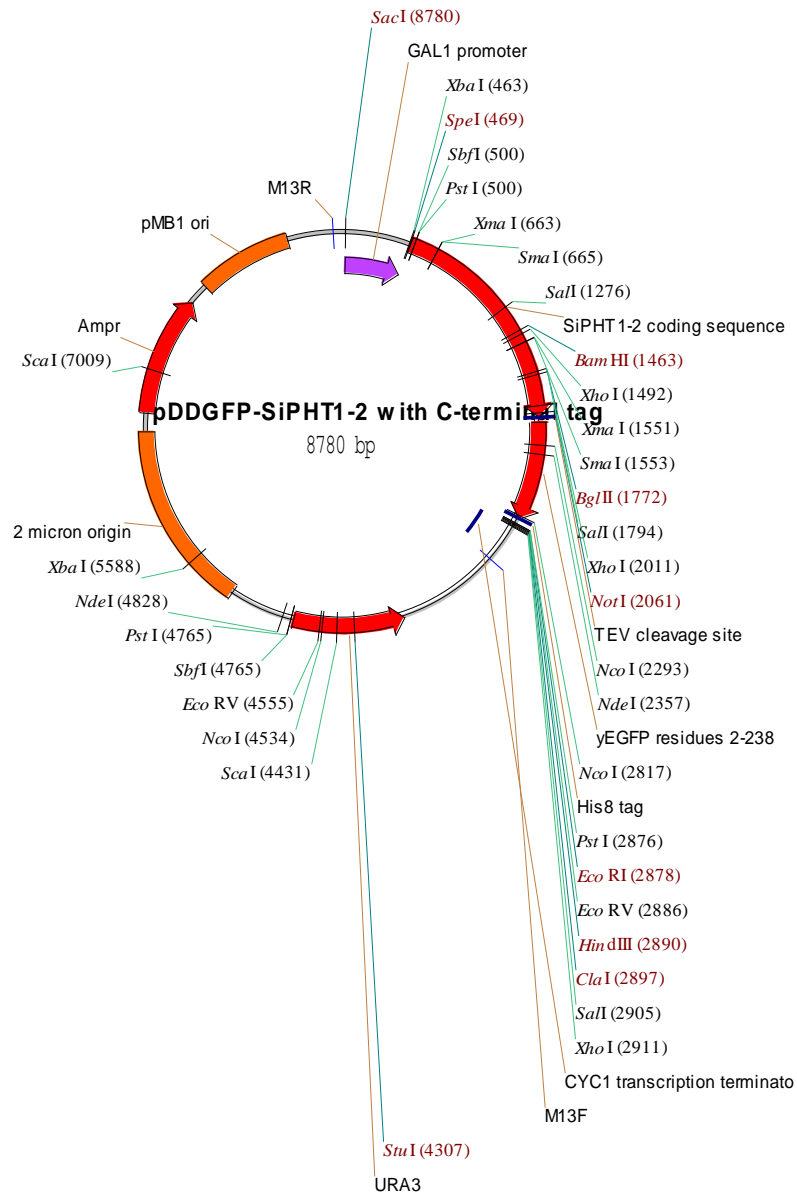
Supplementary Figure S4. Screening of foxtail millet for hygromycin sensitivity. The shoot apex explants were cultured on MS medium containing 0.5 mg/L BAP without hygromycin (A), and 0.5 mg/L BAP with 5.0 mg/L (B), 10.0 mg/L (C), 15.0 mg/L (D), 20.0 mg/L (E) and 25.0 mg/L (F) hygromycin. The response was noted one week after culture in the light. The explants cultured in the medium containing 20 mg/L hygromycin (E) or above (F) showed 100% mortality.



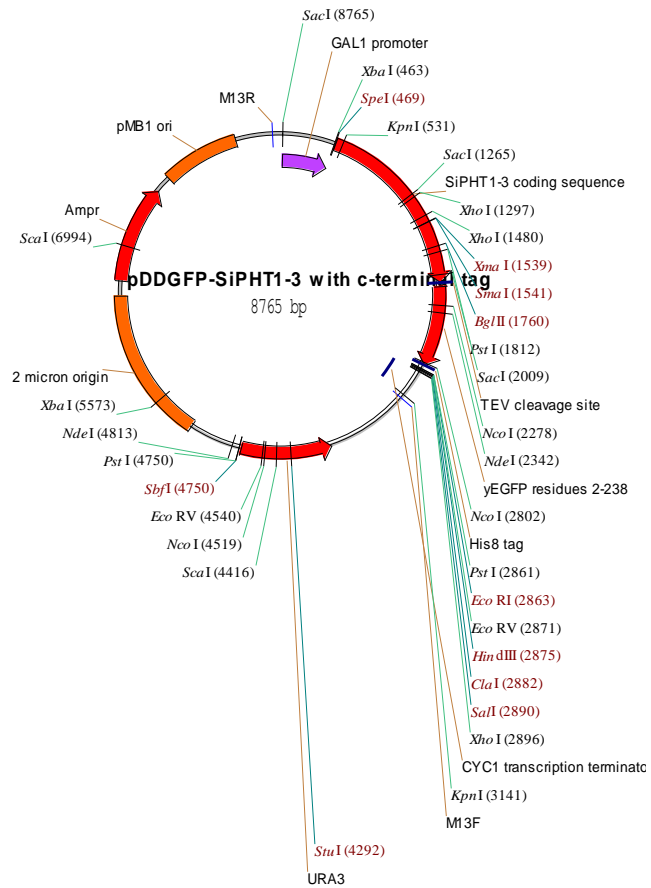
Supplementary Figure S5. Map of the yeast expression vector *PDDGFP-2* (parental plasmid) used for cloning coding sequences of SiPHT1 and PHO84 for yeast complementation assay. The transporters were cloned Gal1 promoter and YGFP regions using *SpeI/AgeI* sites for SiPHT1 transporters and *SpeI/XmaI* sites for PHO84 transporter. The plasmid has Ampr region for bacterial selection and URA3 for yeast selection.



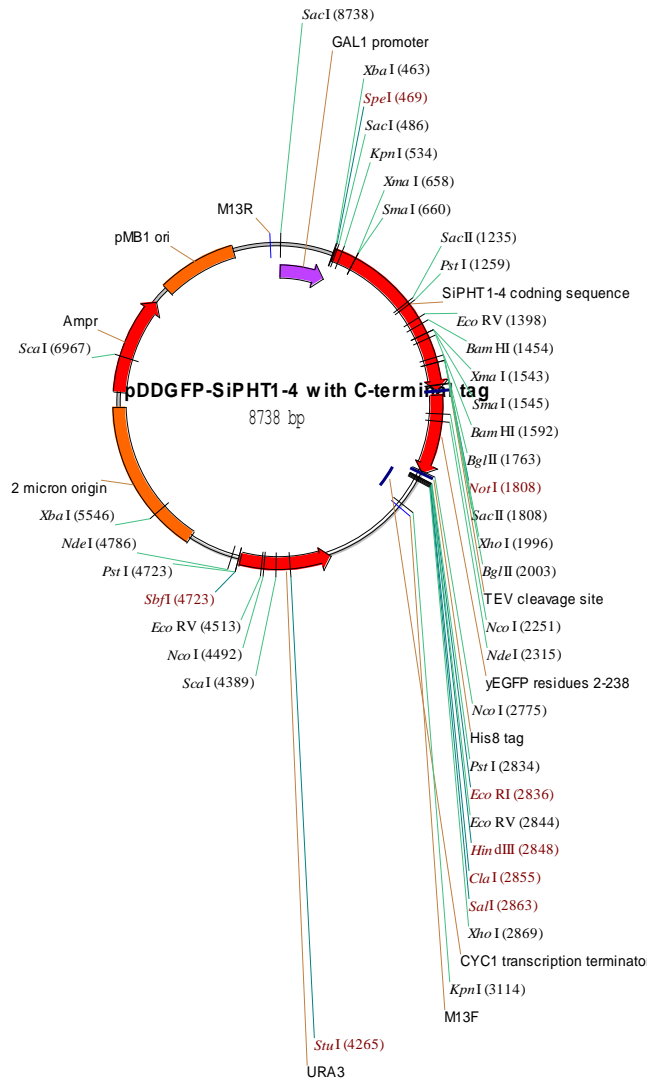
Supplementary Figure S6. Map of the plasmid *pDDGFP-SiPHT1;1*



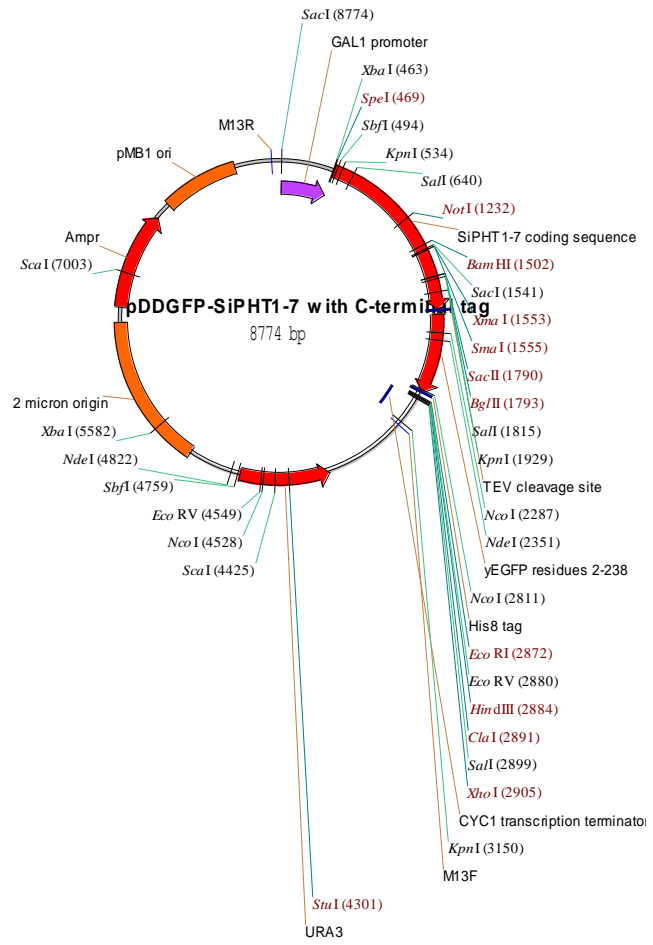
Supplementary Figure S7. Map of the plasmid *pDDGFP-SiPHT1;2*



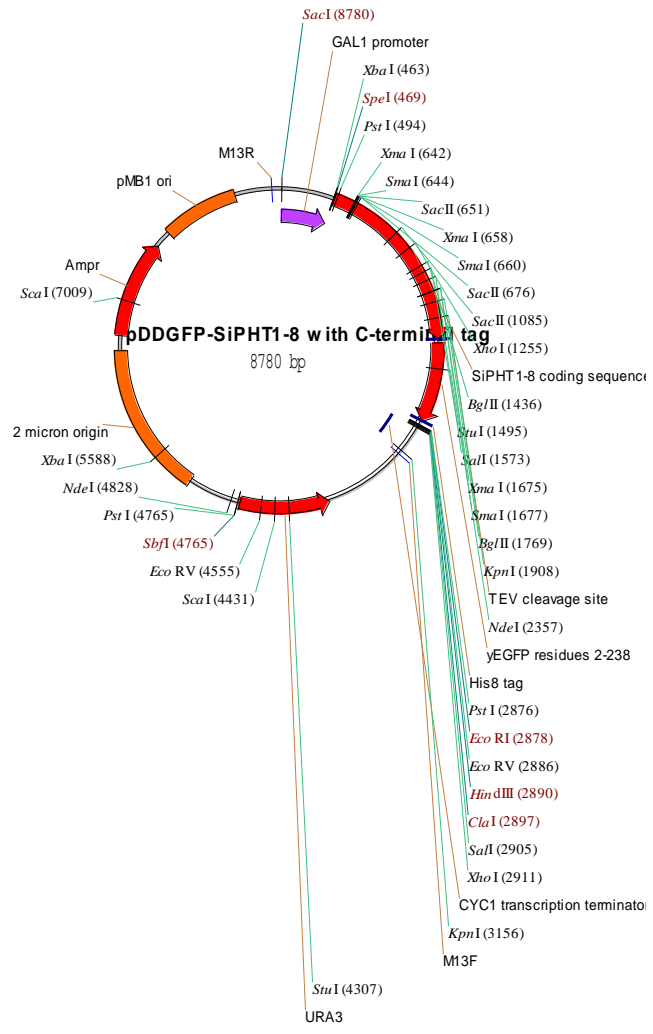
Supplementary Figure S8. Map of the plasmid *pDDGFP-SiPHT1;3*



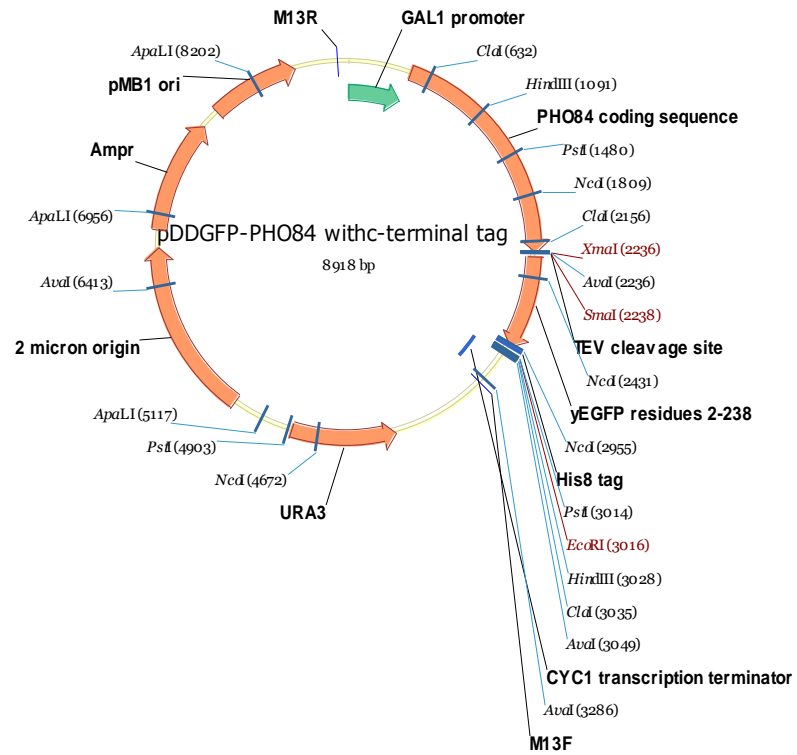
Supplementary Figure S9. Map of the plasmid *pDDGFP-SiPHT1-4*



Supplementary Figure S10. Map of the plasmid *pDDGFP-SiPHT1-7*



Supplementary Figure S11. Map of the plasmid *pDDGFP-SiPHT1-8*



Supplementary Figure S12. Map of the plasmid *pDDGFP-PHO84*

SiPHT1;2 3'UTR sequence used for targeting through RNAi

GGTGTAGTATGACCGTCCGTGGTGATTGGTGATACGTGTAGGCCGGTTCACTTGTTTTTCGTT
TTCCATGTAGAAAGTCAAACCTGCTGTTTCACATGGGCATCTGTTATTTTTATCTCTATATA
AAATATAAAAAAGAAAATATCAAGTACACAAATACATTGGTGAACATACCACGC

SiPHT1;3 coding sequence used for targeting through RNAi

CAAGACGGATGCCGGTTACCCGCCAGGCATCGGCGTGCGCAACTCACTGTTCATGCTCGCCG
GATGCAATGTCATCGGGTTCTTGTTCACGTTCCCTTGTGCCGGAGTCCAAGGGAAAGTCGCTG
GAGGAGCTCTCCGGCGAGAACGACGAGGAGGCAGCACCTGGCCAGAGCATCCAGCAGACTGT
TCCGAC

SiPHT1;4 3'UTR sequence used for targeting through RNAi

CAGAGTCTAGGGGCTGCAGCTCCCCCACACACTTTCTGCGCGCGTGCCCTACATGATGCAC
GGATGGTTTTTCAGGTTCTGTTTGTATGCTTGACTGTGTCCTTGTGTGGTTTACATCATACTC
CTACCTTCCGATTTATTGCATTCTGTGTGATATTTGAAAATATTTTTGGGTAATTTCTCCAC
TGAGAATTTTCGTTTGTGCTAGTGTGTTGTGTCTGATTGACCGACATTCTTATCAATGAATAAA
AAGCTGTTGTACTTCAGCATTCCC

Supplementary Figure S13. Details of gene sequences used for the development of RNAi plasmids for foxtail millet transporters SiPHT1;2, SiPHT1;3 and SiPHT1;4. The binding regions of forward and reverse primers are indicated in blue and red respectively.