Time course experimental design:

Plant Material and Growth Conditions

Arabidopsis thaliana (WS) plants were grown in growth chambers (Percival AR-66) under controlled conditions: 21-22^{o}C, constant light, 25 µmol m^{-2} s^{-1}, and a relative humidity of 70%. Knockout plants deficient in cytosolic *Apx1* (KO-*Apx1*) were obtained as described in Pnueli et al., (2003). Light stress treatments were performed by changing the light intensity from 25 to 250 µmol m^{-2} s^{-1}, all other parameters were maintained constant. At different times, leaves were excised, flash frozen in liquid nitrogen and stored for further analysis at -80^{o}C.

GeneChip® Microarray Experiments

In six independent experiments RNA was isolated from wild type and KO-*Apx1* plants grown under controlled conditions and subjected to a moderate level of light stress as described above (samples were obtained at 0, 0.25, 0.5, 1.5, 3, 6 and 24 h post light stress application; 15 plants per time point; RNA was isolated using Trizol; Pnueli et al., 2003; Rizhsky et al., 2004). For each time point, RNA from 3 independent experiments was pooled to generate 28 RNA pools (2 RNA pools for each time point for a total of 14 wild type and 14 KO-*Apx1* pools). These RNA samples were used to perform chip hybridization analyses (Arabidopsis ATH1 chips; Affymetrix, Santa Clara, CA) at the Virginia Bioinformatics Institute Core Laboratory Gene Expression Facility (https://www.vbi.vt.edu/). Conditions for RNA isolation, labeling, hybridization, and data analysis are described in (Pnueli et al., 2003 and Rizhsky et al., 2004). Data visualization and analysis were performed with the GeneChip mining tool V 5.0, Silicon Genetics® GeneSpring V 5.1 and ArraAassit (IobionLab®). Some of the results were confirmed by RNA blots. See details for data analysis below.

GeneChip® Data Processing and Analysis

All GeneChip® arrays were processed first by RMA (Robust Multi-Array Average) (Irizarry et al., 2003) using the R package `affy` (Gautier et al., 2004). Specifically, expression values were computed from raw *CEL* files by first applying the RMA model of probe-specific correction of PM (perfect match) probes. These corrected probe values were then normalized via quantile normalization, and a median polish was applied to compute one expression measure from all probe values. Resulting RMA expression values were $\log_2$-transformed. (Please see the `affy` manual at www.bioconductor.org /repository/devel/vignette/affy.pdf for details.) Density plots and boxplots of RMA expression value distributions of all arrays were very similar with no apparent outlying arrays (Supplementary Figure 1). Digestion curves describing trends in RNA degradation between the 5' end and the 3' end in each probeset were generated, and all 28 proved very similar, with a downward trend at the 5' end (Supplementary Figure 2).

Pearson correlation coefficients and Spearman rank coefficients were computed on the RMA expression values (log base 2) for each set of biological replicates. Spearman coefficients ranged from .977 to .994; Pearson coefficients ranged between .982 and .995 (Supplementary Table 7). A scatter plot of RMA expression values between the two wild type biological replicates at 6 hours is presented in Supplementary Figure 3.

To determine whether genes were differentially expressed between genotypes across the temporal states, an ANOVA was performed on the RMA expression values. (For an overview on the application of ANOVA to microarray data, please see Kerr et al., 2000.) The following model was used for this analysis: $y_{ijk}=V_i + T_j + (VT)ij + \varepsilon_{ijk}$ where y_{ijk} denotes the $\log_2$ signal measured for variety i, time j, and biological replicate k, with $1 \le i \le 2$, $1 \le j \le 7$, and $1 \le k \le 2$. The terms V_i and T_j measure the effect of the variety and time point, respectively, and the interaction term $(VT)ij$ accounts for the interaction between variety and time. An ANOVA was performed on each gene using the linear model above, and 6 contrasts based on differences of genotypes with respect to the last 6 time points. (Differences between genotypes at time 0 were not of interest in this study.) The R package `limma` was used for ANOVA methods (www.bioconductor.org /repository/devel/vignette/affy.pdf.) A multiple testing correction (Benjamini and Hochberg, 1995) was applied to the *p*-values of the F-statistics to adjust the false discovery rate. Genes with adjusted F-statistic *p*-values < .05 were extracted for further analysis. This resulted in 3,915 genes with significant F-statistics based on the ANOVA above (Supplementary Table 8). Expression values of this selection were then inverse-log transformed and genes with differential expression between wild type and knockout expression values of more than twofold during at least one time point were selected. This selection included 843 genes. Similarly, genes with differential expression between wild type and knockout expression values of less than 0.5-fold during at least one time point were selected as suppressed (Supplementary Table 9). Interestingly no gene was both up-regulated and down-regulated by more than twofold in knockout across the time series, enabling a clear separation between up-regulated and down-regulated genes. Storey multiple testing adjustments (Storey and Tibshirani, 2003) was also performed on the p-values of the F-statistics. All p-values found significant by the Benjamini-Hochberg correction were also found to be significant in the Storey correction. The Storey correction contained 691 additional genes that were not included in the analysis.

Experiment reference:

Davletova, S., Rizhsky, L., Liang, H., Shengqiang, D., Oliver, D., Coutu, J., Shulaev, V., Schlauch, K. and Mittler R. (2004) Cytosolic Ascorbate Peroxidase 1 is a Central Component of the Reactive Oxygen Gene Network of *Arabidopsis*. The Plant Cell, *in press.*