

Additional File 1

In-silico* genome wide identification, expressional profiling and regulation of NHX (Sodium/ Hydrogen Antiporter) gene family in *C. sinensis

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Table S1. BLAST positives table for NHX genes in *C. sinensis*. Protein sequences from Arabidopsis genome database (TAIR) were taken and subjected to BLASTp in the Tea genome database (TPIA).

Arabidopsis NHX gene IDs	Tea NHX gene IDs	Identities	Positives	Gaps
AT5G27150.1 (AtNHX1)	TEA012938.1	74%	85%	0%
	TEA012286.1	73%	84%	0%
	TEA021179.1	75%	87%	2%
	TEA012245.1	63%	76%	10%
	TEA000661.1	64%	76%	12%
	TEA025916.1	65%	79%	1%
AT3G05030.1 (AtNHX2)	TEA012938.1	76%	86%	1%
	TEA012286.1	75%	85%	1%
	TEA021179.1	76%	87%	3%
	TEA012245.1	66%	75%	10%
	TEA000661.1	65%	76%	12%
	TEA025916.1	64%	79%	1%
AT5G55470.1 (AtNHX3)	TEA012938.1	60%	75%	2%
	TEA012286.1	58%	73%	2%
	TEA023041.1	58%	69%	17%
	TEA025916.1	58%	74%	3%
	TEA021179.1	59%	75%	3%
	TEA012245.1	53%	65%	12%
AT3G06370.1 (AtNHX4)	TEA012938.1	75%	83%	3%
	TEA012286.1	74%	83%	0%
	TEA021179.1	73%	83%	3%
	TEA012245.1	61%	72%	12%
	TEA000661.1	58%	71%	12%
	TEA025916.1	66%	81%	1%
AT1G54370.1 (AtNHX5)	TEA012938.1	31%	50%	8%
AT1G79610.1 (AtNHX6)	TEA011468.1	67%	76%	16%
AT2G01980.1 (AtNHX7)	TEA006997.1	62%	75%	4%
AT1G14660.1 (AtNHX8)	TEA006997.1	68%	81%	1%

Table S2. Function specific list of cis-acting elements. These elements have been identified from 2 kbp upstream region of all the identified NHX genes of *C. sinensis*.

Sl. no.	Cis-acting element identified	Sequence	Tea genes	Specific function of the cis element
1.	A-box	CCGTCC	TEA012938.1/TEA006997.1	cis-acting regulatory element
2.	ABRE	CGTACGTGCA/ACGTG/CACGTG	TEA012938.1/TEA012286.1/TEA012245.1/TEA000661.1/TEA025916.1/TEA011468.1	abscisic acid responsiveness
3.	ARE	AAACCA	TEA012938.1/TEA012286.1/TEA021179.1/TEA012245.1/TEA000661.1/TEA025916.1/TEA011468.1	anaerobic induction
4.	Box-4	ATTAAT	TEA012938.1/TEA012286.1/TEA021179.1/TEA012245.1/TEA000661.1/TEA025619.1/TEA011468.1/TEA006997.1	light responsiveness
5.	CCAAT-box	CAACGG	TEA012938.1/TEA021179.1/TEA000661.1/TEA011468.1/TEA006997.1	MYBHv1 binding site
6.	G-box	TACGTGtgACACGTGGCA/ CACGTT/ TCCACATGGCA/ CACGAC/ GCCACGTGGA / CTCCACGTGGCA	TEA012938.1/TEA012286.1/TEA012245.1/TEA000661.1/TEA025619.1/TEA011468.1	light responsiveness
7.	GARE-motif	TCTGTTG	TEA012938.1/TEA000661.1	gibberellin-responsive element
8.	TCT-motif	TCTTAC	TEA012938.1	light responsive element
9.	chs-CMA2a	TCACTTGA	TEA012938.1/TEA012286.1/TEA011468.1	light responsive element
10.	AE-box	AGAAACAA	TEA012286.1/TEA023041.1	light response
11.	CGTCA-motif	CGTCA	TEA012286.1/TEA012245.1/TEA025619.1/TEA011468.1	MeJA-responsiveness
12.	LTR	CCGAAA	TEA012286.1/TEA012245.1	low-temperature responsiveness
13.	MRE	AACCTAA	TEA012286.1/TEA000661.1/TEA011468.1	light responsiveness
14.	O2-site	GATGA(C/T)(A/G)TG(A/G)	TEA012286.1/TEA012245.1/TEA011468.1/TEA006997.1	zein metabolism regulation
15.	TCCC-motif	TCTCCCT	TEA012286.1/TEA021179.1/TEA012245.1/TEA000661.1	light responsive element
16.	TGACG-motif	TGACG	TEA012286.1/TEA012245.1/TEA025619.1/TEA011468.1	MeJA-responsiveness
17.	GATA-motif	GATAGGG/ AAGATAAGATT	TEA021179.1/TEA012245.1/TEA025619.1/TEA023041.1/TEA006997.1	light responsive element
18.	GCN4_motif	TGAGTCA	TEA021179.1/TEA000661.1/TEA023041.1	endosperm expression
19.	Gap-box	CAAATGAA(A/G)A	TEA021179.1	light responsive element
20.	HD-Zip 1	CAAT(A/T)ATTG	TEA021179.1/TEA023041.1/TEA006997.1	differentiation of the palisade mesophyll cells
21.	I-box	gGATAAGGTG	TEA021179.1	light responsive element
22.	TATC-box	TATCCA	TEA021179.1/TEA000661.1/TEA006997.1	gibberellin-responsiveness
23.	AAAC-motif	CAATCAAACCT	TEA012245.1	light responsive element
24.	TC-rich repeats	GTTTTCTTAC	TEA012245.1/TEA025619.1/TEA011468.1	defense and stress responsiveness
25.	TCA-element	CCATCTTTTT	TEA012245.1/TEA000661.1/TEA025619.1/TEA023041.1/TEA011468.1	salicylic acid responsiveness
26.	TCT-motif	TCTTAC	TEA012245.1/TEA025619.1/TEA023041.1/TEA011468.1	light responsive element
27.	chs-CMA1a	TTACTTAA	TEA012245.1/TEA025619.1/TEA006997.1	light responsive element
28.	CAT-box	GCCACT	TEA000661.1	meristem expression
29.	MBS	CAACTG	TEA000661.1	drought-inducibility
30.	TGA-element	AACGAC	TEA000661.1	auxin-responsive element

31.	Circadian	CAAAGATATC	TEA000661.1	circadian control
32.	AT1-motif	AATTATTTTTATT	TEA025619.1/ TEA006997.1	light responsive element
33.	Box II	CCACGTGGC	TEA025619.1	light responsive element
34.	GT1-motif	GGTTAA	TEA025619.1/ TEA023041.1/ TEA011468.1	light responsive element
35.	AT-rich sequence	TAAAATACT	TEA023041.1	maximal elicitor-mediated activation
36.	AuxRR-core	GGTCCAT	TEA023041.1	auxin responsiveness
37.	GA-motif	ATAGATAA	TEA023041.1	light responsive element
38.	P-box	CCTTTTG	TEA023041.1/ TEA006997.1	gibberellin-responsive element
39.	AT-rich element	ATAGAAATCAA	TEA011468.1/ TEA006997.1	binding site of AT-rich DNA binding protein
40.	ATCT-motif	AATCTAATCC	TEA011468.1	Light responsiveness
41.	MSA-like	(T/C)(T/C)AACGG(T/C)(T/C)A	TEA011468.1	ell cycle regulation

Table S3. dN/dS ratios of 9 NHX genes in *C. sinensis*. SNAP v2.1.1 Synonymous Non-synonymous Analysis Program was used to analyse the dN/dS ratios. This ratio is useful to understand the positive or negative selection of the genes.

Compare	Sequence names	Sd	Sn	S	N	ps	pn	ds	dn	dn/ds	ds/dn	ps/pn
0 1	TEA012938.1 TEA012286.1	862.8333	3382.167	1220.833	4692.167	0.7068	0.7208	2.1399	2.4347	1.1378	0.8789	0.9805
0 2	TEA012938.1 TEA021179.1	1462.167	5786.833	2061.167	7991.833	0.7094	0.7241	2.187	2.5242	1.1542	0.8664	0.9797
0 3	TEA012938.1 TEA012245.1	1266.5	4946.5	1758.333	6767.667	0.7203	0.7309	2.4213	2.7529	1.137	0.8796	0.9855
0 4	TEA012938.1 TEA000661.1	1384.333	5460.667	1960.333	7522.667	0.7062	0.7259	2.1299	2.5782	1.2105	0.8261	0.9728
0 5	TEA012938.1 TEA025916.1	1086.333	4473.667	1563	6216	0.695	0.7197	1.96	2.4067	1.228	0.8144	0.9657
0 6	TEA012938.1 TEA023041.1	1531.333	5795.667	2130.667	7922.333	0.7187	0.7316	2.3826	2.7792	1.1664	0.8573	0.9824
0 7	TEA012938.1 TEA011468.1	488.6667	1809.333	674.6667	2520.333	0.7243	0.7179	2.5304	2.3633	0.9334	1.0707	1.0089
0 8	TEA012938.1 TEA006997.1	1480	5750	2068.333	7984.667	0.7156	0.7201	2.3105	2.4174	1.0463	0.9558	0.9936
1 2	TEA012286.1 TEA021179.1	870	3498	1234.833	4678.167	0.7045	0.7477	2.1026	4.3498	2.0688	0.4834	0.9423
1 3	TEA012286.1 TEA012245.1	914	3416	1247.167	4665.833	0.7329	0.7321	2.834	2.8027	0.989	1.0112	1.001
1 4	TEA012286.1 TEA000661.1	919.5	3375.5	1238.667	4674.333	0.7423	0.7221	3.4371	2.4695	0.7184	1.3918	1.028
1 5	TEA012286.1 TEA025916.1	885.1667	3391.833	1210.5	4702.5	0.7312	0.7213	2.7663	2.4469	0.8845	1.1305	1.0138
1 6	TEA012286.1 TEA023041.1	907.6667	3372.333	1244.833	4668.167	0.7291	0.7224	2.6869	2.477	0.9107	1.0848	1.0093
1 7	TEA012286.1 TEA011468.1	495.3333	1821.667	685.5	2509.5	0.7226	0.7259	2.4818	2.5787	1.039	0.9624	0.9954
1 8	TEA012286.1 TEA006997.1	900.5	3348.5	1231.167	4681.833	0.7314	0.7152	2.7735	2.3031	0.8304	1.2043	1.0227
2 3	TEA021179.1 TEA012245.1	1227.167	4960.833	1751.333	6774.667	0.7007	0.7323	2.0417	2.8083	1.3755	0.727	0.9569
2 4	TEA021179.1 TEA000661.1	1366	5457	1943.333	7539.667	0.7029	0.7238	2.0761	2.5149	1.2113	0.8255	0.9712
2 5	TEA021179.1 TEA025916.1	1135.833	4443.167	1571.167	6207.833	0.7229	0.7157	2.4911	2.3145	0.9291	1.0763	1.01
2 6	TEA021179.1 TEA023041.1	2594.667	9668.333	3561.833	13394.17	0.7285	0.7218	2.6628	2.4614	0.9244	1.0818	1.0092
2 7	TEA021179.1 TEA011468.1	505.3333	1832.667	691.1667	2503.833	0.7311	0.7319	2.7619	2.795	1.0119	0.9882	0.9989
2 8	TEA021179.1 TEA006997.1	3142.833	12411.17	4396.667	17074.33	0.7148	0.7269	2.2947	2.6099	1.1374	0.8793	0.9834
3 4	TEA012245.1 TEA000661.1	1215.667	4763.333	1763.333	6762.667	0.6894	0.7044	1.887	2.0994	1.1125	0.8988	0.9788
3 5	TEA012245.1 TEA025916.1	1168.333	4496.667	1583	6196	0.7381	0.7257	3.1045	2.5733	0.8289	1.2064	1.017

3 6	TEA012245.1 TEA023041.1	1302	4904	1789.167	6736.833	0.7277	0.7279	2.6371	2.6447	1.0029	0.9971	0.9997
3 7	TEA012245.1 TEA011468.1	493.3333	1831.667	693.5	2501.5	0.7114	0.7322	2.2245	2.8068	1.2618	0.7925	0.9715
3 8	TEA012245.1 TEA006997.1	1292.333	4860.667	1768.5	6757.5	0.7308	0.7193	2.747	2.3968	0.8725	1.1461	1.0159
4 5	TEA000661.1 TEA025916.1	1115	4483	1577	6202	0.707	0.7228	2.1448	2.4885	1.1602	0.8619	0.9782
4 6	TEA000661.1 TEA023041.1	1421.5	5444.5	2002.167	7480.833	0.71	0.7278	2.198	2.6398	1.201	0.8327	0.9755
4 7	TEA000661.1 TEA011468.1	485	1828	678.5	2516.5	0.7148	0.7264	2.2945	2.5943	1.1307	0.8844	0.984
4 8	TEA000661.1 TEA006997.1	1383.667	5404.333	1958.667	7524.333	0.7064	0.7182	2.1343	2.3716	1.1112	0.9	0.9836
5 6	TEA025916.1 TEA023041.1	1162	4411	1602	6177	0.7253	0.7141	2.5613	2.2795	0.89	1.1236	1.0157
5 7	TEA025916.1 TEA011468.1	478.6667	1808.333	668.6667	2526.333	0.7159	0.7158	2.317	2.3157	0.9994	1.0006	1.0001
5 8	TEA025916.1 TEA006997.1	1109.833	4434.167	1583	6196	0.7011	0.7156	2.0476	2.3126	1.1294	0.8854	0.9797
6 7	TEA023041.1 TEA011468.1	504.1667	1813.833	689.8333	2505.167	0.7309	0.724	2.7509	2.5226	0.917	1.0905	1.0094
6 8	TEA023041.1 TEA006997.1	2623	9721	3612	13344	0.7262	0.7285	2.5875	2.6637	1.0294	0.9714	0.9968
7 8	TEA011468.1 TEA006997.1	455.8333	1843.167	670.5	2524.5	0.6798	0.7301	1.777	2.7225	1.5321	0.6527	0.9311

Table S4. The GO enrichment analysis of all the 9 NHX genes of *C. sinensis*. The potential functions of the genes are divided in 3 major categories based on their biological function, cellular component and molecular function.

BIOLOGICAL PROCESS			
GO Ids	Description	Frequency of genes	Genes
GO:0055067	Monovalent inorganic cation homeostasis	3	TEA012938.1, TEA012286.1, TEA025916.1
GO:1902600	Proton transmembrane transport	6	TEA012938.1, TEA012286.1, TEA021179.1, TEA012245.1, TEA000661.1, TEA006997.1
GO:0035725	Sodium ion transmembrane transport	5	TEA012938.1, TEA012286.1, TEA021179.1, TEA012245.1, TEA000661.1
GO:0055075	Potassium ion homeostasis	5	TEA012938.1, TEA012286.1, TEA021179.1, TEA012245.1, TEA000661.1
GO:0006885	Regulation of pH	5	TEA012938.1, TEA012286.1, TEA021179.1, TEA012245.1, TEA000661.1
GO:0009651	Response to salt stress	5	TEA012938.1, TEA012286.1, TEA021179.1, TEA012245.1, TEA000661.1
GO:0030001	Metal ion transport	3	TEA012938.1, TEA012286.1, TEA025916.1
GO:0042794	rRNA transcription from plastid promoter	1	TEA021179.1
GO:0006355	Regulation of transcription, DNA-templated	1	TEA021179.1
GO:0008380	RNA splicing	1	TEA021179.1
GO:0009793	Embryo development ending in seed dormancy	1	TEA021179.1
GO:0006812	Cation transport	2	TEA012245.1, TEA006997.1
GO:0006811	Ion-transport	1	TEA000661.1
CELLULAR COMPONENT			
GO Ids	Description	Number of genes	Genes
GO:0031224	Intrinsic component of membrane	3	TEA012938.1, TEA012286.1, TEA025916.1

GO:0005774	Vacuolar membrane	5	TEA012938.1, TEA012286.1, TEA021179.1, TEA012245.1, TEA000661.1
GO:0016021	Integral component of membrane	6	TEA012938.1, TEA012286.1, TEA021179.1, TEA012245.1, TEA000661.1, TEA006997.1
GO:0005886	Plasma membrane	5	TEA012938.1, TEA012286.1, TEA021179.1, TEA012245.1, TEA000661.1
GO:0009536	Plastid	1	TEA021179.1
GO:0005739	Mitochondrion	1	TEA021179.1
MOLECULAR FUNCTION			
GO Ids	Description	Number of genes	Genes
GO:0005451	Monovalent cation:proton antiporter activity	3	TEA012938.1, TEA012286.1, TEA025916.1
GO:0015385	Sodium:proton antiporter activity	5	TEA012938.1, TEA012286.1, TEA021179.1, TEA012245.1, TEA000661.1
GO:0003690	Double-stranded DNA binding	1	TEA021179.1
GO:0015299	Solute:proton antiporter activity	2	TEA012245.1, TEA006997.1
GO:0015297	Antiporter activity	1	TEA000661.1

Table S5. Tissue specific expression data of tea NHXs

Gene	Apical bud	Flower	Fruit	Young leaf	Mature leaf	Old leaf	Root	Stem
TEA012938.1	43.76413	121.8748	139.7705	65.48526	62.31895	89.1654	123.3367	89.58724
TEA012286.1	13.32334	19.94589	81.18094	19.25734	195.6898	154.8273	20.42718	38.48513
TEA021179.1	6.507971	5.212446	6.059097	6.496247	18.67248	11.14241	3.025886	6.226808
TEA012245.1	1.826799	3.354445	3.280869	2.031149	5.085746	1.680067	5.336918	6.342119
TEA000661.1	39.60114	52.39562	30.45085	29.85017	45.4933	79.72917	108.1191	44.66389
TEA025916.1	11.08375	9.490869	8.965271	6.847627	18.37551	12.92694	0.793193	11.76175
TEA023041.1	0.202002	0.281211	0.027417	0.239967	0.296978	0.374315	0.19585	0
TEA011468.1	3.117854	10.61571	6.342403	3.530942	6.570635	6.537461	4.768953	6.447821
TEA006997.1	3.776556	4.35877	7.137489	4.473669	6.041643	1.706182	8.539069	10.8873

Table S6. Expression data of tea NHXs under cold stress

Gene	CK	CA1-6h	CA1-7d	CA2-7d	DA-7d
TEA012938.1	29.45019	72.85738	75.17316	96.87901	55.52682
TEA012286.1	126.4097	131.4313	180.7668	107.6854	73.9297
TEA021179.1	16.90728	19.83296	28.62055	22.15093	15.32244
TEA012245.1	3.33917	3.08202	1.960717	2.282841	1.012626
TEA000661.1	50.84357	81.24412	74.82189	118.5099	39.75186
TEA025916.1	18.55783	12.11618	18.69256	15.13589	12.69169
TEA023041.1	0.037597	0.119941	0.163987	0.041164	0
TEA011468.1	7.288367	8.120182	14.04962	12.14041	12.64389
TEA006997.1	8.882562	11.54806	6.637157	3.834754	15.04385

Table S7: Expression data of the tea NHXs under drought stress

Gene	N-0h	PEG-N-24h	PEG-N-48h	PEG-N-72h
TEA012938.1	38.15789	106.9188	97.85543	165.0236
TEA012286.1	75.64702	24.13241	61.51073	28.75782
TEA021179.1	10.77421	3.648387	6.541141	3.283459
TEA012245.1	1.336108	2.915556	2.109869	1.71389
TEA000661.1	40.86235	69.21883	59.24442	81.5388
TEA025916.1	8.871773	3.104651	4.656081	2.715445
TEA023041.1	0	0.558879	0.357562	0.458908
TEA011468.1	6.333739	15.93189	16.78334	17.68513
TEA006997.1	2.21532	1.140223	1.441681	1.182608

Table S8: Expression data of the tea NHXs under salt stress

Gene	N-0h	NaCl-N-24h	NaCl-N-48h	NaCl-N-72h
TEA012938.1	38.1579	72.25577	99.93413	192.6962
TEA012286.1	75.64704	23.65101	35.82729	7.036825
TEA021179.1	10.77421	5.110744	5.70018	3.065808
TEA012245.1	1.336108	0.951984	1.237334	0.879937
TEA000661.1	40.86238	22.79735	32.173	22.55661
TEA025916.1	8.871778	1.774618	3.725586	2.337885
TEA023041.1	0	0.066448	0.458791	0.211543
TEA011468.1	6.333737	15.48246	15.18481	9.151531
TEA006997.1	2.21532	1.802493	1.944796	0.327543

Table S9. Expression data of the tea NHXs under MeJA treatment

Gene	CK	12h_MeJA	24h_MeJA	48h_MeJA
TEA012938.1	34.19849	41.5685	59.2445	51.77012
TEA012286.1	42.00289	59.01399	41.90896	45.30166
TEA021179.1	14.52917	13.38986	13.45105	12.41783
TEA012245.1	1.586705	2.463537	2.125546	1.986136
TEA000661.1	42.73238	48.82838	44.49009	75.10711
TEA025916.1	15.31124	12.53217	15.59798	17.83681
TEA023041.1	0.041174	0.095074	0.07173	0
TEA011468.1	15.59616	23.34664	22.99138	11.62203
TEA006997.1	11.24856	13.71702	13.93264	8.334638