Genome Structures and Halophyte-Specific Gene Expression of the Extremophile *Thellungiella parvula* in Comparison with *Thellungiella salsuginea* (*Thellungiella halophila*) and Arabidopsis

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translation start site.

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Supplemental Figure S1. Comparison of relative DNA contents by flow cytometry. Flow cytometry was performed as described in the method. Relative values to Arabidopsis are represented. Error bars indicate standard deviation from three repeats.



Position in scaffold 00254 (kb)



(A)



Supplemental Figure S2. Analysis of scaffold 00254.

Distribution of repetitive sequences (A) and ORFs/coding sequences (B) predicted by Repeatmasker and FGENESH, respectively, in scaffold 00254.

(C) Homology of predicted ORFs in scaffold 00254 compared to *A. thaliana* reference mRNAs. Total identity was calculated by multiplying percentage of the *T. parvula* predicted ORF sequence covered by the blastn alignment with percent nucleotide identity.

(C)



Supplemental Figure S3. Comparison between *T. parvula* genomic sequences TP-1 and TP-2 with three orthologous genomic regions in *A. thaliana* and *T. salsuginea*.

AT-1 (A) and AT-2 (B) are from *A. thaliana* and TS-1 (C), TS-2 (D) from *T. salsuginea*. Comparisons were made using dot-plot analyses with word size 7. In (A) and (B), boxes indicate regions where linearity is broken. In (C), a and b denote an inversion and the extent of the collinear region, respectively.





Supplemental Figure S4. Syntenic relationships and homologies of sequences represented by percent identity plots (pip).

TP-1 (A) and TP-2 (B) were compared to their orthologous regions in *A. thaliana* and *T. salsuginea* using PiPmaker plot with a word size of 7 (Schwartz et al., 2000). Predicted exons for the ORFs in TP sequences and the direction of transcription are indicated. Dashed box in (A) denote the intergenic space between SOS1 and its 5' neighboring gene. For the explanation of symbols (a-c) see the text.

| (a) TpSOS1 | U A | 7 |
|--|-----------|-----------------|
| | G | U |
| | A | С |
| | C-0 | |
| | U-A | A |
| | U-A | ł |
| | C-0 | 5 |
| | U-A | ł |
| | U-A | ł |
| cnncncnncnncnncnncnncnncnncn | UAGUUGU-A | AUG |
| | Δ | A. |
| (b) TsSOS1 | IJ | ŢŢ |
| | A | U |
| | A | С |
| | C- | -G |
| | U- | -A |
| | U- | -A |
| | C- | -G |
| CUUCUUCUUCAUCGUCAUCAUCAUCAUCAUCAUCAUCAUCAUCAUCAUCAUCAU | AUCAUCAU- | -AAA aug |

(c) AtSOS1

Supplemental Figure S5. Hairpin structures in the 5'UTR of the halophytic *Thellungiella* species.

Hairpin structures that can potentially form adjacent to ATG in the 5'UTR of (a) *T. salsuginea* and (b) *T. parvula*. No such structure exists in *A. thaliana* (c). The ATG codon is located at the 3' end of the sequence. The structures are predicted using RNAfold (Gruber et al., 2008).

| AT | TP | TS | AT | TP | TS | VPI | TS | TP | AT | Сус | AT | TP | TS | HSC | |
|------------------------------|------------------------------|------------------|---|--|--|-----|--|--|------------------------------------|-------------|---|---|---|-------|--|
| А ТАGGAATTAACTAGGAATG | TP TTCCTTTCCTTCCTCCTCCTCCATG | TSGGAGGAGAGAGATG | AT =T G T G A A T A A A T G G T A T G G T A T A A C A T C A C C T T G G A T A A C C T T G G A T A C A T A A C A T A C A T G G A T G G A T A A C A T A C A C C T T G G A T A C A T A C A T A G G A T C C A T G G A T A G C C T T T G G A T A G C C T T C A G C C T T T G G A T C A C C T T C C C A T C C C T T C C C C T T C C C C T T C C C C T T C C C C T T C C C C T T C C C C T T C C C C T C C C C T C C C T C C C C T C C C C C C C C | TP CCCACCACTTCACTACTACTTCCCCCTTTCTTCTTCCCCTTTCATCA | TS GCCTTCATTTCATCATCTCGTCGAAAACACTTCCCTTCC | VP1 | TSCCACGCGTCCGAATTTTTTCTCCGATCCAAAAAAAAAA | TP TTCCACTACTTCGCGTTCCCTCCTCCCTCCGTGATCTACTGCTGCTTCTCCCCTAATCTCACTCTCACACAAATCTTCTCCGATCTCAAAGAAAATG | ATCACACAATCAAACTAAAAAAAAAAAAAAAAAA | Cyclophilin | at ggaaaaaaagaaaacctaacctagccgcaattcactrttccttttcacaatctttccattttcattttcattttcattttcatttttt | TPCCCTAGCCGCCCTCTCGTCTTCTTCATTCTCATTCTTCAGACTCTGTTCTTCGATCTCTTGTAGTTTAA-TCTTTCCGATAAAAATG | TSGTTCTTGATCTCTTGATCTCTTGATCTTTAAATCTTTCCGATAAAAATG | HSC70 | |
| | | | | | | | | | | | | | | | |

gray. The initiation codon ATG is at the 3' end of each sequence. AT reference numbers and TS GenBank accession numbers include HSC70 (AT3G09440 and AY524794), Cyclophilin (AT2G16600 and AY392408), and VP1 (AT4G21550 and GU253905). 5'UTR sequence alignments of selected gene sequences related to abiotic tress. The (CnTn)n repeats are highlighted in Supplemental Figure S6. Comparison of CT tracts in the 5'UTRs of various genes between the three species

| TP-1 ID ^a | Evalue to AT^{b} | Evalue to TS ^b | AT-1 ID ^c | TS-1 ID ^c | Homologous AT locus | AT annotation |
|----------------------|---------------------|---------------------------|----------------------|----------------------|----------------------|--|
| Tp01(+) | 5E-130 | 3E-125 | At01 (+) | Th01 (+) | AT2G01900 | Putative phosphatase family protein |
| Tp02(+) | 6E-60 | 1E-64 | At02 (+) | Th02 (+) | AT2G01905 | CYCJ18; cyclin |
| Tp03(+) | 0 | 0 | At03 (+) | Th03 (+) | AT2G01910 | ATMAP65-6; microtubule binding |
| Tp04(-) | 8E-35 [*] | 2E-38 [*] | At04 (-) | Th04 (-) | AT2G01913 | unknown protein |
| - | - | - | At_a (-) | - | AT2G01918 | calcium ion binding |
| Tp05(+) | 4E-90 | - | At_b (+) | - | AT2G01920 | clathrin assembly protein-related |
| Тр06(-) | 8E-80 | 1E-34 [*] | At05 (-) | Th05 (-) | AT2G01930 | BPC1; transcription factor |
| Tp07(+) | 7E-151 | 2E-149 | At06 (+) | Th06 (+) | AT2G01940 | transcription factor/ zinc ion binding |
| Тр08(-) | 0 | 0 | At07 (-) | Th07 (-) | AT2G01950 | BRL2; transmembrane receptor kinase |
| - | - | - | At_c (+) | - | AT2G01960 | TET14; function unknown |
| Тр09(-) | 0 | 0 | At08 (-) | Th08 (-) | AT2G01970 | endomembrane protein 70, putative |
| Tp10(+) | 0 | 0 | At09 (+) | Th09 (+) | AT2G01980 | SOS1; sodium:hydrogen antiporter |
| Tp11(-) | 4E-83 | 5E-97 | At10 (-) | Th10 (-) | AT2G01990 | unknown protein |
| - | - | - | - | Th_A (+) | (<u>AT1G14610</u>) | TWN2; valine-tRNA ligase |
| Tp12(-) | 0 | 0 | At11a (-) | Th11 (-) | AT2G02000 | GAD3; glutamate decarboxylase |
| Tp13(-) | 0 | 0 | At11b (-) | - | AT2G02010 | GAD4;glutamate decarboxylase |
| Tp14(+) | 0 | 0 | At12 (+) | Th12 (+) | AT2G02020 | oligopeptide transport-related |
| - | - | - | At13 (+) | Th13 (-) | AT2G02030 | F-box family protein |
| Tp15(+) | 0 | 0 | At14 (+) | Th14 (+) | AT2G02040 | PTR2; peptide transporter |
| Tp16(+) | 7E-59 [*] | 8E-19 | At15 (+) | Th15 (+) | AT2G02050 | NADH-ubiquinone oxidoreductase |
| Tp17(+) | 8E-57 | 3E-72 | At16 (+) | Th16-1 (+) | AT2G02060 | transcription factor |
| - | - | | - | Th16-2 (+) | AT2G02060 | transcription factor |
| Tp18(+) | 1e-149 [*] | 0 | At_d^d | - | AT2G02061 | unknown protein |
| Tp19(+) | 0 | 0 | At17a (+) | Th17a (+) | AT2G02070 | AtIDD5; transcription factor |
| Tp20(-) | 0 | 0 | At17b (-) | Th17b (-) | AT2G02080 | AtIDD4; transcription factor |
| - | - | - | - | Th_B (+) | (<u>AT2G02030</u>) | F-box family protein |
| Tp21(+) | 0 | 0 | At18 (+) | Th18 (+) | AT2G02090 | ETL1; helicase |
| Tp22(-) | 2E-20 [*] | 1E-24 [*] | AT19a (+) | Th19a (+) | AT2G02100 | LCR69; peptidase inhibitor |
| Tp23(+) | 2E-20 [*] | 9E-25 [*] | - | Th19b (+) | AT2G02100 | LCR69; peptidase inhibitor |
| - | - | - | At19b (+) | - | AT2G02120 | LCR70; peptidase inhibitor |
| - | - | - | At19c (+) | - | AT2G02130 | LCR68; peptidase inhibitor |
| - | - | - | At19d (+) | - | AT2G02135 | LCR71; peptidase inhibitor |
| Tp24(+) | 1E-18 | 1E-23 | At19e (+) | Th19c (+) | AT2G02140 | LCR72; peptidase inhibitor |
| Tp25(+) | 5E-10 | 2E-22 | At19f (+) | Th19d (+) | AT2G02147 | LCR73; peptidase inhibitor |
| Тр26(-) | 0 | 0 | At20 (-) | Th20 (-) | AT2G02150 | pentatricopeptide repeat-containing |

a, c: signs in parentheses denote orientation of DNA strands.

b: results from blastx to the most highly related *A. thaliana* or *T. salsuginea* protein.

c: named as in Nah et al., 2009.

d: not present in Nah et al. 2009; present in TAIR9.

*: homology found only in part of an ORF.

| Supplemental Table S2. | ORFs identified in TP-2, | AT-2 and TS-2. |
|------------------------|--------------------------|----------------|
|------------------------|--------------------------|----------------|

| TP-2 ID ^a | Evalue to AT ^b | Evalue to TS ^b | AT-2 ID ^c | TS-2 ID ^d | Homologous AT locus | AT annotation |
|----------------------|---------------------------|---------------------------|----------------------|----------------------|------------------------|--|
| Tp01(+) | 4E-6 [*] | N/A | At14(-) | Ts01(+) | AT5G66400 | RAB18; responsive to ABA |
| Tp02(+) | 9E-72 | 2E-73 | At13(-) | Ts02(+) | AT5G66390 | PER72; peroxidase |
| Тр03(+) | 3E-150 | 4E-150 | At12(-) | Ts03(+) | AT5G66380 | ATFOLT1; folic acid transporter |
| - | - | - | At11(-) | - | AT5G66370 | unknown protein |
| Тр04(-) | 2E-164 | 3E-125 | At10(-) | Ts04(-) | AT5G66360 | rRNA adenine dimethylase family |
| Тр05(-) | 3E-152 | 2E-147 | - | Ts05(-) | (<u>AT3G53210</u>) | nodulin MtN21 family |
| Tp06(+) | 5E-122 | 2E-123 | At09(-) | Ts06(+) | AT5G66350 | SHI; transcription factor |
| - | - | - | At08(-) | - | AT5G66340 | unknown protein |
| Тр07(-) | 4E-43 [*] | 2E-43 [*] | - | Ts07(-) | (<u>AT1G77370</u>) | glutaredoxin, putative |
| - | - | - | At07(-) | - | AT5G66330 | leucine-rich repeat family protein |
| - | - | - | At06(-) | Ts08(+) | AT5G66320 | zinc finger (GATA type) family protein |
| Tp08(+) | 0 | 0 | At05(-) | Ts09(+) | AT5G66310 | kinesin motor family protein |
| Tp09(+) | 3E-65 | 4E-59 | At04(-) | Ts10(+) | AT5G66300 | NAC105; transcription factor |
| Tp10(+) | 6E-58 | 8E-60 | At03(-) | Ts11(+) | AT5G66290 | unknown protein |
| Tp11(-) | 9E-45 [*] | 2E-44 [*] | At02(-) | Ts12(-) | AT5G66280 | GMD1; GDP-mannose 4,6-dehydratase |
| Tp12(+) | 6E-110 | 4E-125 | At01(-) | Ts13(+) | AT5G66270 | zinc finger (CCCH-type) family protein |
| - | - | - | At15(-) | - | AT5G66410 | PLP3b; beta-tubulin binding |
| Tp13(-) | 0 | 0 | At16(-) | Ts14(-) | AT5G66420 | unknown protein |
| - | - | - | At17(-) | - | AT5G66430 | SAM methyltransferase family |
| Tp14(-) | 2E-71 | 1E-72 | At18(-) | Ts15(-) | AT5G66440 | unknown protein |
| Tp15(+) | 2E-76 | 3E-86 | At19(-) | Ts16(+) | AT5G66450 | phosphatidic acid phosphatase-related |
| Tp16(-) | 8E-158 | 4E-159 | - | Ts17(-) | (<u>AT3G47840</u>) | pentatricopeptide repeat-containing |
| Tp17(-) | 0 | 0 | At20(-) | Ts18(-) | AT5G66460 | (1-4)-beta-mannan endohydrolase |
| Tp18(-) | 0 | 0 | At21(-) | Ts19(-) | AT5G66470 | GTP binding / RNA binding |
| Tp19(-) | 1E-43 [*] | 8E-25 [*] | At22(-) | Ts20(-) | AT5G66480 | unknown protein |
| - | - | - | At23(-) | Ts21(-) | AT5G66490 | unknown protein |
| Тр20(-) | 0 | 0 | At24(-) | Ts22(-) | AT5G66500 | pentatricopeptide repeat-containing |
| Tp21(-) | 3E-84 | 7E-87 | At25(-) | Ts23(-) | AT5G66510 | GAMMA CA3; carbonate dehydratase |
| Tp22(+) | 0 | N/A | At26(-) | Ts24(+) | AT5G66520 | pentatricopeptide repeat-containing |

a, c, d : signs in parentheses denote orientation of DNA strands.

b: results from blastx to the most highly related *A. thaliana* or *T. salsuginea* protein.

c: named as in Deng et al., 2009.

*: homology found only in part of an ORF.

| Supplemental Table S3. | Comparison of ratio of non-synonymous (Ka) vs. synonymous (Ks) |
|------------------------|---|
| SI | ubstitution rates in orthologous genes and the estimated divergence |
| ti | me of three species. |

| | | | | | Ka/Ks | | Divergence time | | | | |
|---------|-------|-------|-------|----------|----------|----------|-----------------|------------|------------|--|--|
| | TP ID | AT ID | TS ID | TP vs AT | TP vs TS | AT vs TS | TP vs AT | TP vs TS | AT vs TS | | |
| | Tp01 | At02 | Th02 | 0.1840 | 0.2547 | 0.2007 | 14,010,200 | 7,015,467 | 12,621,400 | | |
| | Tp03 | At03 | Th03 | 0.1055 | 0.1124 | 0.1345 | 12,500,933 | 9,075,533 | 10,680,833 | | |
| | Tp07 | At06 | Th06 | 0.1164 | 0.2177 | 0.1536 | 12,928,533 | 6,630,133 | 12,806,067 | | |
| | Тр08 | At07 | Th07 | 0.0719 | 0.0678 | 0.0668 | 17,092,333 | 12,536,000 | 16,977,800 | | |
| | Тр09 | At08 | Th08 | 0.0248 | 0.0325 | 0.0278 | 15,025,200 | 11,721,333 | 14,432,167 | | |
| TP-1 | Tp10 | At09 | Th09 | 0.3300 | 0.2666 | 0.2732 | 10,193,500 | 7,853,767 | 10,862,967 | | |
| | Tp11 | At10 | Th10 | 0.3228 | 0.3657 | 0.3418 | 11,602,333 | 6,453,433 | 10,397,533 | | |
| | Tp14 | At12 | Th12 | 0.1282 | 0.1529 | 0.0878 | 14,891,200 | 9,491,033 | 17,632,533 | | |
| | Tp15 | At14 | Th14 | 0.0532 | 0.0539 | 0.0337 | 11,589,433 | 11,366,833 | 15,499,500 | | |
| | Tp21 | At18 | Th18 | 0.1343 | 0.1145 | 0.0965 | 10,093,333 | 7,624,300 | 11,521,067 | | |
| | Tp26 | At20 | Th20 | 0.1623 | 0.1749 | 0.1592 | 13,631,033 | 10,078,567 | 14,603,867 | | |
| | Tp02 | At13 | Th02 | 0.0856 | 0.1082 | 0.0852 | 13,796,200 | 8,436,267 | 13,231,800 | | |
| | Тр03 | At12 | Th03 | 0.0762 | 0.1226 | 0.1109 | 10,599,900 | 7,403,033 | 9,129,333 | | |
| | Tp04 | At10 | Th04 | 0.2565 | 0.5008 | 0.2903 | 11,557,933 | 9,478,600 | 16,217,800 | | |
| | Tp06 | At09 | Th06 | 0.2304 | 0.2786 | 0.2483 | 13,112,333 | 8,876,467 | 10,081,433 | | |
| | Тр08 | At05 | Th09 | 0.2535 | 0.2438 | 0.3386 | 10,012,700 | 6,591,800 | 9,393,300 | | |
| | Тр09 | At04 | Th10 | 0.1375 | 0.2381 | 0.2022 | 17,666,700 | 10,089,567 | 16,346,767 | | |
| TP-2 | Tp10 | At03 | Th11 | 0.1291 | 0.1799 | 0.1028 | 11,821,433 | 6,101,967 | 10,465,267 | | |
| | Tp15 | At19 | Th16 | 0.2879 | 0.4046 | 0.2865 | 12,458,433 | 6,426,433 | 13,667,167 | | |
| | Tp17 | At20 | Th18 | 0.1734 | 0.1138 | 0.1572 | 11,090,600 | 12,083,967 | 11,764,800 | | |
| | Tp18 | At21 | Th19 | 0.0866 | 0.1075 | 0.0872 | 9,021,300 | 7,735,767 | 10,273,633 | | |
| | Тр20 | At24 | Th22 | 0.2350 | 0.2818 | 0.2094 | 9,366,867 | 8,366,067 | 10,009,867 | | |
| | Tp21 | At25 | Th23 | 0.2372 | 0.2178 | 0.2499 | 8,014,600 | 4,788,100 | 7,309,500 | | |
| Average | | | | | | | 12,264,219 | 8,531,497 | 12,431,583 | | |
| Sd | | | | | | | 2,452,416 | 2,083,382 | 2,821,010 | | |

Supplemental Table S4. Comparison of relative gene expression levels by qPCR.

| Tissue Species | NaCl (mM) | Relative expression levels | | | | | | | | | | |
|----------------|--------------|----------------------------|-----------|-----------|------------|-----------|------------|-----------|-----------|-----------|--|--|
| | | AT2G01950 | AT2G01970 | AT2G01980 | AT2G01990 | AT3G53210 | AT1G77370 | AT3G47840 | AT5G66460 | | | |
| | ۸+ | 0 | 1.00±0.07 | 1.00±0.20 | 1.00±0.09 | 1.00±0.08 | 1.00±0.23 | 1.00±0.14 | 1.00±0.12 | 1.00±0.10 | | |
| Shoot Tp | 200 | 0.15±0.03 | 1.87±0.14 | 1.59±0.16 | 0.39±0.09 | 0.50±0.09 | 1.58±0.10 | 1.06±0.14 | 0.97±0.09 | | | |
| | | 0 | 0.60±0.07 | 0.45±0.04 | 2.63±0.17 | 1.10±0.20 | 2.47±0.17 | 2.49±0.48 | 1.09±0.26 | 0.43±0.03 | | |
| | Тр | 200 | 0.77±0.17 | 0.83±0.05 | 8.62±0.88 | 1.53±0.19 | 5.71±1.10 | 3.41±0.31 | 1.37±0.30 | 1.37±0.16 | | |
| | | 350 | 0.37±0.12 | 1.18±0.11 | 25.10±3.06 | 0.76±0.06 | 11.22±0.64 | 2.44±0.11 | 1.23±0.25 | 0.89±0.24 | | |
| | ۸+ | 0 | 1.00±0.13 | 1.00±0.03 | 1.00±0.25 | 1.00±0.06 | 1.00±0.17 | 1.00±0.03 | 1.00±0.08 | 1.00±0.09 | | |
| | AL | 200 | 0.19±0.07 | 1.00±0.14 | 2.30±0.33 | 0.47±0.19 | 1.49±0.52 | 1.20±0.08 | 1.70±0.23 | 0.35±0.05 | | |
| Root Tr | | 0 | 0.47±0.04 | 0.56±0.03 | 1.48±0.90 | 0.90±0.04 | 0.84±0.08 | 3.24±0.80 | 1.30±0.19 | 0.11±0.01 | | |
| | Тр | 200 | 0.69±0.09 | 0.78±0.06 | 6.97±2.59 | 3.50±0.91 | 0.53±0.07 | 3.96±0.86 | 1.79±0.30 | 0.42±0.04 | | |
| | | 350 | 0.12±0.02 | 0.62±0.02 | 5.22±1.40 | 2.64±0.23 | 0.21±0.19 | 1.80±0.31 | 1.47±0.16 | 0.65±0.10 | | |

Relative mRNA levels compared to those of an EF1 α gene (orthologs of AT5G04810)

Values represented were fold differences normalized with the untreated At samples.

Shaded are relative mRNA levels of SOS1 (orthologs of AT2G01980).

Supplemental Table S5. Known *cis*-elements in SOS1 promoter regions up to 2000bp upstream of the translation start site.

Frequencies in each species followed by positions are given in brackets (for frequencies of four or fewer only); negative numbers show sites on the opposite strand. Positions are counted from 5' end of the sequence and position 2000 refers to the base pair immediately upstream to the start codon. Promoter sequences were searched against PlantCare database (Rombauts et al., 1999), PlantProm database (Shahmuradov et al., 2003), PlantPAN (Chang et al., 2008), and PLACE database (Higo et al., 1999).

| Regulatory element | AT | TS | ТР | Sequence | Function |
|--------------------|--------------------------------|---------------------------|-------------------|--------------------|---|
| ABRE RATCAL | 1 (-902) | 1 (583) | 0 | MACGYGB | "ABRE-related sequence" identified in Ca(2+)-responsive genes |
| L1BOXATPDF1 | 1 (374) | 0 | 0 | TAAATGYA | "L1 box" in AT PROTODERMAL FACTOR1 (PDF1) |
| TBOXATGAPB | 1 (226) | 0 | 0 | ACTTTG | "Tbox" AtGAPB |
| DPBFCOREDCDC3 | 3 (95,902, - 905) | 3 (-224, -426, 828) | 0 | ACACNNG | ABA induced; embryo specific; binds DPBF-1 and 2 |
| ABRE | 1 (1883) | 2 (1562, -1617) | 0 | CACGTG | abscisic acid responsiveness |
| E5-core | 0 | 1 (-772) | 0 | ACATCTG | abscisic acid responsiveness; bDPBF-1; DPBF-2 |
| ANAERO1CONSENSUS | 2 (680, 852) | 0 | 5 | АААСААА | anaerobic genes involved in the fermentative pathway |
| ARE | 3 (-528, - 1526, - 1490) | 1 (- 1942) | 0 | TGGTTT | anaerobic induction |
| ASF1 motif CAMV | 0 | 2 (-243, -975) | 1 (822) | TGACG | auxin and/or salicylic acid responsiveness |
| TGA-box | 0 | 0 | 1 (1791) | TGACGTAA | auxin-responsiveness |
| ARF | 0 | 0 | 1 (754) | TGTCTC | auxin-responsiveness; binds AT1G59750 (ARF1) |
| AGL3 | 1 (614) | 1 (576) | 1 (665) | NNTNCCAWWWATAGNWNN | binds Agamous AT2G03710; F19B11.16. |
| AG | 8 | 5 | 2 (191, - 372) | NTTWCCWAAWNNGGNAAN | binds Agamous AT4G18960. |
| C8GCARGAT | 0 | 1 (147) | 1 (538) | CWWWWWWWG | binds AGL15 |
| PY-Box | 0 | 0 | 1676 | CCTTTTC | binds aleurone layer nuclear proteins |
| AP1 | 1 (382) | 3 (326, - 333, 509) | 0 | TTTTTRG | binds AP1 At1g69120 |
| ARR1AT | 8 | 6 | 9 | NGATT | binds ARR1 |

| PIF3 | 2 (-203, 880) | 1 (-558) | 1 (-159) | GKRGGMCACGTGRMSWCK | binds AT1G09530 /BHLH8/ PAP3 in phytochrome signalling pathways |
|-----------------|--------------------------------|---------------------------|-------------------------|---------------------|---|
| CDC5 | 4 (164, 245, - 782, 922) | 0 | 1 (916) | NGCTCAGCGCN | binds AT1G09770 |
| RAV1 | 5 | 3 (402, 445, - 682) | 2 (-716, -719) | NNGCAACAKAWN | binds AT1G13260 |
| Athb-1 | 8 | 13 | 12 | NYNCAATTATTGSA | binds AT3G01470/ HAT5/ HD- ZIP-1 |
| ANT | 0 | 1 (529) | 1 (89) | CACANWTCCCRAKG | binds AT4G37750 |
| Bellringer | 5 | 0 | 1 (502) | AAATTARW | binds At5g02030/replumless/pennywis |
| Athb-5 | 7 | 8 | 9 | CAATTATTG | binds AT5G65310/ATHB5/ MNA5.4 |
| AtMYB77 | 1 (1024) | 0 | 0 | ATCGACGGTTGG | binds AtMYB77; induced by light, cold, and wounding |
| box b | 1 (410) | 0 | 0 | AGCAAAGCAA | binds DOF1 |
| Athb-9 | 5 | 15 | 5 | NNNNGTAATGATTRCNYBS | binds homeobox HD-ZIPIII proteins |
| MYB4 | 2 (-49, 681) | 0 | 1 (214) | AMCWAMC | binds myb At4g38620 |
| Myba | 1 (1029) | 0 | 0 | CGGTTG | binds Myb proteins |
| PtMYB4 | 1 (-1095) | 0 | 0 | ACCTACA | binds PtMYB4 |
| p33TCP20 BS1 | 0 | 0 | 1 (- 1254) | GCCCG | binds TCP proteins involved in growth and development |
| EVENINGAT | 0 | 1 (-840) | 1 (-891) | AAAATATCT | circadian control; "EE (evening element) motif" |
| CCA1 motif (2) | 0 | 0 | 2 (889-, -928) | АААААТСТА | circadian regulation; binds CCA1 |
| LTR | 1 | 1 (1535) | 0 | CCGAAA | cold induction |
| LTRECOREATCOR15 | 1 (-798) | 0 | 2 (116, 183) | CCGAC | cold responsiveness |
| GA-3 | 0 | 0 | 1 (1202) | AGAAAGGAA | control ovule identity; binds BPC1 |
| ARR10 | 0 | 2 (353 <i>,</i> 840) | 3 (546, 763, 891) | AGATHHKN | cytokinin induction; binds At4g31920 |
| TC-rich repeats | 0 | 2 (560, 890) | 1 (1057) | GTTTTCTTAC | defense and stress responsiveness |
| AtMYC2 | 0 | 3 (-426, 788, 829) | 1 (871) | CACATG | dehydration and abscisic acid responsiveness; binds AT1G32640 |
| DRE 2 | 1 (-1784) | 0 | 0 | GCCGAC | dehydration responsiveness |
| MYCATERD1 | 0 | 6 | 1 (-871) | CATGTG | dehydration responsiveness; Myc recognition |
| MYB1AT | 2 (510, 546) | 1 (462) | 0 | WAACCA | dehydration-responsive; binds myb proteins |
| MYCCONSENSUSAT | 4 (96, 424, 430, 903) | 4 (426, 788, 829, | 2 (248, 871) | CANNTG | dehydration-responsiveness; Myc recognition |

| | - | 893) | - | | |
|-------------------|-------------------------------------|-------------------------------|----------------------------|------------------------|---|
| WA | 1 (336) | 1 (27) | 1 (435) | GGTCAAA | disease resistance; binds WRKY1 |
| PB1 | 0 | 1 (- 1429) | 0 | TGCAAAAG | endosperm specific induction; binds BPBF |
| TA-rich region | 0 | 1 (1460) | 0 | ΤΑΤΑΤΑΤΑΤΑΤΑΤΑΤΑΤΑΤΑΤΑ | enhancer |
| Motif VI | 0 | 0 | 1 (- 1670) | TGGTCC | epicotyl-specific nuclear factor |
| ERE | 0 | 2 (-935 <i>,</i> -1152) | 1 (-818) | ΑΤΤΤΟΑΑΑ | ethylene responsiveness |
| ACGTAT ERD1 | 2 (227 <i>,</i> 904) | 2 (415 <i>,</i> 583) | 4 (71, 99, 186, 824) | ACGT | etiolation-induced expression of erd1 (early responsive to dehydration) |
| CARG AT CONSENSUS | 1 (534) | 1 (506) | 0 | CCWWWWWGG | found in AtSOC1 MADS-box flowering-time gene |
| GAREAT | 0 | 1 (-846) | 1 (-897) | TAACAAR | GA-responsiveness |
| GARE-motif | 0 | 2 (614, 1507) | 0 | AAACAGA | gibberellin-responsive element |
| HSE | 0 | 0 | 2 (- 1601, - 1732) | ААААААТТТС | heat stress responsiveness |
| HEXAMERATH4 | 1 (-45) | 0 | 0 | CCGTCG | hexamer motif of At histone H4 |
| circadian | 1 (1451) | 1 (- 1679) | 2 (1210, -1584) | CAANNNNATC | involved in circadian control |
| GT2 | 0 | 0 | 1 (-784) | GGTAATTA | light down regulation and dark induced; binds DF1 |
| ACE | 0 | 0 | 2 (598 <i>,</i> 1075) | AAAACGTTTA | light responsiveness |
| GA-motif | 0 | 1 (652) | 1 (-854) | AAAGATGA | light responsiveness |
| GT1 motif | 1343 | 0 | 0 | AATCCACA | light responsiveness |
| ATCT-motif | 0 | 0 | -551, - 876, - 915 | ААТСТААТСТ | light responsiveness |
| GAG motif | 1 (-875) | 0 | 0 | AGAGATG | light responsiveness |
| box 4 | 4 (1038, 1712, 1231, 1839) | 2 (845 <i>,</i> 1762) | 1 (938) | АТТААТ | light responsiveness |
| G-box | 0 | 1 (- 1562) | 1 (- 1162) | CACGTA | light responsiveness |
| GATA-motif | 0 | 1 (-727) | 0 | GATAGGA | light responsiveness |
| CATT motif | 1 (1949) | 0 | 0 | GCATTC | light responsiveness |
| GT1CONSENSUS | 14 | 11 | 10 | GRWAAW | light responsiveness |
| TCT-motif | 1 (-1437) | 1 (894) | 0 | TCTTAC | light responsiveness |
| l-box | 1 (1817) | 1 (1678) | -787 | TGATAATGT | light responsiveness |
| box 1 | 0 | 3 (-935, -1152, - 1144) | 5 | ТТТСААА | light responsiveness |
| AT1-motif | 0 | 1 (845) | 0 | ATTAATTTTACA | light responsiveness and shoot specific expression |

| as-2-box | 1 (-1697) | 8 | 0 | GATAATGATG | light responsiveness and shoot specific expression |
|-------------------|------------------|--|--------------------|------------|--|
| AE box 2 | 0 | 0 | 1 (- 1985) | AGAAACAA | light responsiveness; binds AEF |
| AE box 1 | 1 (84) | 0 | 0 | AGAAACTT | light responsiveness; binds AEF |
| GATABOX | 12 | 9 | 14 | GATA | light responsiveness; binds ASF- 2; in LHCII type I Cab genes |
| GBF1 BS3 | 1883 | 0 | 0 | ACACGTGT | light responsiveness; GBF1 |
| GBF1 BS8 | 0 | 1 (1562) | 0 | TTACGTGT | light responsiveness; GBF1 |
| CGTCA-motif | 0 | 3 (-657, 1955, 1223) | 2 (-520, -1791) | CGTCA | MeJA-responsiveness |
| MBS | 0 | 4 (641, 1038, - 1058, - 1088) | 0 | TAACTG | MYB binding site involved in drought-inducibility |
| MYBCORE | 2 (48, - 452) | 3 (-58, 78, 108) | 0 | CNGTTR | myb recognition |
| S1 | 0 | 1 (435) | 0 | ATGGTATT | negative regulation, binds S1F |
| SORLIP3AT | 0 | 2 (492, - 495) | 1 (289) | TGTATATAT | one of "Sequences Over- Represented in Light-Induced Promoters (SORLIPs) |
| CCA1 | 0 | 0 | 1 (763) | ААМААТСТ | phytochrome regulation of an AtLhcb; binds myb AT2G46830 |
| RHERPATEXPA7 | 1 (-227) | 0 | 0 | KCACGW | Root Hair-specificity |
| WBOXATNPR1 | 1 (-504) | 0 | 2 (-129, 821) | TTGAC | salicylic acid (SA)-induction by WRKY |
| TCA-element | 0 | 1 (- 1909) | 0 | GAGAAGAATA | salicylic acid responsiveness |
| SEF3 BS 1 | 0 | 0 | 0 (- 1387) | CACCCA | seed specific enhancer; binds SEF3 |
| SURECOREATSULTR11 | 0 | 1 (-417) | 1 (-755) | GAGAC | sulfur-responsive element (SURE) in SULTR1 |
| SV40COREENHAN | 0 | 1 (388) | 0 | GTGGWWHG | SV40 core enhancer |

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| Primer | Sequence | Used for |
|-----------------------------|------------------------------|-----------------|
| SOS1(1052F) | GTGTTGTCATTGCTGAAGGCATT | Fig 5 (A) |
| SOS1(1399R) | CAAATTGGGTAGTGGATCCATTAA | Fig 5 (A) |
| 1G77370(181F) | CGTTCAGAACGCCATCTTGTCCAA | Fig 6 |
| 1G77370(314R) | CCTCTCTCTGATCAAGCTCCACAACAA | Fig 6 |
| 2G01950(2057F) | GGTCAAATCCCTGAATCTTTCTCGAA | Fig 5 (B) |
| 2G01950(2217R) | TCCGGTAAAGGAACACCGCAAA | Fig 5 (B) |
| 2G01970(1612F) | GAATGGTGGTGGAGATCATTCCTATG | Fig 5 (B) |
| 2G01970(1763R) | AAACCGTAACAAATGCAAGCCATGTA | Fig 5 (B) |
| SOS1(925F) | ATGACTTTGGGCATGTTTTATGCTGCA | Fig 5 (B) |
| SOS1(1075R) | CCTTCAGCAATGACAACACCACTGAGGA | Fig 5 (B) |
| 2G01990(274F) | AGTCAGTTTGTCACTCCACATGTTA | Fig 5 (B) |
| 2G01990(425R) | GATGGTTCTGTTGGAGATTTTTCTCG | Fig 5 (B) |
| 3G47840(816F) | GAGGGATGTGGTTTCGTGGACAAG | Fig 6 |
| 3G47840(994R) | GCTGCTCGCCCCACACAAGTCT | Fig 6 |
| 3G53210(166F) | TCTCTGTTCTTGCTCCCTCTGCTT | Fig 6 |
| 3G53210(313R) | GAGGTATTGTCCAATCCAAAGATGTA | Fig 6 |
| 5G66460(786F) | TGGCTTGGGAGCTCATGAACGA | Fig 6 |
| 5G66460(920R) | CTTCGAGGCCAGCTTCAAGCA | Fig 6 |
| 5G60390(953F) ^a | CAATGTCAAGAATGTTGCTGTCAAGGAT | Fig 5 (B) and 6 |
| 5G60390(1090R) ^a | TCTGACCAGGGTGGTTCATGATGAT | Fig 5 (B) and 6 |

^a used for the reference gene in all qPCR.

Primers for the reference genes shown in Figure 5 (A) were the same as Oh et al. 2009.

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