

## **Journal of Experimental Botany Supplementary Information**

Article title: *Aethionema arabicum*: a novel model plant to study the light control of seed germination

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The following Supplementary Information is available for this article:

- Fig. S1** Germination of dimorphic seed types in response to light.
- Fig. S2** Heatmap of all 87 genes light-regulated in *Aethionema arabicum* CYP seeds and differentially expressed in light-exposed TUR and CYP seeds based on RPKM (reads per kilobase of transcript per million mapped reads) values.
- Fig. S3** Identification of the Arabidopsis orthologue of *Aethionema* AA18G00108 as GA2ox3. (A) Gibberellin 2-oxidase family phylogeny based on protein sequences. (B) Synteny of GA2ox3 position in the genome of Arabidopsis and *Aethionema*.
- Fig. S4** Accumulation of GA forms in *Aethionema arabicum* TUR and CYP seeds under dark and light conditions.
- Fig. S5** Identification and alignments of phytochromes in *Aethionema arabicum*. (A) Phylogenetic tree of phytochromes. (B-F) Phytochrome A, B, C, D, E protein alignments of three *Aethionema arabicum* accessions.
- Fig. S6** Alignment of PIL5/PIF1 protein sequence of three *Aethionema arabicum* accessions.
- Table S1** Information about geographic origin of *Aethionema arabicum* accessions.
- Table S2** List of primers used for quantitative RT-PCR analysis.
- Table S3** List of *Aethionema* accession numbers used for this study.
- Dataset S1** List of differentially expressed *Aethionema arabicum* genes in TUR Dark versus TUR Light.
- Dataset S2** List of differentially expressed *Aethionema arabicum* genes in CYP Dark versus CYP Light.
- Dataset S3** List of differentially expressed *Aethionema arabicum* genes in CYP Dark versus TUR Dark.
- Dataset S4** List of differentially expressed *Aethionema arabicum* genes in CYP Light versus TUR Light.
- Dataset S5** List of common differentially expressed *Aethionema arabicum* genes in CYP Light versus TUR Light and TUR Dark versus TUR Light.

**Dataset S6** List of target genes of Arabidopsis PIL5/PIF1 and transcriptional changes of orthologues in the Aethionema experiments.

**Dataset S7** List of plant species for which protein sequences were considered for phylogenetic tree constructions.

### Supplementary Figure Legends

**Fig. S1** Germination of dimorphic seed types in response to light. Germination of mucilaginous (M+) and non-mucilaginous (M-) seeds were tested from TUR and CYP accessions in dark or under white light ( $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Images were taken 7 days after imbibition.

**Fig. S2** Heatmap of all 87 genes light-regulated in CYP seeds and differentially expressed in light-exposed TUR and CYP seeds based on RPKM values.

**Fig. S3** Identification of the *A. thaliana* orthologue of *Ae. arabicum* AA18G00108 as GA2ox3. **(a)** Phylogenetic tree of gibberelin2-oxidases using Bayesian inference (2000000 generations, standard deviation of split frequencies 0.063371) and allows clear assignment of *Ae. arabicum* orthologues. Sequences of *A. thaliana* (ARATH) and *Ae. arabicum* (AETAR) are marked in green and red, respectively. For detailed assignment of five letter code see Supplemental Dataset 7. **(b)** Synteny of GA2ox3 position in the genome of *A. thaliana* and *Ae. arabicum*.

**Fig. S4** Accumulation of GA forms in *Ae. arabicum* TUR and CYP seeds under dark and light conditions.

**Fig. S5** Identification and alignments of phytochromes in *Ae. arabicum*. **(a)** Phylogenetic tree of phytochromes using Bayesian inference (1688500 generations, standard deviation of split frequencies 0.009992) and allows clear assignment of *Ae. arabicum* orthologues. Sequences of *A. thaliana* (ARATH) and *Ae. arabicum* (AETAR) are marked in green and red, respectively. For detailed assignment of five letter code see Supplemental Dataset 7. **(b-f)** Phytochrome protein alignments of three *Ae. arabicum* accessions. Germination of TUR seeds is light-insensitive while CYP and KM2397 both have light inhibited germination.

**Fig. S6** Alignment of PIL5/PIF1 protein sequence of three *Ae. arabicum* accessions. Germination of TUR seeds is light-insensitive while CYP and KM2397 both have light inhibited germination.

**Table S1 Information about geographic origin of *Aethionema arabicum* accessions**

ID number	Species	Origin	Region	Altitude	Seed source
KM2491	<i>Aethionema heterocarpum</i>	Israel	Golan Heights, Mt. Hermonit	1100 m	S. Cohen
KM2614	<i>Aethionema heterocarpum</i>	Turkey	Belen/Hatay	700 m	K. Mummenhoff
KM2496	<i>Aethionema carneum</i> (Banks & Sol.) Fedts	Israel	Philistine Plain	39 m	A. Singer, Israel Plant Gene Bank 21673
KM2397	<i>Aethionema arabicum</i> Andr. Ex DC.	Turkey	Elazığ, Harput	1200 m	E. Schranz
Iran8458	<i>Aethionema arabicum</i> Andr. Ex DC.	Iran	Mt. Dizin, Karaj	~2600 m	S. Mohammadin
Iran8456-1	<i>Aethionema arabicum</i> Andr. Ex DC.	Iran	Mt. Touchal, Tehran	~2600 m	S. Mohammadin
Iran8456-2	<i>Aethionema arabicum</i> Andr. Ex DC.	Iran	Mt. Touchal, Tehran	~2600 m	S. Mohammadin

**Table S2 List of primers used for quantitative RT-PCR analysis**

Name	Nucleotide sequence
AearACT2_for	AATTGAGCATGGTGTGGTCA
AearACT2_rev	GCTCTTCAGGAGCAATACGG
AearUBQ10_for	GAGGATGGCCGAACATTG
AearUBQ10_rev	TGCCCGTTAGGGTTTTGA
AearAPC2_for	TCTCCTGCAATCGAGGACTT
AearAPC2_rev	GCAGTGAGCAACCGGTATTT
AearNCED5_for	GCCGTTTGATCTTGACGCTC
AearNCED5_rev	ACGGAGTTTAGTTTACGGCGT
AearNCED6_for	GCTTCTTCAGCTCTCGACAA
AearNCED6_rev	GAACCGTTGGATCAGTCGGT
AearNCED9_for	TCCTTTTCTCCGATCAAACCTCT
AearNCED9_rev	TCGAATTCGAGGATTTGGGGA
AearABA1_for	GGAGGAGAAGAAAGGGGAGA
AearABA1_rev	ATCCTTTCTTTTTTCGCAGCA
AearABA2_for	AACATGGCGCAAGAGTCTG
AearABA2_rev	TGGATGAAACAAGCCTCCTT
AearABA3_for	TGGAAGAATTTCTTGAGGAATTTGGA
AearABA3_rev	TCTTGAATTCGGTGTACGGA

AearCYP707A2_for	GCGGTTCCAACAAAGAAAAC
AearCYP707A2_rev	GAGTGGCGAAGAAGGAATTG
AearGA3ox1_for	TCTTCGTCACCTCCCTGACT
AearGA3ox1_rev	GATGAGCGGGAGAGTTGTGT
AearGA3ox2_for	CCCATCCAATACACATTCCA
AearGA3ox2_rev	GCCTTGGCTGAGAAAAGAAG
AearGA2ox2_for	TCACAGCCCCTCACTTTAGA
AearGA2ox2_rev	GGCTTCTGGGTCGGTTAAAT
AearGA2ox3_for	CGCGTCTCTCTTAACCCAAC
AearGA2ox3_rev	TCACATGCCTTGACCATTTG
AearRGA_for	GTCCTCGGCTACAAGGTCAG
AearRGA_rev	TGAGGGAATCCATCTTCAGG
AearGAI_for	TGGTGGTGCTTCTGTACCTG
AearGAI_rev	TCATCCATGTGACCACCATC
AearRGL2_for	GGACCCTGCAACAATACCAT
AearRGL2_rev	CCACGCCTTCAACTTCCTTA
AearSOM_for	CCGCGAAAATTGATATCTCC
AearSOM_rev	TCGATTTGATTTCTCACCA
AearDAG1_for	GCAATTACTCCCCAATAACAACA
AearDAG1_rev	GTTTGCGTTTTCGTTTGAG
AearDAG2_for	GGAACAACAACAACAACAATGA
AearDAG2_rev	CTACCGGAGATGGATGTGGT
AearJMJ20_for	ATGGGAATCGAGATTGTTGG
AearJMJ20_rev	CGGCTGGTTTTTATGCAAGT
AearJMJ22_for	GGGAATGAGCGACCTGATTA
AearJMJ22_rev	TCACTGCATTCCAAGCAGAC
AearPAR1_for	CTCTAGCAACTCCCGACACC
AearPAR1_rev	TCTCCAACAATCTCCGTTTTG
AearPAR2_for	CGTCACTTCAGCGAGTGAAA
AearPAR2_rev	TTCCCGGA ACTATTGTCTGC
AearDOG1_for	CGCGTCACTAAGCGATCTAAC
AearDOG1_rev	GCCGCGTCTTCTTG TAGACTT
AearABI3_for	ATGGCGGAAACCTTCCTTAT
AearABI3_rev	GAGGAAGAGGAGGAGGAGGA
AearABI4_for	CTCAACGCAAACGCAAAGGT
AearABI4_rev	TCACGGATTT CAGCAACCCA
AearABI5_for	GAACGCCGAAGAAAACAATC
AearABI5_rev	TCAACCCGGTTTGGTACATT

**Table S3 List of Aethionema accession numbers used for this study**

<b>Name</b>	<b>Accession number</b>
<i>AearNCED5</i>	AA54G00417
<i>AearNCED6</i>	AA78G00012
<i>AearNCED9</i>	AA31G00716
<i>AearABA1</i>	AA8G00025
<i>AearABA2</i>	AA32G01008
<i>AearABA3</i>	AA37G00095
<i>AearCYP707A2</i>	AA32G00787
<i>AearGA3ox1</i>	AA37G00176
<i>AearGA3ox2</i>	AA31G00895
<i>AearGA2ox2</i>	AA54G00411
<i>AearGA2ox3</i>	AA18G00108
<i>AearRGA</i>	AA14G00090
<i>AearGAI</i>	AA53G00639
<i>AearRGL2</i>	AA10G00264
<i>AearPIL5</i>	AA33G00286
<i>AearSOM</i>	AA7G00098
<i>AearDAG1</i>	AA61G00535
<i>AearDAG2</i>	AA21G00391
<i>AearJMJ20</i>	AA5G00018
<i>AearJMJ22</i>	AA283G00008
<i>AearPAR1</i>	AA21G00074
<i>AearPAR2</i>	AA61G00301
<i>AearDOG1</i>	AA6G00020
<i>AearABI3</i>	AA109G00007
<i>AearABI4</i>	AA29G00257
<i>AearABI5</i>	AA60G00170
<i>AearPHYA</i>	AA65G00005
<i>AearPHYB</i>	AA26G00394
<i>AearPHYC</i>	AA1057G00001
<i>AearPHYD</i>	AA18G00159
<i>AearPHYE</i>	AA57G00083