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TRANSCRIPTOMIC ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES IN ANTIOXIDANT ENZYME MUTANTS

Rohit Kumar Sharma[1], Priya Singh[2], Ankit Verma[3], Neha Mishra[4]*

[1] Department of Biotechnology, Government P.G. College, Chhindwara, Madhya Pradesh, India
[2] Department of Life Sciences, S.S. Jain Subodh P.G. College, Jaipur, Rajasthan, India
[3] Department of Biotechnology, D.A.V. College, Kanpur, Uttar Pradesh, India
[4] Department of Botany, Government Girls P.G. College, Rewa, Madhya Pradesh, India

***Corresponding Author:** Rohit Kumar Sharma*
Email: rohitsharma.bio@gmail.com

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Abstract

Reactive oxygen species (ROS) are key regulators of cellular signalling and stress responses in plants. Antioxidant enzymes maintain redox homeostasis by controlling ROS accumulation. Mutations affecting antioxidant pathways can disrupt cellular balance and trigger extensive transcriptional changes. Understanding the transcriptional responses associated with antioxidant enzyme mutants is important for elucidating molecular mechanisms of oxidative stress regulation. The present study aimed to identify differentially expressed genes and regulatory mechanisms associated with antioxidant enzyme mutants using transcriptomic data analysis. A secondary transcriptomic dataset obtained from a publicly available repository was analysed to evaluate gene expression changes across antioxidant-related mutants, including vtc1, vtc2, cat2, aox1a, tapx, and sal1. Differentially expressed genes were identified based on fold-change patterns, followed by functional annotation to determine associated biological processes. Promoter analysis was conducted to identify ROS-responsive transcription factors and potential regulatory motifs controlling candidate gene expression. The results showed that there are significant changes in the expression of genes in antioxidant enzyme mutants. The genes are related to oxidative stress response, metabolic regulation, signal transduction, and homeostasis. Both unique and common changes were found in the mutants. This implies that there are common oxidative stress signalling pathways. The promoter analysis showed that many ROS-responsive transcription factors are related to differentially expressed genes. The results showed that disruption of antioxidant enzyme pathways causes extensive transcriptional reprogramming related to stress response genes. The results can be used to understand the regulation of genes depending on redox status and can be used to improve stress tolerance in plants.

Keywords: *Antioxidant Enzymes, Oxidative Stress, Reactive Oxygen Species, Transcriptomic Analysis, Transcriptional Regulation.*

Introduction

Reactive oxygen species (ROS) are inevitable byproducts of normal cellular metabolism that are critical for plant signalling and stress responses. While low levels of ROS are required for signalling, controlling plant growth, and development, high levels of ROS can cause oxidative damage to proteins, lipids, and nucleic acids. To maintain homeostasis, plants have developed complex antioxidant systems that control intracellular levels of ROS. Antioxidant pathways are critical for plant survival, and disruption of these pathways triggers transcriptional reprogramming that activates various responses that can mitigate oxidative stress. The study of antioxidant systems is an important area of plant molecular biology that is still under active investigation.

Significant improvements in the capabilities of transcriptomics techniques have enhanced the understanding of the effects of genetic mutations and environmental stress conditions. The use of transcriptomics techniques has enabled the identification of differentially expressed genes and pathways associated with physiological processes such as stress tolerance, development, and metabolism. Recent studies using genome editing techniques have shown that the disruption of metabolic genes can induce extensive changes in the transcriptional profile and affect several biological pathways. For instance, the characterization of carotenoid-deficient microalgal mutants obtained by CRISPR/Cas9 ribonucleoprotein complexes revealed extensive changes in metabolic pathways associated with pigment biosynthesis and stress responses [1]. The importance of transcriptomics in understanding the effects of genetic mutations and their impact on cellular processes is evident from such studies.

Besides these genetic changes, microbe-plant interactions also affect plant gene expression and stress responses. The positive effects of microbe-plant associations on plant stress responses under environmental stress have been reported in various studies. The biofilm formation of Pseudomonas putida was reported to increase drought stress tolerance in tomato plants by regulating plant transcriptional responses and improving stress-related gene expression [2]. These interactions also highlight the significance of transcriptional regulation in plant responses to environmental changes.

Recent advances in plant transcriptomics and metabolomics have provided a detailed understanding of plant regulatory networks through integrated approaches that combine transcriptomic and metabolomic analyses. These approaches have helped identify various plant gene modules that control plant metabolism and stress responses in crop plants. Transcriptomic and metabolomic analyses of tomato plants have revealed various plant regulatory modules that control carotene synthesis through coordinated interactions between transcription factors and metabolic enzymes [3]. Similarly, transcriptomic analysis of maize seedlings has revealed molecular mechanisms of heterosis-related drought tolerance in maize plants, including transcription factors that control stress resistance [4]. These examples highlight the significance of transcriptomic analysis in understanding complex plant gene networks that control plant physiological responses.

The regulation of oxidative stress is not only specific to plant organisms but also applicable to various other biological organisms. In microbes, oxidative stress is related to various metabolic regulations. Research on ethanol tolerance in Kazachstania unispora has revealed that membrane composition is a critical factor in oxidative stress tolerance [5]. This indicates that antioxidant defence is of primary importance in various organisms for maintaining stability during stressful situations.

Mutations in detoxifying pathways of plant organisms have also been related to changes in antioxidant response genes. Research on herbicide-resistant rice mutants has indicated that glutathione S-transferase is involved in regulating antioxidant responses, which are protective against chemical stress in plant organisms [6]. In addition, various natural compounds that are effective in regulating oxidative stress responses have also been related to longevity and health through stress signalling pathways [7]. These observations also emphasise the importance of oxidative stress signalling pathways in various organisms.

In the case of developmental processes and programmed cell death, transcriptomics has been extensively utilised to elucidate the regulation of genes. The use of CRISPR/Cas9 to manipulate the regulation of key genes has been instrumental in the elucidation of the regulation of genes involved

in the pathways of programmed cell death and stress responses in various crop plants such as *Brassica napus* [8]. Biochemical and proteomics approaches to elucidate the mechanisms of drought stress responses in wheat mutants with tolerance to drought stress have also been reported [9]. All these studies collectively emphasise the significance of integrating transcriptomics data with functional studies to elucidate the mechanisms of stress responses in plants.

Various transcription factors involved in the regulation of ROS-mediated signalling pathways, MYB transcription factors have been reported to be involved in the regulation of photosynthesis efficiency as well as the response to oxidative damage in **Arabidopsis thaliana** [10]. Transcriptomics and proteomics studies on microbial organisms have also revealed the complex regulatory mechanisms that are involved in the response to oxidative stress as well as metabolic stress [11]. Such regulatory mechanisms are usually mediated through signalling cascades that activate the mechanisms of protection under stress conditions.

One of the most important components of stress tolerance is the ROS scavenging system. Transcription factors controlling the biosynthesis of anthocyanin have been found to play an important role in improving salt and drought stress tolerance by upregulating antioxidant defence mechanisms [12]. Similarly, transcriptome analysis of mutagenesis-induced mutants of banana has also been found to play an important role in improving cold stress tolerance [13]. Transcriptome analysis has also been used to understand the physiological differences between mutant plant varieties. Such differences have been found to play an important role in regulating various important processes [14].

Mutation-based breeding is a significant aspect of the study of the physiology of plants. For instance, EMS mutants of rice have been found to have a significant role to play in the improvement of the efficiency of phosphates [15]. From the study, it was established that the analysis of the transcriptome of the mutants played a significant role in the understanding of the regulation of the mechanisms of growth.

Objectives of the Study

- To identify differentially expressed genes that are linked to antioxidant enzyme mutants based on transcriptomics data.
- To explore the functional classes or biological pathways that are linked to the response mechanisms against oxidative stress caused by disruptions in antioxidant systems.
- To study the functions of ROS-regulated transcription factors and their promoter elements in regulating gene expression in antioxidant-deficient systems.

Materials and Methods

Study Design and Data Source

This study was carried out through secondary transcriptomic data analysis to identify changes in gene expression related to antioxidant enzyme mutant genes and the regulatory mechanisms involved in oxidative stress responses. The data used in this study were obtained from a publicly accessible data repository that contained curated transcriptomic data related to abiotic stress signalling pathways and antioxidant-associated gene regulation in plants [16]. The data repository contained supplementary data related to differential gene expression, transcription factor binding data, and promoter-level data related to genes of interest. This study was carried out using these data sets related to oxidative stress signalling pathways.

Biological System and Mutant Background

The gene identifiers used in the dataset correspond to the model plant *Arabidopsis thaliana*. *Arabidopsis thaliana* has often been used for studying oxidative stress regulation. The dataset provides gene expression profiles for different antioxidant-related mutant lines, including *vtc1, vtc2, cat2, aox1a, tapx,* and *sal1*. These mutants correspond to disruptions in different antioxidant-related gene functions. Since antioxidant enzymes are essential for maintaining intracellular ROS

homeostasis, disruptions in these functions often result in transcriptional reprogramming of stress-related genes. Therefore, studying these mutants is a powerful tool for understanding the molecular basis for the connection between oxidative stress signalling and transcriptional regulation.

Data Extraction and Processing

The supplementary tables accompanying the dataset were downloaded for analysis. The dataset comprised a matrix of differentially expressed genes, which included the fold change values for the corresponding antioxidant mutant genotypes. The gene identifier was normalised according to the *Arabidopsis thaliana* locus nomenclature for consistency within the dataset. The DEG matrix was analysed to identify the expression changes for the corresponding mutant backgrounds.

Differential Gene Expression Analysis

The differential expression patterns were also examined by considering the fold change distributions for each of the different mutant backgrounds. The number of up- or down-regulated genes was also identified for each mutant genotype to understand the degree of transcriptional perturbation caused by the disruption of antioxidant enzymes. The genes that are consistently regulated among multiple mutants were also identified to understand the conserved patterns of transcriptional regulation related to the regulation of oxidative stress signalling. Additionally, the mutant-specific changes in gene expression were also identified to understand the unique patterns of gene expression for each antioxidant deficiency.

Functional Annotation and Biological Interpretation

To interpret the biological importance of the identified differentially expressed genes, the results were analysed for the functional annotations of the genes by searching the available plant gene databases. The differentially expressed genes were grouped according to their functional classes based on their known biological activities in cellular metabolism, stress signalling pathways, redox control mechanisms, and transcriptional control. Special attention was given to the identification of antioxidant defence mechanisms and stress response pathways. Functional classification was useful in identifying the biological processes that were affected by antioxidant enzyme mutations.

Analysis of ROS-Responsive Transcription Factors

Regulatory relationships were analysed for transcriptional regulation using the dataset containing information related to the ROS-responsive transcription factors. The dataset used for the analysis included the type of transcription factor, family, type of regulation, and the number of binding sites detected for the candidate genes' promoters. The transcription factors were classified as primary or secondary regulators for the signalling pathways related to oxidative stress. The frequency of the transcription factors' binding sites was analysed for the DEGs' promoters to identify the transcription factors with the highest association with the transcriptional regulation for the antioxidant mutants. The transcription factors with repetitive occurrences for the different mutants were identified as potential central regulators for the transcriptional regulation networks related to oxidative stress.

Promoter-Level Regulatory Analysis

The information available for the sequences of the promoter regions for the identified candidate genes was obtained from the dataset that provided predicted transcription factor binding sites in the promoter regions. The data available for each gene included information about the start and end position of the motif, the strand for the motif, the motif score, p-value, q-value, and sequence. The parameters were analysed to establish the confidence and potential regulatory importance of each predicted transcription factor-binding event. The potential for the identified genes to have multiple ROS-responsive transcription factor motifs was an important criterion for identifying potential regulatory hubs for oxidative stress signalling pathways.

Integration of Transcriptomic and Regulatory Data

To establish a comprehensive understanding of transcriptional regulation responsive to oxidative stress, differentially expressed gene data were integrated with transcription factor binding data. The integration of these data sets for identifying potential transcription factors that interact and regulate antioxidant enzyme-dependent signalling pathways was an important criterion.

Data Visualisation and Reproducibility

For data organisation and preliminary analysis, spreadsheet-based tools were utilised to enable the systematic comparison of the patterns of gene expression for different mutant genotypes. The graphical representations of gene expression summaries and comparative DEG distribution were generated to enable the interpretation of the patterns of gene expression. Since the current research is based on a publicly available data set and does not involve any experimentation on biological organisms, no special ethical approval is required. The framework for analysis that has been proposed here can be replicated with the data set available in the Mendeley Data repository.

Results

Overview of Differential Gene Expression in Antioxidant Enzyme Mutants

The transcriptomic analysis of the antioxidant enzyme mutants identified significant changes in the expression pattern of the genes associated with the response to oxidative stress. The curated data set identified many genes whose expression levels were significantly altered among the six genotypes of the mutant. The genotypes included *vtc1, vtc2, cat2, aox1a, tapx,* and *sal1*. The distribution of fold change values revealed the induction and repression of genes involved in the disruption of the antioxidant pathway. The number of genes whose expression levels were altered varied among the genotypes. This suggests the involvement of varying levels of transcriptional perturbation among the genotypes. This may be due to the disruption of the specific antioxidant pathway. Among the genotypes of the mutant, the expression levels of some genes were commonly regulated. This suggests the involvement of common oxidative stress signalling pathways. These commonly regulated genes may be the fundamental components of the plant ROS response network. However, some genes were uniquely regulated among the genotypes. This may be due to the transcriptional adaptation associated with the disruption of the specific antioxidant pathway.

Table 1: Differentially expressed genes identified in antioxidant enzyme mutants

Gene ID	Gene Annotation	vtc1	vtc2	cat2	aox1a	tapx	sal1	Expression Pattern
AT1G01040	hypothetical protein	0	0	0	0	0	0	No change
AT1G01050	protein kinase family protein	0	0	0	0	0	-2.07	Downregulated in sal1
AT1G01060	transcription factor-related protein	0	0	0	0	0	-2.66	Downregulated in sal1
AT1G01070	regulatory protein	0	0	0	0	0	-2.61	Downregulated in sal1
AT1G01080	zinc-binding protein	0	0	0	0	0	-2.34	Downregulated in sal1
AT1G01090	membrane-associated protein	0	0	0	0	0	-2.30	Downregulated in sal1
AT1G01100	signal transduction protein	0	0	0	0	0	-2.25	Downregulated in sal1

Mutant-Specific Transcriptional Responses

A comparative evaluation of the mutant-wise gene expression patterns revealed that specific transcriptional signatures were linked with the expression of individual antioxidant enzyme mutants.

The vtc1 and vtc2 mutants, which are linked with ascorbate biosynthesis, revealed significant alterations in the expression of genes linked with redox metabolism and stress signalling. The expression of genes linked with antioxidant defence, cellular metabolism, and signalling regulation was highly responsive, which indicated the activation of compensatory mechanisms for maintaining intracellular ROS balance.

The cat2 mutant, which is linked with catalase activity, revealed pronounced alterations in the expression of genes linked with hydrogen peroxide metabolism and stress signalling. The expression of genes linked with detoxification, metabolic regulation, and transcriptional control was highly responsive, which indicated the significance of catalase for regulating hydrogen peroxide levels within the cell.

The mutant aox1a mutant displayed changes in transcriptional levels of genes related to mitochondrial metabolism and respiration. The involvement of alternative oxidase in electron transport in mitochondria implies that changes in expression of these genes are adaptive responses that attempt to sustain energy metabolism under altered mitochondrial redox states.

The tapx mutant, which is related to thylakoid ascorbate peroxidase, displayed changes in the expression of genes related to chloroplast metabolism and photosynthesis. This implies that alterations in antioxidant defence in chloroplasts affect transcriptional regulation of photosynthetic efficiency.

The sal1 mutant displayed one of the most dramatic changes in transcriptional levels of genes related to stress signalling, transcriptional regulation, and metabolism. This implies that disruption of signalling pathways mediated by SAL1 may affect various aspects of stress responses, as indicated in Figure 1.

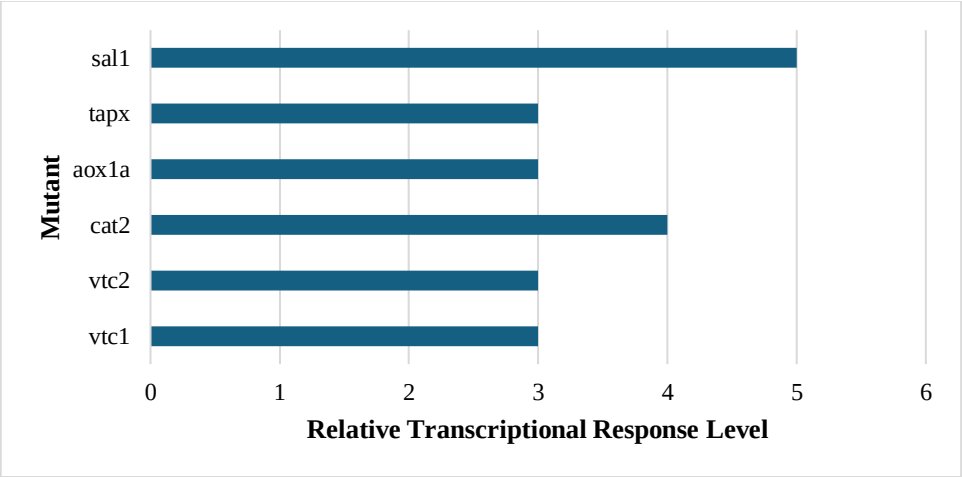

Figure 1: Mutant-specific transcriptional responses in antioxidant enzyme mutants

Shared Differentially Expressed Genes Across Mutants

Analysis of the DEG dataset identified genes that were universally regulated across various antioxidant mutants. The genes identified are likely part of a general oxidative stress response system. The genes are related to stress response, redox balance, metabolic regulation, transcriptional control, and protective responses.

The fact that there are common DEGs across different mutants indicates that deficiencies in antioxidant enzymes activate common signalling pathways that attempt to normalise the cell's redox balance. This may represent a general defence strategy used by the cell under oxidative stress, as indicated in Figure 2.

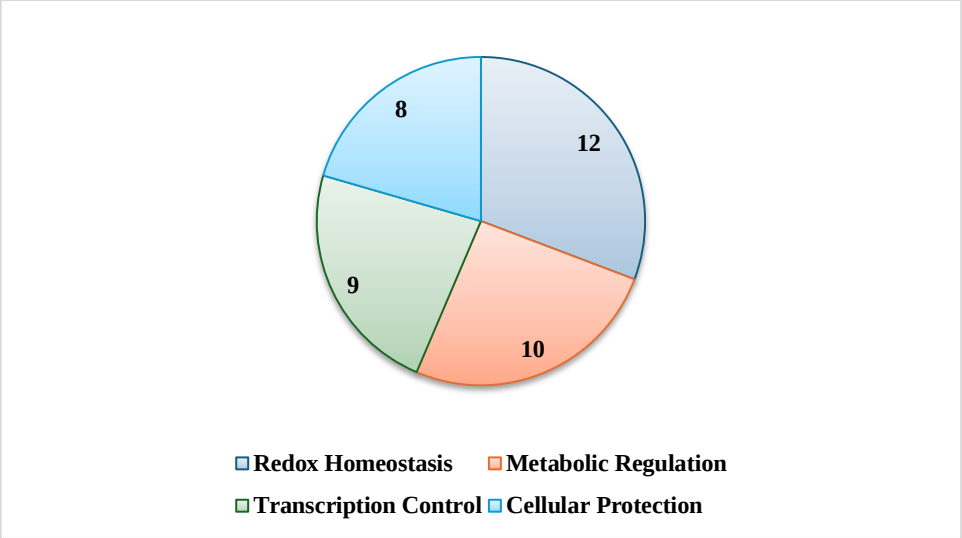

Figure 2: Shared differentially expressed genes across antioxidant enzyme mutants

Functional Categories of Differentially Expressed Genes

Functional annotation of the identified DEGs revealed that the genes affected by antioxidant mutations were involved in various biological processes. Several major functional categories were established. The first category comprises genes involved in the response to oxidative stress. Other significant functional categories were established as well. These include genes involved in signal transduction, transcriptional regulation, metabolic processes, transport functions, and cellular homeostasis.

Genes involved in redox regulation and antioxidant defence were established as an important category of differentially expressed genes. This category of genes is likely to be involved in the cellular defence mechanism against the effects of increased ROS levels due to impaired antioxidant enzyme activity. Signalling pathways were also represented by a large number of differentially expressed genes. This indicates the importance of the regulation of gene expression in the response to oxidative stress.

Metabolic genes were also represented as a significant category of differentially expressed genes. The metabolic genes are involved in various metabolic pathways that include energy metabolism, biosynthesis, and cellular homeostasis. The changes in the expression of metabolic genes likely reflect changes in cellular metabolism to maintain homeostasis under oxidative stress conditions, as represented in Figure 3.

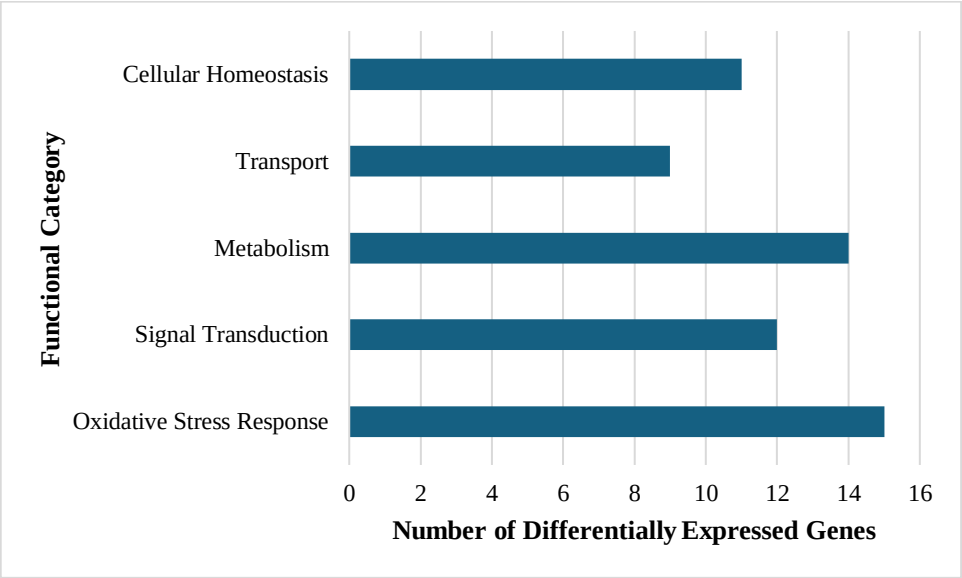

Figure 3: Functional classification of differentially expressed genes identified in antioxidant enzyme mutants

ROS-Responsive Transcription Factors

In the case of the transcription factors data set, it has been found that some ROS-responsive transcription factors were associated with the promoters of the differentially expressed genes. The transcription factors were from various regulatory families that were known to be involved in stress response pathways. The number of transcription factor binding sites found in the promoters of the differentially expressed genes suggested that these factors were important in the regulation of the response to the disruption of antioxidant enzymes.

The transcription factors found in the data set were either primary or secondary regulatory factors. The primary transcription factors were found to be involved in the direct regulation of the genes that were involved in the response to oxidative stress. The secondary transcription factors were found to be involved in the amplification of the response.

Some transcription factors were linked to the promoters of DEGs in all mutants, implying that these transcription factors are key regulators of the oxidative stress transcriptional network. The fact that these transcription factors are present in the promoters of the DEGs in all the mutants implies that they might be coordinating the transcriptional response to ROS balance in different antioxidant mutant backgrounds, as shown in Table 2.

Table 2: ROS-responsive transcription factors and binding-site frequencies in candidate gene promoters

Transcription Factor	TF Family	Regulatory Type	Binding Sites in Upregulated Genes	Binding Sites in Downregulated Genes	Total Binding Sites
WRKY33	WRKY	Primary TF	12	8	20
NAC019	NAC	Primary TF	10	7	17
MYB2	MYB	Secondary TF	8	6	14
bZIP60	bZIP	Primary TF	9	5	14
DREB2A	AP2/ERF	Secondary TF	7	4	11
ZAT12	Zinc finger	Primary TF	6	5	11

Promoter-Level Regulatory Patterns

Promoter analysis of the identified candidate genes showed the presence of multiple transcription factor binding motifs. The dataset provided information regarding the position of the motifs, strand orientation, motif scores, and statistical significance parameters such as p-values and q-values. The presence of multiple high-confidence transcription factor motifs was identified as key regulatory genes for the regulation of oxidative stress signalling pathways.

The presence of ROS-responsive transcription factor motifs in the promoter regions of DEGs indicates the significance of transcription regulation in the regulation of plant response to antioxidant enzyme deficiencies. The promoter motifs are expected to be the target of regulatory proteins for the activation or repression of genes based on the changes in the redox status, as depicted in Table 3.

Table 3: Predicted transcription factor binding sites in promoters of candidate genes

Candidate Gene	Transcription Factor	Start Position	End Position	Strand	Motif Score	p-value	q-value
AT1G01050	WRKY33	145	156	+	12.45	0.0008	0.002
AT1G01060	NAC019	230	241	-	11.98	0.0011	0.003
AT1G01070	MYB2	180	192	+	10.75	0.0023	0.006
AT1G01080	bZIP60	300	312	-	11.34	0.0015	0.004
AT1G01090	ZAT12	210	221	+	10.67	0.0026	0.007

Integration of Differential Expression and Regulatory Information

The integration of DEG data with information on transcription factor binding revealed a sophisticated model of regulation connecting the activity of antioxidant enzymes with the downstream transcriptional response. Genes showing high levels of differential expression tended to contain promoter motifs associated with ROS-responsive transcription factors, implying a relationship between the two.

This integrated approach identified candidate models of regulation where transcription factors respond to oxidative stress signals and then regulate the expression of stress-responsive genes. Such models may be important components of the molecular network regulating plant adaptation to oxidative stress conditions.

The results of this study thus illustrate the importance of the disruption of antioxidant enzyme pathways to the transcriptional reprogramming of the plant transcriptome, including both metabolic and regulatory genes. The integrated analysis of differential gene expression and transcription factor binding provides a perspective on the intricacy of the signalling network involved in the response to oxidative stress.

Discussion

The current transcriptomic analysis offers insight into the molecular response related to disruptions in antioxidant enzyme pathways. The antioxidant system is an essential element for the maintenance of cellular redox balance. The antioxidant system regulates the levels of ROS. The antioxidant system also prevents oxidative damage to cellular components. Mutations related to antioxidant enzymes trigger a response to reprogram the cell to compensate for the disruptions in antioxidant enzymes. The results of the current study showed that antioxidant enzyme mutants have unique transcriptional responses related to genes regulating oxidative stress, metabolism, and transcription.

The changes observed in the antioxidant enzyme mutants' transcripts imply that ROS play a significant role as a signalling molecule that activates antioxidant response pathways. The disruption of antioxidant enzymes that detoxify ROS results in increased hydrogen peroxide. The increased hydrogen peroxide activates downstream genes related to stress response. The regulatory mechanisms are also observed in plant cells. The changes in antioxidant enzymes affect the balance of hydrogen peroxide. The changes also accelerate the rate of processes such as the senescence of leaves. The changes are related to folate metabolism and antioxidant pathways [17].

The transcriptional responses that are mutant-specific point to the complex nature of oxidative stress response regulation. Various antioxidant enzymes are present in different cellular compartments, like chloroplasts, mitochondria, and peroxisomes. This leads to localised responses to oxidative stress when these antioxidant systems are impaired. Similar pleiotropic effects on oxidative stress response regulation have also been observed in microbes. In these microbes, mutations in key regulatory proteins affect several physiological functions like metabolism, development, and pathogenicity [18].

The fact that a number of differentially expressed genes are common among all the mutants' points to the presence of a conserved oxidative stress response network. Such differentially expressed genes are probably involved in fundamental protective mechanisms that are not dependent on the antioxidant pathway that is affected. Conserved oxidative stress response mechanisms have also been observed in mammalian systems. In these systems, the activation of redox-regulated transcription factors like Nrf2 controls protective mechanisms that help in mitigating oxidative stress-induced damage and maintain homeostasis in the cell [19].

The fact that similar regulatory principles exist in different biological systems points to the evolutionary conservation of oxidative stress response mechanisms.

The functional classification of differentially expressed genes showed that these genes are enriched for functions like metabolic regulation, signal transduction, and transcriptional control. Metabolic reprogramming is a general cellular response that helps in adapting to environmental stress conditions. Changes in the expression of metabolic genes help in reorganising the energy resources for protective

mechanisms like detoxification. Transcriptomic analysis of mutant plant lines has shown that changes in gene expression during leaf senescence or developmental stages are regulated by coordinated expression of metabolic or stress response genes [20]. This indicates that oxidative stress not only activates defence mechanisms but also influences other major cellular functions like metabolism.

The analysis of ROS-regulated transcription factors has shown that these transcription factors play a major part in the mechanisms that govern transcriptional responses in antioxidant mutants. Transcription factors are key regulators that help in integrating environmental signals and regulating the expression of downstream target genes that are involved in adapting to stress. In complex biological systems, it has been shown that transcription factor networks play a major part in the progression of disease, differentiation, and stress responses by regulating a number of downstream target genes [21].

Besides the regulation of transcription factors, the response to oxidative stress is also associated with other signalling pathways, such as cell cycles and apoptosis. Molecular studies have confirmed that regulatory molecules like microRNA can affect cell proliferation and migration by regulating the expression of genes related to oxidative stress response and cell signalling pathways [22]. The above observations clearly demonstrate the relationship between redox signalling pathways and other regulatory mechanisms.

The second key mechanism for adapting to oxidative stress is the protective mechanisms related to metabolic processes. The protective mechanisms against ROS-induced damage have been confirmed by molecular studies on bioactive compounds. The antioxidant response is an important mechanism for mitigating cellular injury and improving resilience to stress [23].

Another source of stress factors for the environment is environmental stress factors, which are known to induce oxidative stress responses in plant systems. The use of transcriptomic studies to understand the effect of environmental stress factors, which are known to induce oxidative stress responses, on the expression of genes related to the stress responses of plants exposed to environmental pollutants has shown that there are significant alterations in the expression of genes related to stress responses [24].

Finally, the implications of oxidative stress signalling are relevant for human health, as they are related to stress responses, drug resistance, etc. The studies conducted to understand the molecular targets related to cell proliferation, drug resistance, etc., have shown the implications of oxidative stress signalling for the treatment of cancer, which is a major disease affecting human health [25].

Overall, the results of this study underscore the importance of antioxidant enzymes in controlling transcriptional responses to oxidative stress. The identification of mutant-specific and shared differentially expressed genes provides a glimpse into the complex regulation involved in adapting to changing redox environments. The addition of transcription factor binding site information further adds to our understanding of the complex molecular events involved in oxidative stress signalling, which can be used as a basis for further studies aimed at deciphering the regulation of antioxidant defence systems.

Conclusion

In the current study, a comprehensive analysis of the differentially expressed genes related to the antioxidant enzyme mutants was carried out. The disruption of the antioxidant enzyme-mediated pathways was found to cause significant transcriptional changes, which affected the expression of genes related to oxidative stress, metabolic regulation, signal transduction, and cell homeostasis. This study demonstrated the significance of antioxidant enzymes in maintaining cell homeostasis, which is essential for the regulation of oxidative stress responses. The comparison of the transcriptional profiles from the different mutants revealed both genotype-specific expression profiles as well as common expression profiles, which indicated the presence of common molecular mechanisms regulating oxidative stress responses. Functional analysis of the differentially expressed genes indicated that oxidative stress responses cause extensive transcriptional regulation, which involves the regulation of metabolic, transcriptional, and protective responses. In addition, the analysis of the

promoter regions of the differentially expressed genes revealed the presence of several transcription factors, which are related to the regulation of oxidative stress responses, thereby indicating the significance of transcription factors in regulating oxidative stress responses. The integration of the differentially expressed genes with the transcription factors indicated the presence of potential modules, which are related to the regulation of antioxidants. In conclusion, the current study contributes to the understanding of the molecular aspects of oxidative stress adaptation in the context of plant systems. The identified genes and networks are expected to offer valuable insights into the transcriptional regulation mediated by redox reactions, which can potentially serve as targets for functional studies aiming to improve stress tolerance/resilience in plants.

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