Supplementary Materials

SM1. *cacp* and *cacyt* specific primer design and optimization of RT-PCR condition

To design specific primers for *cacp* and *cacyt*, the ORF of the two sequences were compared using ClustalW. The sequence alignment of *cacp* and *cacyt* ORFs showed high homology (Figure S1) with each other. Two sets of qPCR primers were designed for each gene from the regions of low homology and the specificity of primers were tested by PCR using *cacp* and *cacyt* as the templates for each primer set in separate reactions. Both primer sets (set1 and set2) of *cacp* amplified only *cacp* template but not *cacyt*. However, the band intensity in agarose gel electrophoresis was higher in case of set2 primers as compared to the set1 primers (Table S1). Similarly, the two primer sets of (set1 and set2) *cacyt* amplified only *cacyt* template but not *cacp* template and the band intensity was higher in case of set1 primers as compared to set2 primers (Table S1). The PCR products obtained using set2 primers for *cacp* and set1 primers of *cacyt* were sequenced and 100% homology to the 120 bp and 177 bp cDNA fragments of *cacp* and *cacyt*, respectively were confirmed. The set1 primers for *cacp* and set2 primers for *cacyt* were then selected for expression studies of leucaena *cacp* and *cacyt*, respectively.

SM2. Tissue-specific expression of *cacp* and *cacyt*

For tissue specific expression of *cacp* and *cacyt*, we performed semi-quantitative reverse transcription PCR (RT-PCR) (Tachibana et al., 2011) using the 1µl of 1/10th dilution of cDNA from leaf, stem and root tissues of leucaena in a 15µl of reaction consisting of 0.3µl each of 10µM forward and 10µM reverse primer and analyzed the product in agarose gel electrophoresis. The RT-PCR comparison was made after 28th PCR cycle. The transcript abundance of *cacp* and *cacyt* in different tissues were quantified by densitometry analysis of RT-PCR amplified products using ImageJ software from NCBI (Schneider et al., 2012). The relative density of bands corresponding to *cacp* and *cacyt* in each tissue type were calculated and were normalized against the relative band densities of β-actin, which were used as controls. The transcript abundance of *cacp* was found maximum in leaf tissues followed by stem and root tissues, whereas the transcript abundance of *cacyt* was found maximum in root tissues followed by leaf and stem tissues (Figure S2 a-b). The overall expression of *cacp* was higher than the expression of *cacyt* in leaf and stem tissues but in root tissues the transcript abundance of both *cacp* and *cacyt* were almost similar.

SM3. Identification of suitable internal reference gene

The six housekeeping genes (HKGs) including *β-actin, tubulin-1, ubiquitin-5, 18SrRNA, 5.8SrRNA* and *ef1a* were tested as possible internal reference for each of the treatment and control. The method and primers used for identification of suitable internal reference were the same as we discussed previously (Negi et al., 2011). The qPCR assay for these HKGs performed in two biological replicates and three PCR replicates for each control and treatment group. The primers used for these HKGs were found to be specific as the amplicons for each of the HKGs appeared as a single band in agarose gel electrophoresis and also exhibited single peak in melting curve analysis. The inter-group and intra-group variance for each group was calculated using NormFinder applet (Andersen et al., 2004). The inter-group variance for each HKGs were plotted as a bar and the confidence intervals on the inter-group variance was obtained by plotting the average of intra-group variances as error bars in the inter-group variances. The expression stabilities in leaf tissues under various stress conditions, with respect to control, is represented in (Figure S8a-d). In leaf tissues, *ef1a* was found to be the most stably expressed HKG in the three experimental groups including 'control and drought', 'light and dark', and 'light and bright light'. In the experimental group of 'control and salt', the 5.*8SrRNA* had the most stable expression with the least inter-group and intra-group variance. In case of stem tissues, *18SrRNA*, and *ubiquitin-5*, were found to be the most stably expressed HKGs in 'control and drought', and in 'control and salt' experimental groups, respectively, whereas the expression of *β-actin* was the most stable in 'light and dark' and in 'light and bright light' experimental groups (Figure S8e-h). In root tissues of leucaena, 5.*8SrRNA* had the most stable expression in 'control and drought' group, whereas *β-actin* showed the most stable expression in 'control and salt', 'light and dark' and 'light and bright light' groups (Figure S8i-l).

Table S1. The qPCR primers used in the study and their specificity for the *cacp* and *cacyt* templates

Gene	Primer set	Primer ID	Sequence (5'→3')	Amplicon size	Template	
					cacp	***cacyt***
cacp	Set1	CAcp-F1	TGGCAACCACAGCAGAT	87bp	+	-
		CAcp-R1	AGATTCAGATGATGCGATGC			
	Set2	CAcp-F2	GGTGAGCTCTGCACACA	120bp	+++	-
		CAcp-R2	GTATCCTCCTTTCAGTGAC			
cacyt	Set1	CAcyt-F1	GGGGCGTCGCCGCCG	177bp	-	+++
		CAcyt-R1	AAAACTTTGGGCTTTGGCCGG			
	Set2	CAcyt-F2	TCGGAGCAGTGTACTAA	118bp	-	++
		CAcyt-R2	GAGCACCCTTTAAAACT			

- sign represents no amplification of template

+ sign represents amplification of template but faint band

++ sign represents amplification of template with relatively higher intensity of band

+++ sign represents amplification of template with maximum intensity of band

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cacp   ATGTCGACCGCTTCCATCAACGGCTGCTGCCTCTCTTCTTTCTCTTCCTCTAAGACTTCTCTTCCATCCAAGTTCTCTGTCTCTG 85
cacyt  ---------------------------------------------------------------------------------------


cacp   CAAGGCTCGCCACTCCTCCTCCTTCATCTTCTTCTTCCCCAATCCCTTCTCTCATCCAAAACAGGCCCGTCTTCGCCGCCCCTAC 170
cacyt  ------------------------------------------------------------ATGGCAGGGCAGTC--------------- 14
       *   **** * ***

cacp   TCCCCTTATCACGCCCACCCTGAACGAGGATGCGATCGAGGAAGCTATTGTAGAACTCGAGAAACTGTTCAAGGTGAAGGGTGAG 255
cacyt  ----------------------------ATA----CGAGGAAGCCATTGCAGAGCTGAAGAAGCTTATCAGCGAGAAAGCTGAC 66
       **     ********* **** *** **  **** **  ***  * *** * ***

cacp   CTGGCAACCACAGCAGATGCAAGGGTGGATCAAGTAACAGCTCAGGTGGGAACACCGAC-ATCTGAAGGCATCGCATCATCTGAA 339
cacyt  CTTGGGGGCGTCGCCGCCGCAAAGATCAAGCAGCTGACAGCCGAGTTG--AGCGCCGCCGATTCGAAG--------CCGATTAAA 141
       ** *    *   ** *  **** * *  * **  * *****  ** **  * * *** * **  ****         *   * **

cacp   TCTGTCGAGAGGATCAAGGCTGGTTTTATTCACTTCAAGAAAGAGAAATATGAGAAGAACCCTGCTCTATACGGTGAACTTGCCA 424
cacyt  CCGGACGAGAGGATCCGAACCGGGTTCACTCACTTTAAGAAAGAGAAGTTCGAGAAGAATCCAGATTTGTTTGGGAAACTTGCCA 226
       * * **********    * ** ** * ****** *********** *  ******** ** * * * *  **  *********

cacp   AGGGACAGAGCCCCCCGTACATGGTATTTGCTTGCTCAGACTCTAGGGTCTGCCCATCTCACGTGCTAGACTTCCAACCAGGGGA 509
cacyt  CCGGCCAAAGCCCAAAGTTTTTGGTATTTGCATGCTCAGACTCTAGAGTTTGCCCATCGCATGTACTGGATTTTCAACCGGGTGA 311
         ** ** *****   **   ********** ************** ** ******** ** ** ** ** ** ***** ** **

cacp   GGCTTTTGTCGTCAGAAATGTTGCTAACTTGGTCCCACCATATTGCCAGACAAGGTATGCTGGAGTTGGAGCTGCCGTTGAGTAC 594
cacyt  GGCTTTTGTGGTGCGGAACATCGCCAACATGATCCCGCCTTATGACCAGACGAAATATTCAGGAACTGGGGCAGCCATTGAATAT 396
       ********* **  * **  * ** *** ** **** ** ***  ****** *  *** * ***  *** ** *** **** **

cacp   GCCGTTCTGCATCTCAAGGTGTCGGAAATCGTGGTGATTGGTCACAGTGCTTGTGGTGGTATCAAGGGTCTCATGTCTATCCCAG 679
cacyt  GCAGTGTTGCATTTAAAGGTGGAGAATATAGTAGTGATTGGACATAGCTGTTGTGGAGGTATAAAAGGGCTCATGTCTATCCCAG 481
       ** **  ***** * ******  * * ** ** ******** ** ******** ***** ** ** ****************

cacp   ACAATGGAGCCGTCCCCACCACTGACTTCATAGAGGACTGGGTGAAGATCGGTTTACCTGCAAAGGCAAGAGTGAAATCAGTACA 764
cacyt  ATGATGGGACCA-CTTCA--AGTGAATTCATAGAGAACTGGGTGCAAATTTGTAATCCAGCAAAATCCAAGGTTAAAGCAG-ACA 562
       *  ****  **  *  **  * *** ********* ******** * **  **   ** *****  * *  ** *** ******

cacp   TGGAG-GCGCACCTTTCGGTGAGCTCTGCACACACTGTGAGAAGGAAGCTGTGAACGTGTCACTTGGGAATCTGCTGACATATCC 848
cacyt  CAAAAAGCTTGAGTTTCTCGGAGCAGTGTACTAACTGCGAGAAGGAAGCGGTGAATGTGTCGCTTGCAAATCTGCTGAGCTATCC 647
          *  **     ****   ****  ** **  **** *********** ***** ***** ****  **********  *****

cacp   ATTTGTGAGAGAGGGATTGGTGAACAAGACACTGTCACTGAAAGGAGGATACTATGACTTTGTGAAGGGATCATTTGAGCTGTGG 933
cacyt  GTTTGTTAGAGATGGAGTTGTGAAGAAAACACTAGTTTTAAAGGGTGCTCATTACGATTTTGTTAATGGCACCTTTGACCTCTGG 732
       ***** ***** *** * ***** ** *****     * ** ** *   * ** ** ***** ** **  * ***** ** ***

cacp   GGCCTTCAGTTTGGCCTGTCTTCTTCCCTCTCCGTATGA 972
cacyt  GATCTGGACCTCAAAATGTCT-----CCTCTT----TAA 762
       *  **  *  *     ***** *****     * *
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Figure S1. The sequence alignment of the ORF of *cacp* and *cacyt* using ClustalW. The primers for *cacp* and *cacyt* were designed from the yellow and green highlighted regions, respectively

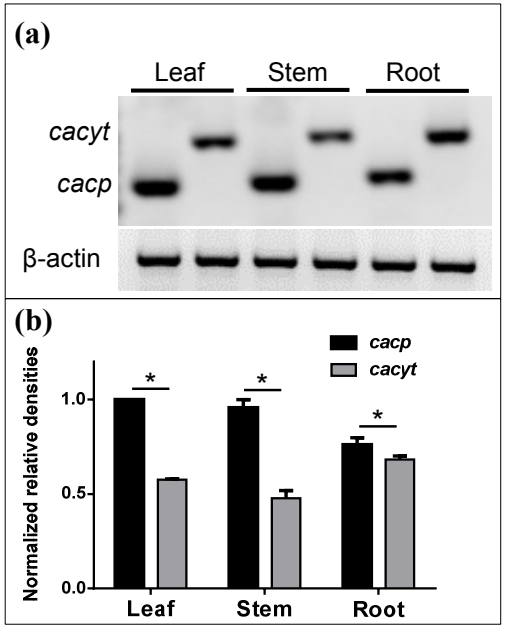

Figure S2. Comparison of transcript abundance of *cacp* and *cacyt* in leaf, stem, and root tissues of *L. leucocephala*. (a) Bands of *cacp* and *cacyt* amplified by RT-PCR from leaf, stem, and root tissues. β-actin was used as loading and PCR control. (b) Quantitative representation of transcript abundance from the same bands in agarose gel. The band intensities were digitized using ImageJ software. The band intensities of β-actin were used to normalize the transcript abundance. Statistical significance was determined using the Holm-Sidak method, with alpha =5.000%

```
GENE ID: 11428425 MTR_6g006990 | Carbonic anhydrase [Medicagotruncatula]
Score =  247 bits (631),  Expect = 1e-79, Method: Compositional matrix adjust.

Identities = 119/146 (82%), Positives = 132/146 (90%), Gaps = 1/146 (1%)
Frame = +3

Query  3    VANLVPPYCQTRCAGVGAAVEYAVLHLKVSEIVVIGHSACGGIKGLMSIPDNGAVPTTDF  182
            VAN+VPPY Q + AG G+A+EYAVLHLKVS IVVIGHSACGGIKGL+S P +GA  +TDF
Sbjct  115  VANMVPPYDQAKYAGTGSAIEYAVLHLKVSNIVVIGHSACGGIKGLLSFPFDGAY-STDF  173

Query  183  IXDWVKIGLPAKARVKSVHGGAPFGELCTHCEKEVVNVSLGNLLTYPFVREGLVNKTLSL  362
            I +WVKIGLPAKA+VK+ HG APFGELCTHCEKE VNVSLGNLLTYPFVREGLVNKTL+L
Sbjct  174  IEEWVKIGLPAKAKVKAKHGDAPFGELCTHCEKEAVNVSLGNLLTYPFVREGLVNKTLAL  233

Query  363  KGGYYDFVKGSFELWGLKFGLSSSLS  440
            KGGYYDFVKGSFELWGL+FGLSS+ S
Sbjct  234  KGGYYDFVKGSFELWGLEFGLSSTFS  259
```

Figure S3. The BLASTx analysis of 515bp partial iSSH clone of *L. leucocephala*. The query sequence exhibited homology with the β-carbonic anhydrase from *M. truncatula* (Gene ID: 11428425 MTR_6g006990)

```
GENE ID: 100500448 LOC100500448 | carbonic anhydrase, chloroplastic [Glycine max]|Length=328 amino acids

Score = 485 bits (1248), Expect = 1e-169, Method: Compositional matrix adjust.
Identities = 249/329 (76%), Positives = 281/329 (85%), Gaps = 7/329 (2%)

Query  1    MSTASINGCCLSSFSSSKTSLPS---KFSVSARLATPPPSSSSSPIPSLIQNRPVFAAPT  57
            MST+SING CLSS S +KTSL     + SV A L TP   SSSS  PSLIQ+RPVFAAP
Sbjct  1    MSTSSINGWCLSSISPAKTSLRKATLRPSVFATLNTPSSPSSSSSFPSLIQDRPVFAAPA  60

Query  58   PLITPTLNEDA---IEEAIVELEKLFKVKGELATTADARVDQVTAQVGTPTSEGIASSES  114
            P+ITPT+ ED     E+AI EL+KL + K EL  TA  +V+Q+TA +GT +S+GI SSE+
Sbjct  61   PIITPTVREDMAKEYEKAIEELQKLLREKSELKATAAEKVEQITASLGTSSSDGIPSSEA  120

Query  115  VERIKAGFIHFKKEKYEKNPALYGELAKGQSPPYMVFACSDSRVCPSHVLDFQPGEAFVV  174
             +RIKAGFIHFKKEKY+KNPALYGELAKGQSP +MVFACSDSRVCPSHVLDFQPGEAFVV
Sbjct  121  SDRIKAGFIHFKKEKYDKNPALYGELAKGQSPKFMVFACSDSRVCPSHVLDFQPGEAFVV  180

Query  175  RNVANLVPPYCQTRYAGVGAAVEYAVLHLKVSEIVVIGHSACGGIKGLMSIPDNGAVPTT  234
            RNVAN+VPPY Q++YAG GAAVEYAVLHLKVSEIVVIGHSACGGIKGL+S P +G   +T
Sbjct  181  RNVANIVPPYDQSKYAGTGAAVEYAVLHLKVSEIVVIGHSACGGIKGLLSFPYDGTY-ST  239

Query  235  DFIEDWVKIGLPAKARVKSVHGGAPFGELCTHCEKEAVNVSLGNLLTYPFVREGLVNKTL  294
            DFIE+WVKIGLPAKA+VK+ HG APF ELC+HCEKE+VNVSLGNLLTYPFVR+GLVNKTL
Sbjct  240  DFIEEWVKIGLPAKAKVKTQHGDAPFAELCSHCEKESVNVSLGNLLTYPFVRDGLVNKTL  299

Query  295  SLKGGYYDFVKGSFELWGLQFGLSSSLSV  323
            SLKGGYYDFVKGSFELWGLQFGL+SS SV
Sbjct  300  SLKGGYYDFVKGSFELWGLQFGLASSFSV  328
```

Figure S4. The BLASTp analysis of full-length deduced amino acid sequence of β-CA1. The query sequence exhibited homology with the chloroplastic carbonic anhydrase from *G. max* (Gene ID: 100500448 LOC100500448)

```
GENE ID: 821134 CA1 | carbonic anhydrase 1 [Arabidopsis thaliana]
Score = 96.3 bits (238),  Expect = 3e-23, Method: Composition-based stats.
Identities = 43/50 (86%), Positives = 47/50 (94%), Gaps = 0/50 (0%)
Frame = +2

Query  2    VLDFQPGEAFVVRNIANMIPPYDQTKYSGTGAAIEYAVLHLKVENIVVIG  151
            VLDFQPG+AFVVRNIANM+PP+D+ KY G GAAIEYAVLHLKVENIVVIG
Sbjct  100  VLDFQPGDAFVVRNIANMVPPFDKVKYGGVGAAIEYAVLHLKVENIVVIG  149
```

Figure S5. The BLASTx analysis of the 151 bp leucaena β-CA obtained as a result of PCR using primers designed from the conserved region of cytoplasmic β-CAs of C3 dicots

```
GENE ID: 11407419 MTR_5g034250 | Carbonic anhydrase [Medicagotruncatula]

Score =  434 bits (1115),  Expect = 2e-151, Method: Compositional matrix adjust.
Identities = 198/252 (79%), Positives = 228/252 (90%), Gaps = 0/252 (0%)

Query  1    MAGQSYEEAIAELKKLISEKADLGGVAAAKIKQLTAELSAADSKPIKPDERIRTGFTHFK  60
            MAG+++E++IA L +L+ EKA+LG +AA KIK+LTAEL A  SKP  PDERIR+GF  FK
Sbjct  23   MAGETFEDSIATLTRLLKEKAELGDIAAVKIKELTAELEANGSKPFNPDERIRSGFVSFK  82

Query  61   KEKFEKNPDLFGKLATGQSPKFLVFACSDSRVCPSHVLDFQPGEAFVVRNIANMIPPYDQ  120
             EKF KNP+L+G+LA GQSPKF+VFACSDSRVCPSH+LDFQPGEAFVVRNIANM+PP+D+
Sbjct  83   TEKFLKNPELYGELAKGQSPKFMVFACSDSRVCPSHILDFQPGEAFVVRNIANMVPPFDK  142

Query  121  TKYSGTGAAIEYAVLHLKVENIVVIGHSCCGGIKGLMSIPDDGTTSSEFIENWVQICNPA  180
            TKYSG GAAIEYAVLHLKVENIVVIGHSCCGGIKGLMSIPDDGTT+S+FIE WVQICNPA
Sbjct  143  TKYSGAGAAIEYAVLHLKVENIVVIGHSCCGGIKGLMSIPDDGTTASDFIEQWVQICNPA  202

Query  181  KSKVKADTKSLSFSEQCTNCEKEAVNVSLANLLSYPFVRDGVVKKTLVLKGAHYDFVNGT  240
            +SKVK +T SLSF+EQCTNCEKEAVNVSL NLL+YPFVRDGVVKK+L LKGAHY+FVNGT
Sbjct  203  RSKVKLETSSLSFAEQCTNCEKEAVNVSLGNLLTYPFVRDGVVKKSLALKGAHYNFVNGT  262

Query  241  FDLWDLDLKMSP  252
            F+LWDL+  + P
Sbjct  263  FELWDLNFNLLP  274
```

Figure S6. The BLASTp analysis of full-length deduced amino acid sequence of leucaena β-CA. The query sequence exhibited homology with the carbonic anhydrase with *M. truncatula* (GENE ID: 11407419 MTR_5g034250)

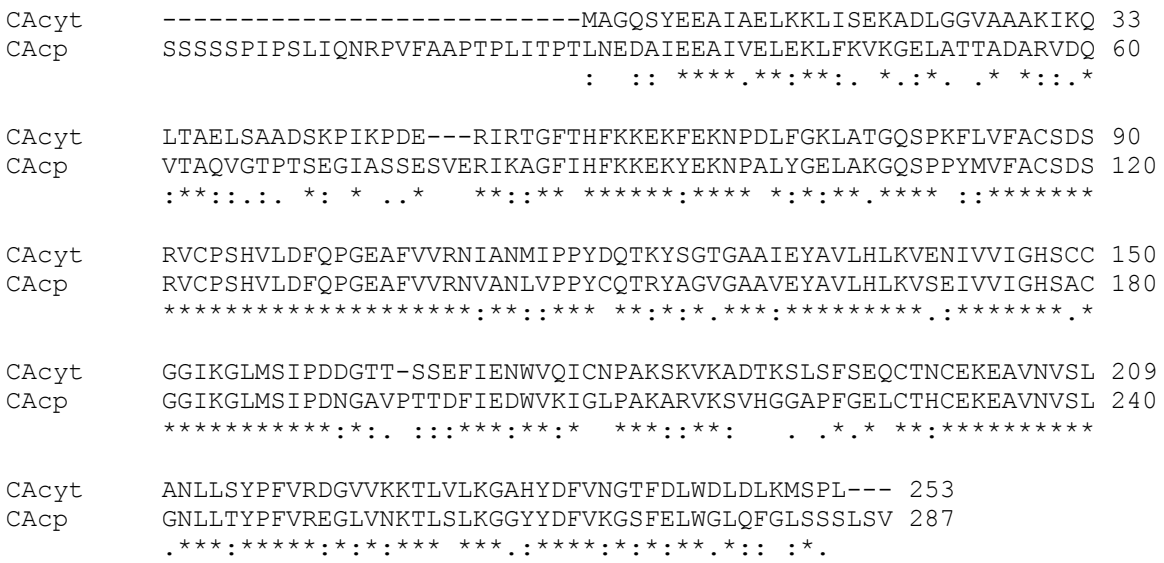

Figure S7. The sequence alignment of the deduced amino acid sequence of CA_{cyt} and CA_{cp} using ClustalW

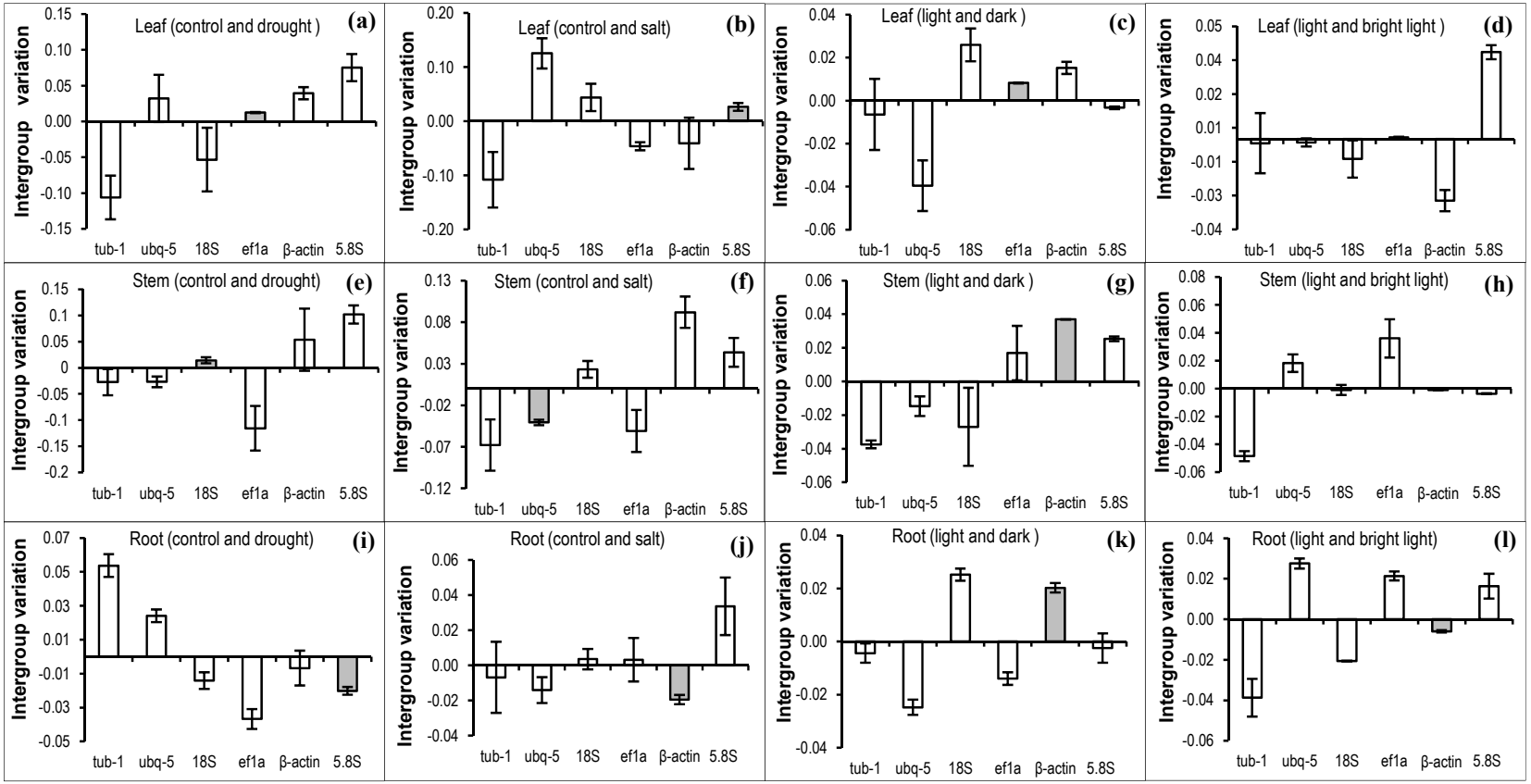

Figure S8. Expression stabilities of six housekeeping genes (HKGs) including *β-actin*, *tubulin-1*, *ubiquitin-5*, *18SrRNA*, *5.8SrRNA* and *ef1a* under stress treatments (drought, salt, light, and dark). Control plants were grown in Hoagland solution under 16 h light and 8h dark photoperiod. The expression stabilities of HKGs were tested in four experimental groups that include control and drought, control and salt, light and dark, and light and bright light. The stability of HKGs expression was studied in three different tissue types including leaf (a-d), stem (e-h), root (i-l). Each experiment has six replicates (n=6). The bars represent inter-group variance whereas the error bars represent the average of intra-group variances. The most stably expressed HKG in each group is represented as the bar shaded in grey color.