Supporting Information

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SI Methods

Metabolite Profiling. Each sample was extracted, derivatized, and analyzed by using GC-TOF/MS as described (1–4). Each sample was used 5 mg fresh weight of tissues for Design 1 (Fig. S2*A*) and 10 mg fresh weight of tissues for Design 2 (Fig. S2*B*) for GC-TOF/MS analysis. Finally, 56 μg fresh weight of the derivatized extract was injected by using GC-TOF/MS for Design 1 and 167 μg for Design 2. Nonprocessed raw data were treated by using a custom script described by Jonsson et al. (1, 2) to perform baseline correction, alignment, and peak deconvolution. Metabolites were identified by comparing their mass spectrum and retention time index (RI) with those generated for authentic compounds analyzed on our instrumentation as well as those in the MS and RI libraries in the Golm Metabolome Database (5–7). The data were obtained from 6 analytical runs in Design 1 and 3 runs in Design 2, performed on a different day. Because changes in signal intensity for each peak generally appear as a result of sample derivatization and/or MS detector sensitivity, a direct comparison among results obtained from different analytical runs is not entirely appropriate. To overcome this problem, the data were normalized by using the mean normalized response calculated for the WT control in each measured batch run, according to a method described in ref. 8.

Transcript Profiling. Comprehensive transcript profiling was performed by using ATH1 GeneChips (Affymetrix). Biological replicates (n = 3) were sampled and analyzed four times [Zeitgeber time (ZT) 8, 10, 12, and 14 h; see Fig. S2*B*] in the diurnal cycle in wild-type (WT) and *d975*. All raw CEL files have been deposited in the Nottingham Arabidopsis Stock Center's microarray database (NASCArrays) under accession number NASCARRAYS-421 (9). Raw CEL files were normalized by robust multiarray average (RMA) (10) with Bioconductor Simpleaffy package (11). Data quality for all GeneChips was assessed by using functions in the Bioconductor AffyPLM package. The mapping of Affymetrix probe set IDs to AGI locus codes was performed by using TAIR7 (2007/05/02) (12). Gene functional classifications and visualizations were based on the ontology tool MapMan (13) and TAIR7.

Evolutionary Analysis. To explore the evolutionary relationships among the distinct pseudo-response regulators (PRRs), phylogenetic analysis was performed on 23 PRR protein sequences from seven plant species (*Arabidopsis thaliana*, *Oryza sativa*, *Populus trichocarpa*, *Carica papaya*, *Vitis vinifera*, *Medicago trunculata*, and *Sorghum bicolor*). Multiple alignments of amino acid sequences and phylogenetic tree creation with bootstrapping were generated by using ClustalW2 and TreeView. The amino acid sequences in *Arabidopsis* and rice were obtained from UniProt: AtPRR9, AtPRR7, AtPRR5, AtPRR3, and AtPRR1 (TOC1) in *Arabidopsis,* Q8L500, Q93WK5, Q6LA42, Q9LVG4, and Q9LKL2, respectively; and OsPRR95, OsPRR73, OsPRR59, OsPRR37, and OsPRR1/OsTOC1 in rice, Q689G6, Q10N34, Q0IUG8, Q0D3B6, and Q689G9, respectively. Other sequences of putative PRRs were from Plant Genome Duplication Database (PGDD) (http://chibba.agtec.uga.edu/duplication/) (14).

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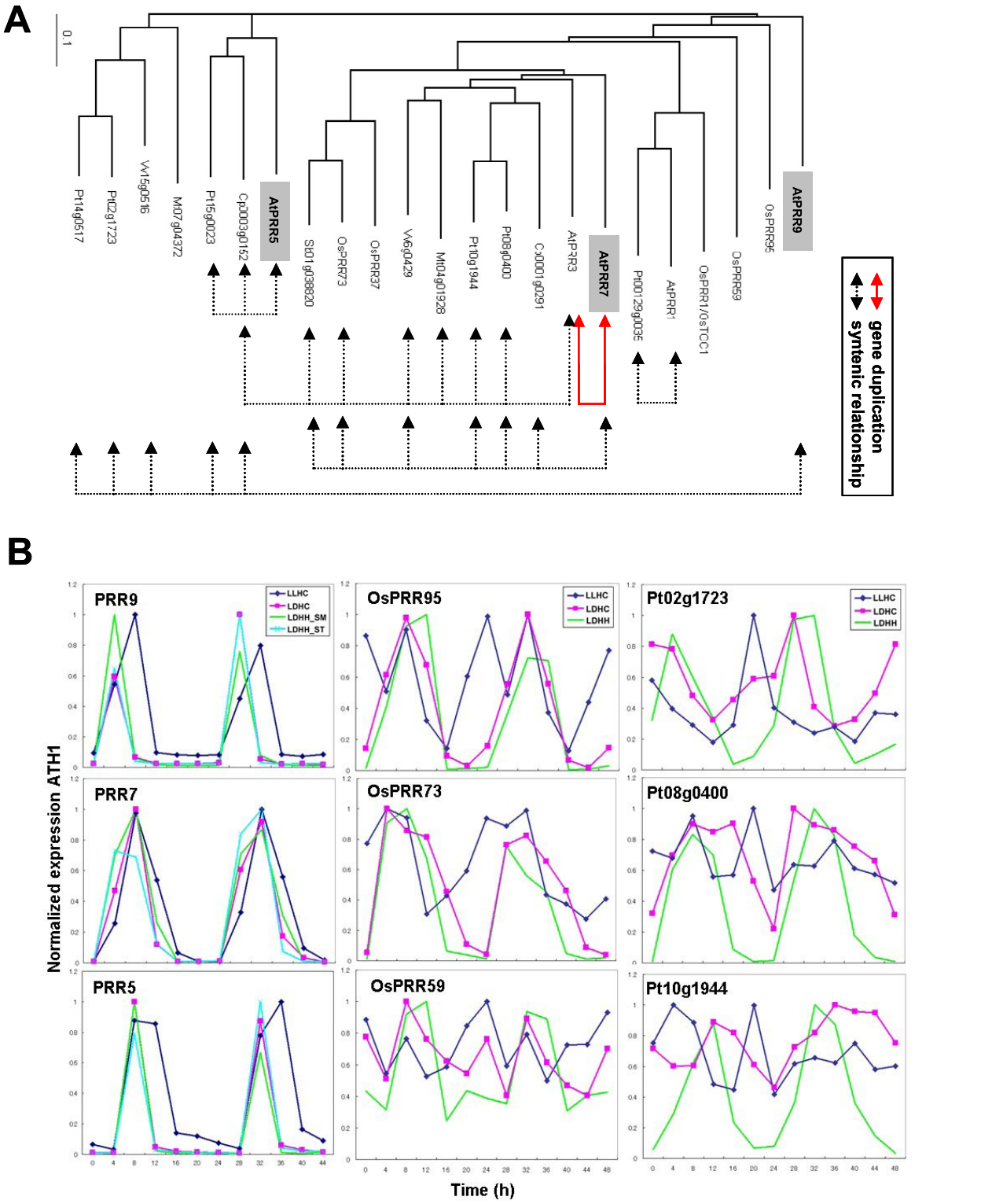

Fig. S1. Evolutionary conservation in PRR sequences and rhythmic expression pattern in higher plants. (*A*) Phylogenetic relationship of the PRR proteins in seven species: *Arabidopsis thaliana* (At), *Oryza sativa* (Os), *Populus trichocarpa* (Pt), *Carica papaya* (Cp), *Vitis vinifera* (Vv), *Medicago trunculata* (Mt), and *Sorghum bicolor* (Sb). (*B*) Comparison of diurnal changes in transcript levels for PRRs using DIURNAL database (15) (http://diurnal.cgrb.oregonstate.edu/), which provided circadian/diurnal gene expression data for *Arabidopsis*, rice, and poplar genes. The retrieval parameters used were; ''Basic Search,'' ''Normalized data,'' and ''Use data.'' The experimental conditions and microarray datasets used were LDHH, LDHC, and LLHC (16). ZT, Zeitgeber time; LD, light/dark cycle; LL, continuous light; HC, hot/cold; HH, continuous hot. LDHH_SM (Smith) and LDHH_ST (Stitt) were described (17, 18). These genome-scale data suggest that the three *PRR* genes are evolutionarily conserved in sequence homology and gene expression patterns.

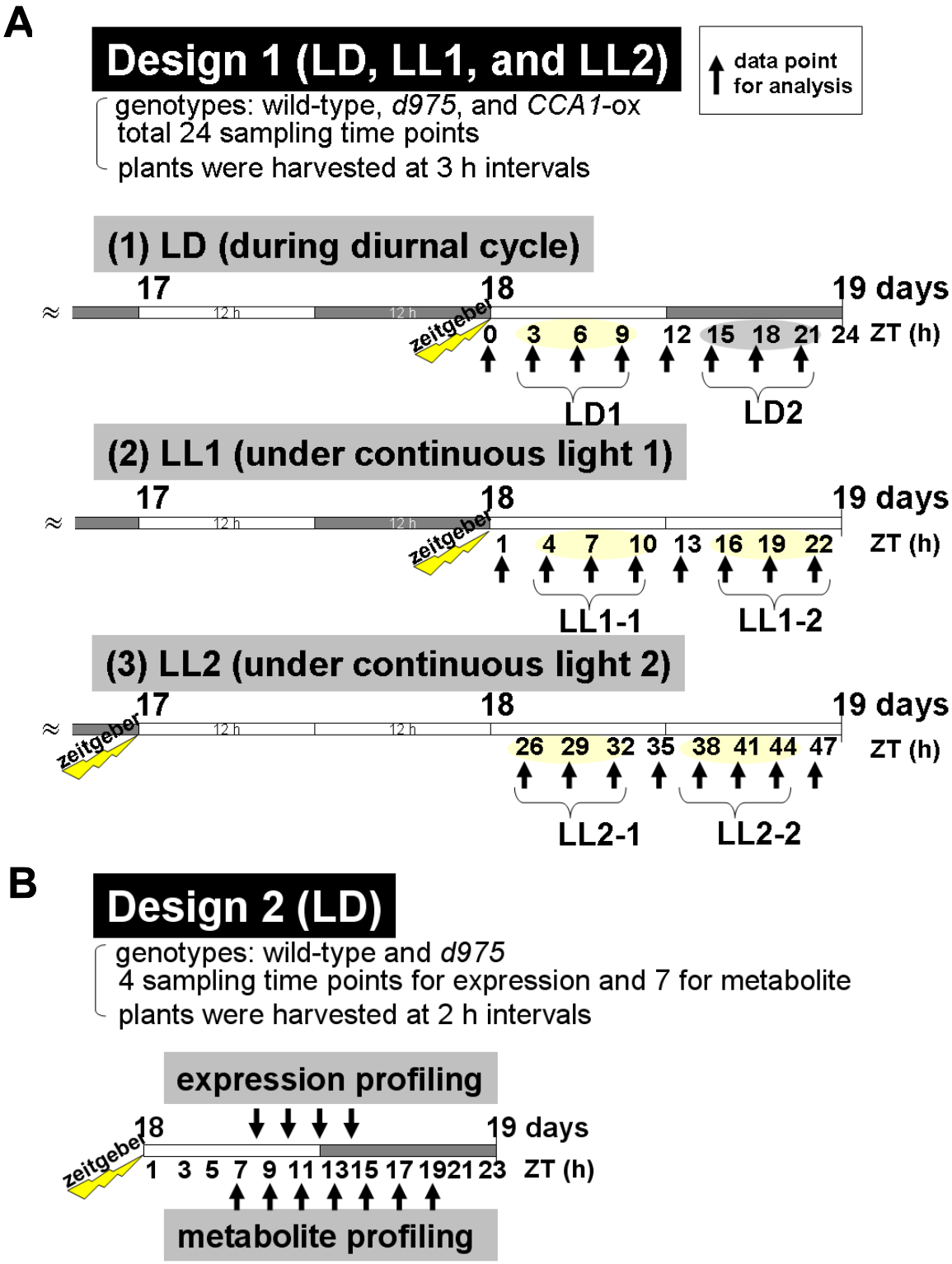

Fig. S2. Experimental design and sampling scheme. The two experiments were designed to perform transcript and metabolite profiling in both LD, light/dark cycle, and LL, continuous light. (*A*) Metabolite phenotyping of three genotypes (WT, *d975*, and *CCA1*-ox) under LD and LL are illustrated (Design 1). (*B*) Both transcript and metabolite profiling from Zeitgeber Time (ZT) 7 to ZT 19 under LD cycle are Design 2. This time series experiment had a higher resolution than that of Design 1.

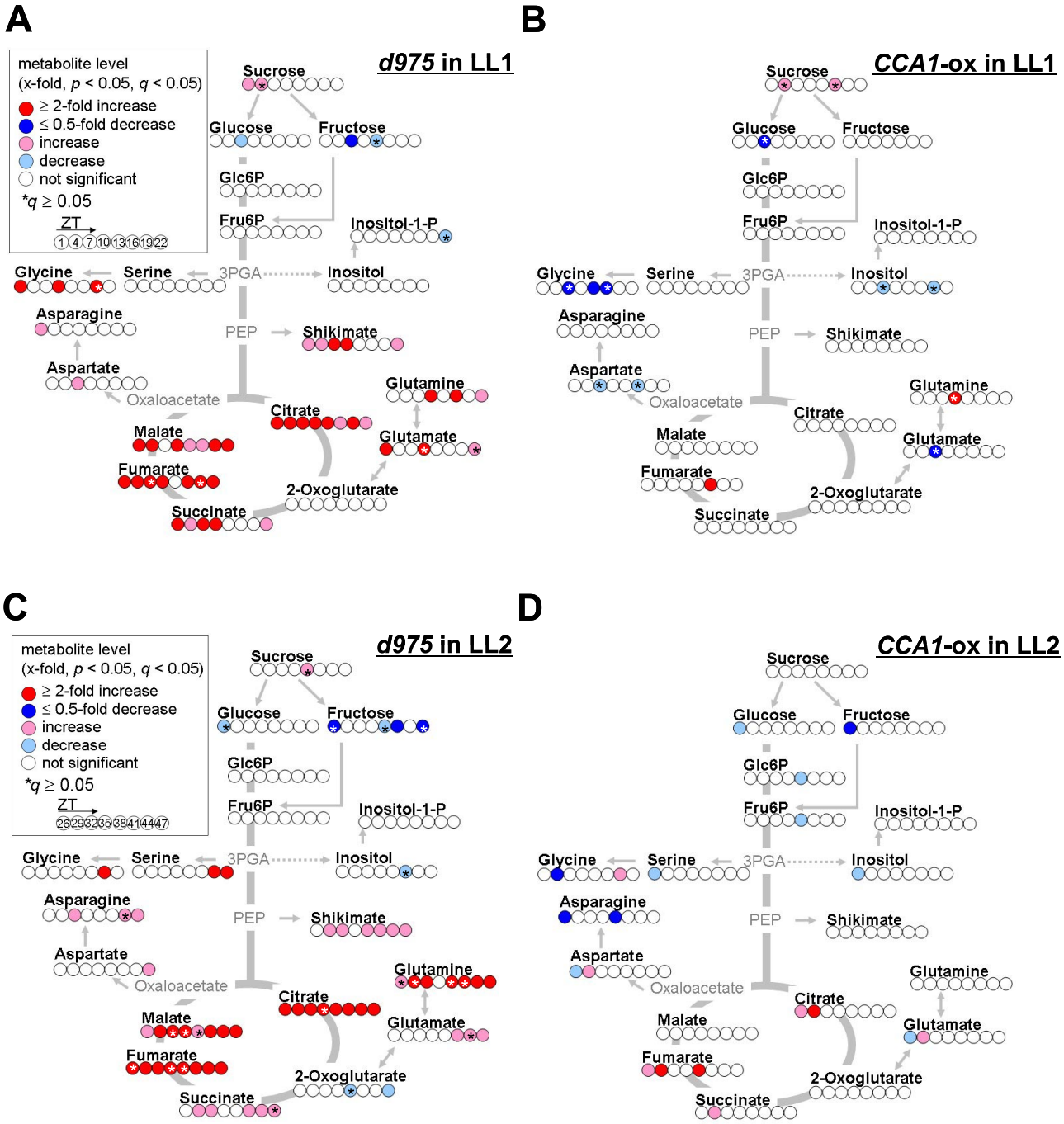

Fig. S3. Changes in metabolite levels of *d975* and *CCA1*-ox plants under continuous light (LL; Fig. S2*A*). The changes were calculated by dividing the metabolite level in the mutant by that in the WT. The level of significance was $q < 0.05$. Red and blue colors indicate that the metabolite level increased or decreased, respectively. The circles indicate the corresponding ZT. *, $q \geq 0.05$.

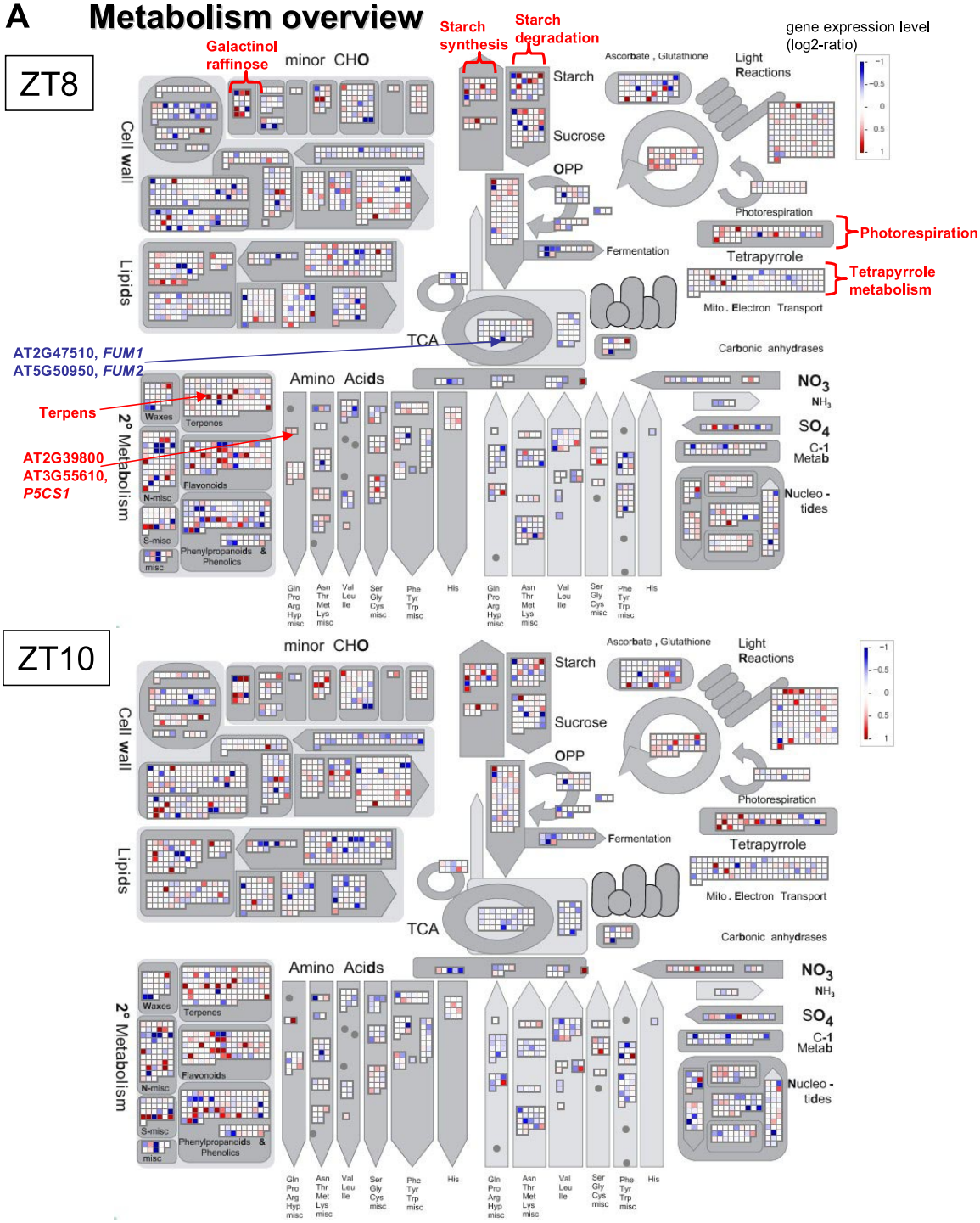

Fig. S4. Changes in transcript level for individual genes involved in central metabolism overview (*A*) and TCA cycle (*B*) using the MapMan software. Red and blue colors indicate that the transcript/metabolite level increased or decreased, respectively. All values indicate log2 ratios (*d975*/WT) at each ZT using ATH1 GeneChips. CHO, carbohydrate; OPP, oxidative pentose phosphate. (*C*) Pathway-level changes in gene expression involved in biosynthetic pathways of tetrapyrrole and α-tocopherol for *d975*. To visualize the qualitative differences in transcript levels between *d975* and WT, we used a hierarchical cluster analysis with ''Euclidean distance'' and the ''average linkage'' method (see the boxes). Metabolite levels of glutamine, phytol, and α-tocopherol (red bold) were increased significantly (*t* test, $q < 0.05$) in *d975*.

A (continued)

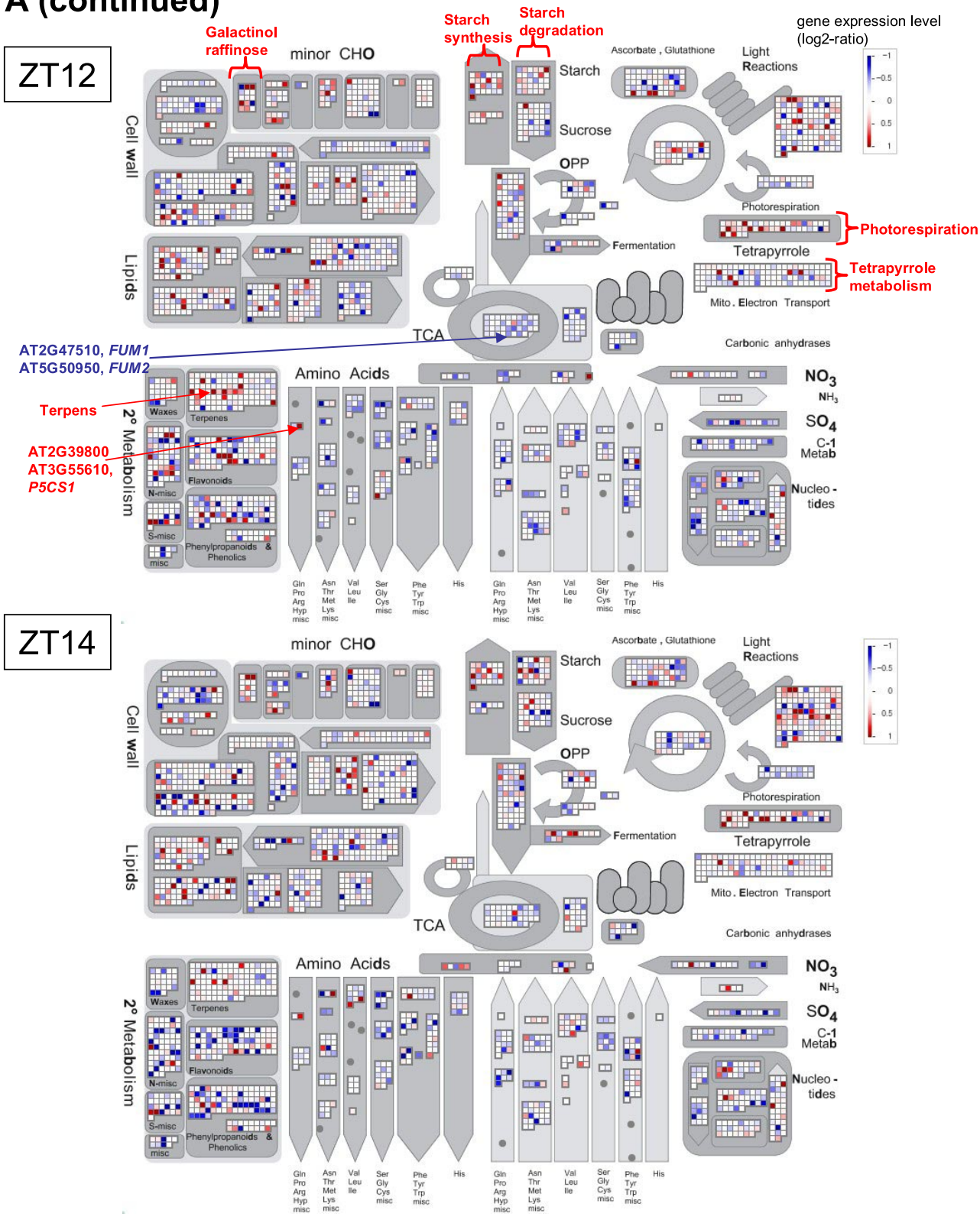

Fig. S4 continued.

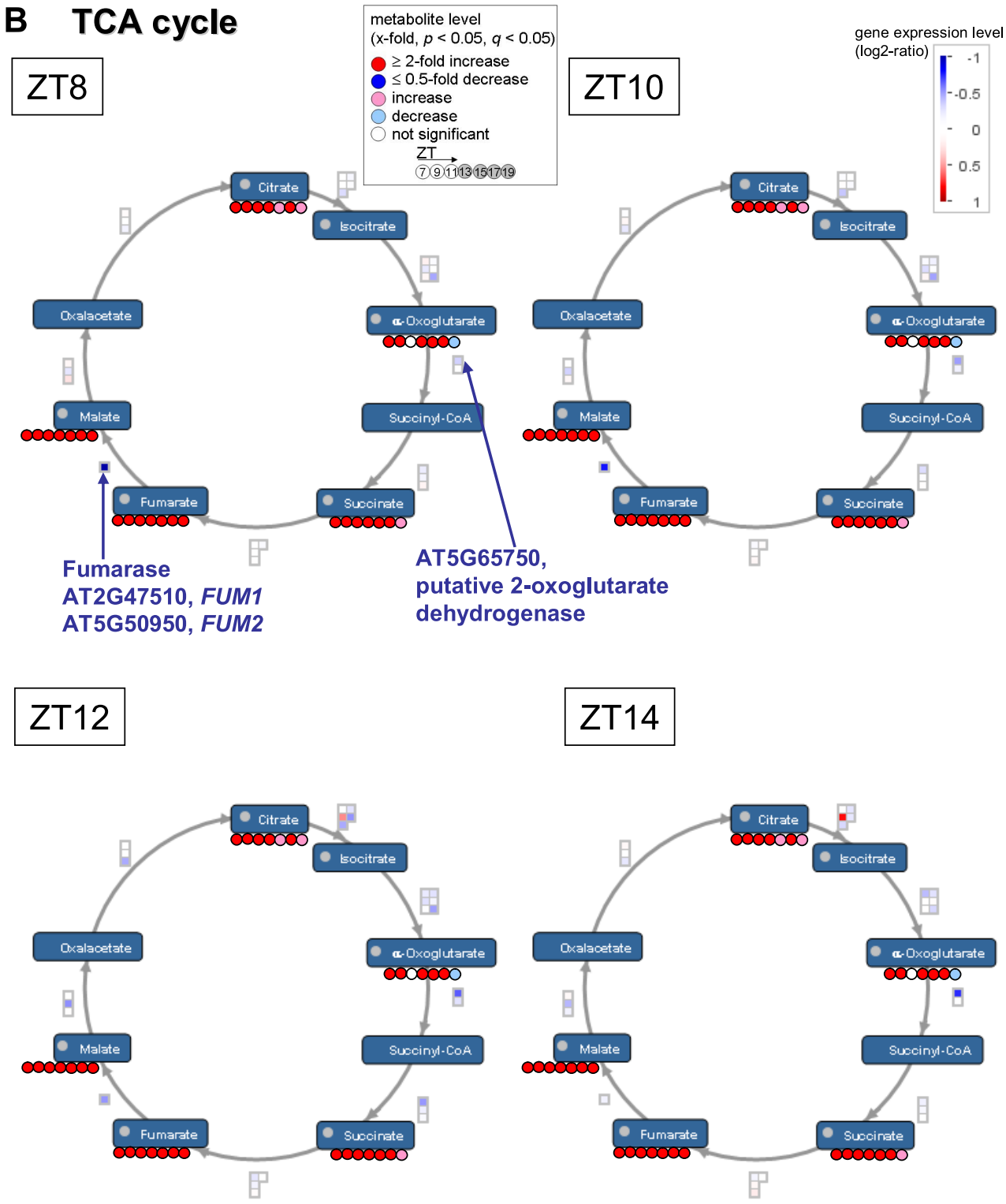

Fig. S4 continued.

C

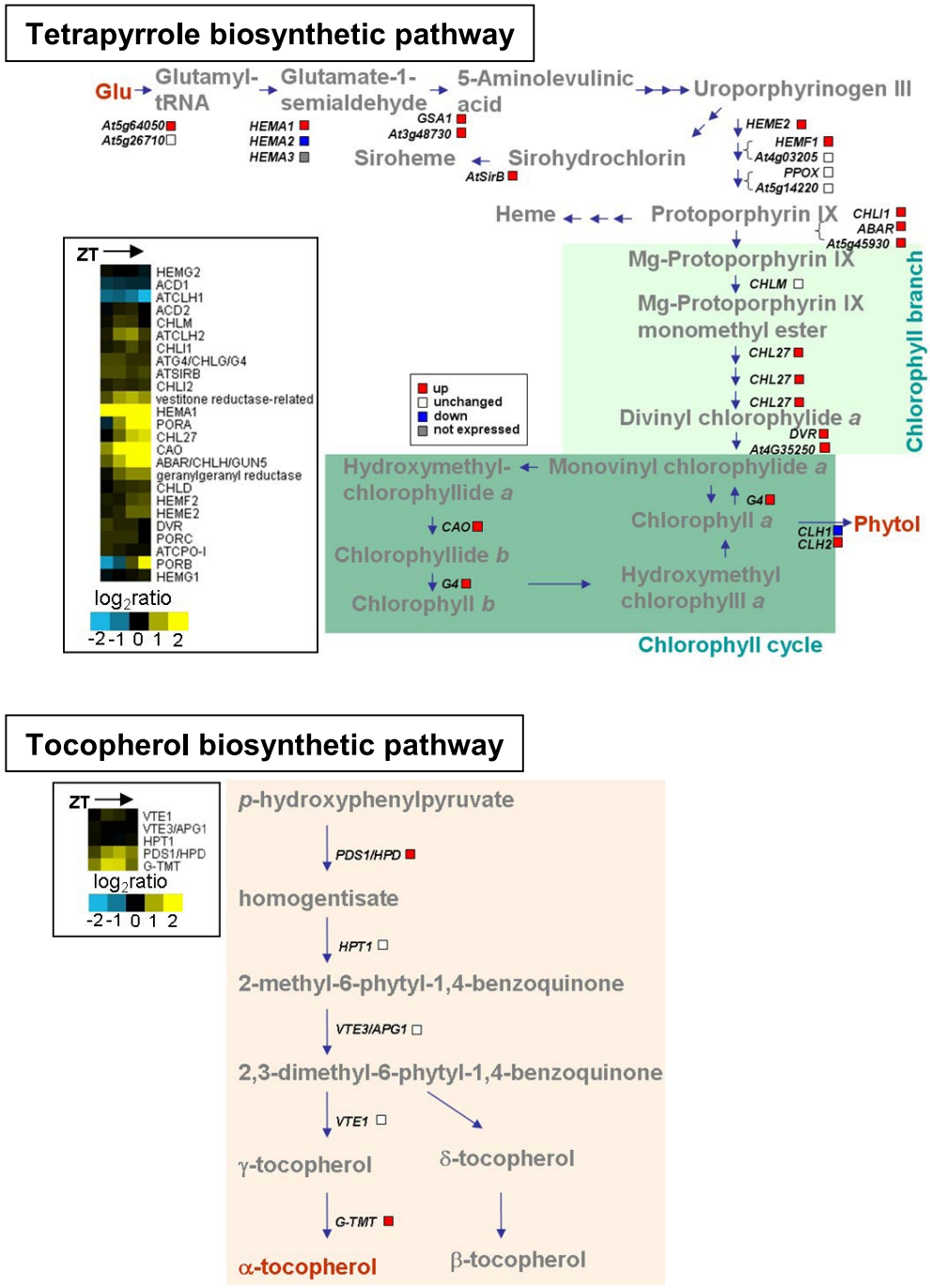

Fig. S4 continued.

Table S1. Phenotypes of two arrhythmic mutants analyzed in this work

Characteristic	*d975*	*CCA1*-ox
Genetic modification	Knockout of *PRR9*, *PRR7*, and *PRR5* genes	*CCA1*-overexpressor
Rhythm of expression	Arrhythmic (in LL or DD)	Arrhythmic (in LL or DD)
Morphological phenotype	Long hypocotyl, dark green leaves (in mature stage), and various stress tolerance	Long hypocotyl and dark green leaves
Light	Hyposensitivity to red light	Hyposensitivity to red light
Flowering	Late	Late
Refs.	9, 20	21, 22

Other Supporting Information Files

Dataset S1

Dataset S2

Dataset S3

Dataset S4

Dataset S5