Light-harvesting chlorophyll a/b-binding proteins are required for stomatal response to abscisic acid in Arabidopsis

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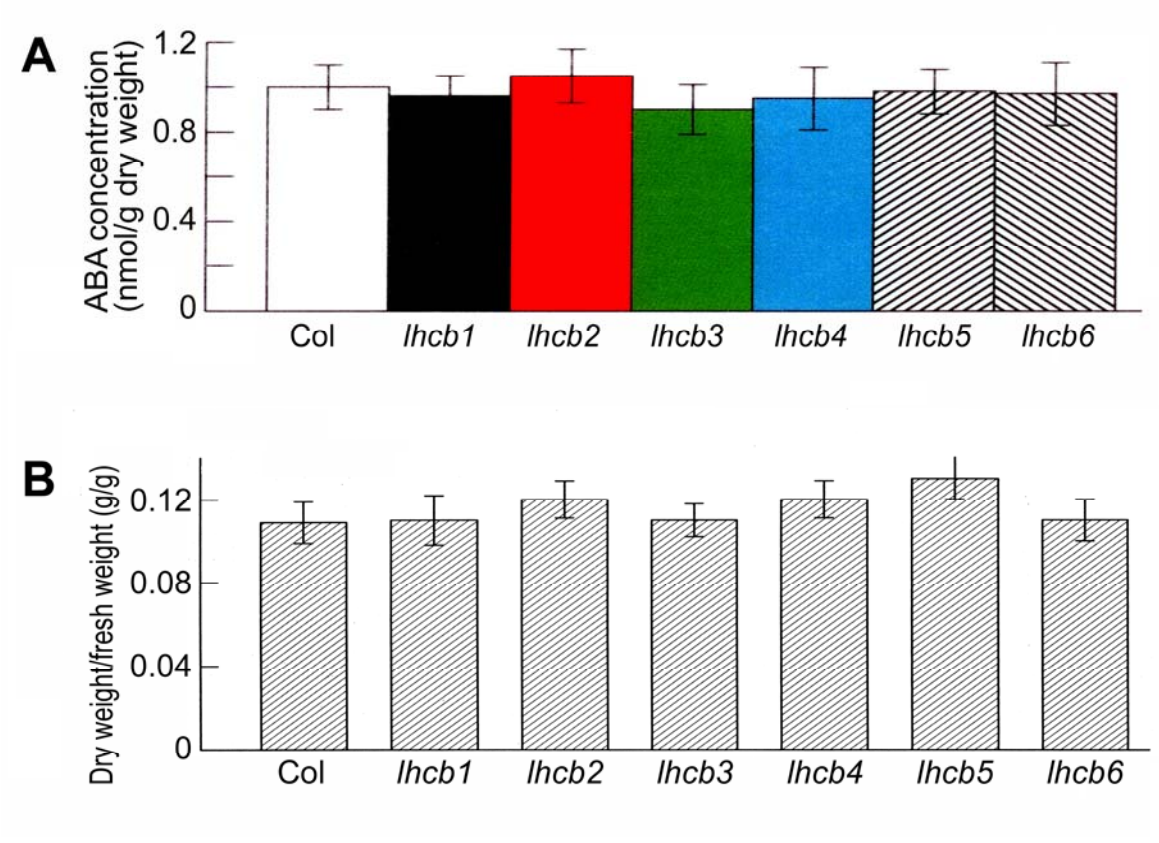
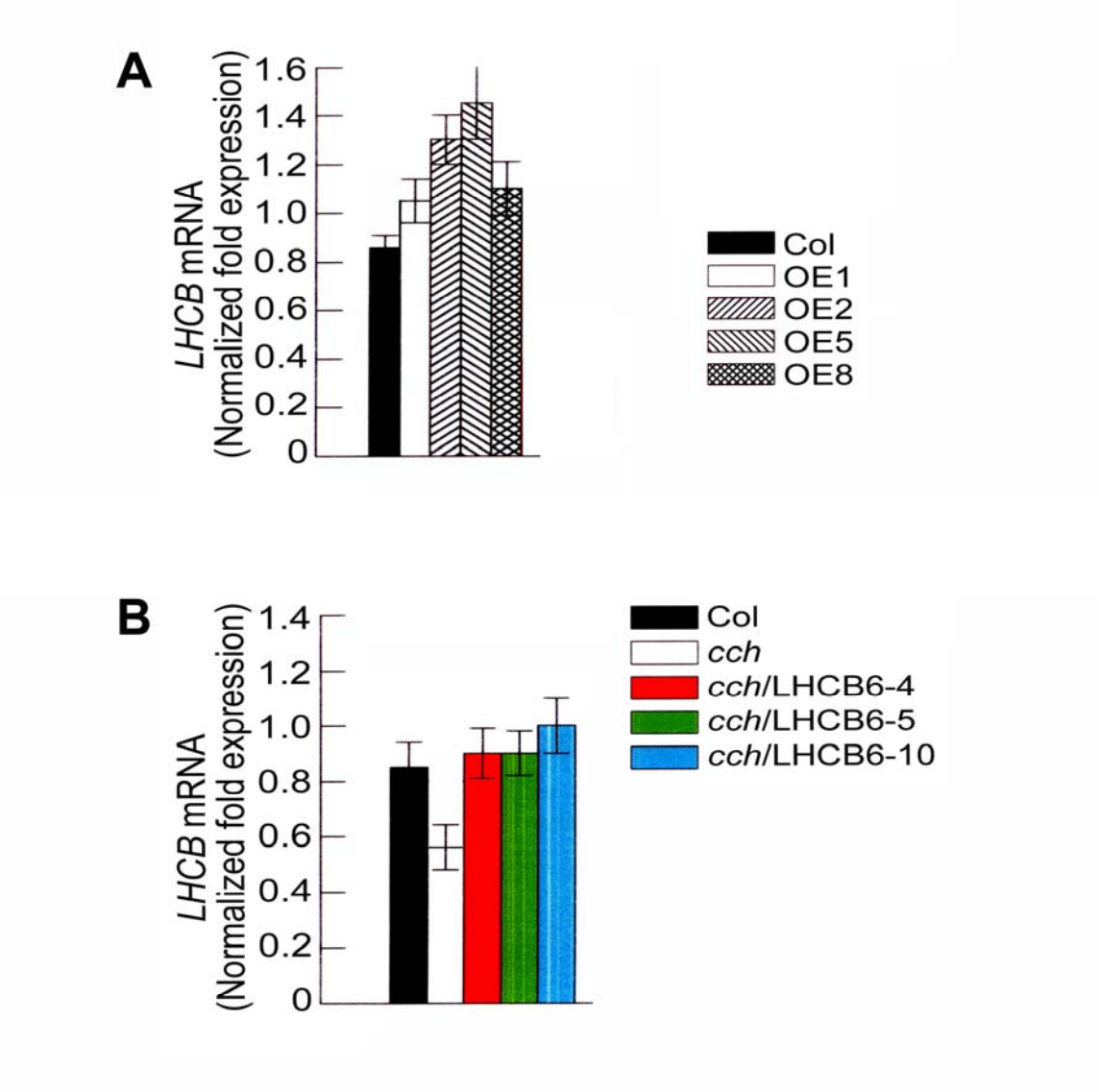
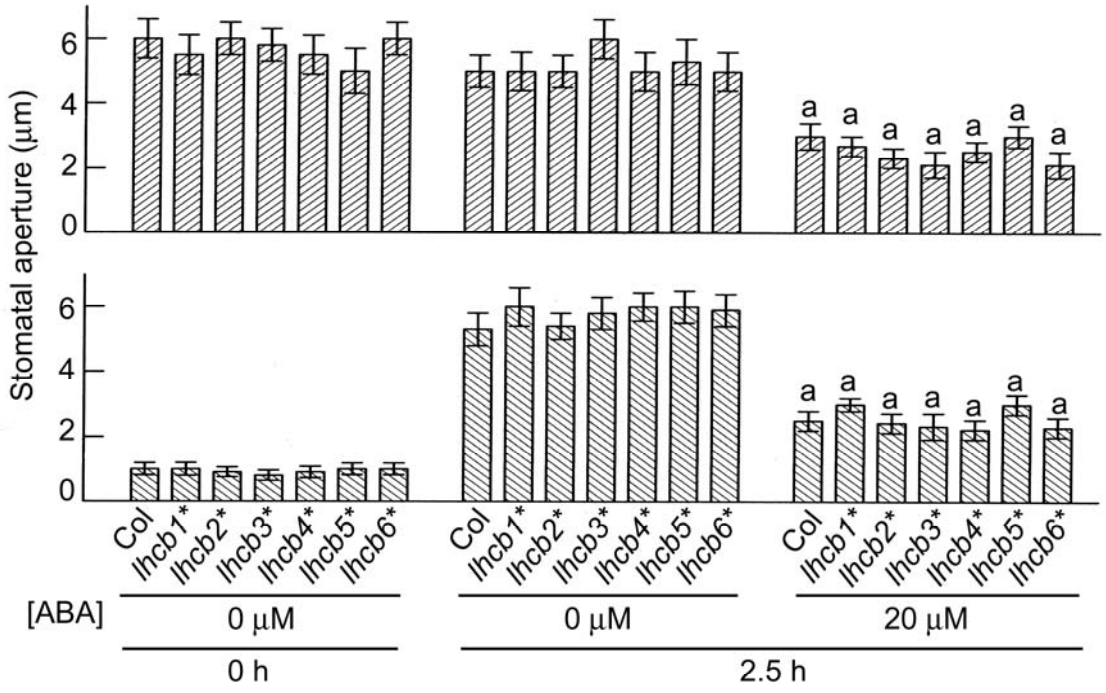
Supplementary Data



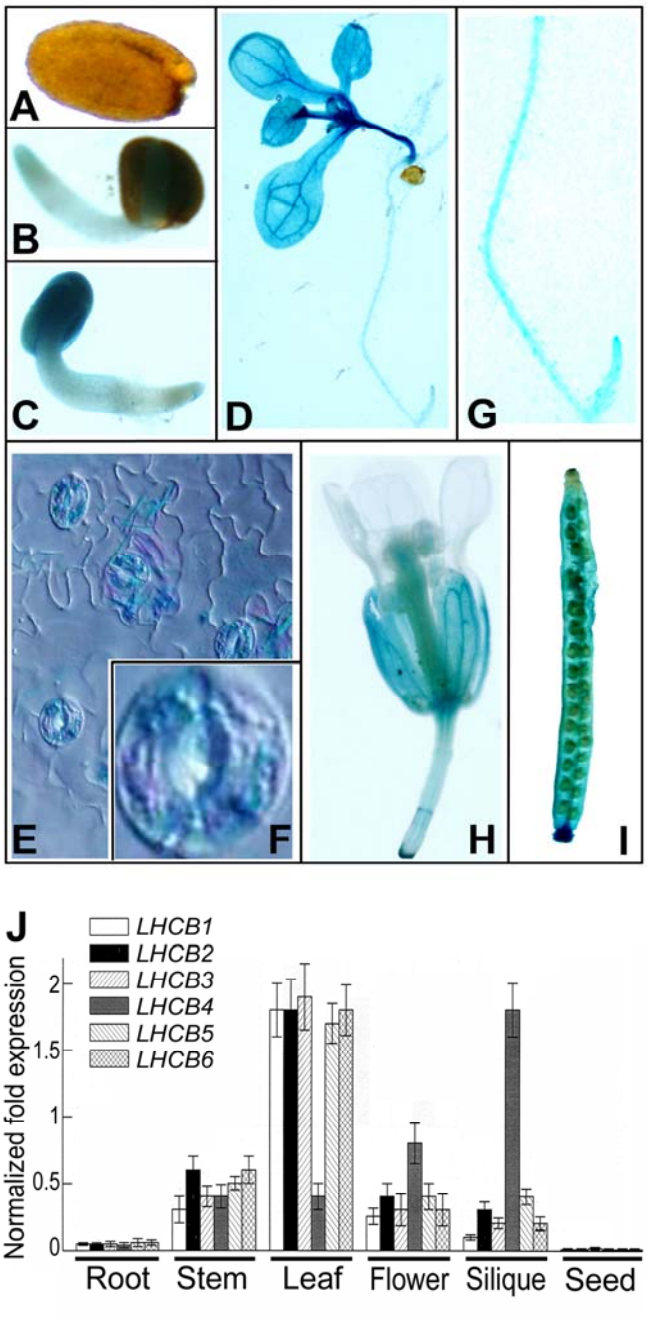
Supplementary Fig. S1. Concentrations of endogenous ABA and accumulation of dry substances of the different *lhcb* mutant plants. Two-week-old plants of the different mutants and wild-type Col plants were used for these assays. **(A)** ABA levels. The rosette leaves were used to assay ABA concentrations by ELISA method using a kit (Plant Growth Regulator Immunoassay Detection Kit, PGR1, Sigma) according to the procedures provided by the kit supplier. **(B)** Accumulation of dry substances estimated by dry weight/fresh weight. Note that there is no significant difference in the ABA levels and accumulation of dry substances between the mutants and the wild-type plants. Each value is the mean ± SE of three biological determinations.



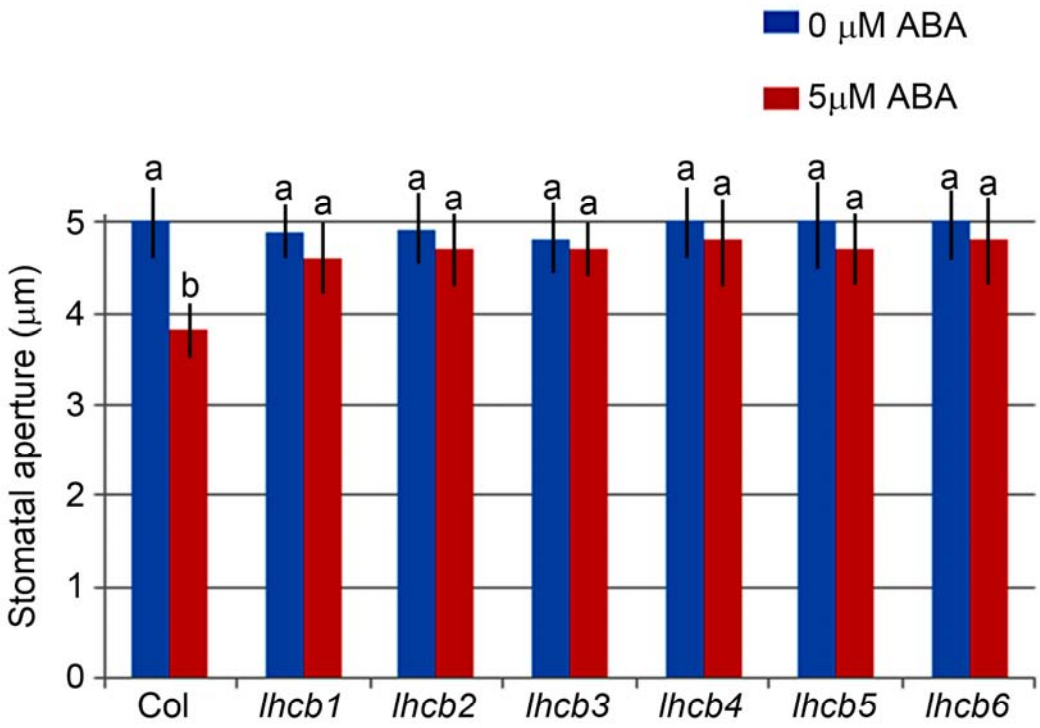
Supplementary Fig. S2. Real-time PCR analysis of the *LHCB6*-overexpression lines. **(A)** *LHCB6* mRNA levels in four overexpression lines (OE1, OE2, OE5, and OE8), showing that the *LHCB6* mRNA levels are higher in the OE lines compared with wild-type Col plants. Each value is the mean ± SE of three biological determinations. **(B)** *LHCB6* mRNA levels in three lines of the *LHCB6*-overexpression in the *cch* mutant background (*cch*/LHCB6-4, *cch*/LHCB6-5, and *cch*/LHCB6-10), showing that the *LHCB6* mRNA levels in these transgenic lines are similar to those in the wild-type Col plants. Each value is the mean ± SE of three biological determinations.



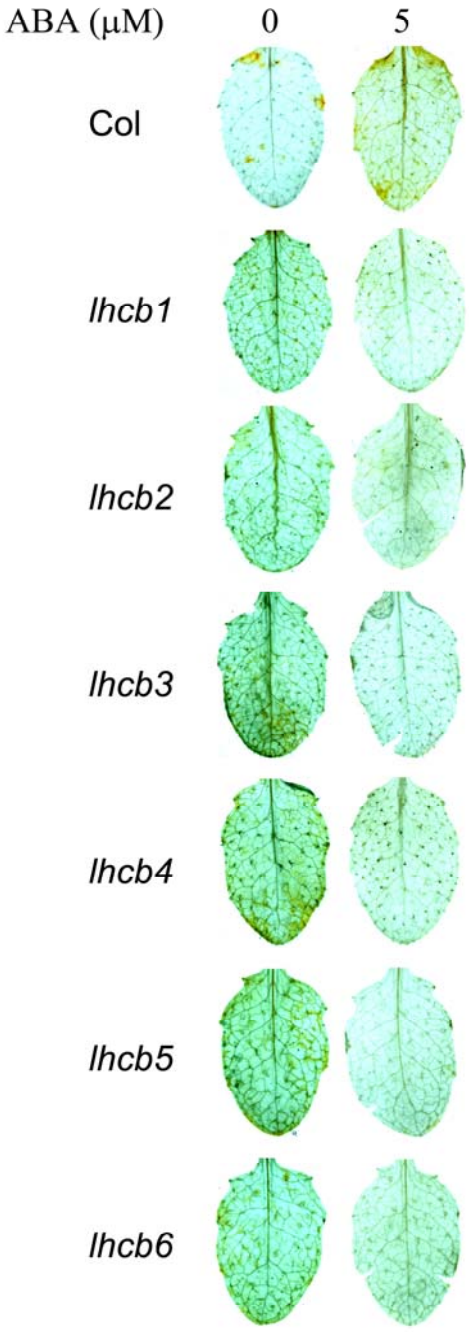
Supplementary Fig. S3. Expression of the 35S-promoter-driven *LHCB*s rescues ABA sensitivity of the *lhcb* mutants. ABA-induced stomatal closure (top) and ABA-inhibited stomatal opening (bottom). 0 μM ABA at 0 h, initial stomatal aperture. Stomatal aperture was recorded 2.5 h after the treatment with 0 μM ABA or 20 μM ABA. Each value is the mean ± SE of five independent biological determinations. n = 60 apertures per experiment. The same letters indicate no significant difference at the level of $P < 0.05$ (Student's t test). Col, wild-type; *lhcb1**, *lhcb2**, *lbcb3**, *lbcb4**, *lbcb5** and *lbcb6** represent the complementation lines expressing, respectively, *LHCB1*, *LHCB2*, *LHCB3*, *LHCB4*, *LHCB5* and *LHCB6*.



Supplementary Fig. S4. Different members of *LHCB*s are expressed ubiquitously in different tissues/organs except for dry seeds. The *LHCB6* expression was shown by the LHCB-promoter-linked GUS-transgenic plants. (**A**) Dry seed. (**B**) and **(C)** Germinating seed. (**D**) 9-day-old seedling. (**E**) and **(F)** Stomata in a matured leaf. Note that LHCB6 is expressed predominantly in the guard cells. (**G**) Roots. (**H**) Flower. (**I**). **(J)** Real-time PCR data for gene expression of *LHCB1*, *LHCB2*, *LHCB3*, *LHCB4*, *LHCB5* and *LHCB6*.



Supplementary Fig. S5. Stomatal aperture of Col plants and *lhcb* mutants when assaying ROS levels in stomata. Each value is the mean ± SE of three independent biological determinations, and different letters indicates significant differences at $P < 0.05$ (Duncan's multiple range test). The data of the ROS levels are presented in Fig. 4C.



Supplementary Fig. S6. ROS homeostasis is altered in *lhcb* mutants. H_2O_2 production in leaves in response to ABA (0 and 5 μM), detected by 3,5-diaminobenzidine (DAB) in wild-type Col and different *lhcb* mutants according to the method as described below. The entire experiment was replicated five times with similar results.
DAB staining (Method described by Miao et al., 2006): The leaves were collected and vacuum-infiltrated with the DAB solution (1 mg/mL, pH 3.8; Sigma-Aldrich). The sampled leaves were placed under high humidity until reddish-brown precipitate was observed (8 h), then fixed with a solution of 3:1:1 ethanol:lactic acid:glycerol and photographed.
Reference: Miao Y, Lv D, Wang P, Wang XC, Chen J, Miao C, Song CP. 2006. An *Arabidopsis* glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. *Plant Cell* **18,** 2749-2766.

Supplementary Table S1. Primers Used in this Study.

1. Primers for Identification of the *lhcb1*, *lhcb2*, *lhcb3*, *lhcb4*, *lhcb5* and *lhcb6* T-DNA Insertion Knockout/Knockdown Mutants

The primers for analysis of T-DNA flanking sequence:
LBa1: 5'-GGTTCACGTAGTGGGCCATC-3'
RBa1: 5'-GTTTCTGACGTATGTGCTTAGC-3'

The primers for analysis of the *LHCB* geneomic sequences:
LP1 (for *LHCB1*): 5'-GCGACTCTGTAACCTTCAACG-3'
RP1 (for *LHCB1*): 5'-AAAGTGACGATGAAATCGATTC-3'
LP2 (for *LHCB2*): 5'-TTGTAAGAGCCATAGCCAAGG-3'
RP2 (for *LHCB2*): 5'-ATGGTCCCAAGTACTTGGGAC-3'
LP3 (for *LHCB3*): 5'-ACCCAACGGGTCAAAGTATTG-3'
RP3 (for *LHCB3*): 5'-TTAAATGGGCAACCAGAAAAG-3'
LP4 (for *LHCB4*): 5'-TGGTCTTAAGAATACGACAGAAGG-3'
RP4 (for *LHCB4*): 5'-GGTTTAGGCTTTTGGTTTTGG-3'
LP5 (for *LHCB5*): 5'-CACGAAGAAGCTTGTGAAAGC-3'
RP5 (for *LHCB5*): 5'-AGGAAGAGACGAAACTGCTCC-3'
LP6 (for *LHCB6*): 5'-TAGACTTACGAGCCATCGAGC -3'
RP6 (for *LHCB6*): 5'-TACCACCGACCGTACAAGATC-3'

2. Primers for Cloning ORF cDNAs of *LHCB1* to *LHCB6* to Rescue the *lhcb1* to *lhcb6* Mutants

The cDNAs were driven by 35S promoter integrated into pCAMBIA1300-221 vector. The restriction sites are indicated in parenthesis.

LHCB1 (804 bp) :
forward primer: 5'-TCCCCCGGGATGGCCGCCTCAACAATGG -3' (*Sma*I)
reverse primer: 5'-ACGCGTCGACTCACTTTCCGGGAACAAAGTTG-3' (*Sal*I)
LHCB2 (795 bp) :
forward primer: 5'-GCTCTAGAATGGCCACATCAGCTATCCAAC-3' *(Xba*I)
reverse primer: 5'-GGGGTACCCTTTCCGGGGACAAAGTTAGTAG-3' (*Kpn*I)
LHCB3 (795 bp) :
forward primer: 5'-GCTCTAGAATGGCATCAACATTCACGAGCTC-3' (*Xba*I)
reverse primer: 5'-GGGGTACCAGCTCCAGGTGCAAACTTAGTTG-3' (*Kpn*I)
LHCB4 (831 bp) :
forward primer: 5'-GCTCTAGAATGGCTACCACCACTGCAG-3' (*Xba*I)
reverse primer: 5'-ACGCGTCGACCTAATTGTTAAAGGTGGCAAGG-3' (*Sal*I)
LHCB5 (843 bp) :
forward primer: 5'-TCCCCCGGGATGGCGTCTTTGGGTGTGTC-3' (*Sma*I)
reverse primer: 5'-ACGCGTCGACTTAGAGAGTGGGAGCTCTCTCGG-3' (*Sal*I)
LHCB6 (777 bp) :
forward primer: 5'-TCCCCCGGGATGGCGATGGCGGTCTCC-3' (*Sma*I)
reverse primer: 5'-ACGCGTCGACTCACAAACCAAGAGCACCGAGAG-3' (*Sal*I)

3. Gene-Specific Primers for Real-Time PCR Analysis of Gene Expression

ABF1 (At1g49720):
forward primer: 5'-TCAACAACTTAGGCGGCGATAC-3'
reverse primer: 5'-GCAACCGAAGATGTAGTAGTCA-3'
ABF2 (At1g45249):
forward primer: 5'-TTGGGGAATGAGCCACCAGGAG-3'
reverse primer: 5'-GACCCAAAATCTTTCCCTACAC-3'
ABF3 (At4g34000):
forward primer: 5'-CTTTGTTGATGGTGTGAGTGAG-3'
reverse primer: 5'-GTGTTTCCACTATTACCATTGC-3'
ABF4 (At3g19290):
forward primer: 5'-AACAACTTAGGAGGTGGTGGTC-3'
reverse primer: 5'-CTTCAGGAGTTCATCCATGTTC-3'
ABI1 (At4g26080):
forward primer: 5'-AGAGTGTGCCTTTGTATGGTTTTA-3'
reverse primer: 5'-CATCCTCTCTCTACAATAGTTCGCT-3'
ABI2 (At5g57050):
forward primer: 5'-GATGGAAGATTCTGTCTCAACGATT-3'
reverse primer: 5'-GTTTCTCCTTCACTATCTCCTCCG-3'
ABI3 (At3g24650):
forward primer: 5'-TCCATTAGACAGCAGTCAAGGTTT-3'
reverse primer: 5'-GGTGTCAAAGAACTCGTTGCTATC-3'
ABI4 (At2g40220):
forward primer: 5'-GGGCAGGAACAAGGAGGAAGTG-3'
reverse primer: 5'-ACGGCGGTGGATGAGTTATTGAT-3'
ABI5 (At2g36270):
forward primer: 5'-CAATAAGAGAGGGATAGCGAACGAG-3'
reverse primer: 5'-CGTCCATTGCTGTCTCCTCCA-3'
Actin2/8 :
forward primer: 5'-GGTAACATTGTGCTCAGTGGTGG-3'
reverse primer: 5'-AACGACCTTAATCTTCATGCTGC-3'
ERD10 (At1g20450):
forward primer: 5'-TCTCTGAACCAGAGTCGTTT-3'
reverse primer: 5'-CTTCTTCTCACCGTCTTCAC-3'
KIN1 (At5g15960):
forward primer: 5'-ACCAACAAGAATGCCTTCCA-3'
reverse primer: 5'-CCGCATCCGATACACTCTTT-3'
KIN2 (At5g15970):
forward primer: 5'-ACCAACAAGAATGCCTTCCA-3'
reverse primer : 5'-ACTGCCGCATCCGATATACT-3'
LHCB1:
forward primer: 5'-ATGGCCGCCTCAACAATGG-3'
reverse primer: 5'-CGGTAAGGTAGCTGGGTGAC-3'

LHCB2:
forward primer: 5’-ATGGCCACATCAGCTATCC-3’
reverse primer: 5’-CTCCAGTTAAGTAAGACGGTGTG-3’
LHCB3:
forward primer: 5’-AATGATCTTTGGTATGGACCTGAC-3’
reverse primer: 5’-CCACACGGACCCACTTTTG-3’
LHCB4:
forward primer: 5’-CAGCCGTACACTGAAGTCTTTGG-3’
reverse primer: 5’-TTCTATCCATATCAACGTCGTCAAC-3’
LHCB5:
forward primer: 5’-GGAGCAGCTGGTTTCATCATT-3’
reverse primer: 5’-ATCAAATGGACCTCCTGGG-3’
LHCB6:
forward primer: 5’-GCGATGGCAGCGGTTCTTG-3’
reverse primer: 5’-CCATGGCGTTGCCCACTCA -3’
MYB2 (At2g47190):
forward primer: 5’-TGCTCGTTGGAACCACATCG-3’
reverse primer: 5’-ACCACCTATTGCCCCAAAGAGA-3’
MYC2 (At1g32640):
forward primer: 5’-TCATACGACGGTTGCCAGAA-3’
reverse primer: 5’-AGCAACGTTTACAAGCTTTGATTG-3’
OST1 (At4g33950):
forward primer: 5’-TGGAGTTGCGAGATTGATGAGAG-3’
reverse primer: 5’-CCTGTGGTTGATTATCTCCCTTTTT-3’
RAB18 (At5g66400)
forward primer: 5’-CAGCAGCAGTATGACGAGTA-3’
reverse primer: 5’-CAGTTCCAAAGCCTTCAGTC-3’
RD29A (At5g52310) :
forward primer: 5’-ATCACTTGGCTCCACTGTTGTTC-3’
reverse primer: 5’-ACAAAACACACATAAACATCCAAAGT-3’
RbohD (At5g47910)：
forward primer: 5’-CTAGCTTTGGATTTTTCTCGA-3’
reverse primer: 5’-GTAACCAACAAAACGGTAGGG-3’
RbohF (At1g64060)：
forward primer: 5’-CTAAAAGACAAGAAGGAAGAAGCC-3’
reverse primer: 5’-CTTCTAGAGTCTCCATCTCATTTGC-3’