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Supplementary Information for

Comprehensive mapping of abiotic stress inputs into the soybean circadian clock

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Datasets S1 to S8

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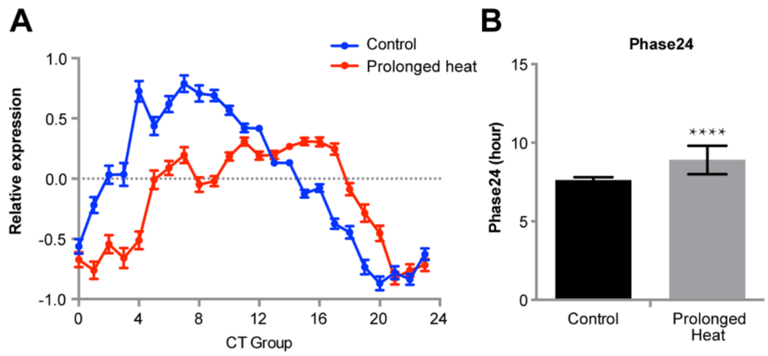

Fig. S1. Prolonged heat stress (5 hours of treatment) causes a mild phase delay. (*A*) Normalized expression levels of time-indicating genes. CT: circadian time. (*B*) Prolonged heat stress causes a mild but significant phase delay. ****, $p < 0.0001$ (Student's *t*-test). Mean and standard error are plotted. (*A*) and (*B*) are derived from E-MTAB-2852.

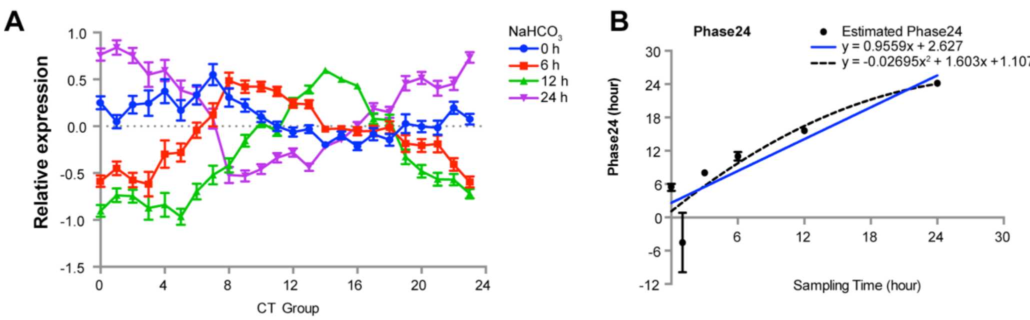

Fig. S2. Short-term alkaline stress does not cause phase advances in roots. (*A*) Normalized expression levels of time-indicating genes. CT: circadian time; h: hour; (*B*) Estimated Phase24 plotted against reported sampling time. A quadratic curve (dashed black line) does not fit the data better than a straight line (solid blue line), $p > 0.05$ (Exact *F*-test). Mean and standard error are plotted. (*A*) and (*B*) are derived from GSE17883.

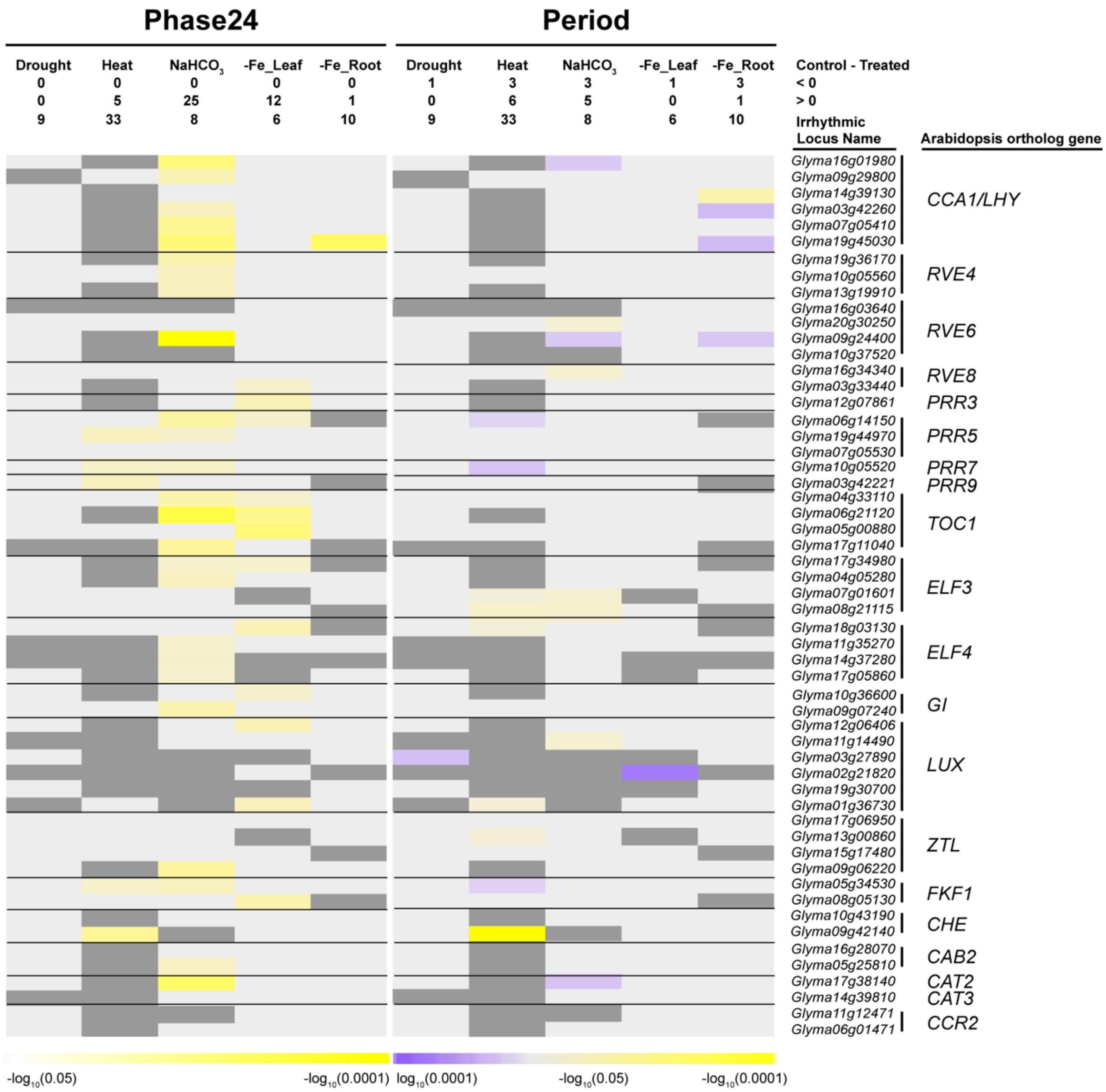

Fig. S3. Summary of soybean circadian clock responses to abiotic stresses under low statistical stringency level. Student's *t*-test with the Benjamini-Hochberg multiple comparison correction was used to compare control and treated samples and derive false discovery rate (FDR). When the control value is greater than the treated value, $-\log_{10}$(FDR) is plotted in yellow. When the control value is less than the treated value, $\log_{10}$(FDR) is plotted in blue. To highlight only the statistically significant changes, FDRs greater than 0.05 are masked and represented in light gray. Genes with non-significant oscillations ($p > 0.05$) in either the control or treated samples are also masked and represented in gray. The total number of genes with statistically significant changes are summarized and listed for each stress. For each clock gene group, the locus names are arranged in the descending order based on their sequence similarity to their orthologs in *Arabidopsis*.

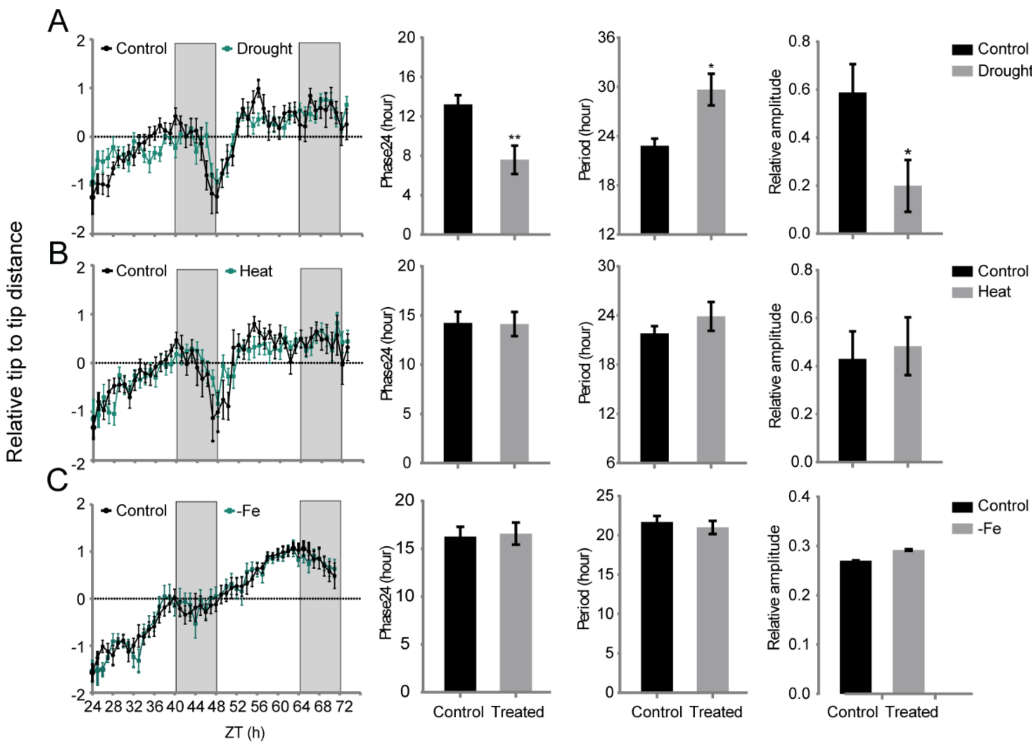

Fig. S4. Assay of soybean circadian leaf movement in response to abiotic stress under constant light conditions. *(A)* Leaf movement of soybean under mild drought stress. *(B)* Leaf movement of soybean in response to heat shock. *(A)* and *(B)* Williams 82 seedlings were grown in soil under 16 h light/8 h dark for 14 days and released into constant light for 24 hours, and then the movement of the first unifoliolate leaf was recorded hourly. *(C)* Soybean leaf movement under short-term iron deficiency. Seven-day-old IsoClark seedlings were transferred from germination paper to a hydroponic system and grown for 10 days in iron-sufficient conditions (100 μM Fe $(NO_3)_3$) under the ambient light condition in the greenhouse. The seedlings were kept in constant light starting on the ninth day. On the tenth day, the treated seedlings were switched to iron-limited conditions (50 μM Fe $(NO_3)_3$) at ZT 24, and then the movement of the first unifoliolate leaf was recorded hourly. Circadian rhythm parameters, including Phase24, period and relative amplitude, were derived via nonlinear regression. The white and gray regions in the trace plot indicate subjective light and dark periods, respectively. *, $p < 0.05$; **, $p < 0.01$ (Student's *t*-test).

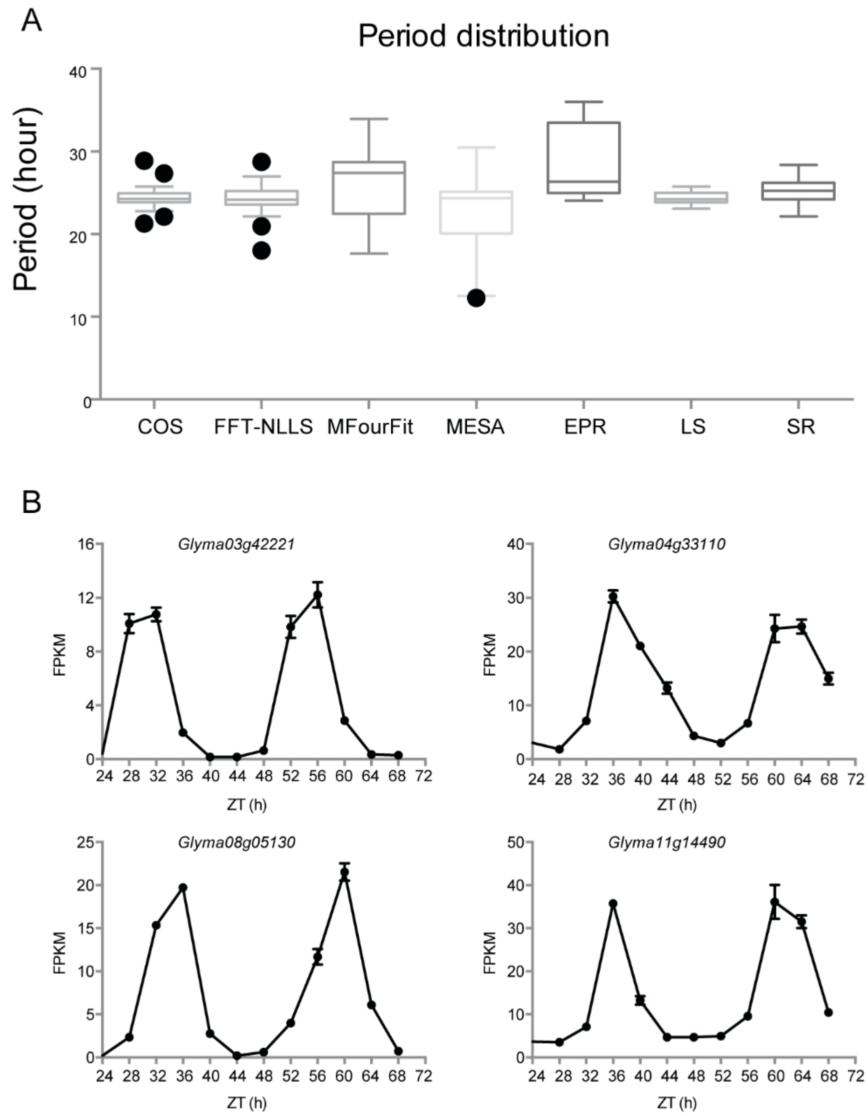

Fig. S5. Comparison of methods for period analysis. *(A)* Period distributions of soybean orthologs of circadian clock components analyzed with different methods. The RNA-seq results from this study were used for period analysis. The COS method was used in this study to derive circadian parameters, including Phase24, period and relative amplitude. The other six methods are available on the BioDare website (https://sourceforge.net/projects/biodare/). FFT-NLLS: fast fourier transform-non-linear least squares; MESA: maximum entropy spectral analysis; EPR: Enright periodogram; LS: Lomb-Scargle periodogram; SR: spectrum resampling. *(B)* Four representative genes from the analysis in *(A)*. The periods of circadian genes with obvious circadian rhythm were accurately estimated using the COS method but were considered arrhythmic with the LS and FFT-NLLS methods.

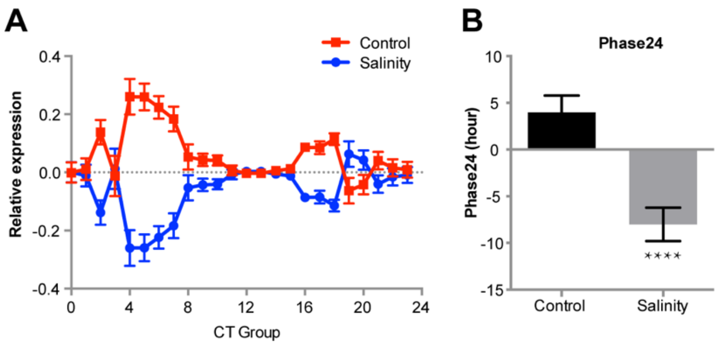

Fig. S6. Salinity stress causes a dramatic phase shift. (*A*) Normalized expression levels of time-indicating genes. CT: circadian time. (*B*) Salinity stress causes a dramatic phase shift. ****, $p < 0.0001$ (Student's *t*-test). Mean and standard error are plotted. (*A*) and (*B*) are derived from GSE41125.

Table S1. Orthologs of key circadian clock and output genes in soybean.

Gene name	Arabidopsis Gene	Soybean ortholog Gene	Score	TBLASTN (e-value)
CCA1/LHY	*AT2G46830*	*Glyma16g01980*	100.5	3.00E-20
		Glyma09g29800	94	3.40E-18
		Glyma14g39130	91.7	1.90E-17
		Glyma03g42260	81.3	3.30E-14
		Glyma07g05410	80.1	1.60E-21
		Glyma19g45030	79	1.90E-13
RVE4	*AT5G02840*	*Glyma10g05560*	76.3	6.10E-15
		Glyma13g19910	75.9	1.10E-13
		Glyma19g36170	74.7	2.40E-13
RVE6	*AT5G52660*	*Glyma16g03640*	79.3	1.30E-14
		Glyma20g30250	79	1.90E-14
		Glyma09g24400	77.4	6.10E-14
		Glyma10g37520	74.3	6.40E-13
RVE8	*AT3G09600*	*Glyma16g34340*	82.4	9.80E-16
		Glyma03g33440	79.7	7.50E-36
PRR3	*AT5G60100*	*Glyma12g07861*	93.2	5.00E-36
PRR5	*AT5G24470*	*Glyma06g14150*	107.5	7.00E-43
		Glyma19g44970	92.8	2.20E-35
		Glyma07g05530	92.8	9.90E-34
PRR7	*AT5G02810*	*Glyma10g05520*	161	6.10E-39
PRR9	*AT2G46790*	*Glyma03g42221*	88.2	2.70E-34
TOC1	*AT5G61380*	*Glyma04g33110*	207.6	3.10E-54
		Glyma06g21120	204.9	2.30E-53
		Glyma05g00880	196.4	1.50E-50
		Glyma17g11040	177.2	2.00E-44
ELF3	*AT2G25930*	*Glyma17g34980*	152.8	2.60E-34
		Glyma04g05280	100.9	3.80E-20
		Glyma07g01601	79	2.30E-13
		Glyma08g21115	75.9	2.10E-12
ELF4	*AT2G40080*	*Glyma18g03130*	117.5	3.70E-29
		Glyma11g35270	116.7	5.50E-29
		Glyma14g37280	83.2	2.00E-17
		Glyma17g05860	79.3	3.70E-16
GI	*AT1G22770*	*Glyma10g36600*	673.7	0
		Glyma09g07240	642.9	0
LUX	*AT3G46640*	*Glyma12g06406*	229.2	1.30E-64
		Glyma11g14490	179.1	2.10E-47

		Glyma03g27890	154.8	3.40E-39
		Glyma02g21820	148.7	4.80E-37
		Glyma19g30700	143.3	2.90E-35
		Glyma01g36730	85.5	1.40E-16
ZTL	*AT5G57360*	*Glyma17g06950*	844.7	0
		Glyma13g00860	842	0
		Glyma15g17480	837.4	0
		Glyma09g06220	833.9	0
FKF1	*AT1G68050*	*Glyma05g34530*	885.2	0
		Glyma08g05130	866.7	0
CHE	*AT5G08330*	*Glyma10g43190*	122.5	3.50E-29
		Glyma09g42140	117.9	1.40E-27
CAB2	*AT1G29920*	*Glyma16g28070*	468.8	1.90E-148
		Glyma05g25810	454.9	1.00E-143
CAT2	*AT4G35090*	*Glyma17g38140*	759.6	0
CAT3	*AT1G20620*	*Glyma14g39810*	723.4	0
CCR2	*AT2G21660*	*Glyma11g12471*	88.6	2.20E-18
		Glyma06g01471	87.8	3.30E-18

Dataset S1 (separate file). CT phase of 3695 time-indicating genes in soybean

Dataset S2 (separate file). Experimental conditions of soybean transcriptome datasets under abiotic stress treatments

Dataset S3 (separate file). Time-indicating genes of soybean transcriptome datasets under abiotic stress treatments

Dataset S4 (separate file). Circadian parameter analysis of soybean transcriptome datasets under abiotic stress treatments

Dataset S5 (separate file). Summary of RASL-seq experiment conditions

Dataset S6 (separate file). Primer list

Dataset S7 (separate file). Results of comparison of 7 period analysis methods

Dataset S8 (separate file). Standardized RASL-seq expression matrix