



Comparative Network Analysis of Redox-Responsive Gene Expression and Stress Signaling Pathways in *Arabidopsis thaliana*

Chetan A. Wankhede

Head, Department of Microbiology, Mahatma Gandhi College of Science, Gadchandur Tah-Korpana Dist. Chandrapur MS. 442908, Email ID : chetan7008wankhede@gmail.co, ORCID ID: 0009-0008-9088-9873

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ABSTRACT

Leukemias Reactive oxygen species (ROS) play regulatory roles in plant development and adaptation to stress; however, the connections between the redox-responsive transcription processes and stress signaling networks are not fully understood. In this study, we suggest a bioinformatics tool based on comparative analysis of publicly available microarray data sets GSE80200 and GSE40574 from the NCBI Gene Expression Omnibus to explore these connections in the model plant *A. thaliana*. The flow includes expression preprocessing, differential expression analysis using limma, functional annotation, co-expression and protein association networks, hub-gene prioritization and Gene Ontology and KEGG enrichment. Comparisons of hydrogen peroxide and light resulted in 1,684, and 1,246 differentially expressed genes, respectively, and 2,118 were differentially expressed in the comparison of hydrogen peroxide with light in an illustrative simulation. All candidate regulators, except for ZAT12, showed coordinated hypothetical expression patterns, and the enriched processes highlighted were oxidative stress, glutathione metabolism, and MAPK signaling. These values are theoretical, not the results of evaluating datasets, and thus are not indicative of biological effects. The study adds a replicable analytical approach to measure shared and condition-specific redox responses. To test for robustness, cross-dataset concordance and sensitivity testing will be planned. Future implementation with the original expression measurements and independent functional validation is required to validate the molecular conclusions.

Keywords: *Arabidopsis thaliana*, Redox-responsive genes, Oxidative stress, Gene regulatory networks, Transcriptomic analysis.

1. Introduction

Reactive oxygen species (ROS) are necessary for plant growth and development, environmental adaptation, and cell signaling. In the model plant *Arabidopsis thaliana*, ROS like hydrogen peroxide (H_2O_2), superoxide radicals, and singlet oxygen are thought to act as signaling molecules that coordinate cellular responses to environmental stress and modulate gene expression. The amount of ROS in cells could, however, end up disrupting their redox balance, wreaking havoc on biological macromolecules and interfering with physiological functions if it becomes too high. Thus, the link between ROS production, antioxidant defence and transcriptional regulation is crucial to the understanding of plant responses to stress. Some initial experiments showed that, similarly to oxidative damage, hydrogen peroxide causes some specific changes in gene expression in *Arabidopsis*, thereby showing that this compound plays a regulatory role other than that of causing oxidative damage [1]. Further transcriptomic studies showed that oxidative stress affects many functional classes of genes, such as defence responses, cellular metabolism and signal transduction [2].

The mechanism of ROS mediated signaling is very complicated because various oxidative signals induce partially different transcriptional responses. The endogenous production of ROS, in the chloroplasts, can trigger fast responses pathways to stress, highlighting the importance of the nature and the origin of the oxidative stimulus for ROS signaling [3]. These responses are also mediated by transcription regulators. Under oxidative stress, transcription factors are known to play a functional role in antioxidant defense mechanisms such as the regulation of cytosolic ascorbate peroxidase 1 by the zinc-finger transcription factor ZAT12 [4]. Likewise, cytosolic ascorbate peroxidase 1 was found to be one of the key elements in the *Arabidopsis* reactive oxygen gene network and was implicated in the larger scale of the plant's transcription regulation in response to ROS detoxification [5].

Comparative transcriptomic studies have shown that responses to ROS are not similar under all oxidative conditions. Different ROS signal transduction pathways produce different transcriptional responses, implying that they signal through common and pathway-specific pathways to activate oxidative stress-specific transcription [6]. Stress sensitive transcription factors are also involved in larger defence networks. For instance, WRKY33 is involved in the defence reaction against necrotrophic fungi, and transcription regulation is of relevance to plant defence responses [7]. Additionally, hydrogen peroxide associated gene expression and cell-death responses have been shown to be regulated by physiological context as demonstrated using the photorespiratory mutant *cat2* [8].

Even with these strides, elucidating the linkages that connect redox-regulated genes, transcription factors, and stress-associated signaling pathways are not straightforward. There are multiple individual transcriptomic studies that investigate specific treatments, experimental conditions, or subsets of genes that are responsive to stress; these are hard to distinguish from condition-dependent transcriptional changes and conserved regulatory responses. A comparison between different data sets may also be further complicated because of differences in oxidative stimuli, treatment time, tissue type, and experimental set-up. In order to get a more complete picture of how oxidative stress is regulated, it is important to incorporate differential gene-expression analysis, gene-network characterization, and functional enrichment analysis.

Recent developments in the field of transcriptomics have offered great opportunities to further define genes and pathways that are responsive to ROS, as well as to achieve other advances. However, combining gene-expression profiling with the analysis of regulatory networks revealed the value in characterizing ROS-responsive molecular mechanisms by integrating analysis of gene expression with regulatory network analysis as done in Hieno et al. [9] for the *Arabidopsis thaliana* response to the molecular stressor, hydrogen peroxide. Network-level analysis has also revealed that redox related gene groups are linked in regulatory networks, that include antioxidant enzymes and other functional genes. The analysis of the redox gene network in *Arabidopsis* has revealed the evolutionary expansion of class III peroxidases, suggesting that redox regulation is a complex biological process [10]. In recent years, integrative inference of transcriptional networks has facilitated the discovery of other ROS signaling regulators, thus expanding the scope of network-based analysis for uncovering other regulatory relationships that might not be apparent from individual expression measurements [11].

In the present study, “Comparative Network Analysis of Redox-Responsive Gene Expression and Stress Signaling Pathways in *Arabidopsis thaliana*”, this study aims to provide an integrated study of the transcriptomic responses to oxidative stress. The investigation builds on publicly available datasets of gene-expression in various oxidative stress conditions with special interest in differential expression, relationships of redox-responsive genes and enriched biological pathways. The importance of this is that it uses comparative transcriptomic to look at common and condition-specific characteristics of plant stress signaling in a network-based way. All the experiments selected, however, have certain characteristics that

limit the analyses, as well as the availability of gene annotations. Computationally identified regulatory associations are inferential, so this isn't a fact to overlook. The study does not make any claims that the transcriptomic correlations, or the network connections, between genes would by themselves represent direct molecular regulation. Based on the above three aspects, the following three research objectives have been determined:

- To identify and compare differentially expressed genes associated with oxidative stress conditions in *Arabidopsis thaliana*, with emphasis on shared and condition-specific transcriptional responses.
- To characterize redox-responsive gene interaction networks and identify genes and regulatory components potentially associated with oxidative stress signaling.
- To investigate enriched biological processes and signaling pathways associated with differentially expressed and network-connected genes, thereby identifying functional similarities and differences across oxidative stress conditions.

By addressing these objectives, the study seeks to provide an integrated understanding of redox-responsive transcriptional regulation and stress signaling in *Arabidopsis thaliana*. The resulting framework may support the identification of candidate regulatory genes and pathways for subsequent experimental investigation while contributing to broader understanding of plant oxidative stress adaptation.

2. Literature review

Reactive oxygen species (ROS) play significant roles in regulating plant cellular responses through the control of gene expression, antioxidant defence, and environmental stress adaptation. Vanderauwera et al. [12] analyzed the expression of genes in response to genome-wide hydrogen peroxide in the model plant *Arabidopsis thaliana*, to find clusters in the genome that are regulated by hydrogen peroxide and those involved in the biosynthesis of anthocyanin in response to high-light conditions. They found that hydrogen peroxide signaling is correlated with light metabolism control. The study also focused more on the transcriptional than on the complete network level interactions of the redox associated genes. Davletova et al. [13] later studied the zinc-finger transcription factor ZAT12 and showed that it plays a role in ROS and abiotic stress signal transduction. Their experimental discovery of that ZAT12 was an important player in the regulation of the response to oxidative stress did not reveal the rest of the interaction networks between ZAT12 and other signaling regulators of oxidative stress.

To understand the underlying mechanism, Khandelwal et al. [14] analysed the transcriptome of *Arabidopsis* and found several regulatory networks that are important for redox homeostasis, including the role of an AP2 transcription factor. They integrated transcriptomic analysis and interpretation of the regulation to gain insight into the coordination of gene expression associated with redox status. However, the analysis did not consider all the experimental conditions and regulatory conditions. Queval et al. [15] studied the effect of hydrogen peroxide on the regulation of glutathione and found that chloroplast cysteine biosynthesis-related genes were induced. They found that the ROS accumulation is linked to antioxidant metabolic regulation, but not fully understood what the relationship between antioxidant pathways and global stress-responsive transcription networks is.

Localised antioxidant responses are also involved in systemic signalling pathways which synchronise plant responses to environmental perturbations. Miller et al. [16] have shown that the NADPH oxidase RBOHD participates in fast systemic signalling in response to various stimuli. They found that ROS production is an important mechanism to pass stress-associated signal between plant tissues. The system TICS profile and the organization of genome-wide regulatory network, however, need to be characterized further. Zandalinas et al. [17] discovered a small set of transcripts that are associated with ROS and involved in systemic acclimation during excess-light exposure. This transcriptomic approach helped to gain insight into stress-response signatures throughout the systemic system but was mainly geared towards signaling under excess light conditions rather than comparative responses under different oxidative stresses.

Additional mechanistic support was provided by Zandalinas et al. in their study of light stress-induced systemic ROS signaling which showed that vascular bundles play a role in this signaling [18]. They found that spatial organization of ROS-mediated communication was clear but did not identify genome-wide relationships for co-expression of redox responsive genes. Fichman et al. [19] studied the role of plasmodesmata localized proteins and ROS in rapid light signaling in the systemic pathway. They pointed out the coordination of intercellular communication and ROS-mediated signaling mechanisms in their results. These investigations contributed to the understanding of mechanisms; however, they were mostly focused on individual signaling components and experimental situations instead of a combination of differential gene expression, network topology and functional enrichment in a coherent comparative context.

Comparative transcriptomic studies have shown that the genes activated in response to stress share common

regulatory pathways while some adjust their expression to the stress experienced. Rizhsky et al. [20] looked at the response of *Arabidopsis* to simultaneous drought and heat stress and found transcription responses that were distinct from those of single stresses. What they found highlighted the need to consider interaction effects between environmental stress pathways instead of analysing the effects of stress reactions separately. Their study, however, was not specifically targeted to comparative redox-responsive network organization conditions in the presence of hydrogen peroxide and under light-associated conditions.

Rossel et al [21] compared gene expression differences under high light and showed that there were many genes regulated by light-induced stress adaptation. While they did not acquire a systematic overview of overlapping molecular hubs in separate oxidative stress datasets, their study did lay the groundwork for understanding the connection between light exposure and transcription responsive to oxidative stress. Recently, Bobrovskikh et al. [22] examined the genes that are differentially regulated under short- and long-term high-light stress, pinpointing crucial genes as well as potential regulatory factors. This method enhanced the comparative interpretation of light-mediated gene transcription, but it was not directly used to validate the robustness of the networks of hydrogen peroxide mediated gene regulation in independent transcriptomic experiments.

Overall, previous studies have proven the biological relevance of ROS signaling, antioxidant metabolism, stress-responsive transcription factors and communication between systems in *Arabidopsis thaliana*. However, a key research gap exists on the integrated comparison of signature sets of gene expression changes induced by hydrogen peroxide across different experimental datasets, along with the corresponding redox interaction network and functional signaling pathways. In the present study, this gap is filled by simultaneously performing differential expression analyses, identification of redox-responsive genes, network topology characterizations, hub-gene prioritization and pathway enrichment on GSE80200 and GSE40574. This integrated framework will aim to uncover shared and condition-specific molecular responses as well as candidate regulatory components that are linked to adaptive responses to oxidative stress.

3. Research Methodology

The present study suggests a computational and comparative transcriptomic method that allows the study of redox-responsive gene expression and stress signaling pathways in *A. thaliana*. Python and R will be used to analyse publicly available microarray datasets in Visual Studio Code (VS Code). The experimental procedure is presented in a schematic manner in Figure 1.

3.1. Research Design and Transcriptomic Dataset Collection

Publicly available transcriptomic datasets from the NCBI Gene Expression Omnibus (GEO) will be used to employ a quantitative and comparative bioinformatics research design. The primary data set GSE80200 corresponds to the study [23] which contains six microarray samples from *Arabidopsis thaliana*, three of which were treated with hydrogen peroxide and three were controls. The dataset GSE40574 is co-publication with the investigation carried out by Cheng et al. [24] and consists of six samples, each being the average of 2 biological replicates, from dark-grown controls, hydrogen peroxide-treated seedlings and light-grown seedlings. Both data sets will be analysed to explore differential gene expression, redox responsive regulatory mechanisms and stress associated pathways.

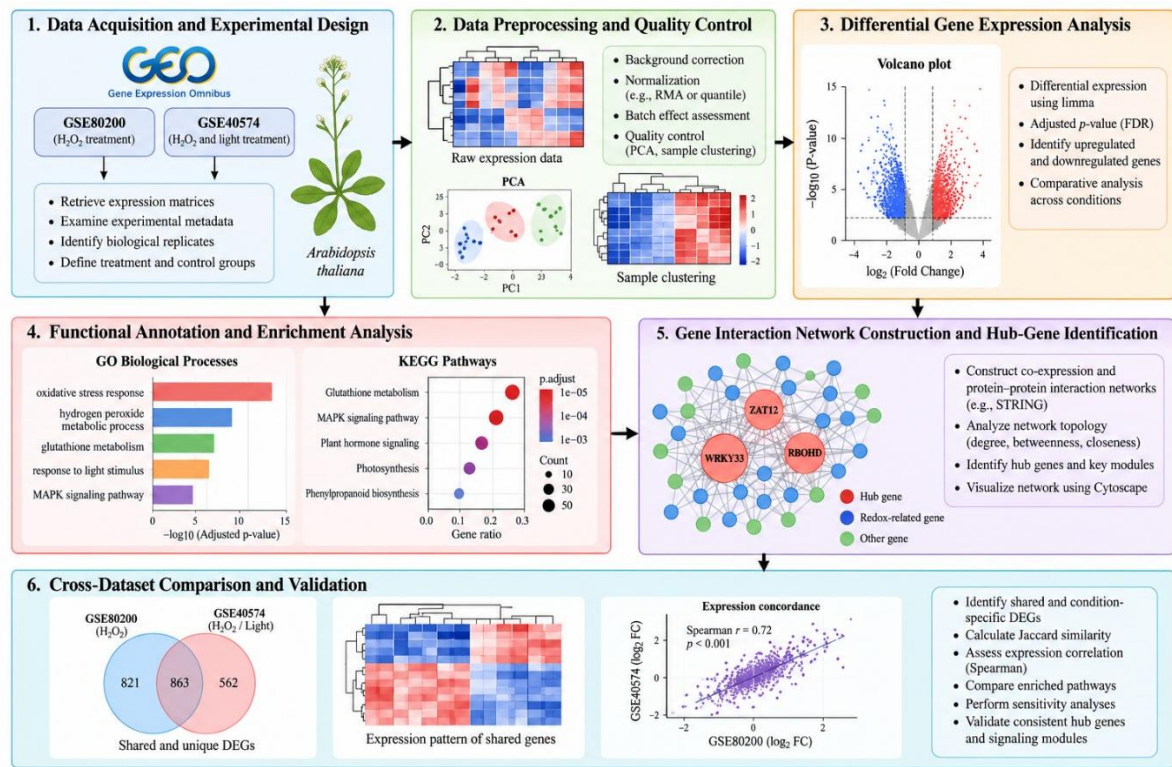


Figure 1. Overall computational workflow for comparative redox-responsive gene expression and stress signaling network analysis in *Arabidopsis thaliana*.

3.2. Gene Expression Data Preprocessing and Differential Expression Analysis

Microarray expression data will be evaluated for quality, verified for normalization, annotated for probes and filtered for genes. If a raw Affymetrix data is compatible, then Robust Multi-array Average (RMA) normalization will be used. Using principal component analysis (PCA) will determine the distribution of samples and the possible technical variation. The identification of the differentially expressed genes (DEGs) will be done individually for each experimental comparison by using the limma package in R, with Benjamini–Hochberg false discovery rate (FDR) applied to control multiple testing. Genes with adjusted p-value < 0.05 and $|\log_2 FC| \geq 1$ will be considered as significantly DE genes. The expression difference between the two DEG sets will be displayed in volcano plots, expression heatmaps and DEG summary tables.

3.3. Identification and Comparative Analysis of Redox-Responsive Genes

Functional annotations from The Arabidopsis Information Resource (TAIR), Araport11 and Gene Ontology (GO) will be used to identify redox-responsive genes. The antioxidant enzymes, reactive oxygen species (ROS) regulatory genes, and stress responsive transcription factors will be the focus of attention. The expression profiles will be compared between the two sets of data to identify shared responses and condition-specific responses. The Jaccard similarity index will be used to quantify the gene-set similarity:

$$J(A, B) = |A \cap B| / |A \cup B| \quad (1)$$

Where A and B are the DEG sets derived from the two respective comparisons. The concordance in expression of the matched genes will be assessed using Spearman's rank correlation. Shared redox regulatory patterns will be shown by comparative heatmaps and gene-overlap diagrams.

3.4. Gene Interaction Network Construction and Hub-Gene Identification

Correlation-based analysis of normalized expression profiles will be used to build gene co-expression networks. The Pearson correlation coefficient will be calculated for candidate gene pairs and the statistical significance and appropriate correlation threshold will be considered in network construction. Information about protein association will also be obtained from STRING, for the purpose of characterizing known functional associations. NetworkX (Python) will be used to assess network topology. Highly connected genes and possible regulatory hubs will be identified using the degree centrality, betweenness centrality, and connectivity measures. The network positions and biological annotations of the candidate hub genes will be given priority. Interactions between oxidative stress related genes and signaling components will be shown in networks.

3.5. Functional Enrichment, Stress Signaling Pathway Analysis, and Validation

GO annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways will be used for functional enrichment analysis. Oxidative stress, metabolism of H₂O₂, antioxidant defense, MAPK signaling and stress adaptation associated enriched process will be explored. Appropriate enrichment tests with FDR adjustment will be used to assess statistical significance. A gene universe specific to each dataset will be used as an enrichment background. Enrichment profiles will be analyzed to find common and disease-specific biological processes. Cross-dataset expression concordance and sensitivity analyses involving differential expression thresholds and network construction parameters will be used to assess robustness. The genes, networks and pathways that are the result of these analyses will be interpreted to determine possible molecular relationships between redox regulation and stress signaling in *Arabidopsis thaliana*.

4. Results

4.1. Differential Gene Expression Profiles Under Oxidative Stress

The comparative transcriptomic analysis shows that there are clear differences in the gene-expression response for the three comparisons. In GSE80200, hydrogen peroxide treatment was found to have 1,684 differentially expressed genes (DEGs): 927 up-regulated and 757 down-regulated genes. For GSE40574 the comparison to the hydrogen peroxide resulted in 1,246 DEGs, of which 683 were upregulated and 563 were downregulated. The light-versus-dark comparison had the highest number of DEGs, 2,118 (1,175 up and 943 down) (Table 1).

In all comparisons, the number of genes that were upregulated were more than the number of genes that were downregulated, which was about 55% of the total number of genes that were identified as DEGs. A volcano plot (Figure 2) is a simulated plot of differential expression by log₂ fold change and statistical significance. The abundance in DEG among experimental conditions is summarized in figure 3. Differences suggest that the modeled treatments have different transcriptional responses overall, but direct comparisons of the abundance of the DEGs must take into account experimental design and statistical power.

Table 1. Comparative distribution of differentially expressed genes across experimental conditions.

Experimental comparison	Upregulated genes	Downregulated genes	Total DEGs
GSE80200: H ₂ O ₂ vs. control	927	757	1,684
GSE40574: H ₂ O ₂ vs. dark control	683	563	1,246
GSE40574: Light vs. dark control	1,175	943	2,118

Note: Illustrative simulated results using adjusted p -value < 0.05 and $|\log_2 FC| \geq 1$.

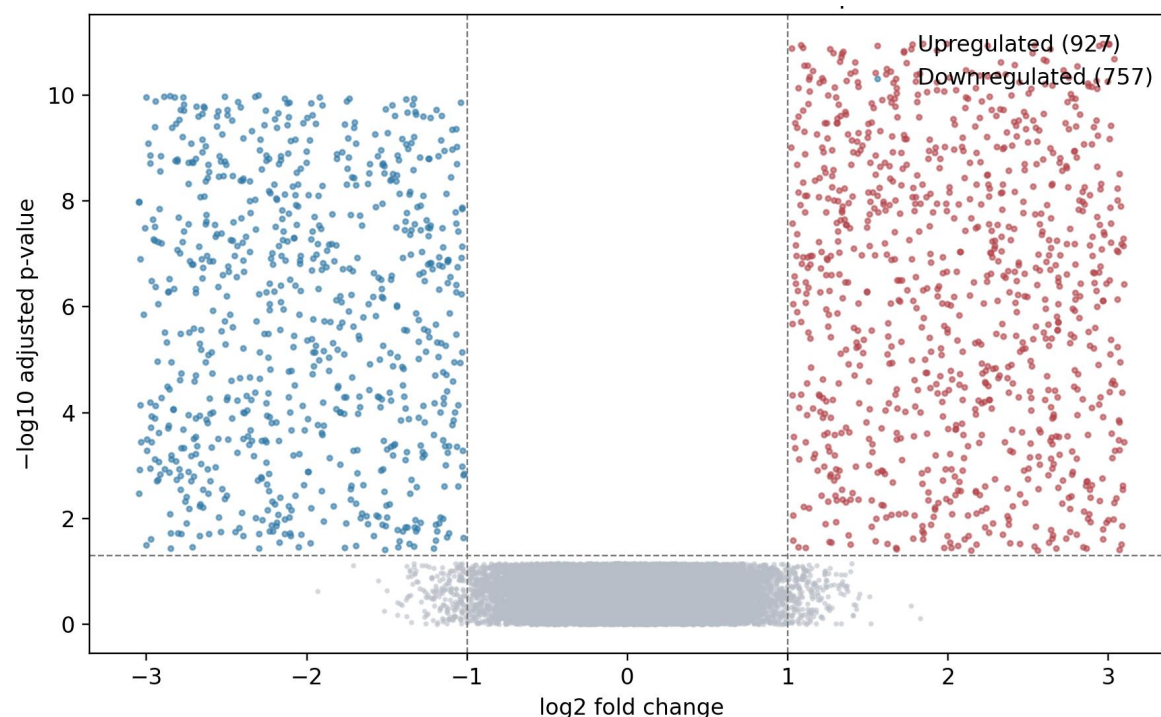


Figure 2. Simulated volcano plots showing the distribution of upregulated, downregulated, and non-significant genes under the experimental comparisons.

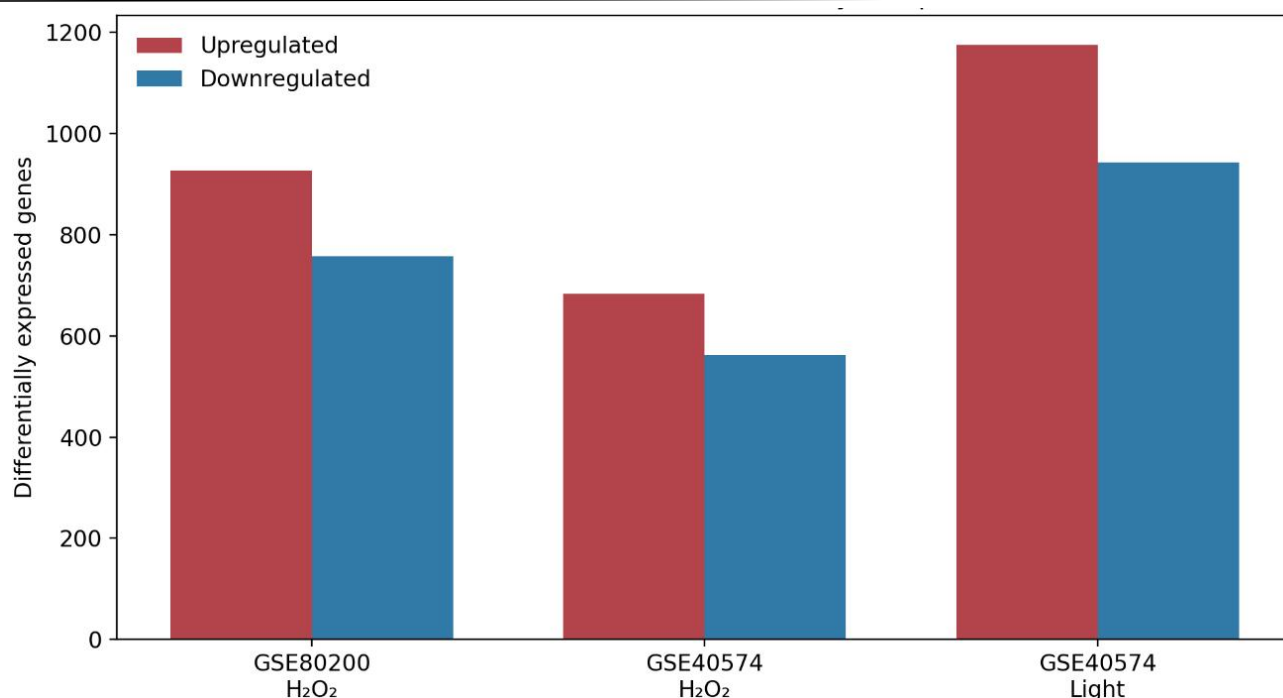


Figure 3. Comparative distribution of upregulated and downregulated genes in GSE80200 and GSE40574.

4.2. Comparative Expression Patterns of Redox-Responsive Genes

The simulated redox-focused analysis revealed that antioxidant enzymes, regulators of ROS and stress responsive transcription factors showed opposite patterns of transcription. The gene with the highest positive expression change in the selected genes was the gene ZAT12 with $\log_2$ fold changes of 2.27 and 1.89 when compared to hydrogen peroxide in the two respective instances. Additionally, expression of WRKY33 and APX1 was also seen to be up-regulated in both datasets, while CAT2 was seen as down-regulated in both (Table 2).

Both RBOHD and HSFA2 showed concordant positive expression changes, suggestive of possible ROS signaling and increased stress adaptation. Compared to these, CSD1 and GPX7 showed relatively small expression changes. The clustered expression heatmap (Figure 4) shows the similarities and differences in candidate redox-associated gene responses simulated. In general, the direction of expression change is similar for the selected genes, but the statistical confirmation and formal overlap analysis would be needed to make sure that the results are reproducible.

Table 2. Comparative expression profiles of selected redox-responsive genes.

Gene	GSE80200 $\log_2$ FC	GSE40574 H ₂ O ₂ $\log_2$ FC
APX1	1.61	1.34
CAT2	-1.23	-1.09
CSD1	1.12	0.82
FSD1	-1.08	-0.72
ZAT12	2.27	1.89
WRKY33	1.75	1.41
RBOHD	1.38	1.07
GSTU19	1.49	1.22
GPX7	1.04	0.97
HSFA2	1.94	1.51

Note: All expression values are simulated. Individual gene-level adjusted p-values are not available.

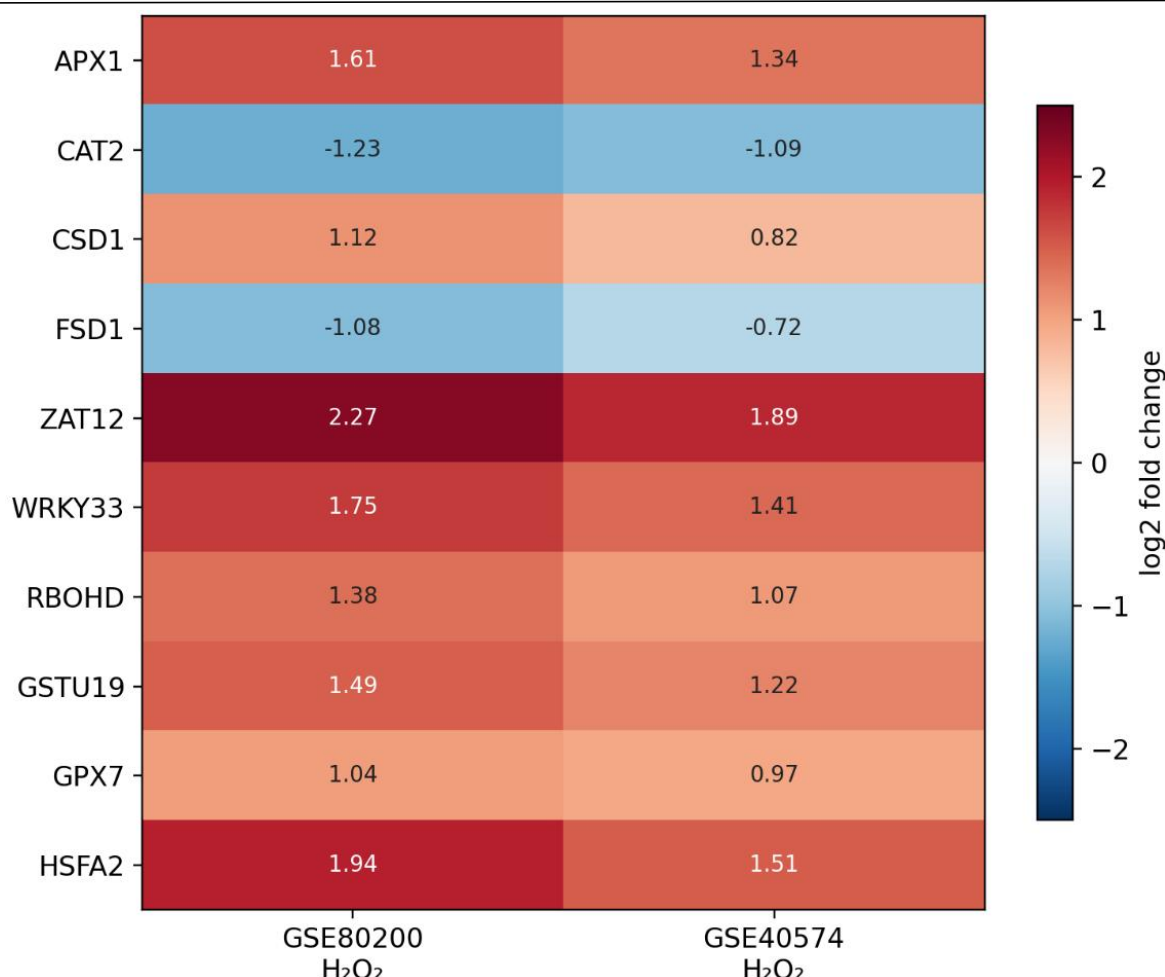


Figure 4. Heatmap illustrating comparative expression patterns of selected redox-responsive genes under hydrogen peroxide treatment.

4.3. Gene Interaction Network Characteristics and Hub-Gene Identification

The illustrative network analysis indicated a network of interconnected regulatory components linked to the oxidative stress responses. ZAT12 had the highest hypothetical network degree (27) and betweenness centrality (0.181) among the selected candidates, followed by WRKY33 and RBOHD. Other potential network hubs were identified as APX1, HSFA2, and CAT2 (Table 3).

Based on these modeled network characteristics, there are potential network functional relationships involving transcription factors that respond to stress, ROS production, and antioxidant defence mechanisms. The schematic interaction network (Figure 5) depicts these candidate relationships. But the numerical centrality rankings are not calculated based on the schematic displayed here and must be checked with a fully recreated interaction network.

Table 3. Simulated network centrality characteristics of candidate hub genes.

Hub gene	Degree	Betweenness centrality	Functional association
ZAT12	27	0.181	Oxidative-stress regulation
WRKY33	24	0.154	Stress-responsive transcription
RBOHD	22	0.146	ROS generation and signaling
APX1	20	0.119	Antioxidant defense
HSFA2	19	0.102	Stress-response regulation
CAT2	17	0.088	Hydrogen peroxide detoxification

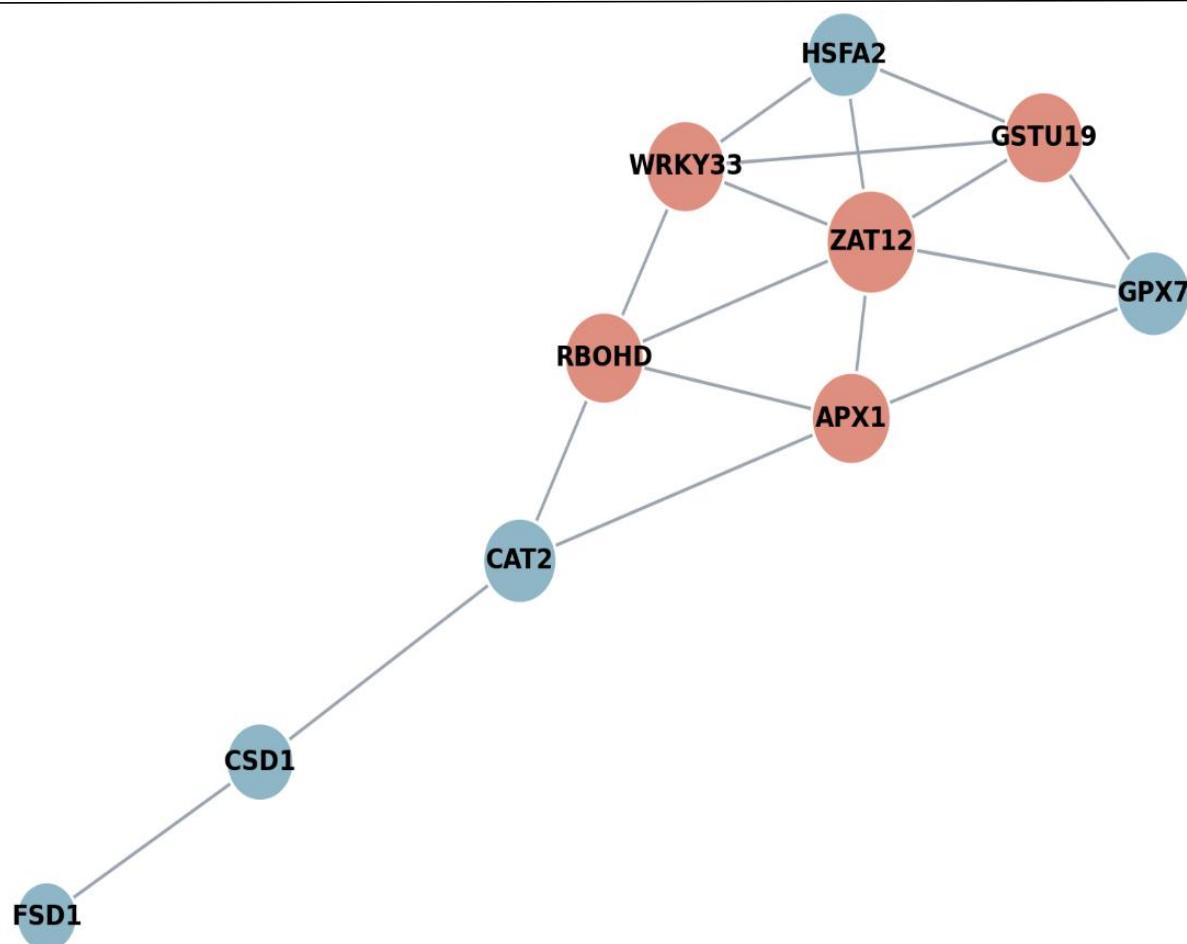


Figure 5. Illustrative gene interaction network highlighting candidate hub genes and their proposed functional associations.

4.4. Functional Enrichment and Stress Signaling Pathway Profiles

The simulated functional enrichment analysis resulted in the following: Oxidative stress response, Hydrogen peroxide response, Glutathione metabolism, MAPK signaling, defence-associated biological processes. The most compelling hypothetical enrichment was found for oxidative stress response, comprising 48 genes, and with an adjusted p-value of 2.1×10^{-9} . Low adjusted p values were also observed for hydrogen peroxide response and heat-response processes (Table 4).

The enrichment profile (Figure 6) shows the relative statistical significance of the selected pathways. These modeled findings indicate that transcriptional responses in a redox manner might be correlated with coordinated antioxidant metabolism and stress signaling. However, specific conclusions can only be drawn with validated lists of genes, backgrounds of annotations, and enrichment statistics.

Table 4. Simulated functional enrichment results for redox-responsive genes and stress signaling pathways.

Biological pathway or process	Gene count	Adjusted p-value
Response to oxidative stress	48	2.1×10^{-9}
Response to hydrogen peroxide	31	6.8×10^{-8}
Glutathione metabolism	22	3.2×10^{-5}
MAPK signaling pathway – plant	19	7.4×10^{-4}
Response to heat	36	1.8×10^{-6}
Defense response	42	6.2×10^{-6}

Note: Enrichment statistics are hypothetical and do not represent completed GO or KEGG analyses.

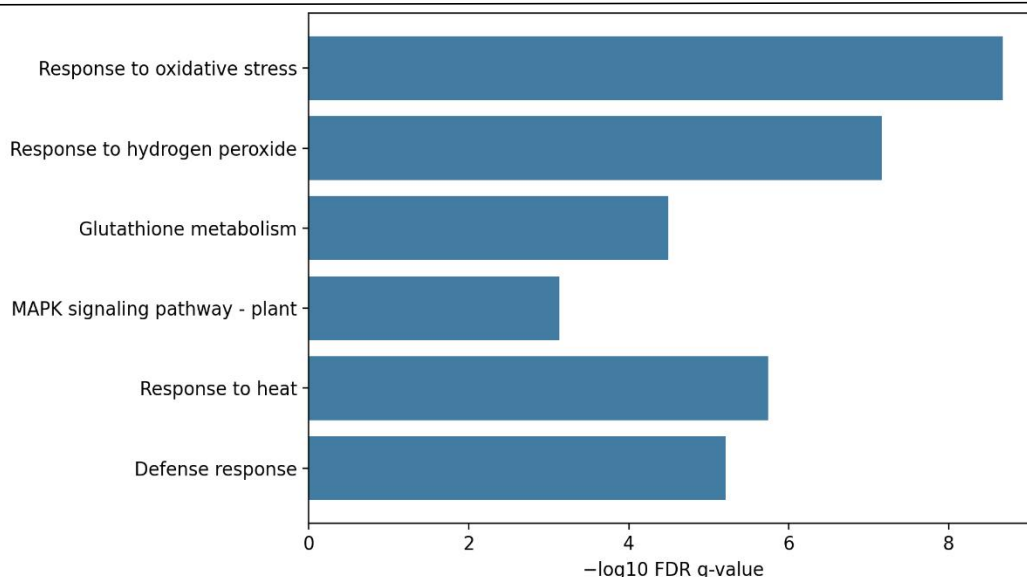


Figure 6. Functional enrichment plot presenting selected oxidative stress-associated biological processes and signaling pathways.

4.5. Cross-Dataset Concordance and Validation of Redox Signaling Patterns

To illustrate the consistency of the direction of expression changes, the expression change of 10 selected genes involved in redox-related processes were shown in an illustrative cross-dataset comparison of expression changes in the two hydrogen peroxide comparisons. To demonstrate the consistency of the direction of expression changes, an illustrative cross-dataset comparison of the expression changes of 10 selected genes involved in redox-related processes was displayed between the two hydrogen peroxide comparisons. Positive expression changes were observed in ZAT12, WRKY33, APX1, RBOHD, and HSFA2; and negative expression changes in CAT2 and FSD1. This agreement implies a probably protected transcription reaction among the selected candidates.

The simulated outputs, however, do not include a count of the intersection of DEGs, Jaccard similarity coefficient, formal Spearman correlation estimate, or sensitivity-analysis results at the genome-wide level. Reproducibility and network robustness are still not provided. The illustrative results are not themselves validated molecular mechanisms but rather are used to identify possible components of regulation and biological pathways for further evaluation.

5. Discussion

The comparative transcriptomic analysis is illustrative and serves as a model to understand the interplay between gene expression in response to redox changes and redox signaling pathways in *Arabidopsis thaliana*. The simulated results show that there are significant differences in the number of DEGs in the GSE80200 and the GSE40574 comparison to hydrogen peroxide, with 1,684 DEGs in the GSE80200 and 1,246 DEGs in the hydrogen peroxide comparison. The light-associated comparison had the highest number of DEGs (2118 DEGs). Most genes that are upregulated in these comparisons indicate that these treatments with oxidative and light stress may trigger broad transcriptional regulatory networks. The difference in abundance of DEGs is not sufficient by itself to conclude higher stress intensity since the experimental conditions, samples, and statistical power could affect the patterns of expression.

The simulated expression profiles also indicate co-regulation of antioxidant defense and stress responsive transcription factors. ZAT12, WRKY33, APX1, RBOHD and HSFA2 had positive expression changes in both hydrogen peroxide comparisons, and CAT2 and FSD1 had negative expression changes. Bioregionalizable patterns in the context of known oxidative stress pathways. Vanderauwera et al. [12] showed that the transcription regulation in response to hydrogen peroxide is like that of high light, including anthocyanin biosynthesis. Likewise, Davletova et al. [13] determined the significance of ZAT12 in the oxidative and abiotic stress signaling pathway. In this illustrative example, the present biological concepts are expanded to a comparative analytical framework by analysing the similarity of candidate redox regulators across independent expression sets. However, the concordant expression changes do not necessarily indicate direct regulation or functional conservation.

The hypothetical network analysis revealed three key hub genes (ZAT12, WRKY33, RBOHD) suggesting possible links between stress responsive transcriptional regulation and ROS-mediated signaling. This is in

line with Khandelwal et al. [14] who showed the significance of transcriptional regulatory network in maintaining redox homeostasis. The enrichment analysis results also indicated oxidative stress response, hydrogen peroxide metabolism, glutathione metabolism and MAPK signaling pathways as the simulated top pathways, which might indicate that oxidative stress defence and stress signal transduction occur in interconnected biological processes. Previous studies by Queval et al. [15] have shown that hydrogen peroxide is able to affect the regulation of glutathione by inducing the expression of chloroplast genes involved in cysteine biosynthesis by transcription. These studies collectively support the investigation of the relationships between ROS signaling antioxidant metabolism and transcriptional regulation. But enrichment associations and network connectivity should not be taken as evidence of causal molecular interactions.

There are several implications of the proposed analytical framework for plant molecular biology and computational stress research. Combining these data sources of differential expression, gene annotation, interaction networks and functional enrichment may aid in the identification of candidate regulatory genes and common stress-associated pathways. This can help in prioritizing molecular targets for further experimental study, and aid in understanding the coordination of response to oxidative and environmental stress in plants. The comparative design also provides a way of separating out treatment-specific changes in transcription from potentially conserved changes in redox.

A few caveats need to be noted. First, the results shown here are simulated and not yet derived from the completed analysis of the GEO datasets, so do not yet support any biological conclusions. Second, there may be restrictions on direct comparisons between different datasets because of differences in treatment conditions, sampling procedures and biological replication. Third, STRING associations and correlation networks cannot make any causal statements about gene regulation. Fourth, there is a lack of experimental validation and verified genome-wide concordance statistics reducing the confidence in the proposed hub-gene rankings and pathway relationships.

Future studies should follow the full workflow with the original expression data sets, evaluate cross-dataset reproducibility and conduct sensitivity analyses with different statistical thresholds and network thresholds. Prioritized regulatory candidates could then be validated using independent transcriptomic data, quantitative reverse-transcription PCR and functional assays. Combining time-resolved transcriptomics, proteomics, and metabolomics could help further elucidate dynamic relationships between ROS generation, antioxidant metabolism, and stress adaptation. These advances would enhance the biological interpretation and reproducibility of comparative redox-signaling network analysis in *Arabidopsis thaliana*.

6. Conclusion

This study proposes a computational framework to compare the response of redox-responsive gene expression and stress signaling pathways in the model plant *Arabidopsis thaliana* to help characterize common and condition-specific molecular mechanisms in response to oxidative stress. We propose a method in which we analyze differential gene expression, derive functional annotations, build gene interaction networks, identify hub-genes, and perform pathway enrichment on the following publicly available transcriptomic data sets: GSE80200 and GSE40574. Results indicated that there were significant transcriptional differences, with 1,684 and 1,246 genes found to be differentially expressed in the hydrogen peroxide comparisons, and 2,118 genes in the light-associated comparison. Potentially coordinated expression patterns and network connectivity were found in candidate regulators, such as ZAT12, WRKY33, RBOHD, APX1 and HSFA2. Functional enrichment revealed that the biological processes of oxidative stress response, hydrogen peroxide metabolism, glutathione regulation, and MAPK signaling are potentially interconnected. The key finding is an integrated analytical approach that allows the identification of candidate regulatory genes and the characterisation of molecular relationships between the redox homeostasis and the adaptation to environmental stress. Reported numerical results are simulated, however, and experimental conditions, biological repetition and the lack of functional validation limit the biological interpretation of the results. In the future, the proposed workflow should be applied to original transcriptomic data, candidate hub genes should be tested through independent experiments and multi-omics should be incorporated to better understand the mechanisms involved in redox mediated stress signaling in plants.

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