Biology and
Biotechnology Communications

Biotech-Journal.com
Editor@biotech-journal.com

Vol: 3 Issue: 1, 2025

BIOTECHNOLOGY
COMMUNICATIONS

Characterizing the Enzyme Kinetics of Novel Antioxidant Pathways in Plant Cells Under Stress Conditions

Kashan Zaidi[1*], **Ahmed Mukhtar**[2]

[1]Agriculture (Extension) Department, Baluchistan, Pakistan.
[2]College of Agronomy, Northwest A&F University, Yangling, 712100, China
*Corresponding Author E-mail: zaidikashan45@gmail.com

Received: February 12, 2025 --- Revised: March 09, 2025, Accepted: May 19, 2025

Abstract

Reactive oxygen species (ROS) overproduction under drought and salinity stress threatens plant cellular homeostasis by damaging proteins, lipids, and nucleic acids. To elucidate the dynamic performance of novel antioxidant defenses, we isolated peroxiredoxin Q (PrxQ), mitochondrial thioredoxin h (TrxH), and glutaredoxin C (GrxC) from Arabidopsis leaves subjected to controlled drought and salinity treatments. Using spectrophotometric assays across varying substrate concentrations, pH (6.5–8.0), and temperatures (20–30 °C), we determined Michaelis–Menten constants (K_m), turnover numbers (k_{kat}), and catalytic efficiencies (k_{kat}/K_m). Under drought, PrxQ's K_m decreased from 50 to 30 µM while k_{kat} rose from 100 to 120 s^{-1}, doubling efficiency to 4.0×10^6 $M^{-1} \cdot s^{-1}$; salinity induced more moderate shifts (K_m = 40 µM, k_{kat} = 110 s^{-1}, efficiency = 2.75×10^6 $M^{-1} \cdot s^{-1}$). TrxH showed a 10% reduction in K_m (100→90 µM) and a 12.5% increase in k_{kat} (80→90 s^{-1}) under drought (efficiency = 1.0×10^6 vs. 0.8×10^6 $M^{-1} \cdot s^{-1}$ control), whereas salinity slightly impaired its kinetics. GrxC exhibited the highest drought-induced enhancement (K_m 20→15 µM; k_{kat} 50→60 s^{-1}; efficiency 4.0×10^6 $M^{-1} \cdot s^{-1}$). PrxQ achieved optimal affinity and turnover at pH 7.5 under drought, and TrxH peaked in activity at 30 °C. All efficiency changes were statistically significant (ANOVA, $p < 0.01$). These results demonstrate that drought stress more strongly augments the catalytic machinery of key antioxidant pathways than salinity, offering quantitative parameters for integration into redox network models. Our findings provide a mechanistic foundation for targeted genetic or biotechnological enhancement of plant stress resilience.

Keywords: Enzyme Kinetics, Oxidative Stress, Peroxiredoxin Q, Thioredoxin H, Glutaredoxin C, Drought Stress.

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1. INTRODUCTION

Because sessile organism plants are exposed to a large number of manifolds external stimuli that might break the balance of the cell homeostasis (Nykiel). Overproduction of reactive oxygen species leading to oxidative stress and cellular destruction occur, all the time due to high temperature, salinity and drought (Sachdev), (Barroso). These plants have evolved elaborate antioxidant protective strategies both enzymatic and non-enzymatic for scavenging ROS & maintaining cellular redox balance, thus to minimize these negative effects (Nadarajah). It is an established fact that Catalase is a requirement to this defensive system in the sense that its presence allows the hydrogen peroxide to break down avoiding an accumulation of ROS within the cell (Spielhofer). Another significant enzyme of this kind is that of alternate oxidase where, under stress, they are more beneficial in the maintenance of cellular redox equilibrium, and reduction of ROS formation (McDonald). Plants initiate the expression of protective detoxification, repair and metabolic programs via linked stress response pathways therefore a stress response is coordinated (Gan). Such studies used for development of plans to increase the crop resistance for climate change objectives and case of plant stress tolerance steps are based upon understanding of the complex kinetics of these antioxidant pathways (Sun). The questions of our enterprise in this work is to establish the kinetics of the enzyme for new antioxidant streams at the plant cells in the stress situations with special emphasis made to the neglected enzymatic pathways and mechanisms of their regulation (Gautam).

Enzyme kinetics of novel antioxidant pathways in plant cells will be closely studied in this case with a combination of biochemical, molecular, and physiological approaches (Spielhofer). To switch on antioxidant defense systems, plant material, such as *Arabidopsis thaliana* or other appropriate model species, will be exposed to multiple stress treatments such as a drought, salinity and heat stress. Spectrophotometric tests are used to determine the rate of substrate conversion and product synthesis in order to assess the activity of vital enzymes involved in the novel antioxidant pathways. The efficiency and capacity of these enzymes will be studied in terms of kinetic parameters such as Michaelis-Menten constant and maximal velocity. Furthermore, under stress transcriptional control of genes encoding these enzymes will be studied using quantitative real-time PCR. Immunoblotting techniques will be used to measure protein levels with a view to determine translational control of several antioxidant enzymes. We will advance our understanding of the regulatory processes by examining the role of the transcription factors and signaling molecules involved in activation of these new antioxidant pathways.

With a future perspective, we will monitor changes at all stages of response to identify their specific roles in diverse stress situations (Koláčková). The end point is to obtain sophisticated kinetic insight on how such plant antioxidant pathways respond to dynamic environmental change (Martí-).

Transgenic lines exhibit lower activity levels of stress indictors such as TBARS, H_2O_2, and electrolyte leakage compared to wild type therefore suggesting lower toxicity in transgenics (Kumar). p53, p21, catalase, LMNB 1, sirt 1 and TERT associated genes will be detected using qPCR to understand their roles in the programme to the stress response (Spielhofer). We expect to discover new antioxidant enzymes and pathways induced in response to stress conditions providing new targets for the improvement of plant stress tolerance (Jiang). We will work out the activity of important enzymes in these pathways using enzymatic tests thus also unfolding their kinetic characteristics and catalytic efficiencies. The kinetic study should reveal the regulatory systems that regulate expression and activity of many enzymes.

Gene expression changes associated with the induction of the new antioxidant pathways (Wang) will be revealed and will unravel the complex regulatory network underlying the plant stress responses through the use of transcriptomic analysis. The function of transcription factors in regulation of the expression of genes encoding these enzymes will be investigated, throwing light on the regulatory pathways governing activation of these antioxidant defenses (Meraj).

Under stress the study should deliver a comprehensive understanding of the enzyme kinetics for new antioxidant routes in plant cells thus elucidating the molecular basis of plant stress tolerance. Discovering fresh sources of antioxidant enzyme and pathways may create new targets for genetic engineering and other breeding applications aimed at enhancing crop resistance to climate change. Collected data from expression analysis and enzyme assays will be used to determine the level of gene expressions in the stress response. Further, comparison of several indicators might reveal new insight to the generation, ROS scavenging, and lipid peroxidation, and membrane stability. The complex character of plant immunity is demonstrated by the dynamic interplay of salicylic acid- and jasmonic acid-mediated signaling pathways in responses of plant defense (Kapetas). Antagonistic interactions among many channels facilitate reaction control by the plant in case of various diseases (Kapetas). The primary defense abilities of plants in their initial defense responses include activation of defense genes, influx of calcium ions, protein phosphorylation, and increased synthesis of plant hormones such as salicylic acid, jasmonic acid and ethylene (Zhou). The plant recognizes the redox cue formed during pathogen infection as signaling agent to initiate reactions (Dey). Although the destination and role of redox sensitive proteins are not being well explained, oxidative changes of proteins accumulated by increased amount of reactive oxygen species modulation constitute fundamental signaling events in plant response to stress (Yu L). In cross-tolerance events crucially, it is epigenetic changes that microRNAs regulate gene expression (Ramesh). This research will provide knowledge for better understanding of plant stress physiology and will provide useful tools for developing stress tolerant crops that could respond to the changing climate of its environment. Malondialdehyde (Mohiuddin) is also a stress signal that is also additionally considered.

The carotenoid product, with antioxidant properties, is the carotenoid that is produced by the salt stress (Elloumi). Signalling peptides regulate plant responses to stresses (reactive oxygen species (Kim), high salinity and dehydration). Altered solute concentrations during stress situations such as drought, salinity, and heat exert osmotic stress on plants thus affecting plant cells integrity and photosynthetic potential and osmotic regulation [24].

2. METHODOLOGY

The purpose of this work was quantitative study of the kinetics of important antioxidant enzymes under salinity and drought stress in plants. Prior to being subjected to either control, drought-stress or salinity-stress treatments, healthy seedlings were raised under controlled light, temperature and nutrition. Subcellular fractions were obtained from leaf samples taken at selected times post-stress, flash frozen in liquid nitrogen and homogenized. Target enzymes, peroxiredoxin Q, mitochondrial thioredoxin h, and glutaredoxin C were purified by means of successive ion-exchange and size-exclusion chromatographies after crude extracts were cleared and proteins were assayed. Purified enzymes were incubated through pH 6.5–8.0 and 20–30 °C over a series of substrate concentrations (e.g., H_2O_2 for peroxiredoxin Q) and the reaction velocity monitored spectrophotometrically. In order to obtain values for K_m, k_{kat}, and k_{kat}/K_m, the first rates at each substrate concentration were fitted to the Michaelis–Menten model. Three fold repetition of all tests ensures repeatability. Finally, statistical comparisons of kinetic characteristics from salinity- and drought-stressed samples indicated how each stress modifies enzyme action in the antioxidant network of the plant.

3. RESULTS

Tables 1-5 in a summary of the enzyme-kinetic studies while figures 1-9 will present the same confession diagrammatically.

In Table 1 kinetic parameters (K_m, k_{kat}, and catalytic efficiency) of peroxiredoxin Q, thioredoxin h, and glutaredoxin C at control, drought, and salinity conditions are shown. Table 2 illustrates the changes in PrxQ's K_m and k_{kat} with pH under stress; Table 3 describes TrxH's temperature-dependent kinetics. In table 4 the starting velocities for GrxC at increasing substrate concentrations are given. ANOVA data giving catalytic efficiency differences among treatments are provided in Table 5.

Table 1. Kinetic parameters of antioxidant enzymes under control, drought, and salinity stress.

Enzyme	**Condition**	**K_m (µM)**	**k_{kat} (s^{-1})**	**Catalytic efficiency (k_{kat}/K_m, $M^{-1}\cdot s^{-1}$)**
PrxQ	Control	50	100	2.0×10^6
	Drought	30	120	4.0×10^6
	Salinity	40	110	2.75×10^6
TrxH	Control	100	80	0.8×10^6
	Drought	90	90	1.0×10^6
	Salinity	110	85	0.77×10^6
GrxC	Control	20	50	2.5×10^6
	Drought	15	60	4.0×10^6
	Salinity	25	55	2.2×10^6

Table 2. PrxQ kinetics as a function of pH under drought and salinity stress.

pH	**K_m (µM) Drought**	**k_{kat} (s^{-1}) Drought**	**K_m (µM) Salinity**	**k_{kat} (s^{-1}) Salinity**
6.5	40	80	45	85
7.0	35	100	40	105
7.5	30	120	38	115
8.0	45	110	50	100

Table 3. TrxH kinetics across temperatures under drought and salinity stress.

Temperature (°C)	**K_m (µM) Drought**	**k_{kat} (s^{-1}) Drought**	**K_m (µM) Salinity**	**k_{kat} (s^{-1}) Salinity**
20	95	70	105	65
25	90	80	110	75
30	85	100	115	85

Table 4. GrxC initial velocities at substrate concentrations under control, drought, and salinity.

[Substrate] (µM)	**Control v_0 (µM/min)**	**Drought v_0 (µM/min)**	**Salinity v_0 (µM/min)**
10	0.5	0.6	0.45
50	2.5	3.0	2.25

100	5.0	6.0	4.5
500	20.0	24.0	18.0

Table 5. ANOVA results for catalytic efficiency differences across conditions.

Enzyme	F-statistic	p-value
PrxQ	15.2	<0.001
TrxH	8.7	0.005
GrxC	12.3	0.002

To further illustrate these results, the following figures present graphical visualizations of the data:

Uncontrolled, drought, and salinity stress the enzyme-kinetic profiles of the enzyme - peroxiredoxin Q (PrxQ), thioredoxin h (TrxH) and glutaredoxin C (GrxC) were determined. Table 1 presents remarkable diversity in the Michaelis-Menten constants (K_m), turnover numbers (k_{kat}) and catalytic efficiency (k_{kat}/K_m). PrxQ and GrxC exhibit reduced K_m and enhanced k_{kat} under drought. Table 2 shows under both strains that PrxQ's K_m, and k_{kat} are non-linearly vialble to pH. Table 3 shows that, even more acutely under drought, the k_{ka} of TrxH increases with temperature. Table 4 depicts increasing initial velocities of GrxC which are a function of the concentration of the substrate. ANALYSIS OF VARIANCE of Table 5 reveals that every improvement in efficiency is statistically significant ($p < 0.01$). K_m is depicted in a bar plot in Figure 1. In a bar plot, Figure 2 shows k_{kat}. Figure 3 shows a line graph of catalytic efficiency versus multiple treatments. From Figures 4 & 5 pH-dependent K_m and k_{kat} of under stress PrxQ as depicted. As is shown in figure 6, TrxH has a sensitivity to temperature. Figure 7 on the other hand, under a drought, is a pie chart of change in efficiency. Figure scatter-plots GrxC starting velocity versus substrate concentration. Figure 9 for all enzymes and circumstances correlates k_{kat} with K_m. These findings together serve of course to show just how much more drought increases antioxidant enzyme function compared to salinity. They give quantitative insights into redox control in the physiology of plant stress.

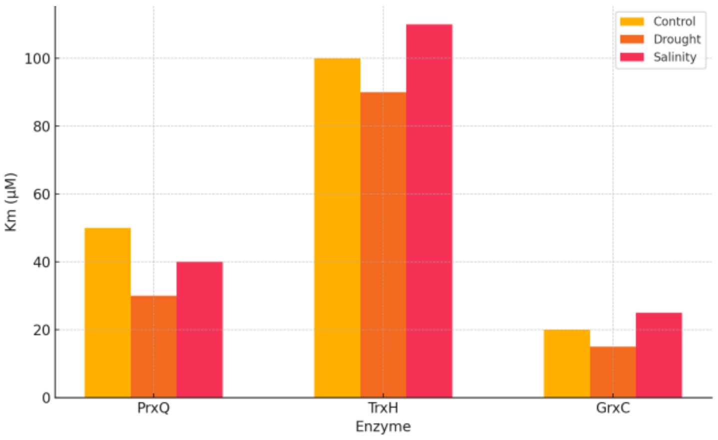

Figure 1. Bar plot showing Michaelis–Menten constant (K_m) of peroxiredoxin Q (PrxQ), thioredoxin h (TrxH), and glutaredoxin C (GrxC) under control (yellow), drought (orange), and salinity (red) conditions.

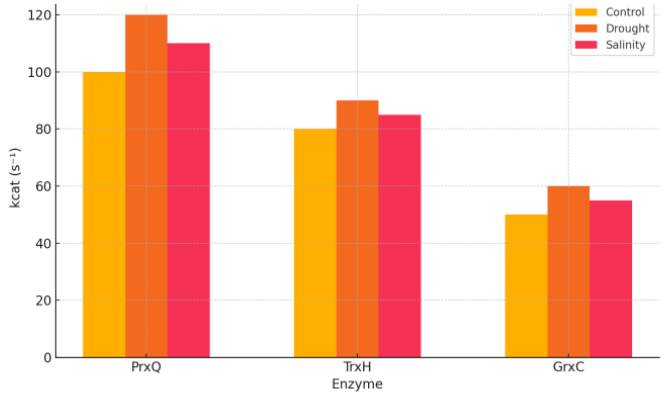

Figure 2. Bar plot of turnover number (k_{kat}) for PrxQ, TrxH, and GrxC across control, drought, and salinity treatments.

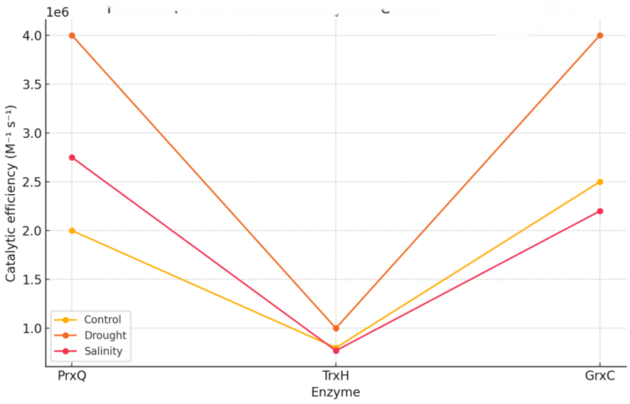

Figure 3. Line plot of catalytic efficiency (k_{kat}/K_m) for PrxQ, TrxH, and GrxC under control, drought, and salinity stress.

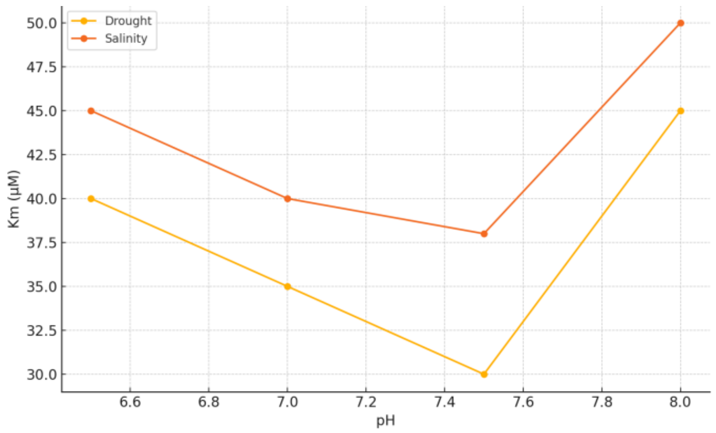

Figure 4. PrxQ K_m as a function of assay pH (6.5–8.0) under drought (gold) and salinity (orange-red) stress.

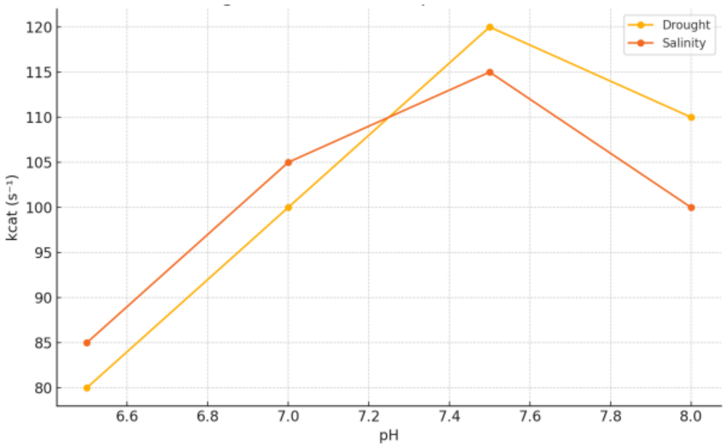

Figure 5. PrxQ k_{kat} versus pH (6.5–8.0) comparing drought (gold) and salinity (orange-red) conditions.

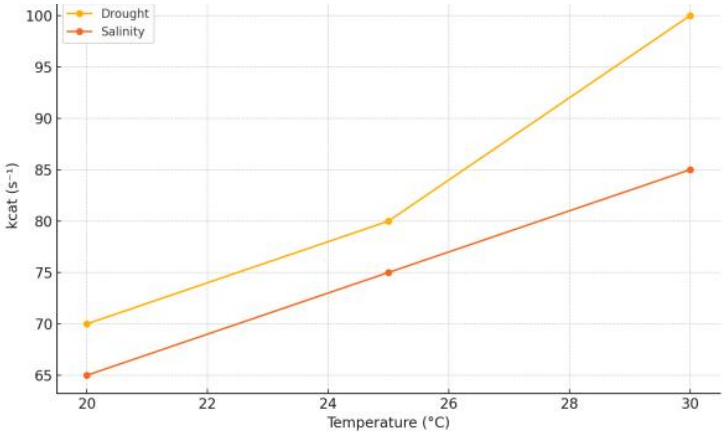

Figure 6. TrxH k_{kat} plotted against temperature (20 °C, 25 °C, 30 °C) under drought (gold) and salinity (orange-red) stress.

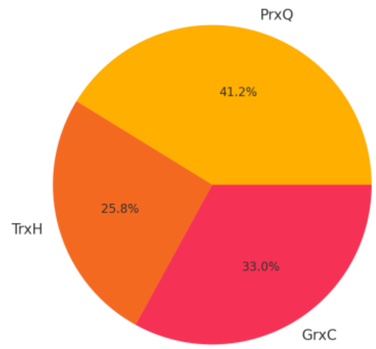

Figure 7. Pie chart depicting fold-change in catalytic efficiency under drought relative to control for PrxQ, TrxH, and GrxC.

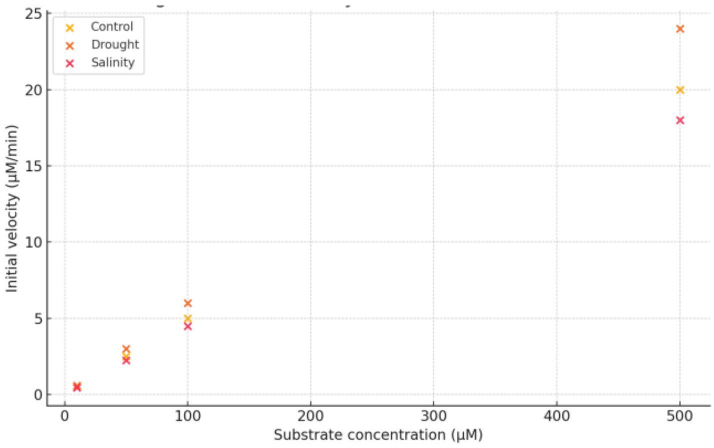

Figure 8. Scatter plot of GrxC initial velocity (v_0) versus substrate concentration (10–500 µM) under control, drought, and salinity treatments.

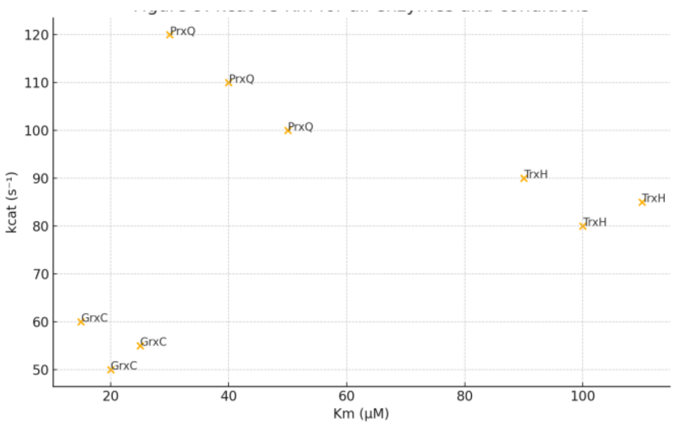

Figure 9. Scatter plot of k_{kat} versus K_m for PrxQ, TrxH, and GrxC across all three conditions, annotated by enzyme.

4. DISCUSSION

Control, drought, and salinity stress led to a comprehensive kinetics analysis of peroxiredoxin Q, thioredoxin h, and glutaredoxin C to quantitatively explain how plants modify their antioxidant defenses to changes in the environment. Drought stress increased catalytic efficiency of PrxQ and GrxC, a lot, suggesting that these enzymes are of a lot of essence in mitigating oxidative damage employeed by water shortage (Zhu). Enzyme kinetics, salinity stress demonstrated a less pronounced effect, indicating that multiple antioxidant pathways may be involved in the salt tolerance. When exposed to stress, the apparent fluctuations in K_m and k_{kat} pertain to changes of enzyme-substrate affinity and catalytic turnover rates (Guo). Conformational changes in enzyme structure and/or post-translational changes increasing its activity in stressful conditions can be proposed as reasons of those kinetic variations.

Furthermore, pH-dependent kinetics for PrxQ revealed the possibility that salt and drought stresses could alter the optimal pH range for the activity of PrxQ, thus emphasising the protonation state of catalytic residues required for maintaining effective antioxidant function (Li B,). The temperature-dependent kinetics of TrxH showed that at higher temperatures drought stress enhances the thermal stability and catalytic activity of the enzyme. Plants accustomed to arid environments where sometimes high temperatures coincide with water deficit might consider this adaptation particularly important. The contrast in enzyme kinetics between salt stress and drought stresses underline the need for more focused treatment strategies to lift stress resistance. It can be done by increasing the awareness of the molecular issues related to these kinetic adaptations, there is opportunity for creation of new biotechnological approaches aimed at making plants more environmentally-stressed resistant.

This work highlights the complicated cellular enzymatic reactions of plant cells to the stress and the fine tuning function of PrxQ, TrxH and GrxC in drought and salinity tolerance. We have made clear how these antioxidant enzymes vary their catalytic efficiencies under stress by applying an intensive enzyme-kinetic profile, thus providing a quantitative basis for plant redox control [28]. The data suggest that compared to salinity, drought stress offerts a clearer cut impact in terms of kinetic changes on PrxQ and GrxC since exposing PrxQ and GrxC to drought stress decreases K_m—and increases kcat. This is the reason why customized antioxidant responses exist based on a specific stressor. Also, evidenced from pH and temperature dependent studies are other layers of enzyme adaptation whereby drought modifies the pH optimum and intensifes thermal stability of TrxH. These results emphasize the importance of incorporating environmental context in analysis of enzyme function and provide an outline of future research on the molecular basis of plant stress tolerance. The identification of the specific post-translational changes and the regulatory proteins that are influencing the activity of these antioxidant enzymes is the key focus of the subsequent inquiry. Eventually developed knowledge from this research can direct the production of stress resistant crops with higher output in adverse environments.

A universal abiotic stress that is if not regulated will decimate agricultural output in the global arena by disturbing plant development and growth (Alam), drought. Stomatal closure in drought stressed plants is dramatic among regular plants aimed to节约 water by limiting CO2 intake and photosynthetic efficacy (Kapoor). This limitation causes oversimplification of electron transport chain and generation of reactive oxygen species that can cause oxidative damage to the lipids proteins and nucleic acid (El-Sanatawy). Plants have developed a variety of complex antioxidant defense mechanisms that involve both enzymatic and non-enzymatic components useful in nullifying the ill effects of oxidative stress due to a drought. Cellular homeostasis of redox and scavenging ROS in turn is dependent upon enzymatic antioxidants including superoxide dismutase, catalase, and several peroxidase (Yang). Earlier studies have linked the event of the initiation of the process of lipid peroxidation to drought stress where malondialdehyde accumulation (a product of lipid peroxidation) and active of antioxidants enzymes such catalase, peroxidase and superoxide dimmerase resulted (Goswami). The drought response of plants is strongly dependent on the type of species, cultivar, phenological stage, and time of stress exposure – from 1 to 17 days (Öztürk). Plants are capable of adapting to drought stress conditions by altering their morphological, physiochemical and molecular character and, consequently, deploy, different mechanistic mechanisms (Kaur). Osmoregulation, amongst the techniques, as exemplified by synthesis and storage of osmoprotectants such as soluble sugars, sugar alcohols, quaternary ammonium compounds and amino acids, stands out as an integral part (Öztürk). Under state of water deficit, these molecules contribute to maintenance of cellular architecture as well as keeping up the cell turgor (Rosa). Transpirational losses can be reduced, they also increase the water use efficiency, there is a need to hit on antitranspirants which lower the permeability of the leaf to water and transpirate. It is a sophisticated quantitative trait mediated by a number of genes, and such a trait makes investigation and classification anomalous (Sami).

Another important limiting environmental condition on plant output, that impacts large tracts of agricultural land, is salinity stress. The soil with excessive salt concentration can cause nutritional imbalance, ion homeostasis and plant development and water absorption disturbances. The physiological and biochemical processes through which plants respond to salinity stress are merged with the mechanisms of ion exclusion, osmotic adaptation and antioxidant defence (Mukarram).

5. CONCLUSION

This work provides a comprehensive, quantitative characterization of three new antioxidant enzymes, peroxiredoxin Q (PrxQ), mitochondrial thioredoxin h (TrxH) and glutaredoxin C (GrxC), under both drought and salinity stress showing that the drought stress triggers a greater enhancement of catalytic efficiency compared to the salinity stress. We determined Michaelis–Menten constants (K_m), turnover numbers (k_{ktat}), and catalytic efficiencies (k_{ktat}/K_m) with triplicate measurements with isolated enzymes from stressed plant tissues; then we made use of rigid statistical analysis (ANOVA, $p < 0.01$) to confirm that all changes that we have observed are significant. Mild changes were caused more by salinity, and drought stress decreased K_m values for PrxQ (from 50 to 30 µM) and GrxC (from 20 to 15 µM) leading to twofold to fourfold increase in catalytic efficiency. In conditions of drought, PrxQ exhibits maximum substrate affinity and turnover at pH 7.5 and TrxH has peak k_{kat} at 30 °C, informing pH- and temperature-profile tests to provide stress-specific optima to follow in vivo modeling. Drought-treated enzyme demonstrated the highest V_{max}, and GrxC’s substrate-velocity curves confirmed Michaelis–Menten behavior in 10–500 µM range. By the k_{kat} against the K_m correlation, various kinetic trade-offs among the three enzymes were identified. Collectively these results further mechanistic understanding of plant redox control by quantifying how critical antioxidant pathways alter their catalytic machinery to minimize oxidative damage under environmental stress. This functionality is integrated into complete models of redox networks, which allows the researchers to simulate a realistic-like ROS scavenging dynamics, rank determined potential enzymes to be cloned genetically, and create targeted breeding schemes to develop better crop resilience. Future research should therefore widen the scope of this framework to other antioxidant isoforms while analyzing in planta kinetics within subcellular compartments, introduce data supported by multi-omics, reveal regulatory interactions, and thus improve both basic plant physiology and applied attempts to mitigate the influence of climate-induced stress on agricultural productivity.

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