Supplementary Protocol S1: Validation of transcript levels determined by RNA-seq

Quantitative real-time PCR (qPCR) was used to quantify transcript levels of selected targets, and thus validate the accuracy of RNA-sequencing. In total, four targets, encoding for components of both CAM pathway and ABA signalling, were analysed.

For qPCR confirmation, the same RNA samples of *Talinum triangulare* were used as for the RNA-sequencing. Synthesis of complementary DNA (cDNA) was carried out using SuperScript™ II Reverse Transcriptase (Thermo Fisher Scientific) according to the manufacturer's instructions. All primer pairs were designed using Primer-BLAST (Ye *et al.*, 2012) and targeting at contigs of currently available assembly of *Talinum triangulare* transcriptome (Brilhaus *et al.*, 2016). Contigs of the transcriptome assembly were annotated based on their homology to Arabidopsis transcripts (Tab. 1)

Luna® Universal qPCR Master Mix (New England Biolabs Inc.) chemistry was used for qPCR and the amplification was monitored in StepOne™ Real-Time PCR System (Applied Biosystems™). The reaction composition was scaled down for a total volume of 10 µl and each reaction contained 1.5 µl cDNA, which was previously diluted 1:10. The PCR cycling conditions were: 95 °C for 60 s, 40 cycles of 95 °C for 15 s and 60 °C for 30 s. The melting curve was measured after 40 cycles to verify primer specificity. For each sample and primer pair, analysis was performed in three technical replicates and average C_T values were used to calculate mean normalized expression (MNE) as described by Simon (2003). Transcript levels of *ATPase, F0/V0 complex, subunit C protein* (AGI: AT2G25610, *Talinum triangulare* contig Tt50206_2) were used for normalization.

To evaluate correlation between RNA-seq and qPCR, RNA-seq reads were additionally quantified against the reference transcriptome using Kallisto (Bray *et al.*, 2016) and the degree of correlation between MNE and transcripts per million (tpm) was evaluated by Pearson's correlation.

Table 1. Primer sequences used to amplify selected targets in *Talinum triangulare* by qPCR and annotation of the targets. *ATPase, F0/V0 complex, subunit C* was used as a house-keeping gene.

Arabidopsis gene	***T. triangulare* contig**	**Forward primer (5'-3')** **Reverse primer (5'-3')**
ABF2 (AGI: AT1G45249)	Tt42370	TAGGGATGGAGTCGTTGGGT CATTCCTCCACCTGGCAACA
ATPase, F0/V0 complex, subunit C (AGI: AT2G25610)	Tt50206_2	GTGTCCCATCGTCCCAAGTT CGCAGACAAGGTTGGCAAAA
PPC1 (AGI: AT1G53310)	Tt63271	TTGGCAACTCTACAAGGCCC TTCACCCCGAATTGCTTGGA
PYL9 (AGI: AT1G01360)	Tt22302	CTACCAGCACGGAGAGGCTA AGGGACACGACGGAAGAGTA
SnRK2.2 (AGI: AT3G50500)	Tt31423_2	AGGCGATGAATCGGGAACAG TGTCGTGCATGATCGGCATA

Results

qPCR was performed and mean normalized expression (MNE) was calculated and correlated with quantification of respective contigs based on RNA-sequencing. For *PPC1*, *PYL9* and *SNRK2.2*, the correlation coefficient was 0.998, 0.705 and 0.91, respectively. In all three cases, the correlation was significant at the 0.01 significance level. For *ABF2*, the correlation coefficient was 0.615 with *p*-value of 0.03 (Fig. 1).

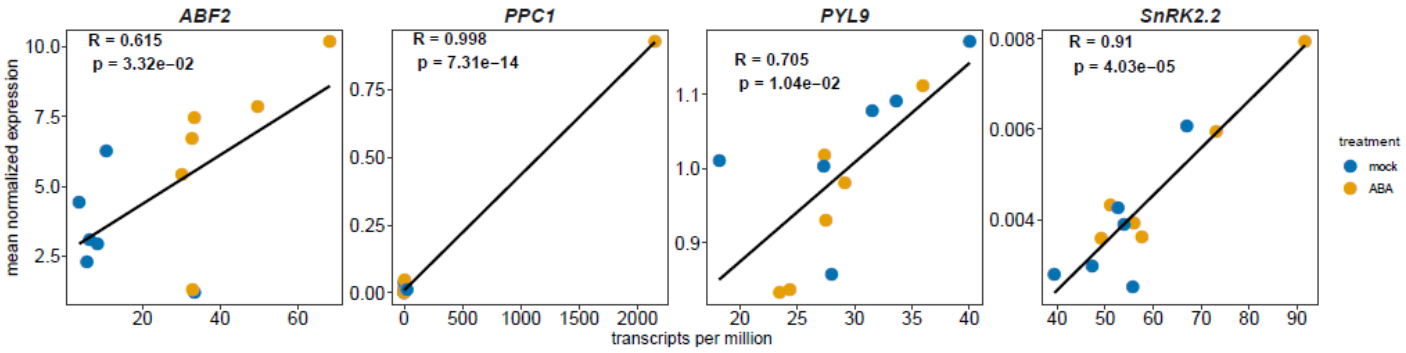

Figure 1. Correlation between mean normalized expression and transcript abundance [tpm] in leave tissue of *Talinum triangulare*. p indicates *p*-value. R stands for correlation coefficient.

References for Supplementary Protocol 1

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Supplementary Protocol S2: Time series RNA-seq analysis

The maSigPro package (version 1.54; Nueda *et al.*, 2014) for R was used to identify genes with significantly different temporal patterns between mock and ABA treatments. Obtained results were used to further support the analysis of differential genes expression at individual time points.

Talinum triangulare reads mapped against *Beta vulgaris* reference transcriptome RefBeet-1.2.2 (Dohm *et al.*, 2014) were normalized to reads per million (rpm) and low abundant genes were filtered prior to the time-course analysis. Different temporal patterns were detected by generalized linear model (GLM) with the following settings: $\theta = 10$ (default), false discovery rate Q = 0.01 and BH adjustment for multiple testing. Profiling of significant differences between the experimental groups was done using `"two.ways.backward"` step method. Additional parameters of the `t.fit()` function included the minimum of 10 true numerical observation per gene and $\alpha = 0.05$. To select temporally differentially expressed genes, the results of the second regression model were filtered using the `"group"` comparison (`get.siggenes()` function) and setting the R^2 cut-off value to 0.85 for the regression model.

Subsequently, hierarchical clustering (`hclust`) was performed to group temporally differentially expressed genes based on similarity in their transcript abundance profiles. The number of required clusters was set to six, which was determined as an optimum by within-cluster sum of squares. Finally, genes within each cluster were tested for enrichment of biological functions based on MapMan categorisation (Thimm *et al.*, 2004). For the enrichment analysis, only transcripts with known Arabidopsis homologs were considered and Arabidopsis-based functional annotations were used. Genes, whose temporal pattern did not differ significantly between mock and ABA, were considered as a separate cluster in the enrichment analysis. Fisher's exact test with *p*-value adjustment according to Benjamini-Hochberg (Benjamini and Hochberg, 1995) was used to find significant enrichments.

Results

With the above specified parameters, temporal patterns significantly different between mock and ABA treatments were discovered for 4,765 genes. The complete list of all genes with significantly altered temporal pattern is available as Supplemental Dataset 2.

References for Supplementary Protocol 2

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Supplementary Protocol S3: Pulse-amplitude modulated (PAM) fluorescence measurements

To investigate whether exogenous ABA induced stress response in *Talinum triangulare* at a physiological level, chlorophyll fluorescence was analysed. Chlorophyll fluorescence ratio F_v/F_m is a robust indicator of the maximum quantum yield of photosystem II. Under stress conditions, the capacity to repair photosystem II is reduced, which translates in the irreversible inhibition of photosystem II, detectable as reduced F_v/F_m (Baker 2008; Murchie and Lawson, 2013).

Two independent experiments were performed, one to investigate a long-term effect of exogenous ABA on photosynthetic performance/stress in *T. triangulare*, and another one to accompany the RNA-seq experiment and focusing on the early response to exogenous ABA. In both experiments, plants were grown under 12 h light/ 12 h dark and 25 °C/ 23°C conditions.

In the long-term experiment, mature leaves of *T. triangulare* were treated daily with 10 µM, 50 µM, 200 µM ABA or mock solution in four biological replicates; non-treated controls were included as well. All treatments were done on a daily basis within the first hour of the light period. F_v/F_m was measured within the last 30 minutes of dark *(i.e.*, in dark-adapted state) using a JUNIOR PAM (Heinz Walz GmbH) device. The same leaves were used during the whole experiment.

For the early response experiment, plants independent of the RNA-seq experiment were used but originating from the same seed batch and grown under identical conditions, and experimental design as used in the RNA-seq experiment (Fig. 1 of the main text). For each time point and treatment, two biological replicates were used and four leaf-discs originating from the treated leaves were analysed per plant. Immediately after cutting, leaf discs were placed in water and incubated in dark for 15 minutes prior to the measurement with an IMAGING-PAM *M-Series* (MAXI version) connected to an IMAG-K6 camera (Heinz Walz GmbH).

Results

Daily ABA treatments (concentration range 10-200 µM) of mature leaves of *T. triangulare* for up to six days did not have any adverse effect on the photosynthetic performance of the treated leaves (Fig. 1). Beginning on day 11, statistically significant decline of F_v/F_m occurred but it could also be a result of growth conditions, as a decline was observed also in mock-treated leaves (day 11, 50 µM ABA).

Consistent with the findings of the long-term experiment, exogenous ABA did not induce damage to photosystem II within 1280 min after the treatment (Fig. 2). The drop of Fv/Fm to 0.59 ± 0.013 and 0.55 ± 0.054 in both mock- and ABA-treated leaves at 640 min, respectively, is likely a result of diurnal changes to the photosystem and not caused by the treatment (Fig. 2).

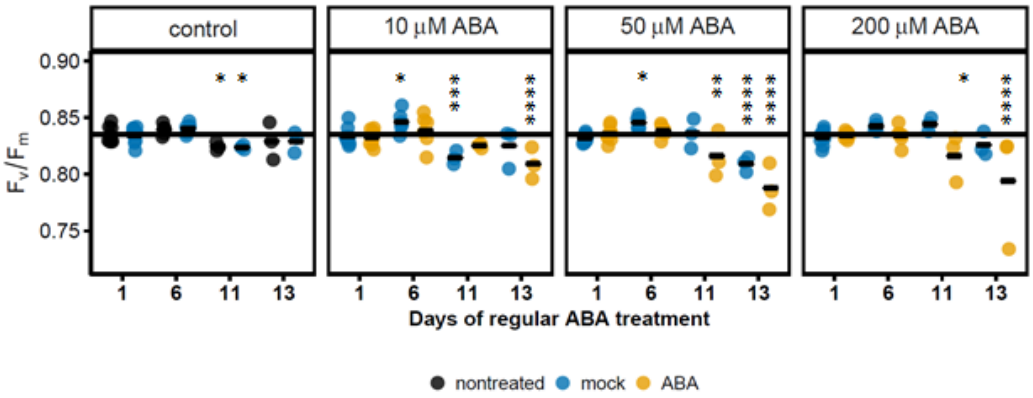

Figure 1. Effect of daily ABA treatments on F_v/F_m of mature leaves of *Talinum triangulare*. Non-treated, mock- and ABA-treated leaves were analysed (n = 4). Treatments were done daily within the first hour of the light. F_v/F_m measurements were done shortly before dawn, while the plants were still dark-adapted. The horizontal line depicts a reference F_v/F_m value (0.835), which is based on measurements of all leaves chosen for this experiment prior to the first treatment. Asterisks indicate difference compared to the reference F_v/F_m value. One sample *t*-test, **** ≤ 0.0001, *** ≤ 0.001, ** ≤ 0.01, * ≤ 0.05.

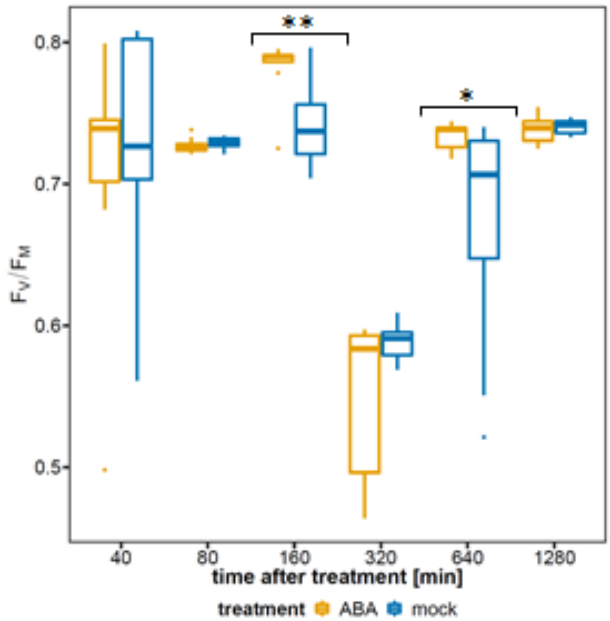

Figure 2. Short-term effect of exogenous ABA on F_v/F_m of mature *Talinum triangulare* leaves. The treatment and harvest schemes were the same as in the RNA-seq experiment (see Materials and Methods). Two biological replicates were used and four leaf-discs per plant were obtained and analysed. After cutting, leaf-discs were kept in water and dark adapted prior to the measurement. Two sample *t*-test, **** ≤ 0.0001, *** ≤ 0.001, ** ≤ 0.01, * ≤ 0.05.

References for Supplementary Protocol 3

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