Systems approach divulges redox regulation of Arabidopsis transcriptome

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Sensors and regulatory circuits that maintain redox homeostasis play a central role in adjusting plant metabolism and development. Plants are unique to operate electron transport chains at two locations in the cell (chloroplast and mitochondria) and are well equipped to adapt changes in redox status in response to environmental signals. To delineate underlying network that is responsible for maintenance of redox homeostasis we chose two perturbations, high light (HL) and DCMU that inhibit photosynthetic electron transport chain in chloroplast. Following perturbations, time series transcription analysis was performed using Agilent's Microarray technology and a redox control network (RCN) was generated.

Redox responsive transcriptome

Arabidopsis leaf discs were exposed to either HL or DCMU. Microarray analysis at different time points revealed that 20% of Arabidopsis transcriptome is under HL regulation while 8% is regulated by DCMU. HL- responsive transcriptome indicates that initial exposure to HL causes Arabidopsis leaves to utilize maximum energy and extended exposure leads to metabolic changes to prevent damage caused by reactive oxygen species (ROS). On the other hand, DCMU induces transcriptional changes to protect against damage caused by ROS. Both perturbations lead to redox imbalance and system tries to maintain homeostasis. The common pool of genes observed under the two perturbations reflects the set of genes responsible for redox homeostasis and is thus termed as redox responsive genes (RRG). We identified 1401 RRGs that have two (or more) fold changes in their expression under at least *ONE* perturbed condition (RRG1). A more stringent criterion to validate RRG was applied and genes with two (or more) fold change in their expression at *ALL* the conditions tested were considered (RRG2).

Redox Control Network (RCN)

We focused on RRG1 to understand systems behavior towards redox perturbations. RCN was generated using co-expression data of RRG1 (fig1).

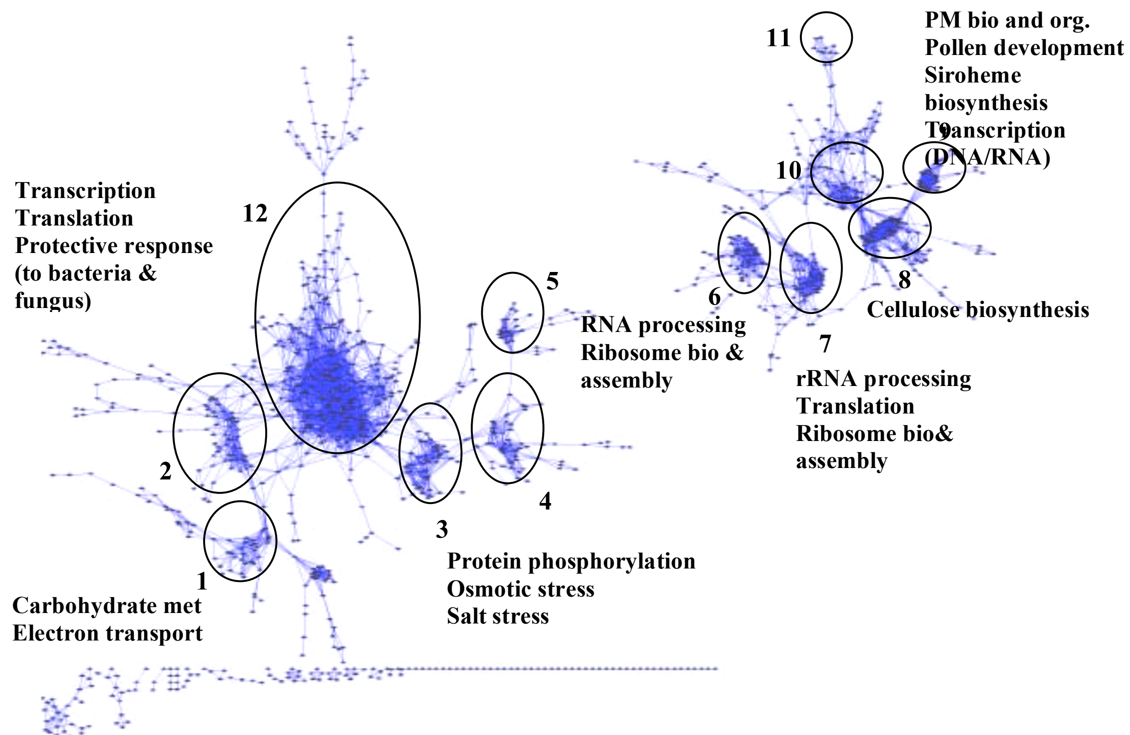

Fig 1: Network diagram of all RRGs perturbed under HL treatment: Each node represents a gene and an edge is drawn between two nodes if Euclidean distance between them is=/< 0.15. Graph is generated using organic layout in Cytoscape 2.3. Sub networks are identified visually and encircled. Each sub network is assigned a number written adjacent to it and Biological processes that are affected in a given sub network are quantified based on Fisher Exact test.

If the genes in a given sub-network (Fig.1) are under a common redox control, we hypothesized that these genes would have a similar expression profile even under a different redox perturbation. To test this hypothesis, we subjected 12 sub-networks to hierarchal sub-clustering using gene expression data from DCMU experiment. This analysis reveals group of genes under common redox control. Motif analysis to identify cis-regulatory elements in upstream sequences (500 bp) was performed on the set of genes with similar expression profile under two perturbations. Our analysis reveals that

G-box (Light-responsive element) is over-represented, ABA-responsive element (ABRE), C-repeat/ dehydration responsive element (CRT/DRE), CArG- box were also identified in upstream sequences. Ribosomal genes are clustered together in cluster 6, 7 and upstream analysis identifies AGGCCC/TGGGCC elements are over represented.

RRG involved in redox homeostasis

RRG2 contains 141 genes. 72 genes encode proteins with previously undescribed function in redox homeostasis. Functional characterization of RRG2 indicates that 23 genes are involved in stress, 19 in protein metabolism, 16 in transcription and 11 in transport. We obtained homozygous null line in one of the transcription factors RRG_KO1, identified in RRG2 to be down-regulated under all conditions. RRG_KO1 was subjected to HL exposure. Under resting state, RRG_KO1 has a higher PSII efficiency than wild type (Wt) indicating an altered PSII structure. Following HL, RRG_KO1 exhibits faster decline in PSII efficiency and by 24h, RRG_KO1 leaves were completely bleached demonstrating loss of protection from redox (Fig 2). Further, RRG_KO1 and Wt were exposed to HL and the subsequent microarray analysis revealed differential expression of 1600 genes in RRG_KO1 and validated 293 genes to be under common redox control.

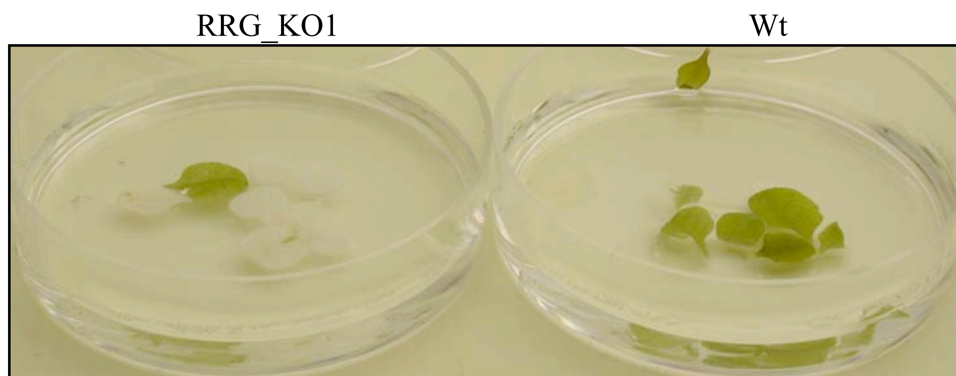

Fig2: Transcription factor knock out (RRG_KO1) and Wt leaves floating in water under HL for 24h.