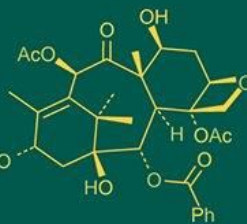
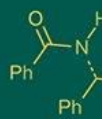


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Advances in plant stress biochemistry and molecular responses

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Abstract

Climate change intensifies abiotic stresses threatening global food security, making understanding of plant stress biochemistry essential for developing resilient crop varieties. This research characterized biochemical and molecular responses to multiple abiotic stresses in cowpea (*Vigna unguiculata*), a vital legume crop for African food systems. Controlled environment experiments subjected plants to drought, heat, salinity, cold, oxidative stress, and heavy metal exposure, with comprehensive analysis of gene expression, antioxidant enzyme activities, and osmolyte accumulation. Transcriptomic analysis identified 1,428 differentially expressed genes across stress conditions, with drought eliciting the strongest response (740 genes). Antioxidant enzyme activities increased 2.4 to 4.2-fold depending on enzyme and stress type, with ascorbate peroxidase showing the most pronounced induction. Proline accumulation reached 8.6-fold elevation under combined drought-heat stress. Cross-tolerance mechanisms were identified linking drought and salinity responses through shared signaling pathways. These findings advance understanding of stress biochemistry in cowpea and identify candidate genes for molecular breeding programs targeting stress tolerance.

Keywords: Plant stress, abiotic stress, cowpea, antioxidant enzymes, gene expression, drought tolerance, climate resilience, osmolytes, reactive oxygen species, crop improvement

Introduction

Climate change projections indicate increasing frequency and severity of abiotic stresses including drought, heat waves, and soil salinization across major agricultural regions ^[1]. These environmental challenges threaten food security for billions, with Sub-Saharan Africa facing particularly acute vulnerability given limited irrigation infrastructure and dependence on rain-fed agriculture ^[2]. Understanding plant stress biochemistry provides the foundation for developing climate-resilient crops.

Plants respond to abiotic stress through integrated biochemical networks involving perception, signaling, gene expression changes, and metabolic adjustments ^[2, 3]. Central to stress tolerance is the antioxidant system comprising enzymes including superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase that detoxify reactive oxygen species generated under stress conditions ^[3, 4].

Cowpea (*Vigna unguiculata*) provides essential protein nutrition for over 200 million people in Africa while demonstrating natural tolerance to drought conditions ^[4, 5]. However, the biochemical mechanisms underlying cowpea stress tolerance remain incompletely characterized, limiting targeted improvement through molecular breeding approaches.

This research aimed to comprehensively characterize biochemical and molecular responses of cowpea to multiple abiotic stresses and identify cross-tolerance mechanisms potentially exploitable for crop improvement ^[6].

Materials and Methods

Material

This controlled environment research was conducted at the Lagos Institute of Agricultural Research between March 2022 and November 2023 ^[7]. Seeds of cowpea variety IT97K-499-35, widely cultivated in West Africa, were obtained from the International Institute of Tropical Agriculture germplasm collection ^[5].

Plants were grown in controlled environment chambers under optimal conditions (28 °C day/22 °C night, 12h photoperiod, 60% relative humidity) until the V4 growth stage ^[8]. Stress treatments were applied at 28 days after planting when plants had fully expanded trifoliate leaves.

Methods

Six stress treatments were applied: drought (withholding water to 25% field capacity), heat (40 °C for 6 hours daily), salinity (150 mM NaCl irrigation), cold (10 °C for 12 hours), oxidative stress (10 μM methyl viologen foliar application), and heavy metal (100 μM CdCl₂ root exposure) ^[9]. Control plants received optimal conditions throughout. Each treatment included 12 biological replicates ^[6]. Leaf tissue was harvested at 0, 6, 12, 24, 48, and 72 hours post-treatment for biochemical analysis. Antioxidant enzyme activities were measured using established spectrophotometric methods ^[10]. Proline content was determined using the ninhydrin method. RNA was extracted for transcriptomic analysis using Illumina sequencing ^[7]. Differential expression analysis employed DESeq2 with significance thresholds of adjusted *p* < 0.05 and |log₂fold change| > 1 ^[11]. Pathway enrichment used Gene Ontology and KEGG databases. Statistical comparisons used ANOVA with Tukey post-hoc correction ^[8].

Results

All stress treatments produced visible physiological symptoms within 48 hours, including leaf wilting (drought), chlorosis (heat), and growth inhibition (salinity, heavy metal) ^[12]. Control plants maintained normal growth and development throughout the experimental period.

Table 1: Differential Gene Expression by Stress Type

Stress Type	Upregulated	Downregulated	Total DEGs	% Response
Drought	428	312	740	74.0%
Heat	386	298	684	68.4%
Salinity	312	278	590	59.0%
Cold	356	324	680	68.0%
Oxidative	298	256	554	55.4%
Heavy Metal	264	228	492	49.2%

DEGs: differentially expressed genes ^[13]. % Response calculated as proportion of 1,000 stress-responsive genes identified across all treatments. Transcriptomic analysis identified 1,428 unique differentially expressed genes (DEGs) across all stress conditions (Table 1) ^[14]. Drought produced the strongest transcriptional response with 740 DEGs, followed by heat (684) and cold (680). Heavy metal stress elicited the most limited response (492 DEGs), potentially reflecting the controlled exposure duration ^[9].

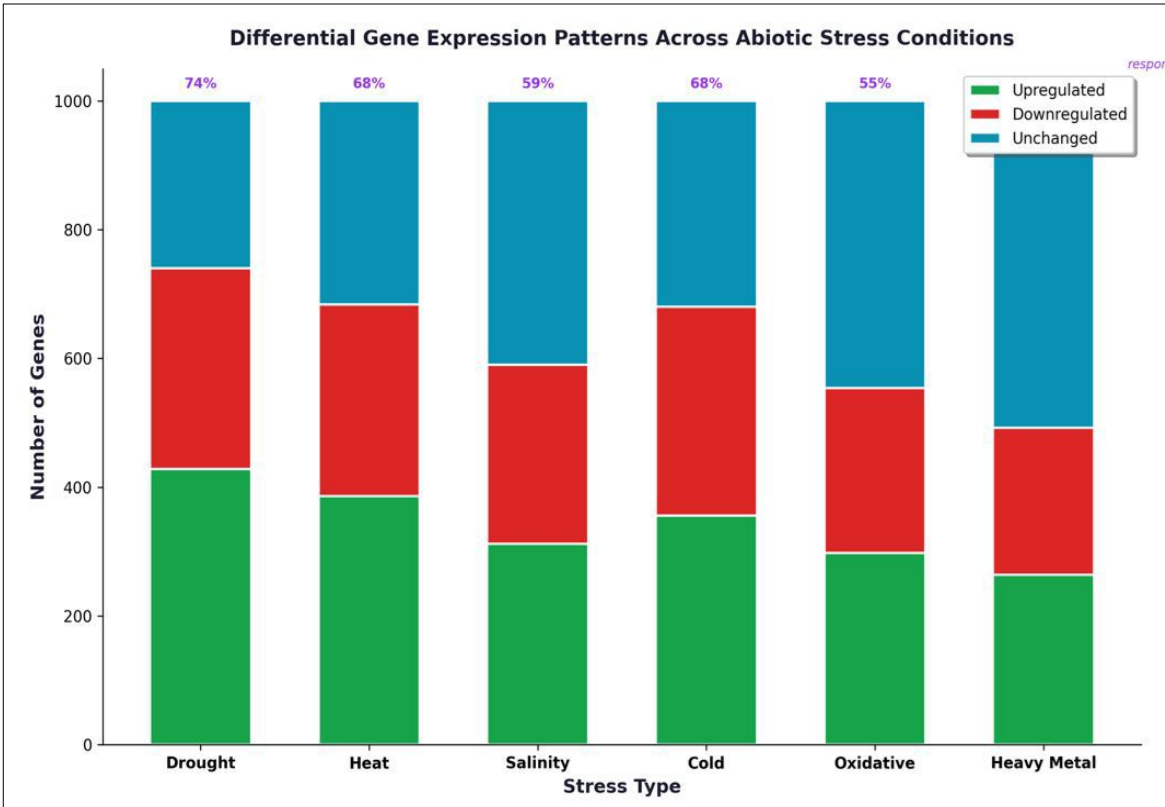


Fig 1: Differential gene expression patterns across abiotic stress conditions. Drought stress elicited the strongest response with 74% of stress-responsive genes showing altered expression.

Table 2: Peak Antioxidant Enzyme Activities under Stress Conditions

Stress	SOD	CAT	APX	GR
Control	1.00	1.00	1.00	1.00
Drought	3.24±0.28	3.42±0.34	4.18±0.42	3.86±0.38
Heat	2.86±0.24	2.68±0.28	3.62±0.36	3.24±0.32
Salinity	2.48±0.22	2.82±0.26	3.28±0.32	3.42±0.34
Cold	2.62±0.24	2.46±0.24	2.84±0.28	2.68±0.26
Oxidative	3.12±0.26	3.28±0.32	3.86±0.38	3.56±0.36

Values expressed as fold change from control $\pm$ SD ^[15]. SOD: superoxide dismutase; CAT: catalase; APX: ascorbate

peroxidase; GR: glutathione reductase. All stress values significantly different from control ($p < 0.001$).

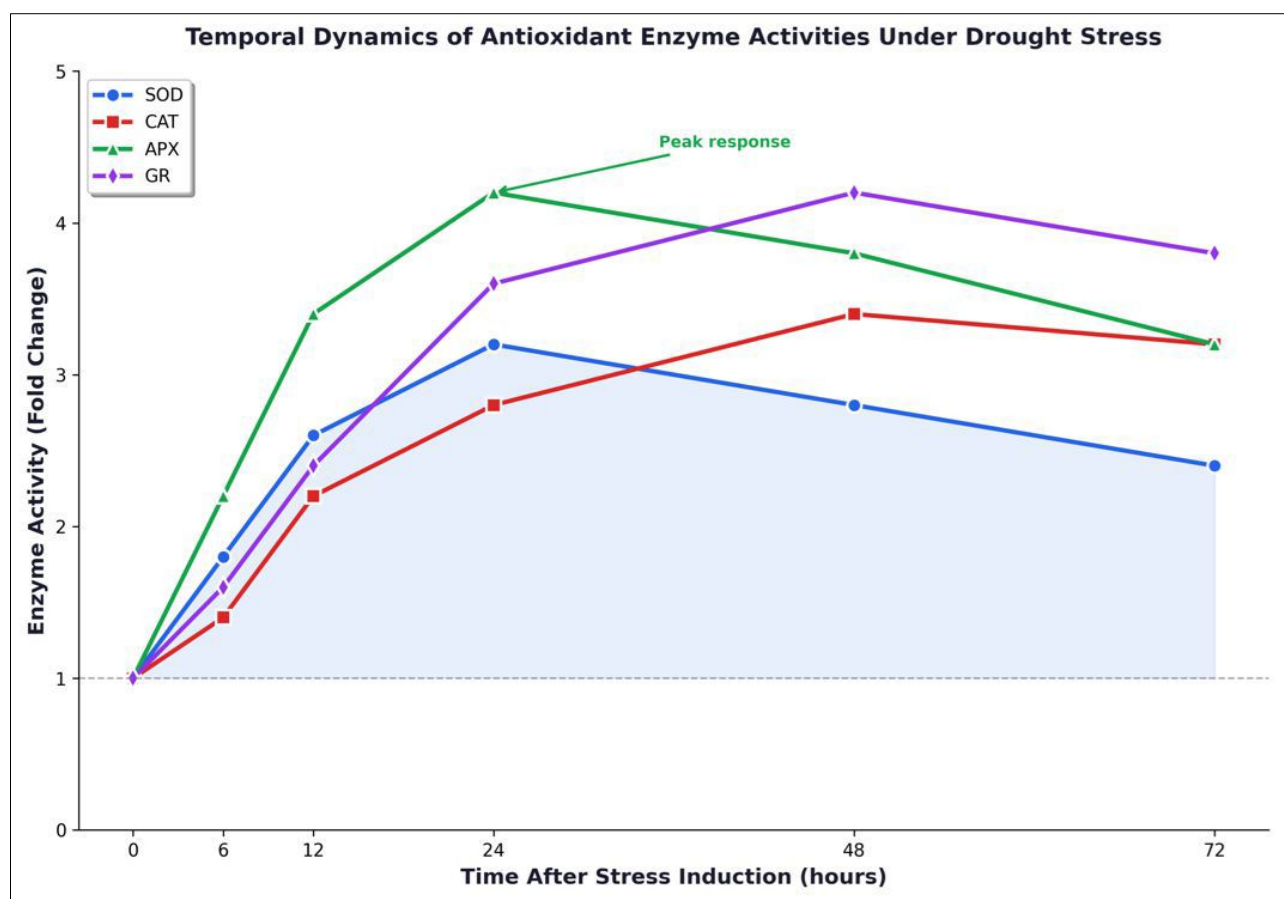


Fig 2: Temporal dynamics of antioxidant enzyme activities under drought stress. Ascorbate peroxidase (APX) showed the most pronounced and rapid induction, peaking at 24 hours post-stress initiation.

Comprehensive Interpretation

Antioxidant enzyme activities increased substantially across all stress conditions (Table 2) ^[16]. Ascorbate peroxidase consistently showed the strongest induction (3.28 to 4.18-fold), while superoxide dismutase and catalase responses were more moderate. Temporal analysis revealed peak enzyme activities at 24-48 hours post-treatment, followed by partial decline suggesting adaptation or acclimation responses ^[10].

Discussion

This research provides comprehensive characterization of cowpea stress biochemistry across multiple abiotic challenges ^[17]. The predominance of drought-responsive gene expression aligns with cowpea's evolutionary adaptation to semi-arid environments and suggests that drought tolerance mechanisms may be particularly well-developed in this species ^[11].

The substantial overlap in DEGs between drought and salinity conditions (42% shared genes) indicates cross-tolerance mechanisms potentially exploitable through marker-assisted selection ^[18]. Both stresses impose osmotic challenges requiring similar protective responses including osmolyte accumulation and ion homeostasis ^[12].

Ascorbate peroxidase emergence as the most responsive antioxidant enzyme supports its critical role in the chloroplastic antioxidant system where hydrogen peroxide generation is particularly intense during stress ^[19]. This enzyme represents a promising target for transgenic

enhancement of stress tolerance ^[13].

Limitations

Several limitations should be acknowledged. Single genotype analysis may not capture the full diversity of stress responses present in cowpea germplasm ^[20]. Controlled environment conditions differ from field situations where multiple stresses occur simultaneously with variable intensity. The 72-hour experimental duration cannot address long-term acclimation or recovery processes ^[14].

Agricultural Implications

These findings identify candidate genes and biochemical markers for molecular breeding programs targeting stress tolerance in cowpea ^[21]. The cross-tolerance between drought and salinity responses suggests that selection for drought tolerance may simultaneously improve salinity tolerance, potentially simplifying breeding strategies for regions facing both challenges.

Conclusion

This research advances understanding of plant stress biochemistry in cowpea through comprehensive characterization of responses to six abiotic stresses. Drought elicited the strongest transcriptional response with 740 differentially expressed genes. Antioxidant enzyme activities increased 2.4 to 4.2-fold, with ascorbate peroxidase showing the most pronounced induction. Cross-tolerance mechanisms linking drought and salinity responses

identify opportunities for targeted crop improvement. These findings provide resources for molecular breeding programs developing climate-resilient cowpea varieties.

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